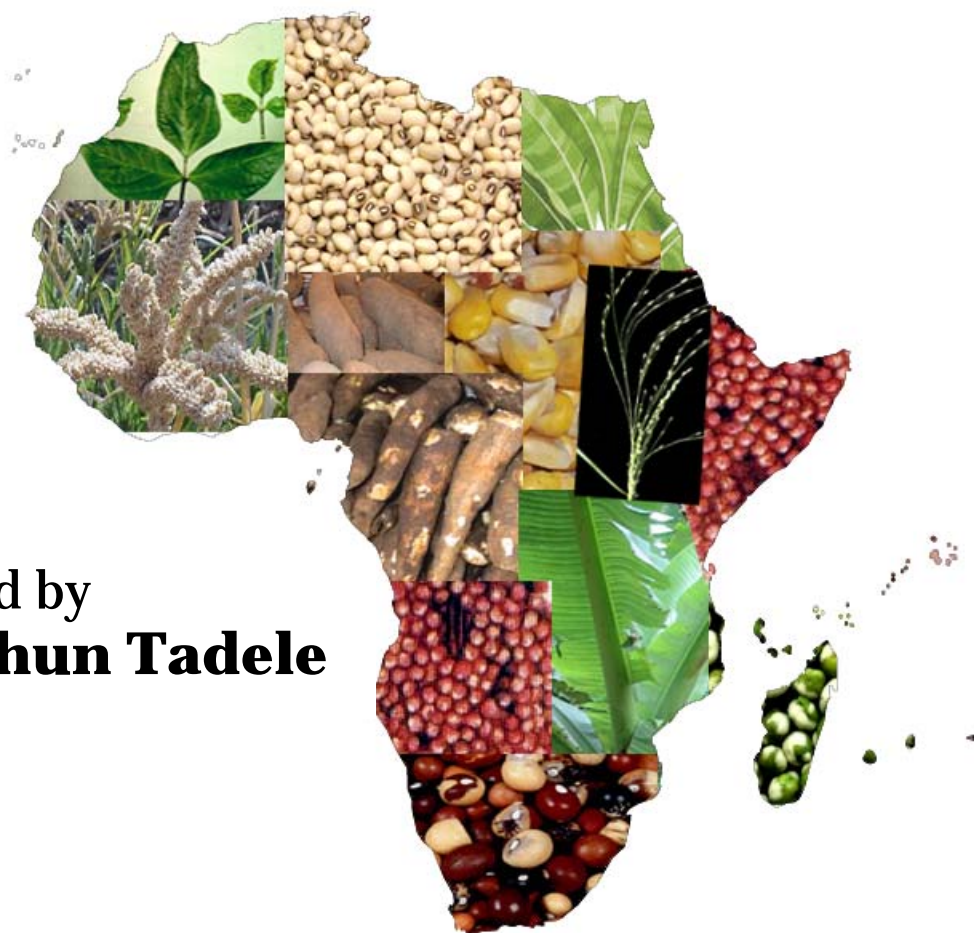


**NEW APPROACHES TO
Plant Breeding of
Orphan Crops in Africa**



Edited by
Zerihun Tadele

**Proceedings of an International
Conference, 19-21 September 2007,
Bern, Switzerland**

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Preface

Orphan- or understudied-crops contribute to the diet of large population of resource-poor consumers and also generate income for smallholder farmers in Africa. These crops perform better than major crops in adapting to extreme environmental conditions such as drought and heat. Orphan crops are mostly neglected by the world scientific community. Due to lack of genetic improvement these largely neglected crops remain to produce inferior yields in terms of both quality and quantity.

In order to develop awareness and devise mechanisms for future improvement of orphan crops, we took an initiative to organize the First International Conference entitled 'New Approaches to Plant Breeding of Orphan Crops in Africa' that was held from 19 to 21 September 2007 in Bern, Switzerland. At the conference scientists from both major and orphan crops discussed the possibilities of transferring modern improvement techniques developed for major crops to orphan crops. Efforts made to bring the awareness of orphan crops to the world community were also presented. At the round-table the panelists discussed the prospects of modern crop biotechnology in future African agriculture. Suggestions were also given to build not only North-South but also South-South collaboration regarding orphan crop improvement.

We would like to thank the following organizations or institutes for sponsoring the conference: IIIème Cycle Romand en sciences biologiques, CTA (Technical Centre for Agriculture and Rural Cooperation ACP-EU), Max und Elsa Beer-Brawand-Fonds, National Centre of Competence in Research (NCCR), Plant Survival Doctoral Programme, Swiss Committee for Molecular Biology (SKMB), Swiss National Science Foundation, Syngenta Foundation for Sustainable Agriculture, and University of Bern. We are also grateful to Prof. Felix Frey, Vice-Rector of the University of Bern for officially opening the conference, and Dr. Andrew Bennett, former Executive Director of Syngenta Foundation for Sustainable Agriculture for closing remarks. In addition, we acknowledge Mrs Franziska Lanz, Dr. Peter von Ballmoos, Mr. Roman Köpfli and Mr. Alexandre Dell' Olivo for their support in organizing the conference.

Zerihun Tadele and Cris Kuhlemeier

Organizers of the Conference

Orphan crops of Africa: their significance and need for improvement

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Summary

Orphan- or understudied-crops are considered as staple food crops in many developing countries. These under-researched crops are categorized under main crop types such as, cereals [e.g., finger millet (*Eleusine coracana*) and tef (*Eragrostis tef*)], legumes [cowpea (*Vigna unguiculata*), and bambara groundnut (*Vigna subterranea*)], root crops [cassava (*Manihot esculenta*), and yam (*Dioscorea sp.*)], and fruit crops [banana and plantain (*Musa spp.* L)]. Orphan crops are more tolerant than major crops of the world, such as wheat and maize, against extreme soil and climatic conditions. However, due to lack of genetic improvement, these crops produce inferior yield in terms of both quality and quantity. The major bottlenecks affecting the productivity of these crops are genetic traits such as low yield (for example, in finger millet and tef), poor in nutrition [cassava 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 needed to increase food production of under-researched crops in order to feed the ever increasing population in Africa. Modern crop breeding utilizes techniques such as Marker-Assisted Breeding, reverse genetics approach such as TILLING (Targeting Induced Local Lesions IN Genome), or transgenics. The application of these techniques to the understudied crops is important in order to boost productivity and feed the largely underfed and undernourished population of Africa.

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

Orphan crops are also known as understudied-, underutilized-, lost-, or disadvantaged crops. These crops play particular role in food security, nutrition, and income generation to resource-poor farmers and consumers in developing countries. 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) proven able to be cultivated either 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 (http://en.wikipedia.org/wiki/Underutilized_crops, accessed 17 August 2009). According to Naylor *et al.* (2004) twenty-seven orphan crops within developing countries are annually grown on about 250 million ha of land.

Orphan 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 such as finger millet and bambara groundnut are extremely drought tolerant while some others withstand waterlogging for longer period than the major crops of the world.

In addition, orphan crops are compatible to the agro-ecology and socio-economic conditions of the continent. However, when these crops were 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 increases whenever exotic crops specifically maize substituted large area of previously occupied by indigenous or orphan crops (Kebede *et al.* 2005, McCann 2005, Ye-Ebiyo *et al.* 2000). Malaria is the major health problem in the world particularly in Africa. In year 2006, there were an estimated 247 million malaria cases causing nearly a million deaths, mostly of children under 5 years (WHO 2008). The study by McCann and colleagues indicated that the pollen from maize facilitates optimum conditions for mosquito breeding. Mosquitoes carry *Plasmodium* parasites that cause 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 (Kebede *et al.* 2005).

Description of selected orphan crops

Orphan crops are many in number, but emphasis is given only to the most important ones in terms of the area they are grown or population they feed. These include cereals (e.g. millet, tef, fonio), legumes (cowpea, bambara groundnut, grass pea), and root crops (cassava, yam, enset). Brief descriptions are given below:

Finger millet (*Eleusine coracana* L.) is 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 (<http://www.icrisat.org/SmallMillets/SmallMillets.htm>, accessed 17 August 2009). The plant is tolerant to drought. The seed of finger millet contains valuable amino acid called methionine (NRC 1996), 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* (Zucc.) Trotter] 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 (Spaenij-Dekking *et al.* 2005). 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 (Ketema 1997).

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 (IPGRI 2004).

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 (<http://www.cowpea.org/node/8>, accessed 17 Aug. 2009). Since cowpea has quick growth and rapid ground cover, it is a useful crop in controlling erosion (<http://www.cowpea.org/node/8>, accessed 17 Aug. 2009).

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) 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 (NRC, 2006).

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 (Yan *et al.* 2006)]. 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 (Getahun *et al.* 2003).

Cassava [manioc; *Manihot esculenta* Crantz] is staple food for about a billion people (<http://www.fao.org/newsroom/en/news/2008/1000899/index.html>, accessed 31 July 2009). 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 their very low protein content and the roots contain poisonous compounds called cyanogenic glycosides (CG) which liberate cyanide (Ceballos *et al.* 2004). 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 (IITA, <http://www.iita.org/>) 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 (Brandt *et al.* 1997). Since enset flour is rich in starch but not in other essential nutrients enset-based diets need heavy supplementation.

Limitations to the productivity of orphan crops

The major bottlenecks affecting the productivity of under-researched crops are genetic traits such as low yield (e.g., tef and millet), poor in nutrition (cassava and enset), and production of toxic substances (cassava and grass pea). Crop productivity is also affected by a variety of abiotic and biotic stresses. The major abiotic stresses in Africa are drought, salinity and acidity. Due to the prevalence of high density and diversity of pests, diseases and weeds in the tropical Africa, the productivity of crops significantly decrease. Other factors that are affecting the production of food crops are the use of agricultural land for biofuel production and environmental challenges such as global warming.

Need for Green Revolution in African orphan 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 (FAO, 2002). 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 (for review Wang and Li, 2006). According to the International Food Policy Research Institute, Green Revolution represented the successful adaptation and transfer of scientific revolution in agriculture (IFPRI, 2002). 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 sequence are not yet available for any orphan crop. 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.

Improvement techniques

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 (2008) 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 (Devos 2005). 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. Transgenic and a modified form known as cisgenesis are widely applied to major crops although only few orphan crops benefited from the techniques so far.

Transgenics 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 125 million ha in 2008 (James 2009). Although in the past there were strong oppositions in Africa towards transgenic crops, the recent trend shows that in many African countries the benefits of transgenics are being realized. The numbers of

countries adopting the Biotechnology and Biosafety laws are also increasing. Many scientists including Norman Borlaug, Nobel Peace Prize laureate and World Food Prize founder, also support the use of transgenics in order to boost crop production so that the world hunger ends (Borlaug 2007). Some of the recent investigations on transgenics dealt with solving the major concerns related to the technology in order to increase the likelihood of acceptance by the public. For instance, the use of plant specific promoters instead of foreign promoters (Jacobsen and Schouten 2007), increasing efficiency of gene targeting in plants using zinc-finger proteins from plant (Shukla *et al.* 2009, Townsend *et al.* 2009), and substituting antibiotic resistant selectable markers with those without adverse effects (Ayalew and Stewart 2005) are some of the breakthroughs. In addition, there is growing interest and demand by African scientists to harness the benefits of transgenics in breeding for crops that can adapt to extreme environmental conditions prevalent in the continent.

The First Orphan Crops Conference

In order to develop awareness of orphan crops to the world scientific community, the First International Conference on Orphan Crops was held from 19 to 21 September 2007, in Bern, Switzerland. Specific objectives of the conference were, i) to address the major crop productivity problems related to orphan crops of Africa; ii) to introduce modern crop improvement techniques developed for major crops and to propose the strategy of implementing these techniques south for orphan crops; 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; and iv) to build the foundation for establishing research collaborations among scientists in the North and South regarding orphan crop improvement. Hence, networking and coordination of orphan crops research is important in order to efficiently use resources. Financial and technical supports for researchers involved in the understudied crops are needed since the majority of research on these crops are dependent largely on locally available meagre resources.

Conclusion

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 crops in other parts of the world. The next Green Revolution for Africa needs to include these locally adapted crops that are mostly known as orphan- or understudied-crops. The implementation of modern improvement techniques on orphan crops has many advantages.

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Comparative Plant Biology:

Opening new avenues for the improvement of orphan crops in a time of rapid and potentially catastrophic change in worldwide agriculture

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Summary

These comments, presented in a seminar and a subsequent roundtable discussion in September of 2007, indicate the status of experiments related to sorghum and tef millet in the Bennetzen lab at that time, and the lead author's thoughts on the near and longer term futures of biotechnology for crop improvement for the developing world. Given the impressive technologies that have been developed for plants like maize and rice, and the potential for facile transfer of knowledge from these model cereals to the lesser-studied grasses by use of comparative genomics, it is surprising how little progress has been made in applying modern tools to such crops as finger millet, foxtail millet, and tef. This deficiency has been primarily caused by an apparently intentional decision by major plant science funding organizations in the developed world that these crops deserve to be ignored and/or abandoned. Two examples are provided, involving disease resistance in sorghum and an attempt to identify semi-dwarf varieties of tef, where a relatively tiny commitment of resources can dramatically alter our understanding of, and potential to overcome, the limitations to agricultural productivity in the developing world.

Introduction

The eventual glory of the Linnaean system of classification has been that organisms with the most shared traits were generally found to be the most closely related when relatively neutral characteristics like synonymous DNA substitutions were brought into systematic analysis. The highly similar gene content and genetic map colinearity of one family within the kingdom *Planta*, the *Poaceae* (grasses), was proposed as a route to allow the nuclear chromosomes of these species to be viewed as different manifestations of a single genome (Bennetzen and Freeling 1993). In more than a decade of subsequent work, this “unified grass genome” hypothesis has provided the conceptual foundation for a great deal of basic research in genome evolution and comparative genomics, and also for applications as wide ranging as map-based gene cloning in surrogate species or identification of cross-species “shared” mutational or allelic variation, including quantitative trait loci (QTL).

However, even with the comprehensive molecular and genomic toolkit developed for species like maize and rice, their use in other grasses requires some investment. Developing a genetic map in finger millet, for instance, was less expensive and more informative than those initially developed in rice or maize, because the finger millet genetic mapping project used heterologous probes and advanced technologies developed in other mapping studies on better-funded organisms (Dida *et al.* 2007). But, the cost of developing a finger millet genetic map was not reduced to zero. Hence, not surprisingly, surrogates like rice have received much heavier use by such major crops as barley and wheat, than by the “orphan” cereals. The orphan cereals, like tef and finger millet, are the staff of life for hundreds of millions of people, often the poorest of the poor, but lack a major presence in developing country agriculture or major seed company portfolios, so they also are deficient in powerful advocates for their continued study and improvement. For social, economic and environmental reasons, the abandonment of orphan crops is precisely the wrong idea at precisely the worst possible time.

This chapter points out two cases where the advanced genetics in maize, rice, and wheat were used to uncover important aspects of plant biology in so-called minor cereals. With minimal additional resources, these discoveries could be converted into crop improvements that could better the lives of millions of people around the world.

Results

The discovery of a new type of highly-targeted disease susceptibility

The *Pc* locus of sorghum provides race-specific susceptibility to toxin-producing isolates of the fungus *Periconia circinata* (Leukel 1948, Arias *et al.* 1983). The *Pc* locus is unusual because it encodes a dominant susceptibility, and this susceptibility naturally mutates to a fully resistant (*pc*) form at a rate of once in a few thousand gametes (Schertz and Tai 1969). The genomic region carrying *Pc* was relatively easily cloned by a map-based strategy using information from the maize and rice genomes (Nagy *et al.* 2007). Although there are many genes in the *Pc* region, a classic NBS (nucleotide binding site) – LRR (leucine rich repeat) disease resistance gene homologue appears to be *Pc*. The natural *Pc* to *pc* mutations change the copy number of these NBS-LRR genes in that region from three to one or two, probably by unequal crossing over, a process that tends to occur at rates of one in a few thousand meioses for many tandem gene families (Nagy *et al.* 2007).

The NBS-LRR genes in plants usually provide race-specific resistance to obligate parasites by encoding a recognition function that leads to hypersensitive necrosis (HR) at the site of pathogen entry (Greenberg 2001). It appears that *P. circinata*, a necrotroph, has used this form of resistance against itself by simulating the signal of a biotroph, and then using the resultant HR as a way to gain entry into the host. This creates a tremendous potential for balancing selection on *Pc* in one direction to better recognize the biotroph and in the other direction to not recognize the necrotroph that is mimicking the biotroph.

The interesting part of this story relative to orphan crops, though, is that neither rice nor maize has any identified example of a similar phenomenon. Will there be cases of balancing necrotrophic/biotrophic selection on NBS-LRR genes in these species, and perhaps in all plants? Almost certainly, but not all phenomena are equally visible in all species, often due to pure chance. Transposable elements, ubiquitous in nature, were first considered by most biologists to be an oddity that was unique to maize, but were first discovered in maize only because of their relatively high activity and dramatic phenotypic contributions, combined with the genius of their discoverer (McClintock 1948).

Semi-dwarfing gene homologues in tef

The “green revolution” was founded on the observation that some crops responded to higher inputs (especially fertilizer) with dramatically improved yields, if the issue of lodging did not become a major limitation on productivity. Lodging in cereals is the uprooting, breaking or bending of plant stalks such that the grain is lost because it is either missed in the harvesting process or is damaged by molds, rots, or pests. In general, increased fertilizer will cause cereals to grow to greater heights, thus causing more lodging, and thereby attenuating or even reversing input-generated yield gains. The green revolutions in wheat and rice both employed specific semi-dwarf mutations (e.g., *Rht-1* and *sd-1*, respectively) to minimize lodging (Khush 1999).

In some crops, however, lodging continues to be a major problem. In tef, for instance, losses of >15% to lodging are routine, and can be greater than 50% in some seasons and regions. This severe problem is manifested in a crop that receives very few inputs. Lodging in tef dramatically limits current yields, but may also block future tef improvement. Breeding to produce bigger grains, more seeds per head, or to improve response to fertilizer are likely to increase lodging.

Is lodging in tef a curable problem? Absolutely yes, and by a simple approach. Semi-dwarf varieties that decrease lodging and increase yield have been found in sorghum, wheat, rice, pearl millet and various other crops. The comparable genes are present in tef and their potential usefulness for tef improvement could be confirmed within a two-year project.

All organisms contain genes that, through natural or induced mutation, yield dwarf or semi-dwarf plants. Most of these genes, however, have negative secondary effects like decreased fertility. A few genes, like *Rht-1* of wheat and *sd-1* of rice, actually increase yield at the same time that they decrease lodging. This is thought to be true because a semi-dwarf plant usually converts a lower percentage of its photosynthate into plant biomass like leaves or stems and more into seed. Hence, improvement of tef will not require an uninformed search for just the right semi-dwarfing mutation. Instead, the tef improvement project can use the genes identified in other grasses, and undertake reverse genetics to see if they yield the appropriate results. This is a particularly important strategy for tef because of its tetraploid nature. Recessive mutations like *sd-1* are not likely to have an observed phenotype in a recent tetraploid, so a forward genetics screen for recessive semi-dwarf mutations is likely to fail.

We have identified and cloned all of the close homologues of the *Rht-1* (Peng *et al.* 1999) and *sd-1* (Ashikari *et al.* 2002, Monna *et al.* 2002) genes from tef. Not surprisingly, given tef's recent tetraploid origins, we found two *Rht-1* homologues and three *sd-1* homologues (unpub. obs.). We sequenced these genes, and used gene-specific primers to amplify these same five genes from each of 31 different tef accessions by PCR. These PCR products were then sequenced. Our hope in these studies was to find native alleles of *Rht-1* or *sd-1* with the appropriate mutant properties, but none were uncovered (unpub. obs). In this limited germplasm sample, most of the genes exhibited only 2-4 haplotypes, although one *sd-1* homologue was associated with ten different haplotypes (unpublished observation).

The next steps in this project are clear and guaranteed to succeed, if undertaken. Many more tef accessions need to be investigated for natural mutations. If discovered, they could be variously pyramided and introduced into high yielding tef backgrounds. Their effects upon tef yield, with various input regimens, could then be tested. Alternatively, reverse genetics could be employed. The simplest form would be to use TILLING (McCallum *et al.* 2000) to identify appropriate mutations in targeted semi-dwarf genes. Alternatively, transgenics could be produced that use RNAi or antisense technologies to silence or lower the expression of these target genes. The molecular components of gene discovery would require about one year, and the verification of their role in tef morphological enhancement would require about one additional year.

Discussion

Orphan crops: missed opportunities, tremendous potential

Because most crop improvement research funding goes into crops with major income potential, at least in some market, it is not surprising that most cereals research has been targeted on maize, rice, wheat and barley. With thousands of applied and basic researchers studying its genetics and yield potential for the last half century, maize is probably the most improved crop in the world. Yields and alternative uses continue to be extracted from this important plant, but at a much higher cost per unit improvement than needed even twenty years ago. From this perspective, maize may also be the single most difficult plant to improve any further. One finger millet breeder may double yields per acre in Uganda in a decade, but a thousand maize breeders working together are needed in the same time period to increase maize yield

10%. This is not, in any way, a scenario limited to maize: all highly improved crops are becoming more difficult to enhance with any current technologies (Ruttan 1999).

Beyond the inherently greater potential of a barely improved crop, the orphan cereals also have the advantages of diversification and adaptation (both environmental and cultural). It has been difficult for many development organizations to resist the temptation to piggyback on the tremendous yield improvements made in developed world maize or rice, and to bring these plants into greater use in the developing world by a process of cultural and genetic modification. In the short term, such an approach may increase incomes, but it will lead to more frequent boom: bust agricultural cycles with these less adapted cereals compared to the lower yielding but more durable and commonly more nutritious indigenous cereals. Moreover, a loss of agricultural diversity may be just as problematic as a loss of natural biodiversity (Tillman 1994). A worldwide monoculture of rice, maize and wheat will guarantee that more serious plant diseases will arise, that they will ramify more quickly, and that they will have more damaging effects on the farmers and societies that rely on a dependable annual production.

The near and distant futures for crop biotechnology in the developing world

Crop biotechnology in the developing world has devolved into an incredible state. A technology with the potential to save millions of lives, and improve the lifestyles of billions, lies unused or under-used because of unproven, unexampled and illogical fears. Moreover, the best forms of plant biotechnology help replace current technologies, like the overuse of pesticides, that are known to be damaging to people and the environment. Many of the opponents of biotechnology use it as a surrogate for "globalization" or corporate malfeasance, both serious issues, but ones that have only a tangential relationship to biotechnology. If opponents of global corporate dominance wish to remove crop biotechnology as an issue, then the best route is to lobby for the support of public sector crop biotechnology.

Some of the most vocal anti-biotechnology voices seem to be taking a Luddite stance. Anything new might be dangerous in unexpected ways, so let us not do anything new. The greatest of all human innovations, the domestication of fire, has been the most lethal technology ever invented, providing the foundation for everything from the weapons with which we fight modern wars to the tractors that clear the rainforests and

the greenhouse gases that pollute the air. Still, without domesticated fire, we'd still be mediocre hunter-gathers with a worldwide population in the tens of thousands and a less than 30-year life expectancy.

In the near term, the opponents of plant biotechnology in the developing world will continue to win their propaganda war. They have the passion and the resources, both money and time, that are not matched by anything in the public or private sector. The big corporations are not willing to invest much on this issue, as they see relatively few profits in developing world agriculture at this time. Developed world agricultural organizations are mostly devoted to serving their clients, the nation's farmers, ag businesses and consumers, and only give token attention to the developing world. The scientists who work in plant genetics and biotechnology understand the current travesty, but have no serious lobbying capability. Moreover, scientists who take any stand on any controversial issue can risk their credibility, and their careers, because we are expected to be "above the fray". For most U.S. scientists, for instance, any time spent on developing world issues (whether doing the science or advocating for more commitment to the problem) is time taken away from the research and teaching that will earn promotions and research support.

This short-term problem is simply tragic. Tens of thousands of people are dying each year, and millions leading lives of poverty and malnutrition, because of yield and quality improvements that have not been made, and could easily have been made, over the last decade by crop biotechnology committed to the developing world. The problem will soon be much worse. For the developing world, the major outcome of the inappropriately precipitous decisions to ramp up conversion of grains like maize into biofuels, funded by extreme subsidization, will be dramatic price increases and a loss of the surplus grain storage that has been the world's backstop against famines. We can expect the next environmental problems in the developing world to lead to far worse famines than would have been imagined possible just a few years ago.

In the long run, biotechnological improvements will become routine all over the world. In many nations, plantings in transgenic plants make up a majority of the acreage for specific crops, with major economic, environmental and social benefits, and no problems yet identified. Some biotechnological advances will be transitory, and will be replaced by new biotechnologies. The current opponents of this technology will be viewed as the comical robed wanderers with the re-usable sign that promises

“the world will end today”. This will be too generous of a treatment, as it forgets the millions, mostly children, who will die in the interim from malnutrition and food shortages.

As scientists, we need to exit our comfort zones to both educate and advocate for crop biotechnology in the developing world. We may receive no rewards, and will certainly receive a hostile reception in some quarters. But this activism should be viewed as an obligation of the privileged lives we lead, motivated by the next experiment and the next discovery, rather than by wondering where the next meal will come from.

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Breeding tef: conventional and molecular approaches

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Summary

Tef [*Eragrostis tef* (Zucc.) Trotter] is an allotetraploid ($2n=4x=40$) cereal with its centers of domestication and diversity in Ethiopia. It is the only cultivated species in the genus *Eragrostis* that contains about 350 species. In Ethiopia, about two million hectares of land is devoted to its production every year. Despite its importance in the Ethiopian agriculture for centuries, there are constraints that have to be addressed through scientific research. The major ones are low yield of cultivars grown by farmers, susceptibility to lodging, and lack of basic genetic knowledge on useful traits of the crop. Conventional breeding on tef started in the late 1960s. Currently, more than 4300 accessions are held at the Institute of Biodiversity Conservation of Ethiopia. Sixteen varieties were developed and released through pure-line selection from germplasm accessions. After the development of the tef crossing technique in 1974, eight additional varieties have been developed and released through trait recombination. Yield gain from tef breeding has been linear with an average annual increase of 0.8%. Varieties developed through crossing produced 9% higher yield than those developed through direct selection from germplasm. Traits such as loose panicle, panicle length and weight, tiller number and biomass yield were identified as components of yield in tef. Lodging has persisted to be bottleneck problem so as to produce tef at a commercial scale. In 1995, tef genomics research was initiated through grant funds from the McKnight Foundation's Collaborative Crop Research Program. Tef has a relatively small genome size of 730 Mbp. The main goal of tef genomics is to provide breeder-friendly PCR-based

markers for important agronomic traits and, thereby, augment the conventional breeding. There are now more than 142 markers available for tef. Four genetic linkage maps have been developed for tef so far. A number of quantitative trait loci (QTLs) were identified for important traits. Currently, greater effort is being exerted to generate more PCR-based markers for a saturated map that can be useful for marker assisted selection (MAS) applications in the tef breeding program.

Introduction

Importance and utilization of tef in Ethiopia

Tef [*Eragrostis tef* (Zucc.) Trotter] is one of the major cereals of Ethiopia. The averages of the recent three years' (2003-2005) production statistics (CSA 2006) indicate that annually tef accounts for about 29% of the total acreage and 20% of the gross grain production of all the major cereals cultivated in the country. The crop species has its centers of both origin and diversity in Ethiopia (Vavilov 1951). Tef domestication predates historical records. As such, tef is not only endemic but also unique to Ethiopia. This is because its cultivation as a cereal food grain, except in very small quantities in some parts of Eritrea and as a recent introduction in some parts of the USA, the Netherlands and perhaps Israel, has been restricted to Ethiopia. However, the plant is known in South Africa, India, Pakistan, Uganda, Kenya and Mozambique mainly as a forage or pasture crop.

In Ethiopia, tef grain is mainly used for food after baking the ground flour into pancake-like soft and sour bread, "*injera*", which forms the major component of the favorite national dish of most Ethiopians. It is also consumed in the form of porridge, and slightly fermented or un-fermented non-raised breads ("*kita*" and "*anebabero*"). Although recent economic feasibilities might have limited such uses, the grain is also used for brewing native beer, "*talla*", and more alcoholic cottage liquor, "*katikalla*" or "*arakie*".

The long-sustained extensive cultivation of tef in Ethiopia can be attributed to a multitude of its relative merits (Assefa 2003, Ketema 1993); 1) broad adaptation and versatility being grown under varied climatic and edaphic conditions, 2) resilience to both low (drought) and high (waterlogging) moisture stresses, 3) fitness for various cropping systems and crop rotation schemes, 4) use as a catch and low-risk crop, and 5) little or no serious threats of disease and pest epidemics, at least, in its major production belts. Its relative merits with respect to utilization

include: 1) best quality “*injera*”, 2) high returns in flour (Ebba 1969) and in “*injera*”, 3) minimal damage from storage pests and diseases coupled with high storage longevity (storability) of the grains, 4) importance of the straw mainly as best quality fodder, and 5) premium market prices of both the grains and the straw (cash crop).

The tef plant: botanical overview

Tef belongs to the grass family, *Poaceae*, sub-family *Chloridoideae* (*Eragrostoideae*), tribe *Eragrostidae*, sub-tribe *Eragrosteae*, and genus *Eragrostis*. Tef is the only cultivated species in the genus *Eragrostis* and together with finger millet (*Eleusine crocana* L.) it constitutes the two species in the sub-family *Chloridoideae* that are cultivated for human consumption. The genus *Eragrostis* comprises about 350 species (Watson and Dallwitz 1992), and of these about 43%, 18%, 12%, 10%, 9% and 6% are native to Africa, South America, Asia, Australia, Central America, North America and Europe, respectively (Costanza 1974). Among the 54 *Eragrostis* species found in Ethiopia, 14 species are said to be endemic (Cufodontis 1974).

The genus *Eragrostis* is generally a complex taxon characterized by the prevalence of polyploidy (about 69%) and presence of cytologic races. Ploidy levels in the genus range from diploids ($2n=2x=20$) to hexaploids ($2n=6x=60$). Tef is an allotetraploid ($2n=4x=40$) forming 20 bivalents at Metaphase I (Tavassoli 1986) with disomic inheritance patterns of qualitative traits such as panicle form and ramification pattern, kernel coat color and color of the floral bracts lemma and glumes (Berhe *et al.* 2001). The putative diploid progenitors have not yet been identified, but recent DNA-based studies (Ayele 1999, Ayele *et al.* 1999, Ayele and Nguyen 2000, Ingram and Doyle 2003) suggested that *E. pilosa* (also allotetraploid) is the closest relative and possibly the immediate wild progenitor of tef.

The chromosomes of tef are minute (0.8-2.9 μm) (Tavassoli 1986); the largest tef chromosome is smaller than the smallest (1D) wheat chromosome (Gugsa *et al.* 2001). The presence of chromosomal races and aneuploidy, unlike some of its related *Eragrostis* species, is not known in tef. The average nuclear genome size of tef is 730 Mbp (Ayele *et al.* 1996a, Hundera *et al.* 2000).

Tef is a herbaceous, annual, C_4 plant requiring 60-140 days to attain physiological maturity (Assefa *et al.* 2001a). On the other hand, tef is relatively waterlogging tolerant possibly due to the occurrence of the

nitrate reductase activity in the root rather in the shoot. The tef plant root system is very thin fibrous (thread-like) rarely emerging from nodes above the base, and growing 4-8 cm deep under field conditions (Ebba 1975). The stems are mostly erect (ascending), but creeping or bending or elbowing (geniculate) in some cultivars, and jointed with hollow internodes separated by nodes. Each culm internodes, except the most basal one, bears one leaf consisting of a sheath and a blade. The paniculate tef inflorescence ranges in form from very compact (whip-like) to very loose and open ones. The panicles ramify into primary, secondary and tertiary branches bearing spikelets. Each spikelet bears a pair of unequal sized glumes at the base and a number of florets (3-17) above. Each floret in turn comprises a tri-nerved lemma, a two-nerved bow- or boat-shaped palea, three stamens (arising from the ovary base and having very fine and slender filaments bearing length-wise opening anthers at the apex), and an ovary or a pistil. The ovary consists of often two or in few exceptional cases three styles each ending in a plumose (feathery) yellowish white stigma. Tef is a highly self-fertilized species with natural out-crossing of about 0.02%. Flowering, anthesis and maturity of florets and grains is basipetal (top-down) on panicle basis and acropetal (bottom-up) on spikelet basis.

The seeds of tef are very minute (hundred kernel mass = 0.18 – 0.38 mg) and vary in the outer caryopsis color ranging from dark brown to yellowish or orange-white. The height of the plant ranges from about 20-155 cm with the culm (11-72 cm) and the panicle (10-65 cm) accounting for about 47-65% and 35-53% of the total aboveground height, respectively (Assefa *et al.* 2001a).

Tef breeding: the phases through time

Scientific tef improvement research in Ethiopia was started in 1956 at the then Jimma Agricultural and Technical High School (now Jimma College of Agriculture). Four years later, the research moved to the then Central Experiment Station at Debre Zeit, and now Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research. In the overall history of tef breeding, five inter-related phases can be distinguished.

The first phase of tef breeding spanning up to 1974 was characterized by germplasm enhancement through collection and acquisition, characterization and evaluation, systematics, and conservation of tef genetic resources from various parts of Ethiopia. During this period, the

genetic improvement relied entirely upon mass and/or pure-line selection directly from the existing indigenous germplasm collection. Tef researchers thought that the tef florets are cleistogamous and not amenable to crossing. As a result, the use of induced mutation techniques was initiated in 1972 in order to create variability in the species.

The discovery of the chasmogamous floral opening behavior of tef flowers and development of the artificial hybridization technique (Berhe 1975) marked the beginning for the second phase (1975-1995). Consequently, the variety development breeding method during this period featured further incorporation of intra-specific hybridization in addition to direct selection from the existing landraces. Furthermore, the use of induced mutation techniques initiated in 1972 also continued during this phase.

The third phase (1995 to present) in tef breeding was marked by the initiation of molecular approaches including development of molecular markers and genetic linkage maps, and molecular genetic diversity analyses. The impetus for the molecular approaches came mainly through the McKnight Foundation Collaborative Crop Research Program funded Tef Research Project, and to some extent also through other external (*viz.* Sida/SAREC and the IAEA) technical and funding assistances.

The fourth phase (1998 to present) of tef breeding featured further incorporation of *in vitro* culture techniques and inter-specific hybridization, and also strengthened re-appraisal of induced mutagenesis particularly for combating the problems of lodging and leaf rust disease resistance due to the insufficient variability noted for these traits in the existing tef germplasm. In the fifth phase (2003 to present), the major additional feature has been the introduction of participatory breeding approaches in the pre-existing overall tef genetic improvement endeavors (Belay *et al.* 2006, 2008).

Tef breeding: conventional approaches

Breeding objectives

The overall tef breeding objectives have been: 1) to enrich and improve the germplasm resource base; 2) to develop suitable cultivars for different agro-ecological zones and various cropping systems; and 3) to generate basic scientific information on tef biology (reproductive) and

apply modern biotechnological tools in tef improvement. The specific tef breeding objectives have been: 1) high grain yield; 2) tolerance/resistance to low moisture stress; 3) improved lodging resistance; and 4) desirable grain quality. Disease resistance, especially to leaf rust (*Uromyces eragrostidis* Tracy), has recently been of little concern as the major tef breeding objective. This is due mainly to the existence of little variation in the existing germplasm with most of the landraces exhibiting high severity and susceptible reaction, while the relatively better tef genotypes show only quantitative resistance/tolerance. Moreover, leaf rust disease epidemics in farmers' fields are generally low and it usually occurs after heading thereby inflicting little losses.

The direct major clients of the tef breeding program are the subsistence tef growing Ethiopian peasants. Indirectly, however, the tef consumers, merchants, food processors, and governmental and non-governmental institutions involved in rural development, and policy makers would also be beneficiaries of the improvement program.

Breeding philosophy, strategy and institutional set-Up

In general, the philosophy of our tef breeding program pivots on "add a little and it makes a difference". Current focal strategic directions of the breeding program are: 1) shift from wide to specific adaptation because of high genotype x environment (G x E) interactions; 2) market orientation in terms of quality and quantity of produce; and 3) expansion of tef to non-traditional production areas.

Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR) houses the National Tef Improvement Project in terms of responsibilities for the overall national coordination, as well as the development and execution of country-wide benefiting tef research projects. Under the umbrella of this institutional framework, the activities, particularly multi-location variety trials, of the tef breeding program are carried out at various federal and regional research centers and testing sites, higher learning institutions, and on farmers' fields. The yield tests starting from the preliminary up to the national variety trials are categorized into two maturity groups as late and early sets with the former targeted for the ample rainfall optimum areas and the early types targeted for low moisture stress areas.

Tef genetic resources for breeding

The total numbers of holdings of tef germplasm accessions in the genebank of the Institute of Bio-diversity Conservation (IBC) in Ethiopia until 2000 was 4395, and of these 1497 accessions were acquired through donations and repatriations and the rest through collections (Demissie, 2001). Nevertheless, the germplasm collections are generally deficient in terms of representations of regions and agro-ecologies, and most of them lack adequate passport data. Out of the total IBC holdings only 2634 accessions amounting to 60% have been characterized and then only for phenologic and morphological traits. Earlier, Ebba (1975) distinguished and described 35 cultivars of tef mainly on the basis of phenology, plant vigor, panicle form, spikelet size, and color of floral bracts and caryopsis. Few attempts have so far been made towards the collection and conservation of the wild relatives of tef.

The ranges of variation in important phenologic, morphological and agronomic traits of tef based on values obtained in different studies have been summarized in Table 1. Generally, the species exhibits broad diversity in most of the traits. However, for some of the most important traits such as lodging resistance and lodging resistance associated traits like culm thickness the observed variation, even considering the maximum values in the desired direction, is not sufficient to make progress in breeding for lodging resistance.

Breeding Methodology and Achievements

The general methodology employed in tef breeding is depicted by the variety development process flow chart in Figure 1. Since genetic variation forms the fundamental basis for breeding, the tef variety development anchors primarily upon germplasm enhancement through three complementary methods.

- 1) The indigenous germplasm constitutes major source of variability for tef breeding. This is because, tef being a native and unique crop to Ethiopia, there have been no opportunities for introductions of germplasm and breeding materials from abroad. The major germplasm enhancement activities include germplasm acquisition through collections and repatriations from genebank or other sources, characterization and evaluation of the germplasm, and mass and/or mostly pure-line selection of desirable genotypes. Selections would be used for nurseries for subsequent evaluation for direct yield tests or as parental lines for hybridization or induced mutagenesis.

Table 1. Ranges for important phenologic, morphological and agronomic traits of tef
(Source: Assefa *et al.* 2001a)

Trait	Minimum	Maximum
Days to panicle emergence	25	81
Days to mature	60	140
Plant height (cm)	20	156
Culm length (cm)	11	82
1st culm internode length (cm)	2.68	8.05
2 nd culm internode length (cm)	4.15	11.45
1 st and 2 nd culm internode diameter (mm)	1.20	4.50
Panicle length (cm)	10	4.50
No. primary panicle branches	10	40
No. spikelets/panicle	30	1070
No. florets/spikelet	3	17
Grain yield/panicle (g)	0.11	2.50
No. tillers/plant (total)	4	22
No. tillers/plant (fertile)	1	17
Grain yield/plant (g)	0.54	21.90
Total phytomass/plant (g)	4	105
Hundred kernel mass (mg)	18.97	33.88
Grain yield (kg/ha)	1058	4599
Harvest index (%)	5	39
Lodging index	20	100

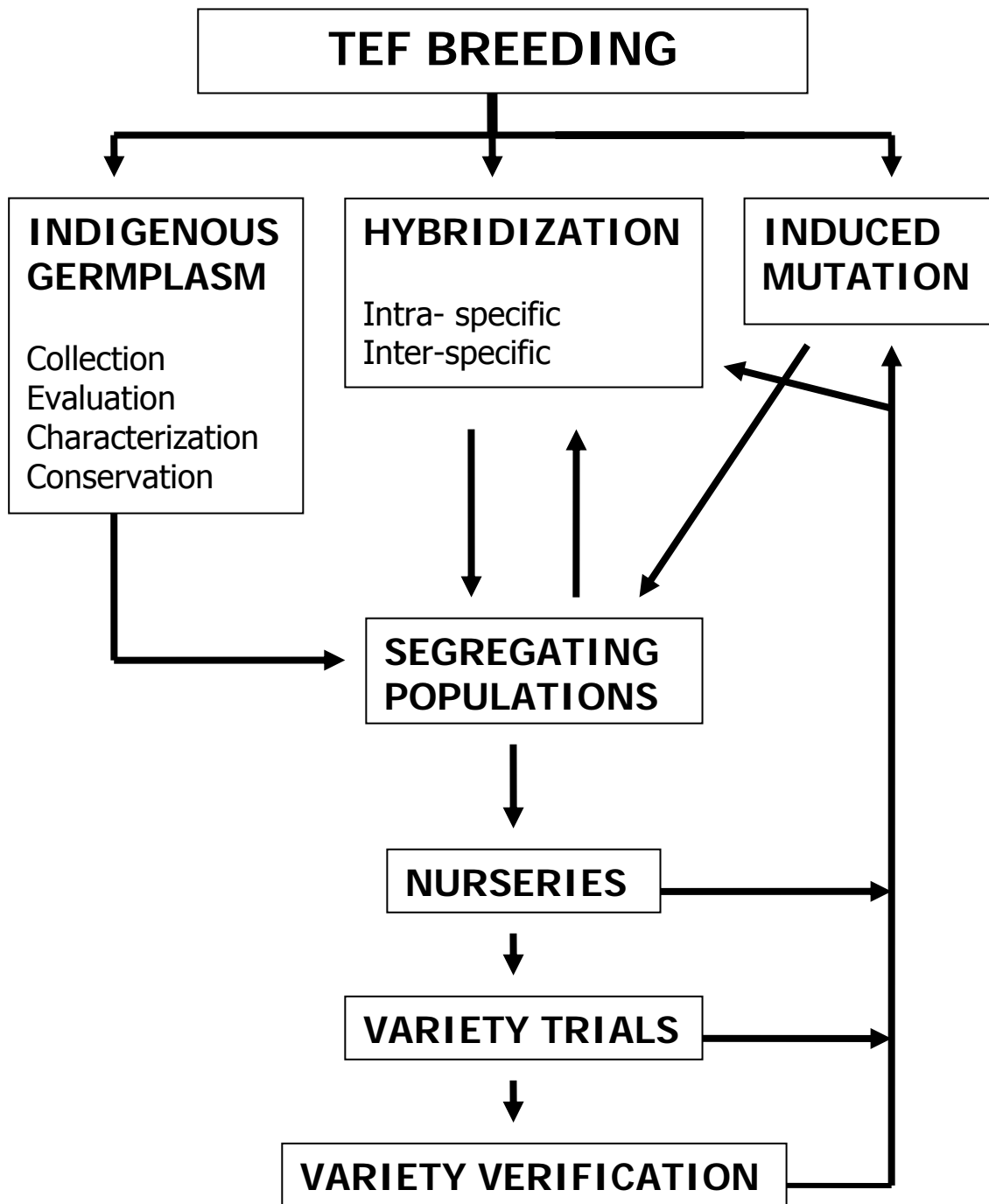


Figure. 1. Schematic representation of the variety development process in tef breeding

- 2) Hybridization mainly intra-specific crosses and recently inter-specific crossing especially with *E. pilosa* (Tefera *et al.* 2003). A total of about 440 crosses have been made so far at Debre Zeit Agricultural Research Center. Particularly in recent years, we largely make simple crosses. Subsequent segregating populations are handled using the modified pedigree or bulk methods.
- 3) Induced mutation techniques are also being used for generation of variability for some important traits such as lodging resistance. The results thus far, however, have been disappointing.

The next stage is the nursery for initial evaluations of selected genotypes from the three germplasm enhancement schemes followed by series of yield trials including preliminary, pre-national and national variety trials. In the variety testing, genotypes are categorized into early and late sets depending on the maturity. The late types are often meant for high yield potential areas with optimum environments, while the early sets are targeted for terminal low moisture stress areas. At the last stage of the process, elite and promising genotypes selected as candidate varieties based on their performance in the various variety trials are entered into a variety verification trial for evaluation by The National Variety Release Committee. At all stages, even after release, genotypes could be selected to be taken back to the earlier steps of hybridization and induced mutation schemes of germplasm enhancement.

Since the initiation of the improvement work in the late 1950's, 17 varieties of tef have been released from Debre Zeit Agricultural Research Center from 1970 up to now (Table 2). Of these, seven were from hybridization and the rest 10 varieties resulted from direct mass or pure-line selections from the local landraces. In addition, tef varieties have also been released by other research centers in Ethiopia. These include seven varieties of which two were released by Holetta Agricultural Research Center in 1999 and 2000, and one variety each released by Melkassa, Adet, Sirinka and Areka and Bako Agricultural Research Centers. Generally, the improved released tef varieties give mean grain yields up to 2.7 t/ha on farmers' fields.

Table 2. Improved varieties of tef released by Debre Zeit Agricultural Research Center in Ethiopia from 1970-2007

Variety	Year of release	Plant height (cm)	Seed color	Days to maturity	Grain yield (t/ha)	
					On-Station	On-Farm
DZ-01-354 (Enatite)	1970	53-115	Pale white	85-100	2.4-3.2	2.0-2.4
DZ-01-99 (Asgori)	1970	53-100	Brown	80-130	2.2-2.8	1.8-2.2
DZ-01-196 (Magna)	1970	50-117	Very white	80-113	1.8-2.4	1.6-2.0
DZ-01-787 (Wellenkomi)	1978	50-110	Pale white	90-130	2.4-3.0	2.0-2.4
DZ-Cr-44 (Menagesha)	1982	85-110	White	15-140	1.8-2.4	1.8-2.2
DZ-Cr-82 (Melko)	1982	96-112	White	112-119	1.8-2.4	1.6-2.0
DZ-Cr-37 (Tsedey)	1984	67-92	White	82-90	1.8-2.5	1.4-2.2
DZ-Cr-255 (Gibe)	1993	63-116	White	114-126	2.0-2.6	1.6-2.2
DZ-01-974 (Ziquala)	1995	84-132	White	76-138	2.4-3.4	2.0-2.7
DZ-Cr-358 (Dukem)	1995	70-109	White	75-137	2.4-3.4	2.0-2.7
DZ-01-1281 (Gerado)	2002	83-100	White	73-95	1.7-2.4	1.6-2.2
DZ-01-1285 (Koye)	2002	80-92	White	104-118	1.7-2.4	1.6-2.2
DZ-01-1681 (Key Tena)	2002	74-85	Brown	84-93	1.7-2.4	1.6-2.2
DZ-01-899 (Dega Tef)	2005	46-68	Pale white	118-137	1.5-2.2	1.6-2.0
DZ-01-2675 (Chefe)	2005	47-91	Pale white	112-123	1.5-2.4	1.4-2.2
HO-Cr-136 (Amarach)	2006	67-81	Pale white	63-87	1.8-2.5	1.4-2.2
DZ-Cr-387 (RIL 355) (Quncho)	2006	72-104	Very white	86-151	2.0-3.2	1.8-2.6

Analysis of the genetic gain through tef breeding from the release of the first varieties in 1970 until 1995 under non-lodging conditions revealed that averaged over the two experimental soil types there has been a steady but slow increase in grain yield from 3.81 to 4.60 t/ha (Teklu and Tefera 2005). This indicates that the breeding resulted in linear ($R^2 = 0.3441$) genetic gain in grain yield with average annual increment of 0.8%, which amounts to about 0.398 kg/ha/year (Figure 2). On the average, varieties developed through hybridization yielded 9% higher than varieties developed through direct selection from germplasm. Analysis of the traits associated with genetic improvement in tef showed that improvement in biomass yield, plant height, panicle length, number of spikelets/panicle, panicle yield, rates of phytomass production and grain filling are characteristic of the improved varieties (Teklu and Tefera 2005).

Our Participatory Variety Selection work has established that tef is a cash crop and seed-colour is the single most important selection criteria by the farmers (Belay *et al.* 2006). We were also successful in releasing a variety named "Quncho" through combinations of both the formal breeding and farmer participation (Belay *et al.* 2008). This combination of breeding methods was necessary in order to meet the regulation requirements of the national variety release system.

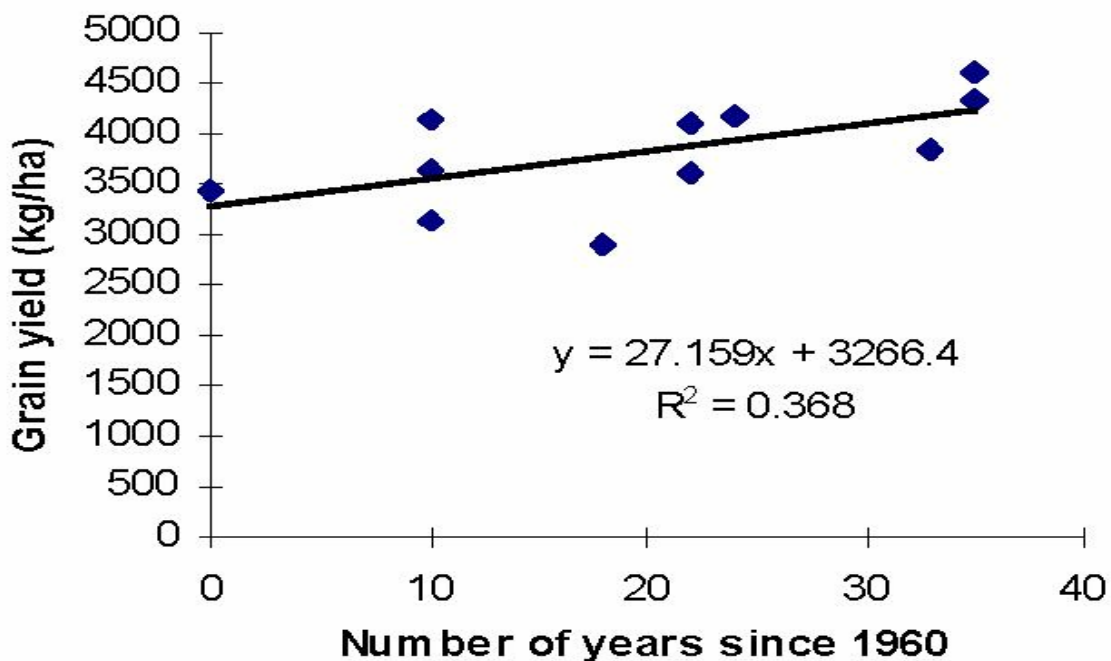


Figure. 2. Relationship between mean grain yield of tef varieties and year of release expressed as the number of years since 1960. (Source: Teklu and Tefera, 2005)

The Molecular-Biotechnological Approach in tef breeding

Generally, the use of the biotechnological and particularly the molecular approach in tef breeding is a relatively recent introduction. However, there have been on-going efforts mainly on developing the pre-requisites towards the use of the biotechnological tools and molecular techniques to complement and hasten the conventional tef breeding.

Genomics

Genetic linkage maps

In the genomics aspect, the major works undergoing are in the areas of molecular genetic map construction. In addition, molecular marker systems have also been used for genetic diversity studies in the crop and related species. The primary objective of the genomics research for the construction of molecular genetic linkage map for tef is provision of user friendly PCR-based markers for important agronomic traits so as to augment the conventional breeding.

For the linkage map construction, three sources of molecular markers have been used: 1) marker information available in other crops; 2) heterologous primers derived from cereal crops such as wheat, rice and finger millet; and 3) new markers developed directly from tef genome. Eighty expressed sequence tag - simple sequence repeat (EST-SSR), 19 single-nucleotide polymorphism (SNP) and 34 intron fragment length polymorphism (IFLP) markers have been developed and used from a total of 170 tef ESTs containing EST-SSRs (Yu *et al.* 2006a). Until now, more than 142 markers have been available and efforts are yet underway to develop more useful PCR-based markers. Four genetic linkage maps have so far been constructed using intra-and inter-specific recombinant inbred lines (RILs). These include the first genetic linkage map for the species developed using AFLP markers (Bai *et al.* 1999a,b); followed by an RFLP map (Zhang *et al.* 2006). Two other subsequent genetic linkage maps have been constructed using combinations of various markers involving: 1) RFLP, EST-SSR, SNPs/insertion and deletions (SNP/INDEL), intron fragment length polymorphism (IFLP), target region amplification polymorphism (TRAP), and ISSR markers (Yu *et al.* 2006b); and 3) AFLP, EST-SSR (wheat, rice and tef), SSR and ISSR markers (Chanyalew 2007).

QTL mapping

In an effort to use the tef molecular genetic linkage maps for eventual marker assisted selection (MAS) conventional breeding program, QTL mapping of important quantitative traits have been attempted with the tef linkage maps so far developed (Zhang *et al.* 2001, Yu *et al.* 2006b). Overall, QTLs have been identified and mapped for important tef traits including lodging, yield and yield components, and other related quantitative phenologic and morphologic traits.

Molecular genetic diversity

Molecular methods have also been used for diversity analyses. In contrast to the broad phenotype (trait) variability evidenced in the crop, amplified fragment length polymorphism (AFLP) (Bai *et al.* 1999a, Ayele and Nguyen 2000) and random amplified polymorphic DNA (RAPD) (Bai *et al.* 2000) analyses revealed relatively low level of DNA polymorphism in the species. In these studies, the Jaccard similarity coefficient among the variable genotypes tested ranged from 84-96% for RAPD and from 73-99% for AFLP, indicating very close similarity, and hence low level of polymorphism. However, inter-simple sequence repeats (ISSR) analysis showed relatively higher diversity among the test genotypes (with Jaccard similarity coefficients ranging from 26-86%), and as such the ISSR technique proved more efficient in deciphering greater (about 20% more) genetic diversity on the basis of mean Shannon-Weaver diversity index (Assefa *et al.* 2003). Although not with genomic nuclear DNA, Pillay (1997) similarly noted considerable rDNA repeats size variation among tef accessions, suggesting that this may provide an effective tool for screening and selecting genetically diverse genotypes for breeding.

In Vitro culture

The development of efficient tef *in vitro* culture and regeneration system would be important for facilitating the conventional breeding program. Directly, doubled haploid or dihaploid production *in vitro* culture systems would hasten the conventional hybridization and induced mutation breeding programs through enabling fixation of homozygosity in one generation. On the other hand, *in vitro* culture systems would also facilitate other modern biotechnological manipulations including *in vitro* mutagenesis, somatic fusion and genetic transformation.

In spite of genotype differences, Tefera (1992), and Tefera and Chapman (1992) reported successful completion of the normal *in vivo* growth and development of florets and kernels from *in vitro* cultures of immature spikelets. The spikelets are detached from the 2-5 mm long

portion of panicles and placed on a wheat spikelet medium (WSM) 8-12 days after tips emerged from the flag leaf with basal florets 5-8 days before anthesis. On the other hand, the development of hybrid kernels with recognizable embryos but smaller embryos than that of the *in vivo* grown kernels was reported with *in vitro* cultures of immature hand-emasculated and pollinated florets cultured on WSM medium 1-72 h after artificial pollination (Tefera and Chapman 1992).

In an attempt to hasten the conventional tef hybridization and induced mutation breeding programs, DH production using *in vitro* culture techniques have been tested with explants involving immature spikelets (florets) and isolated anthers (Ayele 1995, Tefera *et al.* 1999), and isolated ovaries (Tefera *et al.* 1999). These studies generally showed better responses with N₆ than with MS basal media. Although efforts are still underway for the development of DH production anther culture protocol for tef, there has been little success with the technique. Recently, however, Gugsu *et al.* (2006) has reported the regeneration of haploid to octoploid tef plants as revealed from flow cytometric analysis of *in vitro* cultures of immature and un-pollinated spikelets. Those cultures contained hand-emasculated florets on MS basal medium, with the plants regenerated presumed to have been derived from the gynogenesis development of the unfertilized ovules.

Genetic transformation

Generally, three vector systems (viz. electroporation, particle bombardment and *Agrobacterium*) have emerged as the basic tools for the genetic transformation of plants. In studies involving the gene gun transformation method, particle bombardment of chimeric constructs of the β -glucuronidase (GUS) gene under the control of the cauliflower mosaic virus 35S promoter into intact tef cell suspension culture cells, callus tissues and zygotic embryos showed transient expression of the introduced alien GUS reporter gene (Mengistie 1991).

Research on *Agrobacterium*-mediated transformation in tef callus production and growth, suspension culture cell growth, and seed germination and growth sensitivity studies to antibiotics showed leaf base callus production and growth was more sensitive to both Kanamycin and G418 than callus from seeds, and that G418 at low concentrations proved the best selective agent compared to Kanamycin or Carbenicillin (Mengistie 1991). On the other hand, direct transformation attempts through imbibing or soaking of tef seeds and zygotic embryos in a solution of plasmid DNA for half to 24 hours

revealed no uptake of the alien GUS gene. No GUS expression was observed in the progeny seedlings or the callus from the whole seed or embryo culture of the treated seeds (Mengistie 1991).

The lodging problem in tef: the way forward

The tef breeding program in Ethiopia has relied upon the indigenous resources and efforts. The achievements and progresses made to-date notwithstanding, lodging has persisted as the most important bottleneck constraining the production and productivity of tef in Ethiopia both directly and indirectly. Notably, the effects are losses in the yield and quality of both grain and straw produced. Under natural conditions grain yield losses due to lodging are estimated at up to 25% with an average of 17% (Ketema 1993). It also reduces the quality of the seed in terms of germination energy and capacity, color, and nutritional value. Indirectly, lodging hinders the use of high-input husbandry technologies such as increased nitrogen fertilization, and it also interferes with both mechanical and hand harvesting operations. A tangible solution to the problem of lodging in tef would, therefore, increase the yield of both grain and straw through minimization of the lodging-inflicted losses. Lodging resistance would mean great opportunities for commercialization of tef production through mechanization.

The question of combating the problem of lodging has long been a sustaining major objective of the overall national tef improvement program. But the lodging resistant tef varieties have remained elusive. The reasons, among others include, lack of sufficient variability in the available germplasm (including the wild relatives crossable with tef). Also, unfavorable associations/correlations of lodging tolerance and related productivity promoting traits limit breeding progress. There is apparently close linkage between culm thickness and plant height with the dwarf types in the germplasm and recombinants or segregants resulting from hybridization often have very thin stems that succumb to severe lodging. Finally, reduced plant height is associated with the shortening of panicles and panicle length is positively correlated with grain yield.

We predict that the future of tef production in Ethiopia hinges upon the development of successful management packages for lodging and control of weeds. Emerging trends of labor shortages, urbanization effects, and price hike for tef grain attest in favour of our prediction. We envisage a multi-dimensional approach involving uses of genetic

(breeding) methods, cultural practices, anti-lodging plant growth regulators (agronomic) and machinery (Figure 3). Considering the genetic approach, in turn, we are of the opinion that various methods including direct selection, hybridization, induced mutation techniques, and genomics will provide synergistic effect.

The pressing question follows if there would be any sense of optimism to spend resources to solve the lodging problem in tef. We are again positive in our opinion for the following reasons: 1) similar problem has been tackled in the other cereals; 2) the future holds a better scientific promise; and 3) the future generations of tef breeders will be equipped with better knowledge and skills. What is required is a scientific endeavor of a relatively long-term plan, continuous funding with focused implementation of activities.

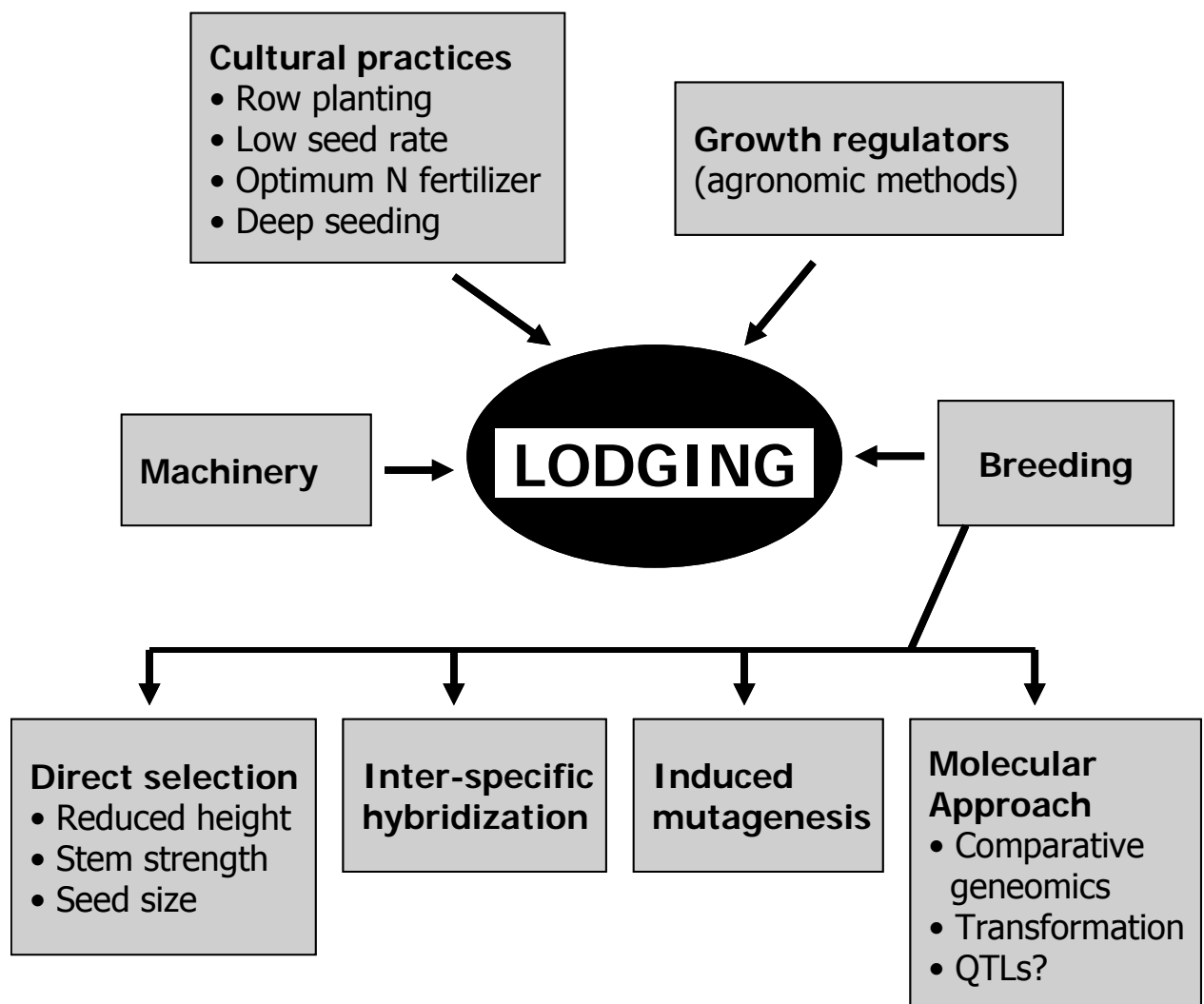


Figure 3. Schematic representation of the approaches for combating lodging in tef

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Genetic resources, breeding and production of millets in Ethiopia

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Summary

In Ethiopia, both finger millet and pearl millet are cultivated to a varying extent with finger millet taking the largest share in terms of area cultivated and total production. There is no statistical data on the area and production of pearl millet, however, it plays insignificant role in the dry land farming system of north and north western regions of the country and the research effort to improve the production of the crop has been low. Whereas finger millet is one of the traditional food grains grown in marginal environments largely by resource poor people. The presence of wide range of variability in local germplasm collections suggests that the crop is one of the indigenous cereals in the country. Much of the variability observed among the landrace collections is associated with the variation in the growing environments such as altitude, temperature regime, rainfall and length of growing period suggesting that different ideotypes that to best fit to the unique environments may be required. Since its inception in 1986 as part of the National sorghum Research program, the millet research activities have focused on addressing this and other production constraints. Over the years, the program has developed and released five finger millet varieties and one pearl millet variety for commercial production.

Introduction

Finger millet (*Elusine coracana*) is one of the important indigenous food crops of Ethiopia. It plays significant role both as food grain and animal feed in areas where production of other cereals are curtailed by marginal environments. According to the national statistical data some 400,000

hectares are put to production of finger millet each year mainly in the northern and north western parts of the country (CSA 2006). As a result of increased drought and soil fertility degradation, a growing number of farmers are resorting to finger millet and thus the area under the crop has significantly increased over the last ten years. Although finger millet is not on the top list of the national priority commodities, the increasing challenge by the environment and the growing acreage under the crop has resulted in some change in research emphasis. Over the last ten years, significant effort has been made by the national program to develop new and improved finger millet varieties and promote the technology to enhance its adoption. As a result of these efforts, finger millet has become one of the preferred crops in the drought prone south, south central and eastern parts of Ethiopia.

Unlike finger millet, pearl millet [*Pennisetum glaucum* (L.) R. Br.] is grown only in pockets of areas in the north and north western parts of the country. Owing to its tolerance to marginal conditions, the crop seems to have been well received by communities living in the dry areas. Its cultivation and use as food grain expanded to drought prone areas in eastern Ethiopia in the last two decades (ICRA 1996). In Ethiopia, research emphasis on millets in general is low and effort to improve pearl millet as food grain has been next to none. Only one variety has been developed and released to the producers to date and feedback on its perception by the community is not available yet. Therefore, this paper primarily emphasizes on finger millet research and production in Ethiopia. It will summarize successes from the previous activities and outline the major challenges in the production and use of the crop as food grain.

Production and uses of finger millet

Finger millet is grown as a staple food grain in parts of Ethiopia where drought takes its highest toll on crop production and as food security crop in several other parts of the country where low and erratic rainfall has been adverse on other food grains. It is grown in wide ecological range of up to 3000m elevation (Doggett 1986). Traditionally, finger millet production has been limited to the north and north western parts of Ethiopia and pocket areas in other parts of the country (CSA 2006). Perhaps due to increased environmental pressure and/or the sustained effort to promote the production of the crop in recent years, acreage under finger millet is

increasing from year to year. Areas where the crop has not been known earlier such as the central rift valley, south central and parts of eastern Ethiopia have seen increased production of the crop since 2001. Figure 1 shows the current millet production areas in the country. A Part of the country where dry land maize has been dominant has seen massive replacement by finger millet in southern Ethiopia (Anchala *et al.* 2006). Its better tolerance to environmental stresses, more versatile use of the grain and the feed value of the straws are some of the benefits of the crop cited by local growers in southern Ethiopia. Besides the acreage, the productivity of the crop over the last 10 years has steadily increased. The national average yield has grown from 0.85 t/ha in 1998 to 1.3t/ha in 2006 (CSA 2006). The introduction and promotion of new high yielding varieties, "Taddesse and Padet" by the national program and the expansion of the cultivation of the crop from its locale in the very dry degraded regions to a relatively productive areas in several parts of the country may have contributed to the increased productivity. Seeds of finger millet can be stored for long time without damage and is less prone to attack by storage pests. Although blast disease caused by *Magnaporthe grisea* (anamorph *Pyricularia grisea*) poses serious biotic constraint in some years, finger millet generally suffers less from diseases compared to common cereals grown in the country.

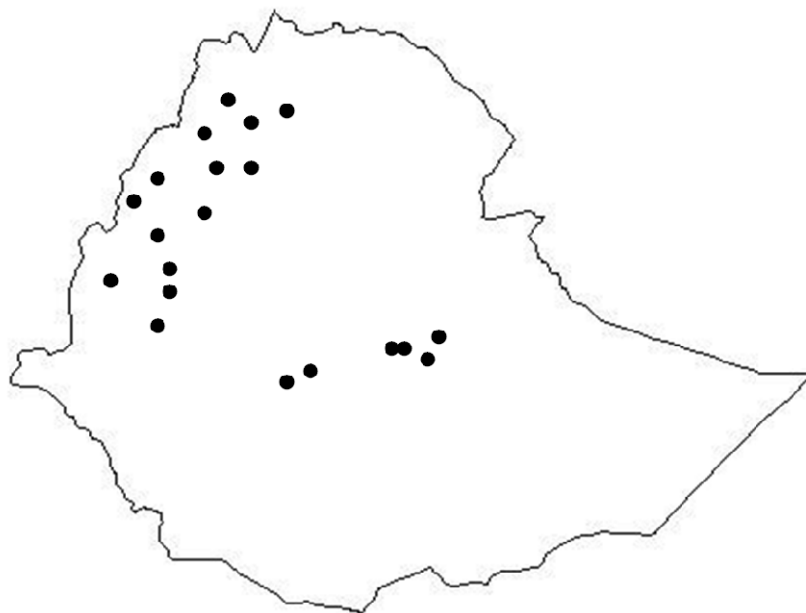


Figure 1. Spatial distribution of millet production areas in Ethiopia.

Finger millet in Ethiopia has a variety of uses. It is used for making *injera*, a thin, pancake-like bread, commonly served in the national dishes, bread and porridge. It is also used for making traditional alcoholic beverages such as 'tella', the local beer and 'arakie', local sprit. The straw is used for animal feed and for thatching roofs. The grains fetch better price than maize (Chimdo *et al.* 2006). Most of the finger millet produced in the country are consumed locally or sold in local markets for traditional uses. The crop is considered as nutritious cereal with enhanced levels of essential nutrients compared to rice, maize or sorghum (Hulse *et al.* 1980). Oil from finger millet is considered to be good quality (polyunsaturated); that it provides health benefits to humans through reducing cholesterol levels (National Research Council 1996). Finger millet is particularly rich in dietary fiber and minerals such as calcium and iron (Table 1). Babu *et al.* (1987) reported that some high-yielding varieties also contain high protein content (8 to 12%) and also rich in calcium content (294 to 390 mg per 100 g).

Table 1. Nutrient composition of finger millet as compared to major food cereals grown in Ethiopia

Contents	Unit	Millet	Sorghum	Tef	Barley	Maize	Wheat
Energy	Cal./100g	326	338	336	334	356	339
Protein	g/100g	7.2	7.1	8.3	9.3	8.3	10.3
Oil	g/100g	1.4	2.8	2.9	1.9	4.6	1.9
Carbohydrate	g/100g	77.1	76.5	75.2	75.4	73.4	71.9
Fiber	g/100g	5.6	2.3	3.6	3.7	2.2	3.0
Calcium	Mg/100g	386	30	140	47	6	49
Phosphorous	mg/100g	220	282	368	325	276	276
Iron	mg/100g	85.1	7.8	59.0	10.2	4.2	7.5

Source: Wondimu and Takebe (2001)

Genetic Resources

Finger millet is cultivated in diverse agro-ecologies in Ethiopia. However, its role as staple food grain is much higher in dry lowlands and degraded areas where other cereals struggle. As such significant variation exist among the local landraces with major variations observed for common agronomic characteristics including maturity, plant height, seed color, plant architecture, etc. Kebede and Menkir (1986) reported the occurrence of vast genetic variability among the Ethiopian finger millet germplasm collection.

Established in 1976, the Ethiopian Plant genetic Resource Center, now Institute of Biodiversity Conservation (IBC) is responsible for collecting and preserving plant genetic materials. Prior to that Debre Zeit Experiment Station collected and preserved genetic materials for various crops including finger millet. Over years, the IBC developed its capacity both in manpower and facilities to evolve as one the biggest plant genetic resource establishment in Africa. At present, over 2000 finger millet germplasm accessions collected from various parts of the country are maintained at the IBC, Addis Ababa (Table 2).

Major problems associated with finger millet production in Ethiopia

Finger millet in Ethiopia is grown in the intermediate-high altitude areas in the north and pockets of dry lowlands in other parts of the country. The following major constraints are identified in finger millet production and consumption:

- ***Drought:*** in the dry lowlands, moisture availability is the major limitation to production of finger millet.
- ***Shortage of improved varieties:*** only few suitable varieties are available particularly for dry lowland areas. Few varieties primarily developed for optimum rainfall areas proved to be adapted to the dry lowlands of central and eastern Ethiopia with average yield of 3 t/ha on farmers field. Future research should target similar environments and use these varieties as potential breeding material to identify new genotypes for similar agro-ecologies.

Table 2. Spatial and altitudinal distribution of finger millet accessions in the national germplasm repository, Ethiopia

Regions	Current holding by regions		Elevation (meters above sea level)	Current holding by altitude zone	
	Total accessions	Accessions characterized		Total accessions	Accessions characterized
Former Eritrea	278	270	Below 1000	140	137
Gamo Goffa	29	26	1000-1500	299	270
Gojjam	294	240	1501-1900	304	199
Gonder	180	106	1901-2300	371	212
Hararghe	6	6	Above 2300	49	35
Illubabor	25	23	-	-	-
Kafa	5	3	-	-	-
Shawa	13	5	-	-	-
Sidamo	3	3	-	-	-
Tigray	212	29	-	-	-
Wollega	127	74	-	-	-
Wollo	6	4	-	-	-
Unknown	873	634	Unknown	888	570
Total	2051	1423	Total	2051	1423

- **Lack of agronomic packages:** little research have been conducted to develop agronomic practices to maximize productivity.
- **Head blast:** this is a major fungal disease of finger millet. The disease affects the peduncle and the grain bearing fingers resulting in significant yield losses. It is particularly severe in the warm and high rainfall areas. Although significant variation exists among germplasm collections, these sources have not been utilized in the breeding program to develop resistant varieties.
- **Lodging:** this poses a formidable challenge to finger millet production. Lodging occurs late after flowering or during grain filling and the problem is particularly severe on tall local cultivars.

- **Utilization:** although the crop is considered highly nutritious, information on its processing are limited. As a result, in many parts of Ethiopia, the use of the crop is highly restricted to production of local alcoholic beverages, although it is often mixed with other grains to make injera. Elsewhere in Africa, the crop is used in several food applications including *Ujji* (thin porridge) that is served in public restaurants.

Finger millet breeding in Ethiopia

Like for many other grains, research on finger millet was initiated at Debre Zeit Agricultural Experiment Station in the late 1950s. Much of the early efforts have focused on collection, conservation and characterization of finger millet germplasm. With the transfer of the national sorghum Research Program from Haramaya University to its current place at Melkassa in 1986, research on finger millet was reinitiated as part of the National Sorghum Program. Since then efforts have been underway to develop high yielding finger millet varieties with improved resistance to major biotic and abiotic stresses outlined in the previous section. Schematic presentation of finger millet variety development process is shown in Figure 2.

Germplasm acquisition and evaluation. Ethiopia is considered as one of the centers of diversity for finger millet. The IBC has over 2000 finger millet germplasm collections in its holding. The national research programs have access to these local germplasm resources. The IBC and the national have over years developed a platform where they jointly conduct germplasm characterization activities at research stations. Through this effort, over 1400 germplasm collections have been characterized for major agronomic traits (Table 3). The joint effort has also created unique opportunity for breeders to conduct preliminary selection among the collections. In addition, through the regional research network, the national program occasionally acquires germplasm sources from the region. The Eastern African Regional Sorghum and Millet Research Network (EARSAM) and later the Eastern and Central African Sorghum and Millet Research Network (ECARSAM) served as an important bridge to bring the regional scientists together and facilitate the flow of germplasm in the region.

Through these networks, over 500 finger millet accessions have been acquired by the Ethiopian program and these along with the local sources are used as key germplasm sources for genetic improvement of the crop.

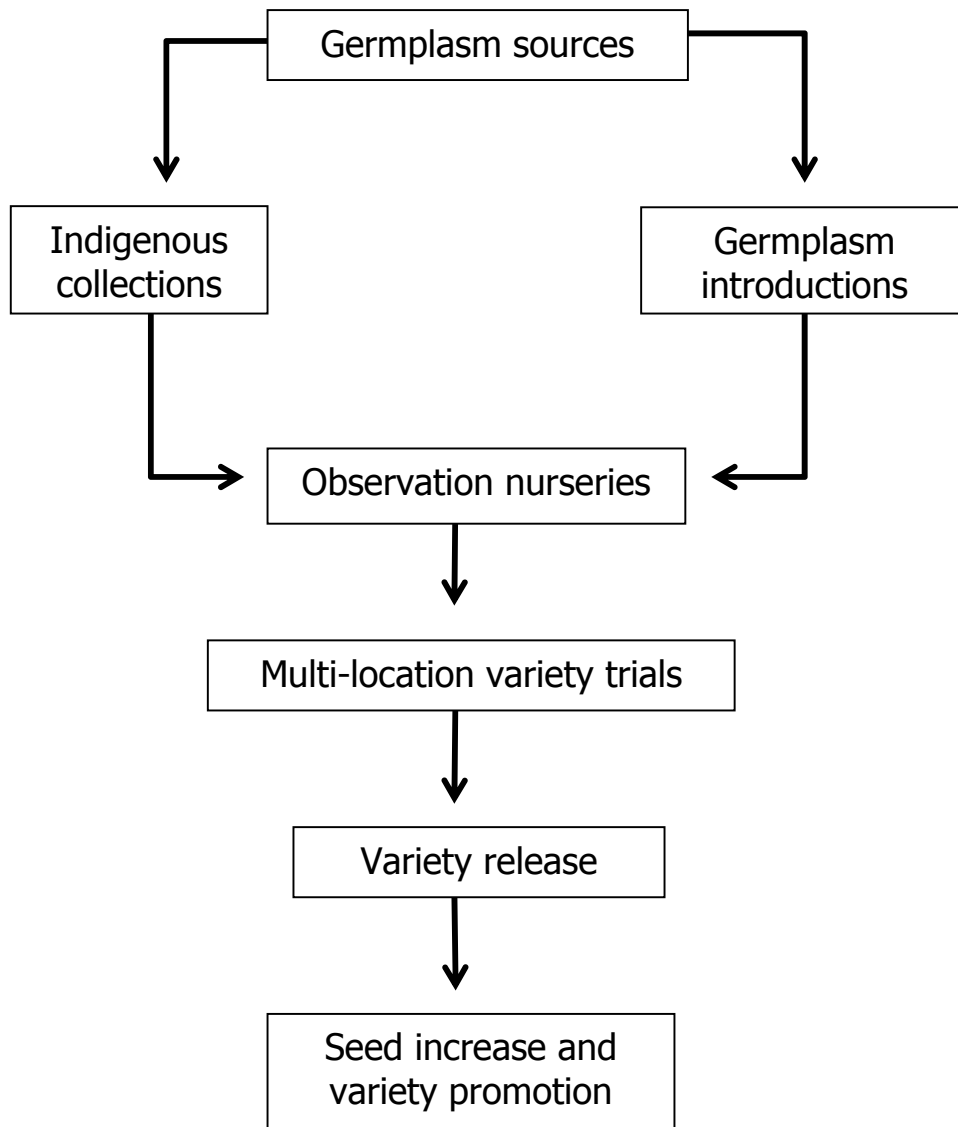


Figure 2. A schematic presentation of the variety development process in finger millet improvement program

Table 3. Major agronomic characteristics of the national finger millet collections in Ethiopia

Characters	No. of Accessions characterized	Minimum	Maximum	Mean	Standard Deviation
Days to flowering	1420	55	184	101	2.68
Number of fingers	1216	1	16	8	1.73
Length of fingers (cm)	524	3	14	7	2.32
Plant height (cm)	1358	34	935	94	64.41
Days to maturity	1344	100	198	147	17.00

The germplasm materials are evaluated in two target environments, the lowland and mid-altitude. Although the country has diverse agro-ecologies with varying crop production potentials and limitation, two broad agro-ecologies are targeted for finger millet germplasm evaluation subsequent testing and release of varieties. The low-land agro ecology includes finger millet production areas located below 1500m elevation and the mid altitude above 1500m. The major characteristics of the lowland environment include low and erratic rainfall, high temperature with drought standing as the major constraint. Early maturity and high yielding potential are the major traits desired for this agro-ecology. The intermediate altitude growing areas receive relatively higher amount of rainfall which in warmer locations leads to high humidity. The key constraint under this condition is the head blast disease.

Performance testing. Based on the results of the preliminary evaluation, promising genotypes are selected for further test under multi-location trails. Genotypes with superior performance across environments are assembled in to a national yield trail and evaluated in several locations across the country. Those that tend to consistently perform better in one

environmental category than the other are assembled to a regional variety trail and evaluated in multiple locations in the target adaptation zone. These tests are often repeated for 3-4 years to capture seasonal weather variability and determine the adaptability of genotypes to these variables. In all cases, the test genotypes are evaluated against the locally grown farmers variety obtained from each of the test areas and recent releases as standard check. Genotypes that consistently outperformed the local and standard check or have the same level of performance but have added advantages in any of the desirable traits, seed quality, disease resistance, lodging resistance, maturity, etc, are selected as candidate for release.

Release of new variety is granted by the national variety release board. Proposal for release of new variety is prepared by the developing breeder and submitted to the board. The proposal should contain information outlining the merits of the candidate variety and performance data from multi-environment tests. The breeder also put together a variety verification trail to be conducted on larger plots on farmers' fields in the target areas. The board assigns a technical team to evaluate the candidate variety while in verification plots. Based on the report by the technical team and information provided by the breeder, the board gives the final decision whether the new variety has to be released. Information on utilization attributes of the candidate variety and the farmers' opinion are taken into consideration for the decision. If a variety is approved for release, the developing program assumes responsibility for maintenance and supply of breeder seed and promotion of the variety.

Over years the National Sorghum and Millet Research Program has released 5 finger millet varieties for different agro-ecologies (Table 4). Efforts to promote some of these varieties has resulted in a nationwide accredited success where the varieties received overwhelming acceptance among communities because of their tolerance to drought and high yield under dry conditions (Anchala *et al.* 2006).

Table 4. Mean grain yield (t/ ha), days to flowering, maturity, number of fingers and length (cm) and plant height (cm) of five finger millet and one pearl millet varieties released in the last decade

Pedigree	Designation	Grain yield (t/ha)		Days to flowering	Days to maturity	Number of fingers	Finger length	Plant height	Special features
		Research station	Farmers' fields						
KNE# 1098	Tadesse	3.2-4.2	2.8-3.4	113	130-145	5-7	5-7.5	98	Drought tolerant, widely adapted, high yielding
KNE# 409	Padet	3.0-3.5	2.8-2.9	104	130-145	5-7	5-7	94	Adapted to cooler environments
KNE# 411	Boneya	2.5 - 3.0	2.0 - 2.4	90-100	130-145	-	-	88	High yielding, blast and lodging resistant
PGRC/E 215874	Degu	2.3-3.0	1.7-2.1	95-105	137-160	-	-	93	-
PW01-075	Baruda	2.5-3.0	-	-	-	-	-	-	Adapted to high rainfall areas
ICMV 221*	Kola-1	4.2	3.0	104-110	80-90	10-12	10-13	150-170	Adapted to low rainfall areas

* Pearl millet variety recently released for low rainfall areas. All the rest are finger millet varieties released for different agro-ecologies over the last ten years.

Source: data from the national and regional variety performance tests and on-farm variety trials.

Major limitations

The major problem to the development and deployment of new finger millet technology is the lack of commitment and priority to improvement of the crop. Given its relatively low importance in the national agriculture, support and commitment by the government and the research community has been low. This has resulted in lack of sustained effort for the improvement of the crop and slow progress in development of new improved varieties and germplasm. Much of the breeding effort is based on exploitation of the existing sources of variability; and genetic recombination through hybridization is seldom conducted. Even though successes have been reported in hybridization of finger millet elsewhere and in an even smaller cereal tef in Ethiopia, no effort has been made to recombine finger millet through hybridization. Although Ethiopia is considered as one of the countries with wide genetic resource for the crop, the number of accessions available and the variation among them are not much when compared with other crops such as sorghum. Hence, the need for use of alternative breeding tools for enhancing genetic variability is imperative.

However, the growing acreage under finger millet has at times attracted attention of policy makers and research administrators that, at one point, millet research had stood as an independent program within the Ethiopian National Agricultural Research System. With its importance as food grain steadily growing, the need to improve the productivity of the crop is mounting. At present Ethiopia is the second largest producer of finger millet in east and central Africa and millions of people directly depend on the crop as major source of energy and protein.

Major areas of intervention to enhance productivity

The declining soil fertility and the ever increasing problem of drought call for development and use of crop species that are resilient in the face of these stresses. Both finger millet and pearl millet hold great potential for the dry lowlands where economic production of other food cereals is constrained by these stresses. We believe that interventions are required from many angles to lift the value of the millets as alternative food and feed crops and strengthen the national effort to develop and deliver improved production technologies. Some of these include:

- i) Research capacity building.* Developing the capacity of research programs working on the improvement of the millets is the first step. This includes formal training of staff assigned on millet research, supporting and strengthening linkage with regional and sub-regional research programs and increasing access to fresh germplasm sources.
- ii) Strengthening transfer of technologies.* The effort by the national program to disseminate improved technologies over the last decade has been encouraging. This effort should also embrace the existing millet technologies. A pilot extension activity conducted over the last five years in south central Ethiopia has resulted in large scale adoption of finger millet in an areas the crop has never been known. These efforts should be scaled up and out to reach more stakeholders in other areas where the crop is expected to have potential impact.
- iii) Developing seed production and delivery mechanisms.* Non hybrid crops rarely attract interest of private entrepreneurs. Researchers, local government and non-governmental development organization should be encouraged to work together and develop a workable format where local communities can participate in production and distribution of quality seeds.

The need for such intervention is not unique to millets and these efforts can be superimposed on the ongoing effort made to promote other food grains.

Acknowledgment

The authors duly acknowledge the Institute of Biodiversity Conservation (IBC) for providing information on finger millet genetic resources. We also thank colleagues who assisted in regional and national testing of varieties over several years.

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Food use and grain quality of Ethiopian barley germplasm

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Summary

In Ethiopia barley has a long history of cultivation and the crop has been evolving with the folk's traditions and consumption habits. In the highlands barley is a reliable staple crop and a major source of food, homemade drinks, animal feed and cash. Today traditional barley landraces are suffering serious genetic erosion. The present paper reports the loss of agro-biodiversity and impacts of end-use on the on-farm conservation of barley landraces in two districts of West Shewa. Moreover, results are presented on important agronomic and quality traits of Ethiopian hull-less barleys which were preserved in various *ex situ* collections. Hitherto the great gene diversity of Ethiopian barleys, especially in regard to resistance genes, was exploited worldwide by modern plant breeding programmes. It is demonstrated that Ethiopian hull-less barley resources carry also valuable quality genes for the improvement of barley's food qualities.

Introduction

In 1927 the Russian botanist and geneticist N. I. Vavilov carried out an expedition to Abyssinia, Eritrea and Somaliland. Due to the exceptional wealth of forms of barley (*Hordeum vulgare* L.) he declared Abyssinia as the center of origin of cultivated barley (Vavilov 1951). Recently Orabi *et al.* (2007) applied diverse DNA markers on a total of more than 500 barley accessions and thereby confirmed that an independent domestication of barley might have taken place at the Horn of Africa. In Ethiopia barley has a long history of cultivation and the crop has been evolving with the folk's traditions and consumption habits. As barley demonstrates a wide ecological plasticity and physiological amplitude

throughout the country, it is cultivated in almost every region of Ethiopia except in warm and lowland areas (Asfaw 1999). In the highlands barley is a reliable staple crop and a major source of food, homemade drinks, animal feed and cash. Barley is a preferred grain in a wide range of recipes that have deep roots in culture and tradition.

Barley was a sustaining food source in the evolution of humans since the Neolithic revolution. It was one of the most important food grains in the ancient world. As other cereals, e.g. wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.), became more abundant barley became mainly a crop for feeding and malting purposes. The food use of barley sustained in a few remote areas of Africa and Asia (Newman & Newman 2006). In the last decades barley was re-discovered as a food grain due to its desirable nutritional composition. Recent research findings showed that barley contains considerable amounts of beta-glucans and tocopherols which can have beneficial health effects (Cavallero *et al.* 2004, Behall and Hallfrisch 2006, Pins and Kaur 2006). Barley foods, snacks and dietary supplements are increasingly available in developed countries. In 2006 the US Food and Drug Administration authorized the use of a health claim for the role of beta-glucan soluble fiber from barley in reducing the risk of coronary heart disease (FDA 2006).

The great biodiversity in Ethiopian barley was demonstrated by various studies (for review, Asfaw 1999). However, as reported by the FAO (1996) traditional barley and durum wheat landraces are suffering serious genetic erosion. Recently Tsegaye and Berg (2007a) reported the genetic erosion of tetraploid wheat landraces. In a follow-up paper they describe the instrumental role of home use in the promotion of on-farm (*in situ*) conservation of landraces in the highlands of Eastern Shewa (Tsegaye and Berg 2007b). The present paper reports the loss of agrobiodiversity and impacts of end-use on the on-farm conservation of barley landraces in two districts of West Shewa, an area known for its extreme richness in barley landraces (Asfaw 1999). Moreover, results are presented on important agronomic and quality traits of Ethiopian hull-less barleys which were preserved in various *ex situ* collections. The potential of Ethiopian hull-less barley varieties concerning the breeding for healthy food is discussed.

Materials and Methods

A field study was carried out in two major barley growing districts (*Woredas*) of the West Shewa Zone in the central Ethiopian highlands, namely Dandi (8°51' N, 38°01' E) and Jeldu (9°02' N, 37°40' E). The study was conducted in January 2007 in a total of five peasant communities (*Kebeles*): three from Jeldu (Hindhe Dora, Edensa Gelan, and Chilanko), and two from Dandi (Galessa Koftu and Galessa Kota Geshir). Per community three villages were studied. The survey area is a rugged and hilly highland with gently to steep, and sporadically very steep slopes. The predominant soil types are Udalfs (Alfisols) (USDA 1999). Most of the area lies at an altitude of 2000–3000 m a.s.l. and is characterized by cool climate; a few places lie above 3000 m a.s.l. and are exposed to very cold temperatures. Mixed crop-livestock subsistence farming is peculiar to the study area. Barley is the dominant crop in both districts at highland areas while its wide area coverage declines in lowland and mid-altitudes. Potato (*Solanum tuberosum* L.) is the dominant crop around homesteads. Primary information was attained in group discussions with men and women farmers.

A collection of hull-less barley accessions obtained from the genebanks of AGES Linz (Austria), IPK Gatersleben (Germany) and the Barley Germplasm Center Okayama (Japan) was grown in 2006 and 2007 under organic conditions in eastern Austria (16°83' E, 48°13' N). The grains were analyzed for 1000-grain mass, test/hectolitre weight, kernel plumpness, total content of polyphenolics, anthocyanins, yellow pigments, beta-glucan and protein. The chemical analyses were carried out on whole grain flour which was milled using a Retsch ZM 100 ultra-centrifugal mill equipped with a 0.5 mm sieve. Crude protein content, yellow pigment content and beta-glucan content were determined according to the ICC Standard Methods Nos. 167, 152 and 166, respectively (ICC 2006). Total anthocyanin content was determined by a modified method after Abdel-Aal and Hucl (1999), total phenol content by means of the Folin-Ciocalteu reagent (Singleton *et al.* 1999). Genotype by environment means were calculated from the multiple measurements of traits and subjected to a principal components analysis (PCA). Subsequently, genotypic scores (eigenvectors) of principal components with eigenvalues >1 were subjected to a hierarchical cluster analysis using Ward's minimum variance method. PCA combined with cluster analysis was applied successfully to describe multivariate variation of germplasm of various crops (e.g. Cartea *et al.* 2002, Granati *et al.* 2003, Kamara *et al.* 2003, Hailu *et al.* 2006). All statistical analyses

were carried out using SAS 9.1 software (SAS Institute, Inc., Cary, NC). Focus was given on hull-less barleys since in this case all the beneficial effects of whole-grain foods can be exploited, while during dehulling of hulled barleys the outer grain layers which contain considerable amounts of minerals and phyto-nutrients are removed.

Results and discussions

Barley landraces in Dandi and Jeldu Woredas (districts)

In total 14 barley landraces were reported by the farmers. The vernacular names are deduced from traits which are used by the farmers to characterize their varieties, e.g. ear/spike type (2- vs. 6-rowed), plant height, maturity, caryopsis cover (hulled vs. hull-less/naked), grain color, specific end-use for foods and/or beverages, or designate the places of their origin. For example *Butuji* is named after its short plant height, *Garbuguracha* after its compact black-seeded spike, *Balame* after its 2-rowed spike, *Muga* after its late maturity, *Luka'a* after its easy threshing/dehulling, and *Kate* after its seed bursting while roasting. Farmers also use the designation *Garbuguarcha* as a generic name. In Table 1 a short description of the reported farmers' varieties of barley is given. It is apparent that the farmers' varieties are often a mixture of diverse grain color types, whereas in regard to spike type and caryopsis cover they are homogeneous.

Currently only four out of the above mentioned 14 varieties are cultivated in the survey area. *Balame* is the dominant landrace, followed by *Garbuguracha*. Despite the described merits the following landraces were lost in recent times: *Ababadhas*, *Abashawaye*, *Butuji*, *Dimicho*, *Hadho*, *Kate*, *Kitankite*, *Muga*, *Sidamo* and *Warkina*. Hence, the estimated loss of agro-biodiversity as defined by Hammer *et al.* (1996) was calculated to be 71.4% for the investigated area. The farmers have listed many natural and social factors that have contributed to the maintenance or loss of barley landraces. Major reasons for the loss of genetic diversity are the decline in soil fertility, changes in climate, and presence of frost and diseases. The degradation of soil fertility is among others due to the increase in human and livestock population. Also torrential rainfalls contribute to the loss of fertile soils (Erkossa *et al.* 2006). Mono-cropping of barley is the main practice in the survey area. Legumes which could contribute to soil fertility are rarely cultivated due to poor adaptation. Biotic factors such as diseases are not the major problem for barley production.

Table 1. Description of barley landraces as reported by farmers in Dandi and Jeldu districts

Name	GLC ¹	CAR ²	ROW ³	End-use
<i>Garbuguracha</i>	<i>Blp</i>	<i>Nud</i>	6	malt, beverage
<i>Balame</i>	<i>blp Blp</i>	<i>Nud</i>	2	<i>Injera</i> , roasted, <i>Kinche</i> , <i>Chiko</i> , <i>Beso</i> , porridge, beverage
<i>Samareta</i>	<i>blp Pre2</i>	<i>Nud</i>	2	<i>Beso</i> , <i>Injera</i> , <i>Shameta</i> , roasted
<i>Butuji</i>	<i>Blp blp</i>	<i>Nud</i>	6	<i>Injera</i> , malt, liquor
<i>Hadho</i>	<i>blp</i>	<i>Nud</i>	6	<i>Injera</i> , <i>Kinche</i> , <i>Chiko</i>
<i>Luka'a</i> (<i>Senefgebs</i>)	<i>blp Blp</i>	(nud)	2	roasted, <i>Kinche</i> , <i>Beso</i>
<i>Shamari</i>	<i>Blp</i>	<i>Nud</i>	2	beverage, liquor, roasted
<i>Kate</i>	<i>blp Blp</i>	<i>nud</i>	2	malt, beverage, roasted
<i>Kitankite</i>	<i>blp</i>	(nud)	6	roasted, beverage
<i>Muga</i>	<i>Pre2</i>	<i>Nud</i>	6	<i>Injera</i> , beverage, liquor, porridge, roasted
<i>Ababadhas</i>	<i>blp</i>	<i>Nud</i>	6	<i>Kinche</i> , <i>Chiko</i> , <i>Injera</i>
<i>Warkina</i>	<i>blp</i>	<i>Nud</i>	6	<i>Beso</i> , porridge, beverage
<i>Abashewaye</i>	<i>blp Pre2</i>	<i>Nud</i>	6	<i>Injera</i> , beverage
<i>Sidamo</i>	<i>blp Pre2 Blp</i>	<i>Nud</i>	6	<i>Injera</i> , beverage

¹ GLC, grain/lemma colour: *Blp*, black lemma and pericarp; *blp*, white lemma and pericarp; *Pre2*, red lemma and pericarp 2 (= purple colour)

² CAR, caryopsis cover: *Nud*, hulled; *nud*, naked; (nud), semi-naked (easy dehulling after roasting of the grain)

³ ROW, Ear/spike type: 2, two-rowed; 6, six-rowed

Since no improved barley cultivar is grown in the area, the chance of competing or threatening the cultivation of landraces is minimum. Farmers reported that improved cultivars have never performed well in marginal conditions, most probably due to the fact that they were selected and developed under more favorable and higher input environments. Other reasons might be that the farmers prefer their own

varieties as a result of their tradition of cultivation and consumption. They cherish their varieties and the indigenous knowledge associated with them as the heritage of their forefathers. The cultivation of barley landraces is especially under pressure by other crops in the areas around homesteads. Especially barley landraces which require more fertile soils and/or more management inputs were given up in favor of potato.

End-uses of barley

Various kinds of foods and beverages are made from barley as reported by farmers from both *Woredas* (Table 2). Each barley landrace has its unique characteristics which might make it a favorable raw material for at least one kind of food or drink. Black and purple-seeded varieties are generally preferred for making malt and beverages, while white-seeded ones are used for different kinds of food preparations. Culture, belief and financial needs of the society have affected the continued cultivation of different landraces that could have otherwise been lost. Barley has a very strong ethnobotanical link with the life of the farming communities of Dandi and Jeldu districts. Barley foods and drinks are not only used as ordinary foodstuffs, but have always been greatly valued and preferably been served during ceremonial events and gatherings in annual religious thanksgiving and society rituals from the past to the present. The multitude end-uses of barley seem to have persuaded farmers to maintain different seed types despite their relatively low yields. The importance of barley in the life of the highlanders is also documented in a wealth of traditional proverbs, poems and songs. Each traditional saying documents a meaningful folk-plant relationship and/or interaction, and the different attributes that barley can give to the household. Many proverbs appreciate the crop for its dependability, wide stability under changing environments and popularity over other crops, or unique qualities of specific landraces (for detailed review, Eticha *et al.* 2009).

Besides home use, market price accounts for a significant impact on the in-situ maintenance of barley landraces. As example the increase in demand and market price of the landrace which is preferred for the preparation of roasted whole grain snacks, encouraged farmers to increase its production although at the prevailing environmental conditions it exhibits poor agronomic performance compared to other landraces. The production of *Luka'a* emerged and replaced in some cases other landraces. Despite low grain yields due to the declining soil fertility many farmers are still interested to produce *Luka'a* since the high price that can be fetched for it is about double the price for other barleys which compensates the reduction in yield.

Table 2. Foodstuffs and beverages prepared from barley in Dandi and Jeldu districts

Product name	Product description
<i>Injera</i> (<i>Budena</i>)	<i>Leavened pancake; likewise soft and spongy bread</i>
<i>Aka'i (Kolo)</i>	<i>Roasted whole grain snack</i>
<i>Dabo</i>	<i>Leavened bread (different forms)</i>
<i>Bikil</i>	<i>Germinated barley (malt); used as starting material for fermentation</i>
<i>Farso (Tella)</i>	<i>Fermented and un-distilled home-made beer</i>
<i>Bukuri</i>	<i>Slightly fermented drink made from flour of lightly roasted grain and malt</i>
<i>Jusi</i>	<i>Non-alcoholic drink made from roasted grains soaked in water after roasting</i>
<i>Areki</i>	<i>Distilled home-made spirit made from fermented ingredients</i>
<i>Marka (Genfo)</i>	<i>Thick porridge</i>
<i>Atmit</i>	<i>Whole grain soup or flour soup</i>
<i>Shorba</i>	<i>Soup-like gruel made from coarsely ground grain</i>
<i>Beso (solid)</i>	<i>Roasted barley flour mixed with warm water to form a solid ball; spiced with salt</i>
<i>Beso (fluid)</i>	<i>Roasted barley flour mixed with cold water to form a liquid mixture; immediately consumed after preferably adding sugar; usually consumed by sportsmen for breakfast after doing physical exercises; medicine for people suffering gastric disease and it has a healing effect if taken regularly.</i>
<i>Chiko</i>	<i>Roasted barley flour, Beso, mixed with spiced butter forming a stiff ball</i>
<i>Kinche</i>	<i>Porridge made from coarsely grounded dehusked barley grain</i>

Characteristics of hull-less barley germplasm

The PCA revealed six principal components with an eigenvalue >1 and which explained 80% of the total multivariate variation. Cluster analysis resulted in three main clusters (Figure 1). Each cluster can be further grouped in at least two subgroups. Cluster A consists of modern German and Czech breeding lines, and in another subgroup of waxy forms of Canadian and US origin. The genotypes of this cluster are characterized by low values for test weight and protein content and high values for yellow pigment content. The grouping for beta-glucan content was inconsistent. While the first subgroup consists of the genotypes with the lowest contents, the waxy hull-less barleys exhibit the highest contents. Cluster B consists of the German (Taiga, Lawina, Nackta) and Italian (Digersano) standard cultivars on the one side, and the Ethiopian germplasm on the other side. Genotypes of this cluster exhibit high test weight and protein content, while the contents of beta-glucan and yellow

pigments are low. Concerning total phenolics and anthocyanins the two subgroups show opposite reaction. The European subgroup has high contents of phenolics but only medium anthocyanins, whereas the contents of the Ethiopian subgroup are vice-versa. Cluster C is the most heterogeneous one, including the Ethiopian germplasm, the old and modern European and Australian varieties, and a black grained Austrian breeding line. The subgroup that includes the Australian cultivars is characterized by low values for the secondary plant metabolites, medium contents of beta-glucan and protein, and high values for grain mass and kernel plumpness. The Ethiopian subgroup is characterized by high grain mass, plump grains, high content of protein and anthocyanins, while the content of total phenolics is low. From the clustering it is obvious that the diversity in the Ethiopian germplasm is huge because the hull-less barleys are partitioned into two main clusters and four subgroups. Interestingly no consistent grouping was obtained for grain color. The narrow genetic base of modern germplasm can be seen by the tight grouping of several subgroups e.g. modern German and Czech breeding lines, Canadian and US waxy varieties, German and Italian standard varieties, and improved Australian varieties. Figure 2 displays the relationship of selected traits for six subgroups from the cluster analyses. Ethiopian germplasm is distributed in groups 4 and 5. It is obvious that Ethiopian hull-less barleys represent valuable genetic resources for the improvement of both quality traits such as protein, beta-glucan or anthocyanins, and agronomic traits such as grain mass.

Conclusions

Ethiopia is a center of origin and diversity for many cultivated crops, among them is barley. The cultivation and use of barley is deeply rooted in the tradition of Ethiopian highlanders. The serious threat of genetic erosion of Ethiopian barley and tetraploid wheat was reported both by the FAO (1996) and various researchers (Asfaw 1999, Worede *et al.* 1999, Tsegaye and Berg 2007a). There are many reasons for the loss of genetic variability. In the present study, the depletion in soil fertility and degradation of soils by erosion are the major causes for the loss of various landraces. Hurni (1993) also indicated as these two soil related factors are the major problem for the sloped cropland of Ethiopia. Alternatives in land preparation can help to minimize the deterioration of soil quality (Erkossa *et al.* 2006), although it might have adverse effect on the diversity particularly when the native landraces are substituted by new crop species. Di Falco *et al.* (2007) recently demonstrated that variety richness increases farm productivity in case of production risk on

degraded lands. Genetic erosion results not only in a loss of probably valuable genes for agronomic or quality traits, or disease resistances, but also in a loss of traditional knowledge on the end-use of specific landraces. Since barley foods are deeply rooted in the cultural and religious heritage their loss will have also sociological impacts.

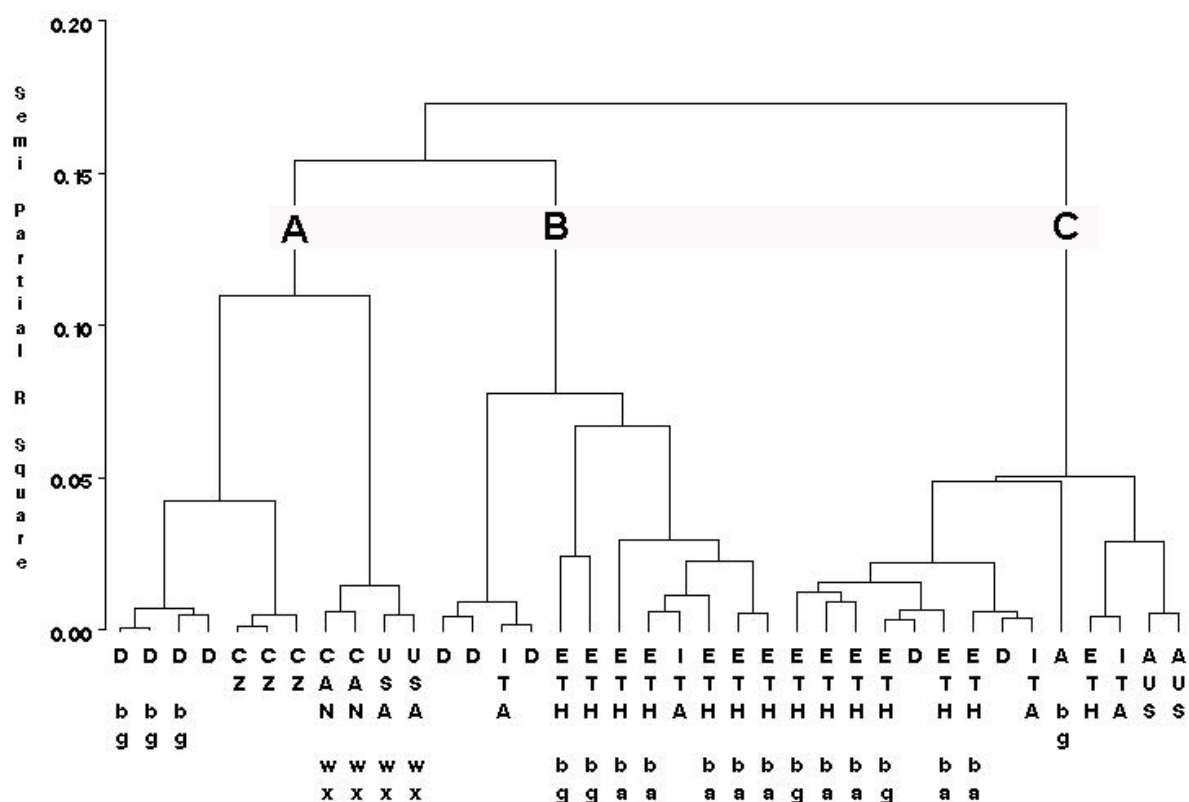


Figure 1. Dendrogram based on genotypic scores of six principal components.

[Country codes: A, Austria; CAN, Canada; CZ, Czech Republic; D, Germany; ETH, Ethiopia; ITA, Italy; USA, United States; Trait codes: ba, blue grain; bg, black grain; wx, waxy starch]

The great gene diversity of Ethiopian barleys was exploited worldwide by modern plant breeding programs, e.g. resistance against barley yellow dwarf virus (Niks *et al.* 2004) and net-blotch (Manninen *et al.* 2006), or higher protein and lysine content (Jonassen and Munck 1981). High protein contents and high grain mass were also confirmed in the present study in hull-less barley accessions of Ethiopian origin. Moreover, genotypes with higher levels of beta-glucan and anthocyanin content have also been identified. Due to high nutritional qualities foodstuffs from whole-grain barley need to be consumed as regular diet. Ethiopian highlanders appreciated for many generations the qualities of foods made from barley without scientific evidence. The consumers of the

Western World have now to be convinced of barley's food qualities by health claims. Hence, the Ethiopian barley may play a role in both cultures – as a sustainable staple crop of the indigenous highlanders or as donors of valuable quality genes in the improvement of barley for healthy food.

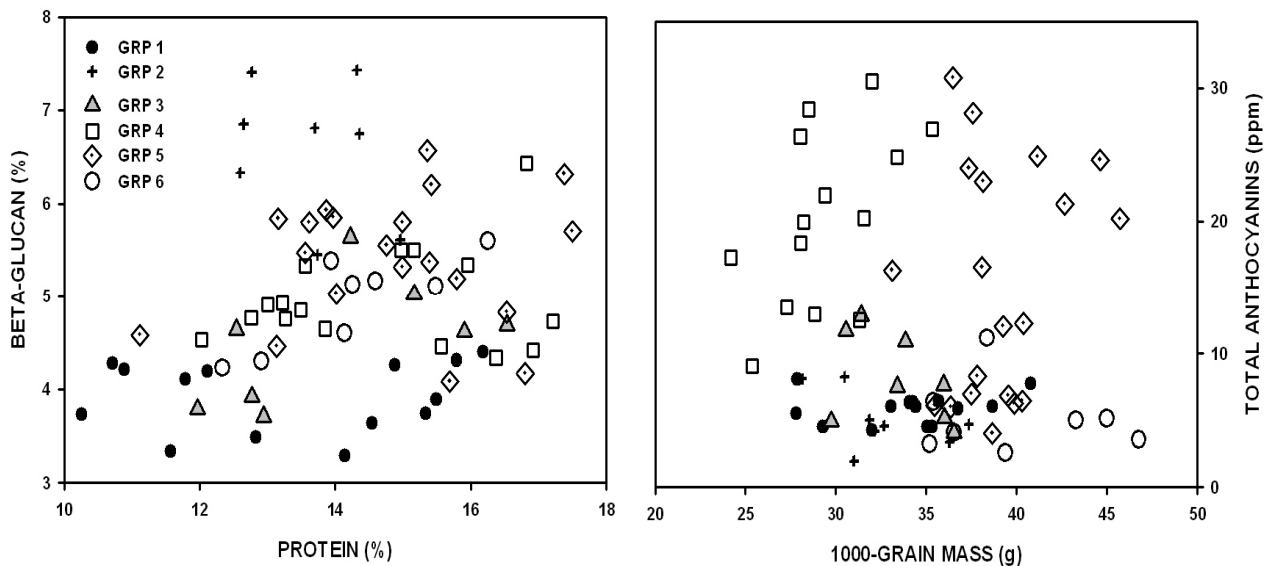


Figure 2. Scatter plots of hull-less barley germplasm in regard to beta-glucan, protein and anthocyanin content, and grain mass. Grouping of germplasm corresponds to the six main subgroups derived from cluster analysis (see Figure 1).

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Strategies in cowpea breeding

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Summary

Cowpea, *Vigna unguiculata* [L.] Walp., is an important grain legume food of the tropics and subtropics. It is a valuable source of protein for developing countries. Despite its widely adapted and stress tolerant characteristics, its production is seriously hindered by numerous constraints that include insect pests, diseases, parasitic weeds, drought and low soil fertility. Breeding programs established both at the national (NARS) and at the international levels (IITA and Bean/Cowpea CRSP) have recorded some encouraging achievements in alleviating these constraints. Important collaborative efforts are being conducted. The present review will focus mostly on the current breeding objectives and the future prospects. Pyramiding genes for resistance to biotic and abiotic stresses is an important ongoing activity. The advent of molecular biology and biotechnological tools offer new approaches for cowpea improvement programs. Hence new initiatives are currently being initiated to take advantage of the latest technologies. The Cowpea Genomic Initiative, as well as Tropical Legumes I and Tropical Legumes II projects are intended to provide opportunities for the application of molecular breeding tools. It is expected that future progress in cowpea improvement will benefit from the use of marker assisted selection and genetic modification.

Introduction

Cowpea, *Vigna unguiculata* [L.] Walp., is an important grain legume grown in diverse and contrasting environments of Africa, Asia, and Central and South America, as well as parts of Southern Europe and the United States of America. In these regions, this crop is cultivated on many types of soils, from highly acid to neutral and under different climatic conditions including low and infrequent rainfall and heat. Cowpea is also known to be part of diverse cropping systems such as monocropping, intercropping, mixed or relayed cropping.

Cowpea is a major source of dietary protein for the poor in many developing countries especially in Sub-Saharan Africa (SSA), South Asia (SA) and parts of Latin America. Young leaves, unripe pods and peas are used as vegetables while the grain is processed to various snacks (akara or kosai, moi-moi or cokie) and main meal dishes. The fodder is a source of quality animal feed. Its role as a cash crop both in terms of grain and fodder is highly appreciated in the Soudano-Sahelian zones of Africa.

Available records show that the crop is cultivated on about 10.1 million hectares in the world, producing 3.76 million tons with average grain yield of 0.41 t ha⁻¹ (FAOSAT, 1996-2006). Of the world production, an average estimate of 94.6% is produced annually in Africa on 97.8% of the total area. World statistics on cowpea production and cultivated areas are not well recorded and quite often merged with dry bean data. Singh *et al.* (2003) have estimated that cultivated area under cowpea is over 14 million hectares and cowpea production is over 4.5 million tons annually. Nigeria is the largest producer and consumer of cowpea with about 5 million hectares and over 2 million tons production annually. Niger Republic is the next largest producer on 3 million hectares and over 560,000 tons. In Central and South America, these authors also reported that Brazil alone produces about 500,000 tons on about 1.5 million hectares. In the southern US about 45,000 tons are produced on an estimated area of 40,000 hectares annually.

Despite its wide adaptation and importance, cowpea productivity is generally low due to several biotic and abiotic constraints as well as agronomic practices (marginal environments, low plant population density, no use of fertilizer and insecticides). Important constraints to cowpea production include insect pests, diseases, plant parasitic weeds, drought and heat, and cultural practices. Numerous breeding strategies are developed in different cowpea breeding programs and appreciable achievements have been reported. The present paper will focus mostly on the recent challenges being addressed in these breeding programs and future opportunities to overcome some of the cowpea production constraints.

Constraints to cowpea production

Farmers in Sub-Saharan Africa get on average grain yield of about 300 kg/ha. The major factors responsible for this low grain yield in cowpea are: 1) insect pests, (2) diseases, (3) parasitic weeds, (4) drought and heat, and (5) low soil fertility. Estimates of yield losses due to these

constraints range from low to complete depending on locations, seasons and resources available to the farmer.

1. Insect pests

Among the biotic constraints of cowpea production, insect pests rank first and can cause total yield failure in cases of severe attack (Jackai and Daoust 1986). There is at least one major insect pest attacking cowpea during each of the different stages of its life cycle from seedling to seeds in storage (Singh *et al.* 1990; Jackai and Adalla 1997). Following a number of farmers interviews carried out in Northern Cameroon (Kitch and Boukar, unpublished), it became obvious that the main limiting factor in cowpea production is insect pests. In general, the insect pest problem is more severe in Africa than in Asia or Latin America (Singh *et al.* 1990) probably because the crop's center of origin is the former. It is very common to find four or more pests on the crop at the same time. Singh and Allen (1980) divided the cowpea pest complex into three groups based on the time of infestation relative to crop phenology:

(a) Pests which are common throughout vegetative growth: leafhoppers, cowpea aphids and foliage beetles. These insects not only feed on leaves but are also vectors to viruses.

(b) Pests which infest at the appearance of flowers: flower bud thrips, lepidopterous larvae, and beetles. They cause flower abortion and destroy buds.

(c) Pests which are prevalent throughout the reproductive period and storage: legume pod borer, a complex of pod sucking bugs and the storage weevil.

The biology and symptoms of insect pests of cowpea have been extensively reviewed by Singh and Jackai (1985), and Singh *et al.* (1990).

2. Diseases

Diseases affecting cowpea are numerous and generally grouped into three main classes: viral, bacterial and fungal diseases. Their incidence and severity vary from region to region although some diseases are found worldwide and cause consistent damage to cowpea crop. Important diseases in each category are given below.

a. Viral diseases

The mainly seed borne viruses are blackeye cowpea mosaic potyvirus (BICMV), cowpea aphid-borne mosaic potyvirus (CABMV), cucumber mosaic cucumovirus (CMV), cowpea mosaic (CPMV) and cowpea severe mosaic (CPSMV) comoviruses, southern bean mosaic sobemovirus

(SBMV), and cowpea mottle carmovirus (CPMoV) (Hampton *et al.* 1997). Some combinations and mixed-infections of viruses have also been reported (Kuhn 1990). Important non-seedborne viruses are cowpea golden mosaic geminivirus (CGMV) and cowpea chlorotic mottle bromovirus (CCMV).

b. Fungal diseases

More than 40 species of fungi are known to cause diseases in cowpea (Singh and Allen 1980). Major fungal diseases include: Anthracnose, Ascochyta blight, Black leaf spot or leaf smut, Brown blotch, Brown rust, Cercospora and Pseudocercospora leaf spots, Powdery mildew, Pythium soft stem rot, Septoria leaf spot, Sphaceloma scab, and Web blight. A complete description of fungal diseases symptoms is reviewed by Emechebe and Shoyinka (1985), Patel (1985) and Emechebe and Florini (1997).

c. Bacterial diseases

Few bacterial diseases of cowpea have been reported in the literature. The major bacterial diseases are bacterial blight and bacterial pustule. A comprehensive description of their characteristic symptoms is given by Emechebe and Shoyinka (1985).

3. Nematodes

Nematodes are found worldwide and in all climatic zones, often in great numbers when environmental conditions are favorable. Caveness and Ogunfowora (1985) have reviewed the life cycle and symptoms of these parasites on cowpea plants. Among the numerous nematodes (more than a dozen genera) which attack cowpea roots, the most important species are the root-knot nematodes, reniform nematodes, root-lesion nematodes and lance nematodes (Caveness and Ogunfowora 1985).

4. Soilborne pathogens

The most damaging soil borne pathogens are macrophomina, phytophthora and pythium. Symptoms of soilborne diseases on cowpea have been described by Emechebe and Shoyinka (1985), and Florini (1997).

5. Drought and heat

In the semi-arid cowpea growing areas, drought and heat are major production constraints. In these regions, drought occurs most frequently and different types of drought can be found (Hall *et al.* 2002). The average period of rainfall can be short or long and occurs with irregular

frequency. In the Sahelian zone of Africa, for example, the rainy season has been getting shorter especially from 1970 to the 1990's, such that the annual rainfall has only partially supported a crop growing season of about two months in most years (Hall *et al.* 2002). Recent climatic changes are characterized by a shift in the period of establishment of the rainfall. The rains arrive late and take longer to become stable as compared to previous years.

Following their studies on cowpea response to temperature, Warrag and Hall (1984a, b) concluded that high temperatures at night can be much more detrimental to grain yield than high temperatures during the day. This conclusion came from the fact that high temperatures that commonly occur at night in the tropics can cause male sterility and substantially reduce grain yield by increasing floral abscission and decreasing the number of pods/m² (Warrag and Hall 1984a, b).

6. Parasitic weeds

The important parasitic weeds attacking cowpea are *Alectra vogelii* [Benth.] and *Striga gesnerioides* [Wild.] Vatke. Both are flowering plants that parasitize cowpea plants in the field. *Alectra vogelii* is restricted to Africa while *Striga gesnerioides* is also found in parts of Asia and USA (Musselman *et al.* 1991, Parker and Riches 1993). Both parasites cause considerable cowpea yield losses. Generally, *Striga* causes severe damage in the Sudan savanna and Sahel of West Africa whereas *Alectra* is more widespread in the relatively more humid Guinea and Sudan savannas of West and Central Africa and in East Africa.

Strategies for cowpea improvement

The numerous constraints to cowpea production have led to the development and implementation of numerous strategies. Broadly, these strategies target three main areas of research activities: pest-control strategies using synthetic chemicals and bio-control agents, and host plant resistance through breeding and biotechnological applications. These subdivisions commonly interact to provide efficient approaches for increasing cowpea productivity. Our discussion here will cover the host plant resistance approaches.

Cowpea breeding

Cowpea breeding activities are carried out in major cowpea growing areas of the world: Africa (Singh and Ntare 1985), Asia (Mishra *et al.* 1985), USA (Fery 1985, Hall and Patel 1985) and Latin America (Watt *et*

a/ 1985). The International Institute of Tropical Agriculture (IITA) with the international mandate for cowpea research and the Bean/Cowpea Collaborative Research Support Program (CRSP) funded by the United States Agency for International Development (USAID) have contributed and still are contributing substantially to the alleviation of cowpea production constraints. In Africa, several National Agricultural Research Systems (including universities) are making considerable progress in establishing functional cowpea breeding programs. In general, Hall *et al.* (1997a) described cowpea breeding activities and progress being recorded as modest given the importance of cowpea on one hand and the improvement works of other crop species on the other hand. These authors pointed out that the reasons given for the limited breeding activities are that cowpea is mainly grown by resource poor farmers for subsistence or sales within local regions hence commercial breeding companies have little interest in this crop. Several funded cowpea improvement activities exist in different regional programs and networks. In Africa, close partnership is established between IITA, Bean/Cowpea CRSP, NARS and the following funding agencies:

- The International Fund for Agriculture Development (IFAD) funds PRONAF (Projet Niébé pour l'Afrique)
- The United States Agency for International Development (USAID) funds Bean/Cowpea CRSP with activities in Africa and Latin and Central America
- The Kirkhouse Trust funds Marker-Assisted Selection and marker development
- The Generation Challenge Program (GCP) of the CGIAR (Consultative Group on International Agricultural Research) supports development and use of molecular markers for cowpea breeding
- The African Agricultural Technology Foundation (AATF) and the Network for Genetic Improvement of Cowpea in Africa (NGICA) link different actors of the cowpea biotechnology
- The Danish International Development Agency (DANIDA) funds crop-livestock systems
- The Gatsby Charitable Foundation and USAID fund the Strategic Seeds Project in Northern Nigeria for crop-livestock integration
- The McKnight Foundation funds the development and promotion of alectra resistant cowpea cultivars for smallholder farmers in Tanzania and Malawi.

The general objectives of all the cowpea breeding programs are to alleviate the numerous cowpea production constraints, improve the grain

and fodder yield and quality and meet the preferences of the end users. Some major achievements and goals of the different breeding programs have been reported by Ehlers and Hall (1997), Hall *et al.* (1997a) and Singh *et al.* (1997, 2002, 2003). The present paper will stress on the major current goals of the breeding programs.

Current cowpea breeding goals

1. Breeding for specific cropping systems

In Africa, cowpea is grown in a sole cropping system or in a combination under different intercropping systems with cereals especially sorghum, millet and to a lesser extent corn. For the sole cropping, the aim is to develop cultivars and management methods that take into consideration not only the effective reproduction period but also the set of potential production constraints. Hall *et al.* (1997a) suggested the development of early-flowering cowpea types with the delayed leaf senescence trait that produce two flushes of flowers or later-flowering cowpea types that produce one large flush of flowers over a long period. Plant spacings and the efficiency of conversion of intercepted photon flux density should also be taken into considerations. Singh *et al.* (1997) described the ideal cowpea variety for sole crop as one that has erect/semi-erect growth habit, with medium sized leaves and short basal branches to avoid lodging and 60-70 day crop duration, long peduncles, and pods displayed above the canopy for easy harvesting by manual or mechanical means. Examples of cowpea lines with these characteristics include IT86D-719, IT89KD-374, IT93K-452-1 and IT99KD-449-35.

Cowpea improvement activities conducted at IITA use two approaches to develop varieties for intercropping: (i) development of existing local cultivars by incorporating several resistance genes and (ii) development of new varieties for both grain and fodder under intercropping (Singh *et al.* 1997).

2. Breeding for drought tolerance

Cowpea is grown most commonly in the drought prone areas of the dry savanna of SSA. Drought tolerance is an important but complex characteristic in any given breeding program. One of the common approaches in developing drought tolerant crop varieties consists of selecting for early flowering as an escape mechanism, and yield testing of advanced lines over several locations and years (Hall *et al.* 1997b). Recent considerations including needs for physiological (osmotic adjustments and desiccation tolerance), and morphological (deeper

rooting systems, delayed leaf senescence) criteria were also discussed by these authors. According to Ehlers and Hall (1997) cultivars that flower early, possess the delayed leaf senescence (DLS) trait, and have an indeterminate growth habit should show drought adaptation and yield stability in many environments.

Instead of using the carbon isotope discrimination method and other physiological measures suggested by Hall *et al.* (1997a, 1997b), Singh *et al.* (1997) proposed the use of the "wooden box" technique for the screening of cowpea lines at the seedling stage and the testing of their field performance at the mature stage under conditions of water deficit. Two groups of drought tolerant lines were identified using latter technique: (i) lines that stop their growth as soon as drought stress is imposed, and survive for 2-3 weeks (e.g., TVu 11979 and TVu 11986) and (ii) lines that remain alive for a longer time, with the lower leaves dying one by one (Dan'Ila and IT90K-59-2).

On-going efforts use field irrigation systems to phenotype breeding lines and germplasm accessions to determine their reactions to drought.

3. Breeding for disease resistance

A lot of efforts were devoted by IITA and other breeding programs to identify and incorporate resistance genes into adapted cultivars. A large number of accessions were screened for resistance to the major cowpea diseases and sources of resistance were identified. The genetics of resistance to the various diseases have been reported by many authors (for review, Boukar 2002). A review of the several studies reported on inheritance of disease resistance in cowpea showed contradictions which probably may be due to strain variations among the pathogens causing the diseases.

Improved cowpea lines with resistance to both bacterial blight and bacterial pustule have been developed. IITA lines TVx 1850-91E, IT90K-277-2, IT86D-715, IT86D-719, and IT81D-1228-14 combine resistance to both of these diseases (Singh *et al.* 1997). Cultivars '*Mouride*' and '*Melakh*' developed in Sénégal have resistance to bacterial blight (Cissé *et al.* 1995, 1997).

Breeding for resistance to viral diseases is important in almost all the cowpea growing areas. The presence of different strains of viruses complicates the efforts of breeders. However, several improved cowpea varieties combining resistances to multiple viruses have also been

developed at IITA (Thottappilly *et al.* 1988). Cowpea cultivars IT82D-889, IT83S-818, IT83D-442, and IT85F-867-5 are resistant to CPMV, CABMV, CGMV, CMV, and SBMV. Selections for lines with resistance to some of these viruses continue as breakdowns of resistance have been observed at some locations.

4. Breeding for insect resistance

Breeding cowpea lines with resistance to insect pests is one of the important strategies in cowpea improvement. Genetics and mechanisms of resistance to several insects were studied and have been reported (Singh 2002). IITA has developed a number of varieties that combine resistance to several insect pests such as aphid, flower thrips, and storage weevils (bruchids) (Singh *et al.* 1997). Despite screening hundreds to thousands of cowpea germplasm accessions, high levels of resistance to *Maruca vitrata*, pod sucking bugs and lygus bug (*Lygus hesperus* Knight) have not been identified. Recurrent selection to increase the levels of resistance to these pests is being pursued (Ehlers and Hall 1997).

5. Breeding for resistance to parasitic weeds

Striga gesnerioides (Wild.) Vatke and *Alectra vogelii* Benth. are the two parasitic weeds that cause damage to cowpea mainly in the dry regions of SSA. More than six races of *Striga gesnerioides* have been identified. Besides race 1, race 2, race 3, race 4 and race 5 located respectively in Burkina Faso, Mali, Nigeria (and Niger), Zakpota (Benin) and Cameroon, a new race has recently been discovered in Senegal. Several sources of resistance have been identified and mode of inheritance has been reported for many of the races (Boukar 2002). Efforts to pyramid the resistance genes are being conducted at IITA. Improved cowpea varieties with resistance to *Striga* developed by IITA include: IT90K-59, IT90K-76 and IT90K-82-2. *Alectra* is more commonly found in the more moist savanna areas than in the dry parts. Although it causes yield losses in cowpea it is not as devastating as *Striga*. IT93K-693-2, IT95K-1090-12, IT97K-499-39, IT97K-497-2, and IT97K-819-154 were identified as the most promising lines with combined resistance to *Striga* and *Alectra*.

6. Breeding for consumer preferences and nutritional quality

Important consumer preferred traits and nutritional qualities such as high protein content in grains, seed coat color and seed size, as well as short cooking time are being incorporated in different breeding programs. Specific efforts are considered for each cowpea growing area

due to the wide differences in consumer needs. For example, large-seeded types are preferred by most of the cowpea consumers whereas rough seed coats are more favored in some regions than smooth seed coats, white seed coat color is more favored than brown or black seed type in some areas. While black seeded cowpea is preferred in some parts of Latin America most consumers in SSA would not eat such cowpea.

7. Breeding for improved N-fixation and efficient use of phosphorus

As a legume cowpea harbors nitrogen fixing bacteria in its roots. The bacteria fix nitrogen which could be utilized by the cowpea plant and some are left in the soil for succeeding crop. The reported genetic variability in cowpea for effective nodulation and N fixation make possible the breeding of cowpea with enhanced N-fixation. Significant relationship between total N and seed yield and nodule weight was reported by Graham and Scott (1983). Sanginga *et al.* (2000) have reported significant varietal differences in cowpea for growth, nodulation and arbuscular mycorrhizal fungi root infection as well as performance under low and high phosphorus. The differences in performance of improved cowpea varieties under different soil fertility levels indicate a good possibility of developing improved cowpea varieties with enhanced nitrogen fixation and higher yields under low phosphorus as well as in soils with aluminum toxicity (Singh *et al.* 2002).

Breeding methods

In general, all the cowpea breeding programs are attempting to develop improved lines with high yield potential, tolerance/resistance to both biotic and abiotic stresses and grain characteristics preferred by both the consumers and the producers. Efforts are made to improve existing lines by incorporating one or more of the desirable characteristics to attain the breeding goals. Because of its global mandate and the various requirements of growers and consumers in different countries or regions, the strategy of IITA cowpea breeding program is to develop new cowpea lines with a range of: maturity (extra-early, early, medium, late); plant type (erect, semi-erect, prostrate); seed quality (color, texture, size); disease resistance; insect resistance; parasitic weed resistance (*Striga*); and adaptability (drought, heat, moisture) among others.

The collection of cowpea germplasm held at the Genetic Resources Unit of IITA with more than 16,000 accessions is continuously characterized

for the identification of traits of interests. Accessions from both cultivated and wild species with desirable traits are chosen to be used as parents in both conventional and molecular breeding approaches. Segregating populations are obtained from single or multiple crosses of these selected lines. Various breeding methods are used depending on several factors, namely the objectives of the crosses, the genetics/nature of the traits to improve, and the environmental conditions (level of inoculum, climate, etc.). The breeding methods include line selection within landraces, pedigree and backcrossing breeding, single-seed-descent method, bulked population method, mutation breeding, and the combination of some or several of these methods or variants of these methods as necessary. A comprehensive review of breeding procedures used for developing improved cowpea breeding lines and varieties was published by Hall *et al.* (1997a).

Biotechnology in cowpea improvement

1. Cowpea transformation

Despite the progress achieved in breeding of improved varieties with multiple pest and disease resistance, cowpea still suffers appreciable grain yield losses from several insect pests including cowpea pod borer, flower bud thrips, and the pod-sucking bug complex. High level of resistance to several insects and diseases exist in wild species, but strong barriers exist which prevent the transfer of these genes into cultivated cowpea. Genetic transformation was suggested as a means to overcome this constraint. Opportunities for biotechnology in cowpea were made possible with the development of suitable bioassays that have facilitated the identification of candidate genes for insect resistance in cowpea, including either *Bacillus thuringiensis* δ -endotoxin (Dean and Adang 1992), protease inhibitors (Gatehouse *et al.* 1991, 1992), cowpea trypsin inhibitors, or α -amylase inhibitors (Shade *et al.* 1994). All existing *in vitro* gene transfer procedures were tried on cowpea with very limited reproducible transformations obtained.

Transformation by *Agrobacterium tumefaciens* or embryo imbibition with or without subsequent electroporation (Penza *et al.* 1992, Akella and Lurquin 1993) has contributed to the development of transgenic cowpea calli or chimeric plantlets from leaf discs, auxiliary buds, or embryos. However, attempts to produce mature transgenic plants failed in all these cases (Kononowicz *et al.* 1997). These latter authors report the development of transformation systems using either microprojectile bombardment or *Agrobacterium* co-cultivation using cowpea cotyledon

segments, immature embryos, and shoot meristems. Ikea *et al.* (2003) reported transgenes transmitted to the progeny but the stability of these was not demonstrated in a homozygous line. Electro-transformation method using intact tissues and organs was thought to be more promising. Chowrira *et al.* (1995) have reported both transient and stable expressions of the *gus* gene using electroporation of auxillary nodal meristems. Southern analysis showed the presence of introduced gene in T₁ plants but not in T₂ plants of cowpea. None of these attempts has produced reproducible evidence of stable transformation.

Recently, Popelka *et al.* (2006) reported the genetic transformation of cowpea and stable transmission of the transgenes to progeny according to Mendelian rules. Their system used cotyledonary nodes from developing or mature seeds as explant and a tissue culture medium lacking auxins in the early stages, but including the cytokinin BAP at low levels during shoot initiation and elongation. Other parameters used included the addition of thiol-compounds during infection and co-culture with *Agrobacterium* and the use of bar gene for selection with phosphinothricin. These authors have now reported the development of cowpea with the *Bt* gene (Higgins, personal communication). The most responsive cowpea line to genetic transformation is IT86D-1010 from IITA.

2. Marker-Assisted Selection

The application of DNA marker technologies in cowpea improvement has been very slow when compared to many other crops. The common applications encountered in the literature are taxonomic relationships and genetic linkage mapping. Cowpea genetic variation was studied using RFLP (Fatokun *et al.* 1993b), RAPD and microsatellite markers (Fatokun and Mignouna 1999) and AFLP (Coulibaly *et al.* 2002). The first genetic map of cowpea based on RFLP, RAPD, and some morphological attributes was developed using a mapping population of 58 F₂ plants, derived from a cross between an improved cultivar IT84S-2246-4 and a wild relative TVn 1963 (*V. unguiculata ssp. dekindtiana*). This cowpea genomic map had 92 markers distributed among 85 loci representing 10 linkage groups, although cowpea has a chromosome number of n=11 (Fatokun *et al.* 1993a). This map has been used to locate two quantitative trait loci (QTL) accounting for 52% of the variation in seed weight (Fatokun *et al.* 1992). The markers flanking these QTLs in cowpea were the same as those identified with seed weight QTLs in mung bean (*V. radiata*). This map also comprises two markers associated with aphid resistance genes in cowpea (Myers *et al.* 1996). An

RFLP marker was found to be very closely linked to one of these genes, thus presenting a potential for cloning this particular resistance gene (Fatokun and Mignouna 1999).

The second genetic map was constructed using 94 F₈ recombinant inbred lines derived from a cross between two cultivated genotypes IT84S-2049 and 524B (Menéndez *et al.* 1997). This map consisted of 181 loci, comprising 133 RAPDs, 19 RFLPs, 25 AFLPs, 3 morphological/classical markers, and a biochemical marker (dehydrin). These markers are assigned to 12 linkage groups spanning 972 cM with an average distance of 6.4 cM between markers. Recently Ouédraogo *et al.* (2002) improved this map based on segregation of various molecular markers (AFLP, RFLP, RAPD) and biological resistance traits (resistance to *S. gesnerioides* race 1 and 3, resistance to CPMV, CPSMV, BICMV, SBMV, *Fusarium wilt*, and root-knot nematodes). The resulting map consists of 11 linkage groups spanning a total of 2670 cM, with an average distance of 6.43 cM between markers.

A third genetic map of cowpea was built using 94 F₈ recombinant inbred lines derived from the inter-subspecific cross between IT84S-2246-4, an improved cowpea line, and TVu 110-3A, a wild relative (*Vigna unguiculata* spp. *dekindtiana* var. *pubescens*) (Ubi *et al.* 2000). This map spanned 669.8 cM of the genome and comprises 80 mapped loci (77 RAPD and 3 morphological loci) assembled into 12 linkage groups, with an average distance of 9.9 cM between marker loci. Location of QTLs for several morphological traits was performed using this genetic linkage map.

With the advent of DNA-based genetic markers, the potential benefits of using markers linked to genes of interest are being constantly reported in different breeding programs. Marker-assisted selection (MAS) is used to speed the selection procedure and to increase the selection efficiency. The application of MAS is being conducted for *Striga* resistance in cowpea. Two Sequence Characterized Amplified Regions (SCARs) markers developed recently, SEACT/MCTM83/84 (Boukar *et al.* 2003) and 61R (Ouédraogo, unpublished) offer an opportunity to use MAS. The latter was further improved into a SCAR marker called Mahse2 (Timko, personal communication).

Recently a West-African co-ordinated Marker Assisted Selection Research and Training Project for cowpea was funded by Kirkhouse Trust to develop improved cowpea varieties using MAS. The institutions involved

in the project are Institut de l'environnement et de Recherches Agricoles (INERA), Ouagadougou, (Burkina Faso); Institut de la Recherche Agricole pour le Développement (IRAD), Maroua, (Cameroon); Institute for Agricultural Research (IAR), Samaru, located within the Ahmadu Bello University (ABU), Zaria, (Nigeria); and The Savannah Agricultural Research Institute (SARI), Tamale, (Ghana). There is a plan to expand the number of countries to be involved. This project aims also (i) to train African scientists in molecular genetic methods as applied to plant breeding for crop improvement, (ii) to increase capacity in African laboratories and universities, and (iii) to enhance trans-national collaboration between African Institutions. Current activities include validation of the two available SCARs for their use in MAS for Striga resistance.

Cowpea breeding opportunities

1. Tropical Legumes I project

To provide food security and alleviate poverty in SSA, the productivity of legume crops including peanut, chickpea, bean and cowpea will be enhanced through the development of efficient breeding programs using modern biotechnology tools. The aim of the project under the auspices of the Generation Challenge Program will be to develop key genomic resources (including cross-legume molecular markers for comparative genomics), identify molecular markers for traits of importance to resource-poor farmers (biotic stresses and drought tolerance), and implement breeding capacities in sub-Saharan Africa. In the case of cowpea, new and efficient molecular marker development approaches will be employed to densely mark the cowpea genetic map at more than 1000 points in the cowpea genome using Single Nucleotide Polymorphisms (SNPs) markers associated with abiotic and biotic stress resistance/tolerance target traits that impact cowpea yields. Selected SNP markers will be converted to easy to use markers for the implementation of MAS by African cowpea breeders to incorporate drought and heat tolerance, and resistance to flower thrips, bacterial blight, fusarium wilt and root-knot nematodes into locally adapted germplasm.

In this project also, a BAC-based physical map and minimal tiling path will be made, and anchored to one or more reference legumes through BAC end sequences and legume synteny. Comparative genomics will be initiated not only within the four legumes studied but also with the two

simple “model” legume genomes, namely *Medicago truncatula* and *Lotus japonicus* as well as with the complicated genome of soybean (*Glycine max*). Knowledge gained in one species can be used in other species and will increase the chance to improve efficiently and rapidly similar crops. Comparative genomics will be the focus in these studies to help in identifying tightly-linked genetic markers for MAS, and thus allow improvement of crop varieties. One important aspect of the project is to involve the collaboration of ARIs (Advanced Research Institutions), CGIAR centers and NARS through a multi-disciplinary approach.

2. Kirkhouse Trust

Kirkhouse Trust (KT) is a UK-based charity established in 2000 to promote education and research in the natural sciences with emphasis on biological and medical sciences. It has dedicated a large amount of resources to crop improvement research in the developing countries through a network of scholarships, fellowships and grants to collaborative research projects.

Besides the MAS project discussed above, KT has also set up a Cowpea Genomics Initiative (CGI) with the collaboration of the University of Virginia (UVa) in order to sequence cowpea genome for gene discovery and cowpea improvement through the use of Orion Genomics' GeneThresher® methylation filtering technology. Under the CGI, an annotation knowledge base, the Cowpea Genespace/Genomics Knowledge base (CGKB), is created to manage, analyze and disseminate information derived from the sequencing of cowpea genespace sequences (GSS). This database and its web interface are accessible at <http://cowpeagenomics.med.virginia.edu/CGKB/>. It is an integrated and annotated resource with features of both homology-based and Hidden Markov Model (HMM)-based annotations, enzyme and pathway annotation, GO term annotation, toolkits, and a large number of other facilities to perform complex queries (Chen *et al.* 2007). This is an invaluable resource for the development of molecular markers and genetic maps for comparing syntenic relationships among legumes and non-legume species. The sequence derived from gene space of cowpea will be used in the development of SNPS. According to Chen *et al.* (2007), a total number of 30,877 SSRs were identified among the GSS with 3,717 SSRs located in GGS with homology to known genes. Thus, breeders can use this database for the design of molecular markers for use in MAS and introgression programs in cowpea and other legumes.

3. National Science Foundation (NSF)

With the support of this organization, a comparative genomics of legume disease resistance gene homologs is being studied at University of California, Davis. Three species were considered: *Medicago truncatula*, *Lotus japonicus* and soybean. Important resources to be produced under this project include (i) 100 orthologous gene-based genetic markers for comparative genomics, (ii) large insert bacterial artificial chromosome libraries (10X genome coverage) and 50,000 BAC end sequence for each species, (iii) about 1,500 simple sequence repeat (SSR) genetic marker for each species, mined from BAC end data, and (iv) a comprehensive analysis (cloning, sequencing, phylogenetic analysis and physical/genetic mapping) of disease resistance genes in each species. The genetic markers generated (orthologous genes, SSRs and disease resistance genes) will be linked to BAC clones that, when mapped genetically, are expected to cover about 50% of the gene-space in each species.

From the comprehensive genetic analysis of disease resistance genes in each species, Tropical Legume I project will have ready access to tightly-linked genetic markers (SSR) for use in MAS for disease resistance traits. An additional benefit of the NSF project to the cowpea research community is the possibility of cloning genes associated with genetic markers for important traits based on the fact that all genetic markers will be anchored to Bacterial Artificial Chromosomes (BAC) clones.

4. Tropical Legumes II

Another perspective is promoted by the project Tropical Legume II which intends to increase the productivity and the production of grain legumes including cowpea. The project aims to develop improved crop cultivars and associated crop management practices for dissemination to farmers thereby enhancing legume productivity in the drought-prone areas of SSA and SA. The strategy is to combine conventional plant breeding associated with farmer-participatory varietal selection and molecular breeding.

The Tropical legumes II project will use markers generated in Tropical Legumes I project to facilitate progress in the development of cultivars tolerant to drought and major pests and diseases. This project also aims at alleviating the problems associated with the availability and quality of seeds to farmers. The seed delivery strategy will emphasize decentralized, pro-poor seed production and delivery. In the case of cowpea, drought tolerant lines will be identified through collaborative

efforts between scientists from CIAT, ICRISAT, IITA and the NARS and farmers in five countries (Niger, Nigeria, Mali, Mozambique and Tanzania). Seeds of promising lines would be multiplied with participation of community based seed producers. Seed production will be supplemented by the seed delivery strategy to be tested. The Tropical Legumes II project would facilitate the promotion of value-added processing and storage, as well as the stimulation of explicit market linkages.

To sustain the efforts of the project, the capacity of NARS scientists, extension personnel and farmers to develop and evaluate improved technologies would be strengthened. The project would also support provision of research infrastructure needed to enhance screening and evaluation of cowpea lines.

Future prospects

Appreciable progress has been achieved in cowpea improvement through conventional breeding programs. The application of molecular tools would be of tremendous help to cowpea breeders. The proposed activities of identifying SNP markers closely associated with drought and heat tolerance in cowpea under the Tropical Legumes I project would contribute to building the needed foundation for the application of MAS. Additionally, important genetic factors and mechanisms would be studied in order to improve other traits of significant importance in cowpea improvement. The development of associated markers will facilitate Marker-Assisted Selection for these traits in cowpea in the near term and could eventually lead to map-based cloning of resistance/tolerance genes that could have broad implications to crop plants in general. The ultimate goal of all the various activities would lead to more efficient development of cowpea varieties with higher levels of resistances to biotic and abiotic stresses which presently devastate cowpea in the resource poor farmers' fields in SSA and elsewhere.

The new initiatives intended to strengthen the capacity of cowpea breeding programs in Africa through support for infrastructural development and training of breeders would enhance productivity and yield stability of cowpea in Africa. IITA cowpea breeding program would play catalytic role in these initiatives by facilitating the transfer of the new technologies to the NARS. Given its global mandate for cowpea, IITA's breeding program has been involved with the development and

identification of markers associated with several important cowpea traits. The program has developed a number of genetic populations which would be phenotyped and genotyped in collaboration with NARS and ARI scientists. The identified markers would eventually be converted into user friendly markers. Protocols for their use would be developed and shared with colleagues in the NARS. Data generated from these various activities would be placed in the public domain thereby making them available to the international community.

With recent success reported on the stable genetic transformation of cowpea, it would now be possible to introduce genes of interest into the crop which could not be done hitherto because of cross incompatibility. The most responsive cowpea line to genetic transformation is not great in its performance. However, the desirable transgenes could be introgressed into well adapted cultivars that are popular among farmers. Molecular markers would be very useful in the introgression as they would quicken the selection process. Deploying genetically modified cowpea would require risk assessment and design of appropriate risk management strategies especially since cowpea is indigenous to SSA where volunteer cultivated types as well as wild cross compatible relatives are found commonly near farmers' fields. Consumer and producer needs would also need to be taken into consideration in the deployment of these genetically improved cowpea lines.

Conclusion

Cowpea is an important grain legume grown and consumed in warm regions of the world. Its ability to grow under diverse agro-climatic conditions in different cropping systems is associated with a wide range of biotic and abiotic production constraints. Considerable progress has been made toward improving cowpea production and future opportunities to further improve cowpea are being established through the setting up of new projects.

Future progress will depend upon the development and the sustainability of multi-disciplinary collaborations between the ARI, CGIAR center, NARS and producers/consumers. Significant increases in cowpea productivity can be obtained by alleviating key constraints to its production. Internationally coordinated efforts would be needed to have better understanding of the crop's genome structure, molecular markers development, and their application to marker assisted breeding of cowpea. IITA cowpea scientists should play very important role in

leading the identification, the collection, the adoption and the transfer of scientific knowledge that will contribute to the overall goal of improving cowpea.

With the advances in crop improvement and associated disciplines (biotechnology, bioinformatics, molecular biology...), the availability of advanced research facilities, the observed building of collaborative efforts between all relevant components of research institutions, the increased engagement of farmers in variety selection, the improvement in the seed sector and the new interest of funding agencies, cowpea breeders have a remarkable opportunity to enhance cowpea productivity and yield stability.

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Field evaluation of bambara groundnut germplasm for important traits and resistance to root knot nematode infestation

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Summary

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is the third important grain legume in most African countries after cowpea and groundnut. In South Africa, although bambara is an important source of protein and family income to rural dwellers who cultivate them, it is one of the neglected crops. Root knot nematode infestation poses serious constraint to bambara production in South Africa. This paper reports the results of field experiment conducted at Agricultural Research Centre - Grain Crops Institute (ARC-GCI) research farm at Vaalharts in 2005/2006, to characterize 215 bambara germplasm accessions for important agronomic traits and response to nematode infestation. The accessions were planted in single rows of two replicates using randomized complete block design (RCBD). Data were collected on leaf shape, petiole color and number of days to fifty percent flowering, grain yield and number of plants attacked by nematodes. Results showed that number of days to fifty percent flowering varied significantly ($P < 0.05$) among accessions ranging between 60-90 days, with 83 % of the accessions flowering between 60-70 days. Three leaf shapes were identified among the accessions namely, broad, narrow and very narrow which represented 83 %, 16% and 1% of the accessions, respectively. There was significant difference in the response of the accessions to grain yield expressed as number of seeds, 100 seed-weight and nematode infestation. Only 45 accessions could overcome the severe damage by nematodes and could produce over 50 seeds per plot.

Introduction

Bambara groundnut, *Vigna subterranean* (L.) Verdc, is an important nutritious source of balanced diet for subsistence farmers and rural

populations in many countries of sub-Saharan Africa (Linnemann and Azam-Ali 1993, Goli, 1997). The crop is cultivated extensively in Africa at a subsistence level and it is the third legume crop after cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogaea*) (Karikari 1971). In many parts of South Africa, it is commonly called jugo beans and the fresh immature seeds or fully ripe seeds are boiled and eaten as a snack or a main dish. Bambara haulm is also a cheap source of animal feeds for rural livestock keepers. Bambara is also important because it is tolerant to drought with high ability to thrive well where cereals would not. However, the crop is still cultivated from landraces characterized with low and unstable yields as well as susceptibility to many diseases. In South Africa, although it is one of the important sources of protein, it is one of the neglected crops. Its production is on the decline in the last three decades because there are no improvement programs put in place to generate pest resistant and high yield genotypes. As a result, the existing landraces are vulnerable to pests due to low genetic variability. One of the important pests of bambara groundnut in South Africa is root-knot nematode. Root-knot nematode infestation poses serious constraint to bambara production (Anchirina *et al.* 2001, Khonga and Kwerepe 2003). Search for bambara genotype(s) resistant to root-knot nematodes is critical and important for the improvement of the crop. Knowledge about the morphological characters of these landraces as well as their response to root knot nematode infestation is crucial to ARC bambara breeding program in order to ascertain their genetic variability and relevance for nematode resistance breeding. This paper reports on results of field experiment conducted at ARC-GCI research farm at Vaalharts during 2005/2006 cropping season, to characterize 215 bambara germplasm accessions for important agronomic traits and their differential response to nematode infestation.

Materials and Methods

The experiment was conducted at ARC-GCI research farm at Vaalharts during 2005/2006 cropping season. The location is noted for high nematode infestation because legumes are grown in the farm every season leading to a high nematode population build-up. Two hundred and fifteen landraces used were obtained from the gene bank at ARC-GCI. The accessions were planted in single rows of two replicates using randomized complete block design (RCBD). The accessions were arranged in rows of three-meter length and inter-row spacing of one meter apart. Intra-row spacing of 20cm was used thus giving rise to 30 plants per plot at the rate of two seeds per stand. During crop growth,

leaf shape, petiole color and number of days to fifty percent flowering were measured while grain yield, 100 seed weight and number of plants attacked by nematodes were taken at crop maturity and percentage nematode incidence was determined. Nematode incidence was calculated as a proportion of nematode infested plants to the total number of plants in the plot and then expressed in percentage. The plant was regarded infected with nematode if it had knots on the roots, especially on the taproots. Grain yield was measured by the number of seeds produced per accession. Data were analyzed and summarized using MS Excel 2003.

Results and Discussion

Results show that number of days to fifty percent flower varied significantly ($P < 0.05$) among accessions ranging between 60-90 days. 83 % of the accessions flowered between 60-70 days which was regarded as early maturity genotypes while 14 % which flowered between 71-80 days, and 3% of the accessions which flowered between 81-90 days were regarded as medium and late maturity genotypes, respectively (data not shown). This implies that there is great potential of utilizing this trait for generating new hybrids with different maturity types to suit South African environments. There was little variation among the accessions for petiole color. Only 3% which represented seven accessions exhibited red petioles while the remaining accessions (97 %) had green petioles (data not shown). Three distinct leaf shapes were identified namely, broad, narrow and very narrow. These were represented by 83 %, 16% and 1%, respectively (data not shown). The majority of the accessions produced broad leaves. These distinct leaf shapes will be very useful as morphological markers for the development of inbred lines and identification of true hybrids arising from artificial crosses (Massawe *et al.* 2004). Response of bambara accessions to nematode infestation was significant and there was high variability in the reaction of the accessions to nematode infestation (Figure 1). Forty-five accessions had nematode incidence less than 45 % while the remaining 170 accessions exhibited reactions ranging from 46 % to 100 % where there was total failure in some accessions. This confirmed the report of Khonga and Kwerepe (2003) in which they reported that total crop failure was due to severe nematode and *Fusarium* wilting. The results also indicated that 45 accessions possess inherent factors to resist nematode infestation. These accessions were selected for further studies and hybridization program. However, the most susceptible accessions (31 in number) explain the reason why farmers could get a total yield

failure should they plant such susceptible landraces. Even though 100-seed weights varied significantly among the accessions (from 1 g to 60g), majority of them had very small weights. Only 27 accessions had selectable range of seed weight (between 31g and 60g). The low seed weight was partly due to severe nematode damage as well as low genetic potential. Similarly, grain yield of all the accessions were very poor. Number of seeds produced by the best 45 accessions ranged from 50 to 600 seeds per accession (Figure 2).

In conclusion, only 45 accessions could overcome the severe damage by nematodes and produced above fifty seeds. The morphological data might suggest narrower genetic variability than might exist among the accessions. The use of molecular markers and finger-printing will be useful to further characterize and group accessions into useful clusters.

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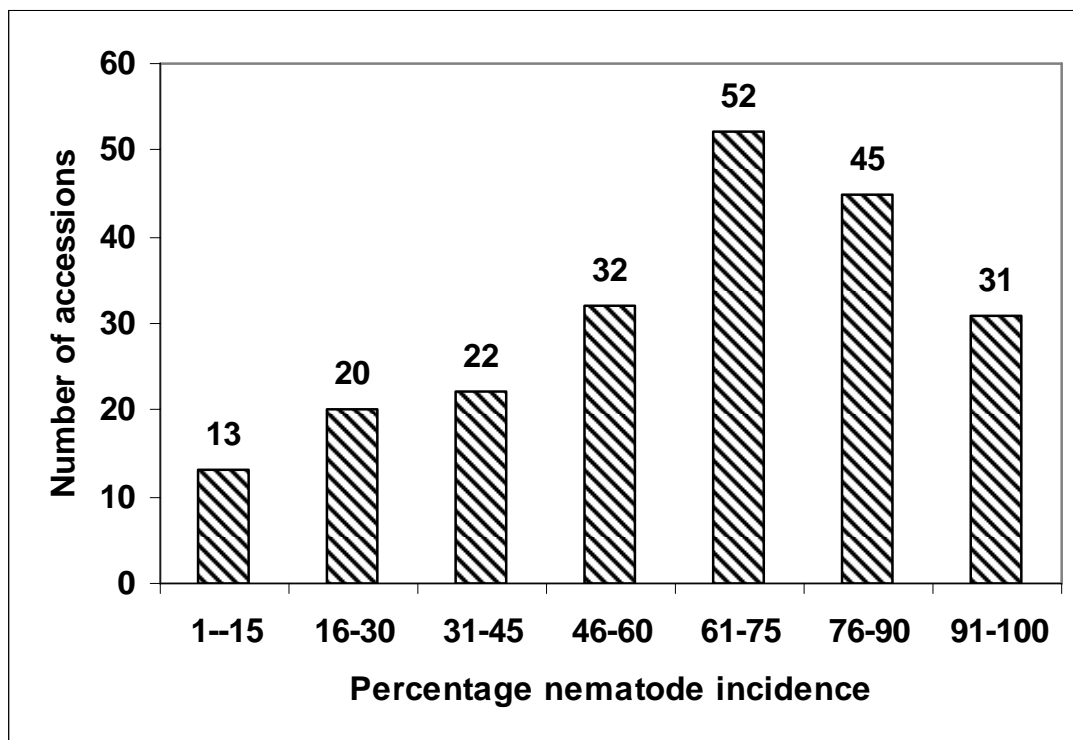


Figure 1. Frequency distribution of percentage nematode incidence among two hundred and fifteen bambara germplasm accessions evaluated at Vaalharts, 2005/06.

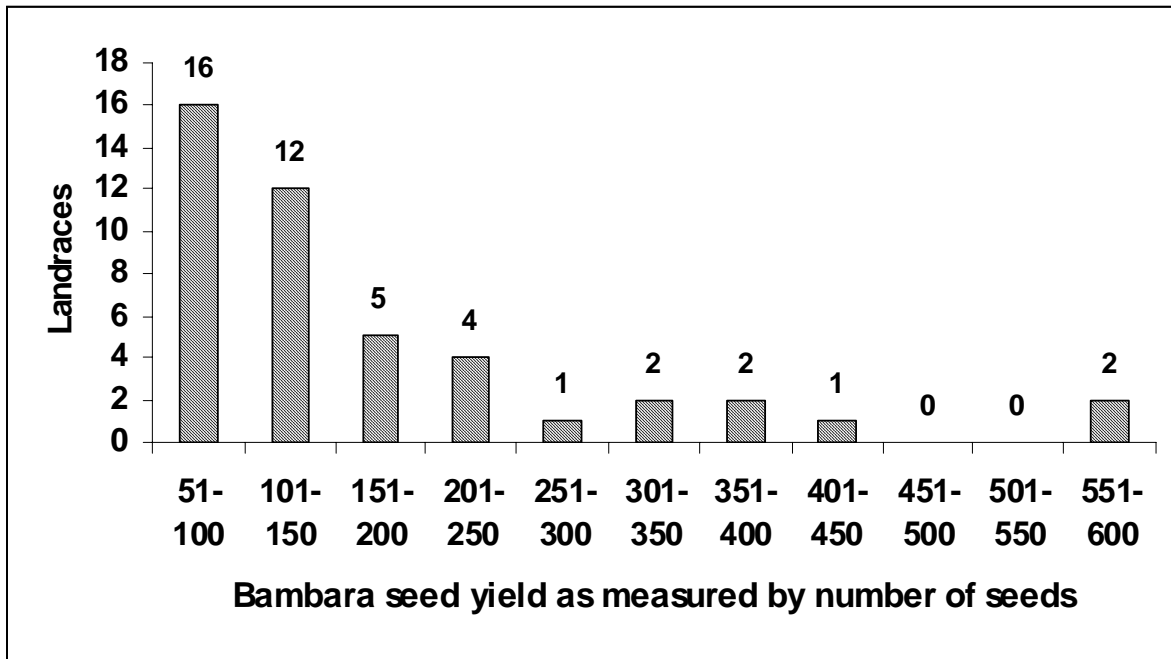


Figure 2. Frequency distribution of grain yield of 45 bambara accessions selected out of two hundred and fifteen bambara germplasm accessions evaluated at Vaalharts, 2005/06.

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Wild species to improve orphan crops: the case of cassava

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Summary

Cassava wild relatives are perennial and vary in growth pattern from nearly acaulescent sub-shrubs to small trees. They have been used as a source of useful characters such as high protein content, apomixis, resistance to mealybug and mosaic disease, and tolerance to drought. Indigenous clones are potential source of β -carotene and lycopene. Apomixis genes have been transferred successfully through interspecific hybridization to the crop, and apomictic clones arising from these hybrids are being now grown at the Universidade de Brasilia. Site-specific hybrids produced early have been polyploidized and have their fertility restored. Different useful types of chimera were also produced.

Wild Manihot species –a botanical review

Wild cassava relatives are perennials and vary in growth pattern from nearly acaulescent subshrubs to small trees. Procumbent, semi-herbaceous sub-shrubs, shrubs, and trees are found in the genus (Figure 1A-F). The branching pattern is typically dichotomous or trichotomous, having at the branching point a terminal inflorescence. Many species such as group tripartita have their stems adapted to dry periods; die back to a root crown regularly and shed their leaves during the dry season. Majority of Manihot species are found on limestone derived and well drained soils (Rogers 1965, Rogers and Appan 1973).

All Manihot species are monoecious and a few are dioecious, which make them obligate out-crossers. In many species, they are protogynous; i.e., pistillate flowers open before staminate flowers of the same inflorescence. Pollination is by insects to whose bodies the sticky pollen adheres. This cross pollination phenomena leads to formation of

extremely heterozygous gene pools. Being allopolyploid species, partially apomictic, and having weak barriers in addition to the allogamous nature, have led to the rapid speciation of this group and formation of the large number of species (Nassar 1999, 2000).

All species of the genus *Manihot* are native to South America (particularly Brazil). The only species found in other tropical regions of the world are those that have been introduced since Columbus voyages to the American continent. The species of *Manihot* are all rather sporadic in their distribution and rarely become dominant of the local vegetation. Many of these species such as *M. pohlii*, *M. zehntneri* and *M. grahamii* are weedy types capable of invading new agitated areas, frequently are found on limestone derived and well drained soils.

According to Rogers and Appan (1973), 98 *Manihot* species have been recognized. Only one species, *Manihotoides pauciflora*, is known in the closest related genus *Manihotoides*. Several of its attributes are not found in any *Manihot* species, including mono-flower inflorescences, which is a primitive characteristic compared with the multi-flowered inflorescence in *Manihot*, and leaves born at the apex of short, condensed stems arising from branchlets. Such evidences suggest this species as a probable origin of all *Manihot* group. Unfortunately this species is on the verge of extinction, and may be eventually extinct (Nassar 1999).

Rogers and Appan (1973) classified *Manihot* species into 19 sections, varying from trees in the section *Glazioviannae* to sub-shrubs, nearly acaulescent, in the section *Stipularis*. The species in this latter section are also characterized by being more dioecious than monoecious, a condition reversed in all other *Manihot* species. Other sections, such as *Tripartitae* and *Graciles*, are perennial sub-shrubs with large woody roots; their stems frequently die back to the root crown in response to dry periods or fires (Nassar 1978 c,d,e, f).

Nassar (1978b, d) defined four centers of diversity for these species: Mexico and northeast, central, and southwest Brazil. Microcenters of diversity of these species exist within central Brazil, where large numbers of species are concentrated in small areas (< 50 km in diameter) (Nassar 1979a, 1979b, 1980a, 1980b, 1982, 1984).

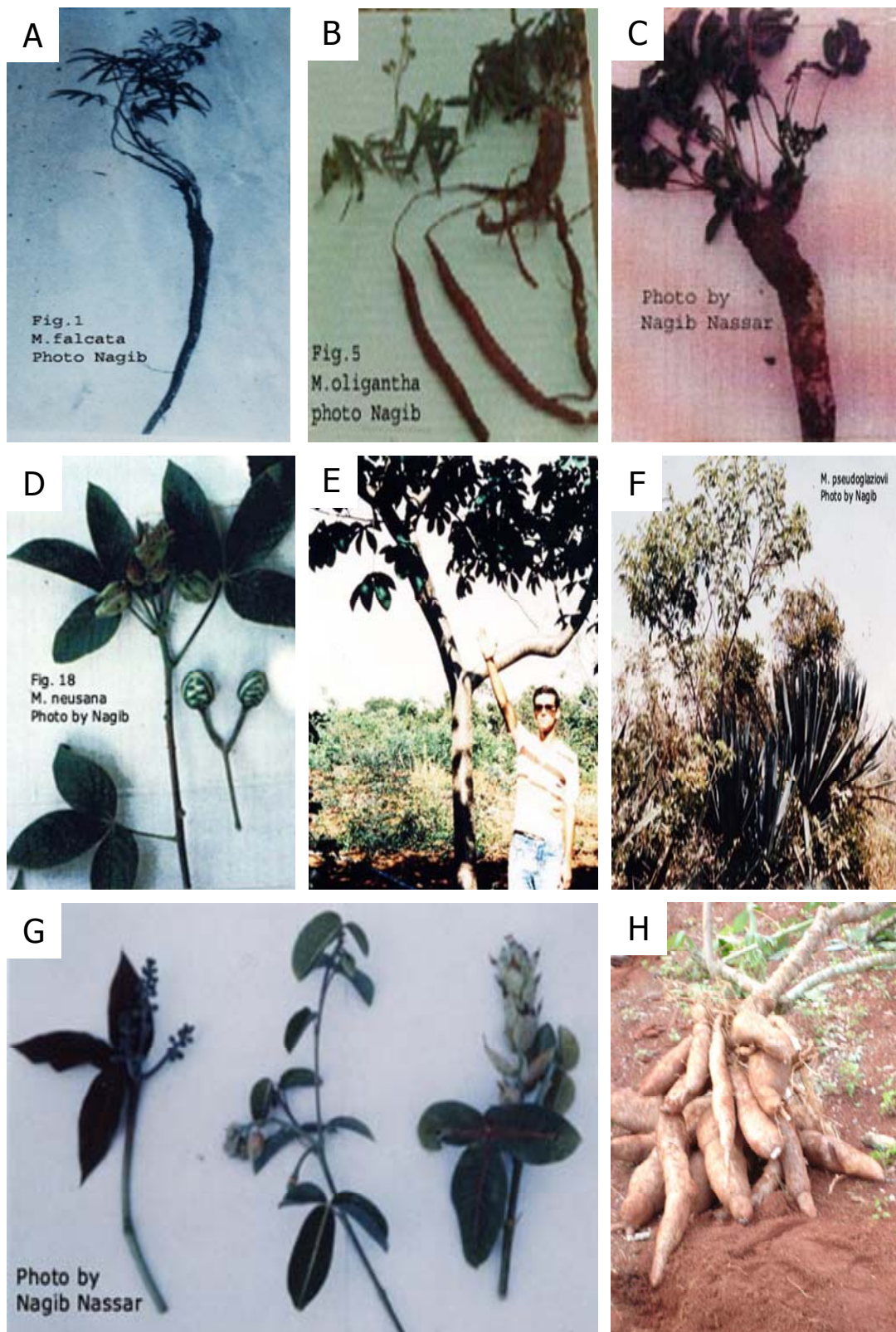


Figure 1. Wild relatives of cassava, (A) *Manihot falcata*, (B) *M. oligantha*, (C) *M. nana* Muell, (D) *M. neusana*, (E) *M. glaziovii*, (F) *M. pseudoglaziovii*, (G) Natural hybrid (right) between *M. alutecia* (left) and *M. reptans* (medium), (H) Clone UnB 120 selected from the interespecific hybrid between *M. cearulescens* and cassava.

These microcenters arose to the frequent hybridization between species and the heterogenic topography of their habitats, which help isolate fragmented gene pools that lead to speciation. Tree-like species, such as *M. glaziovii* and *M. pseudoglaziovii*, are found in northeastern Brazil, whereas short species and sub-shrubs are found in central Brazil (Nassar 1985, 1986).

Wild Manihot species and their interspecific hybrids

Probably the most impressive case is the interspecific hybrid between *M. oligantha* and cassava. This hybrid had high protein content, which reached 4% of peeled roots; i.e., double of protein content in common cassava, combined by low HCN content of 90 mg per kg dry weight (Nassar and Dorea 1982). Recently, genes for apomixis from the wild species *M. neusana* were transferred successfully (Nassar 2000, Nassar *et al.* 2000). Probably the most important utilization of wild Manihot species is the discovery of resistance to mealybug in *M. glaziovii*, and its transfer to cassava gene pool through interespecific hybridization (Nassar 1996). This interspecific hybrid could be polyploidized and its fertility restored (Nassar 2004).

Natural hybridization occurs between wild Manihot species and between them and cassava (Nassar 1984, 1989). Barriers within the genus appear to be weak due to recent evolution of the group. All wild Manihot species examined cytogenetically have a chromosome number of $2n = 36$ (Nassar 1978a). Nassar (1980a) reported frequent hybridization between *M. reptans* and *M. alutacea* in sympatric natural habitats where their population boundaries overlap (Figure 1G). Morphological markers such as leaf color and bract size were used to identify this interspecific hybridization (data not shown). The range of *M. reptans* has expanded during the past 100 years (Nassar 1984) and this is attributed to the continuing gene introgression of Manihot species. Introgression of *M. reptans* with germplasm from other species allowed its ecotypes to penetrate and colonize areas where *M. reptans* (pure) had previously been unable to do so. This phenomenon was also noted in other species such as *M. cearulescens* (Nassar 1980a). From a plant breeding viewpoint, the value of these hybrids lies in their ability to cross with the cultigen.

Morphological markers for lobe shape, the presence of stem nodes, flower disc color, fruit color, and fruit shape were discovered in controlled crosses between cassava and *wild Manihot* species, as well as

in natural hybrids between cassava and different species. These genes were used to identify hybridization. Interspecific hybrids of cassava with *M. glaziovii*, *M. pseudoglaziovii*, *M. aesculifolia*, *M. pilosa*, *M. dichotoma*, *M. pohlii*, *M. neusana* and *M. anomala* were obtained through controlled crosses, although their frequency was low. The meiotic behaviour of several hybrids (cassava with *M. neusana* and *M. pseudoglaziovii*) was studied by Nassar (1992), and results indicated low hybrid fertility between these species and cassava.

Production of cassava cultivars by interspecific hybridization

Cassava cultivars are deficient in many economic characteristics such as resistance to insects, diseases, and drought and have low protein content (Nassar and Dorea 1982, Nassar and Grattapaglia 1986). This can be attributed to the mode of evolution in the species and modifications of the allogamy system of the plant (Nassar and O' Hair 1985). Lost genes can be restored to the gene pool of the cultigen by interespecific hybridization with wild relatives which possess these genes (Nassar and Grattapaglia 1986). Wild species of cultivated crops have frequently been used as an important source of genetic diversity and have been employed effectively in a variety of breeding programs (Stalker 1980). There are interspecific barriers to hybridization but these are weak and can be broken in different ways.

Nassar (1978, 1980, 1989, 1994) reported production of interspecific hybrids of several *Manihot* species with cassava through controlled crosses by insect vectors (Figure 1H). The following morphological markers were used to identify interspecific hybrids: variegated color of the fruit is dominant over smooth, red color of flower disk is dominant over yellow, setaceous bracteole is dominant over foliaceous, and noded stem is dominant over smooth. Observations of growth habit, height, stem texture, and tuber formation were also recorded. Other characters provided indirect evidence of hybridization. The hybrid plants exhibited dominant phenotypes from cassava, namely, ribbed fruit, red color of the flower disk, noded stem, and tuberous root (data not shown). These results show that glabrous stem, setaceous-foliaceous bracteoles, red-creamy color of flower disks, variegated-green color of fruit, and ribbed to no ribbed fruit are simple morphological markers that can be used to recognize interspecific hybridization. It is evident that interspecific barriers between *Manihot* species can be broken by the use of an abundant diversity of pollinator gametes transmitted by insect

vectors. Interspecific crosses were difficult to fertilize manually in the present and in previous crosses (Nassar 1980a).

Interspecific hybrids of cassava with *M. glaziovii* and *M. pseudoglaziovii* were produced (Nassar, 1996), and propagated by cuttings and planted alternately with clone Sonora. Selected clones of backcrossed progeny were characterized morphologically according to Rogers and Appan (1973), and Nassar and Grattapaglia (1986). This characterization was aimed at detecting the association of different morphological characters with tolerance to drought.

Morphological characterization showed that certain characters were associated with tolerance to drought. All selected clones have a notable brown, thick, and rough superficial epiderm. It seems that the brown-colored thick epiderm is associated with tolerance to drought because of its isolative nature, which impedes evaporation. All the wild species investigated by this author have fibrous roots with brown external color and their epidermic layer is thick. This character may be therefore inherited from the wild. Graner (1942) reported that this character behaves dominant to white. Anatomically, the distinct portion of the enlarged root is composed of three sections. Firstly, a layer referred to as the phelloderm which is generally composed of the previously mentioned epidermis, a sub-epidermis, and a thicker inner layer. The phelloderm is thick and easily separated from the next inner layer. Secondly, a layer of parenchymatous cells that constitutes the bulk of the root and is the carbohydrate storage region. Thirdly, a portion called the cortex of flesh at the center of the root is a well-defined central vascular core. As noted previously, the outer epidermis is so thin that it is difficult to measure, but it is possible to evaluate its thickness using the naked eye. It is about 0.5 mm in the thickest types.

The second interesting case in the selected clones is the prominence of leaf scars on stems. All selected clones have a prominent enlarged leaf scar. This character which seems to be well associated with enlarged root formation in the hybrid progeny apparently was inherited from cassava. All wild species studied by this author have a smooth stem without any leaf scar. All selected clones gave deep fibrous roots in addition to enlarged ones. It seems that this character is inherited from the wild. This appears to be a mechanism for cassava to tolerate drought by absorbing water from long distance. Both wild species and their interspecific hybrids produced long and deep roots from the fourth

month onwards, reaching 4 or 5 m long when the plants were one year old.

Another mechanism for tolerance to drought is the thick epiderm layer, probably because of its structure, it impedes evaporation. The dieback of the vegetative parts to the crown in dry season was the third common character shown by all the selected clones. Presumably this habit helps plants to reduce respiration and consumption of carbohydrate deposits. From this study, it is obvious that breeders can make use of morphological characters as a selection criterion to select for drought tolerance.

The transfer of apomixis genes from *Manihot* species to cassava

Apomixis means seed formation without fertilization. In cassava, it is an alternative to reproduction by cuttings which normally is practiced by farmers. The latter type of propagation leads to accumulation of viral and bacterial diseases that reduce productivity and may cause extinction of superior genotypes. Thus, by the use of apomictic plant in propagation, systemic pathogens could be avoided, and the genetic segregation in the progeny is excluded. -Stems produced through apomixis will be cleaned from viral and bacterial contamination. Use of apomixis in preserving superior genotypes and filtering the bacterial contamination provides benefits to international cassava programs that export routinely their germplasm. It is sufficient in this case, for the destination country to produce only one plant and further reproduce it vegetatively to maintain the original superior genotype.

Facultative apomixis was discovered in the wild cassavas such as *M. dichotoma* and *M. glaziovii* (Nassar 1994, Nassar *et al.* 1998). It was noted earlier in *M. neusana* – a species characterized by extreme resistance to bacterial wilt and stem borers (Nassar 1985). Interspecific hybridization was carried out to transfer these useful genes to the cultigen (Nassar 1989). The clearing method was used to detect apomixis (Nassar *et al.* 1996). The anatomical studies of ovules showed that the embryo was formed by apospory from a somatic cell in the nucellus. The megasporogenesis in ovules with aposporous development proceeds normally until nucellar cells enlarge and the nuclei divides to form aposporous embryo sacs. These aposporous embryo sacs appear to develop faster than sexual embryo sacs, probably because they are not delayed by meiotic division (Asker 1979, Nogler 1984). In some cases, development of apospory embryo sacs from cells within the sexual one

was noted. Both the aposporous and sexual embryo grew in parallel and finally coexisted.

This observation confirms results from a previous study (Nassar 1995), where two seedlings grew side by side; one of which was apomictic and one of sexual origin. Nogler (1984) reported that aposporous and sexual processes coexisted in one individual ovule producing several embryos that of *Potentilla*. This study documents the survival of two aposporous embryo sacs beside a sexual one, all of them in a developed stage in the ovule (data not shown).

The embryonic study revealed association between sterility and apomixis since all of the sterile plants were partially apomictic while the fertile plants were sexual. Sterility apparently leads to apomixis. Sterility is caused by consistent defects of meiosis due to lack of pairing. All of these sterile plants showed asynapsis in meiotic metaphase. Formation of univalents ranged from 4 to 6 per cell. The irregular chromosome segregation in these sporocytes must lead to genetically unbalanced and aborted gametes. It seems that this sterility triggers certain genes of apomixis to act. Apomixis will function and be established in such genotypes since it's favored by natural selection as it offers an escape from lethality, providing a perpetuation of the current genotype.

In summary, the nature of apomixis in cassava is different from other types found in other crops since it is present at very low levels (1-2 %). It depends on meiotic irregularity that often causes sterility in plants. This sterility triggers a certain gene in cassava that activates a number of somatic cells in the nucellus or in the sexual embryo sac to form aposporic embryo sacs. Natural selection favors this apomictic genetic system since it is an escape from extinction and a mode of perpetuation for the current genotype.

Polyploidization of the interspecific hybrids

Four interspecific hybrids between cassava and wild *Manihot* species, obtained earlier by this author, were used for polyploidization. These hybrids are *M. neusana* x *M. esculenta*, *M. glaziovii* x *M. esculenta*, *M. aesculifolia* x *M. esculenta*, and *M. pohlii* x *M. esculenta*. Twenty vegetative buds of stem cuttings of each hybrid were soaked in 0.2% colchicine aqueous solution for 24 h. Sprouting shoots were examined for leaf shape. Pollen viability was estimated and pollen mother cells [PMCs] were studied to determine chromosome number.

The colchicine treatments resulted in both complete and chimeral tetraploid tissues having different ploidy levels growing adjacently on the same stem. This is due to the stratified arrangement of cells in the meristem that are treated by colchicine, and derivation of mature tissue from these layers. The derivative cells of the outermost layer of the tunica form epidermis. The second LII layer forms the sub-epidermal tissues while the LIII I form the pith and vascular tissue. The chimeras were distinguished to sectorial and periclinal.

Identification of chimera. It was possible to identify the chimera tissues on the basis of pollen grain viability, leaf shape and stem anatomy. The polyploid section of the stem in sectorial chimeras had short leaves while the diploid side developed narrow and longer leaves. In case of periclinal chimera, pollen viability, leaf shape and stomata size were used as a selection criterion. Pollen formed from LII layer while leaves are differentiated from the LI layer. In periclinal chimeras, leaf is different and stomata enlarged and pollen viability is much higher than diploid plants. All the chimeras in the interspecific hybrid *M. esculenta* x *M. neusana*, and *M. esculenta* x *M. glaziovii* were sectorial while two sectorial, one periclinal chimeras were obtained in the cross cassava x *M. aesculifolia*. In sectorial chimeras, pollen grain viability on one side was low as in a diploid while on the other side it was as high as observed in the tetraploids. The size of pollen grains was notably larger in the later part. The pollen grain size in the periclinal chimeras reflects the ploidy level of this layer.

Instability of chimeras. Little information is available about production of chimera in root crops and much less in cassava, but the use of polyploidy in cassava breeding is frequently reported by Indian breeders. Probably, the most interesting reports came from the Indian team at Thiruvanthpuram (Sreekumari *et al.* 1999). Their article reports production of total tetraploids in cassava but it does not mention chimera induction. Since appearance of chimera is a frequent phenomenon after colchicine treatment, it is possible that the resulting chimeras were overlooked or simply ignored in the above research. Due to stratification of the shoot apex, cytochimeras with different ploidy levels appear in each layer of tissue and their derivatives when the buds are treated by colchicine. A competition between tetraploid and diploid tissues in chimeras was reported earlier (Stewart *et al.* 1974). This competition leads to the loss of desired traits. Only the chimeras in LII (periclinal) layer have a chance of transmitting desirable characteristic to their progeny. In the chimeral sectors observed by my group, the stem

exhibited diploid phenotype after about six months of growth by restoring the normal leaf shape that became narrow, and pollen viability was that of a diploid. It seems that the growth rate of tetraploid tissue is slower than that of the diploid, and tetraploid tissue is often overgrown by diploid tissue. However, it is possible to propagate tetraploid tissue through somatic selection. This was done by cutting the apical buds of the chimeral stem, followed by removal of the lateral shoots grown from the diploid sector and allowing only the tetraploid side branches to grow.

One of the striking features of polyploidization is the restoration of the fertility of the interspecific hybrids. Yet a very small portion of unviable pollen formed in the polyploids due to the formation of 3% multivalents in the polyploidized tissue. Quadrivalent formation occurs in cassava itself. Fertility restoration in the interspecific hybrids through polyploidization improves chances of using the wild species for crop improvement. This means the creation of new tetraploid species with high fertility and capable of self-reproduction maintain their unique characteristics with a new closed gene pool in every interspecific hybrid (Nassar 2002). This technique allows us to incorporate desirable genes in future crosses. The strategy involves backcrossing the polyploidized interspecific hybrids with cassava followed by selection for the desirable traits in the progeny. Preferential autosyndetic pairing between chromosomes of cassava may result in the elimination of the majority of chromosomes from the wild species during meiotic segregation. Even selfing of a fertile hybrid may produce useful recombination between wild *Manihot* species and cassava. One interesting approach in utilizing the induced polyploid types could be to cross them with the facultative apomictic clones, which may lead to the production of apomictic triploid clones that combine both heterosis and polyploidy.

Amino-acids in cassava and the interspecific hybrid ICB 300

Cassava roots are poor source of protein in spite of its quality and the proportion of amino acids therein. Methionine and lysine are, however, limiting amino-acids in the root. If cultivars can be bred with more quantity of these amino acids it would enhance the value of cassava as a food or feed. Only about 60 % of total nitrogen in cassava is derived from amino acids and about 1 % of it is in the form of nitrates and hydrocyanic acid. The remaining 38 to 40 % of total nitrogen remains unidentified (Diasolua *et al.* 2002, 2003).

Cassava proteins are comparable to rice protein in digestibility. The biological value (Block and Michell equivalent) of the total protein is 48 %. The crude protein content of roots appears to be relatively stable and constant with maturity of the plant. According to Close *et al.* (1953) the protein of processed cassava includes the highest percentage of glutamic acid and the lowest of methionine (1%). Osuntokun *et al.* (1968) reported that both cystine and cysteine are involved in cyanide detoxification. Cyanide is produced when the glycoside linamarine is hydrolysed by linase.

Amino acid compositions from *Manihot* proteins were determined by analyzing sample extracts which were dialyzed against water to remove free amino acids, salts, monosaccharide and other small molecules. Tryptophan could not be analyzed since it is degraded upon acid hydrolysis. By adding the amounts of the analyzed amino acids, it was possible to determine the protein content for each sample. Among the six samples analyzed in this study (Table 1), interespecific hybrid ICB 300 offspring 3 Raiz showed the highest amount of protein (1.654 g 100 g⁻¹ of sample powder), followed by ICB 300 Diploid (1.454 g 100 g⁻¹), ICB 300 Progenese 9 (0.922 g 100 g⁻¹). The other samples (ICB 300 Progenese 10 Raiz, ICB 300 Progenese 4 and UnB 01 Raiz) were poorer in protein contents (0.350 g 100 g⁻¹). Essential amino acids (including His, Leu, Lys, Met, Phe and Val) were more concentrated in Progy 3 Raiz while Ile and Thr are more in ICB 300 Diploid. Thus, the two lines (Progênese 3 Raiz and ICB 300 Diploid) would be more interesting for human consumption based on such nutritional characteristics.

Among the essential amino acids arginine, histidine, isoleucine, leucine, phenylalanine, threonine, tryptophan and valine are available in cassava while methionine and tryptophan are lacking. The essential amino acids profile of cassava seems to be deficient in sulphur-containing amino acids (methionine, cystine and cysteine) (Bailey 1961). Osuntokun *et al.* (1968) pointed out that both cysteine and cystine are involved in the cyanide detoxication (cyanide is produced when cyanogenic glucoside - linamarine present in cassava is hydrolyzed by linamarinase or by acid). The cysteine is mainly detoxicated by conversion to thiocyanate, in the process of which it reacts with cysteine and cystine. Excessive detoxication may be responsible for the low concentration of sulphur-containing amino acids.

Efforts were made earlier to increase the level of protein in cassava roots by interspecific hybridization with wild species such as *M. saxicola* and *M. mefanobasis*. Although Bolhuis (1953) obtained few lines from crosses between and *M. saxicola* with 2% protein level in fresh roots, successive progenies had much lower level of protein. Moreover, the efforts made by Jennings (1957) to double the protein content was not successful as the offsprings from his work were further progressed may due to poor root yield.

Barros and Bressani (1967) and researchers at the Centro Internacional de Agricultura Tropical (CIAT) reported cultivars with high protein content (7%). It is, however, not clear whether the nitrogen level indicated for these cultivars is converted to protein or breakdown to cyanogenic glucosides.

Table 1. Amino acid (AA) profile in peeled roots of cassava cultivar UnB, its inter-specific hybrid with *Manihot oligantha* –namely ICB 300 Diplóide, and ICB 300 Diplóide offspring (Progênese 3, Progênese 10, Progênese 4, Progênese 9 and Diplóide)

AA	Sample mass (g per 100 g)					ICB 300 Diplóide
	UnB 01	Progênese 3	Progênese 10	Progênese 4	Progênese 9	
Ala	0.02	0.093	0.017	0.019	0.04	0.098
Arg	0.037	0.261	0.061	0.082	0.32	0.108
Asp	0.016	0.146	0.023	0.033	0.052	0.137
Cys	0.027	0.029	0.026	0.025	0.026	0.025
Glu	0.039	0.222	0.044	0.065	0.151	0.221
Gly	0.012	0.078	0.012	0.015	0.037	0.075
His	0	0.038	0.01	0.01	0.027	0.036
Ile	0.008	0.068	0.01	0.01	0.018	0.069
Leu	0.016	0.131	0.013	0	0.041	0.127
Lys	0.01	0.098	0.02	0.019	0.034	0.079
Met	0.014	0.041	0.004	0	0.019	0.037
Phe	0.016	0.129	0.058	0	0.065	0.12
Pro	0	0.054	0	0	0	0.066
Ser	0.012	0.088	0.013	0.018	0.033	0.078
Thr	0.008	0.061	0.007	0.013	0.022	0.066
Tyr	0	0	0	0	0	0
Val	0.019	0.115	0.027	0.025	0.039	0.112
Total	0.254	1.654	0.344	0.336	0.922	1.454

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Mutation induced genetic improvement of neglected crops

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Summary

Neglected and underutilized crops are often considered as “minor crops” due to their lesser importance in terms of global production and consumption systems. Researchers have recognized their great potential towards food security, sustainable agriculture and improving the socio-economic aspect in the poor rural sector. Plant tissue culture has a great potential in plant improvement, provided plants can be readily regenerated in large numbers. It provides the options to reduce costs in generating the useful traits and pre-breeding materials for plant breeders, as well as shortening the screening programs. Induced mutations with physical and chemical mutagens have been quite effective for a significant increase in plant production among both seed and vegetative propagated crops. More than 2600 mutant varieties have officially been released in many countries (<http://www-mvd.iaea.org/>), however, there is not enough work done on mutagenesis in neglected and underutilized crops. The role of molecular tools has become very crucial in understanding the molecular basis of differentiation and genetic variability; gene identification, isolation, function and transfer.

Introduction

Human population is growing at an alarming rate in the developing countries, and food availability is under pressure. The agriculture production is also affected due to environmental factors, lack of improvement of local crop species, erosion of high genetic diversity, soil erosion, water scarcity, and dependence on a few crop species for food supply. Only a few crops produce the majority of the world’s food, yet many neglected and under-utilized crops are extremely important for food production in the developing countries (Ochatt and Jain 2007). Basically, neglected crops are those which are traditionally grown in their

centers of origin or centers of diversity by farmers, where they are still important for the subsistence of local communities. These crops continue to be maintained by socio-cultural preferences and traditional uses. A few of them may be distributed more widely, but tend to occupy special niches in the local ecology and in production and consumption systems. Neglected crops remain inadequately characterized and, until very recently, have been largely ignored by research and conservation. Under-utilized crops are those which were once more widely grown such as bambara groundnut, buckwheat, sesame and safflower, but are now falling into disuse for various agronomic, genetic, economic and cultural factors. Farmers are losing these crops because they are less competitive with improved major crop species.

In vitro culture techniques have a vast potential in plant improvement provided plants are readily regenerated from explants (Jain and Maluszynsk 2004, Jain and Spencer 2006), use cost reduction parameters in generating the plants with useful traits and pre-breeding materials for plant breeders, as well as shortening the screening period, e.g. for salt and drought tolerance. These techniques together with nuclear technology are effective in generating genetic variability, selection of useful mutants and their multiplication in large numbers, especially in vegetative propagated crops. It is highly desirable to regenerate plants with *in vitro* techniques before inducing mutations. Most of the major crops are amenable to *in vitro* culture techniques for plant regeneration both via organogenesis and somatic embryogenesis. *In vitro* plant regeneration is very much dependent on type of explants; culture medium, plant growth regulators etc., and these factors hinder wider application of *in vitro* plant regeneration. Radiation treatment of somatic embryogenic cell suspension cultures is suitable for mutation induction, mutant selection, and plant regeneration. The advantage of this approach is that millions of cells can be irradiated at one point, and probability of getting desirable mutations becomes higher.

Nuclear techniques for mutagenesis

Nuclear technology has benefited greatly in genetic improvement of seed and vegetative propagated crops worldwide (Ahloowalia *et al.* 2004, Jain 2005). Even though nuclear technology has benefited greatly agriculture, it has still a great potential in genetic improvement of cassava and other crops. More than 2600 mutant varieties have officially been released in many countries (<http://www-mvd.iaea.org>). Both chemical and physical mutagens are used to induce mutations. Among them, gamma rays and

ethyl-methane sulphonate (EMS) are widely used for mutation induction. Initially LD₅₀ dose is determined, which is used as an optimal dose for mutation induction. By ignoring this step, mutagen dose can either be high or low in mutation frequency. Fine embryogenic cell suspension cultures are most suitable for inducing mutations by transferring to the filter paper and plated on the agar-solidified culture medium for gamma irradiation. Irradiated cells are transferred to the fresh culture medium for cells to recover from radiation treatment. Irradiated cells are further cultured to the fresh medium for the development, maturation, and germination of mutated somatic embryos. This approach provides mutated somatic seedlings in a short period and also prevents chimera problem which otherwise requires to multiply plants up to M₁V₄ generation for chimera dissociation. The problem with this approach is low rate of somatic embryo germination, which is highly genotypic dependence. A combination of somatic embryogenesis and organogenesis would be a realistic approach for mutation induction and multiplication of mutant plants in large numbers (Jain 2006a, 2006b, 2007).

Shoot tips are used to induce direct shoots, preventing callus formation, in order to micropropagate plants in large numbers. This would be ideal system for mutant plant multiplication in large numbers for further evaluation. Shoot tips can also be irradiated and cultured on a medium containing appropriate plant growth regulators. By this approach, regenerated plants will be chimeras, and mutations will be unstable, and would require chimera dissociation by micropropagation of shoot cultures up to M₁V₄ generations to make sure mutated plants are stable. This approach would be suitable to follow in crops recalcitrant to somatic embryogenesis.

Mutagenesis in some selected neglected crops

A 5-year Coordinated Research Project entitled 'Genetic Improvement of underutilized and neglected crops in low income food deficit countries (LIFDCs) through irradiation and related techniques' was initiated in 1998 at the International Atomic Energy Agency (IAEA), Vienna. The overall objective of the Project was to improve food security, enhance nutritional balance, and promote sustainable agriculture in LIFDCs by using radiation technology. Special emphasis was given for the following seed- and vegetatively-propagated crops.

1. Seed-propagated crops

a. Amaranth

Amaranth is a multipurpose crop, used as vegetable, grain crop, medicinal, forage, and for ornamental purposes. The crop is particularly suitable for marginal areas and is known to be resistant to both drought and salinity. In most developing countries the crop is not grown for commercial purposes, but rather as part of a local sustainable agriculture in the traditional system. Amaranth is very rich in vitamins (A, B), minerals (Ca, Fe, P, Mg), proteins (particularly lysine and methionine), fibres (5-25%) and lipids (4-10%). A greater drought tolerance trait is among those desirable characteristics needed to enhance the use of this crop in South Africa along with day-light insensitive genotypes that would allow its cultivation in winter time when food insecurity is more severe in poor rural areas.

The following achievements were made on Amaranth:

- i) 15 selected M5 drought tolerant leafy mutants (*A. tricolor*) were selected in the field for their comparatively higher performance in artificially created drought conditions.
- ii) Five day-insensitive mutant lines were identified.
- iii) There were 48 mutants of *Amaranthus cruentus* selected and 18 mutants of K-433 hybrid were obtained, that bore various useful traits, i.e. determinate growth, uniformity in flowering and seed maturity, leaf-less inflorescences, and an increased seed size.

b. Bambara groundnut

Bambara groundnut is a major source of inexpensive protein in sub-Saharan Africa. The crop has high lysine and methionine content. Seeds are consumed by humans, pigs, and poultry while the haulm is used as fodder for livestock. The crop also features prominently in cropping systems in Ghana and makes a significant contribution to soil fertility through symbiotic nitrogen fixation. The project in Ghana was aimed at collecting and evaluating bambara groundnut germplasm for desirable agronomic traits and select superior ones.

In spite of the importance of bambara groundnut, there has been little research on the crop. Its long life cycle (about 6 months) and floral biology do not facilitate improvement of the crop. A shortening of the generation cycles would allow rapid progress in breeding. In addition,

knowledge of the *in vitro* requirements for regeneration would help fasten mutagenesis and biotechnology-based generation of genetic novelties in this species. The French scientists focused on developing *in vitro* techniques for plant regeneration and shortening of the generation cycle.

Some of the achievements made on bambara groundnut are:

- i) Identification of mutants resistant to *Cercospora* leaf spot with desirable agronomic traits.
- ii) A new technique has been developed for the shorten of the generation cycle of bambara groundnut using a combination of *in vitro* and *in vivo* methods (Ochatt *et al.* 2002b)
- iii) An efficient *in vitro* shoot regeneration system has been developed in bambara groundnut by combining phytohormones and using embryonic explants (Lacroix *et al.* 2003).
- iv) True-to-typeness of regenerants were established (Lacroix *et al.* 2003).

c. Grass pea

The grass pea (*Lathyrus sativus*), with its large eco-physiological plasticity and very low production costs, is a cheap source of dietary protein available for subsistence farmers in LIFDCs where vast surfaces of this crop are grown. However, there are some limits to grass pea consumption deriving from the sometimes relatively high (up to 0.76 %) seed content of β -N-Oxalyl-L- α,β -diaminopropionic acid (ODAP), the toxin responsible for neurolathyrism. It is therefore needed to develop cultivars with decreased levels of this toxin. In India, this project was aimed at mutagenesis, and *in vitro* regeneration techniques to introduce transgenes blocking the biosynthesis of oxalic acid.

The grass pea is a seed legume that tolerates extreme environmental conditions and has high protein content and a significant resistance to anthracnose. Grass peas would also be a useful genetic resource for resistance breeding of peas (*Pisum sativum* L). Unfortunately, grass peas have a long generation cycle, are cross-incompatible with most other cultivated seed legumes and insufficient research input has been devoted to them in the past, so that little is known on their floral biology, cytogenetics and breeding in general. In France, *in vitro* selection, a shortening of generation cycles and somatic hybridization were developed and a series of fundamental, cytological, genetic and biophysical studies were performed.

The following achievements were made on grass pea:

- i) Fertile plants were regenerated from various explants, with or without callusing (Ochatt *et al.* 2001).
- ii) The genetic mechanism underlying hyperhydricity was identified (flow cell cytometry) (Ochatt *et al.* 2002a).
- iii) Two tetraploids and one mixoploid were obtained and several more variants were identified and characterized (isozymes, RAPDs, flow cytometry).
- iv) The cross-incompatibility between pea and grass pea was confirmed.
- v) Leaf protoplast-derived calluses were obtained, and after fusion of grass pea and pea protoplasts, somatic hybrid calli were produced (Durieu and Ochatt 2000).
- vi) Cytogenetic tools were developed for the characterization of *Lathyrus* metaphase chromosomes using fluorescent *in situ* hybridisation.
- vii) A novel strategy for shortening of generation cycles *in vitro* gave 7 and > 4 generation cycles/year for pea and grass pea, respectively (Ochatt *et al.* 2002b).
- viii) Early predictors (cytological and bio-physical) of somatic embryogenesis competence have been identified.
- ix) The shortening of generation cycles developed permits a faster breeding and ensures an absolute self-pollination of plants, useful for single seed descent.

d. Naranjilla

This is a traditional high altitude culture in Ecuador, that is also found in other South American countries but also in New Zealand and the USA. However, the species is very much in danger of genetic erosion due to its floral biology, and the main problems for cultivation are diseases and pests, especially nematodes. In the absence of adequate phytochemical treatments to avoid these, farmers are contaminating the soil and the fruit and causing a negative ecological impact. There is therefore an urgent need to obtain novel, nematode resistant genotypes of Naranjilla, and this project is aimed at the development and use of mutation induction through gamma rays to induce nematode resistant plants from the landrace Baeza, which is characterized by its valuable organoleptic traits and high market demand, but also by its high susceptibility to nematodes.

Among the achievements made on naranjilla:

- i) Mutations were induced by gamma ray irradiation of true seeds and axillary buds of the traditional Naranjilla variety Baeza.
- ii) Seed-derived mutants were observed in the greenhouse and 35 nematode resistant genotypes were kept for further analyses.
- iii) Tissue culture-derived mutants were also evaluated in the greenhouse and plants are available for future field evaluation.

e. Quinoa

Quinoa is one of the pseudocereals which are widely recognized nowadays as having great potential to contribute to solve the malnourishment problems affecting great sectors of various populations in marginal rural zones, as this species is also able to cope with low soil fertility, drought and frost. However, the main problem in this crop is the high saponin content and one goal of this project was the application of radio-induced mutagenesis techniques to the locally cultivated variety Barandales of quinoa, to obtain low-saponin, high yielding lines.

Saponins are natural detergents that are effective antimicrobial, cholesterol-lowering, anticancer chemicals.

In addition, the exploration of valuable germplasm of *Chenopodium berlandieri ssp muttaliae* native landraces was performed and some genotypes were recollected to be incorporated as progenitor in future breeding programmes of quinoa. A prerequisite to this was the detailed cytological characterization of such materials and this was therefore another main goal in this project.

In general, the following results were obtained on quinoa:

- i) A large number of *Chenopodium quinoa*, including radio-induced mutants, and two accessions of *C. berlandieri ssp. Muttaliae* were characterized in terms of morphology, productivity and saponin content.
- ii) Several groups of quinoa germplasm were distinguished on the basis of plant architecture, stem diameter and yield.
- iii) Three mutant lines exhibiting high yield and low saponin content in the M₇ generation were obtained.
- iv) Molecular markers (RAPDs) for the identification of low- and high-saponin content quinoas were preliminary developed.
- v) Cytological studies with *C. quinoa* var. Barandales permitted to determine its chromosome complement, as well as details on the chromosome length, total chromatin, nuclear DNA content and genome size.

2. Vegetatively propagated crops

a. Bitter potato

Potato and other Andean tubers are the most important crops in Bolivia. Bitter potatoes are cultivated in high lands (around 4000 m above sea level) and are among the few species that tolerate extreme eco-physiological conditions, including freezing. A major challenge to enhance the use of bitter potatoes is their high glycoalkaloid content. Another problem of this crop is the gradual loss of many local, native varieties which are being replaced by sweeter genotypes. The objectives of this project were to collect such valuable genetic material, and also to develop improved varieties with low solanine and solasodine content through the application of mutations induced by irradiation of *in vitro* cultures.

The achievements made on bitter potato include:

- i) Conditions for *in vitro* culture and virus cleaning for several genotypes were optimised (Murillo 1998).
- ii) Dosimetry studies were carried out and optimum doses were 22Gy for Bola Luck'y and 28Gy for Luck'y Kheto (Murillo 2002).
- iii) Chimaeras were dissociated by multiplication through to M₁V₄.
- iv) Mutants were acclimated in the greenhouse for further characterization.
- v) A total of 14 potentially useful mutants were kept for further field experiments.

b. Cocoyam

Cocoyams (*Xanthosoma sagittifolium* and *Colocasia esculenta*) are important starchy staples in Ghana and Costa Rica providing people with carbohydrates, proteins and vitamins. It is a neglected crop and its cultivation is hampered by root rot disease caused mainly by *Fusarium* spp. Cultural practices and chemical treatments have failed to control the disease and no resistant varieties have been developed so far. The corms of *Colocasia* and the cormels of *Xanthosoma* are used in various food preparations whilst the leaves of *Xanthosoma* are consumed as a vegetable. Despite their importance, they have received little attention by research and development groups. The major constraints of these crops are low yields and diseases (e.g. root rot and leaf blight). The project was aimed at characterizing the genetic resources of cocoyams, generating genetic variability and selecting superior genotypes for

mutagenesis to develop varieties resistant to the root rot and leaf blight diseases by mutagenesis induced through radiation of shoot bud apices followed by their culture *in vitro*.

Some of the achievements on cocoyam are:

- i) A mutation induction methodology for cocoyam using gamma rays was achieved: recollection of plant material, virus cleaning, *in vitro* cultures, determination of the LD₃₀, dissociation of chimaeras.
- ii) Establishment of a greenhouse screening methodology for *Fusarium* spp.
- iii) 17 putative tolerant mutants were obtained after irradiation and screening.
- iv) Development of a DNA extraction method and standardization of RAPDs Molecular and greenhouse phenotypic characterization of collected and mutated material.
- v) Establishment of a greenhouse germplasm bank of cocoyam.

c. Okra

Okra (*Abelmoschus* spp.) is a multipurpose vegetable crop in Thailand, whose fruits are an important source of Vitamins (A, C) and minerals (particularly Ca). The plant is also used for many other purposes, including medicinal (in treating peptic ulcers). The total area under okra cultivation in Thailand increased to 1,600 ha in 1996. However, due to severe disease attack, both area and production decreased dramatically in 1997 (up to 50%). Infections by okra yellow vein mosaic virus are among the most damaging ones, causing stunting in the plant and reduced number of fruits. This disease is transmitted by the tobacco white fly. To address this problem, a breeding program on okra was established in 1996 in Thailand and activities were undertaken using resistant commercial varieties originated in India. Induced mutation using gamma radiation to develop resistant varieties was selected as a viable breeding option.

The achievements made on okra include:

- i) The optimum dose for gamma irradiation technique (LD₅₀ 400-600Gy) in okra was identified.
- ii) The white fly transmission technique was developed in greenhouse conditions in order to reduce environmental interferences to a minimum.

- iii) 22 mutant lines with resistance to OYVMD in greenhouse and field conditions were obtained, 12 of these gave satisfactory yields but fruits had an undesirable shape, the remaining 10 mutant lines produced fruits of a desirable shape but the yield is still to be determined.

d. Taro

Taro (*Colocasia esculenta*) is an important staple crop in several regions of Indonesia. The corms and leaves are major sources of carbohydrates, proteins, minerals and vitamins. However, there are production constraints including the leaf blight disease caused by *Phytophthora colocasiae*, low yields and acidity of varieties. The limited genetic diversity is a major constraint in crop improvement.

Significant achievements made on taro are:

- i) Protocols for *in vitro* propagation of taro have been developed.
- ii) The LD₃₀ of *Colocasia esculenta*, cultivar Bentul was established as 10Gy.
- iii) Plants were multiplied through M₁V₄ and transplanted to the greenhouse and in the field.
- iv) Three mutants tolerating leaf blight and more palatable have been identified and screened for early maturity and improved yield.
- v) Three variants (B43, B63 and B133) had the desired characters: high tolerance to leaf blight disease, early maturing, heavy corms and good taste.

Conclusions and prospects

Neglected crops are getting attention to realise to meet the challenges in feeding the world in the changing environmental conditions. The world food supply has been dependent on few crop plants mainly cereals including rice, wheat, barley, maize and so on. So far, food crops have gone through a lot of selection cycles for tailor made variety development, and as a result the gene pool has narrowed down. There is a great need to diversify crops by growing underutilized crops for food production to feed ever growing human population in the changing climatic conditions.

Induced mutations are necessary to enhance rate of genetic variability since spontaneous mutation rate is very slow and that prevents breeders to exploit them in plant breeding programs. The major advantage in

induced mutations is that multiple trait mutants can be isolated as compared to transgenic approach where single trait can be introduced in the crop and moreover there is a lack of acceptance of genetically modified (GM) food.

The new gene discovery with reverse and forward genetics will open the way for developing functional genomic plant breeding. The general strategy for reverse genetics is called TILLING (Targeting Induced Local Lesions in Genomes), or coming together of traditional mutagenesis with functional genomics. EcoTILLING, a variation of this technique, represents a means to determine the extent of natural variation in selected genes in crops (Till *et al.* 2007). It may be a cost effective approach for haplotyping and SNP discovery. Furthermore, DNA sequence information of crop plants facilitates the isolation of cisgenes (Jacobson *et al.* 2007), which are genes from crop plants themselves or from crossable species. The increasing number of these isolated genes, and the development of transformation protocols that do not leave marker genes behind, provide an opportunity to improve plant breeding while remaining within the gene pool of the classical breeder or mutation breeding.

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TILLING and Ecotilling: towards high throughput tools for understudied crops

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Summary

Induced mutation and natural nucleotide variation are powerful tools for probing gene function and developing new crop varieties. Traditional mutagenesis using chemical mutagens has been widely used as a forward genetics strategy in plants. Mutagens such as ethyl methanesulphonate (EMS) cause stable point mutations and thus produce an allelic series of truncation and missense changes that can provide a range of phenotypes. TILLING (Targeting Induced Local Lesions IN Genomes) uses traditional mutagenesis and SNP discovery methods for a reverse genetic strategy that is high in throughput, low in cost, and applicable to most organisms. For TILLING, target fragments of approximately 1.5-kb are amplified from mutant individuals by PCR with gene-specific oligonucleotide primers that are 5' end-labeled with fluorescent dyes. Throughput is increased by pooling samples prior to PCR. After amplification, samples are denatured and then annealed to form heteroduplexes between strands of DNA harboring nucleotide polymorphisms. Heteroduplexes are digested using a single-strand specific nuclease and then size-fractionated by denaturing polyacrylamide gel electrophoresis and visualized by fluorescence detection. Over the past six years, TILLING has moved from proof-of-concept to production with the establishment of publicly available mutation discovery services for several plant species, including maize. Pilot-scale projects have been completed on many other plants, such as rice and soybean. The low cost and generality of the TILLING method mean that it is ideally suited for crops that have not previously benefited from the development of sophisticated genomic tools. With the utility of TILLING as a reverse genetic tool well established, a next challenge is to integrate the technology into a crop improvement pipeline. The same methods used for TILLING can be applied for the discovery of natural

nucleotide diversity, a process termed Ecotilling. Like TILLING, Ecotilling is general, can be applied to a variety of crops, and has the potential to facilitate crop improvement.

Introduction

Increasing world population, change and variability in climate, and competition for non-food applications are pressuring agricultural output and threaten food security, especially in developing nations. Breeding superior crop varieties is one way to ameliorate these problems. For traditional mutation breeding, plants are treated with mutagen to create novel genetic diversity that can cause superior phenotypes. This method has been used for decades and has led to the release of ~3000 mutant crop varieties (Maluszynski *et al.* 2000, <http://mvgs.iaea.org/>). Inducing heritable genetic changes is also a powerful tool for assaying gene function *in vivo* (for example, Page and Grossniklaus, 2002; Candela and Hake, 2008). In recent decades, the accumulation of large amounts of genomic sequence information from a variety of different species has fuelled the development of reverse genetic strategies to target the disruption of specific genes. As both sequence data and knowledge of gene function expands, reverse genetic approaches can be an efficient method for validating gene function and creating useful phenotypes in lesser studied species such as orphan crops. In addition, reverse genetic strategies can be used to uncover phenotypes or traits that are either inefficient or impossible to produce through standard mutation breeding due to polyploidy or genome duplication.

While reverse genetic strategies can be quite powerful, many are either difficult to transfer from species to species, or require the use of transgenic methods that come with regulations or restrictions when applied to food crops. TILLING (Targeting Induced Local Lesions IN Genomes) is a general reverse genetic technique that combines traditional mutagenesis, as is used for mutation breeding or forward genetic screens, with high throughput nucleotide polymorphism discovery methods (McCallum *et al.* 2000a, Colbert *et al.* 2001). It is a non-transgenic method that is easily adapted to many species. For seed propagated crops, seeds are typically mutagenized and plants are propagated to the first non-chimeric generation (M_2) through self-fertilization and a single seed descent procedure when possible (Figure 1). DNA is collected from the M_2 , and M_3 seed is collected that represents the mutant line. To screen for a mutation in a target gene, the gene is first amplified by PCR using gene-specific primers, and

products are then subject to a mutation discovery assay. A variety of mutation discovery methods can be used to detect mutations for TILLING (for example (McCallum *et al.* 2000b, Colbert *et al.* 2001, Wienholds *et al.* 2002, Caldwell *et al.* 2004). A common method is enzymatic mismatch cleavage of heteroduplexed DNAs followed by fluorescence detection (Till *et al.* 2006a). In this method, amplicons are prepared using gene-specific primers that are fluorescently end-labeled. PCR products are then denatured and annealed to create heteroduplexed molecules that are mismatched at the site of the mutation. Mismatches are then cleaved by incubating heteroduplexed molecules with a single strand specific nuclease such as CEL I (Till *et al.* 2004a). Samples are visualized by size fractionation using denaturing PAGE and fluorescence detection with an apparatus such as the Li-Cor DNA analyzer. Throughput is increased and costs are reduced by pooling samples prior to screening (Figure 2).

High mutation densities and a broad spectrum of allele types have been reported for plant populations treated with chemical mutagens (For example, Till *et al.* 2003, Greene *et al.* 2003, Cooper *et al.* 2008). The efficacy of using physical mutagens is largely unknown; a low density of presumably induced single nucleotide mutations has been reported for rice treated with gamma irradiation (Sato *et al.* 2006), although gamma irradiation is expected to cause larger lesions (Naito *et al.* 2005). A high density and broad spectrum of induced mutations means that useful alleles can be obtained by screening relatively small populations of ~3000 to 6000 mutant lines. This has allowed the establishment of high-throughput TILLING services for a variety of organisms including maize, Arabidopsis and Drosophila (<http://genome.purdue.edu/maizetilling/>, [http://tilling.fhcrc.org:9366/files/Welcome to ATP.html](http://tilling.fhcrc.org:9366/files/Welcome_to_ATP.html), [http://tilling.fhcrc.org:9366/fly/Welcome to Fly-TILL.html](http://tilling.fhcrc.org:9366/fly/Welcome_to_Fly-TILL.html)).

Protocols for inducing mutations have been established for many species and are easily transferable to many others. Methods for mutation discovery such as enzymatic mismatch cleavage tolerate a wide range of GC content in amplicons, and thus can be applied to PCR products from most species. Together, this makes the TILLING strategy broadly applicable. Indeed, TILLING has been described for a variety of species including Arabidopsis, barley, *C. elegans*, Drosophila, *Lotus japonicus*, maize, rice, soybean, wheat, and zebrafish (McCallum *et al.* 2000b, Caldwell *et al.* 2004, Gilchrist *et al.* 2006a, Bentley *et al.* 2000, Perry *et al.* 2003, Till *et al.* 2004b, Till *et al.* 2007, Cooper *et al.* 2008, Slade *et al.* 2005, Winkler *et al.* 2005, Wienholds *et al.* 2003).

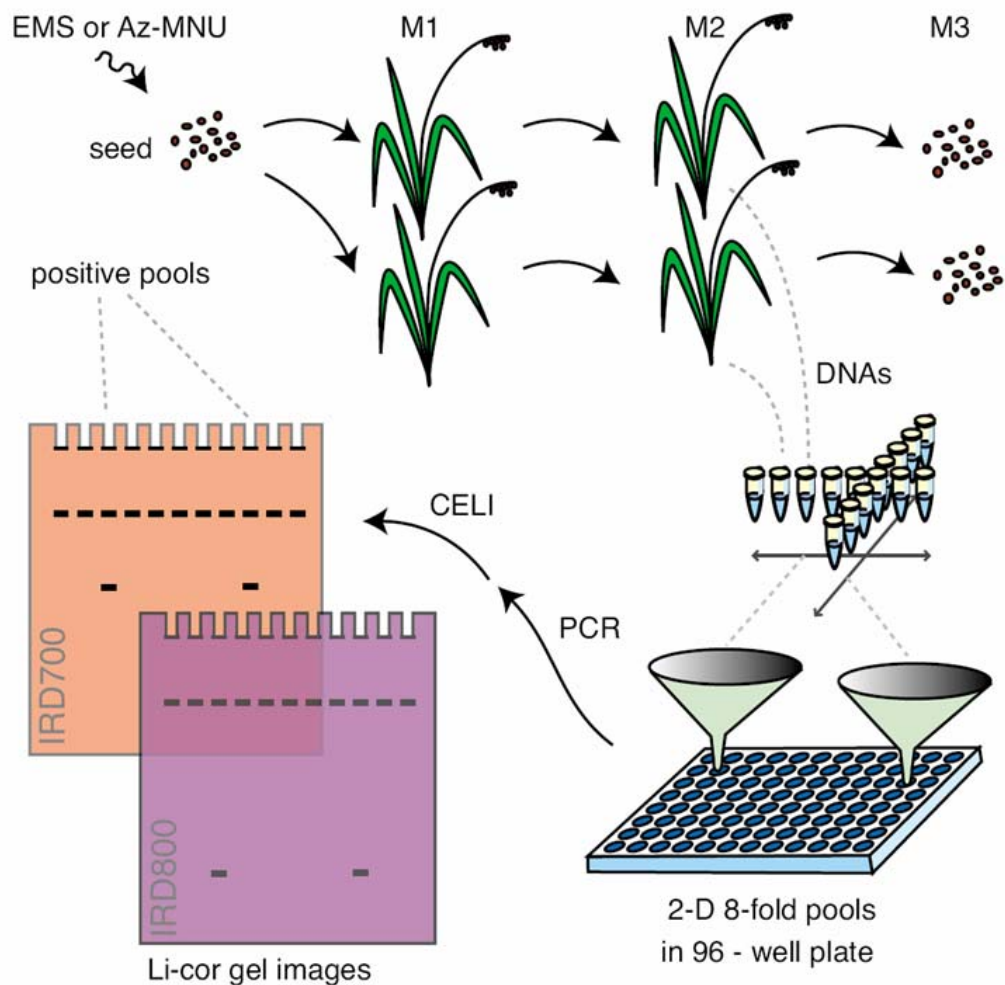


Figure 1. A typical TILLING strategy using chemical mutagenesis of seed. Seed are mutagenized by soaking with chemical mutagen for a set amount of time. The first generation (M_1) is chimeric because of the multicellular composition of the seed at mutagenesis and is therefore not suitable for screening. Following self fertilization, M_2 plants are grown. These are non-chimeric and suitable for TILLING. A single M_2 individual is selected from each M_1 parent to establish the line. The M_2 is selfed and M_3 seed is collected and stored as the germplasm stock. DNA from each M_2 is collected. DNAs are normalized to a common concentration; samples are arrayed in 96 well plates, and pooled to increase screening efficiency (a two-dimensional eight fold pooling strategy is used in this figure). DNAs are screened for mutations by gene-specific PCR followed by mutation discovery by enzymatic mismatch cleavage followed by fluorescence detection using denaturing polyacrylamide gel electrophoresis and the Li-cor DNA analyzer. This figure of a rice TILLING strategy is from (Till *et al.* 2007).

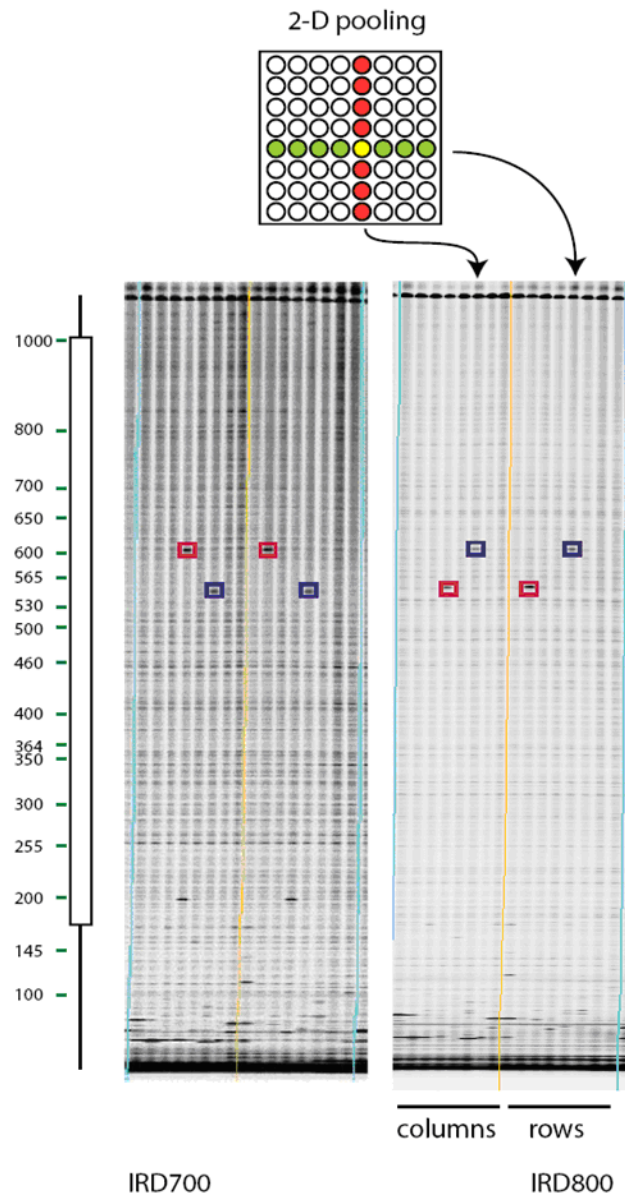


Figure 2. An example of TILLING mutation discovery using enzymatic mismatch cleavage followed by fluorescence detection. PCR products are prepared with primers containing fluorescent IRDye 700 (forward primer) and IRDye 800 (reverse primer) covalently attached to their 5' ends. Following mismatch cleavage, products are size fractionated on a denaturing polyacrylamide gel and bands visualized using the Li-cor DNA analyzer. Two images are produced; one corresponding to DNA containing the IRDye 700 label and one containing IRDye 800 (left and right, respectively). Boxed bands correspond to mutations identified in the assay. The size of the band in the IRDye 700 image plus the size of the band in the IRDye 800 image will equal the size of the full length undigested PCR product (about 1200 base pairs). This allows an estimation of the approximate location of the mutation and greatly reduces false positive errors. Samples in this example of rice TILLING are arrayed in a two-dimensional format so that the individual harbouring a mutation in a pool of eight samples can be deduced in a single assay. This figure is taken from (Till *et al.* 2007).

The same methods developed for TILLING can be used to discover and genotype natural nucleotide diversity, a process called Ecotilling. Studies with *Arabidopsis*, black cottonwood trees, and humans, among other species, has shown Ecotilling to be a robust, accurate, and low cost method using pooled or non-pooled samples (Comai *et al.* 2004, Gilchrist *et al.* 2006b, Till *et al.* 2006b). It is therefore suitable to consider Ecotilling for, among other applications, mapping, haplotype analysis, diversity studies and the discovery of rare disease alleles.

Developing TILLING and Ecotilling for understudied crops

Rapid accumulation of genome sequence information, concurrent development of hypothesis regarding gene function based on homology to other species, and the sometimes slow development of biotechnological tools makes TILLING and Ecotilling ideally suited for gene function studies and mutation breeding applications in understudied crops. The application of such technologies need not be considered completely academic. Understudied crops represent a large range of biodiversity and local adaptation that may prove very important for food security as the population grows and the climate is subject to variation and change. While robust, locally adapted species can suffer from low yields and may be susceptible to existing or emerging threats. Therefore, using TILLING or Ecotilling to direct improvements in yield or to enhanced biotic or abiotic resistance in orphan crops may prove beneficial.

The Plant Breeding Unit of the Joint FAO/IAEA programme on Nuclear Techniques in Food and Agriculture is focused on helping its Member States improve food security by providing laboratory training, mutagenesis and genetic analysis services, and adapting technologies to agronomically important, but understudied crops or crop varieties. To develop a TILLING and Ecotilling platform at our facility, we chose to start with three species that were considered paradigms for technology development due to their unique properties: banana, cassava, and rice. Edible banana is a triploid, infertile and must be propagated vegetatively. We are using accessions from the International Network for the Improvement of Banana and Plantain (INIBAP, now Bioversity International), for assay development and are in the process of developing mutagenized populations (Table 1). We aim to learn about the density and spectrum of mutations that can be obtained from both chemical and physical mutagens.

Table 1. Plant populations for TILLING

Organism	Genotype	Mutagen	Dose	# mutagenized	# survive	Stage
Banana	Grande Naine AAA	EMS (plantlet)	1%, 3hr 0.5%, 6hr 0.125%, 24 hr, 0.06%, 48 hr	4000 (1000 each treatment)	< 1000	Dissolved chimeras
	Giant Cavendish AAA	Gamma (plantlet)	20 Gy, 30 Gy	800	N.A.	Dissolved Chimeras
	Calcutta 4 AA	Gamma (seed)	50 Gy	1000	N.A.	M ₂
Cassava	Bf-red	Gamma	15 Gy	500	302	M1V4
	01/1277	Gamma	15 Gy	1200	~700	M1V4
	01/1277	EMS	0.5%	1100	260	M1V4
Rice	Cisande	Gamma	210 Gy	2000	NA	M1 planted
	Cisande	EMS	1.5%	1000	0	
	Witra4	EMS	1/5%	1000	0	

In the longer term, we hope to understand the utility of different mutagens and different mutagenic treatments in the context of developing useful phenotypes. For example, one might hypothesize that inducing mutations in a vegetatively propagated triploid would be largely inefficient if a mutagen causing a high frequency of recessive mutations is used. Yet phenotypes have been reported when mutagenizing triploids (for example, Roux, 2004), and so further study into the genetic and epigenetic effects of mutagenesis and the functional capacity of the different genomes in a triploid is required. We are taking a similar approach of platform development for cassava and rice, starting with Ecotilling accessions while concurrently developing mutagenized populations (Table 1). Cassava is typically vegetatively propagated and like with banana, we aim to understand the utility of different mutagens to provide useful phenotypes. Edible cassava can also be seed

propagated and we will be exploring the logistics of sexual propagation to uncover recessive phenotypes. For rice, the utility of TILLING has already been shown, but there may be a strong genotypic component in the ability to achieve a high density of induced mutations using chemical mutagens (Wu *et al.* 2005, Till *et al.* 2007). We hope to understand this further by testing different genotypes with different doses and types of mutagens. Together, this knowledge should allow the provision of expert advice to Member States who wish to begin a program of mutation breeding and reverse genetics. At the same time we are focusing on technology development and adaptation, we are also focusing on key biological processes that are important to each crop. For banana, we are focusing on resistance to *Mycosphaerella fijiensis*, the causative agent of black sigatoka disease. For cassava, we are focusing on improved starch quality, and for rice we are focusing on improved drought and salinity resistance.

A look towards the future

Whole genome sequencing of a variety of crops is currently planned, including understudied crops such as cassava and banana (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj>). With improvements to throughput and reductions in costs for sequencing, the trend is likely to continue and we can expect within decades to have available whole genome sequence for many of the crops now considered understudied. Improvements and cost reductions in next generation sequencing technologies may pave the way to advances in mutation discovery that will allow a broad spectrum of point mutations and larger chromosomal deletions and rearrangements to be discovered with a single machine, increasing the choices for the type and dosage of mutagen for TILLING. These advances, together with dedicated efforts to develop suitable mutagenized populations, will allow high-throughput TILLING for many important crops.

Due to the cross-platform nature of TILLING, screening for mutations can be efficiently performed at key centralized facilities. This allows the development of collaborative research networks where multiple labs with specialized expertise can be linked together to provide an efficient pipeline for gene discovery and mutation breeding. Such networks will exploit developed expertise in plant propagation, high-throughput phenotyping, mutation discovery, post discovery characterization including transcriptomics and proteomics, and development of improved varieties. In addition to the capacity to efficiently tackle the current

problems facing agriculture, such networks will be able to rapidly respond to yet unforeseen agricultural dilemmas.

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Employing Green Revolution Genes to improve orphan crop tef

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Summary

Tef [*Eragrostis tef* (Zucc.) Trotter] is the most important cereal crop in Ethiopia and is grown extensively under various climatic and soil conditions. However, the crop produces low yield compared to other cereals due to lack of cultivars with desirable agronomic traits. Lodging is a major agronomic problem in tef production and causes direct and indirect losses in grain yield and quality. The Tef Biotechnology Project aims at improving the grain yield of tef by reducing the losses caused by lodging. We implement a non-transgenic method known as TILLING in order to obtain semi-dwarf candidate plants that are resistant to lodging and also respond to fertilizer application in order to increase grain yield. In this report, we present our strategy and some progresses made so far.

Introduction

Tef is extensively grown in Ethiopia on over 2.6 million ha of land. The crop is ecologically and agronomically versatile. It is preferred both by farmers and consumers because farmers benefit since tef is more tolerant to abiotic stresses such as drought and water-logging, and also biotic stresses such as pests and diseases particularly to storage pests, than other cereal crops. Consumers prefer tef because of good quality bread, in addition to providing high protein. Moreover, unlike wheat and other cereal grains, the seeds of tef are free of gluten for which many people are allergic (Spaenij-Dekking *et al.* 2005).

However, tef is one of the low yielding cereal crop in the world. Lodging is considered as the major yield limiting factor (Ketema 1997). The causes of lodging in tef are related to the standing power of the plant

(morphological) and environment (fertilizer, weather). Tef has a tall and tender stem that is susceptible to lodging caused by wind and rain. In addition, when optimum amount of fertilizer is applied to increase the yield, high incidence of lodging occurs. As a consequence, the yield from the crop is severely reduced in terms of total grain yield and quality. The lodged plant also poses a great problem for harvesting.

The Tef Biotechnology Project, based at the Institute of Plant Sciences in the University of Bern, focuses on solving the problems related to lodging in tef. The immediate objective of the project is to develop lodging resistant tef lines. The so-called 'Green Revolution Genes' that are known to boost grain yield for rice and wheat during and after Green Revolution era are the major targets for the project.

Green Revolution Genes

The Green Revolution occurred in 1960's and 1970's in Asia and Latin America and dealt with optimum use of agronomic packages including agricultural mechanization, irrigation, fertilizers, pesticides, and improved seeds in order to boost crop production. The key components of this agricultural revolution were improved seeds. The main characteristics of the seeds are, i) high yielding, ii) responsive to fertilizer application, iii) semi-dwarf in stature, and iv) the plants are lodging resistant.

The dwarf plants of wheat that tremendously increased the yield during the Green Revolution contain the mutated Reduced height-1 (Rht-B1 and Rht-D1) gene (Peng *et al.* 1999). Rht-B1 and Rht-D1 are the orthologs of Gibberellin Insensitive (GAI) gene from *Arabidopsis*. In addition, *semi-dwarf (sd-1)*, a commercially important variety of rice is defective in gibberellin 20-oxidase (GA20ox, Spielmeier *et al.* 2002). As a result, sd1 mutants are characterized by short culm that improved lodging resistance (Sasaki *et al.* 2002, Spielmeier *et al.* 2002).

Various genes in brassinosteroid pathway were also shown to regulate plant height in rice. One of the first dwarf mutants involved in this pathway was *dwarf61 (d61)*, a rice mutant containing a mutation in a BR-receptor that affects internode elongation (Yamamuro *et al.* 2000). The loss of function in two cytochromes P450 genes, namely CYP90D2 and CYP724B1 results in dwarf rice mutants, *dwarf2 (d2)* and *dwarf11 (d11)*, respectively (Tanabe *et al.* 2005, Hong *et al.* 2003). The maize *brachytic2 (br2)* mutants and its ortholog in sorghum *dwarf3 (dw3)* are also characterized by compact lower stalk internodes (Multani *et al.*

2003). The height reduction in these plants results from the loss of a P-glycoprotein (PGP) that modulates polar auxin transport in maize stalk (Multani *et al.* 2003). Another mutation affecting plant height is *high tillering dwarf1 (htd1)* in rice. In a model plant Arabidopsis, the orthologous gene for HTD1 is *AtMAX3*. *AtMAX3* encodes a carotenoid cleavage dioxygenase which is required for negative regulation of the outgrowth of axillary buds (Zhou *et al.* 2006). The D35 (Tan-Ginbozu) mutants of rice also exhibited a semi-dwarf phenotype (Itoh *et al.* 2004). D35 encodes for ent-kaurerene oxidase (KO) that is involved in early step of gibberellin biosynthesis (Itoh *et al.* 2004).

Although semi-dwarfism is proved to be a valuable traits in increasing lodging resistance, other trait such as stem strength and root anchorage also play major role. Some genes or loci responsible for these traits were already identified in rice (Ishimaru *et al.* 2008).

Strategies of the Tef Biotechnology Project

TILLING and ecoTILLING

The project aims at improving the grain yield of tef by reducing the direct and indirect losses caused by lodging. In order to achieve the objective, we implement a recently developed reverse genetics approach known as TILLING (Targeting Induced Local Lesions in Genomes). TILLING is proved to detect useful mutations in crops such as wheat (Slade *et al.* 2004), barley (Caldwell *et al.* 2004), maize (Till *et al.* 2004), rice (Sato *et al.* 2006, Till *et al.* 2007, Suzuki *et al.* 2008), and sorghum (Xin *et al.* 2008).

The Tef Project has so far generated over 10 000 M₁ and 6000 M₂ tef populations mutagenized by ethyl-methanesulphonate (EMS), a chemical known to induce point mutations. Leaf samples were collected from 2-3 weeks old M₂ seedlings and the seeds of M₃ lines were harvested. DNA isolation was made from M₂ samples. Two genes known to influence plant height in rice, namely HTD1 (HIGH-TILLERING DWARF1, Zou *et al.*, 2006) and DWARF4 (Sakamoto *et al.*, 2006) were isolated from tef. Based on the sequence information each of these genes is represented by two copies having high homology in the exon region but divergent in the intron region. The divergent region is used to design copy specific labelled primers in order to amplify only one copy at a time.

EcoTILLING is a modified form of TILLING used to detect mutations in the natural population (Comai *et al.* 2004). We implement ecoTILLING on about 500 tef accessions collected from different growing regions. Detailed description on the methodologies of TILLING and ecoTILLING are presented by Till *et al.*

The major problems we have encountered in TILLING and ecoTILLING tef is related to the polyploid nature of the plant. Tef is an allotetraploid with $2n=4x=40$. Since each gene is present at least in two copies, PCR amplification and detection of a single copy is difficult. In order to tackle this problem, we first investigate the number of gene copies present in the genome. Then, we design primer(s) that can preferentially amplify one copy at a time.

Direct screening for semi-dwarf phenotype

In order to directly screen for semi-dwarf lines, we grew 6-8 plants each from over 4000 M₂ mutagenized tef population in the glasshouse of Syngenta AG at Stein where environmental factors such as light, humidity and temperature are properly regulated. Evaluations were made twice during the growing season, one month and two months after sowing. Families with at least 2 semi-dwarf lines were considered as candidate. Ten candidate lines selected from the screening are currently being investigated for fertility, productivity, etc.

Conclusions

The TILLING technology could be applied to crops like tef which are affected by lodging. Once the problem of lodging is tackled, optimum amount of fertilizer can be applied and the plant can also allocate its resources to producing more grain instead of long stalk. The implementation of TILLING and ecoTILLING to the understudied crops has many advantages. The techniques do not require prior knowledge of the genome sequence except for the gene of interest. The other advantage is for the polyploid species like finger millet and tef where most mutations do not show obvious phenotypes due to the existence of unmutated gene copies. Since TILLING and ecoTILLING reveal many allelic mutations, through double or multiple crossings expected mutant phenotypes could be obtained.

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Initial results from applying Diversity Arrays Technology (DART) and Massively Parallel Signature Sequencing (MPSS) to bambara groundnut

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Summary

The EU-funded BAMLINK project aims to develop new concerted strategies to improve and promote the use of underutilised crops for sustainable food production in semi-arid Africa and India. Bambara groundnut (*Vigna subterranea* (L.) VERDC.) is a tropical legume featuring the capacity to produce reasonable yields of high nutritional value even under adverse environmental conditions. As part of the molecular characterisation of bambara groundnut, an initial Diversity Arrays Technology (DART) DNA marker discovery array was developed and tested on a subset of 32 genotypes, representative of the genetic variability in cultivated landraces. Initial results indicate the discovery of potentially useful markers through this technique which allow unambiguous discrimination and fingerprinting of the samples used. However, the overall genetic diversity appears to be low in domesticated material. In addition to this, eight cDNA pools (four genotypes vs two environments) were produced and sequenced through Massively Parallel Signature Sequencing (MPSS) in order to gain insight into cellular mechanisms of the crop's response to water-deficit and discover candidate genes for breeding varieties with improved drought tolerance. Initial results from both techniques are presented.

Introduction

The multi-disciplinary EU-funded Framework 6 INCO-DEV BAMLINK project was launched in 2006 to facilitate the development and use of bambara groundnut (*Vigna subterranea* (L.) VERDC.) for sustainable food production in semi-arid Africa and India. It is an important case study for research approaches on underutilised crops which are integrated across different disciplines, ranging from basic genetics and physiology, through nutritional studies, to end-user benchmarking and value-added products.

Bambara groundnut is an annual legume originating from Western Africa, and grown throughout sub-Saharan Africa as well as in Southeast Asia. The crop's key advantage is the capacity to produce reasonable yields of pods of high nutritional value even under low intensity agriculture and periods of drought.

One aim of the present study is to develop molecular markers for the characterisation of germplasm and to complete genetic linkage maps (Basu *et al.* 2006). Diversity Arrays Technology (DArT) relies on hybridisation of defined DNA fragments and is considered to be highly suitable to apply in neglected crops as it offers robust markers and high throughput without depending on access to sequence information.

The identification of genes differentially expressed under drought stress plays an important role in understanding the plant's physiological response to water deficit. For this, the novel method known as Massively Parallel Signature Sequencing (MPSS), another cost-efficient high-throughput approach based on counting thousands of short sequence tags (from the Next Generation sequencing technology of 454 system) as a measure of gene expression, was applied to test its performance in orphan crops.

Materials and Methods

DArT

In order to produce a core set of germplasm representative of the genetic diversity in bambara groundnut, thirty-eight bambara groundnut landraces (37 domesticated accessions, *V. subterranea* ssp. *subterranea*; and one wild form, *V. subterranea* ssp. *spontanea*) were selected based on their geographic origin and their AFLP fingerprints in a previous study (Singr un and Schenkel 2003). Plantlets were raised in a home-made

germination device and genomic DNA was extracted by the CTAB method (Saghai-Marooof *et al.* 1984).

The construction of an initial discovery array carrying polymorphic fragments from the bambara groundnut metagenome was carried out by Diversity Arrays Technology Pty Ltd, Yarralumla, Australia. In brief, DNA samples were digested with the restriction enzymes *Pst*I and *Alu*I to obtain genomic representations of lower complexity. Amplified fragments were pooled and cloned. 1536 colonies were picked; inserts were PCR amplified and spotted onto slides. The same complexity reduction method was applied to a subset of thirty-two landrace samples. Target genomes were fluorescently labelled and hybridised onto the slides. Signals were detected by a scanner and results were processed through the DARTSoft analysis software. Initial results were analysed for levels of inter-landrace polymorphism and a phenetic tree constructed. For a deeper investigation of markers, cloned inserts were PCR amplified using pBL2SK-1/2 primers and sequenced.

MPSS

This study was conducted using single plants from four landraces adapted to either drought prone areas (namely DipC from Botswana, and AS17 from South Africa), or humid environments (Swazi red from Swaziland, and LunT from Sierra Leone). Plants were grown in a controlled environment (day/night temperatures 30°C/25°C, daylength 12 hours, relative humidity 50%) with drought stress applied by the reduction of irrigation from non-limiting down to 30% of non-limiting volume for a period of seven days. The youngest fully developed leaves were used for RNA extraction (Chang *et al.* 1993). Non-normalised cDNA libraries for 454 sequencing were prepared by vertis Biotechnologie AG and sequenced using a Genome Sequencer 20 System (Roche Applied Sciences). Data analysis (clustering) was performed with the aid of the SeqClean script and the software package TGICL (Pertea *et al.* 2003; downloaded from <http://compbio.dfci.harvard.edu/tgi/software>). NCBI's netblast (Altschul *et al.* 1997; <ftp://ftp.ncbi.nih.gov/blast/executables/LATEST>) was used to search for homologies to the published sequences, applying the *blastn* algorithm and a threshold E-value at $1e^{-3}$. Functional categorisation of the clusters was assigned manually according to the functional catalogue of the Munich Information Center for Protein Sequences (<http://mips.gsf.de/proj/funcatDB/>) by means of bibliographic searches.

Results and discussion

DArT

A set of 76 high-quality polymorphic markers were obtained through the first discovery array. However, around 70% of these markers showed identical discrimination patterns across the genotypes scored. Therefore, all polymorphic clones were sequenced in order to eliminate redundant markers. Finally, data of 26 markers (mean PIC value 0.32) of unique sequence information were included in cluster analysis. Polymorphism content between cultivated landraces in the total *PstI* + *AluI* library is thus estimated to be at a low level at 2% which is consistent with results for cultivated pigeonpea, another underutilised legume (Yang *et al.* 2006). These data support the conclusion of Pasquet *et al.* (1999) who reported low overall genetic diversity in bambara groundnut and moreover, a loss of polymorphism during domestication of the crop as assessed through isozyme analysis, but are in contrast to previous AFLP and RAPD results (Massawe *et al.* 2002, Massawe *et al.* 2003) which revealed high levels of polymorphism. More recent data from initial microsatellite analysis also suggests reasonable levels of polymorphism within landrace material (Basu *et al.* 2007 and unpublished data).

Nevertheless, cluster analysis of 32 accessions showed, apart from a few out-grouped genotypes, three groups that tend to reflect sites of collection (Western Africa, South-Eastern Africa, and Southern Africa; Figure 1). The two Indonesian accessions did not cluster together, but show similarity to Southern and Eastern African landraces, respectively. Furthermore, the fairly high PIC values of the 26 DArT markers allow unambiguous discrimination of the accessions used in this study and can thus form the basis of an initial set of markers for fingerprinting of germplasm collections.

However, further array development is necessary to confirm these initial results and to complete high-density genetic linkage maps.

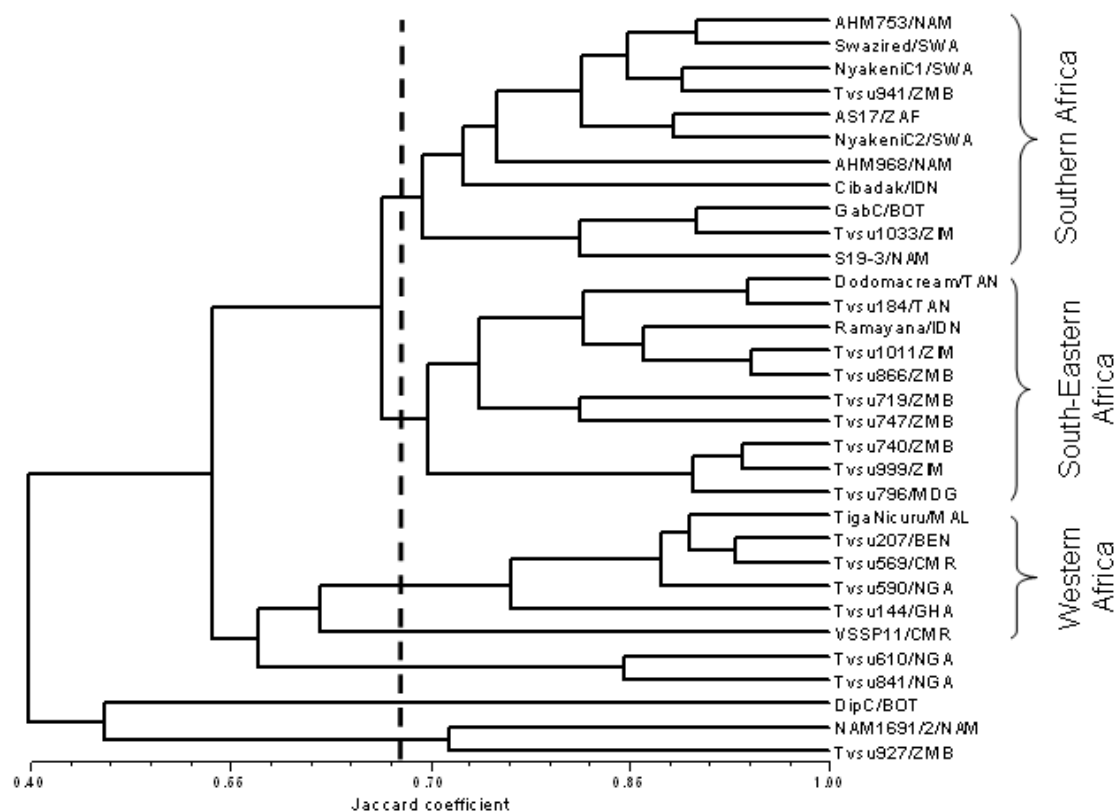


Figure 1: UPGMA dendrogram of 32 bambara groundnut accessions based on Jaccard's coefficient of similarity of 26 DARt markers constructed using NTSYSpc 2.1 software.

MPSS

The 454 sequencing run resulted in 216,022 sequence tags. After cleaning the sequences by removal of poly-A tails, of reads of low quality and removal of the 454 adapters (7 nucleotides each), 197,400 tags with an average length of 84 nucleotides remained. These were clustered into 10,583 clusters ranging from 3,026 to 2 contained sequences. Dividing clusters into landrace and treatment specific groups generally showed good consistency within irrigated and drought-stressed landraces and some significant differences between treatments (Figure 2).

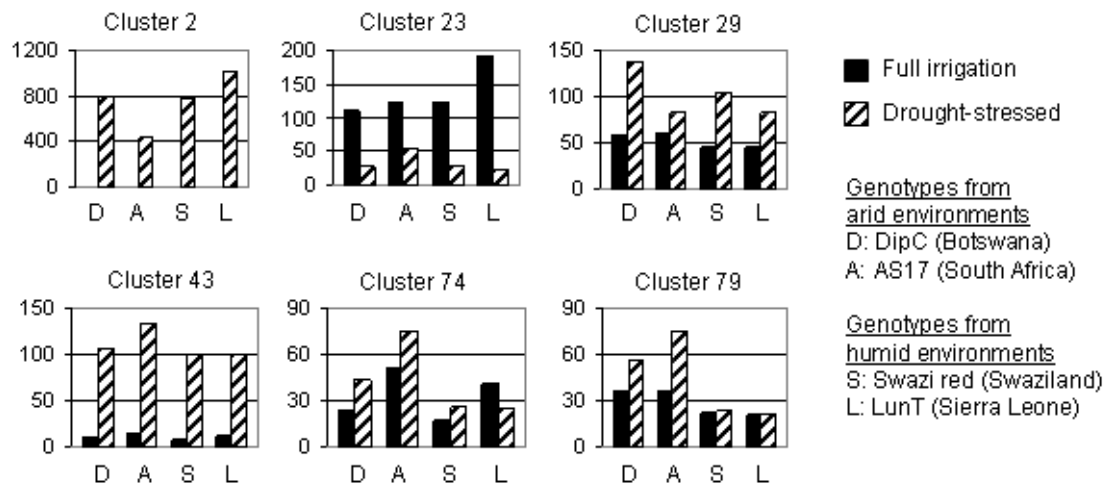


Figure 2: Expression profiles of selected genes (clustered sequence tags). Values on the y-axis indicate the number of sequence tags. Cluster 2 is a good example of a gene which is significantly induced under drought stress across all genotypes. Cluster 23 are tags of a gene which is repressed under water-deficit. Both of these effects appear to genotype independent. However, some Clusters also show genotype-specific responses, such as Cluster 74 and Cluster 77.

Blastn search hits of assembled contigs (Table 1) revealed various genes associated with (drought) stress in plants. Examining clusters consisting of least 50 sequences ($n=455$), 167 genes up-regulated (at least +1.5-fold change) in all four genotypes under water-deficit conditions can be identified. Among these, 26 fall into the category "cell rescue, defense and virulence", most of which have been described playing a role in drought stress adaptation in legumes (e.g. Iuchi *et al.* 1996, Boominathan *et al.* 2004, Kavar *et al.* 2007). However, 64 genes did not show homology to known sequences. This indicates the MPSS technique being a suitable tool to discover a significant number of novel genes in under-researched crops such as bambara groundnut. The majority (47 of 160) of categorised down-regulated genes (at least -1.5-fold change) are involved in energy supplying processes (photosynthesis), followed by repressed primary metabolism (21 of 160 genes). Genotype-specific differences, both qualitative and quantitative, can be detected in a similar way. For example, 27 genes are up-regulated in DipC, which is adapted to dry conditions (Botswana), but not in LunT, originating from a rain-fed area (Sierra Leone), and 34 generally up-regulated genes are more than 1.5-fold higher expressed in DipC compared to LunT.

From a subset of tags displaying promising differences in their expression profiles, an oligo-based hybridisation microarray will be

created. Temporal expression patterns will thus be evaluated in different genotypes and physiological states in order to identify candidate genes for drought stress tolerance in bambara groundnut. Interesting sequences without significant BLAST hits can be elongated through sequencing the original cloned cDNA population using MPSS-derived primers.

Table 1. Sequence homologies of the genes shown in Figure 2.

Cluster	Query length [bp]	Blastn homology	Blast match length [bp]	E-value	Identity [bp]	Function
2	131	CPRD22 protein; <i>Vigna unguiculata</i>	1129	$3e^{-33}$	116/128	Cell rescue
23	106	Photosystem II reaction center; <i>Retama raetam</i>	442	$2e^{-37}$	100/106	Energy
29	101	ADP-ribosylation factor; <i>Vigna unguiculata</i>	841	$1e^{-37}$	91/94	Signalling
43	85	None	-	-	-	Unknown
74	128	None	-	-	-	Unknown
77	98	Ubiquitin-conjugation enzyme; <i>Glycine max</i>	929	$3e^{-18}$	68/74	Protein fate

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Molecular analysis of castor and cassava hybrids using PCR-based markers

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Summary

Cassava (*Manihot esculenta*) is a multiple-use crop adapted to a wide range of agroecologies with a remarkable tolerance to an array of biotic and abiotic stresses. In order to overcome the limitations of the commonly used F₁/BC₁ mapping population, an alternative mapping population such as recombinant inbred lines (RIL) or doubled haploids (DH) is necessary. Towards this goal, we launched a project to develop DH population by crossing cassava (*Manihot esculenta*) with castor (*Ricinus communis*). As a first step of validation of crossing, different types of molecular markers (RAPD, AFLP, and SSR) were screened to distinguish the two species and the hybrid at the DNA level. Out of the many AFLP, RAPD, and SSR primers screened, few showed that some hybrid might have combined alleles from both parents. However, these results were not consistent with most hybrids and results were inconclusive. In the latest approach, we used bioinformatics tools to design castor specific sequence tagged site (STS) primer. Results show that castor-specific STS primer pairs could not amplify products in the putative hybrids while cassava-specific primers amplify products. Comparison of relative nuclear DNA content with flow cytometry showed that putative hybrids have similar DNA content with cassava suggesting that the tested genotypes may not be true hybrids. The development of markers to verify such wide crosses, complemented by cytological analysis, opens an opportunity to use DH mapping population thereby accelerating the development of saturated linkage map in cassava, with the possibility of introgression of novel traits of commercial value from castor to cassava.

Introduction

Cassava (*Manihot esculenta*) is a multiple-use crop adapted to a wide range of agroecology with a remarkable tolerance to an array of biotic and abiotic stresses. In addition to its vital role as a staple food for hundreds of millions of people, its use as animal feed and industrial raw material is steadily growing. Recently, there is a growing interest in cassava as an alternative source of renewable energy (biofuel) that prompted genome sequencing initiative.

Sparked by recent advances in molecular technology, efforts to enhance cassava improvement through marker-aided breeding are rapidly gaining momentum at the International Institute of Tropical Agriculture (IITA). Emphasis was placed on the development of SSR-based framework linkage-map to aid in tagging qualitative and quantitative traits of importance. However, since the publication of cassava genetic linkage map a decade ago, (Fregene *et al.* 1997), the work to saturate the map with PCR-based markers such as SSRs (simple sequence repeats) has not progressed considerably. Even though, close to a thousand SSR markers are developed by different groups, only about 200 SSRs have been placed on the linkage map (Lokko *et al.* 2005, Mba *et al.* 2001, Okogbenin *et al.* 2006). One of the reasons for the low proportion of mapped SSRs is the genetics of the crop and the type of the mapping populations. The mapping populations commonly used to develop linkage map in cassava were F_1 , F_2 , or BC_1 populations derived from the crossing of two heterozygous parents. When such mapping populations are used, after eliminating monomorphic markers, only markers that are single-dose restriction fragments (SDRF) (Wu *et al.* 1992) that meet the expected segregation ratio are selected. As a result of these screening, the number of useful SSR markers are considerably reduced. Experience shows that less than 20% of the total cassava SSR markers assayed can be unambiguously scored for linkage analysis (unpublished data).

In order to enrich the repertoire of markers to saturate the existing framework map, an alternative mapping population such as recombinant inbred lines (RIL) or doubled haploids (DH) is necessary. At IITA, there is an ongoing effort to develop inbred lines by selfing selected genotypes. However, due to the long generation cycle and the accumulation of heterozygosity over a long time, homozygosity has not been achieved (Dixon, unpublished data). Therefore, we launched the double haploid approach to instantly develop pure homozygous clones by crossing cassava (*Manihot esculenta*) with castor (*Ricinus communis*) to

be followed by chromosome doubling in verified hybrids. As a first step of verification of crossing, different types of molecular markers (RAPD, Random Amplified Polymorphic DNA; AFLP, Amplified fragment length polymorphism; and SSR) were screened to distinguish the two species and the hybrid at the DNA level. Therefore, we examined the utility of two approaches, the first involving bioinformatics tools to design castor specific primer whereas the second was comparison of nuclear DNA content with flow cytometry.

Doubled haploids, besides saving a number of generations in development of pure lines, provide numerous advantages in mutation studies, genomics, and transgenics. Doubled haploids have been successfully developed and utilized in other crops to construct genetic linkage map, to perform bulk segregant analysis, and QTL mapping (Forster and Thomas 2003). One of the established methods of developing haploids is wide hybridization (e.g. *Hordeum vulgare* x *H. bulbosum*) that results in chromosome elimination after which the embryos were treated with colchicine to double the haploid genome of the hybrid (Hayes *et al.* 2003). In this study, we hybridized castor bean ($2n=2x=20$) with cassava ($2n=2x=36$), both of which belong to the Euphorbiaceae family. Castor bean is cultivated for its seed oil that has various uses in industrial products and traditional medicines. In addition, castor bean exhibits resistance/tolerance to various biotic and abiotic stresses including disease, pests, drought and low fertility among others. Inter-generic hybridization of cassava and castor may open the opportunity to incorporate the gene for oil production and better fitness into cassava, which, in turn, make it possible to derive more uses for cassava and enhance its commercial value, especially of the botanical seed, that presently has limited uses.

In this pilot study, the status of hybridization was assessed by developing and using DNA-based markers, morphological characterization, and determination of nuclear DNA content by using flow cytometry.

Materials and Methods

Plant material and crossing

Elite cassava lines were chosen to be the female parent and crossing performed according to the established method at IITA Cassava Breeding Program. The embryos of the hybrid seeds were cultured and

the plantlets obtained were multiplied. Five plants from each progenies were planted in the nursery beds and in the field.

DNA extraction and molecular markers

DNA was extracted from the parental lines of cassava and castor as well as the progenies by using Qiagen DNeasy plant mini kit or by using in-house reagents as described (Lokko *et al.* 2005). In the first phase of molecular marker analysis, various primers and combinations of RAPD, SSR, and AFLP were screened. RAPD was performed as reported before (Akinbo *et al.* 2007) whereas AFLP and SSR were performed according to Lokko (Lokko *et al.* 2005). In the second phase of the study, sequence tagged site (STS) primers were designed from two ESTs that were derived from castor bean cDNA library. The first sequence (GenBank acc. EG65914) was a 1.7kb cDNA derived from leaves whereas the second sequence was a 1.4 kb EST from seeds (GenBank acc. EG693595). Primers were designed from the two sequences by the web program FastPCR (Kalendar R. 2007) using the default criteria with emphasis on annealing temperature and absence of primer-dimer or loop formation. Primer sequences were then blasted against available cassava sequences to ensure specificity to castor bean. Primers with sequence similarity with cassava, particularly those with sequence identity at the 3'-end were eliminated. Three forward and three reverse primers matching the above criteria were selected for the assay.

PCR amplification

PCR was performed in 25 μ l reaction volume containing about 100 ng of genomic DNA, 200 μ M dNTP, 10 pmole primers, 1.5mM MgCl₂, one unit of Taq polymerase. After initial denaturation at 94°C for 2 minute, 35 cycles of 94°C for 15 sec (denaturation), 56°C for 20 sec (annealing), and 72°C for 45 sec (extension) was performed on BIORAD DNA Engine thermocycler. Amplification products were analyzed on 1% agarose gel stained with ethidium bromide.

Flow Cytometry

Nuclear DNA content was determined by a PA flow cytometer (Partec GmbH, Germany) according to Awoleye (Awoleye *et al.* 1994b) with minor modification. Two methods of sample preparation, chopping leaf tissues with razor blade or Geno/grinder (Spex CertiPred Ltd, UK), were used for isolation of nuclei in Otto buffer (Dolezel and Gohde 1995). Nuclear DNA was stained with DAPI (Awoleye *et al.* 1994a).

Results and Discussion

In the first experiment, a cross was made between a castor and a cassava clone (94/009) whereas, in the second experiment, 88 F₁ progenies were produced from a cross between 27 improved and land race cassava clones as a female and 2 castor (large- and small-seeded) as male parents. The pictures of the parents and hybrids are shown in Figure 1. Seeds obtained from the crosses were recovered through embryo rescue techniques. Plantlets obtained from tissue culture were then transplanted into nursery beds and leaves harvested for DNA extraction and subsequently analyzed with RAPD, SSR, and AFLP markers.

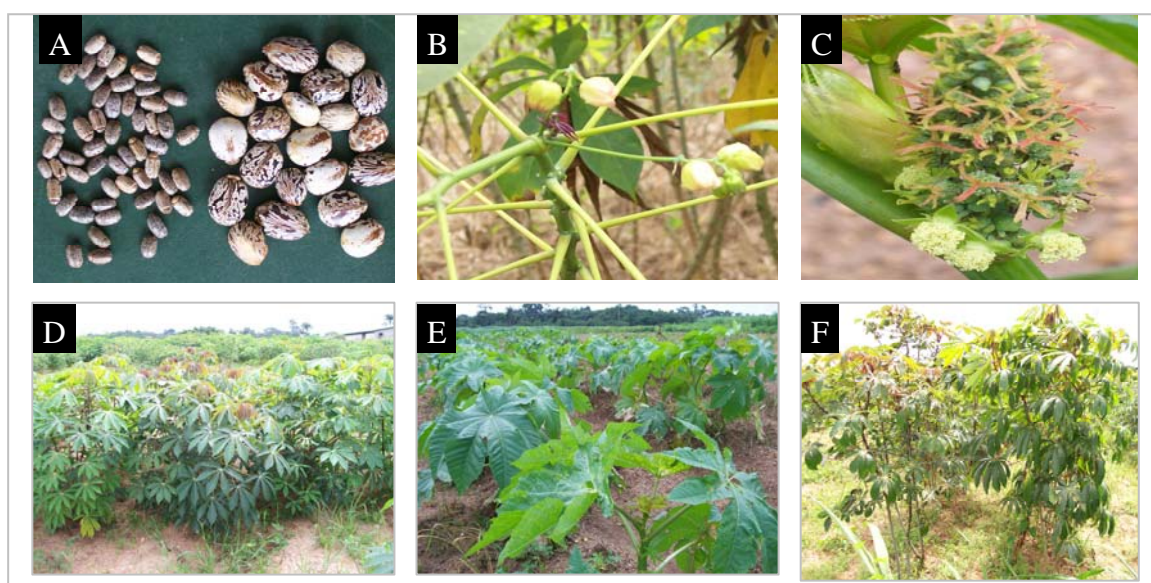


Figure 1. Cassava (left) and castor (right) seeds (A), cassava flower (B), castor bean flower (C), cassava plant (D), castor bean plant (E), hybrid (F).

RAPD analysis

Out of the 40 RAPD primers used to screen the two parents and the hybrid, OPAA1, OPAB5, OPT05, OPAB18, and OPAA10 showed that the hybrid combined alleles from both parents while OPAB5 showed a castor specific band that is absent in both cassava and hybrid (data not shown).

SSR analysis

About 100 polymorphic cassava SSR primers were initially screened on a panel of parental lines and progenies out of which only 28 amplified in castor, suggesting that some of the SSRs are conserved between the

two species. Only six primers (SSRY26, SSRY40, SSRY47, SSRY 61, SSRY52, and SSRY 189) were selected for further analysis of the parents and the progenies in a larger panel. Products were resolved on 1.4% agarose gel or 6% polyacrylamide gel stained with silver nitrate. The six SSR primers used in this study produced amplification products with alleles ranging from 1 to 2 for the parents and the hybrids. Some alleles, e.g. SSRY40- 231bp, identified HY40 and HY58 as likely hybrids but could not detect all hybrids (data not shown). Three bands (A, B, and C) were observed. In this case, band A was common to cassava and some of the hybrids whereas band B was castor-specific and was shared by three hybrids. HY-81 inherited a single band (C) from the cassava parent and none from castor whereas HY-26 inherited a one of the two castor bands but none from cassava. Band (B) was completely absent from the cassava (P1) profile. Lack of reproducibility of this pattern in other hybrids limits the utility of this SSR primer to identify hybrids at the DNA level. Some of the results obtained might be an artifact.

AFLP analysis

A total of 20 AFLP EcoRI-MseI primer combinations produced 353 bands/fragments. Hybrids usually combined the alleles from the two parents and sometimes new ones were observed that were suspected to have resulted from either recombination or mutation. Some castor and cassava specific bands were detected which were also found to be in the hybrid, when using primer EACT/MCTG for selective amplification (data not shown). Primer combination - EACA/MCAT - was used to show the banding pattern in parental lines and progenies (data not shown). From the data analysis, the percentage contribution of alleles/fragments from cassava into the hybrids is higher than that of castor. Similar to RAPD and SSR markers, the AFLP analysis was not robust enough to detect all hybrids or results were not consistent across hybrids. Similar to RAPD and SSR assay, these results were not reproducible in all of the hybrids.

STS marker analysis

In the first phase of the study, screening of large number of RAPD, SSR, and AFLP marker could not identify a robust marker to detect hybrids as the observed result was compounded with lack of reproducibility and consistency across the test panel. To overcome this problem a new approach was devised in which castor ESTs that are available in public database were utilized to design STS primers unique to castor bean. A total of 14 castor-specific primer pairs, derived from two Genbank castor bean sequences, were assayed on several cassava, castor, and potential castor x cassava hybrids. Two primer pairs, which consistently amplified

castor but not cassava, were used to screen the hybrids. Primer pair 2F1-1R1 amplified a product size of 1100 bp in all castor bean genotypes tested while no product was amplified in all cassava lines that were tested. The second potential DNA marker was primer pair 3F5-2R5 which produced a fragment of 1600 bp in most castor bean lines. However, this primer pair amplified smaller product of about 500 bp in some cassava clones and hence excluded. Repeated test of the primer 2F1-1R1 proved reliability of this marker in detecting castor genome. Therefore, all available DNA samples isolated from the putative hybrids were tested. As shown in Figure 2, screening of the hybrids with 2F1-1R1 primer pair could not reveal the presence of castor bean DNA in the putative hybrids. Figure 2A shows the amplification of the 1.1kb fragment in 22 different castor bean lines. Lack of amplification in the cassava sample (Figure 2A, lane 21 (note: the faint band in this lane is due to contamination during loading) and Figure 2B, lanes 1-8) provides a strong evidence of the specificity of the primer. As shown in Figure 2B (lanes 9-16), there was no amplification in the hybrids and the corresponding cassava parental lines (lanes 1-8). A different set of 24 putative hybrids were assayed with the same primer but none of them were positive for castor DNA (Figure 2C, lanes 1-24). On the other hand, amplification of the same 24 hybrids with a cassava SSR primer (NS33), produced the expected product size (Figure 2D), suggesting that the hybrids contain cassava DNA but not castor bean's DNA.

Even though, this result is unambiguous and reproducible, additional castor-specific primers which scan different regions of the castor genome need to be designed and tested to draw conclusion. Tens of thousands of castor as well as cassava EST and genomic DNA sequences are available in public databases. Further bioinformatics analysis is required to identify more castor sequences with no or low similarity to cassava sequences to develop more markers.

Nuclear DNA content

Nuclear DNA contents of the parental lines (cassava and castor bean) as well as the progenies were determined on flow cytometer (PA, Partec). Nuclear DNA extracted from leaf tissues were stained with DAPI (Awoloye *et al.* 1994b). Several samples of the parental cassava genotypes, castor bean, and progenies were assayed. Figure 3 depicts representative histograms of cassava (Figure 3A), cassava and castor (Figure 3B), two hybrids (Figure 3 C, D). The expected nuclear content in the hybrids is somewhere between channel 50 (castor) and channel (100), approximately around channel 75 assuming both species

contributed a haploid set of genome. However, most of the tested hybrids contained the same nuclear DNA content as the cassava parent. This result confirms the PCR experiment in which no castor DNA was detected by the STS marker.

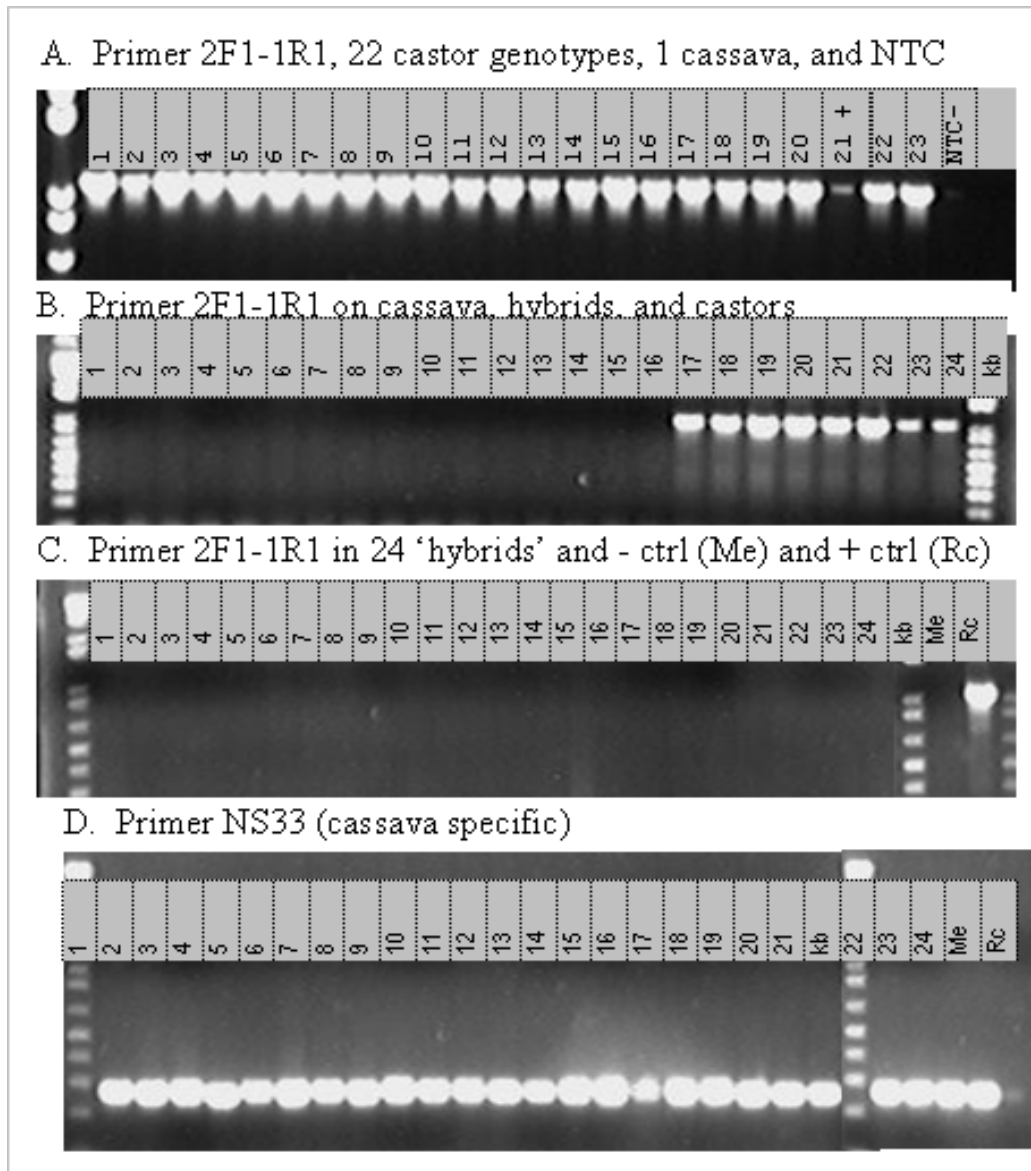


Figure 2. Amplification of different castor, cassava and progenies with castor- and cassava-specific primers. A=22 castor genotypes, 1 cassava (lane 21) and no template control (NTC); B= amplification of a different set of 8 cassava (lanes 1-8), hybrids (9-16) and castor (17-24); C=24 hybrids along with cassava (Me) as negative control and castor (Rc) as positive control; D=amplification of the same 24 hybrids (Figure 4C) with cassava primer, along with cassava (Me) as positive control and castor (Rc) as negative control.

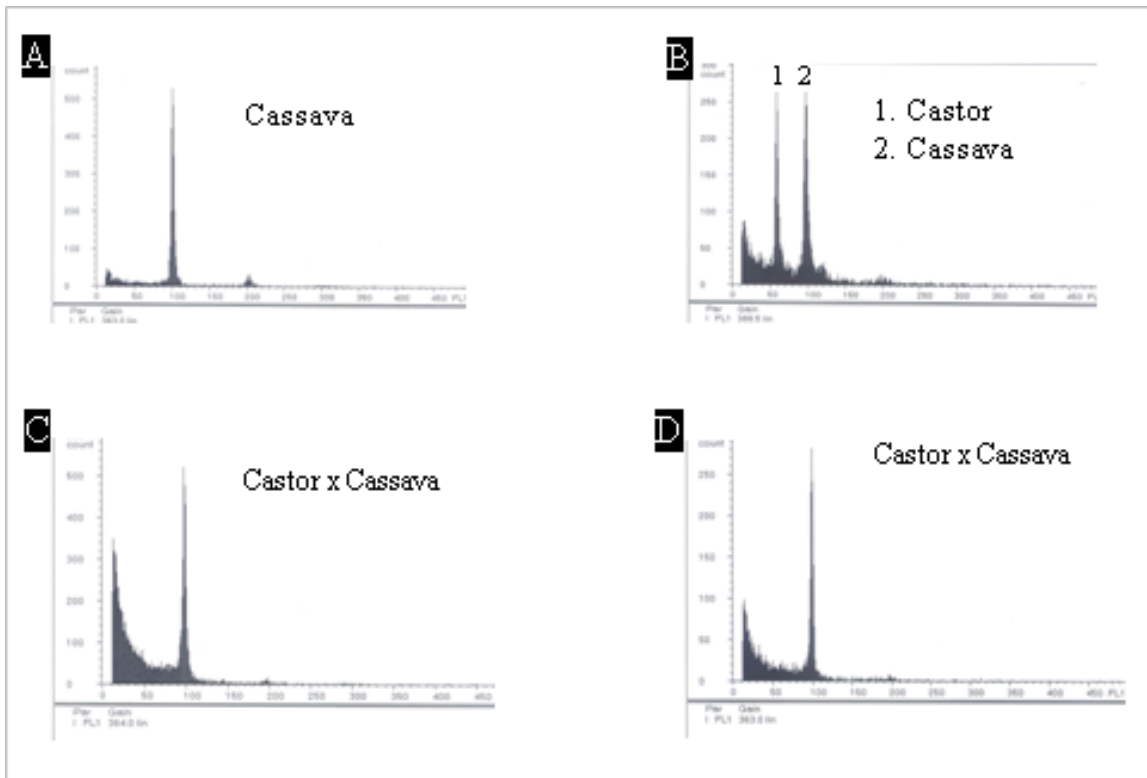


Figure 3. Histogram representing the relative nuclear DNA content of G0/G1 cells isolated from the leaf tissue of cassava (A), castor bean (B), and hybrids (C, D).

Conclusion

From the results of the above experiments, it is difficult to conclude that the two species were hybridized. Since RAPD and AFLP are low resolution markers, these assays could not provide conclusive results without isolating and sequencing the candidate bands. Despite the reliable and reproducible result of the STS assay, the absence of STS detection might have occurred due to the loss of the primer target site as a result of recombination in the progenies. Therefore, more primers, representing different regions of the genome should be developed and assayed.

The finding with the STS primers is in agreement with the flow cytometry result. Ploidy analysis of selected hybrids and corresponding parental lines showed that progenies have similar nuclear DNA content as the cassava parent as the DNA content of castor is distinctly lower than that of cassava (Figure 3). However, all the existing and forthcoming hybrids need to be analyzed with flow cytometer.

Detailed morphological characterization and classical genetic analysis involving segregation ratio in the progenies could be used to support the molecular evidence. Furthermore, since most of the progenies exhibit morphological similarity to cassava, it is essential to determine the ploidy level in the plant, in case chromosome elimination and/or other karyotypic events such as aneuploidy have occurred.

The development of markers to verify such wide crosses, once confirmed by cytological analysis and morphological characterization, opens an opportunity to use DH mapping population thereby accelerating the development of saturated linkage map in cassava, in addition to the introgression of novel traits of commercial value from castor to cassava.

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Influence of culture medium on callus proliferation and morphogenesis in finger millet

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Summary

In cereal crops, somatic embryogenesis is the most common pathway of plant regeneration. We describe a protocol for high frequency regeneration of finger millet (*Eleusine coracana*) via formation of callus produced from scutellar portion of seeds of five genotypes (ML-181, ML-322, MR-1, PR-202 and VR-708). The induction of callus occurred on MS medium supplemented with different auxins (2,4- Dichlorophenoxy acetic acid) and cytokinins [6-bezyl amino purine (BAP)]. The frequency of callus induction was found to be highest in cultivar MR-1 (93%) on medium supplemented with 2,4-D (3 mg/l) plus BAP (0.5 mg/l). The plantlets regenerated on BAP (1 mg/l) were found to be more favorable giving rise to highest mean number of shoots (94.2) for the cultivar MR-1. After four weeks well-developed plants with healthy roots were transferred to field condition, where 100% plantlets acclimatized successfully.

Introduction

Millets rank as the world's sixth most important food crops among cereals and are primarily grown in Asian and African countries (Goldman *et al.* 2003). There are major and minor millets; minor millets are a group of grassy plants with short slender culms and small grains endowed with the rare ability to survive in infertile soils under harsh and severe drought conditions. Among eight minor millets, finger millet (*Eleusine coracana* (L.) Gaertn.), also known as African millet, has outstanding attributes as a subsistence food crop. It is the primary food source for millions of people in tropical dry land regions grown globally in more than 4 million ha. Finger millet (Ragi) contributes about 81 per

cent of the minor millets produced in India. Its seeds can be stored safely for several years without any insect damage, and is a traditional component of farmer's risk avoidance strategies in the drought-prone areas (Latha *et al.* 2005). Finger millet also have nutritional qualities superior than that of rice and is on a par with that of wheat.

The genetic engineering of millets is desirable because in spite of their small size, they are capable of surviving in some of the most inhospitable ecosystems of the world providing food and fodder to the millions where other quality cereals cannot be grown. Hence, with an ultimate aim of supplementing conventional breeding efforts, the genetic transformation protocols for millets need to be developed so that important quality traits may be incorporated across the barriers of incompatibility (Gupta *et al.* 2001). Most of the genetic transformation protocols are dependent on the callus induction and plant regeneration potential of the species. Preliminary investigation on *in vitro* response of a genotype provides the route for recovery of transgenic plants. These protocols are tuned to suit the needs of gene transfer, selection and regeneration (Kishore *et al.* 2006).

A repeating theme in cereal tissue culture is the genotypic variation in culture response (Wernicke and Milkonits 1984). Conditions optimal for plant regeneration in one cultivar fail to produce plants in another cultivar of the same species. This has been a nagging problem in cereal tissue culture, and may have contributed to the abundant literature on cereal tissue culture, as investigators try to optimize conditions for individual cultivars (Bhaskaran and Smith 1990).

Many cereal explants express embryogenic competence on the presence of 2,4-D. A combination of auxin and cytokinin found to be suitable for embryogenic callus initiation in several cultures. Responses to plant growth regulators are similar in other cereals. A combination of auxin and cytokinin was found to be suitable for embryogenic callus initiation in several cultivars of rice (Grimes and Hodges 1990) and Sorghum (Rathus *et al.* 2001).

Mature seeds are the most preferred explants for *in vitro* protocols as they can be stored, available round the year and can easily be handled. Kothari *et al.* 2004 and Wakizuka and Yamaguchi 1987 have reported callus induction and regeneration in finger millet. Development of efficient regeneration system is an essential prerequisite for successful production of transgenic plants. So far, limited attempts have been made

in finger millet to standardize protocols for callus induction and plant regeneration. In the current study we evaluated 5 genotypes of finger millet seeds for various concentrations of plant growth regulators and also to identify ideal genotype for genetic transformation.

Materials and Methods

Mature seeds of five genotypes (PR-202, MR-1, VR-708, ML-181 and ML-322) of *Eleusine coracana* (L.) Gaertn were obtained from All India Coordinated Research Project (AICRP) on Small millets, University of Agricultural Sciences, GKVK, Bangalore. Seeds were dehusked, surface-sterilized with 0.1% (w/v) mercuric chloride solution for 5 minutes and then rinsed thrice for 5 minutes each in sterile distilled water and inoculated aseptically on MS basal medium (Murashige and Skoog 1962) with 3% sucrose supplemented with various growth regulators singly or in combination. The medium was solidified with 0.8% agar (Bacteriological Grade, Qualigens, India), pH adjusted to 5.8 and then autoclaved at 1.2-1.3 kg/cm² pressure and 121⁰C for 15 min. All the growth regulators were added before autoclaving and the cultures were incubated in dark conditions.

Induction and maintenance of embryogenic callus

Callus was induced from the germinating embryos of the cultured seeds (in Petri plate with 25 ml of the medium) 4-5 weeks after incubation. The media used for callus induction were MS basal media, MS medium supplemented with 1,2,3,4, and 5-mg/l 2, 4-D individually or in combination with 0.5 mg/l BA.

An average of 50 seeds were cultured in each Petri plates. After inoculation, the cultures were incubated under photoperiod of 16h light (1400 lux) and 26 ± 1 °C temperature. After 4 weeks of incubation, the compact, green, nodulated embryogenic sectors of callus were separated from non-embryogenic, watery callus and then sub-cultured on MS medium with lower level of 2,4-D (0.2 mg/l). The embryogenic callus was sub-cultured every 3-4 weeks on fresh medium for maintaining the same in embryogenic state. The embryogenic calli developed after 5-6 passages, were used for subsequent plantlet regeneration.

Plantlet Regeneration

The embryogenic callus which gave highest frequency in each genotype was used for plant regeneration on MS basal medium, MS basal medium supplemented with 1.0 and 2.0 mg/l BA or with NAA and incubated

under photoperiod of 16h light (1400 lux) and 26 ± 1 °C temperature. Each treatment was replicated five to six times. After 5-6 weeks of incubation, regenerated plantlets were counted. Plants with well-developed root system were transferred to jiffy cups containing vermiculite for hardening. Finally, the plants were transferred to pots containing soil for establishment and grown to maturity in glass house.

Results

During the present investigation on seed culture, different growth regulators were supplemented to the MS basal medium at varying concentrations. The selection of these concentrations was on the basis of related works carried out in our laboratory and elsewhere, previously.

Induction and maintenance of embryogenic callus

The first response of the seeds to culture media was similar upto 10 days and mostly independent of genotypes and culture media. Although seeds were swollen, no callus proliferation was observed during first few days. During second week of culture, callus formation was observed from the scutellar portion of seeds. Only embryos initiating callus were counted 4 weeks after incubation. Although, callus initiation was recorded from all the genotypes in the 10 culture media tested, its frequency varied among genotypes as well as culture media. The frequency of callus induced among genotypes ranged from 30 to 93%.

At 30 days, distinct calli organized to compact structures appeared in all the genotypes on both auxin alone and in combination with cytokinin media. Medium supplemented with slightly higher concentration of 2,4-D alone (3-4 mg/l) showed good percentage of callus induction. However, higher concentration (5 mg/l) was found to be inhibitory for callus induction. Whereas maximum callus induction was seen when low levels of cytokinin was combined with 2,4-D. Response to callus induction was influenced by medium and genotype independently and thus their interaction (data not shown) had a stronger effect.

Culture medium supplemented with both 2,4-D and BA was superior to 2,4-D alone. Responses to different culture media were significantly different, being utmost on MS medium supplemented with 3 mg/l 2,4-D + 0.5 mg/l BA in MR-1 (93%) genotype followed by 2 mg/l 2, 4-D + 0.5 mg/l BA in PR-202 (80.2%) and VR-708 (68.8%). ML-181 (51.2%) and ML-322 (60%) showed highest percentage of callus induction in 1 mg/l

2,4-D + 0.5 mg/l BA (Table1). However, only germination response was observed on growth regulator free media.

Morphogenic callus formation

After 2-3 weeks of culturing on the same medium, the calli could be distinguished on the basis of their morphogenic features. After 4 weeks of incubation, two types of calli were distinguishable: a compact nodulated embryogenic callus with green and white patches (Figure 1a) and white, friable, watery, apparently non embryogenic callus were formed. On microscopic examination, the compact nodulated callus was found to contain few embryoids whereas the soft watery callus contained no embryoids in it. Non- embryogenic callus (Figure 1b) was not subcultured. This type of callus was predominant in the media containing 2,4-D alone. But the media supplemented with both auxin and cytokinin favoured embryogenic callus. A mixture of greenish and light green calli with dense and glossy texture was taken as morphogenic calli for observation.

Table 1. Percent callus induction for five genotypes of finger millet grown on MS media supplemented with various levels of growth regulators (mean \pm S.D.)

Hormones supplemented to MS media (mg/l)	Genotypes of finger millet				
	PR-202	MR-1	VR-708	ML-181	ML-322
0	NR	NR	NR	NR	NR
2,4-D (1)	45.2 \pm 1.51	50.4 \pm 3.31	30.4 \pm 3.45	30.7 \pm 3.93	45.9 \pm 1.23
2,4-D (2)	50.0 \pm 1.06	60.5 \pm 2.22	30.6 \pm 3.31	35.8 \pm 4.45	45.6 \pm 3.34
2,4-D (3)	51.1 \pm 1.36	63.7 \pm 3.31	55.7 \pm 2.23	45.5 \pm 4.45	50.8 \pm 2.25
2,4-D (4)	61.5 \pm 1.89	75.5 \pm 3.24	48.9 \pm 1.45	42.9 \pm 3.44	35.8 \pm 3.34
2,4-D (5)	54.2 \pm 1.23	55.8 \pm 2.23	40.5 \pm 2.56	39.2 \pm 2.22	30.9 \pm 2.34
2,4-D (1) + BA (0.5)	65.2 \pm 1.03	65.9 \pm 2.21	41.8 \pm 1.98	51.2 \pm 3.34	60.0 \pm 2.90
2,4-D (2) + BA (0.5)	80.2 \pm 1.11	80.2 \pm 2.77	68.8 \pm 1.76	45.5 \pm 4.45	51.1 \pm 3.34
2,4-D (3) + BA (0.5)	70.4 \pm 2.56	93.0 \pm 1.91	66.6 \pm 1.99	41.3 \pm 3.45	52.5 \pm 1.12
2,4-D (4) + BA (0.5)	70.6 \pm 2.67	79.5 \pm 2.37	55.8 \pm 2.00	40.9 \pm 2.23	40.4 \pm 1.45
2,4-D (5) + BA (0.5)	65.7 \pm 1.34	60.6 \pm 2.56	54.9 \pm 1.56	40.2 \pm 4.45	35.8 \pm 2.23

NR = No response

The embryogenic callus was maintained and proliferated on medium with lower level of 2,4-D (0.2 mg/l). The embryogenic sectors of the callus were selectively subcultured after every 3-4 weeks. With each subculture, the number of embryoids increased profusely. Different culture media varied significantly for this. Maximum embryogenic calli formation was recorded on the medium supplemented with 3 mg/l 2, 4-D + 0.5 mg/l BA with genotype MR-1 (81.4%) followed by 2 mg/l 2, 4-D + 0.5 mg/l BA in PR-202 (73.8%) and VR-708 (67.9%). ML-181 and ML-322 showed highest percentage of embryogenic callus 50.5% and 57.1% respectively in 1 mg/l 2,4-D + 0.5 mg/l BA (Table 2).

Plantlet regeneration

Most of the calli gave rise to plantlets after a prolonged culture on the maintenance media. However, transfer of embryogenic calli on to regeneration medium exhibited higher plantlet regeneration and growth (Figure 1c). Along with proliferation of the embryogenic callus differentiation of additional embryoids was also observed. Regeneration took place by germination of embryoids. The plantlets formed were healthy, green and with well developed root-shoot axes. Plantlet regeneration required lower concentration of BA (1 mg/l) and higher concentration of NAA (2 mg/l), suggesting that lower concentration of cytokinin and higher concentration of auxin is essential for efficient plantlet regeneration from seed culture of finger millet.

The percentage of plantlet regeneration frequencies varied among genotypes from 20.8 to 94.2 % (Table 3). Higher percentage of regeneration was observed on medium MS + 1 mg/l BA being maximum from genotype MR-1 (94.2%) and VR-708 (58.9%) followed by MS + 1 mg/l NAA in PR-202 (60.6%). However, plant regeneration frequencies were maximum in growth regulator free media in ML-181 (48.8%) and ML-322 (45.1%). Among the five genotypes studied, the ideal genotype for callus induction is MR-1 (93%), which also showed highest percentage of embryogenic callus (81.4%) irrespective of culture concentrations. The same genotype also gave the best plantlet regeneration followed by genotype PR-202.

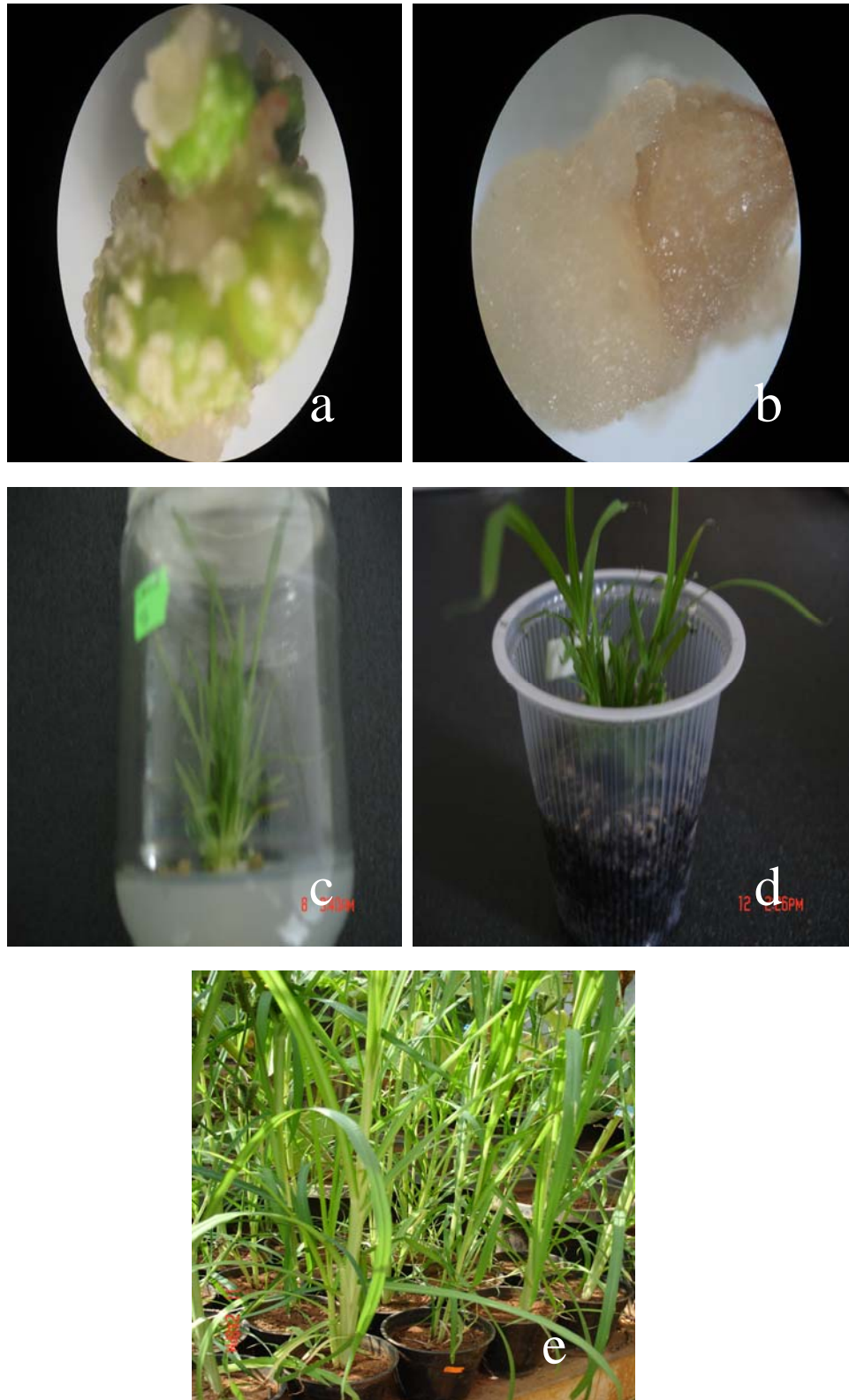


Figure 1. *In vitro* regeneration from Finger millet; a) embryogenic callus; b) non-embryogenic callus; c) Plantlet regeneration; d) Hardening of the regenerated plantlets and e) Plants established in glass house.

Table 2. Percent embryogenic callus induction for 5 finger millet genotypes on MS medium plus growth regulators (mean \pm S.D.)

Hormones supplemented to MS media (mg/l)	Genotypes of finger millet				
	PR-202	MR-1	VR-708	ML-181	ML-322
0	NR	NR	NR	NR	NR
2,4-D (1)	31.2 \pm 2.16	30.5 \pm 1.51	15.6 \pm 2.34	31.9 \pm 2.34	24.6 \pm 1.98
2,4-D (2)	35.5 \pm 1.51	33.6 \pm 1.06	20.6 \pm 1.16	32.5 \pm 3.14	26.8 \pm 2.34
2,4-D (3)	36.8 \pm 3.16	35.3 \pm 1.36	25.6 \pm 1.16	37.6 \pm 1.37	31.4 \pm 3.34
2,4-D (4)	40.5 \pm 2.16	40.2 \pm 0.89	20.4 \pm 1.45	35.9 \pm 1.67	25.6 \pm 4.67
2,4-D (5)	30.6 \pm 3.16	38.8 \pm 2.10	20.2 \pm 1.36	30.4 \pm 2.34	18.8 \pm 3.45
2,4-D (1) + BA (0.5)	50.6 \pm 2.10	57.9 \pm 1.63	40.8 \pm 2.10	50.5 \pm 1.36	57.1 \pm 2.23
2,4-D (2) + BA (0.5)	73.8 \pm 1.16	66.8 \pm 1.16	67.9 \pm 1.34	41.9 \pm 3.39	43.5 \pm 3.32
2,4-D (3) + BA (0.5)	46.9 \pm 1.34	81.4 \pm 1.40	45.9 \pm 1.76	42.7 \pm 4.34	39.9 \pm 1.23
2,4-D (4) + BA (0.5)	40.5 \pm 1.18	46.2 \pm 1.47	35.2 \pm 3.32	35.8 \pm 5.89	25.1 \pm 3.34
2,4-D (5) + BA (0.5)	40.5 \pm 1.36	40.3 \pm 1.34	31.7 \pm 2.22	31.9 \pm 3.47	22.9 \pm 4.34

NR = No response

Table 3. Frequency (%) of plant regeneration in five finger millet genotypes exposed to different media (mean \pm S.D.)

Hormones supplemented to MS media (mg/l)	Genotypes of finger millet				
	PR-202	MR-1	VR-708	ML-181	ML-322
0	35.5 \pm 2.25	70.9 \pm 3.32	20.8 \pm 3.36	45.1 \pm 2.21	48.8 \pm 1.97
NAA (1)	60.6 \pm 1.89	40.4 \pm 2.21	25.6 \pm 1.21	21.9 \pm 3.31	30.2 \pm 1.78
NAA (2)	40.5 \pm 1.03	39.6 \pm 1.03	30.6 \pm 1.10	18.9 \pm 1.23	28.6 \pm 1.23
BA (1)	45.9 \pm 2.56	94.2 \pm 1.45	58.9 \pm 2.33	31.6 \pm 2.24	40.9 \pm 3.31
BA (2)	35.8 \pm 2.67	80.8 \pm 1.23	40.7 \pm 3.34	25.4 \pm 4.40	32.5 \pm 2.34

Complete plantlets regenerated were transferred to jiffy cups containing vermiculite up to one month for hardening (Figure 1d). Finally, the plants were transferred to pots containing soil where 100% plantlets acclimatized successfully and grown to maturity in glass house (Figure 1e)

Discussion

Various components of culture medium exert their influence on development pathways to be taken by a culture. The culture medium has a major function to direct the growth and development pattern of the explants--the hormonal control (Tripathi and Tiwari 2004). The type and relative proportion of growth regulators present in the initial culture medium largely decide the pattern of growth and regeneration potential of a culture system. Hormonal control on growth and development is exerted by the kind of hormones or growth regulators, their concentrations and the sequence in which they are supplied (Eapen and George 1990).

For seed culture, 2,4-D in combination with BA facilitated callus. Higher callus induction on culture medium of 3 mg/l 2,4-D + 0.5 mg/l BA as compared to MS + 2,4-D (4 and 5 mg/l) suggested that neither too high nor low concentration of auxin is required for this purpose. Inferior results with only 2,4-D alone as compared to combinations of 2,4-D and BA revealed that auxin alone was insufficient for high degree of callus induction from scutellar portion of the seed. Morphogenic calli initiation was pre-eminent with combination of 2,4-D and BA. However, plantlet regeneration varied among growth regulators used suggesting that lower concentration of cytokinin and higher concentration of auxin is essential for efficient plantlet regeneration from seed culture of finger millet. Some genotypes showed higher percentage of plantlet regeneration in growth regulator free medium.

Major factors, which produced considerable variation in the pattern of development in culture, were genotypes and media. Genotypic differences may be related to variation in endogenous hormone levels (Norstog *et al.* 1970). Different explants from a single genotype do not respond identically in cultures, most likely due to varying gradients of endogenous hormones (Kumar *et al.* 2001).

As reported earlier, composition of basal medium does not play a major role in deciding the *in vitro* response as the type and concentration of growth hormones (Chawla and Wenzel 1987). Sensitivity to growth hormones is probably determined by the endogenous levels of hormones in the cells (Bhaskaran and Smith 1990). Thus the genetic basis of variability in tissue culture response and morphogenesis is most likely due to differences in hormonal metabolism within the explants that are established by the level of gene expression for individual hormone and genotype (Kishore 2006). Significant differences in genotype by medium interaction observed in the present investigation suggest further enhancement of *in vitro* response by modifying the culture medium.

To conclude, the establishment of finger millet callus cultures, which are competent to express embryogenesis, has become more routine. An explant on a simple medium containing 2,4-D and low levels of cytokinin generally produces the white, nodulated callus. Visual selection and separation of this callus is important for maintenance of embryogenic potential. It could be inferred, that all genotypes are capable of producing embryogenic cultures and initial exposure to the appropriate plant growth regulator are critical (Bhaskaran and Smith 1990). In sorghum, Ma *et al.* (1987) found that the ability to form regenerable callus varied among genotypes, was heritable, and acted as a dominant trait; they suggested at least two gene pairs were involved.

In summary, from the present study we have determined optimum level and type of media required for callus formation and plant regeneration in finger millet. The technique can be used efficiently for raising somaclonal variations and *in vitro* selection. Among five genotypes used for the study, MR-1 is considered as the ideal genotype for gene transfer in addition to the isolation of totipotent protoplasts.

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Nutritional improvement of grass pea and cassava to prevent neurolathyrism and konzo

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Summary

Grass pea (*Lathyrus sativus*) is the most drought tolerant legume and 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 an irreversible crippling disease 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, where it may be the cheapest source of dietary carbohydrates. Over-consumption of cassava roots in a monotonous diet can cause an irreversible crippling disease 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 question arises how such different food intake can give rise to such similar symptoms of toxicity and whether the common deficiency in essential amino acids can be the real cause of these crippling diseases. Nevertheless, the focus of breeding has always been the reduction of the neuro-excitatory amino acid β -ODAP (β -N-oxalyl-L- α,β -diaminopropionic acid) blamed to be the cause of neurolathyrism in grass pea and the reduction of the cyanogenic glucosinolates in cassava. Developing varieties with higher content in methionine and cysteine may be more relevant in improving nutrition without jeopardizing the tolerance for biotic stress of these crops. An animal model for both diseases does not exist but is essential and urgently needed in order to assess the safety of existing and new varieties.

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. 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. It grows on marginal land and is tolerant to biological and environmental stress better than other legumes. It is also the most efficient nitrogen fixer comparing to other food legume crops (Biederbeck *et al.* 1996), and thus improves the fertility of the soil for subsequent crops. 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 %, Campbell 1997), and produces the cheapest protein available for the poor. The seeds of this legume crop are consumed in large quantities especially during periods of drought and famine when other crops fail. 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 the degeneration of upper motor-neurons and the irreversible paralyzing of the legs: neurolathyrism. A non-protein free amino acid β -ODAP (β -N-oxalyl-L- α , β -diaminopropionic acid) with neuro-excitatory properties is blamed as causal agent. This metabolite was identified in 1964 (Rao *et al.* 1964) and during the past 40 years research to improve the dietary value of grass pea was focused on the reduction of β -ODAP. This multifunctional metabolite (Lambein *et al.* 2007) is however highly affected by the environment. Varieties developed in Canada with very low levels of β -ODAP loose this trait when planted in the iron-rich vertisol soil of volcanic origin in the highlands of Ethiopia (Tadesse 1995).

Epidemiological surveys have now indicated risk factors and protective factors for neurolathyrism (Getahun *et al.* 2003, 2005). These should indicate the directions for improvement of the nutritional quality of grass pea. 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 reduces the content of β -ODAP by 90% and improves the balance of essential amino acids that are deficient in

unprocessed grass pea (Kuo *et al.* 2000). 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 (Fikre *et al.* in preparation). Young men are more affected by lathyrism than women, the ratio being about 3/1. A number of risk factors for neurolathyrism such as heavy physical labour and fever seem to also be involved in oxidative stress that is often mentioned in the aetiology of neurodegenerative diseases (Haque *et al.* 1996). Recently identified protective factors are the addition of onion, ginger or garlic to the preparations, or the addition of one third cereals richer in sulphur amino acids than grass pea (Getahun *et al.* 2005). In Bangladesh the people in the coastal areas where fish is available are free of neurolathyrism, although they consume grass pea as much as in the north. In Ethiopia only a minority tribe is consumer of fish and also this tribe is relatively free of neurolathyrism (Fikre *et al.* in preparation).

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 move from rural areas to the city under socio-economic pressure. 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 (Haque 1990). Recently the price of grass pea is higher than the price of rice and no victims of neurolathyrism are 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 and chick pea. The poor who cannot afford the more expensive commodities are the main consumers. 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 funeral offerings (Vaz Patto *et al.* 2006). 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 inmates had about 0.5 to 1 g of β -ODAP 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 (Lambein *et al.* 2001). 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 β -ODAP) per day without any symptoms, there seems to be no correlation between intake of β -ODAP and the incidence of neurolathyrism. This lack of correlation also makes it impossible to determine a threshold toxic level for β -ODAP. Other factors must have sensitised the prisoners to the grass pea toxicity. These aggravating factors might be heavy physical labour, under-nutrition or malnutrition of the prisoners in Vapniarca.

Interestingly, the metabolite β -ODAP in grass pea held responsible for neurolathyrism is also present in the longevity promoting Ginseng root where it is named dencichine in Chinese herbal medicine and considered as a haemostatic agent (Kuo *et al.* 2003, Xie *et al.* 2007). β -ODAP is a multifunctional plant metabolite for which over 30 different physiological or biochemical activities have been described in the plant or in animals (Lambein *et al.* 2007). Remarkable activities are the facilitation of the uptake of zinc ions during zinc deficiency (Lambein *et al.* 1994) and the protection of enzymes of photosynthesis against high light intensity (Zhang *et al.* 2003). Besides the well known excitation of the AMPA-receptors on the neurons it also affects other receptors and transporters in brain cells (Kusama-Eguchi *et al.* 2000).

The prolonged consumption of a diet deficient in methionine can deplete glutathione from the body, one of our main defences against oxidative stress. Any additional stress such as heavy physical labour, micronutrient imbalance (Kuo *et al.* 2007) or the neuro-excitation by β -ODAP can contribute to this oxidative stress and jeopardize the integrity of motor neurons.

Cassava (*Manihot esculenta* Craz, 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 (Diasolua-Ngudi 2002). 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* family is consumed in such quantities as cassava.

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 period, the integrity of the root cells is broken and the enzyme linamarase, stored in the cytoplasm, comes into contact with the cyanogenic glucosinolate linamarine that is stored in the vacuole of intact cells. 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 (Diasolua-Ngudi *et al.* 2002). 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 neuroexcitatory activity that in turn can again contribute to oxidative stress (Diasolua-Ngudi 2005).

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 neurolethyrism. 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 (Diasolua-Ngudi *et al.* 2003). This deficiency of sulphur containing amino acids was suspected to be part of the aetiology of konzo as these amino acids promote cyanide detoxification (Tylleskar *et al.* 1991). 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. Higher incidences occur during the dry season when less other food is available (Tylleskar *et al.* 1991). 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.

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 (Getahun *et al.* 2002, Diasolua-Ngudi 2005). Both diseases occurred in areas where drought, poverty, illiteracy and malnutrition are prevalent and both are under-reported.

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 1), and the physiological effects of a neuro-excitatory amino acid β -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.

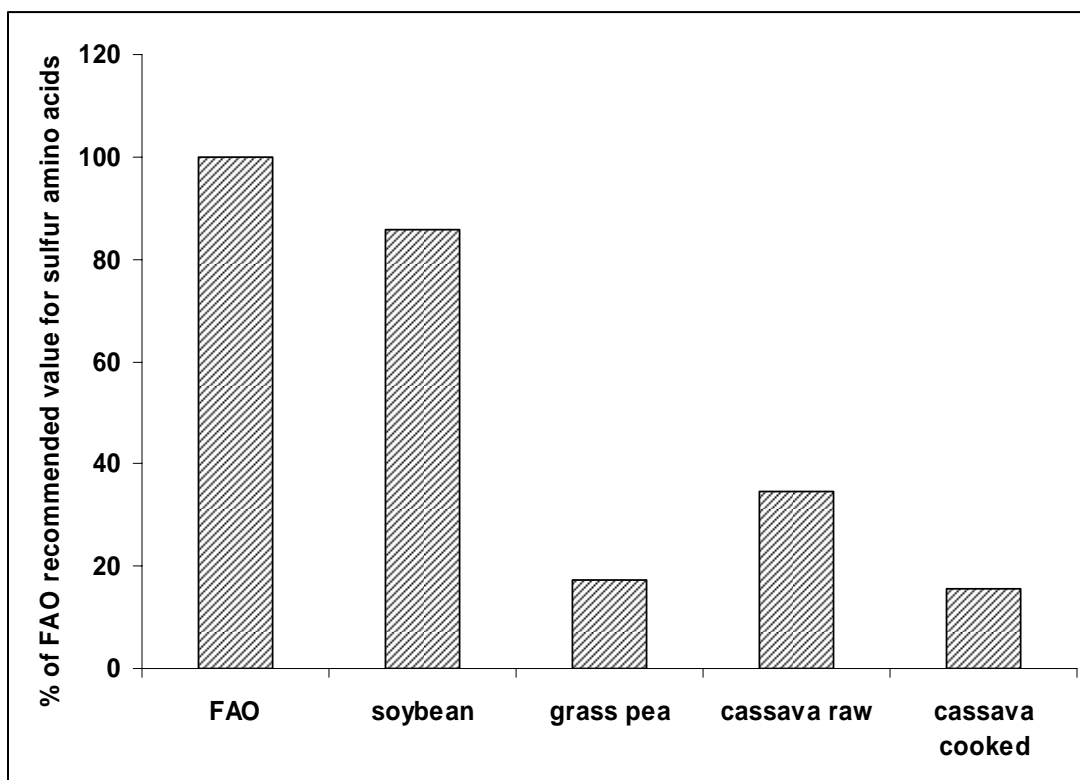


Figure 1: Grass pea seed and cassava leaf preparations (raw and cooked) are both deficient in methonine and cysteine

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 (Defoort 2004). In some cases the physical activity 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. Better collaboration between researchers studying these diseases may help to solve such preventable problem.

As of now, there is no geographical overlap of the production of grass pea and cassava. Considering the present interest in drought tolerant crops, there is a real risk that these crops spread and overlap. This could really cause a socio-medical disaster 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. 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 Congo is not a good idea, because only soybean has an acceptable level of methionine and cysteine albeit not enough to balance the deficiency in cassava.

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 really 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 effect on the consumer. However, while a single meal of unprocessed bitter cassava can be suicidal, great amounts of grass pea are needed to be consumed as a monotonous diet during several months to risk an up to 6% chance to develop 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 for 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 β -ODAP or new varieties of cassava with lower levels of linamarine.

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Genetic engineering of plant disease resistance

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Summary

Genetic modification (GM) can contribute to yield and quality of crop plants and, thus, to food security, and, in addition, can help to minimize ecological side effects of agriculture. Currently, monogenic traits such as herbicide tolerance and insect pest resistance are the main traits of genetically modified crop plants on the market. However, besides of insects and weeds, also microbial pathogens and saprophytes including viruses, fungi and bacteria are major threats to yield and storage of crops. Genetics of resistance against these pathogens is complicated, and thus more difficult to achieve by gene technology. Consequently, no commercial product is available yet. Biotechnology can contribute to overcome these limitations by molecular breeding and by genetic modification. This review provides a brief survey over the promising approaches and the status of their development into discrete GM crops and does not claim completeness.

Gene technology

Traditional breeding has its limitations, among others, due to (i) limited availability of useful resistance genes within the crossable gene pool, (ii) limited genetic loci to combine the alleles in one genome, (iii) undesired side effects of the resistance genes or the accompanying genes on yield or quality, and (iv) genetic diversity of highly heterozygous genomes of certain important crop species, which generates a new variety by each crossing. This group of crop plants is multiplied by vegetative propagation, in order to maintain the genetic stability of the variety. Cassava is an example for these crop plants in developing countries.

GM (or also known as Genetic engineering) has certain advantages over classical breeding, provided it is embedded in a breeding program and

the genetically modified plants are part of a proven agronomic practice. The advantages of genetic engineering can be summarized as follows:

- i) On the level of the genome, genetic engineering
 - a) can transfer the incorporated genes to a genetic background without changing it;
 - b) is not restricted to available genes in the gene pool of the crossable species. Genes from all organisms including bacteria and viruses can be used to increase the genetic diversity of crop plants; and
 - c) can pyramide alleles which could not be combined in one organism, since they share the same genetic locus.
- ii) On the molecular level,
 - a) the transgenes are well characterized, their activity and specificity of the gene product can be modified by targeted mutations in the coding region of the transgene; and
 - b) the expression pattern of a gene can be modified by combining it with a non homologous promoter to create a chimeric operon, an event which is extremely rare in classical breeding by natural mutation or recombination.

From scientific point of view there are hardly any drawbacks of GM. GM plants which have passed the deregulation process are at least as safe - if not safer - as conventionally bred crop plants since they are more rigorously tested than any other crop plant before their release to the market. In this respect there is no difference between transgenes (genes from non crossable organisms) and cisgenes (genes originating from the same species). The risk of GM plants is a merely felt risk with little to no scientific rational. After growing GM crop plants for about 20 years no event became known which would have been or actually represents a danger to health or environment. All the so called scandals have been economic issues due to the fear of the consumers or legal issues due to the high regulation density of GM plants, which is a direct consequence of the public concern.

Particularly the European public is highly concerned about GM plants and even refrains from feeding the livestock. Thus, the European public mood prevents currently the adoption of GM crops in Africa. The governments fear a boycott of European feed importers when GM plants are grown in the donor country since a strict separation technology is not available or established. Insect resistant Bt-cotton impressively demonstrated that the GM technology has advantages particularly for

small-scale farmers in developing countries (Qaim and Zilberman 2003). Farmers adopted the technology more rapidly than any other new agronomic technology before. This is mainly because cotton is not a food crop and consumers in Europe are not worried by the expansion of transgenic cotton in Africa. As a result, African farmers are benefiting a lot from growing transgenic cotton particularly the one with Bt-technology that enables them to drastically reduce the number of pesticide sprays.

Plant diseases

Plants are hosts or victims of a large variety of diseases caused by pathogens from different kingdoms. Members of viruses, bacteria, and fungi are the most important pathogens and consequently the most important targets for disease control. Over the millennia during human breeding of crop plants mostly those varieties have been selected which lost important resistance genes since many of their anti-microbial products are also toxic or at least bitter for human consumption. Moreover, the rich nutritional content of crop plants is an additional attraction for pathogens. Potato might serve as an example: The tubers can only be eaten since they do not contain the highly toxic alkaloids like all the green parts of the plant. Each crossing could reactivate this alkaloid production in the tubers. Fortunately, this breeding risk is not a risk for the agronomic production. All the traditional potato varieties are multiplied by vegetative propagation and thus the varieties are clones. This loss of endogenous resistance has to be replaced by agronomic practice, i.e. mostly crop rotation or chemical treatment, and by breeding. Since about two decades breeding efforts include molecular techniques and genetic engineering.

Resistance against bacterial and fungal pathogens in plants is a complicated process which is accomplished by different approaches and can involve several pathways. For a recent review see e.g. Eichmann and Hüchelhofen (2008). The most important defense pathways are summarized in Figure 1 (A through F). Briefly, pathogen resistance according to Flor's gene-for-gene theory (Flor 1946) is based on elicitors (Figure 1A) and single resistance genes which in many cases are lysine-rich receptor membrane proteins (Figure 1B). Upon specific recognition of an elicitor these resistance gene products trigger expression of pathogen related genes in the nucleus *via* map kinases (Figure 1C). This gene-for-gene resistance usually leads to hypersensitive reaction (HSR) and cell death in the infected cells and their direct neighbors and triggers

signaling for systemic acquired resistance (SAR) towards not yet infected parts of the plant (Figure 1). The advantage of gene-for-gene resistance is in most cases complete resistance, the disadvantage is its often very high specificity against one or few races of the respective pathogen species. In mixed populations naturally occurring in nature, diversity of these resistances in different individuals provides resistance in the population. However, in monoculture agriculture this type of resistance is overcome sooner or later by selection against specific pathogen strain.

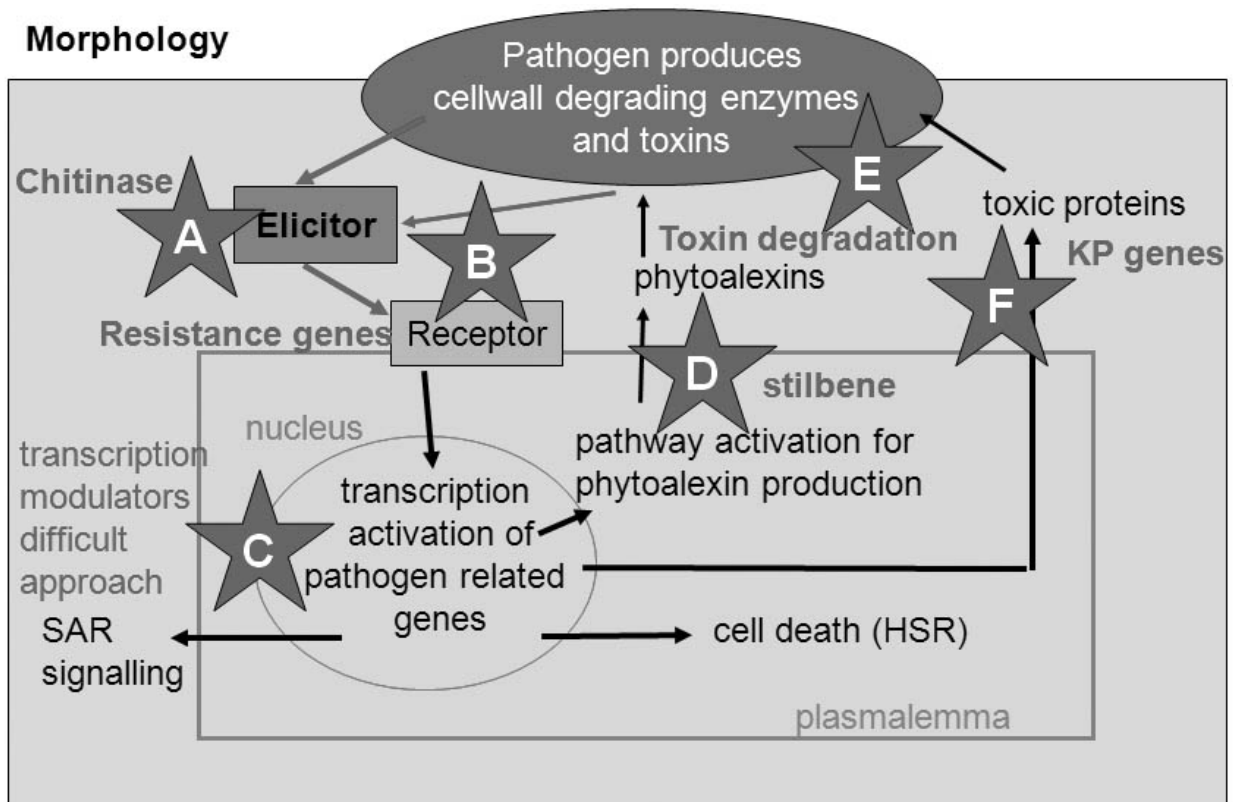


Figure 1. Important defense pathways in brief.

The alternative approach of plants to defeat pathogens is broad spectrum, quantitative resistance. This usually involves a number of genes which are known by breeders as quantitative trait loci (QTL). Often such genes encode for enzymes producing elicitors (Figure 1A), enzymes which are involved in phytoalexin metabolism (Figure 1D) or direct inhibitors of toxins produced by the attacking pathogen (Figure 1E) or toxins which impair growth of the pathogen (Figure 1F). Advantageously, such resistance has a broad spectrum of efficacy against all races in a number of related pathogen species. On the other hand, this resistance is usually not complete leaving a certain level of

symptoms in the population. This might be an advantage since it reduces the selection pressure and, thus reduces also the chance for overcoming the resistance by the pathogen.

In general, this very complicated defense strategy of plant hosts makes it much more difficult to support defense system of plants. For genetic engineering, in order to defeat bacteria and fungi it needs probably several transgenes or an impact with complete signal transduction pathways as compared to e.g. insect resistance or herbicide tolerance where usually single, dominant genes provide significant improvement (Roh *et al.* 2007, Tan *et al.* 2006).

Viruses

The difficult strategies are true for bacteria and fungi, but due to the different life style of viruses single gene approaches have been successful. Viruses differ from bacteria and fungi in their way to attack the host, since viruses depend on the host transcription and translation machinery. This makes it conceivable to interfere with these processes. In the past most successful was the ectopic expression of the virus coat protein in the host plant prior to infection. Once the virus particle is delivered to a cell by its vector, and the virus removes its coat in order to replicate its genome, the cell contains already enough coat protein, such that the virus genome is immediately coated again. This approach was first very successful for papaya in Hawaii (Gonsalves 2007). Papaya ring-spot virus (PRSV) was threatening papaya production in the island before genetically engineered papaya plants producing the coat protein were developed. These completely resistant plants are grown in Hawaii for the past 10 years with great benefit to the farmers. Nevertheless, it is apparently difficult to apply this successful technology in Thailand due to more complicated market and a GM-reluctant public discussion (Davidson 2008).

Other approaches to defeat virus infections in plants include direct interference with the (i) transcription, e.g. by expression of defective viral transcriptases which compete with the virus-own polymerases, or (ii) with the movement of the virus from cell to cell to prevent systemic spread of the virus in the host. All these approaches do not prevent infection, but inhibit multiplication and systemic movement of the pathogen.

Bacteria

The possibilities are very similar to support the plant in its defense efforts by genetic engineering against bacterial and fungal pathogens. Elicitors, e.g., which trigger pathogen response are often flagellin in case of bacteria (Boller 2005), whereas in fungal infections the elicitors are often cell wall fragments from the pathogen or specific cell wall fragments from the host cell, that are produced by pathogen enzymes to degrade the host cell wall. In this review, we focus on the fungal pathogens. Similar approaches could be applied to bacteria.

Fungi

In order to establish defense mechanism against fungal diseases a variety of genetic engineering approaches have been tested. The first approach was using chitinase and glucanase encoding transgenes (Figure 1A). In this case the chitinases and glucanases are cloned into plants so that they specifically can be deposited in the cell wall where they can attack and degrade the cell wall of the pathogen. The major effects of chitinases and glucanases on the pathogen are, (i) to weaken the cell wall of the pathogen which often needs turgor to penetrate into the host cell, and (ii) to create elicitors from the cell wall of the aggressor, specific for the attacking pathogen. These approaches provided an increase in quantitative resistance in a number of plant species and pathogen combinations e.g. in silver birch (Jongedijk *et al.* 1995, Parsonen *et al.* 2004). In other cases promising greenhouse results could not be confirmed in field tests (Anand *et al.* 2003). Apparently, the success of this defense mechanism depends on the type and developmental stage of the host plant, the type of the pathogen, and suitability of environmental factors for infection.

Genes encoding for lysine-rich receptors are also promising for establishing pathogen resistance through genetic engineering (Figure 1B). Different alleles, originally described by classical breeding, have been isolated and transferred to susceptible varieties (Gessler and Patocchi 2007, Feuillet *et al.* 2003). The positive effect on pathogen resistance is impressive, provided the correct pathogen strain is used (Wicker *et al.* 2007). In the particular case reported by Wicker and colleagues, the approach still has to be proven in field tests which are under way in Switzerland, however these field tests have been destroyed by anti-gene technology activists in 2008. The disadvantage of these resistance genes for agronomy, i.e. their race specificity can be overcome by pyramiding a number of these alleles in a single genotype. Conventional breeding, however, could not provide this possibility.

Additional advantage related to this approach is that it uses cisgenesis instead of transgenesis. Further information on cisgenesis is available in another chapter. The public accepts cisgenes more readily than transgenes although there is currently no scientific reason known for this assumption in risk assessment.

Trials to engineer the expression of pathogen related genes directly by the transfer of transcription modulators (Figure 1C) are very difficult unless specific pathogen related promoters become available. Currently available promoters are either not completely repressed without a pathogen attack or activated not only by pathogen attack but as well by other stresses. This could lead to cell death similar to HSR as a consequence of abiotic stresses such as heat and wind. Thus, the plants do not have desirable agronomic trait. No report is yet available in which the resistant plants are not affected by stresses apart from pathogen.

Easier to handle are approaches using transgenes encoding for an enzyme which is active in a single metabolic pathway (Figure 1D). This arrangement keeps the chance of pleiotropic effects low. For example, resveratrol is a phytoalexin and occurs naturally in a wide variety of plants including grape wine, raspberries and peanuts. Ectopic expression of stilbene synthase increases the amount of resveratrol in the plant and, thus, resistance against certain pathogens (Oldach *et al.* 2001).

Many pathogenic organisms produce toxins which weaken the host and, thus, its defense response. Inactivation of such toxins is often part of natural defense mechanisms (Figure 1E). Fusarioses, e.g., in feed and food are particularly unhealthy for the consumer, since they produce mycotoxins, known as aflatoxins which are not only toxic to the host plant but as well to vertebrates including human. One approach to defeat Fusarioses uses antibodies (Peschen *et al.* 2004). Alternatively, enzymes which degrade the aflatoxin dioxynivalenol have been used to genetically engineer wheat (Okubara *et al.* 2002). At least in greenhouse tests the result was promising. Field tests of Fusarium resistant GM wheat have been destroyed in Europe by anti-gene technology activists. It is not known whether the project is still pursued.

The same fate might wait for a project which uses a toxic protein from a virus to defeat fungal diseases including smut and bunt (Figure 1F). The so called killer proteins (KP) from certain viruses contain strains of corn smut (*Ustilago maydis*) that inhibit specifically growth of other smuts and bunts. The virus gene is transcribed and translated in the fungus and

secreted into the surrounding tissue of the host plant. There it inhibits growth of other non virus-infected strains of corn smut. Thus, the virus protects its host, the fungus, from competition in a limited substrate. This system offers several non-homologous antifungal virus genes and it is highly specific for species from the order *Ustilaginales*, which contains exclusively pathogens. Transferred into the genome of genetically engineered wheat, KP4 reduced the number plants with symptoms of common (or stinking) smut (*Tilletia caries*) by 30% in the greenhouse (Clausen *et al.* 2000) and by 10% in the field (Schlaich *et al.* 2006). Even though this result might be improvable, already a 10% increase in yield would make such a gene attractive for breeders, provided it would not be a transgene. The negative perception of consumers makes the development of this useful agronomic line fruitless, despite the fact that there is no indication for any toxicological or allergenic risk towards human or environment. All organisms tested so far are not susceptible, including many bacterial species, fungi, plants, and mammalian cell cultures (Schlaich *et al.* 2007). The KP4 protein just inhibits cell division and longitudinal growth of hyphae but it does not even kill the pathogen. The KP4 treated fungal cells can be rescued by addition of external calcium.

Unpublished greenhouse tests indicate that - expectedly - loose smut is also defeated by KP4 and even with a much higher efficacy. Loose smut is a seed transmitted disease and, thus, particularly a problem for small-scale subsistence farmers in developing countries. These farmers have to use part of their harvest for sowing, an agronomic practice which accumulates seed transmitted diseases within few vegetation periods. The burden of an unpleasant though not justified name of the transgene and the fact that we work with wheat makes it very difficult to acquire funding and field test permissions for this system in the well-fed Europe. This is a situation ethically close to a scandal, although not recognized and communicated as such. Sadly, without recognizing how things are connected, many colleagues in research support this kind of "genetic imperialism", since it is politically more convenient, thus supporting skeptic NGOs with a scientific touch.

Conclusion

Many different approaches to defeat pathogenic organisms in support of plant pathogen defense have been explored. Often the results of various approaches depend on the host plant species, the pathogen species, the environmental conditions or the developmental stage of the plant.

Apparently, a magic bullet does not exist in which a single transgene completely controls a wide variety of pathogens and at the same time without any adverse effect to non-target organisms, human and the environment. In order to solve these problems and obtain a plant with all desirable traits, financial support and conducive working environment should be given for researchers involved in such type of investigation.

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Cisgenesis for crop improvement

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Summary

One of the main activities in plant breeding is introgression of desired traits from wild germplasm into high quality varieties. However, introgression is seriously hampered by simultaneous inheritance of unwanted traits from the wild germplasm. This requires several generations of breeding, leading to removal of the unwanted traits, yet keeping the desired gene(s) of interest. This process can be very time consuming, especially if genes underlying unwanted traits are genetically tightly linked to the genes of interest. This problem is even more serious for crops having a long generation period such as trees and bananas, or in case several genes for different wild sources have to be accumulated, e.g. for durable resistance. These problems would be prevented if only the gene(s) of interest would be added, leaving the undesired genes in the wild germplasm behind. This is feasible by means of 'cisgenesis'. Cisgenesis is meant to fasten introgression of alleles from wild germplasm used in classical breeding. Here the concept of cisgenesis is explained, and apparent objections are discussed.

Definition

A cisgenic plant is a crop plant that has been genetically modified with one or more genes isolated from a crossable donor plant. A cisgene contains its native introns and is flanked by its native promoter and terminator in sense orientation. In contrast, a transgenic plant contains genes from non-crossable organisms such as bacteria, or contains synthetic genes, or artificial combinations of a coding gene with a regulatory sequence such as a promoter from another gene. Cisgenic plants contain solely genes that have been present in the conventional breeder's germplasm (Schouten *et al.* 2006a, Schouten *et al.* 2006b). Cisgenic plants can in principle also be obtained by means of classical

breeding, as far as the phenotype is concerned. This indicates clearly its limits regarding breeding possibilities, but also its limits regarding possible biosafety risks.

Improvement of existing cultivars

An extra advantage of cisgenesis in comparison with cross breeding is that cultivars can be used that already have a proven high quality. This is especially interesting for self-incompatible crops. Crossing scrambles the genetic composition of good cultivars, and restoring such a cultivar through crossings is virtually impossible in case of self-incompatibility. However, cisgenesis preserves the genetic assembly of the high quality cultivar, and adds one or several well-defined alleles.

Cisgenesis can be applied to all kinds of crops. It is particularly attractive to crops that are cross-fertilized, and are propagated vegetatively, such as banana, grapes, strawberry and potato.

The treasure chest of isolated genes

A prerequisite of cisgenesis is the availability of isolated functional genes. Currently, the amount of DNA sequence information is increasing exponentially. Large EST-databases are available to everybody. After the whole genome sequencing of *Arabidopsis thaliana* and rice, many other crops are following. This increasing knowledge of functions and DNA sequences of plant genes of model plants can be used for identification of similar genes in orphan crops. In the coming ten years, a vast number of major genes and their alleles for desired traits will be isolated in many crops and their wild relatives. This will hold also for orphan crops. So, the treasure chest of isolated genes is being filled at an increasing rate. These alleles can be used directly for cisgenesis. This treasure of knowledge should be used also for breeding of orphan crops, to the benefit of Africa. Cisgenesis is a valuable approach for this.

Apparent objections

We presented advantages of cisgenesis. However, objections can be raised too, but these can be rebutted quite easily.

Objection 1: Unpredictable insertion site and mutations

Cisgenes can be inserted by means of *Agrobacterium tumefaciens*. At the moment we are not able yet to direct the cisgene to a specific location in the plant genome. Therefore, the cisgene is inserted at a site that is not known in advance. The position may affect the expression of the cisgene. For that reason, several transformation events have to be carried out, leading to separate cisgenic individuals. Plants that show a good expression of the cisgene have to be selected, and the others discarded. At the site of insertion, a mutation will occur, possibly disturbing a functional gene or regulatory sequence. This may affect the phenotype. For that reason, the plants that show a good expression level of the inserted cisgene, have to be phenotyped thoroughly for unwanted side effects.

Actually, phenotypic selection is very normal in plant breeding, as well as in mutation breeding in which many mutations are induced at unknown sites in the genome. Phenotypic selection of varieties from induced mutation breeding has led, in the past 70 years, to more than 2500 cultivars (Maluszynski *et al.* 2000, <http://www.infocris.iaea.org/MVD/default.htm>). Many of these cultivars are grown, eaten and used world wide (Ahloowalia *et al.* 2004), without reports on biosafety problems due to these mutations (Schouten and Jacobsen 2007).

Another example is translocation breeding. In wheat, introgression breeding with diploid wild species is complicated because of the lack of meiotic crossing-over between homeologous chromosomes (Chen *et al.* 1994). Introgression breeding with the wild species led, in these cases, to addition or substitution lines, with the complete donor chromosome containing the gene of interest. Gamma-radiation of the seeds of addition or substitution lines, led to chromosome breakages and random insertion of a piece of donor chromosome with the desired gene in one of the receptor chromosomes (Friebe *et al.* 1996). This translocation was accompanied by linkage drag. Similar translocation breeding approaches have also been applied in tobacco, sugar beet, oat and radish (Aung and Thomas 1976, Savitsky 1975, Caplin and Mann 1978, Kaneko 1992). All these plants are treated like conventionally bred plants. No biosafety problems have been reported to be caused by these induced translocations to unknown positions in the genome. The thorough phenotypic selection, which is normal in classical breeding, apparently suffices in preventing such problems (Jacobsen and Schouten 2007).

Spontaneous mutations at unknown positions in the genome are very common in crops, without any recorded biosafety problems. Many widely grown cultivars have been replaced to a significant extent by their mutants. For the apple cultivar Gala, several registered mutants are present, such as Royal Gala, Imperial Gala, Mitchgla, Regal Prince, Tenroy, and Galaxy. Similarly, for the apple cultivars Elstar, Golden Delicious, and Jonagold many mutants are registered. Apparently, mutations are not regarded as dangerous in view of biosafety in apple breeding. It would be unscientific and unbalanced to regard mutations caused by insertion of a cisgene as dangerous, but accepting induced mutations in mutation breeding or translocation breeding or spontaneous mutations, such as in apple, without raising the biosafety issue.

Objection 2: Safety of the inserted gene

Transgenic plants contain genes from non-crossable organisms (e.g., a selection marker gene originating from a micro-organism), synthetic genes or artificial combinations of a coding gene with regulatory sequences from another gene. A transgenic plant may have a phenotypic trait that did not occur before in that species or its crossable relatives. Such a novel trait could in theory affect fitness in ways new to the species. Gene flow to wild relatives could potentially spread this fitness effect to feral populations. Further, transgenes, again theoretically, might cause toxic effects or allergic reactions that are new to the host species. For these reasons, regulations have been put into place to control these risks (European Parliament 2001, Cartagena Protocol 2000). Much attention has been paid to controlling these possible risks, sometimes to exaggerated levels.

However, in case of cisgenic plants, no novel genes are introduced and, therefore, no novel traits are added compared to conventional breeding. For cisgenesis, the introduced allele with its native promoter has already been present in the species or in crossable relatives for centuries. A cisgene only invokes fitness changes that could occur also through traditional breeding or in nature. The same holds true for other environmental risks, such as effects on non-target organisms and soil ecosystems, and risks for food or feed. As a result, deliberate release of cisgenic plants into the environment is as safe as the deliberate release of traditionally bred plants.

Gene flow between a cisgenic crop and wild relatives may occur. This does not invoke a risk to the wild relatives, as the cisgene was already present in the wild relatives, as it has been isolated from there, and it may have been used already in classical breeding. Cisgenesis does not add extra genes to the gene flow and, therefore, does not induce an extra risk to wild relatives and feral vegetations.

An important advantage of cisgenesis is food safety. The use of wild sources, e.g. *Solanum* species in potato breeding as source of genetic variation is bringing back different kinds of glycoalkaloids that have been removed during the breeding process in the past (van Gelder 1989). The use of wild species as source of genetic variation is accompanied by the re-introduction of these compounds. These have to be removed again by means of back-crosses. Before release, new potato varieties have to be tested for the amount of glycoalkaloids in the potato tuber in view of food safety. Existing varieties with a long history of safe use are the best source for the application of cisgenesis. It is expected that the cisgenic approach for insertion of e.g. resistance genes to the devastating pathogen *Phytophthora infestans* (Haverkort *et al.* 2008) will not increase the existing glycoalkaloid content and that no new types of glycoalkaloids will appear. These expectations are important and can easily be tested in tubers of cisgenic potato plants.

Therefore, it makes sense to state that cisgenic plants are as safe as or safer than classically bred plants or plants from mutation breeding (Schouten *et al.* 2006a, 2006b, 2006c, Jacobsen and Schouten 2007, 2008).

Objection 3: GMO regulation

The regulations for GMOs (genetically modified organisms) have been put into place to control possible risks of introduction of foreign genes in new genetic backgrounds. The regulations do not distinguish between transgenesis where foreign genes are introduced, and cisgenesis where only genes are introduced that can be introgressed by means of classical breeding. As a result, cisgenic plants still have to be regulated like transgenic plants. In the European Directive 2001/18/EC for Deliberate Release of GMOs into the environment a GMO is defined as an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination (European Parliament 2001). In this directive mutagenesis is regarded as a method of genetic modification, however, this technique is exempted from the

regulation. Therefore, plants from induced mutation breeding are treated like classically bred plants. The same holds for plants that are derived from fusion of protoplasts from plants that can exchange genetic material in classical breeding. Plants from such a protoplast fusion are formally regarded as GMOs, but they are exempted from the GMO regulation, and treated like conventionally bred plants. Cisgenic plants fit perfectly in this list of exempted breeding techniques and, therefore, we propose adding cisgenesis of plants to this list of exempted GM technologies (Schouten *et al.* 2006a, 2006b).

Objection 4: The unnaturalness of the process

The product from cisgenesis, i.e. the cisgenic plant, is similar to the product of conventional breeding. However, the process of cisgenesis differs significantly from the process of conventional breeding. In case of a cisgenic plant, an allele is amplified by means of PCR and brought into *A. tumefaciens* (or through particle bombardment), and subsequently inserted into a cultivar without linkage drag. This is a highly technological process, which differs from meiosis and fertilization. Some citizen groups have objections against this technical process. For that reason, cisgenesis may be abandoned in organic farming (Lammerts van Bueren *et al.* 2007), like the use of synthetic pesticides or artificial fertilizers have been abandoned, due to their "unnaturalness".

However, also in organic farming, fungicides are applied frequently against fungal diseases. Instead of synthetic fungicides, sulphur and copper may be used, that have a negative impact on the environment too (Anonymous 2005). Resistance to pests and pathogens may lower the environmental impact considerably, even more than the shift from synthetic fungicides to organic compounds, sulphur or copper (Sukkel *et al.* 2007). Both organic farming and cisgenesis deserve respect as means to allow durable cultivation crops, for the sake of ecologically sound agriculture.

Objection 5: Acceptance by consumers

Inquiries among consumers in the USA indicated that between 15 and 20 % of the consumers have been willing to eat a vegetable that is genetically modified with a gene from a virus, bacterium or fungus (Lusk and Sullivan 2002). The acceptance rose to approximately 50 % if the vegetable was modified with a gene from another non-crossable vegetable. However, 80 % of the consumers were willing to eat the

vegetable if it was genetically modified with a gene from that vegetable. So, the acceptance increased from less than 20 % in case of transgenesis using a gene from a micro-organism, to 80 % in case of cisgenesis (Lusk and Sullivan 2002).

Conclusions

The time is ripe now for applying cisgenesis for speeding up the classical breeding considerably. The number of isolated genes and alleles from crops and their wild relatives is increasing rapidly, and methods are available for inserting these isolated alleles into the crops. Cisgenesis is as safe as classical breeding or mutation breeding. Consumers generally prefer cisgenic plants compared to transgenic plants. Cisgenesis can be applied to all kinds of crops. It is particularly attractive to crops that are cross fertilizers, and are propagated vegetatively, such as banana, grapes, apple, strawberry and potato. Cisgenesis should not be hampered by GMO regulation that is designed for transgenic crops, not for cisgenic crops. In case cisgenic crops for Africa remain to be approved under the current GMO regulations, it is likely that these approvals can be afforded by non-African large multinationals only.

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Clean Gene Technology and its application to crops

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Summary

The World's population is increasing daily and this situation has created two major problems. First, there are many more mouths to feed and secondly, there is less arable land to farm to produce more food needed by the rapidly swelling population. While conventional breeding has performed wonderfully well in meeting this challenge, a lot more is still necessary to meet the hunger threat. In this respect, transformation biotechnology can help especially where conventional breeding lacks solution. Plant transformation technologies require selectable marker genes to produce transgenic plants. However, after selection events, selection marker genes are of no value thereafter except when used as gene of interest (e.g. selectable markers that provide herbicide tolerance). In fact, selection marker genes in transgenic plants are perceived to pose potential bio-safety problems. In all genetic transformation technologies based on direct gene transfer (electroporation of protoplasts, particle bombardment, etc) the selectable marker genes generally co-integrate with the gene of interest(s) in one Mendelian locus in the plant genome; hence, their removal is highly desirable. This may also help in the acceptability of transgenic plants by society. Transgenic plants that contain the desired gene of interest but lack the selection marker gene used in its production are termed "clean" and the methods utilized in their production are referred to as Clean Gene Technology. There are several proved methods of eliminating selectable marker genes: (i) marker gene excision consisting of, (a) intra-genomic relocation of transgenes via transposable elements, and (b) site-specific recombination systems; (ii) intra-chromosomal recombination; (iii) Gene replacement or Targeted gene replacement; and (iv) transformation with multiple T-DNAs, which could result in linked and/or unlinked co-integration of transgenes. Unlinked transgenes are then segregated out during meiosis.

Introduction

Conventional breeding has been of extraordinary help in meeting the challenges of food production to feed the ever-increasing World's population. Examples include increase in cowpea production in Nigeria - from an average of 300kg/ha to 1,200kg/ha (IITA, 1999), doubling of rice production world-wide between the mid sixties and the 1990's and the development of inter-specific hybrids "NERICA" by WARDA: new rice plant types combining advantages of both the African and Asian rice types (Jones *et al.* 1997, WARDA 1999, 2000). However, a lot more is still needed if the growing world population must adequately be fed. In this respect, transformation biotechnology can be of significant help especially where conventional breeding lacks solution. Such areas include limited availability of stable and durable genetic source of resistance, enhancing existing traits or the introducing new ones (e.g. disease/nematode resistance, addition of nutritional value to food), where and when chemical treatment not economically feasible and/or cause environmental damage.

All plant transformation protocols routinely use various antibiotic regimens and herbicide as selection agents to identify transformation events in a wide range of crop species. With the exception of when selectable marker gene is used as a gene of interest e.g. herbicide tolerance (HT), the marker genes generally have little agronomic value after selection events. Moreover, once the HT trait has been introduced into germplasm of a plant species, retention of the HT gene in the genome becomes problematic and an alternative marker system must be used to incorporate subsequent transgenes.

Additionally, consumer and environmental groups have expressed concern on the use of antibiotic- and herbicide-resistance genes from an ecological and food safety perspective. Some went to the extent of "labeling" genetically modified crops as "Frankenstein Food". Some of these public concerns include the potential danger of selectable marker genes in transgenic plants on health and safety grounds. One of the major apprehensions with the commercialization of transgene products has been the concern that selectable marker genes or their products may be toxic or allergenic when consumed. When antibiotic selection markers having wide clinical and veterinary application are used, the marker gene could be transferred into microorganisms in the human and

animal gut, which could, render such antibiotics functionally useless for both the clinical and veterinary application due to bacterial resistance to such antibiotics.

Other perceived legitimate concerns include the possibility of (a) a marker encoding herbicide resistance changing the transgenic plant into a weedy pest, (b) the spread of the selectable marker to other organisms which may upset the balance of the ecosystem, and (c) the horizontal transmission of the marker into wild relatives may transform them into weedy pest. The spread of the antibiotic selection marker to other organisms may upset the balance of the ecosystem (Gressel 1992, Dale 1992, Nap *et al.* 1992).

While no scientific basis has been determined for these concerns (FDA 1994, Flavell *et al.* 1992, Fuchs *et al.* 1993, Nap *et al.* 1992, Redenbaugh *et al.* 1993, 1995), it is apparent that there is a need for the removal of selection marker gene in transgenic plants. Perhaps, the production of marker-free transgenic plants, may certainly contribute to alleviating these concerns and lead to the public acceptance of transgenic crops. Alternative selection marker genes not based on antibiotic or herbicide resistance [e.g. phosphomannose isomerase (PMI), aspartate kinase (AK), and dihydrodipicolinate synthase (DHPS)] genes for lysine inhibition will not be discussed here because they are alternative markers and not selection marker removal techniques.

Proven strategies for eliminating selection maker gene

There are a few proved techniques for removing or eliminating selection marker genes in transgenic plants (Hare and Chua 2002, Jaiwal *et al.* 2002, Ebinuma *et al.* 2001, Puchta 2000, Yoder and Goldsbrough 1994). Such methods include:

i) Excision of selectable marker gene

a. Intra-genomic relocation of transgenes via transposable elements

In this instance, the selection marker gene is inserted between the two Ds elements of the maize Autonomous *Ac* element with the *Ac* a transposase coding gene. Sequences cloned between the inverted repeats of Ds elements can then be mobilized to new genomic location in the presence of an *Ac* gene. The selection marker gene is then lost (Figure 1). This technique is useful for marker removal from vegetatively

propagated crops such as grapes, chrysanthemums, potatoes, strawberries, cassava, etc. Marker-free transgenic rice containing insect resistant *Cry1b* gene, between the Ds elements has been produced (Cotsaftis *et al.* 2002). Transposons can also be combined with positive selection marker like the isopentenyl transferase (*ipt*), cytokinin type from *Agrobacterium tumefaciens* P022) and the rol-proliferation of "hairy" roots (auxin type from *A. rhizogenes* NIAES1724) whereby enhancement of "ipt-shooty or" "hairy roots" of regenerates' are obtained (Ebinuma *et al.* 1997).

Disadvantages of transposon-based systems include low efficiency partly due to the tendency of transposable elements to reinsert elsewhere in the genome. The excision is frequently imprecise and repeated cycles of insertion and excision may lead to the generation of mutations at numerous unknown loci. Finally, the continuous presence of heterologous transposons may also lead to genomic instability in transgenic plants.

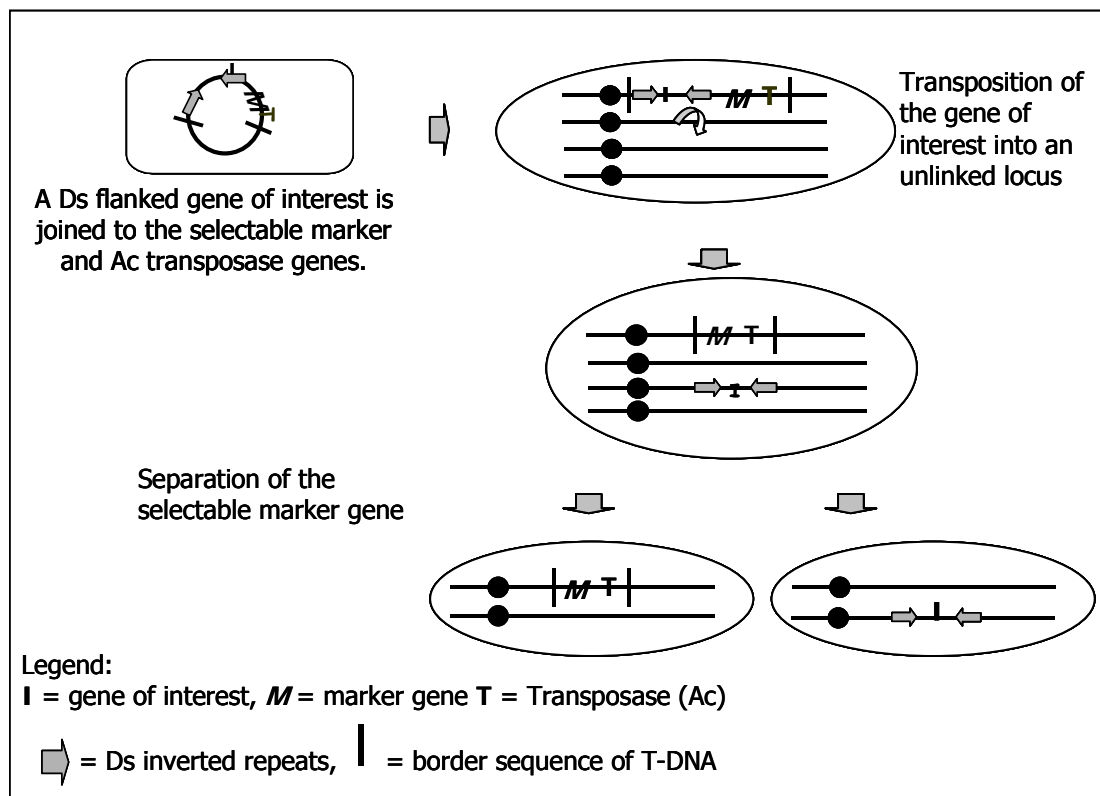


Figure 1. A Ds flanked gene of interest is joined to the selectable marker and Ac transposase genes. After transposition of the gene of interest into an unlinked locus, it is segregated from the selectable marker gene by sexual crossing.

b. Site-specific recombination systems (microbial recombinases)

Recombination is a common phenomenon that can occur at any place along two homologous DNA molecules. In bacteriophages (temperate ones), there is a second type of recombination called site-specific recombination, which takes place only between defined excision sites in the phage and in the bacterial chromosome. Three well-described site-specific recombination systems have been useful for the production of marker-free transgenic plants, (1) Cre/*loxP* system from bacteriophage P1, where the Cre enzyme recognizes its specific target lox P sites (Sternberg and Hamilton 1981, Hoess and Abremski 1990); (2) FLP/*FRT* recombination system from *Saccharomyces cerevisiae*, where the FLP recombinase acts on the *FRT* sites (Cox 1989, Huang *et al.* 1991); and (3) R/*RS* recombination system from *Zygosaccharomyces rouxii*, where R and *RS* are the recombinase and recombination site, respectively (Matsuzaki *et al.* 1991).

Recognition sites for recombinases consist of palindromes, which are flanked with 7-12bp core sequences. Cleavage of the sites occurs at the borders between the recombinase binding elements and the core sequence. The recombinase gene cassette can be introduced into transformed plants that contain the selection marker gene between two recognition sites. Alternatively, a transgenic plant of interest can be crossed with a plant that expresses a recombinase gene. After segregation, marker-free transgenic progeny plants can be identified (Figure 2).

Sugita *et al.* (1999, 2000a) replaced the Ac/Dc system with the R/*RS* system. Also replaced was the 35S CaMV promoter of *ipt* gene (in the Ac/Dc transposase system) with glutathione-S-transferase (GST-II-27) promoter, a chemical inducible promoter from maize. The herbicide antidote "Safener" was used to induce the expression of GST-II-27 promoter. Within three months of "Safener" induction normal shoots (instead of the *ipt*-shooty) appeared. Matsunaga *et al.* (2002) utilized this method for gene pyramiding in woody plants.

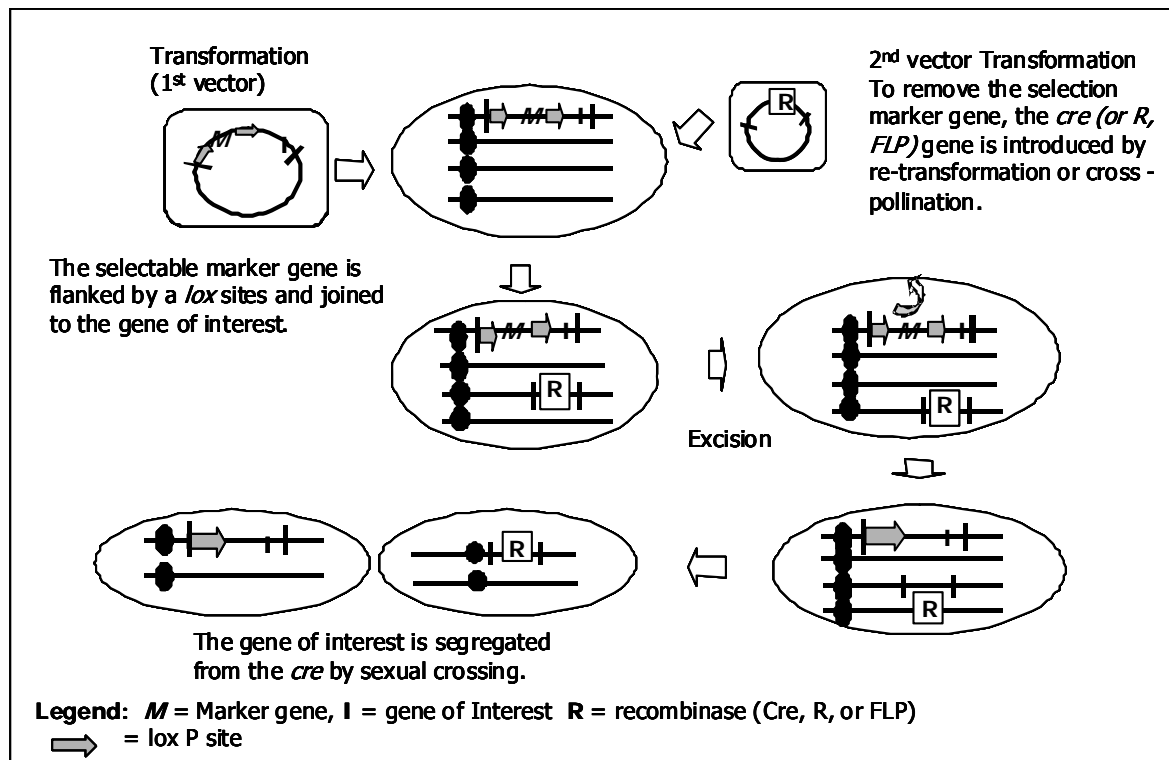


Figure 2. Site-specific recombination system (*Cre/lox*) of removing a selectable marker gene. The selectable marker gene, joined to the gene of interest, is flanked by a *lox* sites. To remove the selection marker gene, the *cre* gene is introduced by re-transformation or cross-pollination. The gene of interest is segregated from the *cre* by sexual crossing.

ii) Intra-chromosomal recombination system (ICR)

ICR is based on intra-chromosomal recombination between two homologous sequences. The procedure exploits the natural nuclear recombination systems present in plants. Attachment sites of bacteriophage λ (*attP*) used for integration at attachment sites of *E. coli* genome (*attB*) is used to generate deletions. The addition of Transformation booster sequence (TBS) from *Petunia hybrida* enhances homologous and illegitimate recombination.

Zubko *et al.* 2000 developed vector (*pattP-ICR*) that contains selectable marker *nptII*, reporter gene *GFP*, and *tms2* genes inserted between two *attP*. Sugita *et al.* (1999, 2000) replaced the *Ac/Dc* system was with the *R/RS* system. *tms2* is negative marker gene to identify deletion in presence of naphthalene acetamide. In this case, Sugita *et al.* (1999, 2000) replaced the *Ac/Dc* system was with the *R/RS* system. Oryzacystatine I gene served as example of gene of interest for transfer into the genome of the tobacco plant. After transformation,

molecular analysis showed that 13% of transgenic plants had lost the *tms2* and the *nptII* genes contained the *oryzacystain I* gene. The advantages offered by the ICR system are the following:

1. Expression of a heterologous recombinase and sexual reproduction are not necessary.
2. There is a one-step selection procedure for transgenic calli (lengthy propagation might increase the risk of somaclonal variation).
3. The system utilizes a natural nuclear recombination systems present in plants.
4. The frequency of intrachromosomal recombination between two homologous sequences in plants can be increased by stimulation of repair systems.
5. The efficiency of homologous recombination is directly correlated with the size of the homologous regions.

iii) Targeted gene replacement

Targeted gene disruption by homologous recombination in rice has also been developed (Terada *et al.* 2002) using the *Waxy* gene which encodes granule-bound starch synthase as a target. About 1% of selected calli and their regenerated fertile plants were heterozygous at the targeted locus, and only one copy of the selective marker was found at the targeted site in their genomes.

iv) Co-cultivation with multiple T-DNA

Multiple T-DNAs can be transferred into plant genome by *A. tumefascens*. Integration of a T-DNA, containing the gene of interest at a different locus to another T-DNA carrying the selection marker gene, opens the possibility of producing transgenic progenies free of the selection marker genes. To achieve this, however, two conditions must be met. First, the co-transformation frequency of both T-DNAs (one carrying the gene of interest and the other selection marker gene) needs to be reasonably high, such that if one is selected for, one is reasonably sure that the second one is there. Secondly, but more importantly that the co-transformed genes must integrate at genomic locations sufficiently far apart from each other (unlinked) to allow effective segregation during meiosis (Figure 3).

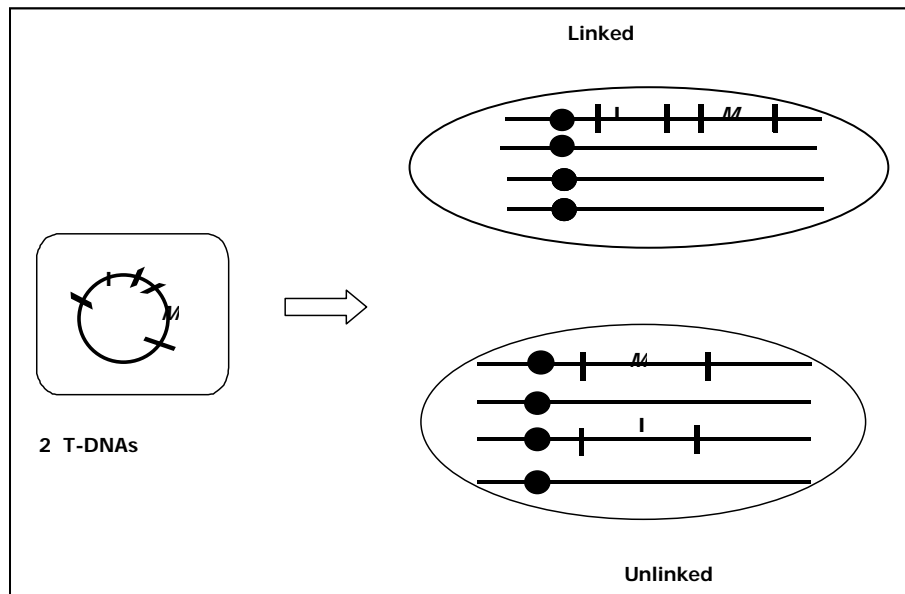


Figure 3. Linked and unlinked co-integrated genes in *Agrobacterium*-mediated transformation with multiple T-DNAs. *Agrobacterium* strain carrying two different T-DNAs. One T-DNA contains the gene of interest and the other T-DNA the selectable marker gene. T-DNAs can be integrated at either genetically linked or unlinked locations.

Agrobacterium-mediated transformation is the only technology that has been reported to-date that allows integration of different transgenes at different Mendelian loci with little transgene rearrangements in plants (Komari *et al.* 1996, Daley *et al.* 1998, Afolabi *et al.* 2004, 2005). The frequency of segregation of the transgenes is determined by their location in the plant genome relative to each other. A unique dual binary plasmids such as pGreen/pSoup that was developed at the John Innes Centre in UK meet these two criteria (Hellens *et al.* 2000, www.pgreen.ac.uk).

Major disadvantages of this system are that only a proportion of plant carrying the selectable marker will also carry the desired trait at an unlinked site and the method cannot be used for vegetatively propagated plants. Figure 4 shows all the strategies of *Agrobacterium* mediated multiple T-DNA co-cultivations.

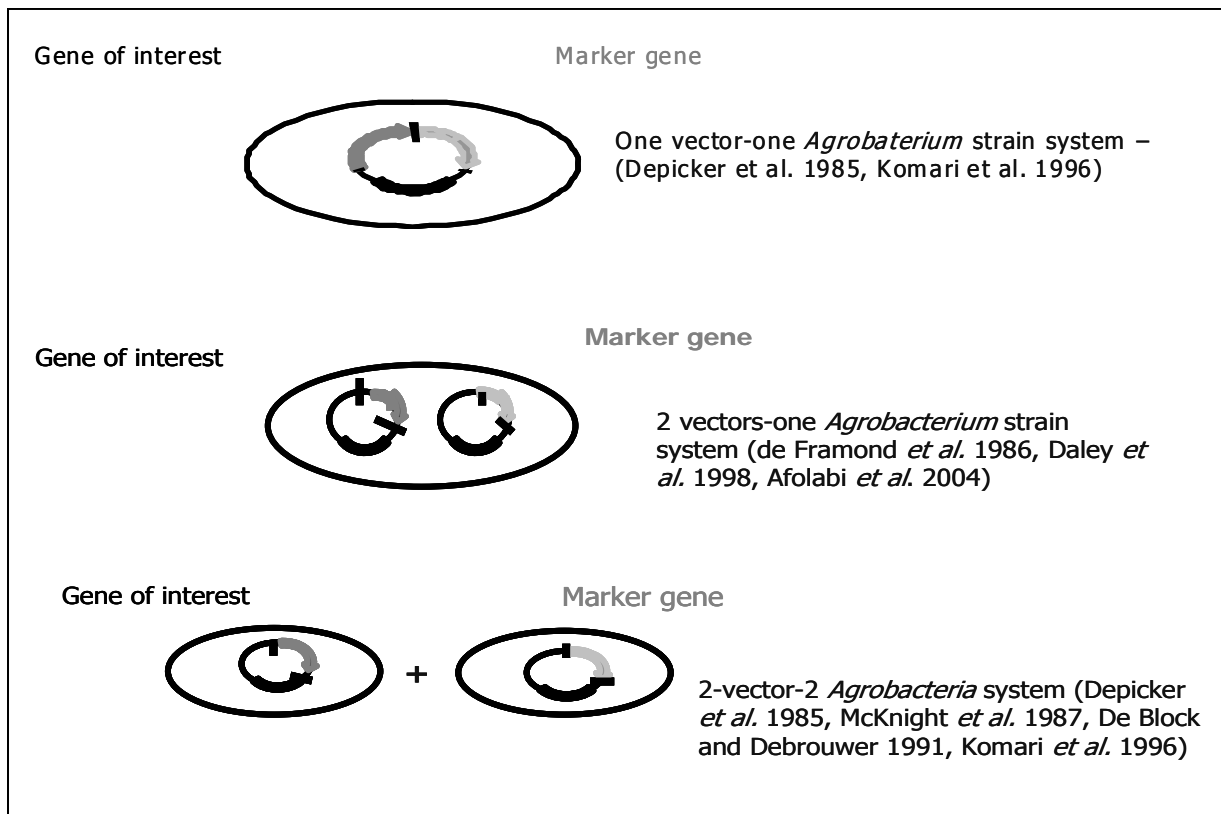


Figure 4. Strategies available for multiple T-DNA co-cultivation with *Agrobacterium*-mediated transformation.

v) Transformation without selection marker gene

Due to the availability of high-throughput screening, it is possible today to transform a plant with the gene of interest only and to screen the product for the plants carrying and expressing the new trait. High-throughput screening techniques include dot-blot analysis and high stringency PCR. These techniques have been used for both cross-pollinating and vegetatively propagated crops (de Vetten *et al.* 2003).

Conclusion

In recapitulation, there are many compelling reasons to produce transgenic plants with as little foreign DNA as possible. Plant regeneration is one of the limiting factors in orphan crops transformation, because there are not many people (due to lack of resources) working on such crops. Today, there are different several proven methods of removing selection marker genes. Removal of selection marker genes may encourage consumers and offer solid evidence that GM crops are safety contrary to the emotion-based opinion of the "Green" groups.

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Policy responses to agricultural biotechnology and their impact on African development

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Summary

Many European states and retailers continue to be unimpressed by the growing body of experimental and empirical evidence about the positive environmental, health and economic impact of Genetically Modified Organisms (GMOs) worldwide. They stick to their bans on GMOs and encourage many African countries to do so too. This European pressure on African countries is not just exerted through aid and trade policy but also by generally cutting funding for the genetic improvement of orphan crop research. The justification for these decisions is that the perception of agricultural biotechnology in Africa would be negative and therefore GM crops should not be introduced in African countries. A perception survey conducted in South Africa indicates however, that stakeholder perceptions in the national debates in African countries may be shaped by the interests and attitudes of foreign, rather than domestic stakeholders. South Africa is nevertheless an exception. In spite of well-organized opposition groups, the country grows GMOs for almost a decade and its positive experience may eventually induce other African countries think twice whether they want to say no to this new technology.

Introduction

Worldwide controversy over the use of modern biotechnology in agriculture has led to many public protests, acrimonious debates and impressive political lobbying by biotech advocates and critics. As a result, regulatory policy in many countries has been captured by non-state actors and their respective interests. This applies most notably to the United States and the European Union, the world's two largest

economies. In the US, the corporate sector has successfully pushed for rather lax regulatory standards. In the EU, non-governmental organizations (NGOs) have left the strongest impression on the Union's highly restrictive agri-biotech regulation (Bernauer 2005).

Egged on by non-state actors, both parties are also pressure governments in African countries to side with their particular cause. In addition to pressuring their own governments, these Western non-state actors are also building alliances with local non-state actors in Africa in order to exert political influence from within in these countries. A quick look at the current preventive global regulatory environment on GMOs indicates that the opponents of this new technology were more successful than the supporters in influencing biotechnology policies worldwide and in Africa in particular (Cohen and Paarlberg 2004). This seems counterintuitive from a political economy point of view because the supporters of this new technology would be expected to have more financial and political resources available to lobby for favorable regulation. Moreover, the potential benefits of GMOs in agriculture are being increasingly confirmed in the field whereas their potential risks have turned out to be rather speculative.

This article argues that non-state actors that oppose GMOs tend to have more political success with the public than supporters of agricultural biotechnology because of the general belief that they act in the public interest. This political success manifests itself in Europe where they were able to persuade governments to stick to their preventive policies towards GMOs and make them exert pressure on other countries to do so too. The same applies to large retailers who are scared to face protests in front of their supermarkets if they would offer labeled GM food products. This generally hostile climate against GMOs in Europe has decreased the amount of funding even for research on the genetic improvement of orphan crops and the plant scientists who use genetic engineering for crop improvement (conducted in the public interest) are often portrayed in the mass media as irresponsible. This has induced many plant scientists to abandon this important research or circumvent the use of genetic engineering by designing approaches that are based on the same principles but sound different. Yet, the assumption that Africans would not accept GM crops may be wrong in view of the fact that it is European trade and aid pressure that largely shapes the attitude of African decision-makers. This was confirmed in a stakeholder perception survey in South Africa that illustrates that foreign stakeholders in favor and against agricultural biotechnology try to

actively shape the national public debate. Yet, national academia is still considered to be most trustworthy actor in the debate and this may help explain why South Africa has embraced agricultural biotechnology research and the cultivation of GM crops. The positive experience in South Africa indicates that the hitherto successful strategy of GMO-opposing NGOs to avoid any sort of political compromise may eventually backfire. The increasing assertiveness of African leaders to fight for their 'freedom to innovate' is reflected in a recent report published by a high-level panel of African experts organized by the African Union (AU) and the New Partnership for Africa's Development (NEPAD). The report concludes that African countries should mobilize the potential of this new technology to address urgent economic, nutritional and environmental problems (Juma and Serageldin 2007). In addition to that, the ruling of the World Trade Organization (WTO) dispute panel 'European Communities – Measures Affecting the Approval and Marketing of Biotech Products' published in September 2006, clearly states that the safeguard measures invoked by the EU to justify its moratorium on GMOs from June 1999 to 2003 were not based on risk assessment that satisfy the definition of the SPS Agreement of the WTO (WTO Panel Report, 2006). This ruling may not change the highly restrictive policies of EU member countries and Switzerland toward GMOs but it may nevertheless undermine their moral authority and the general credibility of their policies in the rest of the world.

The political economy of environmental regulation

The political economy of regulation rests on two basic assumptions derived from positive analysis and broad empirical research. The first assumption argues that political agents primarily seek to maximize their utility (within the limits of bounded rationality). The second assumption claims that regulation of an industry works like a market: the demand for regulation comes from industry who would like some state subsidies and trade protection, and the supply comes from the regulators who are willing to provide such regulation, as long as they will get something in return from industry – for example a financial contribution to the next election campaign (Bailey 1985).

These two assumptions are certainly unable to explain the emergence of environmental regulation in the 1970s. At that time, environmental problems were largely ignored by the political establishment even though there was evidence that certain environmental hazards were not just harming the environment but also are also affecting human health. As a

result, a large bottom-up environmental movement against the industrial pollution of air, water and soil emerged, especially in the United States where the early efforts at environmental regulation were pioneered. Public pressure subsequently enabled a broad political compromise across all political parties to clean up the environment (Kern *et al.* 2005).

Today, however, environmental policy has become a more mature policy area with many interest groups and lobbying organizations trying to maintain access to scarce public resources. Therefore, the issue is no longer 'regulation' versus 'deregulation' but 'bad regulation' (that only serves special interests and is ineffective) versus 'good regulation' (that serves the public interest and is effective).

Puzzling regulation of agricultural biotechnology in Europe

GMOs as toxic waste?

A example of 'bad regulation' (driven by special interest groups and largely ineffective in addressing environmental and consumer health issues) may be the way most European countries are currently regulating agricultural biotechnology, namely as an unnecessary risk to human health and the environment comparable to toxic waste regulation. This bad regulation may largely be the result of highly influential non-state actors who oppose any compromise on the issue but are generally perceived to act in the public interest.

Even though more than 100 million hectares in 22 countries were cultivated with GM crops in 2006 (corresponding to an annual growth rate of 13% according to ISAAA 2007) and Americans consume GM food for more than a decade, Europeans still tend to believe that the decision whether this technology is to be rejected by mankind or not, is still pending.

EU Member States continue to invoke the strong version of the precautionary principle (PP) and the Principle of Subsidiarity in EU regulation in order to maintain their ban on GMOs (Cantely 2004). In December 2006, the Council of EU Environment Ministers once again backed Austria's illegal ban on the cultivation of EU-approved GM crops (Farrelly *et al.* 2006). All this is puzzling in view of the fact that EU-sponsored risk assessment projects on GM crops were also unable to find any indication that transgenic crops are inherently more risky to human health and the environment than conventional crops (Morris 2007).

No political compromise in the public debate on GMOs in Europe

Apart from Spain where the cultivation of Bt corn was approved on a limited area of land, European agriculture is still largely devoid of transgenic crops and the number of field tests to learn more about potential risks has decreased significantly (Lheureux *et al.* 2003). In this context, Gaskell and Bauer (2001) argued that agricultural biotechnology is a typical example of a policy area where bargaining and political compromise have largely failed in Europe.

In a government-sponsored participatory project conducted with political stakeholders on the risk and benefits of herbicide-resistant soybeans in Germany in the early 1990s (van den Daele 1996), environmental organizations refrained from even signing any joint statements that acknowledged the validity of subcontracted research results obtained during the project. Wolfgang van den Daele, the coordinator of this project, therefore concluded that certain interest groups might benefit from avoiding bargains and political compromise. But if this is true it would clearly contradict the basic tenet of political economy and game theory that an actor would always agree to a political compromise or bargain if it resulted in a net material benefit to him/her or his party (Becker 1976).

Trade Dispute on GMOs between Europe and the United States***Economic Cost***

The deadlock situation in Europe on GMO regulation has resulted in political tensions in relations with US food exporters and biotech firms. Exports of US corn to the EU declined from US\$ 420 million in the mid-1990s to around US\$ 3 million in 2002. Exports of US soybeans to the EU also decreased from a peak of US\$ 2.6 billion to US\$ 1.1 billion in 2002 (Bernauer 2005).

As a consequence, in summer 2003, the US together with Argentina and Canada requested the WTO to establish a WTO dispute settlement panel on the EU's policy concerning GMOs. They consider the EU Member countries' *de-facto* ban on GMOs (since 1998) to be illegal under WTO rules.

Even though this *de-facto* ban on GMOs was replaced by stringent labeling and traceability requirements for GM food and feed, compliance with this new regulation will be a heavy burden on US producers, and the negative public attitude towards GMOs in Europe is likely to keep GM

food out of the EU market (Bernauer 2005). Therefore, there seems to be a political deadlock in Europe that is unlikely to be solved by the ruling of the WTO dispute panel.

Environmental costs

The dogmatic struggle within the EU against agricultural biotechnology may do more harm than good to the environment because biotechnology is not just about food — it may become the key technology in a general transformation from a troubled agro- and petro-chemical industry to a more efficient and cleaner biological industry in the decades to come (CSIS 2005). The severe constraints on funding for basic research in plant biotechnology (which also constitutes an important pillar of basic genomic research) and public hostility towards the new technology are inducing many leading researchers in biotechnology to move to the United States or Asia where their work is more appreciated and better funded. This will have serious long-term effects on the European economy because European countries will increasingly be forced to import the new products and processes derived from biotechnology research (Lheureux 2003).

The welfare costs in developing countries

The efforts of many EU member countries to influence public debates and biosafety regulations in developing countries may have significant political consequences. Developing countries have different priorities regarding risk reduction and they can less well afford to entirely reject a new technology that has the potential to address existing risks related to economic, agricultural and environmental problems (Aerni and Bernauer 2006). This is particularly true when modern technology is used for the genetic improvement of orphan crops and the fight against poverty in rural areas (Aerni 2006).

The issue of public trust

Existing economic and political theory has so far proved unable to come up with a simple and persuasive argument as to why it should be in the interest of the Europeans to reject agricultural biotechnology and, in addition, to ensure that developing countries reject it as well. A plausible explanation might be less related to moral concern than to the increasing importance of public trust in the politics of agricultural biotechnology (Aerni 2003).

European governments tend to be very sensitive to changes in public opinion. Since public opinion on agricultural biotechnology has become hostile, European government agencies have responded by assuming a critical stance towards GMOs and by funding anti-GMO activists not because they are convinced that human health and the environment are at risk, but because they realize that it is easier to get re-elected by simply endorsing public concerns raised by non-state actors that claim to represent the public interest. So why were European NGOs so successful in shaping public opinion? The changing meaning of 'risk' in affluent societies and the association of agricultural biotechnology with 'US interests' may be important reasons (Luhmann 1993, Douglas 2002, Aerni and Bernauer 2006). In the public controversy over GMOs where 'risk' has been politicized and corporate interests are perceived to prevail over public concerns, public trust has become a crucial political resource in agri-biotech policy. A political actor who is trusted to act in the public interest gains in public legitimacy at the expense of those actors who are not trusted because they are believed to pursue private interests. Protest groups in industrialized countries that are still trusted to act in the public interest have therefore recognized that public trust, which used to be a public good that facilitated cooperation among actors with different interests in politics (Hirsch 1976), has become a private good that can be managed through public-attention-seeking activities. In other words, protest groups have to appear regularly in the mass media to remind the public to trust their good motives. The more they win the public's favor, the more political influence they gain and the higher the donations and the numbers of members they can attract (Luhmann 1993).

The private management of public trust has become the key instrument in the arsenal of biotech advocates and critics. Public trust, mostly ignored as a political resource in public choice, has proved to be a valuable asset in a world characterized by uncertainty and complexity. In other words, public trust is a strategic asset in politics like money or political power. However, unlike money and political power, public trust is not tradable (fungible) in politics. It is based on the belief that a given stakeholder has 'good' motives (having the public interest in mind), in contrast to other stakeholders that would act only in a self-serving manner (i.e. are perceived to be interested only in obtaining more money and political power) (Aerni 2003). Because public trust (managed as a private political resource) cannot be exchanged for money or political power, political bargains eventually become impossible and political polarization increases. For example, professional advocacy groups whose main political resource is public trust, as confirmed by

various surveys such as Eurobarometer (2006) and WEF (2003), it represents a crucial political currency that has to be used strategically. They may act as if they are seeking consensus and dialogue but, in fact, hardly ever agree to a compromise with those who are accused of pursuing self-serving goals.

Public attention seeking vs investigative journalism

Even though international NGOs and environmental advocacy groups in particular may have an intrinsic motivation to make the world a better place and indeed contribute significantly to sustainable grass-roots initiatives and people's empowerment, the sudden increase in funding for these organizations due to their popularity as spontaneous actors in the mass media, has led to a sudden increase in the number of advocacy groups specialized in public-attention-seeking. The increased competition for the favor and trust of the public is strongly linked to the competition in the market for donations (the main source of revenues). The struggle to survive in a more competitive funding environment has induced many of these organizations to focus on the management of a portfolio of protest topics with high public resonance and to neglect their intrinsic motivation and their core mission of uncovering malpractices in government and industry through investigative journalism. In other words, many NGOs have become normal political stakeholders who try to preserve their interests in the political arena (Luhmann 1993).

The leadership gap in agricultural biotechnology

This explains why the global agri-biotech debate has become more acrimonious, personal and moral whereas public policies show few signs of leadership. Neither NGOs nor corporations are able to provide public goods (because both have first and foremost a private interest in surviving and expanding as organizations), nor do they have the political legitimacy to do so (they are not elected). It is the task of democratically elected governments to manage public goods in collaboration with NGOs, international organizations, corporations and other stakeholders. However, nowadays politicians in government who want to be re-elected do not think that this will be achieved simply through a good performance record but rather by exhibiting good motives. As a consequence, politicians tend to either passively endorse the views of trusted public interest groups or even actively support their preferred regulation. This strategy allows them to be popular and get votes without bearing the costs and frustrations of designing and advocating an elaborate public policy that may alienate them from their traditional

constituency on the short run but provide benefits for the public at large on the long run.

Move to Developing Countries

In their struggle to appropriate and leverage public trust, political stakeholders from rich countries have not only shaped domestic public policy but have created a new battleground in poor parts of the world. They have done so in part because of the increasing importance of being perceived at home as acting in the best interest of people in developing countries, and therefore, implicitly, in the general public interest. To be perceived as acting in the interest of the poor and the environment carries the promise of generating public trust as a political asset. This also explains the increasing importance of foreign policy used as a trump card in domestic politics to legitimize or de-legitimize a new technology. Corporate press releases and NGO position papers in Europe and the United States almost always contain a reference to the role of agricultural biotechnology in developing countries, portrayed either as a curse or a possible means of salvation for poor and hungry people in Africa.

Claiming to prevent Africa from committing a mistake

The most vulnerable countries to foreign interference in domestic policy are the Least Developed Countries (LDC) because they are most dependent on foreign aid and agricultural exports as the primary source of foreign exchange. Since the majority of these LDCs are located in Africa it is not astonishing that African countries support some of the most restrictive and expensive regulations of agricultural biotechnology. Apart from Egypt and South Africa, the commercial cultivation of GM crops is therefore still banned in Africa. Moreover, some LDCs like Zambia have even invoked the right to say no to food aid that contains GM food with the argument that unnamed European experts told them that the stuff is dangerous (Steffens 2007, Paarlberg 2003).

This highly restrictive regulation of GMOs in developed and developing countries has also discouraged many donor agencies to fund research on the genetic improvement of orphan crops if it involves genetic engineering (Aerni 2006). In response, many researchers have developed new approaches such as 'Cisgenesis' (transgenic plants containing solely genes that have been present in the conventional breeder's germplasm) and 'Clean gene technology' (transgenic plants that contain the desired gene but lack the selection marker) in the hope that this would appeal more to the public taste. Yet, it is unlikely that

GMO opponents would suddenly endorse a more politically correct sort of genetic engineering because the debate is already framed between 'bad' agricultural biotechnology on the one side and 'good' organic farming on the other side. The successful political stance that is based on a black and white portrayal of the issue would certainly be undermined if NGOs would suddenly have to differentiate between bad and less bad genetic engineering.

South Africa, the exception

Yet, the future of GMOs in Africa may not be all that gloomy in view of the case of South Africa. South Africa started to regulate issues related to biotechnology as early as the late 1970s through the establishment of the South African Genetic Experimentation Committee (SAGENE) as the national advisory body on biotechnology research and development. This prior biotechnology regulation was gradually replaced in the 1990s by a new regulatory system of which the GMO Act, passed in 1997 and implemented in December 1999, is the main piece of legislation dealing with trade, production and R&D of genetically modified organisms. The GMO Act is administered by a Registrar, located in the National Department of Agriculture (NDA). The Registrar receives all applications for permits to conduct field trials with transgenic crops and to market commercial products derived from GMOs in South Africa. The regulatory system is praised by proponents of agricultural biotechnology for its scientific approach and its method of checks and balances between government departments, academia, and commercial producers as well as the concept of conditional approval, which obliges applicants to consider possibilities of technology transfer to local companies in South Africa (Aerni 2005).

South Africa approved the first field trials with genetically modified crops in 1992 and the first conditional commercial releases started in 1997. Today it grows transgenic cotton, corn and soybean with built-in pest resistance and/or herbicide resistance. In spite of a largely positive experience, vocal opponents to agricultural biotechnology in South Africa criticize the current National Biotechnology Strategy (DACST 2001) and legislation on GMOs for being too supportive towards industry interests and not paying sufficient attention to consumer health and the environment. Yet, the opposition seems less strong than in other African countries mainly because South Africa has sufficient economic and political power to set its policy priorities without having to give too much of a say to foreign stakeholders.

The influence of different stakeholders in the public debate on agricultural biotechnology in Africa has been investigated by means of a stakeholder perception survey conducted in 2000 (Aerni 2005).

Stakeholder attitudes towards agricultural biotechnology in South Africa

The relevant stakeholders in the South African debate on agricultural biotechnology were selected with the help of local key informants, press articles and academic literature. The stakeholders who participated in the survey were evenly spread across the political spectrum. They included 11 respondents from academia (representing departments of agronomy, biotechnology, environmental and social sciences), 10 respondents from NGOs (including Africa Bio, anti-GMO groups and many GMO opposing environmental organizations), 8 respondents from government institutions (representing agriculture, the environment, public health and research), 7 respondents from business and 4 respondents representing producer organizations. Moreover, churches, consumer organizations, and patent lawyers are represented with 2 respondents each. The return rate of the questionnaires was 55%. Altogether 44 respondents from 38 institutions participated in the survey.

The stakeholders were asked to complete a semi-standardized questionnaire in which they had to assess statements and pre-structured answers to certain questions on a scale from 1–5 (e.g. ranging from 1 = not important at all to 5 = very important). The results of the survey reveal that, on average, the respondents in South Africa believed that drought was the most important problem in agriculture and biotechnology has a potential to solve this problem. Moreover they saw further potential agricultural biotechnology with regard to problems such as high use of pesticides, fluctuating yield, pest infestation, plant disease and poor eating quality (Figure 1).

Figure 2 shows the average respondent rating of the potential impact of 5 GM products on small- and large-scale farmers. It reveals that drought-resistant corn is believed to have a positive impact for small-scale and large-scale farmers alike. The same applies to lesser extent for Bt cotton and Bt corn. These assessments confirm recent empirical research on the positive socioeconomic impact of Bt cotton and Bt corn on small-scale farmers in Africa (Gouse *et al.* 2005, Morse *et al.* 2005).

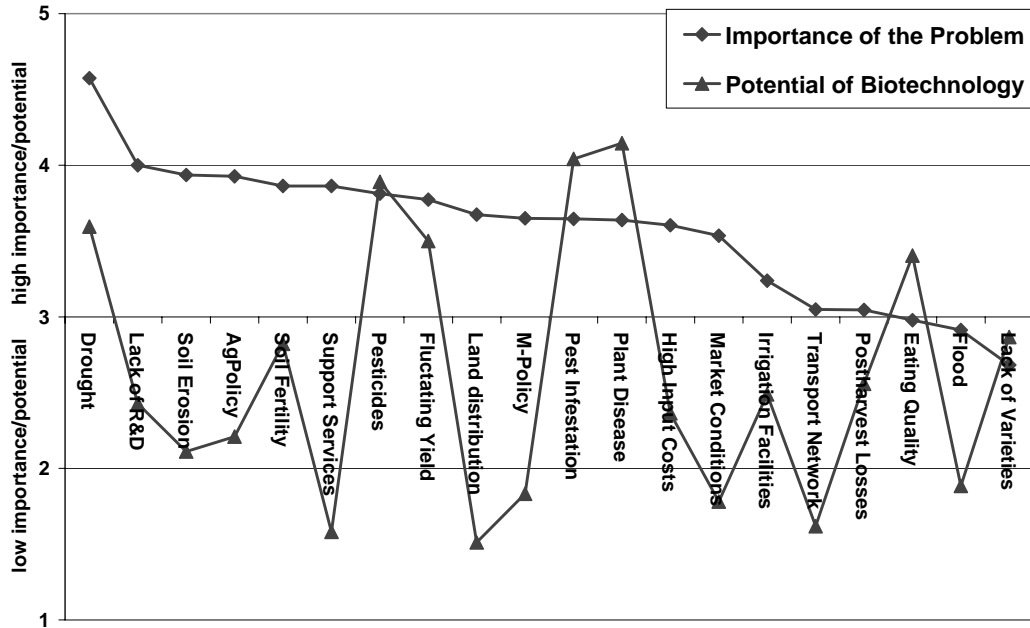


Figure 1. The assessed potential of biotechnology in agriculture

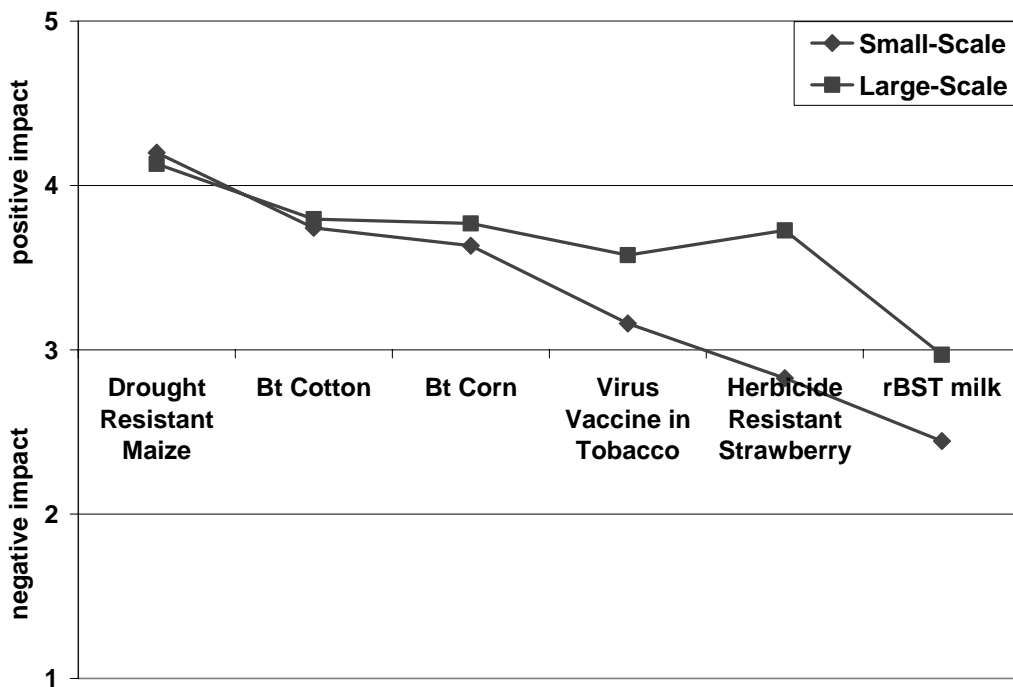


Figure 2: Assessed benefit for small and large scale farmer of 6 selected products

The results of the perception pattern analysis and the social network analysis showed that one of the three perception groups identified was opposed to the use of GMOs in South Africa. It consisted mainly of academics and NGO leaders. However, the other two perception clusters tended to be positive about genetically modified crops. These two positive clusters do not just contain government officials and business executives but also the representatives of farmer and consumer organizations. This is in strong contrast to the European debate where these organizations strongly oppose agricultural biotechnology.

Finally the policy network analysis revealed that national academia is still the most trusted stakeholder in the public debate on agricultural biotechnology in South Africa (Aerni 2005).

National academia as a potential force of change

Judging from the stakeholder survey assessments in South Africa, Mexico and the Philippines (Aerni and Bernauer 2006), national academia has the greatest potential to become a force of political change in developing countries because they have better expertise about local problems and how to use the new tools of biotechnology to address them. They are also of key importance in shaping national biotechnology policies into a more pro-active direction. The stakeholder surveys indicate that national academia may be able to restore public trust as a genuine public good by building bridges between the different interest groups and facilitate political compromise on the sustainable use of agricultural biotechnology.

A Political Bubble?

The strategy of European stakeholders to recruit stakeholders in Africa to mobilize against agricultural biotechnology and win public trust in the public debate back home may turn out to be a political bubble if academia in Africa eventually asserts its scientific authority and uses its public trust to facilitate political compromise.

This bubble manifests itself in a growing divergence between, on the one hand, the political rhetoric of interest groups from rich countries on what developing countries want and need, and, on the other hand, the empirically observable demand for and expectations in regard to agricultural biotechnology on the part of farmers, scientists and other stakeholders in developing countries.

The artificial inflation of the debate on GMOs was fuelled by a cultural paternalism. It indirectly endorsed radicalism and uncompromising behavior and forced many African countries to take sides in the transatlantic dispute on GMOs. This led to a crowding out of any efforts to discuss the different types of agri-biotech applications in developing countries and how to address the local problems of African agriculture with these tools.

The bubble also generated regulatory schemes, often indirectly imposed by rich countries, whose implementation is costly and incongruent with domestic interests and capacities (Steffens 2007). It also prevented the adoption of agri-biotechnology applications that may have been useful for particular developing countries, and it imposed the acceptance of applications that have only limited benefits for poor countries. Finally, the bubble also reduced overall public and private investments in R&D in agricultural biotechnology.

This bubble may eventually burst because it will become increasingly difficult for biotech-averse interest groups to sustain public fears of the technology, particularly as bottom-up demand for certain agri-biotech applications in developing countries grows (e.g Bt cotton) (Gupta and Chandak 2005). Conversely, it will become increasingly difficult for biotech proponents to sustain their predictions of large-scale benefits for developing countries as it becomes clear that the dream of leapfrogging in agricultural development through a doubly green revolution or 'gene' revolution (Conway 2005) would require a much more serious funding commitment from the different national and international stakeholders.

Consequently, the public may eventually withdraw its trust in the most vocal non-state actors who expound their views on agricultural biotechnology in the public arena, because their motives will be increasingly questioned. This may force them to focus more on their core activities again: NGOs may reassume their core role as reliable watchdogs in society and multinational companies will return to concentrating on profit-seeking (within the boundaries of regulation) rather than public-attention-seeking (corporate social responsibility activities) (The Economist 2005). The subsequent loss of influence of vocal non-state actors in regulatory policy would decrease political polarization and enable a more pragmatic and comparative approach of regulating agricultural biotechnology.

Responses in developing countries: building up homegrown capacity

The South African stakeholder survey revealed that most stakeholders in the national public debate tend to have pragmatic views about the use of genetic engineering in agriculture. If there was skepticism, it was related to the concern that insufficient home-grown capacity for biotechnology research may increase economic dependence and make it harder to ensure the satisfactory implementation of biosafety guidelines. This viewpoint coincides with public opinion in developing countries that have achieved advanced home-grown capacities in biotechnology research, such as South Africa, Cuba, Brazil, China, and India. These countries tend to be more confident about the prospect of agricultural biotechnology and apparently have a more positive attitude towards GMOs than in the United States (Hoban 2004). Eventhough it is still very difficult in these countries to convert the results of interesting crop research conducted at local research institutes and universities into useful products for farmers, more investment in training, collaboration with the private sector, and experience in complying with the regulatory requirements may help change the situation soon (Cohen and Paarlberg 2004).

New initiatives to promote biotechnology for development

The international calls for re-defining the role of universities in development strategies and to promote science, technology and innovation for sustainable development (Juma and Yee-Chong, 2005, Juma and Serageldin 2007, Gore *et al.* 2007) also put special emphasis on exploring the opportunities offered by biotechnology. There is great hope that this is a first sign, that the real demands of the developing world will at last receive more attention from the international donor community. Yet, it is not clear to what extent donors are again willing to give financial support to orphan crop research initiative and local empowerment through the promotion of local entrepreneurship and innovation. The donor agency habit to merely fund workshops on the issue will have to be dropped in favor of real action. Moreover, there is a danger for merely relying on global initiatives of private foundations because they are likely to be even more concerned about their public image. The Rockefeller/Gates Foundation Alliance for a Green Revolution in Africa demonstrated this in 2007. After having announced their intention to use modern agricultural biotechnology to increase agricultural productivity, they were attacked by some NGOs for attempting to introduce GMOs in Africa. Promptly, the Alliance feared

about its image as a public interest group and assured the public that they do not intend to use genetic engineering for crop improvement (Odhiambo 2007).

The risk of cultural paternalism became also apparent when the high-level AU Panel on Biotechnology called for more investment in the home-grown capacity of biotechnology in Africa (Juma and Serageldin 2007). In spite of the fact that all the Panel Members were independent African expert, Biowatch SA promptly attempted to portray them in public as stooges of US interests (Fig 2007) and thus induced donors to shirk once again.

Conclusions

This article discussed the public responses to agricultural biotechnology and their repercussions for biotechnology policies in Africa. We observed that the classic laws of political economy are unable to explain the increasing political polarization on GMOs and, in particular, the success of anti-GMO activists in affluent countries to shape not just public opinion and regulation in their home country but also in most African countries.

Results obtained in a stakeholder perception survey on agricultural biotechnology conducted in South Africa suggested however that pragmatic attitudes towards GMOs still prevail there. South Africa is not as dependent on European aid and trade as other African countries and its national academia, which was assessed to be the most trustworthy stakeholder in the survey, tends to be positive about GMOs. The favorable attitude is also due to the largely positive experience with GM crops such as Bt cotton and Bt corn that are cultivated in South Africa for many years already.

South African NGOs that strongly oppose GMOs, did obviously not succeed in shaping the policy agenda in South Africa because the real experience with GM crops in the country made it obvious that there is no scientific basis for their claims that transgenic crops are more risky than conventional crops. As a result, other African countries may become increasingly unwilling to support the Europeans in their efforts to make international biosafety regulation as restrictive and costly as possible. The South African experience may convince them that biotechnology is not just a risk but also an opportunity to address the urgent economic and environmental problems in their particular country.

This would be even more true in the case of the genetic improvement of orphan crops that would contribute significantly to the reduction of poverty and malnutrition. Orphan crop researchers would however have to learn to speak with one voice rather than split between agro-ecologists and molecular biologists, and within the group of molecular biologists, between those who use genetic engineering in crop improvement and those who use alternative methods of modern agricultural biotechnology. These politically motivated divergences may distract from urgent joint action.

A possible shift towards a more positive attitude toward agricultural biotechnology in Africa may eventually burst the political bubble produced by the increasing gap between European risk rhetoric and the real facts on genetically modified crops in developing countries. Yet, many of the least developed countries in Africa still adhere to the European view because they are highly dependent on European aid and European special treatment on market access. That means that the political bubble may have to burst in Europe first in order to facilitate changes in the regulatory environment of least developed countries and make the benefits of agricultural biotechnology available to the poor.

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Role of the media in agricultural research

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Summary

Researchers play a huge role in shaping the destiny of humankind. However, quite often, not much of their work comes to light and even when it does, there seems to be a disproportionate level of attention accorded to their findings, a situation that is a direct consequence of flawed communication. This paper describes the issues surrounding communication of agricultural research findings through the mass media. It highlights the obstacles that hinder a smooth interaction between researchers and journalists. Noting that both researchers and media practitioners are equally culpable for the poor communication of research work, the paper offers insights into the mindset of both parties and provides explanations for the status quo. Motivated by the verity that research serves little purpose if results are not communicated to the ultimate consumers, the paper concludes that whereas media practitioners ought to change their perception of what constitutes news, researchers too must embrace openness and learn to communicate in a way that could be understood by the lay person. Suggestions on how each party could improve in order to have a seamless communication process have been offered.

"Agricultural research profits no one if its results are not communicated fruitfully to the farmer" FAO.

Introduction

African countries continue to grapple with the seemingly incurable problem of food shortages occasioned by widespread drought and a combination of socio-economic and political factors. Today, a large number of people across the continent, particularly in Sub-Saharan Africa, are staring starvation in the face. Yet, this is a situation that can be avoided, at least to some extent without having to resort in food aid from the West. The solution to this recurrent problem may lie in the

promotion of traditional crops that were grown on the continent long before the advent of “modern” crops. These include cassava, sweet potatoes, sorghum and others.

Unfortunately, these crops, which are now referred to as orphan crops or abandoned crops, have not been given adequate attention not only by the population that stands to benefit from cultivating them, but also by governments and research organizations in these countries and elsewhere in the world. As it is, these organizations have their hands full with “modern” crops such as maize, rice, wheat, coffee, tea and cocoa. It is therefore encouraging to see that there is a cross-section of researchers who have taken a keen interest in orphan crops and have dedicated significant time and other resources to conduct research in this critical area.

But there is another problem that is rarely highlighted whose consequences in the adoption of research findings, however useful they might be, are far-reaching. It is the apparent absence of a proper link between researchers and the ultimate consumers of the research – farmers. How does the small-scale farmer out there get to know that a researcher has developed a new variety of sweet potato that is resistant to a certain disease? Is it enough to publish findings in a journal or at a conference and sit back? Is it justified to spend enormous amounts of resources including years of laboratory and field work only for the results of the toil to be known by peers and not celebrated and used by farmers? Agriculture research is of little use if it cannot be adopted by the end users. On one hand, research findings need to be transferred to farmers to enable them solve their problems. On the other hand, farmers’ problems and concerns need to be fed to the researchers. This linkage has to be fundamental, but in reality, the practice is problematic (Mudannayake 2006).

The mass media provides a unique forum for researchers to communicate their results to farmers. In particular, newspapers and radio, which have a far much wider reach in Africa than television and the internet, are critical in passing on information. Some of the alternative methods used by researchers to reach farmers in sub-Saharan Africa are posters, flyers, field days and extension services. In several countries, radio is also used, albeit occasionally.

So why are scientists not making full use of the common mass media to share their findings with the people who need this information? Two

theories have been advanced to explain this situation. One theory casts blame on the media, pointing out to the media's ignorance in science matters. The other censures the scientists, arguing that they are aloof and too technical to be understood.

Theory I: It is the media's fault

While indicting researchers for their failure to interact with the mass media, it would be unfair to absolve media houses from blame. In fact, a casual look at news coverage on any given day in Africa will betray how near or far matters of research are to the hearts of news editors.

Although most of Africa's economy is agriculture-driven, few editors have space for agricultural research news, unless of course there is some sort of controversy surrounding the issue. *Agricultural Review's* observation in parts of eastern and southern Africa where the publication circulates is that there is hardly any meaningful coverage of issues related to agricultural research both in the print and electronic media. In this region, only South Africa and Kenya appear to have some publications (periodicals) dedicated to agriculture but even these hardly devote much space to research findings. The mainstream media comprising daily newspapers and the electronic media seldom pay attention to matters of agricultural research.

To understand the reasons for the status quo from the perspective of a news editor, it is important to understand what could be called the journalist's mindset. This is essentially about what constitutes "news" in the eyes of a journalist. There are several indicators that the media all over the world use to determine what to put out to the consumers.

- **Prominence.** The involvement of persons who tower above the rest on the social, economic or political arena is considered news. Consequently, a pop star will make news when she adopts a Malawian child but few ordinary people would get a line in any newspaper anywhere in the world if they sought to adopt 20 Malawian children at a go.
- **Magnitude.** The scale of an event has a great deal of influence on the coverage it receives. For instance, the 9-11 atrocities were on an unprecedented scale.

- ***Ramifications.*** The extent to which an event is likely to influence other events or change existing order is also important in the eyes of the news editors. For instance, if the United States pulled out of Iraq, this would be a major news event. The act of pulling out is significant in itself but the interpretation of such a move would probably be even greater.
- ***Human interest.*** This refers to the scenarios of dog biting man/man biting dog. The latter is news; the former is not.
- ***Proximity.*** If an extreme weather situation happens in Bern causing unprecedented havoc, it would be headline news across Switzerland. But the story would probably be in the inner pages of newspapers in South America or Africa and would certainly not make headline news on news broadcasts in far-flung places.
- ***Availability of dramatic footage or photographs.*** If a TV cameraman captures a gun battle between gangsters and the police, that item could make it to the headlines despite the fact that it is an ordinary occurrence in some countries.

So, where does agricultural research fall among these categories? Probably in the third category. Still, one is likely to find little being said on this subject unless, as already stated, there is some sort of controversy surrounding it.

Why research news hardly makes headlines

A closer look at this state of affairs yields interesting facts. There seems to be clear reasons why many news organizations especially in Africa shun not just agricultural research data but generally all scientific research. The main ones are:

- Agricultural research hardly falls into any of the six categories that guide the mass media. Science is not appealing to the masses. Journalists would rather report political opinion polls than research findings on the safety of genetically modified foods.
- Many media houses in Africa do not have dedicated science journalists. Often, one will find that the reporter who does the political beat is the same one who will be asked to report on a science-based story. Most reporters have general first degrees

followed by post-graduate studies in journalism. Their first degrees may be in education or even psychology, meaning that they may not always be able to gather and disseminate science information accurately.

- Scientists produce complicated reports that are full of jargon that neither the ordinary journalist nor the typical newspaper reader can comprehend.

Theory II: It is the scientists' fault

Communication among scientists, policy makers, analysts and the common people in society is often fraught with ambiguity, anxiety and sometimes clear confusion. (Gupta 1999). The following are some fundamental barriers to effective articulation of research in public policy:

- Use of jargon that is only understood by peers
- Use of foreign languages, meaning a large group of would-be consumers of the information is disconnected.
- Mindset of researchers about their mandate. Researchers believe that their role is to generate technologies while dissemination is the role of extension systems.

Other reasons as to why many scientists do not engage the media in conveying results of their research findings include:

- Fear of misrepresentation and inaccuracy
- Fear of loss of control of their research findings
- Feeling that journalists are exploitative and manipulative
- Perception that the media is likely to trivialize the research in their attempts to repackage the information for their audiences.

Many researchers interviewed for this paper were unanimous that their relationships with the media would improve immensely if the journalists showed a greater grasp of the subject at hand. There was also a feeling that the media generally underreported research activities and that too few media houses had conscious policies regarding science coverage.

What needs to be done:

The media

- Media practitioners should appreciate the role of research and the impact it has on the society of which they are members.

- The media must undergo a paradigm shift and realize it is not only tragedies, conflicts and controversy that can make news. Society has a right to learn about scientific breakthroughs.
- Media owners should invest in training so that their journalists are able to understand specialized disciplines. Alternatively, they should explore the possibilities of working with researchers who are also capable of doubling as their correspondents in the latter's respective fields.
- Journalists should network with researchers to stay informed on new developments just as they mingle with politicians and celebrities.

Scientists

A study at the Sokoine University of Agriculture in Tanzania came up with several recommendations on how communication between scientists and other stakeholders could be enhanced (<http://forum.waterandfood.org>). Among the key ones were:

- Research projects should include communication strategies at the design stage.
- Develop capacity of researchers to engage in promotion of research information
- Researchers should use simple language that would be easily understood by farmers/ lay persons.

With respect to the role of the media, the following should be done:

- Scientists/researchers should appreciate that the media wields huge influence in society. Many in the society obtain their information from the media and make decisions based on that.
- Researchers should establish a close working relationship with the media to sensitize journalists on their work and its implications in society.
- Researchers should view the media as partners and not as possible agents of misinformation.
- Researchers should take time to explain their work to the media in a way that could be understood by journalists who may not be specialists. They should keep in mind that if the media do not understand the information provided, the ultimate users of the information will be disadvantaged.

Some notes and caution

But while challenging researchers to open up to the media, it is imperative that the former understand the basics of journalism and not throw all caution to the wind when releasing information to the press. To this end, the Royal Society of the United Kingdom developed some guidelines for scientists to use when dealing with journalists (The Royal Society, 2000). Here are a few of them:

- Scientists should know who the journalist represents.
- Journalists work with strict deadlines. If they are not briefed adequately, they'll probably publish what they have, which may not always represent what the researcher intended to send out.
- Science stories compete with others for limited space. The scientist must therefore clearly show the significance of the work and possibly provide catchy photos or demonstrations.
- As mentioned before, scientists must repackage their information for consumption by the general audience – in other words, avoid jargon.
- Since the journalist may not understand the scientist's field of research even if he/she is a science reporter, the scientist must therefore explain in simple terms.
- Scientists should avoid the temptation to exaggerate the significance of their work.
- Researchers should let the journalist know if the work has been subjected to an external quality control mechanism e.g. appearing in a journal, peer review etc.
- If the work is a joint effort, this should be disclosed.
- Scientists should prepare for disappointment: a journalist may spend three hours with them but the result is half a column in the papers or two minutes of air-time.

Conclusion

The media can play a huge role in communicating research findings to the end users. However, as noted, various obstacles prevent researchers and media practitioners from working together. These hindrances are largely artificial and must be urgently addressed because without close interaction between researchers and the media, far-reaching findings that could transform farming may never be fully implemented, thereby rendering the entire research effort a futile exercise.

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Abstracts

Genomics of orphan crops

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Research on minor crops that rank low in world-wide importance but are regionally very important as staple foods is traditionally underfunded and hence lags substantially behind that of the major crops. One such crop is finger millet, *Eleusine coracana* subsp. *coracana*, which is grown mainly in East Africa and Southern India. Finger millet is an allotetraploid that was domesticated some 5000 years ago from the wild subsp. *africana*. Finger millet yields in farmer's fields, which are often sown with unimproved landraces, are commonly between 400–2000 kg per hectare, which is less than 50% of the yield potential of finger millet. To aid breeders in improving finger millet, we developed a series of genetic tools, including genetic markers and maps, and comparative knowledge on the relationship between the finger millet and rice genomes which allows the exploitation of rice resources for finger millet enhancement. We also trained two finger millet breeders in the use of molecular markers. To widen the germplasm base for breeding applications, a biodiversity study was conducted on a set of 79 cultivated accessions from a broad geographical range and 13 wild subsp. *africana* accessions using 45 SSR markers. The molecular analysis was complemented with a phenotypic study. The study shows that Indian and African finger millet accessions belong to different subpopulations, and are also morphologically distinct. We will discuss the results of the biodiversity study, the organization of the finger millet genome, and how the information and resources can be exploited in breeding.

Genomics approaches to improve drought tolerance in small-grain cereals

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Most of the morpho-physiological traits involved in the adaptive response to drought are polygenic and controlled by quantitative trait loci (QTLs). Using biparental mapping populations, we have identified QTLs for grain yield under water-stressed conditions in barley (Talamè *et al.* 2004, *Annals Applied Biology*, 144:309–319) and in durum wheat (Maccaferri *et al.* 2007, *Genetics*, in press). Additional QTLs have been identified in durum wheat using association mapping based on the evaluation of a panel of 189 accessions (Maccaferri *et al.* 2006, *Plant Genetic Resources* 4:79-85; see also the website of the EU-funded project IDuWUE: <http://www.distagenomics.unibo.it/iduwue/index.html>) tested in 15 different environments in Mediterranean countries. Validation of these QTLs identified in durum wheat is underway as a prerequisite toward marker-assisted selection and, eventually, QTL cloning (Salvi and Tuberosa, *Trends in Plant Science*, 10:297-304). It is expected that sequence data coupled with the analysis of gene products through post-genomics platforms (e.g. transcriptomics, proteomics, metabolomics, etc.) will facilitate the identification of candidate genes and QTL cloning (Tuberosa and Salvi 2006, *Trends in Plant Science*, 11:415-412). Reverse-genetics approaches such as TILLING provide additional opportunities to investigate gene function. Under this respect, we have developed a TILLING platform in barley based on cv. Morex (*TIILMore*; Talamè *et al.*, submitted). An effective deployment of genomics to improve drought tolerance will only be possible within an agronomically sound context, particularly in terms of the dynamics of the drought-stress treatments (Talamè *et al.*, 2007, *Journal Experimental Botany*, 58:229-240). Most importantly, harnessing the full potential of genomics-assisted breeding will require a close and adequate collaboration of molecular geneticists with the breeders.

Diversity Arrays Technology (DArT) for crop improvement

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Molecular genetic markers offer a powerful tool to accelerate and refine the process of genetic improvement of crops. Existing marker (genotyping) technologies have been applied successfully to agricultural species, but their cost remains prohibitive for most breeding applications. This is particularly true for species for which no molecular data and very limited resources, such as all "orphan" crops. Because of the limitations of existing marker technologies, we have developed Diversity Arrays Technology (DArT), a novel method to discover and score genetic markers. DArT is a sequence-independent, high-throughput method, able to discover hundreds or thousands of markers in a single experiment. DArT offers at least an order of magnitude lower cost of marker discovery and also of routine genotyping costs, once the array of markers is developed. DArT markers are typed in parallel, using high throughput platforms, increasing automation of data production and reducing the risk of errors compared to technologies used currently in crop genetics and breeding. We have developed DArT successfully for rice, barley, wheat, cassava, sorghum, pigeon pea and approximately twenty other species. We have developed a dedicated Laboratory Information System (LIMS) called DArTdb and an automatic data extraction package, DArTsoft, the key components of the technology. We also developed and continue to deliver a genotyping service for a number of crops, with over 100,000 samples profiled for hundreds or thousands of markers each. Progress in applying DArT to crop genetics and breeding, with special emphasis on "orphan" crops, will be presented, together with the new technological developments in "wet science" and in software tools. The bottlenecks in capturing the value of marker technologies, including DArT, for resource-poor farmers will be also discussed.

Breeding By Design

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Breeding by Design™ (BBD) is a new concept of plant breeding. By using extensive phenotyping, ultra dense genetic maps, and bioinformatic tools, BBD brings closer to reality the opportunity to create new varieties with improved agronomic and novel traits. The ultra-dense maps and the extensive phenotyping provide information about markers associated to all agronomically important alleles present in the species under investigation. This knowledge allows the breeder to create an ideal variety *in silico*. Later, BBD provides the best approach to obtain the variety from existing lines without the need of phenotyping and in just a few seasons of field work. At the end of the process, elite lines go through extensive phenotyping to confirm all selected-for traits are present in the lines. All tools and technologies necessary for BBD are currently available; the difference is that BBD uses them in new and complementary ways. The BBD approach brings closer to reality the ultimate goal of the breeder: to optimally exploit all natural available genetic resources to improve crop performance.

Molecular research on orphan crops in South Africa

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The African Centre for Gene Technologies (ACGT) is an initiative by the CSIR and the University of Pretoria to create a South African national centre of expertise in 3rd generation biotechnology. As a world-class platform in gene technologies with increasing involvement by other South African organizations, the ACGT has a particular focus on gene and genome analysis and applications thereof. The talk will highlight projects undertaken by ACGT member institutions on the orphan crops pearl millet, sorghum, cassava and cowpea. CSIR Biosciences has an interest in cereal crop improvement, and research performed includes optimization of pearl millet transformation, introduction of an antifungal gene into pearl millet and subsequent evaluation/screening for improved downy mildew resistance. This institution has also undertaken a gene mining project to isolate defense response related genes from pearl millet. Subsequent cDNA microarray analyses were performed to determine which pearl millet genes are involved in conferring tolerance to the biotrophic rust pathogen *Puccinia substriata*. CSIR Biosciences is also a partner in a Gates Foundation funded project that aims at enhancing the nutritional quality of sorghum. This will be achieved through transgenic approaches to improve digestibility and the bioavailability of iron and zinc, as well as to elevate levels of lysine, provitamin A and vitamin E. Proof of concept studies for selected traits is currently underway in transgenic sorghum. The Universities of Pretoria and Witwatersrand are partaking in a Generation Challenge Program (GCP) project to develop genomic resources for molecular breeding of drought tolerance in cassava. This project builds on a previously funded GCP project, and will develop single nucleotide polymorphism (SNP) markers throughout the cassava genome to identify favorable alleles related to drought tolerance in developed mapping populations. Finally, a collaborative project between the University of Pretoria and the University of Eduardo Mondlane, Mozambique will be highlighted which aims at phenotyping local cowpea landraces in Mozambique under normal and drought stress conditions.

Understanding the genetic basis of post attachment resistance to *Striga* species; insights from Quantitative Trait Loci (QTL) Mapping and Transcriptomics

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Understanding the molecular basis of host resistance to *Striga* species is a critical step in the identification of genes that can be used for improving crop productivity either via biotechnology based approaches such as crop transformation or via the development of molecular markers for use in marker assisted selection programs. We have screened the 'model' cereal rice for post attachment resistance to *Striga hermonthica* and we have identified cultivars that exhibit varying degrees of resistance to this parasite. In order to begin the identification of the molecular genetic basis of post-attachment resistance in rice we have taken two complementary approaches. First we have carried out a QTL analysis using a mapping population (Nipponbare/Kasalath//Nipponbare) of Backcross Inbred Lines and second we have profiled changes in gene expression in roots of a susceptible and resistant cultivar of rice following infection by *S. hermonthica*. QTL explaining a large proportion of the resistance were discovered on five chromosomes; 4 alleles providing resistance from Nipponbare and 1 allele from Kasalath. Each of these QTL were statistically significant at the stringent genome-wide $P < 0.001$ threshold. All of these QTL were of magnitude in excess of the usual definition of a major gene, thus although the resistance trait is polygenic, it is likely that this may indicate a few genes of major effect. To dissect the Nipponbare QTL into their underlying genetic determinants, an analysis of changes in gene expression in *Striga*-infected roots has been carried out using the Affymetrix whole genome rice oligonucleotide array. To date a small number of genes have been identified that are significantly differentially expressed within the major resistance QTL; such genes are potential candidates for *Striga* resistance and could also be used to develop molecular markers of resistance.

Lessons from Golden Rice

Ingo Potrykus

Chairman Humanitarian Golden Rice Board, Professor Plant Sciences ETH Zürich, retired since 1999

Rice is the major diet for over 2 billion poor in developing countries. It is an excellent source of calories, but poor in micro nutrients. The consequence is widespread micro nutrient deficiency in rice-dependent poor societies. The most cost-effective and sustained intervention to reduce micro-nutrient deficiency is the improvement of the micronutrient content of basic crops, using the potential of genetics: 'biofortification'. This concept may require use of GMO-technology and 'Golden Rice' is the first successful case. Since the first 'proof of concept' (Ye *et al.* 2000, *Science* 287:303-305), science has progressed such that 'Golden Rice' provides the amount of provitamin A necessary, to prevent vitamin A malnutrition from a traditional standard diet, if it replaces ordinary rice (Paine *et al.* 2005, *Nature Biotechnology* 23:482-487). Within the framework of a humanitarian project 'Golden Rice' is carried through 'product development' and 'deregulation' and will be made available, to subsistence farmers in developing countries, free of costs. Ex ante studies have indicated that adoption of 'Golden Rice' would have dramatic health and economic benefits (Anderson *et al.* 2005, *Journal of Economic Integration* 20:771-788). In India alone it would save 40 000 lives per year and the economic benefit to developing Asia would amount to USD 15.2 billion p.a. Extreme precautionary regulation delays use of 'Golden Rice' for a minimum of 8 years and is, therefore, responsible for many avoidable deaths (e.g. 8 x 40 000 lives in India). And there is no scientific justification for this delay. Other, even more important, micro nutrient deficiencies relate to iron, zinc, and essential amino acids. This challenge has been taken up since the middle of the nineties (Lucca *et al.* 2001, *Theor. Appl. Genetics* 102:392-397), and a breakthrough should be expected within the next five years from the 'Grand Challenges in Global Health' initiative financially supported by the Melinda & Bill Gates Foundation (www.grandchallengesgh.org). International research consortia have been established to work on 'Golden Rice' complemented by 'high iron', 'high zinc', and 'high quality protein'. And this concept is extended to cassava, sorghum, and banana (www.goldenrice.org).

Progress and prospects of millet research in Sudan

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Pearl millet (*Pennisetum glaucum*= *P.typhoides*) is grown in western Sudan; and Finger millet (*Eleusine corocana*) is mainly grown in western Equatoria. Finger millet has at least two wild relatives, *E. indica* and *E. flagellitera* present in Sudan. It is believed that Sudan is the centre of origin for pearl millet as part of the Sahel zone which extends from Western Sudan to Senegal. About 18 wild species of *Pennisetum* are found in the country. The national genebank collection contains 835 millet accessions. It is maintained by the Agricultural Research Corporation in Sudan. Despite investigations proving the existence of considerable variability in Sudanese pearl millet landraces, the valuable germplasm resources are still largely under exploited. We highlight parts of the current situation and outcomes of the collection program, improvement programs for millet yield, breeding programs for drought tolerance, insect and disease resistance. We also address the research priorities for millet, based on these findings. Very recently, new technologies started at low scale adopting the DNA based methods. Moreover, Sudan with its wide geographical area, harbor several neglected crops that were used as "famine food" and that are no longer studied. I believe that Cooperation and scientific network of various institutions throughout the world are the only chance to boost research of those neglected varieties.

Compatibility studies between *Sorghum bicolor* and its wild relatives in Zimbabwe

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Zimbabwe has a diverse population of wild sorghum, some of which show robust characteristics like drought tolerance, disease resistance and even higher yields per plant than their domesticated counterparts. Compatibility tests between eight wild varieties and fifteen cultivated varieties and a male sterile line were carried out by artificial as well as natural pollination. Some experiments used wild varieties as pollen recipients while some used them as pollen donors. All the cultivated varieties were compatible with the wild varieties studied and produced viable seeds. Morphological differences between the offspring and their parents clearly showed successful introgression. The hybrids exhibited the characteristics of both parents like stay green qualities of *Sorghum bicolor* subsp. *arundinaceum*, lack of shattering of the cultivated sorghums, higher yield by the hybrids than both parents. Differences observed include size and colour of grains, head shape, length and thickness of nodes and plant height, increased number of tillers per plant, changes in flowering times, and juiciness of stems. We conclude that cultivated sorghum readily crosses with its wild relatives in Zimbabwe and as such this can be taken advantage of in improving cultivated sorghum.

Gynogenic plant regeneration from unpollinated flower explants of tef

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Tef [*Eragrostis tef* (Zucc.) Trotter] is the most important cereal in Ethiopia. In its wild relative *E. mexicana*, regeneration of six green plants resulted from culture of 121 non-pollinated immature pistils. In the allotetraploid crop species tef, however, only callus and root formation was obtained by this method. By contrast, immature spikelets and panicle segments of *E. tef* proved amenable to gynogenic plant regeneration. Upon step-wise optimization of the protocol, efficient plant formation was achieved in all three cultivars tested. In cv. DZ-01-196, culture of 1305 immature spikelets resulted in formation of 159 green plants. Flow cytometric analysis revealed (di)haploid, triploid, tetraploid and octoploid regenerants, from which the vast majority was tetraploid. Tef breeding programs will likely benefit substantially from efficient generation of true-breeding plants.

Acha (fonio) genotypic diversity and management in Nigeria: challenges for sustainable food security

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The germplasm of fonio commonly known, as acha in Nigeria was first assembled at National Cereals Research Institute (NCRI) and has henceforth been characterized. The crop is presently cultivated in six states of Nigeria and the Federal Capital Territory (FCT), Abuja. Fonio production status has been observed to vary across locations depending on several factors. Crop production and husbandry is typically traditional farmers' practices. Several landraces and extensive genotypic variability and diversity has been found to exist across these locations. However, the scientific replacement of primitive cultivars and wild species with genetically improved or uniform varieties is very weak. Farmers therefore maintain a heterogeneous population of seed stock for many generations. It is even possible that this traditional conservation allowed coexistence of Acha with related cultivated species and intercrossing has created or generated new variations. Studies on morphological and genetic characters of different genotypes have confirmed the nature of genotypic diversity among Fonio communities in Nigeria. These are discussed in relation to the challenges for sustainable food security of this native African food which has remained a staple food to over 4 million Africans. Indeed an aggressive desire to promote the research and development of this crop should be the thrust of the International scientific community.

Evaluation of genetic diversity in two fonio species native to West Africa

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Fonio millets (*Digitaria exilis* Stapf, *D. iburua* Stapf) are valuable indigenous cereal crops grown for centuries in arid and semi-arid areas of West Africa. However, the crops have been neglected by research and their potential for food and nutrition remains largely underexploited. For efficient conservation and exploitation of these important genetic resources in breeding programmes, germplasm accessions from diverse agro-ecological areas through the region have been characterized using AFLPs to assess their genetic diversity, its structure and distribution pattern. The results revealed a clear-cut differentiation of the two species and a clustering of *D. exilis* accessions in three major genetic groups fitting to their geographical origins. Shannon's diversity index detected in *D. iburua* was low ($H=0.02$). In *D. exilis*, the most widespread cultivated species, moderate level of genetic diversity (Shannon's diversity $H=0.267$; Nei's gene diversity $H'=0.355$) was detected. This diversity is unequally distributed with the largest part detected in Upper Niger River basin (Guinea, Mali, Burkina Faso) while a very low diversity was found in Atacora Chains zone (Benin and Togo). Genetic differentiation in *D. exilis* was large with 70% and 56% of the total genetic variation detected among the genetic groups and among countries of origin, respectively. Using laser flow cytometry analysis, average 2C genome sizes of $1.848 \pm 0.031\text{pg}$ and $1.956 \pm 0.004\text{pg}$ were detected for *D. iburua* and *D. exilis*, respectively. The landraces investigated were found to be tetraploid with $2n = 36$ chromosomes identified. Intra-specific variations were found slight and insignificant, suggesting genome size stability mainly within the cultivated gene pool. The results of the present study provide highly relevant information that can be exploited for future breeding work on these neglected but valuable genetic resources in West-Africa.

Comparison of orthologous loci from small grass genomes *Brachypodium* and rice: implications for wheat genomics and grass genome annotation

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Brachypodium sylvaticum and *Brachypodium distachyon* were recently proposed as new model plants because of their small genomes and their phylogenetic position between rice and Triticeae crops. We sequenced a 371-kb region in *B. sylvaticum*, the largest genomic sequence available so far from this species, providing quantitative data on gene conservation, collinearity and phylogeny. We compared it with orthologous regions from rice and wheat. *Brachypodium* and wheat show perfect macro-collinearity of genetic markers, whereas rice contains an approximately 220-kb inversion. Rice contains almost twice as many genes as *Brachypodium* in the region studied, whereas wheat has about 40% more. Through comparative annotation, we identified alternative transcripts and improved the annotation for several rice genes, indicating that approximately 15% of rice genes might require re-annotation. Surprisingly, our data suggest that 10-15% of functional sequences in small grass genomes may not encode any proteins. From available genomic and expressed sequence tag sequences, we estimated *Brachypodium* to have diverged from wheat about 35-40 Mya, significantly more recently than the divergence of rice and wheat. However, our data also indicate that orthologous regions from *Brachypodium* and wheat differ considerably in gene content, thus the *Brachypodium* genome sequence probably cannot replace genomic studies in the large Triticeae genomes.

Development of simple sequence repeat markers specific for the *Lr34* resistance region of wheat using sequence information from rice and *Aegilops tauschii*

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Hexaploid wheat (*Triticum aestivum* L.) originated about 8,000 years ago from the hybridization of tetraploid wheat with diploid *Aegilops tauschii* Coss. containing the D-genome. Thus, the bread wheat D-genome is evolutionary young and shows a low degree of polymorphism in the bread wheat gene pool. To increase marker density around the durable leaf rust resistance gene *Lr34* located on chromosome 7DS, we used molecular information from the orthologous region in rice. Wheat expressed sequence tags (wESTs) were identified by homology with the rice genes in the interval of interest, but were monomorphic in the 'Arina' x 'Forno' mapping population. To derive new polymorphic markers, bacterial artificial chromosome (BAC) clones representing a total physical size of similar to 1 Mb and belonging to four contigs were isolated from *Ae. tauschii* by hybridization screening with wheat ESTs. Several BAC clones were low-pass sequenced, resulting in a total of similar to 560 kb of sequence. Ten microsatellite sequences were found, and three of them were polymorphic in our population and were genetically mapped close to *Lr34*. Comparative analysis of marker order revealed a large inversion between the rice genome and the wheat D-genome. The SWM10 microsatellite is closely linked to *Lr34* and has the same allele in the three independent sources of *Lr34*: 'Frontana', 'Chinese Spring', and 'Forno', as well in most of the genotypes containing *Lr34*. Therefore, SWM10 is a highly useful marker to assist selection for *Lr34* in breeding programs worldwide.

Identifying useful biological traits in indigenous African legumes

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Africa is home to about 43% of all the legume genera in the world. As a result, many indigenous legumes exist that are under-utilized, though with great potential for use as food crops, nutraceuticals, and/or phytomedicines. These under-researched and under-exploited African food legumes include *Vigna unguiculata* (Cowpea), *Vigna lobatifolia*, *Vigna subterranea* (Bambara groundnut), *Vigna vexillata*, *Sphenostylis stenocarpa* (African yam bean), *Tylosema esculatum* (Marama bean), and *Macrotyoma geocarpum* (Kersting's bean). In South Africa, two indigenous legumes, namely *Aspalathus linearis* subsp. *linearis* and *Cylophia* species, produce tea that is rich in flavonoids, and other antioxidants and are therefore consumed as a health supplement. Data collected so far show that relative to commercial species such as soybean and groundnut, these orphan legumes exhibit high content of protein and oil in grain, as well as nutritionally-significant levels of protein in tubers when compared to conventional tuber crops. Detailed studies of N nutrition in Marama bean has shown that this legume does not nodulate with N₂-fixing rhizobia, despite the high content of protein in both grain and tubers. There is further evidence that some genotypes of these unimproved legumes such as cowpea and Kersting's bean are tolerant of nitrate in the rhizosphere, a trait important for greater symbiotic performance in N-rich soils. This multifactor approach of identifying useful biological traits for legume improvement has proved successful, at least, for cowpea. This study was supported with grants from the McKnight Foundation and the European Union.

Towards developing bambara groundnut as a potential food crop for Semi-Arid Africa and India

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The current BAMLINK project on bambara groundnut (*Vigna subterranea* L. Verdc.; $2n = 2x = 22$) aims to promote this crop for sustainable food production in semi-Arid Africa and India. BAMLINK is coordinated by the University of Nottingham (UK) in collaboration with African, European and Indian institutes and is the third EU funded project on bambara groundnut. Current genotypes of bambara groundnut, a highly drought tolerant annual legume, are in fact mixtures of landraces. Before EU supported projects no concerted effort had been made into understanding the physiology, genetics and breeding of bambara groundnut targeted towards varietal development of the crop. In BAMLINK we have successfully crossed (under controlled conditions) several landraces as well as a wild and cultivated accession (wide cross) of bambara groundnut. The wide cross was taken further into the F_2 generation (comprising 100 progenies) through self-fertilization of a single F_1 plant. A range of agronomic and domestication traits were studied in the segregating population. The molecular marker data (AFLPs and SSRs) obtained from this population was used to construct the first genetic linkage map of bambara groundnut and a preliminary QTL analysis was done using the trait data. The development of microsatellites markers for the bambara groundnut genome is one key to the future genetic improvement of this crop. A microsatellite-enriched genomic library was constructed and work is underway to map approximately 200 SSR markers to the existing AFLP based map. A detailed diversity analysis will also be carried out using the newly mined SSR markers to understand the extent of inter and intra landrace variations present in the population.

Characterization and sustainable utilization of sorghum and cowpea in the Eastern Cape, South Africa

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Approximately 30 % of the area of the Eastern Cape (EC) province of South Africa is occupied by smallholder farmers. They mostly produce maize and beans, including an assortment of other field crops and vegetables. Food insecurity, unemployment and poverty are rampant in the smallholder sector. Dryland crop production is constrained by a number of factors, one of which is low rainfall potential. Cultivated soils have a poor fertility status, in addition to being generally acidic. The objective of this project is to collect landrace varieties of sorghum [*Sorghum bicolor* (L.) Moench] and cowpea (*Vigna unguiculata*), characterize, improve and promote increased production and utilization of these crops in the EC. These crops generally have good tolerance to drought, poor fertility and acidic soil conditions, in addition to numerous other benefits. The idea is to conserve the landraces and associated indigenous knowledge systems. Germplasm collection will be combined with the undertaking of a baseline survey. Among other things, the survey will help to establish strengths and weaknesses of the different varieties of each landrace. Priority traits that require improvement for each variety will thus be identified using participatory approaches. Priority traits will form the basis for crossing germplasm with complementary characteristics. Farmers will then participate in selecting desirable types in segregating generations that will be grown in both on-farm and on-station nurseries. Subsequently, farmers will be trained in seed production and multiplication of successful varieties of each crop. Community based seed production and distribution networks will be established. A positive image towards the neglected crops will be portrayed through promotional activities such as field days, identifying marketing opportunities, promotion of alternative uses of the two crops, and provision of information brochures on available varieties, etc. Information brochures will be written in both English and vernacular languages. This should improve the potential contribution of these orphaned crops to sustainable nutrition and food security in South Africa.

Physiological, biochemical and agronomic parameters to improve adaptation of cowpea to drought

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Five cowpea genotypes, Gorom local (Go), K VX61-1 (KV), Mouride (Mo), Bambey 21 (B21) and TN88-63 (TN), differing in their susceptibility to water stress, were studied under glasshouse and field conditions, to determine their physiological, biochemical and agronomic responses to water deficit at flowering stage. Effect of water deficit on leaf water potential (ψ_l), canopy temperature, gaseous exchange, leaf proline content, total protein and starch contents, maximal quantum yield (ϕ_{p_0}) and yield components was examined. Water deficit significantly increased the canopy temperature and the proline content of the five genotypes while ψ_l , gaseous exchanges, ϕ_{p_0} and starch content decreased significantly. Yield components, with the exception of seed number per pod, of the five genotypes, were also significantly affected. Under glasshouse and field conditions, the results showed that stomata closure is the common strategy used by the five genotypes to avoid dehydration. Go, Mo and TN tolerated water stress better than B21 and KV. Furthermore, Go and Mo recovered more rapidly after re-watering than B21 and KV. These latter genotypes are revealed to be sensitive with low recovery capacity. The results suggest that the maintenance of net photosynthesis and solute accumulation seem to be traits conferring water stress tolerance in Go, Mo and TN. These traits and recovery capacity could be valuable selection criteria for higher yields under water deficit conditions.

Molecular and physiological responses to drought stress in the mutant germplasm of cowpea

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Drought is a major abiotic stress limiting crop production in many developing countries. Food crops such as cowpea have been naturally selected to adapt and grow in conditions where there is limited water supply. However, different species exhibit different levels of drought tolerance. Breeding for such crops, will lead to the improvement of a myriad of traits that are of agronomic importance. With the advent of molecular-marker technology, research on drought resistance in crop plants has shifted from physiological descriptions of the phenomenon to the molecular dissection of the mechanisms involved. The effect of progressive drought stress was investigated over time (0, 4, 8, 12, 17 & 24 days of without water) on the two mutant lines of Cowpea, *CP-M217* and *CP-M447*, using both molecular and physiological tools. As early as 12 days, the wild-type started to wilt, showing symptoms of water stress. At the peak of the drought stress, the mutants outperformed the wild-type in terms of stomatal conductance and exhibited a significantly high percentage of the relative water content. We used the differential expression technique cDNA-AFLP to identify transcripts that accumulated at the different time points in the mutant lines of Cowpea subjected to drought stress. The expression patterns of the mutants and the wild-type differed from day 4 of water stress. The sequences of some of the isolated transcript derived fragments were highly similar to published sequences, including a leucine rich protein, an NADH dehydrogenase subunit, a GTP binding protein and a transducin-like protein, indicating possible involvement in plant cell defence, energy and signal transduction, respectively. RT-PCR was also used to validate the differential gene expression profile displayed by the cDNA-AFLP. These gene-derived molecular markers described here will serve as a basis to further dissect and improve the drought tolerance mechanism in Cowpea.

Research on a strategic crop enset to enhance food security in Ethiopia

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The indigenous enset-farming complex of the south and southwestern highlands of Ethiopia has supported a higher population density than any other farming system. Enset (*Ensete ventricosum* (Welw.) Cheesman) has been cultivated as (co-)staple food for about 7-10 million people. Since the last three decades, however, because of population pressure, recurrent drought and diseases there has been degradation of natural resources and thus the system failed to sustain the population. In this study, the indigenous enset production methods, farm-based enset biodiversity, nutritional contents of enset corms and the plant characteristics and environmental factors influencing productivity were analyzed to identify yield potentials and constraints in Sidama, Wolaita and Hadyia. The ultimate goal was to develop improved agronomic practices and enhance the use of the existing genetic diversity to reduce the gap between the actual yield and yield potential. Among 146 morphologically diverse enset clones, a total of 180 AFLP fragments was scored of which 104 (58%) appeared polymorphic. The AFLP-based dendrogram showed more redundancy and more duplication groups than the farmers' characterization method suggesting that farmers overestimate the genetic diversity. Eleven enset landraces suitable for corm production were identified and collected from the study areas, and planted at Hawassa University. The result of nutritional analysis of corms showed a marked significant variability in the nutritional composition of enset, which makes possible the selection of nutritionally rich enset landraces. Large differences in protein content (ranging from 0.90 to 2.39 %), crude fiber (ranging from 0.833 to 1.117 %), carbohydrates (ranging from 27.84 to 39), Fe (ranging from 1.11 to 4.33 mg/100 gm), Zn (ranging from 6.16 to 15.55 mg/100 gm) and Beta carotene (ranging from 1.473 to 22.233 µg/100 gm) were observed. This study combines indigenous technical knowledge, agronomic, physiological, nutritional and molecular studies. It has contributed significantly to the understanding of the production methods and the genetic diversity.

BioCassava Plus Project

Peng Zhang

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Inadequate nutrition is the single greatest cause of excess mortality, morbidity and suffering in sub-Saharan Africa. Two hundred and fifty million Africans rely on the starchy root crop cassava (*Manihot esculenta*) as their staple food. Cassava-based diets, however, are deficient in both macro and micronutrients. BioCassava Plus is an international, multidisciplinary team of world-class scientists whose objective is to improve the health of Africans through development and delivery of novel cassava germplasm having increased bioavailable levels of zinc, iron, protein and vitamins A and E, and reduced levels of toxic cyanogenic glycosides. The effective delivery of the enhanced cassava will be achieved by linking optimal nutritional traits with improved post-harvest durability and elevated resistance to viral disease; characteristics required to provide ample amounts of foodstuffs and the incentive for farmers to adopt and sustain the use of cassava cultivars developed within BioCassava Plus. The BioCassava Plus program is distinguished by an innovative combination of approaches that draw upon transgenic plant science, effective collaboration with African partners and field and human feeding tests to demonstrate efficacy of the improved cassava. In the last two years, BioCassava Plus team made significant progress on genetic improvement of cassava, from prove-of-concept to product development. In this presentation, I will highlight recent results from the consortium, including increased bioavailable levels of iron and zinc levels, increased protein content of cassava roots, increased levels of both vitamin A and vitamin E, reduced cyanogen levels in cassava foodstuffs, suppression of the rapid post-harvest physiological deterioration and Generation of robust geminivirus resistance in susceptible, farmer-preferred cultivars.

Controlling post-harvest physiological deterioration in cassava

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Cassava (*Manihot esculenta* Crantz) is the most important source of cheap food energy for close to 30 to 70% of sub Saharan Africans. Its use has tremendously increased as an important raw material for starch and animal feeds as well as strong potential for biofuel production. One of the major constraints of cassava production as cultivation intensifies is post-harvest physiological deterioration (PPD). Cassava roots generally start to deteriorate 24-48 hours after harvest. This phenomenon causes high marketing margins and risks by farmers and small processors whose roots would have to be sold a day after harvest at very low prices or process immediately in terrains with poor road access and technologies. Increased yields from farmer-preferred cassava varieties tied to growth markets will boost the income of small cassava farmers and will benefit trade intermediaries as it has been demonstrated in the transformation of cassava from a rural subsistence crop to a cash crop and urban staple in West Africa (especially in Nigeria). The ability to extend the shelf-life of the roots to two to three weeks would be of benefit to resource-poor farmers in Africa and elsewhere, and it would open up the full potential of this crop. Breeding materials derived from cassava's wild relatives could improve some of the intractable constraints of cassava but the long reproductive cycle of cassava slows introgression and pyramiding of useful genes. There is limited genetic variability for PPD and most varieties have poor shelf life. However, a new source of genes for dramatically delayed post-harvest physiological deterioration was identified in an interspecific hybrid between cassava and a wild relative *Manihot walkerae* in collaboration with the International Center for Tropical Agriculture (CIAT), Colombia. This interspecific hybrid (CW 429-1), whose roots could stay for 15 days without post-harvest deterioration, is currently being used as donor parent in an advanced backcross scheme to develop segregating populations for genetic mapping for the introgression of delayed PPD into cassava gene pools. We have established the second backcross derivatives with this trait and will be evaluating them in mid-2008.

Biotechnology approaches to modulate post-harvest physiological deterioration of cassava storage roots

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Cassava storage roots play an important role not only as a basic food source for the developing countries but also as starch reserve for the starch industry. Cassava roots undergo post-harvest physiological deterioration (PPD) within 24 hours after harvest, thus reducing the crop's palatability and marketability. Cassava PPD is due to wound initiated oxidative stress, therefore efforts to enhance the capacity for ROS scavenging as well as increasing the levels of antioxidant enzymes serves as logical starting point for PPD modulation. Information regarding changes in gene and protein profiles during post harvest physiological deterioration is currently scarce. To gain more insight into the molecular events occurring during PPD we have undertaken a study to determine changes in protein complement during PPD. Using a proteomics approach, the protein expression profiles of cassava during PPD was studied. Proteins were extracted from cassava roots 0, 12, 24 and 48 hours after harvesting and separated by differential gel electrophoresis (DIGE). Changes in protein profiles were found in cassava roots in the different PPD time points. Gel image analysis identified up-regulated and down-regulated proteins during PPD with annotated functions in protection against oxidative stress and regulation of reactive oxygen species. The characterization of differentially expressed proteins in cassava storage root during PPD is an initial step towards understanding the mechanisms underlying PPD and will deliver useful tools to modulate the process via genetic engineering. Additionally, in an effort to improve the antioxidant scavenging capacity of cassava roots, transformation vectors targeting over-expression of three antioxidant molecules in cassava root: dehydroascorbate reductase, (DHAR) glutathione reductase (GR) and glutathione peroxidase (GPX) have been constructed and are being used to transform cassava.

Engineering Cassava Mosaic Disease resistance in cassava

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In the last decade, cassava mosaic disease (CMD), caused by several geminivirus species, has emerged as the most important disease of cassava in Africa. In order to test the efficacy of the RNA interference approach against bipartite geminiviruses, we have used the naturally occurring plant-virus system (African cassava mosaic virus (ACMV)—cassava). In transgenic cassava expressing hairpin double-stranded RNA (dsRNA) cognate to the viral DNA A bidirectional promoter, improved plant recovery from ACMV infection was observed. A similar pattern of 21 to 24 nt geminivirus-derived short dsRNAs was detected from ACMV infected wild-type plants and non-infected dsRNA transgenic plants*. No qualitative differences among short dsRNAs from infected wild-type and dsRNA transgenic plants could be detected. The production of these different classes of short dsRNAs might involve several distinct silencing pathways in the geminivirus-plant interactions. The high level of geminivirus-derived short dsRNAs observed in transgenic cassava plants upon infection might indicate their potential antiviral defense function. The 22 to 24 nt small RNAs, which are predominant in infected wild-type plants, display relatively strong signals in dsRNA transgenic cassava plants. In transgenic cassava expressing hairpin dsRNA cognate to the ACMV-Rep sequence, total ACMV resistance could be observed for several independent cassava lines. Interestingly, the resistance level was positively correlated with the expression level of hairpin-derived AC1-homologous short RNAs. The AC1-homologous hairpin was processed into small RNA classes that were also found in the infected WT plants. Resistant transgenic cassava lines selected in a first infection assay remained resistant under increasing viral load via biolistic delivery and agroinoculation procedure. Our results show that total ACMV resistance can be achieved in cassava by expression of AC1-homologous dsRNA hairpin. This work has been done in collaboration with Professor T. Hohn's Lab (University of Basel, Switzerland).

Genotyping of yam core collection

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A core set of yam germplasm made up of 342 accessions representing six economically important yam species (*Dioscorea alata*, *D. bulbifera*, *D. cayenensis*, *D. dumentorum*, *D. esculenta* and *D. rotundata*) with diverse geographical origin in West and Central Africa were evaluated for inter- and intra-specific variability using SSR markers. The accessions were analyzed using 23 SSR primers that showed polymorphisms. Pairwise distance matrices using Jaccard coefficients resulted in similarities varying from 58% to 93%. Principal components analysis showed distinct separation between *D. rotundata* and *D. alata*. A close genetic association was observed between *D. rotundata* and *D. cayenensis*. Genetic diversity as revealed by tree construction of the SSR data using unweighted neighbour-joining method showed large number of inter- and intra-specific polymorphisms that enabled us to reliably discriminate between the samples. The wide genetic variation observed constitutes a good basis for development of hybrid yams.

Farmer Participatory Breeding: the foster parent in the breeding of yam in Ghana

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Yam (*Dioscorea spp*), have been aptly described as an orphan crop. It has suffered institutional neglect from time immemorial. Until this study there were no formally released yam varieties in Ghana. The farmer participatory breeding approach was adapted to fast-track the development and release of three new yam varieties in Ghana. This paper uses the breeding process as a case study and conducts a SWOT analysis to provide guidelines for breeding for crops in Low External Input Agriculture. It was faster than conventional breeding in varietal development. Scientific quality of Farmer Participatory Breeding is as good as conventional breeding. Farmer Participatory Breeding is a must for breeding yam in Ghana. Ultimately, three genotypes 2000/001, KUP2000/001 and TDr89/02665 were accepted and released as new varieties "CRIKukrupa", "CRIPona" and "Mankrong Pona" respectively in March 2005. Danger of omission of some promising genotypes in the course of varietal development and danger of opinion group cabalizing the group discussions were identified as potential threats to the approach. Effective research-extension-farmer linkage was identified as extremely vital to the success of the approach. Farmer participatory breeding process was also sustainable and more efficient than conventional breeding in varietal selection in yam breeding.

Biosafety regulations in Africa

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African activities in modern biotechnology/genetic engineering and biosafety legislation are under constant development. Since more than ten years, South Africa is the only country that grows GM crop plants commercially. About nine other countries have reported field trials (incl. Kenya and Burkina Faso) and about 20 countries are engaged in research on GMOs. To date, 40 African countries have ratified the Cartagena Protocol on Biosafety and are Parties to the Protocol. Of these, only eleven countries have biosafety regulations - mainly in draft form, some already in force. Seven countries currently develop biosafety legislation. Even in Kenya and Burkina Faso where field trials with GM plants already took place, legally binding biosafety regulations are still under debate (expected to be tabled in Kenya this summer in Parliament). In fact, the vast majority of African countries has no biosafety legislation in place yet and will not have so for years to come. An overview of on-going activities and associated problems will be presented. Modern biotechnology requires high technical and academic skills for proper handling to ensure the benefits, this also includes operational legislation. To date this is hardly a reality in any African country. For instance, except for South Africa, almost no country is currently in the position to systematically screen and detect GMOs growing in or imported into their countries. This lack of detection capabilities and facilities creates serious enforcement and oversight problems. With regard to orphan crops and modern biotechnology, careful choices will have to be made regarding crop and traits to be introduced. It will require solid assessments of the problems to be solved, the targeted group of the society and the required enabling conditions. A new breeding technique or cultivar, GM or not, is not sufficient *per se* to ensure access by those who most need it. GM, however, adds a dimension of complexity in required capacity for proper handling in order to achieve the broader goals of hunger alleviation and long-term development.

Application of biotechnology on selected African underutilized crops: experiences using the BecA/ILRI research platform

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International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)

Increased productivity of orphaned crops among small-scale farmers may hold the key for Africa's agricultural production, if the continent is to feed the rapidly expanding population. Biotechnology has been identified as a potent technology that can contribute effectively in alleviating chronic food shortages in Africa. Advances in the biosciences promise powerful new ways of improving crop productivity of these crops using research platforms such as Biosciences eastern and central Africa (BecA). This is an initiative endorsed by the New Partnership for Africa's Development (NEPAD) to support eastern and central African countries develop and apply bioscience research and expertise to produce technologies that help poor farmers. It provides a focal point for the African scientific community to support the activities of national, regional, and international agencies as they address agriculturally related problems of the highest priority for alleviating poverty and promoting development. BecA consists of a *hub* that provides a common biosciences research platform, research-related services and capacity building and training opportunities; and a network of regional nodes located throughout eastern and central Africa for the conduct of research on priority issues affecting Africa's development. Over the last several years, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has been using the BecA research platform and its Centre of Excellence on Genomics to improve the productivity of sorghum and millet, which are two of the most important orphaned cereal crops in Africa. ICRISAT has been working closely with National Programs in Africa in the use of molecular markers (SSRs) for studying the genetic diversity and improving resistance to both biotic and abiotic stresses in sorghum and pearl millet using Marker Assisted Selection and genetic mapping. In addition, the high-throughput facilities at BecA are being used for training, capacity building and provision of genotyping services for breeding programs in African NARS.

Global Facilitation Unit for underutilized species: enabling the deployment of underutilized plant species

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Global Facilitation Unit for Underutilized Species (GFU), Italy

The Global Facilitation Unit for Underutilized Species (GFU), set up in 2002, is a multi-institutional initiative of the Food and Agriculture Organization of the United Nations (FAO), the International Fund for Agricultural Development (IFAD), the International Centre for Underutilized Crops (ICUC) Bioversity International and Global Forum on Agricultural Research (GFAR). It is funded by the German Ministry of Economic Cooperation and Development (BMZ) and based at Bioversity International in Rome. Its mission is to promote and facilitate the sustainable deployment of underutilized plant species to increase food security and alleviate poverty among the rural and urban poor. The GFU is not promoting particular plant species and is not involved in the implementation of projects of national partners. Rather, its objective is to create an enabling environment for stakeholders engaged in enhancing the deployment of underutilized species. The Unit, therefore, deals with cross-cutting issues that are relevant to most stakeholders and most underutilized species. Its activities focus on networking and knowledge sharing, awareness creation, and formulation of policy recommendations at the national and international level. Major achievements are: i) development of a web-based portal on topics related to underutilized species; ii) creation of a platform for discussion and information exchange; iii) in partnership with ICUC and Bioversity International the development of a strategic framework for Research and Development of Underutilized Species to support priority setting and international collaboration; iv) sensitization of national policy makers for the need to create legal and policy environments in which underutilized species can be better utilized for the benefit of the poor through policy analysis and formulation and dissemination of policy recommendations; and v) establishment of a multi-institutional task force to lobby for an amendment of the EU Novel Food Regulation and the submission of recommendations for its revision to the European Commission in order to ease access of underutilized species based foods to the EU market.

The Indo-Swiss Collaboration in Biotechnology

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The Indo-Swiss Collaboration in Biotechnology (ISCB) promotes research partnerships between Swiss and Indian institutions in various areas of biotechnology and fosters technology transfer to the private industry. The programme is jointly funded by the Swiss Agency for Development and Cooperation (SDC) and the Department of Biotechnology (DBT) of the Government of India. The overriding goal of the ISCB is the establishment of equitable research partnerships between Indian institutes and their respective counterparts in Switzerland. Its mandate comprises three main components: first, to develop products and biotechnological processes which have an impact on poverty reduction and the sustainable management of natural resources in India; second, to focus on innovative technologies in agriculture and environmental research; and third, to build capacities and R&D partnerships between Swiss and Indian Institutions and private companies with strong economic, social and ecological relevance. Within this frame, five topics have been identified as priority research areas for the ISCB: the improvement of stress resistance and pest control in pulses, the improvement of resistance against fungal diseases in wheat, the monitoring and *in-situ* degradation of pesticide residues, the improvement of soil quality, and trans-sectoral issues (e.g. biosafety, need assessments, intellectual property rights). The project portfolio of the programme ranges from the development of a product enabling the biological control of a chickpea pest to genetically engineered plants with improved insect resistance. The exploration of alternatives to genetic engineering (multi-optional approach) forms an integral part of the programme. Detailed information on the individual projects is available at <http://iscb.epfl.ch>. The ISCB programme is organized in phases. Under the current second programme phase (2004-2007), three research networks and four collaborative projects are supported, involving 23 Indian and 13 Swiss research groups. The framework for the third programme phase (2007-2011) has recently been approved by the funding agencies SDC and DBT.

Future of underexploited plants and orphan crops 2030 and beyond

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Nature's storehouse provides an unlimited reservoir of gene wealth to meet food, nutrition, health and other human needs associated with global changes. There is a pressing need to conserve biodiversity, plant genetic resources and genetic variability for their sustainable use. In agriculture, the productivity of major crops cannot be maintained, let alone expanded, without a constant infusion of fresh genetic variability. Agriculture in the past has relied on few species of crops to meet food, nutrition needs of the world. African countries with a large proportion of people living in marginal and extreme environments continue to face the challenge of providing basic livelihood security and dignified living to its people. In times of famines, emergencies and disasters local people depend for their food-dietary, medicinal needs on locally used species also referred to as "Life Support" species. What the continent needs is not just gene revolution but continuation of their traditional farming wisdom of diversified agricultural system relying on their indigenous plant species diversity with high socio-economic and ecological value. Future of underexploited plants and orphan crops in the region will depend on creating adaptive science and technology. Some thoughts on new ways of understanding the biology of "Underexploited" plants, "Orphan" crops are discussed. Model and role of a carefully designed new International Program on Biodiversity and Genetic Resources of Underexploited plants and Orphan crops in designing and launching future approaches to meet the emerging needs is given.

Roundtable discussion

Panelists

- Dr. Andrew Bennett, Executive Director, Syngenta Foundation for Sustainable Agriculture; Chairperson
- Prof. Jeff Bennetzen, University of Georgia, Athens, USA
- Prof. Felix Dakora, Tshwane University of Technology, Pretoria, South Africa
- Dr. Andrzej Kilian, Diversity Arrays, Technology Ltd., Yarralumla, Australia
- Prof. Mohan Jain, Helsinki University, Finland
- Prof. Roberto Tuberosa, Department of Agro-environmental Sciences and Technology, Bologna, Italy
- Prof. Julie Scholes, University of Sheffield, UK
- Prof. Fernand Lambein, Institute of Plant Biotechnology for Developing Countries, Gent, Belgium
- Dr. Dan Kiambi, ICRISAT-Nairobi, Kenya
- Dr. Irmgard Hoeschle-Zeledon, Global Facilitation Unit for Underutilized Species, Italy

Rapporteurs:

- Dr. Afolabi Abolade, Biotechnology Advanced Laboratory, Abuja, Nigeria
- Dr. Obidiegwu Jude, International Institute of Tropical Agriculture, Ibadan, Nigeria

Overview

Prior to the roundtable discussion, the following overview was given by Dr. Zerihun Tadele, co-organizer of the conference.

Agricultural revolution particularly, the famous Green Revolution played key role to boost crop yields of wheat and rice in certain Asian countries and Mexico in 1960s and 1970s. The Green Revolution was successful because of the availability of improved crop varieties along with inputs such as fertilizers, herbicides and fungicides. Expansion of arable area supplemented with irrigation facilities was also implemented. Nowadays, there are some criticisms to the way Green Revolution was carried out because only rich farmers had access to irrigation and other inputs; hence the gap between poor and rich farmers became bigger and bigger. Africa is believed to be bypassed from the Green Revolution due to a number of reasons. The unique nature of African agriculture is that majority of farmers are resource poor and grow orphan crops. Although

orphan crops have advantages in tolerating extreme environmental conditions and fit to the diet of the African population, they produce inferior yield either in quality or quantity. The following points are suggested for the roundtable discussion:

1. Need for agricultural revolution

- Can we achieve Green- or Gene-Revolution in Africa using orphan crops?
- How can we transfer modern crop improvement techniques to orphan crops? For example, can plant tissue culture and mutagenesis technology be applicable to orphan crops?
- Can we obtain cost-effective technology that can be absorbed to local conditions?

2. Research collaborations

- How should research on orphan crops be collaborated between North-South or between South-South?
- Is networking orphan crops research useful?
- How can we involve International Agricultural Research system such as CGIAR to orphan crops research?

Andrew Bennett (chairperson): We need to move crops from the state of being orphan to a higher level where it is no longer neglected. Scientists need to translate their findings to end users and how to go about it is the question. There is a need for partnership in production flow like marketers and processors. The challenge before us is what agriculture can offer to society. It could be seen from the point of service delivery. Do agricultural findings take into consideration cost-effectiveness? How best can we define cost-effectiveness in the midst of societal priorities and demand? Orphan crops provide different goods (food, medicine, etc.). Do we need agricultural revolution or evolution of agriculture? There are many ways of seeing agriculture (wealth creation, food, good land). Have we got a clear view of what we want from orphan crop improvement? Cost effective technology, what is it? Find way of plant project to move us forward? The question to the panelists is how do we get ourselves organized to get the information across to lift the crops up? Who are the people among the stakeholders who are our allies to help? It should be known that the value of orphan crops is beyond food; some are used in medicine, cosmetic, etc.

Mohan Jain: Genetic technology offers hope and needs to be emphasized in developing countries. Genetic variability is important and

use of mutants and technologies that can be absorbed by the people and supported by their governments should be cost-effective. From personal experience, manpower capacity is lacking and should receive emphasis and not equipment which is cost-effective. Africa has potentials in food production with bio-diversity and good weather if fully exploited. African scientists and government need to evolve a clear cut direction to attract help. Emphasis must also be given to conservation or utilization of local germplasm.

Andrew Bennett to Mohan Jain: Is there a need for regional laboratory? Are you personally in favor of regional laboratories?

Mohan Jain: Yes, I am in favor of regional centers of excellence.

Andrzej Kilian: Our meeting today covers only a segment of a broader problem in respect of being technologists. Technology is a small component of our challenge. We must exist in holistic manner. Technology has a role but we must ensure we detect variation in its optimal potential. We must work with people who have positive approaches to development and market expansion. We need to advance orphan crops using genetic/information science. As scientists, we must be fully committed beyond publication and salary and think of service delivery or product development.

Felix Dakora: Currently Africa is not in charge of its own research agenda. Since the majority of African research is externally funded, research agenda is formulated externally. These externally made decisions will not help Africa to attain food self-sufficiency. The intentional promotion of exotic cash crops over the orphan crops is affecting the improvement of indigenous orphan crops of Africa. Highly adapted indigenous crops are neglected. However, African research needs collaborators and/or partnership from the north. As CGAIR (Consultative Group on International Agricultural Research) is expanding, hunger and poverty persists. Could CGAIR be not sensitive enough?

Andrew Bennett to Felix Dakora: How can the dynamics of agenda setting be changed in Africa?

Felix Dakora: Some of the African agricultural organizations have done better than others. Personnel or manpower planning is limited to certain individuals. Doors need to be opened for more sincere interaction. It is

not easy to judge but ASARECA (Association for Strengthening Agricultural Research in Eastern and Central Africa) has done well.

Jeff Bennetzen: Africa's agriculture is a challenge and about to get worse especially with climate change and global warming. This could further be worsened by the introduction of biofuel. American priorities have changed towards biofuel and food surpluses will cease. Orphan crops simply mean crops that were abandoned and we need to re-engineer with an adoption agency. There is more agricultural coordination for African agriculture. Emphasis must be placed on crop improvement instead of coordination. I think GM (genetic modification) is a big hope for Africa to catch up being a perceptual issue.

Roberto Tuberosa: All issues raised are correct. Having evolved this far Africans need to learn from the past mistakes and enough mistakes have already been made. Science needs to be translated to science delivery. Participatory breeding is an option. The research package needs to be more sensitive. There should be clear-cut definition of expertise within scientific community. Laboratories are not aesthetic rooms but should be functional. Towards science delivery, there should be rational spending. Basic research, good coordination, good scientist/experts should concentrate on drought tolerance. Avoid duplications; be more focused and investigate for better solutions. GM is an option Africans should look for.

Julie Scholes: Scientists need to define biological problems. Scientists need not to duplicate efforts. There should be room for collaboration and integration. There is a gap between researchers in universities and NGO's as stakeholders. Stakeholders need to unite based on present circumstances. Public perception need to change to GM. More collaboration and communication will elucidate the perception of GMO by the people. Currently, seeking funds to study striga, the major parasitic weed in Africa, is difficult.

Andrew Bennett to Julie Scholes: Is there a new perception that science is far from delivery? Do you have any method of improving top-down approach to science?

Julie Scholes: Yes, in-terms of problem definition. Funding is a problem which hinders continuity.

Fernand Lambein: Africa needs to speed up green- and gene-revolution and empower women; and also reduce bureaucracy. With too much subsistence farming, African farmers lack inputs. Africans are not sensitive to research as emphasis is placed on infrastructure and not maintenance and utilization. Food improvement entails diet improvement and hence women need to be empowered.

Andrew Bennett to Fernand Lambein: What is the role of women in science? How can the dynamics of agenda setting be changed in Africa?

Fernand Lambein: Regarding the role of women, things are changing gradually.

Irmgard Hoeschle-Zeledon: Research committees need to complement orphan crops with other indigenous food and vegetables to ensure healthy living. Community conservation of crops needs to be supported. It is very nice that CGIAR is moving into underutilized crops which are outside their mandate. There is great potential in underutilized crops, however, we need to make sure the benefits are enjoyed by the farmers. Coordination should be overtaken by partnership to ensure maximal use of limited funding.

Andrew Bennett to Irmgard Hoeschle-Zeledon: Is there any process to create demand for these crops? Any comment on market development?

Irmgard Hoeschle-Zeledon: A case study in Europe shows that market is improving for these crops. There are a growing group of consumers that are health-conscious. Public awareness needs to be made.

Dan Kiambi: African agricultural set-up is diverse and a good base for orphan crop growth. African government needs to be more sensitive to agricultural research taking into consideration the strategic role of agriculture in gross domestic product (GDP). Orphan crops are localized in regions and are tied to traditional systems. The list of these crops is long. There are a lot of opportunities and potentials for orphan crops. Therefore, regional priority is important and the most important ones must be selected. We need to ask ourselves how best to meet with expertise in setting priorities. There is a need to have a consortium of underutilized crops. I also support the need for centers of excellence as suggested by Mohan Jain because they attract synergy and ensure that

the cost of service delivery lowers. Donors are also more inclined to invest to regional centers. The impact of CGIAR centers regarding nutritional programs needs to be addressed. Over the years there have been fruitful partnerships with NARS (National Agricultural Research System) in Africa in releasing improved seeds.

Philip Aerni (participant): Why is CGIAR does not create or open market for the orphan crops? CGIAR should help in the market outlet.

Dan Kiambi: such partnership is in progress in some CGIAR centers.

Otoo Emmanuel (participant): Assistance from external donors should be based on what the local people know best and have the skill to produce, rather than importing what is exotic. Local priority should be respected. Technology should be effective and adaptable. There is a need for fair play between CGIAR and NARS scientists.

Admasu Tsegaye (participant): There should be a clear distinction between an orphan and under-utilized crop. For example, tef (*Eragrostis tef*) is an orphan crop but extensively utilized or grown crop. CGIAR centers have financial advantage over those of NARS. On a personal assessment they are doing well based on the funds they get. However, their impact needs to be appraised. Capacity building in NARS should be given priority instead of CGIAR. We need to treat each country in Africa individually rather than as a unit to solve problems.

Promila Kapoor-Vijay (participant): There is a need for biologists and experts to generate information about the importance and conservation of crop germplasm.

Joseph Asiwe: Who neglected the crop? Scientists, media or government? The scientist should work in collaboration with media and government to improve the awareness, funding and research of orphan crops.

Andrew Bennett (conclusion): the main points raised in the discussion are why we need to set-up research agenda and need for regional centers versus national centers. The other value of the meeting is also how to collaborate, to know people and to solve problem. When people share a common goal, they collaborate and things work. Some things have been previously under-appreciated, which we are now clear about, e.g. vegetables. Thank you everyone!

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