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Chapter 4

Parallel sessions: Sustainable food systems and nutrition

4.1 Report of outcomes from the three parallel sessions dedicated to the theme of sustainable food systems and nutrition

Margaret Gill

CGIAR Independent Science and Partnership Council, Rome, Italy
E-mail: m.gill@abdn.ac.uk

Our first session was on “Improving productivity through enhanced resource use efficiency”, the second on “Post-production value addition and food safety” and the third on “Nutrition and food quality”. So you can see that over the three days we covered a huge range of different opportunities for agricultural biotechnologies to make a difference. Our focus throughout was on smallholders.

In the first session, we looked at the application of biotechnologies not just to staple crops, but also to vegetables and livestock and this was a theme throughout our three sessions, where we were saying that it is not just about the staple crops. There is the potential for biotechnology to actually redress some of the balance in terms of the relatively cheap price of the staple crops compared with vegetables, livestock and fish. Trees were also an important part of our sessions and they do contribute to nutrition, as I will briefly cover.

The aim was to look at the application of technologies to those commodities to achieve sustainable intensification, meaning higher yields per hectare or greater efficiency of use of inputs such as water. So we started with a presentation on that key crop, rice, and heard about a project from China, where there are 27 partners, on “Green Super Rice” which hopefully can use less pesticides, less fertilizer, is water-saving, drought-resistant and, at the same time, high-yielding and of superior quality. And this shows the ambition you can have with biotechnologies. Genes have been identified to improve nitrogen use efficiency and tolerance to phosphorus deficiency. Partnerships were important here, but you will see the importance of partners throughout our other sessions as well.

The next presentation was on vegetables. I was very keen to get vegetables into this series of sessions because, as became clear during these three days and as many people have already stated in the literature, the emphasis on staple crops means there has been less research on fruits and vegetables and, from a nutrition point of view, fruits and vegetables should be getting greater focus. The whole concept of diversification of diets was much discussed in the third session, this morning.

One thing that came up in this talk about vegetables was about looking along the value chain and the potential for employment opportunities for youth. We need to put more effort into the value chain, adding value as you go from production through to consumption. Another important point from this talk was the importance of understanding farmers’ needs.

Our next talk was on aquaculture and genetic improvement. As in the opening plenary session, we heard about the potential of biotechnology to control sex ratios. Issues raised in the talk were the use of genomic analysis and the importance of context. That is another theme that came up throughout the sessions; matching our technologies with the needs of different countries and different contexts.

The fourth talk was on bridging the biotechnology–livestock productivity gap in East Africa. We went through dairy cows, pigs and goats in a very interesting talk and, again, we had this concept of matching the breed to the farm context. In pigs we were looking at disease resistance and in goats we heard about the potential for identifying prolific does. But again there was the message: investing in breeding alone, using biotechnologies alone, will not work without paying attention to other issues such as basic management or hygiene.

In terms of trees, I learned in the fifth talk there is a tree species *Allanblackia* whose oil is used for margarine. Also we heard about the nutritive value of food from the forests and the diversity in terms of nutritional context and the use of marker-assisted or genomic selection to match seed to site.

So out of that session, common messages were:

- Biotechnologies exist which can help smallholder farmers.
- Genetics alone does not guarantee increased productivity.
- There is no "one size fits all" solution. We have to match the nature of the improvement, the focus of that type of improvement, to the site and context.
- Potential for different sectors to learn from each other.
- Importance of partnerships in defining research questions and conducting research. But what do smallholder farmers get from research?

The second session, on Tuesday, was on post-production value addition and food safety. It was in two parts. The first was on enhancing value in the post-production phase. We heard about feed additives generated by fermentation, a traditional biotechnology that has been around for a long time, and the opportunities it presents through, for example, efforts to reduce the use of antibiotics in livestock production. The potential for genomics to actually advance the selection of feed additives – to target them more closely – was also discussed.

In the second talk, biotechnologies to increase storability and shelf-life of fruit were discussed. The short shelf-life of fruit causes marketing issues that can be a problem for smallholder farmers trying to get into this area. What I learned is that harvesting early to improve transport has a negative effect on taste and that comes back to another common theme about acceptability. If we target too tightly, then there can be unintended consequences which are negative. We also heard about gene editing, which has the potential to switch individual biochemical reactions on or off, such as ethylene production, particularly important in relation to ripening. We were, very importantly, also reminded that while there is a lot of excitement about all the potential here, there is quite a long time line to take biotechnologies through to the field.

The third talk was about biotechnology tools and capabilities. We heard about the African Orphan Crops Consortium, also mentioned elsewhere in the symposium, which is a good example of the type of partnership we could build on more. And, going back to orphan crops, we heard again about the need to avoid focusing just on the staples but to broaden the focus. If we want to deal with nutrition, if we want to have value addition, we need to be working on more than just the staples. We also heard about the growth and potential of the clustered, regularly interspaced, short palindromic repeats (CRISPR) technology.

The second part of the session was on using biotechnologies to ensure food safety. The first talk was about milk fermentation, where some of the traditional technologies, not just genetically modified organisms (GMOs), were covered. We also heard a talk about Aflasafe, used to reduce aflatoxins in crops. It is a good success story because Aflasafe is a product that works and developing countries, at least some of the governments, are actually investing in its production in their countries. It is fine to get investment in something where you can actually see immediately the response. But, I think it is very significant that there is investment in reducing aflatoxins which cannot be seen and whose impact is often not seen for some time except, as was brought to our attention, in the poultry industry where you immediately see the impact on productivity of the birds.

The final talk was about diagnostics for tuberculosis in cattle in Brazil. Issues covered included problems associated with regulation; differences between testing methods; and the practicality of actually bringing cattle in from the range twice, if you have a complex test.

Common messages from the second session were:

- Biotechnologies exist which can help to enhance post-production value.
- Genomics and gene editing provide new opportunities.
- Biotechnologies on their own are not a "silver bullet". We need communication on all those other aspects which are going to help biotechnologies actually deliver, such as on hygiene/best management practices.
- Time scale from innovation to impact in the field can be 15–20 years.
- GMO discussion needs: we need to have a participatory approach; option of a high-profile champion; use of evidence-based results; separation of the discussion from commercial benefits.
- Importance of policy and regulation and multistakeholder investment.
- Need to consider nutrition, resilience and yield simultaneously.

The focus of the third session was on nutrition and food quality. The first talk was an excellent introduction in terms of giving us the context of the two billion people who suffer from hidden hunger and micronutrient deficiencies. We also heard about the economic cost of obesity and the global figures were similar to those for smoking. Hidden hunger and micronutrient deficiencies are so important for the development of young children. Biotechnology can increase food production, increase nutritional content and, here, biotechnology was mentioned as a potential rallying point across disciplines. One of my key passions is about trying to get scientific disciplines working together.

The second talk was on biotechnologies applied to improve the quality of wheat and rice. We heard about the potential of golden rice, but saw photos of a field trial actually being trashed and heard what that meant to the PhD student involved. Current examples of what can be achieved in rice include beta-carotene, high iron and folate in grains – the potential again is there. One of the key things though is market acceptability. There is no point in producing all these crops if people will not actually buy them. One of the things about rice is the importance of aroma in terms of peoples' acceptance of it. So that is important in terms of food quality.

The third talk was on biofortification of staples. We heard about the success already achieved with some of the biofortified crops such as vitamin A fortified orange sweet potato and also maize. Again, acceptability came into that in terms of different countries having different preferences for orange or white maize, and making it clear that if it is going to be acceptable to the farmers, yields should not be sacrificed to biofortification.

The final talk was on biotechnology to improve nutrition through fish. So, the focus was on aquatic species that need feed. Again, fermentation biotechnology, already out there, is important. Micro-algae or genetically modified yeast can contribute to replacing fish oil in aquaculture feed. This is very important in terms of long-term sustainability so we are not just catching fish to feed fish. Fermentation biotechnology is also important in improving the shelf-life and nutritional value of fish which, again, comes back to taste acceptability and the importance of fish sauces in some parts of the world.

Common messages from the third session were:

- Biotechnology is not new. Fermentation technologies are a traditional way of dealing with many nutritional issues both on the quality side and in terms of food safety.
- Importance of acceptability of new products to consumers – regarding their colour, taste, quality, perception of risk – and to farmers – regarding the economic importance of yield.
- The potential for biotechnology to redress the balance of past high investments in staples through a focus on other healthy crops, as a result of the increasing appreciation and recognition of the importance of a diverse diet.
- Cost of regulations or deregulations to enable new products. If we are moving from more traditional technologies to high-tech ones like transgenics, that cost-effectiveness needs to be built in.
- Dialogue early and with multiple stakeholders – recognizing "language" differences and providing definitions. This point came up throughout the symposium: dialogue early and with multiple stakeholders. I use the word "dialogue" deliberately; it often came up as "communication", but a key thing is to listen as well as to actually get a message across. And also to recognize that if a scientist talks to policy-makers they use different languages. Scientists have to make the effort to get their messages across in a way that policy-makers, the public and farmers understand. It is not enough to say we have a technology let us push it out there. We need to listen and we need to learn.

I hope that I have covered all points from the sessions. Thank you very much.

4.2.1 *Report of the parallel session* Improving productivity through enhanced resource use efficiency[9]

The world will need to improve the efficiency of food production systems in order to feed over nine billion people by 2050. Recognizing the important contributions genetic biotechnologies make to improved resource use efficiency, this session contained a diverse collection of presentations on Green Super Rice (GSR) in China, groundnut development in Malaysia, genetic improvement of fish in tropical areas, livestock in East Africa and forest genetics in Africa. It was chaired by Sergio Feingold, co-chaired by Thuy Nguyen and moderated by Dominic Glover.

Sibin Yu presented a case study on GSR in China where genomic design, traditional selection and marker-assisted selection (MAS) helped identify desirable genes towards the development of new varieties of rice with increased disease resistance, water use efficiency, nutrient use efficiency and growth. A network of research institutes, universities and private industry was created and has developed numerous lines and cultivars for distribution outside of China to Africa and South Asia.

Although major crops have helped improve food security, underutilized crops are important but often have not been the subject of genetic improvement programmes. Sean Mayes described how underutilized groundnut can be improved by the use of genetic markers to assess pollen transfer, genetic diversity and seed quality. By examining related and ancestral species, the research chain and farmers' needs, and by recognizing that many farming systems are low-input or on marginal lands, hundreds of lines of groundnut were developed for worldwide distribution.

David Penman highlighted the numerous genetic technologies that have the potential to improve resource use efficiency in tropical fish. Genetic technologies to improve growth and to control maturation and sex determination include chromosome set manipulation and mono-sex production to take advantage of sexual dimorphism in desirable farming traits, e.g. male tilapia grow faster than females and female rainbow trout grow better than males. Genetic mapping, sex-linked markers, DNA markers and quantitative trait loci (QTLs) are being used to identify superior breeders in both farmed and wild populations while more precise gene editing allows for more targeted modification of the fish genome. Cryopreservation of fish sperm has not had wide application, but can facilitate breeding programmes.

Denis Mujibi presented three case studies from East Africa where cross-breeding is a common strategy. Single nucleotide polymorphism (SNP) markers were used to match cross-breds with their production environment in dairy cattle. Genes from local breeds performed well under local conditions and a high percentage of exotic genes from supposedly improved breeds did not perform significantly better in low- and medium-input systems. SNPs were further used to identify the genetic basis for resistance to African swine fever (ASF). Indigenous pigs were shown to be resistant to ASF; the more

9 This report was prepared by the FAO rapporteurs - Devin Bartley and Julie Belanger

"African" genes a pig contained the higher the resistance. Fecundity in goats is an important farming trait, but the genetic basis for fecundity and the goat's general adaptability to diverse and marginal environments are poorly understood. MAS is being planned to help understand the genetic basis for these traits.

Judy Loo highlighted the special characteristics, advantages, disadvantages and the many uses of trees. Trees are largely undomesticated and genomic selection, genetic modification, cloning and QTLs have only been applied to a handful of species. A case study from Kenya and the African Orphan Crops Consortium highlighted the *Allanblackia* species complex, which is targeted by the industry for its edible oil, timber and medicine. Partnerships have been created to establish supply chains, and for domestication and breeding efforts with the objective to decrease time for seed production, improvements in propagation and in seed dormancy period. Challenges in public–private partnerships were highlighted. Genetic markers are being used in forest management to look at impacts of illegal logging on important traits and in forest restoration, e.g. to match source to site.

Discussion summary

Discussion topics included:

- Clarification of breeding strategies in GSR as to whether they included GMOs: In the case of GSR, conventional breeding of plants with desirable genes was used – genetic modification was not used.
- The probable acceptability of precision breeding and genetic modification for the African Orphan Crops Consortium.
- The value of reducing variability of cross-bred populations to reduce unknown diversity with unpredictable inheritance; productivity of the breed is the important factor.
- Partnerships between scientists and farmers should be sought, as farmers are often innovators/scientists themselves – they try the technologies and form farmer/research networks.
- The value of providing phenotypic information on breeds to link with genotypic data and the vital role farmers play in this process.
- How farmers will benefit or be repaid from biotechnology research when they share knowledge, seeds, wood, etc. with scientists? What in the case patents are developed?
  - Although past examples do not encourage confidence the session stressed that there should be a mutual learning experience with feedback and tangible benefits.
  - Farmers should get improved varieties and provide advice on management.
  - In the case of underutilized species, the knowledge comes from the farmers; therefore it is a moral obligation that sharing information should benefit them.
  - In intellectual property, protection for minor species may not be an issue because large companies may not be interested, but any intellectual property rights should be afforded to farmers.

The session demonstrated that:

- An integrated approach to the application of biotechnology is important.
- Genetic biotechnologies can speed up progress and are useful across sectors to help:
  - produce more food with less land, water and other inputs;
  - identify the genetic basis for important farming traits, e.g. disease resistance, nutrient uptake,

drought tolerance and growth;
  - manage wild and farmed populations;
  - match species and genetic biotechnologies with the environment and growing system, noting that a different collection of genes will be needed for different growing conditions, e.g. high- and low-input systems, or optimal or marginal environments.
- Different sectors can learn from each other.
- It is important to ensure technologies are usable, appropriate to smallholders.
- Orphan crops, breeds and underutilized species, as well as undomesticated species, e.g. many fish and trees, are potential resources for the future.

Partnerships and cooperation are necessary between actors (civil society organizations, academia, private sector, governments, farmers, etc.) for breed development, testing and for wider dissemination of plants and animals with greater resource use efficiency. Finally, biotechnology is not a magic bullet but must be used in conjunction with other aspects of food production.

4.2.2 "Green Super Rice" to be resource saving and environment friendly

Sibin Yu and Qifa Zhang

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University,
Wuhan 430070, China
Presenter's e-mail: ysb@mail.hzau.edu.cn

Sustainable crop production is of predominant concern for food security. The global demand for crop production is expected to double by 2050 as the growing world population is estimated to be over 9.6 billion. This forecast would require the global increase of crop yield to be around 1.5 percent per year, which is a massive challenge in the face of climate change and diminishing resources. Increasing crop production to meet the demand must be achieved in a sustainable manner from less land and fewer inputs of pesticides, fertilizers, water and other resources.

Rice is the main staple food for more than half of the world's population, particularly in Asia where 90 percent of rice is consumed. In the past half century, the increase in rice production has made great contributions to global food security. However, crop production with high-yielding cultivars required high inputs of nutrients, water and labour. For example, in recent years, China has been consuming 55–60 million tonnes of chemical fertilizers annually, which accounts for more than one third of total fertilizers consumed globally. China has also been consuming 31 percent of the total pesticides produced worldwide. In addition, irrigation water is used annually in agriculture, accounting for 70.4 percent of the total water consumption in China, of which 70 percent is used for rice production alone. Excessive use of fertilizers, insecticides and water has resulted in severe problems such as the deterioration of soil, water and the environment, as well as in reduced crop productivity. To achieve continuous enhancement of crop production in a sustainable manner, Chinese scientists proposed the notion of "Green Super Rice" (GSR) with the key characteristics of "less input, more production and better environment", as the goals for agricultural research and crop improvement.

GSR aims to produce more rice of good quality to meet the consumers' demands with higher resource use efficiency and resilience to climate change in crop production. GSR focuses on promoting resource saving and environmentally friendly rice production, while still achieving yield increases and quality improvements. Thus, the new varieties should possess the following characteristics: resistance to major insects and diseases in various rice producing regions, improved nutrient use efficiency, and resistance to drought and other stresses in areas. Furthermore, GSR is not only varieties with the above desirable traits, but also advocates an efficient and environment-friendly crop management. Labour-saving, mechanization and less intensive field management in crop production are now emerging as necessary with the rapid urbanization in China. Such changes require the GSR varieties to have those traits suitable for labour-saving and mechanized crop cultivation.

In the past decade, on the basis of the GSR concept and breeding strategies, we have made tremendous achievements in development and adoption of GSR in rice production in China. For the development of GSR, a combination of strategies has been formulated by integrating germplasm, genomic resources, molecular technology, traditional and genomic breeding with target traits of insect and disease resistances, N- and P-nutrient efficiency, drought tolerance, good quality and high yield. With the rapid advances in functional genomics, a large number of genes identified so far have provided a rich source for developing GSR cultivars. For example, several genes related to root growth are currently available for developing N and P nutrient use efficient rice. Many genes for drought tolerance hold promise for the development of water use efficiency cultivars. Accumulation of these desired genes by genomic design and marker-assisted or genome-based selection would result in progressive improvement of the rice varieties, eventually leading to GSR.

Currently, the Chinese government has launched the mega-project of GSR with the goal to benefit billion farmers to boost rice productivity by 8 percent in the target regions with 30 percent less input. A national network for the GSR project has been established, comprising more than 360 scientists from 21 research institutions, four universities and two seed companies with the strongest expertise in rice breeding and genetic research in China. By using the GSR strategies, the GSR network team has developed thousands of pre-breeding lines with resistance to major insects and diseases, improved drought tolerance, high N- and P-use efficiencies, and other desirable traits in many elite rice genetic backgrounds. The first version of GSR cultivars with stacked favourable genes has accelerated in recent years. Several hundred varieties with the green traits have been tested in many regions of China as well as in other countries.

Almost 100 GSR varieties are now widely available across various rice growing ecological areas in China, where they are helping farmers produce more rice using fewer inputs of pesticides, fertilizers and water, and thus increasing their incomes. So far, about 50 first-version GSR cultivars have been released by national or provincial Crop Variety Approval Committees. Utilization of these cultivars will result in increased rice productivity with much reduced inputs to ensure a great sustainability of rice production. With an international collaborative project supported by the Bill and Melinda Gates Foundation and the Ministry of Science and Technology of China, GSR practices have also spread to other countries in Asia and Africa. Some developed varieties are now being trialed and demonstrated in African and Southeast Asian countries.

Development and demonstration of GSR has set up a successful model with its emphasis on sustainable agricultural production. The notion of GSR has influenced the prioritization of research direction and changed agricultural policies in China. Further activities are underway including determination of key traits for GSR in each target region or country, integration of platform of green genes and pre-breeding lines for developing GSR, procedures for evaluating and releasing GSR varieties in target countries, establishment of efficient crop management for the development and adoption of GSR varieties, and capacity building for widespread application and dissemination of the GSR technologies in agricultural production.

4.2.3 Resource use efficiency in vegetables: Application of molecular breeding to bambara groundnut, an underutilized crop for low-input agriculture

Sean Mayes[1,2], Presidor Kendabie[2], Wai Kuan Ho[1,3], Hui Hui Chai[1,3], Razlin Azman[1], Aryo Feldman[1], Asha Karunaratne[1], Lim Miang Hoong[1], Lim Yin Sze[1], Patrick O'Reilly[1], Festo Massawe[3] and Sayed Azam-Ali[1,2]

[1] Crops For the Future, Jalan Broga, Semenyih 43500, Selangor, Malaysia
Presenter's e-mail: sean.mayes@cffresearch.org

[2] Plant and Crop Science, Sutton Bonington Campus, University of Nottingham, Loughborough, Leicestershire, LE12 5RD, United Kingdom

[3] University of Nottingham Malaysia Campus, Jalan Broga, Semenyih 43500, Selangor, Malaysia.

Intensive agriculture will continue to help in the quest for food and nutritional security. High inputs of nitrogen, growth regulators, irrigation, mechanization and monoculture of yield-selected genotypes have a valid role for the future. Nevertheless, multiple drivers have forced a partial re-think, with efforts underway to reintroduce resource use efficiency traits into major crops, while maintaining yields. Progress is being made at the pre-breeding level with the introduction of exotic germplasm from ancestors and relatives of current major crops. However, re-engineering "resilience" into major crops still leaves food and nutritional security vulnerable to abiotic and biotic stresses associated with extensive and intensive monoculture. In marginal environments often found in the global South (where subsistence or small-scale farming still produces the majority of the world's food), inputs, infrastructure and finance are often not available for high-input, high-return farming, while degraded soils may not support such agricultural systems.

A complementary approach is to evaluate the potential of many of the minor and underutilized crop species which have been grown under low-input agricultural systems for millennia. Such traits and crops may have potential to support agricultural resilience in the face of climate change. In addition, there is some evidence that the dietary nutritional value of such crops can be greater than many of the comparator major crops. However, underutilized crops often suffer from a range of factors which limit their potential. Often very little breeding work has been carried out, the pollination systems of the plants are poorly understood and relatively simple genetic problems remain unsolved. Yet, breeding "elite" cultivars (if that is the aim) is unlikely to be the critical step towards further uptake of such underutilized crops. Farmer requirements and preferences, the existence of markets, value-added products, validated nutritional data and sustainable agricultural systems to grow these species, are all required and their lack can significantly limit progress. Moreover, given that it is estimated that around 7 000 species of plants have been used at some point by humankind, a critical question is how to choose the crops that should be the focus of future collaborative efforts? A sensible approach is to identify crops which already have outstanding trait values, whether for drought tolerance, nutritional content or another characteristic which makes these species potentially valuable. Even in their "unimproved" state such crops have some potential to complement or, in specific circumstances, replace major crops and are likely to exhibit good resource use efficiency after many centuries of selection in low-input systems.

Crops For the Future (CFF) is the world's first research centre with a specific focus on underutilized and minor crops for food, feed, fuel and industrial uses. Based next to the University of Nottingham Malaysia Campus near Kuala Lumpur, Malaysia, it has adopted a research value chain approach, which requires disciplinary input from across the range of academic subjects impacting the growth and use of a crop; from biotechnology to socio-economics. BamYIELD (www.bamyield.org) is a programme within CFF which is focused on using bambara groundnut as an exemplar crop, with the lessons learned in this species being tested in other species. Bambara groundnut (*Vigna subterranea* (L) Verdc) is an African drought tolerant legume which is widely grown in sub-Saharan Africa by subsistence and small-scale farmers. As a legume, it also contributes nitrogen to the agricultural system and non-animal protein to the human diet. We are beginning to apply the approaches developed in winged bean (*Psophocarpus tetragonolobus*), *Amaranthus* ssp. and Proso millet (*Panicum miliaceum*).

Biotechnology and crop genetics

We have developed and used markers to assess pollen transfer, genetic diversity of accessions and for quality control as we develop breeding lines and carry out field work. We concluded that seed from a single plant represents an inbred line. With the International Institute of Tropical Agriculture (IITA), we are developing an extensive collection of lines (500) with genotypes which will be available worldwide to researchers and farmer groups. Working with DArT Pty Ltd, a genotype-by-sequence marker system has allowed us to map the order of markers on the chromosomes of bambara groundnut. Using this approach we have bred lower sensitivity to long day lengths into a number of lines – a trait which can be an issue for pod filling – for testing in 2016 in South Africa, Tunisia and the United Kingdom. The markers also allow us to compare marker and gene positions in related species such as the common bean, which has been far more extensively studied. A coordinated effort to supply germplasm for the African Orphan Crops Consortium through the BamNetwork (www.bambaragroundnut.org) will ensure that sequenced genomes are linked to known genetic lines available worldwide.

Breeding and agronomy

Matching the genotype to the environment is a critical aspect of introducing new crops to new environments. A grant from the third round of the Benefit-sharing Fund of the International Treaty on Plant Genetic Resources for Food and Agriculture will allow common protocols and a core of common genotypes to be trialled with local material in Ghana, Malaysia, Nigeria and Indonesia. This will generate robust data on the environmental influence on nutritional and processing traits. By including the major crop equivalents (peanut, cowpea) in the trials, we will also produce robust data to determine under what circumstances it makes sense to grow the underutilized crop, rather than the major crop. The network of field partners will also be used for selection and breeding work alongside developing collaborations with seed companies in Southeast Asia and Africa.

Meteorology and ecophysiology

Given the predictions of climate change, it is also important to understand how any introduction of a new crop is likely to be impacted by climatic change over time, as well as to know that it is suitable for planting now. Based on trial data from Gaborone (Botswana) we predicted that bambara groundnut has potential in Malaysia, both today and also in the future. We are now generating a comparable set of data in Malaysia alongside actually selecting genotypes within Malaysian field environments, to allow validation of the models. This approach can be applied worldwide.

Nutrition and bioproducts

Many underutilized crops are held back by a lack of nutritional and processing knowledge and how to produce value-added products. These can limit a crop to remaining purely a commodity. We have developed a number of potential products, including vacuum-fried and extruded products, soy-replacement drinks and supplemented wheat flour (which has a better balance of amino acids than either wheat or bambara groundnut flour alone). We are working (non-exclusively) with a major manufacturer in Southeast Asia to develop the supply chain needed for large scale supply of raw materials for such products, with the potential to expand approaches to multiple countries.

Social, economic and policy

Ultimately, the development of underutilized crops must contribute to social and economic benefits for producing communities. Currently, many of these communities are made up of subsistence farmers growing crops in small-scale low-input farming systems, with limited resources, little leverage over the supply chain and a very limited ability to effectively market and/or capture added value which could come from the processing of the raw materials. Work currently underway in United Republic of Tanzania is directly addressing the question of devising appropriate economic metrics for underutilized crops employing bambara groundnut as an exemplar. Further projects are being undertaken with partners in Ghana, Indonesia and Nigeria to develop and test approaches to increasing our understanding of the current role of bambara groundnut in smallholder systems and evaluating its current and potential contribution to smallholder welfare.

4.2.4 Resource use efficiency: Applications of biotechnology in genetic improvement in tropical aquaculture

David J. Penman

Institute of Aquaculture, University of Stirling, Scotland, United Kingdom
E-mail: d.j.penman@stir.ac.uk

Aquaculture is the most rapidly growing food production sector, but modern aquaculture has a short history, of about 40–50 years. Many aquatic species are farmed (541 are reported in FAO production statistics) and others are being tested on a pilot-scale level; several are becoming major global aquaculture species, while some have fallen out of use. Among the dominant fish groups in world aquaculture are the tilapias and carps, which are mostly farmed in tropical and subtropical regions. Catfishes have also seen rapid recent growth in production. Seaweeds, clams and oysters are dominant groups in tropical marine aquaculture.

Several biotechnologies have been developed that have potential to assist genetic improvement and resource use efficiency in aquaculture. These include: chromosome set and sex ratio manipulation to overcome problems with differential growth of the sexes, early maturation and reproduction before harvest; DNA markers, linkage mapping and QTLs are useful in tracking pedigree and identifying genetically superior individuals; cryopreservation can assist with gene banking, storage and transport, and assessing genetic gain; and genetic modification allows for targeted genetic improvement based on the function of specific genes.

The first group (chromosome set and sex ratio manipulation) are appropriate for species-specific problems rather than having generic application for long-term genetic improvement. Thus, there is very little need for such techniques in most large carp species, which reach harvest size before maturation and show relatively little sexual dimorphism at this stage. However, in tilapias, the second ranking group in world aquaculture by production volume, maturation and reproduction before harvest constitute significant problems in most farming environments. Energy is wasted in reproduction and the resultant fry compete with the originally stocked fish, leading to wasted resources and reduced market prices due to lower harvest sizes.

The most commonly used way to overcome this is by dietary treatment of fry with 17α-methyltestosterone, although this is banned in some countries. Triploidy, induced through the application of pressure or temperature shocks to newly fertilized eggs, renders most females sterile and prevents males from producing functional sperm, and has been shown to result in increased yields in experimental trials. However, it is not feasible to use this in commercial tilapia hatcheries due to their reproductive biology: females spawn small batches of eggs frequently and asynchronously, making it impossible to obtain large quantities of unfertilized eggs to induce

triploidy. The production of genetically male tilapia (from crosses between YY males and XX females) has been commercialized on a small scale, but the management of this is complex, partly due to several generations of breeding to produce large numbers of YY males and partly due to the complexity of sex determination in tilapia. The recent discovery of a master sex-determining gene (*amhy*) may help to overcome these problems through the application of marker-assisted selection (MAS). Other species in which these techniques have potential include the African catfish (triploidy to reduce female egg mass at harvest), giant freshwater prawn (all-male production to take advantage of faster growth rate) and oysters (triploidy to reduce maturation and allow all-year marketing).

The second group (DNA markers, linkage mapping and QTLs) is linked to the rapidly developing science of genomics. The genomes of several important aquaculture species (including the Nile tilapia) have been sequenced in the last few years, and more are underway. Polymorphic DNA markers (microsatellites and, more recently, SNPs) have been used in studying the structure of the wild populations of many species (aquaculture still relies on wild fish as broodstock and in some cases for juveniles), for parentage assignment (can be achieved from communally reared mixed families, particularly important in mass spawning species and also to allow communal rearing of families in breeding programmes to reduce environmental effects on selected traits), and in the development of QTL mapping and MAS in aquaculture species. While high-value, high-latitude species have led the way in the application of such techniques (the first QTL to be applied in commercial breeding programmes in aquaculture was one for resistance to infectious pancreatic necrosis, a viral disease of Atlantic salmon; this only happened a few years ago), the potential for the application of DNA markers and MAS in tropical aquaculture species is there and starting to be realized.

Unlike mammals and many plants, gene banking in fish is currently only possible for sperm. The inability to cryopreserve eggs and/or embryos is a serious limitation. However, sperm cryopreservation has been used for gene banking at the start of several breeding programmes as a tool in assessing genetic gain (progeny from the current generation of selection can be compared with crosses between eggs from current generation females and cryopreserved sperm from earlier generation males), and as a way of transferring genetic material between aquaculture programmes. Cryopreservation has not, however, found large-scale use in routine seed production.

Genetic modification in fish was first achieved in the 1980s in China, but progress towards use in aquaculture has been limited due to the negative image of GM animals for consumption. Recently, the United States Food and Drug Administration (FDA) approved a long-running application by AquaBounty for Atlantic salmon with modified growth hormone expression. These fish are produced in a hatchery in Canada and currently grown on in tanks in Panama to reduce the risk of impact from accidental escapes. This case may act as watershed for other potential applications, although public acceptance remains in doubt and is likely to vary from country to country. These GM fish were produced by microinjection of many copies of the DNA construct into fertilized eggs, with random integration of a very low proportion, often creating mosaic insertion patterns. The recent emergence of highly targeted gene editing offers the potential for more accurate genetic modification, which is now being realized at an experimental level.

Although the current impact of biotechnology in genetic improvement in tropical aquaculture is limited, the increasing level of application in temperate aquaculture, the development of breeding programmes for tropical species, current research on tropical aquatic species and the decreasing costs of sequencing and genotyping all indicate increasing impact on production. As will be outlined in the presentation, it is to be expected that this will improve the efficiency of resource use.

4.2.5 Resource use efficiency in livestock: Bridging the biotechnology–livestock productivity gap in East Africa

Denis Fidalis Mujibi

Institute for People, Innovation and Change in Organization (PICO Eastern Africa),
Suite 1, Spring Court, Watermark Business Park, Ndege Road, Karen, P.O. Box 1094,
00502 Nairobi, Kenya
E-mail: fmujibi@gmail.com

Introduction

Africa's indigenous cattle populations are the backbone of the continent's livestock industry, providing sources of income, nutrition and livelihoods to millions of people in marginal areas. However, unit productivity of these animals lags behind most other regions of the world. Recent studies predict significant contraction of mixed farming systems, which represent the majority of food producers in sub-Saharan Africa. Changes in the growing length, disease vector habitat as well as water distribution and availability, require that sustainable intensification of livestock productivity be given priority.

Biotechnology, and specifically genetic improvement, can play a significant role in increasing productivity of livestock in Africa. There is a wealth of information and tools available for such improvement, but they have been poorly utilized for most livestock species because of lack of infrastructure, high initial investment and the long time required for impacts to be made. We present three case studies that illustrate our efforts in applying genetic tools in improving livestock productivity in Africa.

Identification of appropriate cross-breeding levels for smallholder dairy enterprises

The use of cross-bred animals continues to be the basis of most dairy enterprises in Eastern Africa. However, the indiscriminate cross-breeding practised in these systems produces highly admixed animals resulting in huge differences in productivity. Additionally, since the breed composition is unknown, there is often a mismatch between production environment and animal breed type, a situation which cannot sustain the growth and expansion of the local dairy sector.

We have recently applied SNP markers in order to match breed composition with production environment for smallholder dairy cows. The results indicate that there is an optimal level for cross-breeding beyond which increased upgrading to exotic breeds does not result in additional yields. In high-input smallholder production systems, higher grade exotic breeds would be ideal to maximize returns. However, for mid- and lower-input systems, high grade cross-breds do not perform any different from lower grade crosses. In these systems, the overriding considerations would be the percentage of exotic genes that an animal harbours, which needs to be balanced against the unfavourable effects of lower survival and higher disease incidence.

There are opportunities to identify bulls that sire more adapted offspring that produce higher yields. We intend to develop genomic tools that allow mass profiling of animals to identify and propagate tropically adapted high-yielding cows.

Unveiling the genetic basis of African swine fever virus tolerance

Diseases are responsible for enormous productivity losses in Africa. In hotspots where several livestock and zoonotic diseases are endemic, the net effect of disease can be very large, especially when disease control systems are poor. African swine fever (ASF) is a viral hemorrhagic disease of pigs (*Sus scrofa*) that results in complete mortality of infected animals. The disease is endemic in Africa and Sardinia where it is largely restricted. Spread of the disease into major pork producing regions of the world could lead to massive losses to farmers. There is anecdotal evidence that indigenous pigs in Africa are less susceptible to African swine fever virus (ASFv) infection compared to improved international breeds. Apparently healthy pigs have tested positive for the virus or viral antibodies, without clinical symptoms of the disease. However, the determinants of this tolerance are not known.

We used genome-wide SNP markers to investigate the genomic structure of indigenous and improved international breeds in comparison with wild pigs. Wild pigs are resistant to ASF. Results indicate that village pigs that tested negative for ASFv have significantly higher indigenous ("African") ancestry (54 percent and above) compared with those testing positive, which had higher proportions of international commercial breeds. This has significant implications in disease surveillance and diagnosis of infection status. A genome-wide scan detected several regions having signals that indicate preferential selection for genes within those regions. These results point to a possible underlying genetic control to ASF tolerance.

Understanding of the genetic basis for ASFv tolerance will be a great boost in the management of ASF.

Harnessing fecundity in goats

Goats play an important role as a source of meat and income for large numbers of African families. The biology of the goat, particularly its small size (10–80 kg), short generation interval (15 months), prolificacy (up to five live births possible) and short age to market makes it a very attractive prospect for many households, and especially for women and youth. Owing to it being one of the hardiest animals adapted to a host of harsh environments, the goat has been integrated into the social fabric of Africa. The goat's dominance and distribution is testament to the high regard it receives.

The specific genes underlying the capacity of the goat to adapt easily to diverse agroecologies are poorly understood. Current efforts are trying to reverse this lack of knowledge, and activities targeting genomics of disease and adaptation are already underway. One of the research projects underway is looking at improved fecundity in goats. Given the nature of goat production systems, reproduction equals production. Success of the enterprise depends on the number of kids raised, weaned and sold.

West African dwarf goats are extremely fecund. Super does that produce multiple kids have been observed, with twins and triplets being quite common. We are studying the genetic determinants underlying this trait with the aim of increasing the utility of high prolificacy. We intend to produce a marker panel applicable in a MAS framework to identify bucks that sire does that produce more triplets and quadruplets. Access to highly-fecund super does will not only increase incomes for families, but also improve household nutritional status.

Conclusion

Despite the promise that biotechnology presents, there are still many challenges to be contended with, including the high cost of genomic tools, marker systems that are biased towards exotic breeds and the lack of phenotypes to aid in associating performance in smallholder systems with genomic determinants. These limit the application, utility and potential benefits accruable from the use of genomic tools.

4.2.6 Resource use efficiency in forestry: Utilization of tree genetic resources

Judy Loo[1] and Ramni Jamnadass[2]

[1] Bioversity International, Via dei Tre Denari, 472/a, 00057 Maccarese, Rome, Italy
Presenter's e-mail: j.loo@cgiar.org

[2] World Agroforestry Centre (ICRAF), United Nations Avenue, Gigiri, Nairobi, Kenya P.O. Box 30677, 00100 Nairobi, Kenya

What is different about trees?

Among the 80 000 or more species of trees, thousands are important for livelihoods, and for food, fuel, construction, fodder, medicine and other purposes. Tree species differ from agricultural crops in obvious and less obvious ways that affect the use and management of their genetic resources. Trees are large, long-lived organisms that are late maturing, highly diverse and still mostly undomesticated. Gene flow occurs over long distances and trees may produce millions of seeds throughout their lifetime. In addition, they are often managed in some form of the commons; on public land or in community forests.

Table 1. Evolutionary advantages and drawbacks of the tree growth form (from Petit and Hampe, 2006)

Advantage	Drawback
Great potential of biomass gain	High maintenance costs
High competition after successful establishment	Extremely high recruit mortality
Endurance to short-term resource depletion	Increased vulnerability to catastrophic events
Great lifetime fecundity	Delayed maturity
Little dependence on particular reproductive events	Trade-off between present reproductive output and future growth, survival and reproduction
Large pollen and seed production and release height facilitate gene dispersal	Low plant density complicates mating and increases pollen limitation
Local adaptation favoured by strong selection during early life stages	Local adaptation hindered by high gene flow
Low accumulation of mutations per unit of time	Increased mutation rate per generation
Strong inbreeding depression increases outcrossing rate and maintains genetic diversity	Lifelong accumulation of somatic mutations results in susceptibility to inbreeding depression

Tree species make direct contributions to food and nutritional security; for example if 11–13 food-producing tree species were cultivated on each farm in East Africa, a constant supply of vitamins A and C could be assured, including the period known as the hunger gap between harvests of agricultural crops.

At the global scale, forestry investments are increasing; $US80 billion worth of forestry investments were made in 2014 and investments are predicted to continue to rise with increasing returns. In part, this is driven by the need to tackle climate change and deforestation. Investors are not philanthropists, however; they must see returns, and some of the highest returns are such as those described by the Brazilian company, Fibria which is the biggest producer of Eucalyptus pulp. Using the latest biotechnologies, after four generations of cloned genetically improved Eucalyptus, pulp productivity has almost doubled and it continues to increase.

Biotechnology has many applications for trees, including the use of genetically modified *Populus* spp. in China, the increasing possibilities posed by genomic selection, sequencing entire genomes and resequencing, mass production of clones, application of molecular tools for management and improving returns on planting by choosing the right tree for the right place.

Taking advantage of the increasing availability of gene technologies for trees, the African Orphan Crops Consortium (AOCC) was conceptualized in 2010–2011 as "an uncommon public–private partnership under the leadership of Mars, the World Agroforestry Centre (ICRAF), the University of California, Davis (UCD) and the New Partnership for Africa's Development (NEPAD)". The vision of AOCC is "to improve the nutritional content, productivity and climatic adaptability of some of Africa's most important food crops (including 47 tree species); providing a fundamental step in helping to eradicate chronic hunger, malnutrition and stunting in the children of Africa", and it has been endorsed by African Heads of State at an African Union Assembly. ICRAF hosts the AOCC's Genomics Laboratory and the African Plant Breeding Academy where 250 plant breeders will be trained over a five-year period.

A large number of orphan crops in Africa are under study, including several tree species. Genomes for 24 out of 101 species of African orphan crops, including baobab, are already being sequenced by the Beijing Genomics Institute. The ultimate aim is to use the genomic information to improve productivity and nutritional value. For example, just 20 grams of baobab fruit pulp provides twice the amount of calcium as spinach, three times the vitamin C of oranges and four times more potassium than bananas. Increasing production of baobab could significantly improve nutritional outcomes for many people. Twelve of the orphan crop species are undergoing resequencing.

Allanblackia is an important tree genus in Africa; oil from nine species has been used for centuries for cooking and medicine. ICRAF and partners conduct research on these species to establish supply chains and domesticate promising species and genotypes, aiming to reduce the time to fruiting and increase production of oil which is ideal for margarine. In September 2014, Becel Gold margarine, containing *Allanblackia* went on sale in Sweden. More than 10 000 farmers have planted *Allanblackia* with support from 15 rural resource centres that provide training and seedlings. About 250 local buyers provide a link with harvesters and local supply chains operate in the three countries.

Public–private partnerships (PPPs) have been and will continue to be crucial to allow *Allanblackia* to reach its full potential. In agroforestry research, PPPs are not always easy because of differing cultures and expectations between scientists, farmers and investors.

Genetic technologies are also employed in forest management and restoration. Studies carried out by Bioversity International scientists examine how selective logging affects genetic diversity and viability of populations of high value timber species using molecular markers. If tree density is reduced too much by logging, inbreeding increases, reducing seed viability and regeneration success. Genetic diversity may decrease, reducing the evolutionary potential for adaptation to changing conditions. For example, in the Maya Biosphere Reserve in Guatemala harvesting mahogany in community forests significantly increases incomes for members, often making the difference between subsisting below the official poverty and escaping poverty. The community forestry managers must demonstrate that harvesting operations are sustainable as a condition for maintaining their rights to the forest and molecular markers are employed for this purpose. Likewise, a study in the Congo Basin was designed to determine whether harvesting practices in high value hardwood concessions are sustainable; results showed that species vary in their population viability at low densities.

Both ICRAF and Bioversity are working on tools to help choose the right species and right seed source within species for planting. This is particularly urgent considering the massive amounts of seed that will be required to meet the demands for restoration.

Reference

Petit, R.J. & Hampe, A. 2006. Some evolutionary consequences of being a tree. *Annu. Rev. Ecol. Evol. Syst.,* 37: 187–214.

4.3.1 *Report of the parallel session* Post-production value addition and food safety[10]

Innovation in the agricultural sector is essential for feeding a growing world population as greater amounts of food and energy are needed; climate change has also altered the way we grow crops. Concerns over food safety, health and nutrition present new challenges to our societies. The world needs innovations now more than ever as growing demands on global food production supplies require new solutions for our farmers.

The session was organized in two sub-sessions, chaired by Sergio Feingold (INTA, Argentina) and Delia Grace (International Livestock Research Institute, Kenya) respectively. It focused on the issues arising post-harvest, which are as important as production issues, since the spoilage of food or feed (by bacteria or toxin contamination, or natural decay in the case of fruits) has serious implications, diminishing the total yield available. Moreover, these issues have a greater impact on smallholder farmers and their environment, where conditions for storability and shelf-life are often far from ideal. This is especially true for underutilized crops and tropical fruits, where market opportunities are limited by a very short shelf-life.

Addressing these issues, Cadaba Prasad presented the case of animal feed additives to add value to animal welfare and meat quality. An overview was provided on the use of biotechnology for: genomic-based knowledge of gut microbes to advance the selection of feed additives; potential combinations of prebiotics and probiotics to reduce the risk of intestinal diseases; antioxidants from microbial sources; and food additives for modifying gut bacteria for mitigating enteric methane emissions. However, more research is needed to obtain information on standardizing the preparation and delivery of microbial enzymes, probiotics and prebiotics to take full advantage of their potential for using food additives in animal feed to address animal welfare, food safety and environmental issues.

Traditional milk fermentation as a potential tool for sustainable improvement of food safety was discussed by Kohei Makita as an approach to address food-borne diseases. Staphylococcal food poisoning is one of the most common food-borne diseases in the world, and is caused by ingestion of staphylococcal enterotoxin produced in food by certain strains of *Staphylococcus aureus*. Participatory methods were used to investigate the use of fermentation and its efficacy in reducing staphylococcal poisoning. Models were then run on the information gathered. Traditional milk fermentation was found to reduce the risk of staphylococcal food poisoning by 93.7 percent in Debre Zeit, Ethiopia. The study showed the importance of supporting traditional food preparation. Indeed, complementing these methods with provision of efficient strains of fermentation bacteria would be one way to support this.

The use of diagnostic tools to detect pathogens that cause tuberculosis in cattle and prevent their transmission through dairy products to humans was presented by Flábio de Araújo. Mycobacterial species that cause tuberculosis in humans and animals belong to the *Mycobacterium tuberculosis*

10 This report was produced by the FAO rapporteurs - Arshiya Noorani, Devin Bartley and Julie Belanger

complex, including *M. bovis*. An overview of the different methods for detecting infected animals was presented, including intradermal tuberculin tests and interferon-gamma tests, using an enzyme-linked immunosorbent assay (ELISA). The increasing importance of DNA-based approaches, using polymerase chain reaction (PCR) for identification of *M. bovis* in tissue samples and milk, and sequencing the genomes of strains of *M. bovis*, was highlighted.

The session also discussed the use of biotechnologies to increase the storability and shelf-life of fruit. Eric van de Weg discussed the assessment of cultivar diversity of climacteric fruits (i.e. require ethylene for ripening, such as tomato, apple, banana, mango, peach, pears, avocado, and melon). The study showed the genetic basis for range of shelf-life and storability in apple and tomato cultivars. The huge potential that new gene editing technologies offer, in introducing changes to an ethylene gene, for example, was underlined. They may provide great opportunities for crops like mango and banana which are not easy to improve through classical breeding approaches.

Howard-Yana Shapiro discussed the need to focus on the minor crops and highlighted the work of the African Orphan Crops Consortium (AOCC), with diverse stakeholders. The AOCC is working on 101 minor crops of Africa, which are important as sources of micronutrients. The use of host-induced gene silencing for fungal pathogens could significantly reduce levels of aflatoxin contamination. He also described the many applications of gene editing using clustered, regularly interspaced, short palindromic repeats-CRISPR associated protein 9 (CRISPR-Cas9), including as a powerful technology against pathogens. He underlined the enormous potential the breakthrough technology represented.

The discussion on aflatoxins was continued by Ranajit Bandyopadhyay, who described use of the Aflasafe biocontrol product to reduce aflatoxin contamination. The approach focused on the use of native non-toxin producing strains of *Aspergillus flavus* to naturally outcompete aflatoxin-producing strains. Field testing of country-specific Aflasafe products in Burkina Faso, Kenya, Nigeria, Senegal, the Gambia and Zambia for several years has produced extremely positive results in reducing aflatoxin contamination of maize and groundnut consistently by 80 to 90 percent, and even as high as 99 percent. The biocontrol strains carry over through the value chain, discouraging contamination in storage and transport even when conditions favour fungal growth. The study showed how adapting and applying biocontrol solutions to address aflatoxin contamination in Africa could dramatically improve nutrition, health and livelihoods of millions of families while reducing commodity losses due to contamination.

Discussion summary

Discussion topics included:

1) Concern for targeted science for nutrition and resilience and yield. Feed additives, especially for ruminants, should be developed to address the need for decreasing GHG emissions, especially in the face of climate change.
2) There is a need for multistakeholder investments, such as in the AOCC to highlight the value of biotechnology to all sectors.
3) The shelf-life of Aflasafe, which is officially two years, and whether the non-toxic strains revert to

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producing toxins: This is unlikely as the strains are clonal and recalcitrant.

4) Acceptability of genetic modification strategies and how to change public awareness that genetic modification does not equate to biotechnology.
   - One way forward would be to develop initiatives in a participatory manner, with greater dialogue and inputs needed from farmers. The dialogue between farmers and scientists needs to be iterative and continuous.
   - The fear of the new is present. Another option might be for a high-profile champion to convince the public of the safety of biotechnology.
   - The application of the precautionary principle exists but is not applied uniformly, for example to vaccines, rennet, wine yeast and medicine.
   - Need to highlight evidence-based results.
   - There is a need to also dissociate the commercial aspects of biotechnology from the technical aspects.
5) What is the greatest challenge to getting biotechnology to smallholders?
   - The obstacles are often affecting change in policy and regulation, which can in turn be linked to other sectors such as international trade.
   - The knowledge gap is a major challenge. Scientists are not aware of alternative, often traditional, methods to reduce risks of contamination. Greater dialogue is needed among the policy-makers, researchers and smallholder farmers to promote exchange of information.
   - Another challenge concerns the formal versus informal production sectors. Regulation and technology transfer are often effective in the formal sector but many smallholder farmers are within the informal sector. Thus, there is a need to affect change through dialogue with policy-makers to promote awareness-raising and education across the board. Sensitization workshops held for regulators have been shown to be for the entire biotechnology development pathway.

6) The issues of food safety are problematic as they are often unseen, as in the case of the presence of aflatoxins.
   - Integration of various practices is essential and creating incentives for change are also needed through policy innovation. Linked to that is how to reach smallholders. Public interventions are necessary. For example, in Kenya the government funded initiatives in raising awareness of aflatoxin poisoning. Private sector interventions in Senegal were also effective.
   - Therefore, it must be borne in mind that no one size fits all.
7) There is a need for incentives (in terms of costs and benefits) for smallholder farms to increase the uptake of biotechnologies. When farmers are actually shown the benefits of biotechnological approaches, there have been major success stories and high levels of uptake.

4.3.2 Use of feed additives generated through fermentation technologies for livestock feed

Cadaba Srinivas Prasad and Ashis Kumar Samanta

National Institute of Animal Nutrition and Physiology, Bengaluru, India
Presenter's e-mail: cadaba_prasad@yahoo.co.in

The primary aim of the livestock industry is to produce safe and high-quality animal products after due consideration of consumer awareness, public health, ethical issues and the environment. Feed additives occupy a significant niche amongst the list of scientific interventions that guide the enhancement of livestock production for augmenting the growing demands for animal products by the global population. However, application of some of those feed additives, such as antibiotics, raises grave concerns for both the animal industry as well as public health. As a result, several countries have already imposed bans on the addition of antibiotics to feed. This emphasizes the need to develop alternative strategies to enhance nutrient availability for achieving higher productivity and/or quality. Evidently, feed additives sourced through fermentation technologies seem to be the front runner to cater for the new and emerging challenges of the livestock sector.

Put simply, feed additives are defined as non-nutritive ingredients that stimulate selectively the growth or enhance the performances (quality/quantity) of animals through improvement of nutrient utilization and uptake. Presently, feed additives are also advocated for maintaining the health of animals. The use of feed additives is much greater in poultry and pig production than in ruminants. One of the simpler ways of producing feed additives is through microbial fermentation. The feed additives generated through microbial fermentation technologies can be classified broadly into prebiotics, probiotics, enzymes, yeast embedded minerals and amino acids.

Prebiotics

Prebiotics are non-digestible bio-active molecules that affect the host beneficially by stimulating selectively the growth and/or activity of one or a limited number of bacteria in the gastrointestinal tract of an animal. Although, most feed additives target only one or a limited number of functions, prebiotics target a range of different physiological functions such as increase in gut health, mineral absorption, immune stimulation and pathogen exclusion, and reduced cholesterol. Commercially available prebiotics are inulin, fructo-oligosaccharides, galacto-oligosaccharides, mannan-oligosaccharides, xylo-oligosaccharides etc.

Supplementation using prebiotics in the diets of poultry and swine enhance the population of beneficial gut microflora followed by decreasing pathogenic microflora in addition to improving mineral absorption, immunity and growth performance. As the demand for prebiotics is increasing, researchers around the world are enriching the "nutraceutical basket" with newer ones, and attempts to use agricultural residues like straws, coconut husk and grasses for production of prebiotics are gaining importance.

Probiotics

A probiotic is defined as "a live micro-organism which, when administered in adequate amounts, confers a health benefit on the host". The beneficial effects of probiotics are manifested by regulation of intestinal microbial homeostasis, stabilization of the gastrointestinal barrier function, secretion of bacteriocin and immuno-modulatory effects. The probiotic organism may originate either from prokaryotic (bacteria) or eukaryotic (yeast) cells. The bacterial probiotics are *Lactobacillus, Enterococcus, Bacillus, Streptococcus* spp., among others. In the eukaryotic class of probiotics, the important genera are *Saccharomyces, Kluyveromyces* and *Aspergillus*. Among the several species of yeasts, *Saccharomyces boulardi* is a commonly used probiotic in both ruminant and non-ruminant species. Administration of a *Lactobacillus*-based probiotic reduced the population of *Salmonella enteridis* in challenged broiler chicks. Daily dosing of piglets with *Enterococcus faecium* as a probiotic supplement reduced the incidence of diarrhoea and improved daily weight gains.

Enzymes

The rearing of animals and the use of enzymes have been distinct parts of human life for many thousands of years but it is only recently that their use is increasing due to drastic reductions in the costs of enzyme production. The major enzymes that are being used in animal feeds are classified broadly into four categories namely: 1) fibre-degrading enzymes; 2) protein-degrading enzymes; 3) starch-degrading enzymes; and 4) phytic acid-degrading enzymes. As the name implies, each enzyme can act specifically on a particular substrate and ensure benefits to the animals either by unlocking the energy, by releasing amino acids, by complementing the animal's own enzyme activities or by releasing nutrients from unbreakable linkages such as phosphorus from phytate using phytase. Phytate removal by phytase also enhances the availability of minerals to animals.

Yeast embedded micronutrients

Chromium (Cr) and selenium (Se) have been found to be essential for ruminants as well as monogastrics due to their anti-oxidation properties. They also improve immunity and reduce thermal stress. However, inorganic forms of these minerals are less bio-available and use of organic Cr and Se in the form of Cr-yeast and Se-yeast is being extensively studied due to their enhanced bio-availability. Cr-yeast has been found to both improve immunity and also reduce cholesterol content in eggs. Se-yeast supplementation in poultry during peak summer temperatures reduced the ill-effects of thermal stress and improved the anti-oxidant status.

Amino acid production through fermentation

Inefficient utilization of absorbed protein in ruminants and monogastrics can lead to high nitrogen losses. Matching the animal's dietary amino acid levels with their biological needs minimizes nitrogen excretion to the environment. Providing the essential amino acids (lysine, methionine, threonine, glutamic acid) would help in reducing the protein content in the feed leading to better utilization of nutrients, less wastage of nitrogen and reduced feeding cost.

Use of feed additives is limited in smallholder ruminant production systems because their effects are not consistent. Also, high cost prohibits their use. However, their use in smallholder poultry and pig production system is higher. Future research to understand better the conditions under which they produce consistent, long-term and greater effects would enhance their use in animal diets.

4.3.3 Use of biotechnologies to increase the storability and shelf-life of fruit

Eric van de Weg, Ernst Woltering and René Smulders

Wageningen University and Research Centre, Droevendaalsesteeg 1, P.O. Box 386, 6700 AJ Wageningen, the Netherlands

Presenter's e-mail: eric.vandeweg@wur.nl

Storability and shelf-life are major determinants of the economic value of fruit and determine the risk for loss of produce due to decay. Extended storability and shelf-life supports the delivery and consumption of high-quality fruit during a longer time period, thus improving market prices (avoiding dumping prices due to over-supply), food availability, income, job opportunities and economic sustainability, especially of small producers.

Improved storability and shelf-life have been successfully addressed through the breeding of new, well-adapted cultivars in a series of crops following classical breeding approaches. New biotechnology techniques that recently came available may further accelerate the coming availability of new cultivars, especially for tropical crops for which little breeding activity is currently ongoing.

For the application of biotechnology in breeding, especially climacteric fruit may have potential. In climacteric fruit, ripening is accompanied by a burst of ethylene production and respiration. The ethylene produced stimulates ripening. Being a gas, ethylene from a ripe fruit can also stimulate the ripening of other (unripe) fruit (e.g. at the household level, a ripe banana may be wrapped together with unripe mangoes). Tomatoes, apples, bananas, mangoes, peaches, avocados and melons, among others, are climacteric. Non-climacteric fruit like citrus, grapes, watermelons and strawberries do not produce ethylene and respiration bursts and do not need ethylene for ripening.

In view of storability, shelf-life and transport over long distances to, for example, export markets, climacteric fruit is harvested unripe: the earlier harvested, the better the storability. Later, close to the point of sale, ripening is forced by the external application of ethylene. This is common practice for crops like banana, mango and avocado. However, the early harvesting of unripe fruit has negative effects on flavour attributes including texture, juiciness, sweetness and aroma. When harvested too unripe, the fruit will never become as tasty as a tree-ripened fruit.

Thanks to breeding and biotechnology, the shelf-life of tomatoes has been extended from seven days to more than a month. Freshly-picked apples have a shelf-life of 2–4 weeks, depending on the cultivar. Apples can be stored for another two weeks and up to four months under temperature-controlled conditions, and even for up to 13 months when the atmosphere in the cold room is also controlled (low oxygen, high CO_2). This variation in storability results from variation in the genetic composition of cultivars. More precisely, it is based on natural variants of a very limited number of genes that are involved in ethylene production or the ethylene perception pathway, or in the production of specific cell wall-degrading enzymes. In tomato, natural variants of three specific genes are involved. The taste of tomato is "repaired" by enhancing other taste-related pathways. In apple, seven such genes are involved which slow down ripening without compromising on taste.

Classical breeding approaches include the search for the desired trait in wild germplasm or raising the desired trait by the creation of gene variations through, for instance, radiation. Next, these new sources are used in crosses to raise new progenies, the best of which are selected and released as cultivars. Through time, diagnostic molecular markers have been developed to support breeding: these allow the desired trait to be traced more efficiently in the progenies.

Classical genetic modification approaches have led to the approval of a limited number of GM cultivars in melon and tomato which target storability and shelf-life. They were introduced between 1992 and 1999 and none of them are on the market anymore. Besides lack of public acceptance, the techniques of that time were still crude so that too many other traits may have been affected negatively. Other fruit crops for which GM cultivars have been approved are apple (2015), eggplant (2013), plum (2007) and papaya (1998), all of which except plum are in commercial production. That of plum is held in stock deliberately until an outbreak of plum pox virus shows up in the United States of America.

Recently, new biotechnologies have been developed that are now turning out to be true game changers. They allow removal or change of just a few nucleotides of a gene in a very precise way. Such minor changes in an ethylene gene, for example, may have major effects on ethylene production and hence on storability and transportability, especially when applied on the promotor region of such a gene. This is because each promotor region has several on/off switches that determine the activity of the gene in a tissue and in a time-specific manner. In principle, this would make it possible to hamper or delay the production of ethylene in only that fruit which is close to maturation. At that time, the fruit is not only sensitive to external ethylene applications, but is also ready to produce all the specific components that give it its delicious taste. Moreover, in the near future, modifications brought about through this new procedure may not fall under GM regulation.

These technologies may offer great opportunities for crops like mango and banana which are not easy to improve through classical breeding approaches. Small-scale growers may be the first to benefit through immediately extended periods of sale, whereas large-scale export production will follow when production volumes allow this. Still, it may take 15–20 years from application to marketable fruit because of the various successive stages in production that have to be passed, from the actual application of this technology (years 1–3), to the cultivation of small *in vitro* plantlets to fruit-producing trees (years 3–6), selection of the best performing new plants (years 6–8), multiplication of the few initial copies of these best plants to 10 000s of trees, completion of the intellectual property and admission procedures (years 9–12), plantation of orchards (year 13) and first production of fruit (year 15). An excellent overview on new biotechnology and breeding techniques can be found in Schaart *et al.* (2015).

Reference

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4.3.4 The promise of biotechnology tools to help control mycotoxins, especially aflatoxins

Howard-Yana Shapiro[1,2,3,4]

[1] Chief Agricultural Officer, Mars Advanced Research Institute Fellow, Mars, Incorporated

[2] Senior Fellow, Plant Sciences, College of Agricultural & Environmental Sciences, University of California, Davis, the United States of America

[3] Science Advisor, MIT Media Lab, Massachusetts Institute of Technology, Cambridge, MA 02139-4307, the United States of America

[4] Distinguished Fellow, World Agroforestry Centre, Nairobi, Kenya

Mycotoxins represent an ongoing threat to global food safety and security. Aflatoxins are a common food contaminant representing 20 chemically-related toxins produced by *Aspergillus flavus* and *Aspergillus parasiticus.* Twenty-five percent of food crops are contaminated by aflatoxin, exposing 4.5 billion people annually. The greatest impact is on the impoverished, malnourished and young. Although a global problem, sub-Saharan Africa has the greatest prevalence of mycotoxin-related health issues – premature deaths in women, stunting in children and a high prevalence of liver cancers. Mycotoxins appear to have a greater impact on those who are immune-compromised and may contribute to a worsening of this compromise. The impact of chronic low-level exposure has not been well documented. In the United States of America, food limits are 0.5 micrograms per kilogram.

Sixty percent of agriculture is produced by smallholder farmers who represent five out of six producers. Of those smallholders, most are struggling economically and half are women. Mycotoxin contamination threatens the food supply, reduces the amount of available food product and degrades its value. Estimates place food loss and waste at about 1.3 billion tons annually with 40 percent occurring in developing countries at post-harvest and processing. Mycotoxins, in particular aflatoxins, are present in many raw materials and are not destroyed by normal food processing.

Industry focus for controlling mycotoxins has been directed: 1) at the field level, with interventions such as improved agricultural practices, better crop varieties and specific treatments during production to control fungal growth and insects; 2) at harvest, by controlling moisture content, assuring crop maturity and reducing plant diseases; 3) at and during storage, by controlling temperature, moisture and insects, improving sampling methods and increased training of personnel to detect contamination; and 4) at and during manufacture, by maintaining clean and sanitized equipment, controlling process temperatures and humidity and improved processing of raw products. With the information available, little progress has been made beyond the rejection of raw materials and refusal to utilize contaminated raw products in human foods or animal feeds. Aflatoxin production is dependent on temperature, moisture, pH, low oxygen, high CO_2, and oxidatively-damaged lipids and available sugars.

New methods for controlling mycotoxins, and specifically aflatoxins, need development. Progress in the areas of gene editing, mechanisms of innate plant immunity, and specific applications of RNA interference (RNAi) concepts provide an opportunity to approach the problem at an earlier point of production in the field. Definitions of RNAi and other biotechnology-related terms used in this summary are provided in Gaj, Gersbach and Barbas (2013).

Since the first reports of RNAi in 1998, a new understanding of how cells modulate gene expression using RNA has created numerous opportunities for modulating the host-pathogen relationship (Fire *et al.,* 1998). A number of naturally occurring systems have been identified that utilize RNAi as a basis for controlling gene expression in both plants and animals. Dicer, an endonuclease, cleaves double stranded RNAs (dsRNA) into small interfering RNAs (siRNA) and microRNAs which induce the RNA-induced gene silencing complex (RISC) and is essential for the induction of innate immunity in plants (R resistance) (Bernstein *et al*., 2001; Baker *et al*., 1997; Fang *et al*., 2011). CRISPR/Cas9 controls the expression of plasmids and phage genes in microbes; CRISPR processes DNA and forms microRNAs through transcription to use siRNAs to control the expression of these newly introduced genes (Ishino *et al*., 1987; Marrafini and Sontheimer, 2010). P-element induced wimpy (PIWI) testis silences transposons in eukaryotic stem cells by detecting and eliminating dsRNA (Cox *et al*., 1998; Saito *et al*., 2006).

A number of additional pathways use similar mechanisms ultimately for gene silencing. RISC represents a variety of systems that are triggered by dsRNAs and which are fragmented subsequently into short nucleotide sequences (10–20 nucleotides) and complexed with the Argonaute protein allowing for the identification of complimentary RNA transcripts, initiating the gene silencing process. Gene silencing occurs by blocking translation and stopping protein synthesis, degrading targeted mRNA, altering the reading frame or by degrading DNA (Hsu, Lander and Zhang, 2014).

Recently, guide RNAs that target specific complimentary sequences have been produced providing new methods for utilizing CRISPR/Cas9 and Argonaute protein within an organism through RNA-guided DNA endonucleases. Advances using zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALEN) are providing gene identification and sequence-specific DNA binding modules that are coupled to a non-specific DNA cleavage domain allowing gene editing and silencing. DNA cleavage may be double-stranded or single-stranded depending on the endonuclease (Gaj, Gersbach and Barbas, 2013).

A number of research teams have been working with RNAi systems to control host-pathogen interactions. Host-induced gene silencing (HIGS) has been used in a variety of leaf and grain crops to limit damage from fungal diseases, nematodes and insects. Several studies seek to inhibit specific pathogens. Transgenic plant varieties have been developed that produce siRNAs that target specific gene sequences unique to a specific pathogen's survival. The host produces the siRNA, which is taken up by the pathogen with the silencing of essential genes within the pathogen with no impact on the host. Demonstration studies have been conducted in lettuce against downy mildew, *Bremia lactucae.* In this study, two genes in the pathogen were successfully inhibited resulting in a significant reduction in fungal growth and sporulation (Govindarajulu *et al*., 2015). Another recent study demonstrated the induction of resistance to *Fusarium* in Arabidopsis and barley using the dsRNA directed at the three gene forms of cytochrome P450 lanosterol C14 alpha demethylase (Koch *et al.,* 2013). Similar early demonstrations have been published indicating the effectiveness of RNAi on insects (Huvenne and Smagghe, 2010). The control of nematodes is also feasible using RNAi silencing (Huang *et al*., 2006).

These studies clearly demonstrate a maturing area of science where toxin production by contaminating fungi can be controlled in food crops, improving the amount of food produced and safely consumed. Populations in the developing world will have significant health benefits in those who are in greatest need of relief.

HIGS is an emerging mechanism that needs development in the area of mycotoxin production and control. Focused studies on the potential of gene silencing of the twelve genes associated with toxin production in *Aspergillus flavus* are required if better food safety is to become a global reality. The morbidity and mortality of aflatoxicosis justifies the development of a progressive research programme to reduce mycotoxin contamination to near zero in the next 10 years. Motivation, not the maturity of the science or technology, seems the current limitation in mycotoxin eradication.

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4.3.5 Traditional milk fermentation as a potential tool for sustainable improvement of food safety

Kohei Makita[1,2], Fanta Disissa[3], Akafete Teklu[3], Girma Zewde[3] and Delia Grace[1]

[1] Food Safety and Zoonoses, International Livestock Research Institute (ILRI), P.O. Box 30709, Kenya

[2] School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, 069-8501, Japan
Presenter's e-mail: kmakita@rakuno.ac.jp

[3] Faculty of Veterinary Medicine, Addis Ababa University, P.O. Box 34, Debre Zeit, Ethiopia

Every year, 1.5 million people die due to diarrhoea, and animal-source foods are an important source of food-borne diseases (FBDs). FBDs include non-diarrhoeal serious zoonoses such as brucellosis and tuberculosis. A large proportion of FBDs occur in developing countries where informal markets dominate in the food supply and improvement of food safety through implementation of public services is a challenge in these settings. The objectives of the study were two-fold: to prove that participatory risk assessment can be applied to informally marketed foods; and to assess the risk of staphylococcal poisoning through consumption of raw milk and home-made yoghurt in Debre Zeit, Ethiopia.

Staphylococcal food poisoning is one of the most common FBDs in the world, and is caused by ingestion of staphylococcal enterotoxin (SE) produced in food by certain strains of *Staphylococcus aureus*. *S. aureus* starts producing enterotoxin in milk when the population density reaches about $10^{6.5}$ colony-forming units per millilitre (CFU/ml) of milk and thereafter the amount of SE increases linearly with time. A small amount of SE, 100–200 ng, can cause illness. Intoxication is characterized by the sudden onset of nausea, vomiting, abdominal cramps and diarrhoea. The optimum pH for *S. aureus* growth is 7 and the minimum pH is reported to be 4.9. In this summary, contribution of traditional milk fermentation to food safety found by the risk assessment is described.

The study sites were urban and peri-urban areas of Debre Zeit, Ethiopia. Rapid urban appraisals were combined with conventional interviews to identify and quantify formal and informal milk value chains and to collect information on consumers' food preparation and consumption behaviour. Milk was sampled in 170 dairy farms and five milk collection centres and microbiological tests were conducted. Published data on milk fermentation in Ethiopia were used to estimate the time when pH became lower than 4.9 after milking, to model the stop of growth of *S. aureus*. A published mathematical growth model of *S. aureus* was used to model the competition between SE production by *S. aureus* reaching $10^{6.5}$ CFU/ml, and stop of bacterial growth due to traditional milk fermentation reaching low pH values. The growth of *S. aureus* was dependent on the initial bacterial population in milk, temperature (storage of milk in room or refrigerator) and length of storage time before milk consumption. A system from production to consumption was stochastically modelled in @Risk, and Monte Carlo simulation was run for 10 000 iterations. Sensitivity analysis for all the uncertainty

parameters was run for 1 000 iterations.
Prevalence of *S. aureus* in five milk collection centres (72 percent, 18/25) was significantly higher than in bulk milk samples at dairy farms (43.5 percent, 74/170, x^2 = 5.99, df = 1, p = 0.014). No dairy farmers boiled milk for sale. Consumption of raw or fermented milk was common and there was no significant difference in the probability of boiling between farmers (68.2 percent, 116/170) and consumers (64.0 percent, 16/25, x^2 = 0.038, df = 1, p = 0.85). The annual incidence rate of staphylococcal poisoning was estimated to be 20.0 (90 percent CI: 13.9–26.9) per 1 000 people. When the effect of fermentation was removed from the model, the annual incidence rate increased to 315.8 (90 percent CI: 224.3–422.9) per 1 000 people, showing the importance of traditional food preparation methods in risk mitigation; traditional milk fermentation reduced the risk of staphylococcal food poisoning by 93.7 percent. Sensitivity analysis found two very sensitive factors: the initial population of *S. aureus* and the storage temperature of milk.

Improving the safety of milk and dairy products could be achieved through supporting appropriate traditional food preparation and consumption where an industrial risk mitigation system is not feasible. For example, provision of good strains of fermentation bacteria to farmers and consumers can be a highly effective and sustainable intervention for improving food safety. In industrialized settings, communication to avoid mixed use of refrigerator and natural fermentation of raw milk may be necessary. Improvement of milk hygiene was another important factor to reduce illness.

4.3.6 Aflasafe: A case study for aflatoxin reduction in crops

Ranajit Bandyopadhyay[1], Peter J. Cotty[2], Adebowale Akande[3], Juliet Akello[4], Joseph Atehnkeng[5], Joao Augusto[6], Abuelgasim Elzein[1], Lawrence Kaptoge[1], George Mahuku[7], Charity Mutegi[8] and Lamine Senghor[9]

[1] International Institute of Tropical Agriculture (IITA), Oyo Road, Ibadan, Nigeria
Presenter's e-mail: R.Bandyopadhyay@cgiar.org

[2] United States Department of Agriculture Agricultural Research Service (USDA-ARS), School of Plant Sciences, University of Arizona, Tucson, the United States of America

[3] IITA, Abuja farm, Kubwa Village, Abuja, Nigeria

[4] IITA, Lusaka, Zambia

[5] IITA, Lilongwe, Malawi

[6] IITA, Nampula, Mozambique

[7] IITA, Mikocheni, Dar-Es-Salaam, United Republic of Tanzania

[8] IITA, Nairobi, Kenya

[9] Direction de la Protection des Végétaux (DPV), Dakar, Senegal

Food quality and safety issues resulting from aflatoxin contamination are significant obstacles for improving nutrition and agricultural production while linking smallholder farmers to markets. Aflatoxin exposure is frequent and widespread in most African countries where the key staples maize and groundnuts are particularly vulnerable to aflatoxin contamination. Aflatoxin poses a significant public health risk in many tropical developing countries and is also a barrier to the growth of domestic, regional and international markets for food and feed. The most documented health impact of chronic exposure to aflatoxins is liver cancer. Broader health effects such as child stunting and immune suppression with higher rates of illness have also been associated with aflatoxin exposure. Aflatoxin-contaminated feeds decrease livestock productivity. Aflatoxin contamination has also led to the destruction of hundreds of thousands of tonnes of grains, contributing to huge losses of much-needed income, food and trade with health and food security consequences.

An innovative scientific solution in the form of a natural biocontrol has been developed by USDA-ARS. This breakthrough technology, already in wide use in the United States of America, reduces aflatoxins during both crop development and post-harvest storage, and throughout the value chain. Atoxigenic strain based biological control is a natural, non-toxic technology that utilizes the ability of native atoxigenic (incapable of producing aflatoxins) *Aspergillus flavus* to naturally outcompete their aflatoxin-producing cousins. IITA and partners have successfully adapted this competitive displacement technology for use on maize and groundnut in various African countries using native microflora, developing biocontrol products called Aflasafe. We describe progress made with the development of biocontrol of aflatoxins in Africa, the current status and prospects for further scaling up in maize and groundnut value chains.

Field testing of country-specific Aflasafe products in Burkina Faso, Kenya, Nigeria, Senegal, the Gambia and Zambia for several years has produced extremely positive results in reducing aflatoxin contamination of maize and groundnut, consistently by 80–90 percent, and even as high as 99 percent. The biocontrol strains carry over through the value chain, discouraging contamination in storage and transport even when conditions favour fungal growth. Positive influences of atoxigenic strain applications carry over between crops and provide multiyear benefits. A single application of atoxigenic strains may benefit not only the treated crop but also rotation crops and second season crops that miss a treatment. Additionally, because fungi can spread, as the safety of fungal communities within treated fields improves so does the safety of fungal communities in areas neighbouring treated fields. The excellent efficacy of biocontrol in reducing aflatoxin in these countries has led to the expansion of the programme to other countries in East (Burundi, Rwanda, Uganda and United Republic of Tanzania), West (Ghana) and Southern (Malawi, Mozambique and Zambia) Africa.

To make the biocontrol product available to farmers and other end users, a manufacturing plant (capacity 5 tonnes/hour) has begun to produce Aflasafe in Nigeria. A small-scale modular manufacturing plant is under construction in Kenya. A model for creating sustainable market demand for Aflasafe in maize value chains is being piloted under the AgResults Aflasafe Initiative in Nigeria where farmers have purchased Aflasafe to treat about 30 000 ha of maize crop (application time: 2–3 weeks before crop flowering; application rate: 10 kg/ha; cost of product: US$12–US$18.75/ha). Farmer groups that treated their maize crop with Aflasafe sold grains at a 13–15 percent premium to food and feed processing industries. These farmers also retained a portion of the treated crop for home consumption thereby improving the safety of food for their families.

The Kenyan Government is providing a Kenyan Aflasafe product to smallholder farmers to treat almost 23 000 ha in aflatoxin-prone areas as a public good in public health interest and to improve marketability of maize grains. Initial results are very encouraging – all the maize harvested from several hundred ha of treated maize crops in the government's food security initiative in the Hola/Bura/Galana irrigation scheme met the stringent European Union standard (four parts per billion). A Senegalese agribusiness firm provided 20 tonnes of Aflasafe to its contract growers in 2014 and 2015 to improve the safety and marketability of groundnuts procured from the farmers.

Smallholder farmers harvest, store and consume home-grown crops. The deployment of Aflasafe can profoundly improve safety of food of smallholder farm families and reduce post-harvest losses since the technology dramatically reduces the source of contamination in the field before harvest, during storage and until maize/groundnut is consumed. Reduced crop contamination could translate into improved food security and better access to domestic, regional and international markets that pay premium for aflatoxin standard-abiding maize and groundnuts. Scaling up of biocontrol has also the potential to revitalize exports and to increase smallholder farmers' opportunities to access premium export markets where aflatoxin-safe grain is a prerequisite for trade. For realizing health and income improvements, the biocontrol technology must be scaled up to reach the various players in the maize and groundnut value chains by developing sustainable product manufacturing and delivery mechanisms. Several challenges remain for scaling up. To address these challenges, the next phase of Aflasafe development is geared towards technology transfer to the private or public sector (as per situational analysis) and commercialization of the product in 11 countries.

4.3.7 Diagnostic tools to detect pathogens causing tuberculosis in cattle and prevent their transmission through dairy products to humans

Flábio R. de Araújo[1], Nalvo F. Almeida[2] and Martin J. Zumárraga[3]

[1] Embrapa Beef Cattle, Campo Grande, MS, Brazil
Presenter's e-mail: flabio.araujo@embrapa.br

[2] School of Computing, Federal University of Mato Grosso do Sul, Brazil

[3] Biotechnology Institute, National Institute of Agricultural Technology (INTA), Buenos Aires, Argentina

Mycobacterial species that cause tuberculosis in humans and animals belong to the *Mycobacterium tuberculosis* complex (MTBC), including: *M. tuberculosis*, *M. africanum* and *M. canettii*, which are mainly human pathogens; *M. bovis* and *M. caprae,* which are mainly ruminant pathogens; *M. microti*, a pathogen of small rodents; *M. pinnipedii,* from marine mammals; *M. mungi*, from mongooses; and *M. orygis*, from oryx.

Members of the MTBC have been associated with food-borne transmission to humans. The consumption of contaminated raw dairy products has been recognized as a major cause of transmission of *M. bovis* to humans, generally associated with the development of extra pulmonary tuberculosis. Another MTBC species that infects man is *M. caprae*. Although the transmission to humans by raw dairy products has not been proven formally for *M. caprae*, the relatedness of the pathogens and the epidemiological settings suggest that this is probably the case.

Current bovine tuberculosis (bTB) eradication programmes are based on a screening and slaughter policy, using mainly the intradermal tuberculin test, which detects the cell-mediated immunity (CMI) to the injection of purified protein derivative (PPD), a mixture of proteins prepared after a heat treatment and lysis of *M. bovis* AN5 (bovine PPD) and *M. avium* D4ER or TB56. The single intradermal tuberculin test (SITT) and the caudal fold tuberculin test (CFTT) both use bovine tuberculin, while the comparative intradermal tuberculin test (CITT) uses both bovine and avian PPD. The CITT is used to differentiate between animals infected with *M. bovis* and those responding to bovine tuberculin as a result of exposure to other mycobacteria.

Advantages of the intradermal tuberculin tests and reasons for their wide use are low costs and low logistical demands, and a well-documented use. Limitations include difficulties in administration and interpretation of results, need for a second-step visit, low degree of standardization and imperfect test accuracy. False-negative reactions are also a concern, since infected cattle may remain in herds. In Brazil, there is strong evidence of a resurgence of bTB in accredited-free herds due to infected cattle not being responsive on CITT. Also, when results of intradermal tests are inconclusive, it is necessary to wait at least 60 days before repeating the SITT or applying a CITT. This mandatory interval requires cattle to be kept in quarantine and it increases the risk of spreading the disease to herdmates and potentially to humans.

The interferon-gamma (IFN-γ) assay also detects the CMI on tuberculosis-infected cattle. In this test, sensitized lymphocytes from infected cattle are incubated *in vitro* for 16–24 hours with PPD and the released IFN-γ is detected with a sandwich enzyme-linked immunosorbent assay (ELISA) that uses two monoclonal antibodies to bovine gamma interferon. In animals that are difficult or dangerous to handle, the advantage of the IFN-γ test over the skin test is that the animals need to be captured only once. Another advantage is that since lymphocyte stimulation is done *in vitro*, it is not necessary to wait 60–90 days to repeat the test when the initial test is inconclusive.

Bovine tuberculosis is an infection that triggers predominantly a CMI during early and intermediate phases of the infection. Therefore, the main diagnostic techniques used worldwide in eradication programmes are based on the detection of the CMI: intradermal tests and interferon-gamma (IFN-γ) assay. As the disease progresses, there is a decrease in CMI and the development of serological responses. The importance of antibodies for the diagnosis of bovine tuberculosis has been debated because of the variable sensitivity (18–73 percent) reached with serological assays in preliminary studies, although high specificity has been observed (88–96 percent)

Recent studies have re-established interest in serological assays as diagnostic tests to detect false-negative animals in the intradermal tests and the IFN-γ assay. In animals with experimental infection, the serological response has been shown to increase after performing intradermal tests (anamnestic effect), leading to an improvement of the sensitivity of these techniques. The serological response varies depending on the different antigens. The Brazilian Agricultural Research Corporation (Embrapa) has developed an ELISA for detection of *M. bovis* antibodies based on a fusion recombinant antigen with the hydrophilic domains of 6 kilodilation early secreted antigen test (ESAT-6), MPB70 and MPB83 proteins. This ELISA has been used to detect infected animals missed by the CITT.

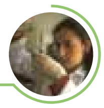
In Brazil, the control of bTB is regulated by the Brazilian National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis (PNCEBT). These regulations involve the slaughter of cattle with positive reactions to the intradermal tuberculin test (*ante-mortem* diagnosis) and the inspection of carcasses for gross lesions in abattoirs (*post-mortem* diagnosis). However, there is increasing pressure from beef markets for a definitive diagnosis of tuberculosis in cattle exhibiting lesions compatible with tuberculosis (LCT). Since 2012, the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) determined that farms with cases of bovine/bubaline tuberculosis cannot export beef to the Customs Union of Belarus, Kazakhstan and Russia. All lots of animals from a farm with suspicious animals are sequestered and the LCT are submitted to an official laboratory for aetiological diagnosis.

The culture is considered to be the "gold standard" and definitive test for the confirmation of bovine tuberculosis. However, the microbiological diagnosis of *M. bovis* is an extremely slow procedure which may take as long as 2–3 months. An additional 2–3 weeks are required for the biochemical identification of isolates. Therefore, the need for more rapid diagnostic systems is evident. Molecular diagnostic systems, particularly those based on real-time PCR technology, are faster.

A nested-PCR technique was developed by our research group that showed a clinical sensitivity value of 76 percent with tissue samples from animals that exhibited positive results in the CITT, as well as from those with LCT that rendered positive cultures. A clinical specificity value of 100 percent was detected with tissue samples from animals with CITT-negative results, with no visible lesions and negative cultures. Nested-PCR allowed the identification of *M. bovis* in tissues with a performance that was similar or superior to the culture. Individual results from the nested-PCR were obtained in a short period of time (two days), in contrast with the culture which took up to 90 days.

It is a priority to improve and simplify diagnosis of bTB. The excretion of mycobacteria in milk is intermittent, and up to 30 percent of infected cows eliminate it by milk. Because milk samples are very easy to collect, a new strategy based on PCR in bulk tank samples has been developed at INTA in order to detect herds infected with *M. bovis*. The touchdown (TD) modification programme of PCR was used to amplify *M. bovis* insertion sequence IS6110, since sensitivity increases significantly when compared with conventional PCR. In individual milk samples, 55 percent of PPD-positive cows were shown to be positive and 95 percent of PPD-negative cows were negative to TD-IS6110 respectively. Besides, in infected herds, 47 percent of samples were positive whereas in herds with official free-of-tuberculosis-certification (TFC), 62 percent were negative and 38 percent were positive by TD-IS6110 PCR, respectively. TD-IS6110 PCR in bulk tanks could be used as a vigilance strategy for negative skin test in herds with official TFC since the negative predictive value was 95 percent. This method has been incorporated since 2012 in the Plan of Control and Eradication of Tuberculosis of Santa Fe province, Argentina, which produces 41 percent of the total milk production of the country.

To the extent that programmes for the eradication of bTB advance, more effective genotyping techniques are required in order to trace back the remaining outbreaks. A research group which involves Embrapa Gad de Corte, INTA, Universidade Federal de Mato Grosso do Sul, LANAGRO-MG, Instituto Biológico and Universidade de São Paulo has been working on the sequencing of genomes of South American strains of *M. bovis* and comparing these with genomes from the United States of America, in conjunction with the USDA-ARS. These studies will give us a better understanding of bTB and its relationship to specific phenotypes of all strains investigated; they will also generate important data for local epidemiological studies.



4.4.1 *Report of the parallel session* **Nutrition and food quality**[11]

This parallel session on nutrition and food quality was chaired by Margaret Gill (CGIAR Independent Science and Partnership Council, Rome, Italy). Anna Lartey set the stage by introducing the context of increasing population; changing economic conditions; climate change; current trends towards staple food production and consumption; low dietary diversity; hidden hunger and the double burden of nutrition and their impacts on human health; and the ways in which biotechnologies can be used to promote sustainable food systems. Biotechnologies can be used to enhance human nutrition by, for example, making foods more available by increasing food production and by enhancing the nutritional content of foods, or in transforming food and prolonging shelf-life through fermentation. In line with the Sustainable Development Goals agenda and the 2nd International Conference on Nutrition (ICN2), biotechnologies have to be considered to address current nutritional challenges, in collaboration with all disciplines.

In the context of population growth, climate change and increasing proportion of middle class, the application of biotechnologies, including cross-breeding, genetic transformation and genome editing, presents opportunities to improve the quality of rice and wheat. Melissa Fitzgerald reported that the demand for high-quality food (physical appearance, sensory quality and nutritional traits) would increase along with the need to develop new high-yielding varieties adapted to new climates and stresses (agronomic traits). Currently, the nutritional traits being investigated are for beta-carotene, zinc and folate in rice grains, and iron and carbohydrates with low digestibility for diabetics in both rice and wheat. One of the quality traits of rice is fragrance. Using metabolomic profiling and sensory evaluation, a number of compounds have been identified that determine the high-quality jasmine fragrance and the genetic regions (QTLs) for several of these compounds for use as markers in breeding programmes.

Howarth Bouis presented biofortification as an efficient and cost-effective solution contributing to addressing micronutrient malnutrition. Through conventional plant breeding or transgenic techniques, biofortification increases the density of minerals and vitamins in food. Challenges to the success of biofortification include ensuring acceptability and bio-availability (uptake of the nutrient across the gut). It has now been demonstrated that high mineral and vitamin traits can be combined with high yields and high profits. Peer reviewed published data demonstrate that biofortified foods improve nutritional status and reduce disease incidence and duration. Already more than 15 million people in 30 developing countries are growing and eating biofortified foods. To date, all released biofortified crops have been developed using conventional plant breeding techniques. Yet, transgenic techniques are very powerful tools for adding vitamins and minerals to food staples, as exemplified by the golden rice and transgenic iron and zinc-dense rice.

[11] This report was produced by the FAO rapporteurs - Julie Belanger, Arshiya Noorani and Devin Bartley

Albert Tacon presented examples of agricultural biotechnologies based on the use of micro-organisms for the improved nutrition of the aquaculture feeds used for the production of farmed aquatic species, the reduction of potential environmental impacts of aquaculture feeds and the improvement of the nutritional content of aquaculture produce for direct human consumption. He also discussed the traditional and current use of fermentation technologies for the processing and conservation of small lower-value fish within most Asian countries, including the common production of fish sauces for food seasoning as well as the more recent trend toward the fortification of farmed fish produce with supplemental nutrients for the benefit of consumers prior to harvesting.

One of the key messages from this session was that agricultural biotechnologies provide promising tools for contributing to improved nutrition and food quality. However, efforts are needed to convince consumers and influence policies in favour of their development and application. Communications must be well managed in presenting the facts, especially around food safety.

Discussion

The discussion addressed a number of interrelated key themes related to biotechnologies, nutrition and food quality.

Communication:
- Commercial information provided on certain products, such as probiotics, sometimes made it difficult for farmers and consumers to understand their real impact on nutrition.
- The perception from the crop sector that biotechnologies benefit private companies, not farmers or consumers, can negatively impact the perception of biotechnologies in other sectors. Communication is needed to show that biotechnologies can benefit consumers and farmers.
- Key terms, like biofortification, need to be defined early and clearly.
- What is the balance between safety of food derived from biotechnologies and risks represented by malnutrition?

Safety:
- Issues about safety should be discussed early in the development of the product and with all relevant groups.

Acceptability challenges:
- Regulation and potential benefits depend on the degree of acceptability and adoption of the products. If there is political opposition, costs are not likely to make it worthwhile.

Diversifying diets:
- Concerns were voiced about the replacement of dietary diversity by a few foods derived from biotechnologies.
- Diversified diets are recognized as a key solution to good nutrition.
- Foods derived from biotechnologies are part of the solution.
- Price of non-staple foods is high due to past under-investment in research. Investments in research are needed to improve productivity of non-staple foods.
- To help farmers, economic incentives would be needed to improve the profitability of growing non-staple foods.

4.4.2 Our foods, our diets, our health: Where do we go from here?

Anna Lartey

Nutrition and Food Systems Division, Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, 00153 Rome, Italy
E-mail: Anna.Lartey@fao.org

By 2050, the world's population is expected to increase to about nine billion and this is expected to call for a 60 percent increase in food production. For developing countries where the bulk of the population growth will occur, food production needs will increase by 100 percent from current levels. Increase in cereal production will far outweigh other food crops. The production of fruits, vegetables and pulses over the past five years has increased little. While the prices of staple foods are dropping, the prices of non-staple foods such as fruits and vegetables, pulses and animal-source foods are increasing. Under the circumstance, for poor households it is cheaper to meet food needs on staple foods, even if less nutritious, than to use scarce resources on the more expensive non-staple foods.

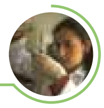
Many developing countries are going through the nutrition transition characterized by improved economic conditions, but increased consumption of highly processed foods high in fat, sugar and salt. As economic conditions improve, many countries are moving into a state of the double burden of malnutrition – a situation where undernutrition including micronutrient deficiency co-exist with overweight and obesity. The food environment is changing globally. Access to healthy diets affordable to the majority of the population is becoming an issue. The consequence is the slow progress made in addressing malnutrition. Although significant progress has been made in reducing hunger from over one billion people affected in 1990–1992 to under 800 million in 2015, the progress made in reducing stunting and micronutrient deficiency has been slow. Micronutrient deficiency affects about 30 percent of the world's population. Anaemia, a major contributor to maternal death, affects over 500 million women of reproductive age. The other side of malnutrition has crept up on us. About 1.9 billion are overweight or obese; this is about 30 percent of the global population. The economic burden of obesity and its associated non-communicable diseases is estimated to be US$2.0 trillion, comparable to that of armed conflicts and smoking (US$2.1 trillion each). Not surprisingly, concerns about broken food systems dominate the discourse around our diets.

The 2nd International Conference on Nutrition (ICN2), hosted by FAO and WHO in November 2014, drew the world's attention to actions needed to promote sustainable food systems. Among these are:

- the need to review national policies and investments and integrate nutrition objectives into food and agriculture policy, programme design and implementation to enable healthy diets (recommendation 8);

- strengthen local food production and processing, especially by smallholder and family farmers, giving special attention to women's empowerment, while recognizing that efficient and effective trade is key to achieving nutrition objectives (recommendation 9);
- promote the diversification of crops including underutilized traditional crops, more production of fruits and vegetables, and appropriate production of animal-source products as needed, applying sustainable food production and natural resource management practices (recommendation 10);
- improve storage, preservation, transport and distribution technologies and infrastructure to reduce seasonal food insecurity, food and nutrient loss and waste (recommendation 11);
- establish and strengthen institutions, policies, programmes and services to enhance the resilience of the food supply (recommendation 12);
- develop, adopt and adapt, where appropriate, international guidelines on healthy diets (recommendation 13);
- encourage gradual reduction of saturated fat, sugar and salt and trans-fat from foods and beverages to prevent excessive intake by consumers and improve nutrient content of foods, as needed (recommendation 14);
- explore voluntary instruments to promote healthy diets (recommendation 15);
- establish food or nutrient-based standards to make healthy diets accessible (recommendation 16).

The knowledge and technologies to tackle the current nutritional and food system problems exist. One of these tools is biotechnology. This tool can be used to enhance human nutrition by making foods more available and by enhancing the nutritional content of foods. There are many examples where modern biotechnology has been used to improve the nutrient content of foods such as orange-flesh sweet potatoes, iron- and zinc-rich millet, and vitamin A-rich cassava. Biotechnology in the form of fermentation has been employed in traditional cultures to transform food and prolong shelf-life. Modern science has opened up a wide range of techniques for the application of biotechnology to agriculture and nutrition. The evidence around the potential of biotechnology to improve nutrition has built up. It is up to us to use it well to our advantage.



4.4.3 Application of biotechnologies to improve the quality of rice and wheat

Melissa Fitzgerald and Venea Dara Daygon

School of Agriculture and Food Science, University of Queensland, Slip Rd., 4072, Queensland, Australia
Presenter's e-mail: m.fitzgerald2@uq.edu.au

Background

Rice and wheat account for most of the global grain harvest and together these two grains supply around half of all calories consumed by the entire human population. Human consumption accounts for 85 percent of all rice produced and 72 percent of wheat produced, illustrating 1) the importance of these two crops for human sustenance; and 2) the need to ensure that production is able to overcome future challenges.

Current trends of population growth, middle class expansion and climate change have consequences for crop improvement programmes and agricultural production into the future. Social science also informs us that as developing countries undergo economic transition, the demand for high-quality food will increase. High quality can include physical appearance, sensory quality and nutritional traits. Breeding programmes have already embraced the challenge of developing new high-yielding varieties adapted to new climates, and scientists are searching for the genes and physiological pathways that lead to crops reaching and exceeding yield potential and maintaining this yield under stress. However, with demand for quality increasing, success of a new variety of any cereal is more likely to be measured by consumer acceptance of the food derived from the harvested grain, rather than adoption by farmers of the plant and its suite of agronomic traits.

Biotechnology

"Climate change, Urbanization, Biotechnology. Those three narratives still taking shape, are developing a long arc likely to dominate this century" (Brand, 2010).

Biotechnology applies scientific and engineering principles to living organisms in order to produce products and services of value to society. Biotechnology encompasses features from many different disciplines such as chemistry, mathematics, physics, engineering, microbiology and genetics. It can drive transformational changes for agricultural production, food security and nutrition security for our future generations. Much biotechnological research reveals genes with important functions, and biotechnology enables those genes to be mobilized into different varieties either through conventional breeding and marker-assisted selection or through more rapid techniques, such as genetic transformation or the newer technique of genome editing. Transformation leads to the insertion of a gene into the DNA of another variety or organism, and genome editing refers to the insertion, deletion or replacement of DNA within the genome of an organism.

Equipping the genome of rice or wheat with a valuable gene by either transformation or genome editing is a translational outcome of biotechnology research because it places the research outcome into farmers' fields for maintaining yield and therefore income; if the new gene increases the nutritional content of the grains, then the value is found in the diets of people with chronic micronutrient deficiency or those with Type II diabetes. Currently, the nutritional traits being investigated are for micronutrients of beta-carotene, zinc and folate in rice grain, and in both rice and wheat iron and carbohydrates with low digestibility for diabetics. Not all consumers accept that value can be derived from using the tools of biotechnology to modify the genome of animals or plants that are intended to be food. This is powerfully illustrated by the destruction of field trials of plants genetically modified to express traits for health and nutrition, which means that it will take a lot longer to place these nutritionally beneficial foods into the food bowls of the people most affected by these chronic nutritional conditions. In order to avoid such devastating impacts in the future, it is important to: 1) understand what drives the fear, and 2) initiate a dialogue supported by science to explain the value of equipping plants with a valuable gene when evolution did not.

As with improving plants with genes for agronomic traits, a new variety is unlikely to be accepted with a nutritional trait unless it also meets quality traits for the cooked rice or the processed wheat.

Using biotechnology to discover genes for quality

The expansion of middle classes is most strongly seen in the rice-consuming countries of Asia. One of the quality traits of rice that all consumers rate important is fragrance, and this is demonstrated by the cost of fragrant rice in all markets. The compound giving fragrance is 2 acetyl-1-pyrroline (2AP), and the gene leading to the presence of the compound is known. Both jasmine and basmati rice contain 2AP, and no other compounds have been identified that combine with 2AP to give the characteristic jasmine or basmati aroma, and no genes have been discovered that lead to different amounts of 2AP.

Using metabolomic profiling and sensory evaluation, we have identified a number of compounds that determine the high quality jasmine fragrance, compounds that associate negatively with fragrance, genetic regions (QTLs) for several compounds, and three QTLs that associate with the concentration of 2AP. Using different tools of biotechnology, these QTLs can be delivered as markers to breeding programmes to enable them to use conventional breeding with genetic selection. Using that pathway, breeders must keep track of the new QTLs as well as the host of other agronomic and quality traits, so a new, highly-fragrant variety will take many years to reach a market. If consumers can be persuaded to accept genetic engineering technologies, and Brand's long arc can be shortened, a new, highly fragrant version of currently accepted varieties could be fast-tracked to market by editing the genomes to insert or replace the genes required for high fragrance for the many rice consumers that value fragrance highly, and for the new varieties with nutritional benefit.

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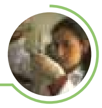

4.4.4 Biofortification of staple food crops: Justification, progress and future activities

Howarth E. Bouis

HarvestPlus, c/o International Food Policy Research Institute (IFPRI), 2033 K Street, NW, Washington, DC 20006, the United States of America

E-mail: h.bouis@cgiar.org

An estimated two billion people in the developing world suffer from the effects of micronutrient malnutrition, caused primarily by poor quality diets which are characterized by high food staple (e.g. rice) intakes. Inadequate intakes of essential vitamins and minerals lower disease resistance, increase mortality, compromise cognitive development, stunt growth and lower work productivity. Pre-school children and mothers of reproductive age are most vulnerable due to their higher requirements.

The density of minerals and vitamins in food staples eaten widely by the poor may be increased either through conventional plant breeding or through use of transgenic techniques, a process known as biofortification. For example, HarvestPlus seeks to develop and distribute varieties of food staples (rice, wheat, maize, cassava, pearl millet, beans, sweet potato) which are high in iron, zinc and provitamin A through an interdisciplinary, global alliance of scientific institutions and implementing agencies in developing and developed countries.

Biofortified crops offer a rural-based intervention that, by design, initially reach these more remote populations which comprise a majority of the undernourished in many countries, and then extend to urban populations as production surpluses are marketed. In this way, biofortification complements fortification and supplementation programmes, which work best in centralized urban areas and then reach into rural areas only in areas with good infrastructure. Initial investments in agricultural research at a central location can generate high recurrent benefits at low-cost as adapted biofortified varieties become available in country after country across time at low recurrent costs.

In broad terms, three things must happen for biofortification to be successful. First, the breeding must be successful – high nutrient density must be combined with high yields and high profitability. Second, efficacy must be demonstrated – the micronutrient status of human subjects must be shown to improve when consuming the biofortified varieties as normally consumed. Thus, sufficient nutrients must be retained in processing and cooking and these nutrients must be sufficiently bio-available. Third, the biofortified crops must be adopted by farmers and consumed by those suffering from micronutrient malnutrition.

First greeted with scepticism by plant breeders who worried that adding micronutrients would reduce yield, it has now been demonstrated conclusively that these high mineral and vitamin traits can be combined with high yields and high profits. More than 15 million people in 30 developing countries are already growing and eating biofortified foods, and the number continues to grow rapidly. *Ex ante* and *ex post* cost-effectiveness analyses have been conducted for several micronutrient-crop-country-crop combinations. Cost-effectiveness of biofortification interventions has also been compared with other micronutrient interventions within several countries. All of these analyses indicate that biofortification is highly cost-effective and has the potential to engender significant reduction in micronutrient deficiencies.

Peer reviewed published data demonstrate that these nutritious foods improve nutritional status and reduce disease incidence and duration. For example, a study in Mozambican children (Jones and de Brauw, 2015) who ate biofortified vitamin A-rich sweet potatoes showed an impressive 42 percent reduction in the incidence of diarrhoea in children under five, and a 52 percent reduction in children under three. The same study showed a reduction in the duration of diarrhoea among children who fell ill – 10 percent in under-fives and 25 percent in under-threes. These were comparisons between children in intervention villages four years after orange sweet potato was first introduced and two years after all extension activities were discontinued, as compared with children in control villages where white sweet potato was continued to be grown.

To date, all released biofortified crops have been developed using conventional plant breeding techniques. However, transgenic techniques are very powerful tools for adding vitamins and minerals to food staples. Transgenic biofortified crops could be of great benefit in reducing mineral and vitamin deficiencies if political barriers to their development and release could be overcome.

The example of golden rice is well-known. Milled rice contains no provitamin A. Potrykus and Beyer have demonstrated that transgenic techniques can result in high levels of provitamin A in milled rice (Ye *et al.,* 2000).

Likewise, recently Trijatmiko *et al.* (2016) demonstrated that high levels of both iron and zinc can be added to milled rice using transgenic techniques. Substituting this biofortified transgenic rice one-for-one for non-biofortified rice on any given day would result in an additional 30 percent of the estimated average requirement (EAR) of iron in the diet and, at the same time, an additional 60 percent of the EAR of zinc.

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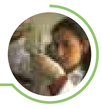

4.4.5 Fish matters: Role of biotechnology in improving nutrition

Albert G.J. Tacon

Aquatic Farms Ltd, Kaneohe, HI 96744, the United States of America
E-mail: agjtacon@aquahana.com

Aquaculture has been the world's most rapidly growing food sector for over a quarter of a century, with total global production (including all farmed aquatic plants and animals) increasing over nine-fold from 10.2 million tonnes in 1984 to a new record high of 97.2 million tonnes in 2013. Valued at over US$157 billion, global aquaculture production has been growing at an average annual rate of 8.1 percent per year since 1984, compared with 0.66 percent per year for total capture fisheries landings, and 2.6 percent for terrestrial meat production over the same period. Moreover, with over 95.2 percent of total global aquaculture production being produced within developing countries, aquaculture is viewed as an important weapon in the global fight against hunger and malnutrition as a much-needed provider of high-quality food and essential dietary nutrients.

According to FAO, agricultural biotechnology includes "Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use". For the purposes of this paper, agricultural biotechnology is used here to include those activities related to improving nutrition, including the improved nutrition of the aquaculture feeds used for the production of farmed aquatic species, the reduction of potential environmental impacts of aquaculture feeds, and the improvement of the nutritional content of aquaculture produce for direct human consumption.

The paper presents examples of microbially-produced feed additives commonly used within commercially-formulated aquaculture feeds (total global production currently estimated at about 42 million tonnes), including microbially-produced dietary essential amino acids (lysine, threonine, tryptophan), dietary enzymes (proteases, phytases, complex carbohydrate digesting enzymes), vitamins (vitamins B_{12}, riboflavin), trace minerals (selenium yeast, zinc yeast, chromium yeast, iodine), carotenoid pigments (algae-produced astaxanthin), nucleotides and immune enhancers (derived from bacteria and/or yeast), organic acids and probiotics.

Particular emphasis is given to the use of microbially-produced phytases to increase the utilization of indigestible plant-based phytates, and by so doing reducing phosphorus excretion and pollution to the aquatic environment. Similarly, the important role played by the microbially-produced essential amino acids lysine and threonine (these two amino acids usually being the second and third limiting amino acids in most aquaculture feeds) within compound aquafeeds has helped the aquaculture feed industry move away from its reliance on fishmeal with alternative more sustainable feed in gradient sources.

In addition to feed additives, agricultural biotechnologies have recently placed particular effort to the mass production of microbial biomass for use as dietary fishmeal replacers (including yeast and bacterial single cell proteins) and/or as a source of long-chain polyunsaturated fatty acids (algal docosahexaenoic acid [DHA]). The paper also discusses the traditional and current use of fermentation technologies for the conservation of small lower-value fish (from a marketing viewpoint) within most Asian countries, including the common production of fish sauces for food seasoning, and the more recent trend toward the fortification of farmed fish produce with supplemental nutrients for the benefit of consumers prior to harvesting, including long-chain omega-3 polyunsaturated fatty acids and essential trace minerals.

Finally, particular emphasis is given to the fact that aquatic food products represent one of the world's most nutritious and healthy food sources. Thus, according to the FAO/WHO Joint Expert Consultation on the Risks and Benefits of Fish Consumption (FAO, 2010), there is convincing evidence that: 1) fish consumption reduces the risk of death from coronary heart disease and that fish consumption by women reduces the risk of suboptimal neuro-development in their offspring; 2) fish consumption may reduce the risk of multiple other adverse health outcomes, including ischaemic stroke, non-fatal coronary heart disease events, congestive heart failure, atrial fibrillation, cognitive decline, depression, anxiety and inflammatory diseases; and 3) fish consumption may provide a greater nutritional impact than the sum of the health benefits of the individual nutrients consumed separately.

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