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UNTAPPED RICHES
Molecular breeding to improve orphan crops

KEY THEMES

- Need to concentrate on orphan crops.
- Molecular breeding methods.
- Prospects for realising innovations.

Orphan crops: the case for improvement

For centuries, those resources we call orphan crops have provided food, animal feed, fibre, oil and medicinal products. They often manage to thrive in harsh conditions where they have evolved and adapted to the environment, making them ideal for agriculture in these conditions, and playing a major role in feeding the poor in the developing countries of Sub-Saharan Africa and South Asia. But they remain, compared to the most high-yielding crops, neglected and under-researched. They have never received the attention of plant breeders that has been enjoyed by major players such as wheat, maize and rice.

This lack of genetic improvement makes them much more susceptible to pests, weeds and diseases than they could be. So even though a large area in developing countries – some 250 million hectares – is used to cultivate just 27 of the crops defined as orphan, their yields tend to be much lower than those of major crops.

In short, the enormous potential of orphan crops in helping to meet the growing needs of the expanding human population remains unrealised.

Valued for its nutritious fruits and anti-viral properties, the baobab *Adansonia digitata* is the first orphan crop whose genomes are being sequenced by the African Orphan Crops Consortium.

Increasing molecular breeding

This unsatisfactory state of affairs could well be remedied by more investment in modern genetic technologies to bring these orphans in from the cold. Advanced molecular techniques have demonstrated their usefulness for the major crops by giving us unprecedented knowledge of key genes in their DNA sequences. It is now time to deploy these transformational tools more widely, and there are several ways in which this could be done.

Next-generation sequencing for orphan crops

The latest sequencing technologies – next-generation sequencing (NGS) – can produce thousands or millions of sequences concurrently. Genes with desirable properties can be identified far more quickly than in the past, which makes highly accurate reference genome sequences affordable, and promises more effective molecular breeding for trait improvement. So it is now realistic to consider advanced breeding programmes for orphan crops.

Marker-assisted selection

The use of marker-assisted selection (MAS) in improving rice is a major success story. Could this DNA-based technology for increasing our ability to identify genetic variation and develop new combinations of genes be extended to orphan crops?

The speed and affordability of NGS have begun to make this feasible. Researchers have, for example, used NGS with the medicinal plant *Artemisia annua*, which produces the potent anti-malarial compound artemisinin in microscopic structures on the surface of its leaves. They rapidly discovered a large number of genes responsible for artemisinin production, going on to identify thousands of markers associated with the expressed genes. This method of identifying molecular markers has the advantage that they all reside in gene sequences that are switched on – expressed – and therefore actively affecting the function of the genes. Within less than five years, a toolkit was created for marker-assisted breeding of an orphan crop for which, previously, little or no genetic information had been available. More importantly, the researchers began to see incremental improvements in the crop's performance.

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Just a kilo of teff grains is needed to cultivate a hectare of the crop, while some 100 kilos of wheat grains are needed for a hectare of wheat.

Likewise, with the perennial plant *Jatropha curcas* – an oil-producing orphan crop that initially excited the interest of researchers as a potential source of biodiesel, and therefore a replacement for petroleum – large-scale production was hampered by lack of detailed knowledge of its genetic make-up. This resulted in very little breeding to improve its characteristics. Again, researchers have, in a similar fashion to their work on *Artemisia annua*, used NGS to build up a fuller picture of the genetic machinery of *Jatropha curcas* and are now working on a molecular breeding programme to identify high-yielding varieties that can be used for biodiesel production.

The use of MAS and NGS has done much to develop plants with potential as medicinal and biofuel crops, and now looks set to have a huge impact on improving the traits of orphan food crops.

Mutation breeding by TILLING

During the second half of the 20th century, breeders of the major crops made use of ionising radiation or chemicals to increase the amount of variation in their stocks by inducing mutations – sudden random changes in the genetic material of the cell. The methods were quite successful, though they did have a few technical drawbacks that limited their usefulness. Over the past decade, however, an updated version of mutation breeding has been developed that extends its applicability, especially to orphan crops. TILLING (Targeting Induced Local Lesions IN Genomes), and a variation called ecoTILLING, have been used in conjunction with NGS for rapid identification of numerous candidate genes associated with specific traits.

Currently, a number of TILLING projects are under way to improve orphan crops such as banana and cassava. Another centres on teff (see Case study), a staple cereal grown on more than 2.5 million hectares, mainly in Ethiopia.

It may well be that new NGS-based screening methods could replace TILLING and ecoTILLING in the hunt for valuable mutations in target genes.

Engineering variation

Genetic modification has already proved its worth in enhancing the yield, quality and environmental impact in terms of pesticide and fuel use of some of the major crops, including soybeans, maize, rice and rapeseed. Indeed, GM foods have been a significant component in global markets for some years.

CASE STUDY Improving teff by TILLING

Teff can grow where many other crops cannot thrive and its light-weight seeds – less than a millimetre in diameter, similar to a poppy seed – make it ideally suited to semi-nomadic life in areas of Ethiopia and Eritrea where it has long flourished.

Teff has a number of desirable characteristics: tolerance to drought and water logging; exceptional nutritional value; and very few post-production problems with pests or diseases. One major weakness, however, is a tendency to stem displacement, or lodging, when the plant falls to the ground under wind and rain, impairing both the quality and quantity of the ensuing harvestable grain.

So far, conventional breeding has failed to find a remedy to the problem. However, a new genetic approach has been to look at several genes that have boosted the yields of wheat and rice while conferring resistance to lodging. These are dwarfing genes that code for reduced height.

Next-generation (high-throughput) sequencing of the teff genome is currently in progress, with promising results. It does appear to carry some of the dwarfing genes, which could be used as targets for TILLING, with the aim of engineering semi-dwarf, higher-yielding varieties of the orphan crop.

CASE STUDY BioCassava Plus

A number of scientists from around the world pooled their expertise in the BioCassava Plus programme, a team effort to reduce malnutrition among the 250 million people in Sub-Saharan Africa who rely on cassava as their staple food.

Over the past five years or so, the team has developed cassava plants with 30 times as much beta-carotene (a source of vitamin A), four times as much iron and four times the protein content of traditional plants. Such enhanced levels would go a long way to meet nutritional needs.

From the laboratory, these crops have gone on to be field-tested and assessed for safety in Nigeria and Kenya – the necessary preparation for getting regulatory approval and, eventually, release for use by farmers.

While not yet fully tested or approved, BioCassava Plus may well influence regulators in Africa. Like their counterparts in Europe, African authorities have been cautious in their acceptance of genetically modified foods, but such high nutritional value may be hard to ignore in the face of Africa's burgeoning population.

However, it has not all been plain sailing: GM foods and crops have incited considerable controversy, especially in Europe, despite widespread scientific confidence in their proven benefits and safety for both human health and the environment. So it is hardly surprising that orphan crops, by definition the relatively neglected contributors to food production, have received little investment for GM development. Nonetheless, the work on GM-improved cassava (see Case study) gives some cause for optimism.

Can the potential be realised?

The experience of, and attitudes to, GM technology in Africa show that there are still some hurdles to the wholesale adoption of molecular plant breeding in order to improve orphan crops.

The cost of complying with regulatory processes, for instance, can deter investors from trying to develop these new crops. But this may change. When analysis was carried out on four GM products in the Philippines – insect-resistant eggplant and rice, and virus-resistant papaya and tomato – the regulatory costs, although significant, were generally lower than the cost of developing the technology. What is more, regulatory costs tend to decline as more experience is gained in these kinds of crops, so should not be an insurmountable barrier.

On the positive side, molecular plant breeding clearly has huge potential for rapidly improving orphan crops. Next-generation sequencing technologies are making it possible to set up molecular breeding initiatives to target improved crop traits very quickly. Indeed, some of these technologies have reached the stage of "plug-and-play" usability since they are capable of being readily and routinely carried out in laboratories.

The use of new-generation sequencing looks set to have a huge impact on improving orphan food crops such as guava.

One final prospect is that the lowering of cost and the increases in throughput brought about by NGS and associated technologies will enable future molecular breeding work on orphan crops to be carried out in research institutes, academic laboratories and small to medium-sized companies. Its development will not have to depend solely on large multinational organisations.