

Smart Breeding

Marker-Assisted Selection:

A non-invasive biotechnology
alternative to genetic engineering
of plant varieties

EXECUTIVE
SUMMARY
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Executive Summary by Greenpeace International

While genetically-engineered plants have gained much public attention, another modern breeding technique called MAS (marker-assisted selection) has gone through a silent revolution in recent years. MAS is a technique that does not replace traditional breeding, but can help to make it more efficient. It does not include the transfer of isolated gene sequences such as genetic engineering, but offers tools for targeted selection of the existing plant material for further breeding.

MAS has already proven to be a valuable tool for plant breeders: it requires less investment, raises fewer safety concerns, respects species barriers, and is accepted by the public. This report highlights dozens of examples of already marketed MAS-bred varieties, demonstrating its high potential to meet challenges such as a changing climate, disease resistance or higher nutritional qualities.

In contrast, genetic engineering - even after 25 years of global efforts - has basically only delivered two single-trait types of plants: herbicide-tolerant and pest-resistant.

What is Marker-Assisted Selection?

In traditional breeding, individual plants expressing desired new traits, such as sweeter strawberries or bigger potatoes, are selected from crosses of a wide range of strawberries or potatoes. While simple traits such as sugar content or size can easily be measured, more complex traits such as disease or drought-resistance are much more difficult for the breeder to see when choosing individuals expressing those traits from among a large pool of plants: for example, it is cumbersome, if not impossible, for breeders to identify those potatoes that are more drought-resistant than others.

Marker-assisted selection (MAS), also called marker-assisted breeding (MAB), avoids this problem by using genetic markers that are linked to the desired trait. Once they are able to identify a genetic sequence that is always linked to disease resistance they can, for example, avoid testing every single offspring plant for this complicated trait – they just need to look for the marker with a rapid DNA test, and they know immediately which plants have the trait and which do not. No DNA is altered and no new gene introduced during this process – it is traditional breeding with molecular help. MAS is often used as a synonym of SMART breeding, which stands for Selection with Markers and Advanced Reproductive Technology.

MAS can be more efficient, effective and reliable than phenotypic selection. Furthermore, MAS can shorten the development time of varieties significantly, so in some cases it will be more cost-effective than selection based on phenotypes. MAS also allows the breeding of complex traits not feasible through previous conventional methods. Although certainly not the silver bullet for all problems, MAS is a promising approach to conventional plant breeding.

image Farmer Sella Mansores, 45, with corn damaged by severe drought, Surallah, Cotabato, Philippines. Since the extreme drought struck farmers report that the harvest is less than one third the normal yield. Since October 2004, the country's worst drought in 50 years has affected around 700,000 people. Greenpeace links rising global temperatures and climate change to the onset of one of the worst droughts to have struck the Philippines, Thailand, and Cambodia in recent memory.



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Beyond breeding techniques

Breeding techniques such as MAS are only one part of the solution to the food crisis. Key for the future of food security are farming systems that are ecological and bio-diverse, rather than continuing with chemical-intensive farming. In April 2008, a collective effort of over 400 scientists worldwide, coordinated by the World Bank and the Food and Agriculture Organisation – the International Assessment of Agricultural Science and Technology for Development (IAASTD) – took stock of the current global state of farming. Their final report, which was adopted by over 60 governments, is a sobering account of industrial farming's failures. It concludes that chemical-intensive agriculture fails to meet local communities' needs for building their livelihoods and achieving a healthy, varied diet. The report calls for a systematic redirection of agricultural research, to better address hunger, severe social inequities and environmental problems.

Rapid changes are needed in agricultural production, and new, bio-diverse and low-input farming systems must be developed and employed. There is also a need for new crop varieties that are fit for change: tolerant to drought, heat or other climate challenges, adapted to bio-diverse farming systems and providing healthier products for farmers and consumers.

Ecological Farming

Ecological Farming ensures healthy farming and healthy food for today and tomorrow, by protecting soil, water and climate, promotes biodiversity, and does not contaminate the environment with chemical inputs or genetic engineering.

A new paradigm in biotechnology

Since the human genome was sequenced, many experts talk about the post-genomic era, in which isolated genetic elements play only marginal roles and the focus is on the complex regulation of genes, their interactions and the sophisticated cellular signalling systems that are yet to be understood.

Scientists using MAS techniques consider plants and their genome as a coherent system and do not interfere with regulation at the genetic level. To achieve desired qualities in plants it is not necessary to identify certain genes that directly influence the desired features. No knowledge or understanding of the underlying regulation is required. It is enough to know that certain structures in the genome can be correlated with some qualities of the plants. Coming from the era of post-genomics, MAS perfectly represents the new paradigm in biotechnology.

The techniques of MAS make use of the natural systems that were established and refined by the mechanisms of evolution. In fact – as this report shows – normal gene regulation can be much more reliable than genetic engineering: transferring a single isolated gene that confers flood tolerance was unable to deliver the same result as targeted crossing and selection within conventional breeding supported by MAS (see Chapter 3 of this report).

MAS is superior to genetic engineering

With respect to the most relevant traits for world food security, the genetic engineering of plants has not performed very well in its first 20 years. Those genetically engineered (GE) crops that are on the market are producing insecticides and/or tolerating herbicides. They hardly contribute to higher yield, they do not aim to address climate change conditions, and their value for sustainable agriculture is highly questionable: the use of GE herbicide-tolerant plants has been responsible for the development of new herbicide resistant weeds (Servive, 2007). Genetically engineered Bt plants contribute to the increase of new insect pests that have adapted to new ecological niches (Catangui, 2006).

Even in the technical parts of patent applications of companies like Monsanto and Syngenta, one can find clear indications of the technological barriers and general disadvantages of the genetic engineering of plants. By way of illustration, one can read this telling quote in Monsanto's patent application WO 2004053055:

"Nonetheless, the frequency of success of enhancing the transgenic plant is low due to a number of factors including the low predictability of the effects of a specific gene on the plant's growth, development and environmental response, (...) the lack of highly predictable control of the gene once introduced into the genome, and other undesirable effects of the transformation event and tissue culture process."

MAS outcompetes genetic engineering

Marker-assisted breeding has a much higher crop improvement potential than genetic engineering. Two examples from this report:

- **Disease resistance:** In rice, 28 genes conferring resistance to bacterial blight have been identified and are now amenable to molecular breeding. Whereas several rice lines have been genetically engineered with these resistance genes, none of these lines has yet been commercialised. In contrast, several MAS-developed bacterial blight resistant rice varieties are currently available for farmers, demonstrating that MAS creates a competitive alternative to the genetic engineering approach.
- **Drought tolerance:** In 2007, MAS 946-1 became the first drought-tolerant aerobic rice variety released in India. To develop the new variety, scientists at the University of Agricultural Sciences (UAS), Bangalore, crossed a deep-rooted upland japonica rice variety from the Philippines with a high yielding indica variety. Bred with MAS, the new variety consumes up to 60% less water than traditional varieties.

In contrast, the growing importance of new techniques in conventional breeding such as MAS is also reflected in statements by some of the most important – and conservative – research centres in plant breeding such as those of the CGIAR (Consultative Group on International Agricultural Research), like IRRI, the International Rice Research Institute in the Philippines. The CGIAR centres promote techniques such as MAS as a major tool in their work to breed new plant varieties: “Today, new techniques enable us to create gene maps, discover precise information about the roles genes play, and mark individual genes. As a result, plants with desirable characteristics can be bred much faster.”

(<http://www.cgiar.org/impact/agribiotech.html>)

Similarly in IRRI's official 'strategic plan 2007-2015', much emphasis is given on smart breeding, while in comparison genetic engineering is not mentioned as crucial technology:

“Recent IRRI research has shown that the drought tolerance trait is strongly influenced by genes and gene networks with large effects. The project will scale up their detection, analysis, and delivery for use in marker-aided breeding.”

Outlook

This report focuses solely on the technical possibilities of MAS, its strengths compared to genetic engineering, and its potential impact on plant breeding. Technically, MAS has a huge potential to contribute to ecological farming, but it is not a panacea. The future of farming lies in farming systems that are bio-diverse and accompanied by a range of policy cornerstones, to support rural livelihoods and long term, sustainable farming. MAS and other breeding techniques are but one technical contribution in this regard.

But most importantly, it is still an open question as to who will control this technology. If MAS or other plant breeding techniques are used to gain patents on a wide range of plants and plant varieties, farmers – and whole countries – would lose control over the seed and food supply, with potentially devastating consequences for food security and rural livelihoods. Hence, it is still an open question whether MAS will be able to deliver its technical potential in the real world, or whether it will rather be abused to secure seed monopolies for a few international agrochemical companies.

The benefits of ecological farming:

- 1) Ecological farming provides the ability of communities to feed themselves and ensures a future of healthy farming and healthy food to all people.
- 2) Ecological farming protects soils from erosion and degradation, increases soil fertility, conserves water and natural habitats and reduces emission of greenhouse gases.
- 3) Ecological farming is both a climate change mitigation and adaptation strategy. Ecological farming can provide large-scale carbon sinks and offer many other options for mitigation of climate change. In addition, farming with biodiversity is the most effective strategy to adapt agriculture to future climatic conditions.



Marker-Assisted Selection:

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PREPARED FOR
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BY BENNO
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Overview

What is Marker Assisted Selection?

In traditional breeding, new crop traits such as sweeter strawberries or bigger potatoes are selected from crosses of a wide range of strawberries or potatoes. While simple traits such as sugar contents or size can easily be measured, more complex traits such as disease or drought resistance are much more difficult to determine for the breeder. It is time consuming for breeders to identify those potatoes that are more drought resistance than others. Smart breeding or marker assisted selection (MAS, also called marker assisted breeding - MAB) avoids this problem by using genetic markers that are linked to the desired trait(s). Once they are able to identify a genetic sequence that is always linked to disease resistance, for example, they can avoid testing every single offspring plant for this complicated trait – they just need to look for the marker with a rapid DNA test, and they know immediately whether or not which plants have the trait. No DNA is altered and no new gene introduced during this process – it is breeding with molecular help.

Unlike genetic engineering it does not involve the transformation of isolated (foreign) genetic material into the genomes of plants. Basically smart breeding works like conventional breeding. Because of the speed and accuracy of MAS, smart breeding can dramatically fast-track conventional breeding efforts.

The use of genetic markers in plant breeding is based on the assumption that the presence of particular markers in the plant genome is linked with the presence of a particular trait. The information about links between markers and traits is delivered by genomic research.

Smart breeding is the use of genetic markers in conventional breeding programs to predict the presence of the desired traits. Different from genetic engineering, it does not involve gene isolation, direct modification and asexual insertion of genetic material.

State of development and future prospects of MAS

In the last decade public and private plant scientists heavily invested in the development of MAS. Today, molecular markers are available for a wide range of traits and crop species. Examples of successful applications of MAS are: disease resistance varieties in barley, bean, pearl millet, rice, soybean, wheat and tomato, varieties with enhanced quality in maize and rice, and varieties with complex traits such as drought-tolerance in aerobic rice and yield in tomato. The more basic genomic research advances, the easier it will become to apply MAS for many different traits including traits depending on multiple gene involvement and complex gene interactions, functions and regulations.

Feature I: MAS for superior rice

Rice is the world's most important food crop and a primary source of food for more than half the world's population. As rice production has to be improved to satisfy growing demand, much efforts has been put into the development of MAS and genetically engineered rice varieties. Whereas there are no genetically engineered rice varieties commercially grown at the moment, the first MAS-developed rice varieties are already available for farmers. In China, India and Indonesia, for example, rice varieties have been released, which are resistant to bacterial blight, a widely distributed rice diseases. In the USA MAS-rice with enhanced quality was developed and in India a drought-tolerant rice variety is available. The release of salt and submergence tolerant MAS-rice is expected for 2009/2010.

image Mushroom Risotto at Kinabuhayan Cafe, Luzon, Quezon. Leading heritage restaurants joined the Greenpeace 'GMO-free rice restaurants' campaign to protect rice from the threat of genetically-modified organisms or GMOs. The 'GMO-free rice restaurants' campaign aims to gather the commitment of restaurants around the country to serve only GMO-free rice.



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Feature II: Drought tolerance

Drought is one of the stresses that threaten worldwide crop productivity most severely. The polygenic nature¹ of drought resistance makes the breeding of drought tolerant varieties extremely difficult. Whereas no genetically engineered drought-tolerant crop has been commercialised to date, conventionally bred drought tolerant varieties have been released to the market. Examples are the open pollinated maize variety ZM521 and the wheat varieties Drysdale and Rees. A wheat variety based on Drysdale and improved by MAS should be ready for commercialisation in 2010. Promising opportunities for improving drought tolerance by MAS also exist in rice, maize and sorghum.

Feature III: MAS harnesses biodiversity

Despite the value of genes or alleles originating from wild species and landraces conventional breeders have been reluctant about the use of non-cultivar (i.e. from landraces) germplasm in their breeding programs due to complex, long-term and unpredictable outcomes. Marker assisted selection now enables to precisely introgress small sectors of wild species or landraces into elite varieties. Two successful examples of this approach are the yield-increased tomato variety AB2 and the maize variety Vivek QPM 9 with improved protein quality.

Feature IV: Breeding for better nutrition

Given the genetic variation in concentrations of zinc, iron, and vitamins among plant varieties, conventional and marker-assisted breeding have great potential to increase the micronutrient contents of staple crops. One successful example of conventional breeding is the release of sweet potato varieties with enhanced levels of provitamin A in African countries. In near future the first biofortified MAS-variety is believed to come to the market. Scientists recently identified genetic markers in maize, which are associated with higher levels of provitamin A in kernels. With the help of these markers provitamin A concentration in kernels can now be assessed in an easy and cost-effective way.

¹ Polygenic means it is based on many genes, i.e. there is not one single gene that confers drought resistance (which could be theoretically transferred with genetic engineering), but several genes have to act together to convey drought resistance.

Introduction

Today, there is serious concern about sufficient future global production of food from crop plants. In addition to an increasing world population, there are several reasons for this concern. Firstly, global climate change will affect crop growth and threaten the conservation of cultivated lands. Secondly, freshwater resources are declining and the competition for this water between urban areas, the industrial sector and agriculture is increasing. Thirdly, the availability of arable land is decreasing because of soil erosion and degradation, land conversion to other uses and non-sustainable farming.

There are several vital ways to cope with these emerging problems, including management adoptions and politico-economic reforms. The International Assessment of Agricultural Science and Technology for Development (IAASTD) has recently called for a greater support of agroecological approaches, which it considers a great potential for world agriculture. One approach to improving global food production is the enhancement of the raw material – the crops themselves. For the last two decades, biotech companies - as well as some politicians and public sector scientists - have promoted genetic engineering (GE) as the best plant-breeding approach to solving the problems. However, production of the first genetically-engineered varieties is being driven by available technical possibilities and economic interests rather than by the central needs of the market. So far, genetic engineering has failed to meet the emerging problems. Today, genetic engineering is mostly limited to a handful of major crops and a small number of traits that are of interest to large-scale commercial, industrial farmers, mainly developed by a few global life science companies.

Although genetic engineering has captured much attention, many scientists believe that greater progress can be made in improving plant varieties through applications of biotechnologies that do not involve genetic engineering². Such a tool is marker-assisted selection (MAS). MAS is used to speed up and widen the scope of crop breeding around the world and has become a valuable alternative to genetic engineering, as it has great crop improvement potential without threatening irreversible harm to the environment or long-term human health effects.

While genetic engineering is in the public spotlight, the development and achievements of MAS have gone largely unnoticed. The aim of this paper is to highlight some of the current outputs of MAS. The first section describes how MAS works and what advantages it offers compared to conventional breeding. The second section compares MAS to genetic engineering. Following this is a description of the state of development and future prospects of MAS. Subsequently, attention is drawn to MAS in rice breeding, including several examples of successfully developed new rice varieties. The last three sections feature MAS for developing drought tolerance, harnessing biodiversity and improving nutrition.

² Biotechnology in plant breeding covers at least four areas of work (Dawson et al. 2009): (i) tissue culture and micropropagation, (ii) molecular marker characterisation of genetic diversity, (iii) genetic maps, marker-assisted selection and genomics, and the related disciplines of proteomics and metabolomics, and (iv) genetic engineering and the production.

1) Marker-assisted selection: Fast-tracking plant breeding

“Where breeding goals cannot be achieved using traditional approaches, there is now considerable scope for using molecular markers to develop new varieties.”

Peleman & Rouppe van der Voort 2003b

“Marker-assisted selection has held promise for impacting, perhaps revolutionising, plant breeding disciplines.”

Cahill & Schmidt 2004

“One of the most powerful tools in the emerging biotechnology toolkit is marker-assisted breeding.”

Naylor & Manning 2005

The performance of a given plant variety is the end result of the action of thousands of genes and their interactions with environmental conditions and cultural practices (Collins et al. 2008). Selection of the plant varieties with the desirable performance under given environmental conditions and cultural practices is the fundamental basis of plant breeding (Collard & Mackill 2008). Traditionally, plant breeders have selected plants based on their visible or measurable traits, called the phenotype. As the direct target of the selection is the trait itself and its phenotypic expression, the specific genes behind the trait are selected indirectly. Through the development of molecular markers it has now become possible to directly target genomic regions that are involved in the expression of traits of interest (Babu et al. 2004). The use of molecular markers in plant breeding is called molecular marker-assisted selection, often also simply referred to as marker-assisted selection (MAS), marker-assisted breeding or ‘smart breeding’.

Molecular markers represent genetic differences between individual organisms or species. They are a sequence of nucleic acid, which makes up a segment of DNA. Generally, they do not represent the target genes themselves but are located near the DNA sequence of the desired genes and therefore act as ‘signs’ or ‘flags’ (Collard et al. 2005). Since markers and genes are close together on the same chromosome, they are disposed to stay together during the breeding process. This linkage helps breeders to find out whether an individual plant has desired genes or not. Breeders can scan new varieties for the presence of the markers and if they can find the markers, it means the desired genes themselves are present.

image After finishing a half day harsh farm work, a Hani rice farmer is eating his rice from a traditional bamboo rice bowl, Yunnan Province, China.



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The process of phenotypic selection used in conventional breeding programmes can be difficult, slow, costly and influenced by the environment (Francia et al. 2005, Babu et al. 2004). MAS now offers a solution to these constraints. The advantages of MAS over conventional phenotypic selection depend on several parameters, including type of crop species and kind of traits to be improved. In general, MAS can be more efficient, effective and reliable than conventional breeding and can shorten the development time of varieties significantly (Babu et al. 2004, Collard et al. 2005, Francia et al. 2005, Foolad 2007, Brennan & Martin 2007, Xu & Crouch 2008). Furthermore, in some cases MAS can be more cost-effective than conventional phenotypic selection (Dwivedi et al. 2007, William et al. 2007, Brennan & Martin 2007, Bernardo 2008).

A major breakthrough brought to plant breeding by the use of molecular markers is that genotypic selection has become possible not only for qualitative (that are based on single genes) traits but also for complex traits that involve a broad range of genes. Most of the traits of agronomic importance, such as yield, abiotic stress tolerance, quality traits and some classes of disease resistance, are complex and regulated by several genes (Francia et al. 2005). It has been estimated that 98% of the important traits in domesticated crops are of quantitative nature (Utomo & Linscombe 2008). The regions within plant genomes that contain genes associated with a quantitative trait are called quantitative trait loci (QTL). The identification of QTLs based only on conventional phenotypic evaluation is not possible (Collard et al. 2005). As Young (1999) wrote, "Before the advent of DNA marker technology, the idea of rapidly uncovering the loci controlling complex, multigenic traits seemed like a dream." Now, this dream is becoming a reality and with the help of molecular marker genome regions can be flagged now allowing the selection for QTLs.

Today molecular markers can be employed to assist a wide range of components of modern plant breeding programs, including the following applications: improved access and utilisation of germplasm resources, genetic analysis of breeding populations, parental selection, marker-assisted selection, marker enhanced backcross breeding, pyramiding genes, protection of plant breeder's rights and comparative mapping (Crouch & Ortiz 2004, Collard & Mackill 2008, Hospital 2009). With respect to important MAS schemes, three main uses of molecular markers in plant breeding can be emphasised:

Marker-assisted evaluation of breeding material: The first selection step in plant breeding is the choice of lines to mate as parents of new populations. Conventionally, the selection of such parents is based on a combination of phenotypic assessments, pedigree information, breeding records and chance. Now, the use of molecular markers enables a marker-assisted germplasm evaluation. This type of evaluation has the potential to make parental selection more efficient, to expand the gene pool of modern cultivars and to speed up the development of new varieties (Xu & Crouch 2008, Edwards & McCouch 2007).

Marker-assisted introgression: The process, where a gene or a QTL from a population A is introduced to a population B by crossing A and B and then repeatedly backcrossing to B, is called introgression (Hospital 2009). Here, molecular markers can be used to control the presence of the target gene or QTL and to accelerate the return of background genome to recipient type. Marker-assisted introgression is very effective for introgressing genes or QTLs from landraces and related wild species, because it reduces both the time needed to produce commercial cultivars and the risk of undesirable linkage drag with unwanted traits of the landrace or wild species (Dwivedi et al. 2007).

Marker-assisted pyramiding: Pyramiding is the process of combining several genes or QTLs together into a plant variety. Using phenotypic selection methods it is extremely difficult and sometimes impossible to pyramid the desired traits. A striking example is the breeding of durable disease resistance. When a variety is protected by one gene with a major effect against a disease, it is often not possible to introgress additional resistance genes to the same disease because they show the same phenotype. However, if resistance genes can be tagged with markers, the number of resistance genes in any plant can be easily determined (Collard & Mackill 2008).

Taken together, MAS can significantly reduce the time needed to develop plant varieties with desired traits and enthusiasts claim that MAS could offer to plant breeding what the jet engine has brought to air travel (Knight 2003). Furthermore, since the desired genes occur naturally in the plant and are simply selected for during the breeding process, no foreign gene introduction is involved. MAS also allows the breeding of complex traits that were not feasible by previous methods. Although not a panacea, MAS is a promising new approach to conventional plant breeding.

2) Molecular breeding: MAS outcompetes genetic engineering

“Fortunately, biotechnology has provided additional tools that do not require the use of transgenic crops to revolutionise plant breeding.”

Dubcovsky 2004

In the last two decades, genomics research in plant species has generated a wealth of information about gene structure and function. This information is now accessible for crop improvement and is applied in two broad areas of molecular breeding. The first area is marker-assisted breeding, the second area is genetic engineering (Naylor et al. 2004). Some still promote genetic engineering but increasingly plant breeders are waking up from this genetically-engineered dream (Zamir 2008).

Whereas genetically-engineered plants have gained much public attention, MAS has gone through a silent revolution. Today, MAS has become a realistic option to develop new varieties and many scientists assume that MAS is a valuable alternative to genetic engineering (Fernie et al. 2006, Schauer et al. 2006, Wenzel 2006, Herdt 2006, Naylor & Manning 2005, Naylor et al. 2004, Dubcovsky 2004, Peleman & van der Voort 2003a, Zamir 2001).

Marker-assisted breeding has a much higher crop improvement potential than genetic engineering, and it can do what GE does with out threatening to cause irreversible harm to environment or to human health.

This is illustrated by the breeding of disease resistance. In rice, for example, 28 genes conferring resistance to bacterial blight have been identified and are now amenable to molecular breeding. Several MAS-developed bacterial blight resistant rice varieties are currently available for farmers showing that MAS creates a competitive alternative to the genetic engineering approach (Collinge et al. 2008; examples see section 4.1). As marker-assisted breeding of disease resistance is even more promising than genetic engineering in various situations, the EU Commission-funded project ‘Bioexploit’ gives high priority to MAS for the exploration of natural disease resistance in wheat and potato (Anonymous 2007).

Another example of MAS being more effective than genetic engineering is the development of submergence tolerant rice varieties. The sub1a gene is known to make rice plants survive under flooded conditions. Although flooding-intolerant rice lines genetically engineered with the sub1a gene show submergence tolerance, they also show a pleiotropic phenotype including reduced plant height (Xu et al. 2006). In contrast, the incorporation of the sub1a gene into popular flooding-susceptible varieties by marker-assisted backcrossing resulted in improved varieties showing submergence tolerance without compromising on yield or other agronomic important traits (Neeraja et al. 2007; see also section 5.5).

MAS also seems to be a very promising approach against insect pests, such as the brown plant hopper on rice. There is a lot of research activity in this area, and recently three scientific papers have been published by Chinese researchers detailing advances for plant hopper resistance using MAS and detailing on new genes found in rice that give resistance to BPHⁱ.

Probably the most important advantage of MAS over genetic engineering lies in the potential of improving quantitative traits. MAS is able to select and pyramid QTLs, even without knowing the specific genes conferring the traits. In contrast, a major drawback of genetic engineering is that breeders must know which genes should be transferred to the plant genome. The identification of key genes, whose manipulation affects quantitative traits, is highly complex because of the polygenetic nature of quantitative traits. While genomics has identified many sequences that correlate with phenotypic changes, the question as to which are the best candidate genes related to quantitative traits continues to baffle genetic engineers (Singh et al. 2008, Zamir 2008, Vij & Tyagi 2007, Ghandilyan et al. 2006). Furthermore, the choice of the right promoters is critical for tailoring and fine-tuning the expression of candidate genes to improve quantitative traits (Venter 2007). However, the ability to make wise predictions about which promoters to use is still very limited (Zamir 2008). Another hurdle to the improvement of quantitative traits by genetic engineering is that the coordinated manipulation of multiple genes is difficult to achieve (Halpin 2005). Although engineering systems are available for the transfer of multiple genes into plant cells, several questions still remain open and technical issues need to be addressed before these systems can be routinely used (Dafny-Yelin & Tzfira 2007). A further handicap: the use of multiple genes is likely to make safety assessment of genetically-engineered plants more complex, as not only the safety of each gene transferred has to be assessed but also potential interactions among them.

ⁱ Su CC, Wan J, Zhai HQ, Wang CM, Sun LH, Yasui H, Yoshimura A. 2005 A new locus for resistance to brown planthopper identified in the indica rice variety DV85. *Plant Breeding* 124: 93-95.

Yang HY, You AQ, Yang ZF, Zhang F, He RF, Zhu LL, He G. 2004. High-resolution genetic mapping at the Bph15 locus for brown planthopper resistance in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 110: 182-191.

Ren X, Wang X, Yuan H, Weng Q, Zhu L, He G. 2004. Mapping quantitative trait loci and expressed sequence tags related to brown planthopper resistance in rice. *Plant Breeding* 123: 342-348.

image Fish and Co.'s kitchen crew display a dish of beef tapa served with java rice during the launch of Greenpeace's 'GMO-free rice restaurants' campaign in The Philippines.



In summary, genetic engineering continues to be a restricted technology that can address a few simple traits, but remains inadequate for improving the often far more valuable complex traits.

Given the great plant-breeding power of MAS, it becomes obvious that MAS offers several advantages over genetic engineering: MAS respects species barriers, raises less safety concerns - especially about irreversible environmental harm and long-term negative health effects intrinsically associated with GE - is accepted by the public, and is not harming or endangering organic farming.

MAS respects species barriers: All genes that are incorporated into crops by MAS are present within the natural gene pool of a particular crop and reside at their natural chromosomal locations. In contrast, most genetic engineering applications involve the transfer of genetic material originating outside the natural gene pool of a particular crop and the transformation results in random genomic integration. As MAS respects species barriers, it provokes less ethical concerns regarding 'naturalness' and 'plant integrity' than genetic engineering.

MAS raises fewer safety concerns: MAS mainly involves backcrossing and introgression. As both breeding processes have a long history of safe use in conventional plant breeding, MAS-derived cultivars are generally as safe as conventionally-bred varieties. In contrast, genetic engineering is unfamiliar with most of the conventional breeding approaches and the long safety history of conventionally-bred varieties cannot be translated to genetically-engineered varieties. Specifically, it is widely documented that the process of transferring isolated genetic material into plant genomes frequently leads to random genomic integration and multiple insertions of gene and vector sequences resulting in insertional mutagenesis, thus causing unintended effects (Latham et al. 2006).

MAS requires less investment: Genetic engineering is an expensive technology. While the cost for the development of a conventional variety is approximately USD 1 million (Goodman 2004), genetic engineering requires an investment of anywhere between USD 20 million to USD 100 million for developing a commercially released event (Goodman 2004, Powell 2007, Trommetter 2008). Although no exact figures can be found about costs of developing MAS-varieties in the literature, MAS is certainly less expensive than genetic engineering technologies (Trommetter 2008). For public breeding programs, the costs for genetic engineering are usually beyond the resources, and therefore genetic engineering approaches are not currently used in most cultivated crops (Dubcovsky 2004). Also, for private companies, costs for genetically engineering varieties can be prohibitive. For example, Seminis, a major fruit and vegetable seed company, is using marker-assisted breeding because the high investment in genetic engineering does not make sense for the small scale of fruit and vegetable costs (Powell 2007).

MAS is accepted by consumers: Although there is only one study available indicating that consumers favour MAS over genetic engineering (van den Heuvel 2008), it is generally assumed that MAS-developed varieties will be accepted by consumers, as they are not genetically engineered (Fernie et al. 2006, Dubcovsky 2004, Peleman & Rouppe van der Voort 2003a, Zamir 2001, Pollack 2001). This is substantiated by the fact that naturalness and intrinsic value of plants are explicitly mentioned as consumer concerns in several reports (Lammerts van Bueren et al. 2003).

MAS poses no threat to organic farming: Genetic engineering is not accepted in organic farming, as it contrasts with the concepts of naturalness and integrity (Verhoog 2007, Lammerts van Bueren et al. 2007). In contrary, although discussion is still going on about the use of marker technology in organic farming (Verhoog 2005, Lammerts van Bueren 2005, Haring 2005, Backe & Østergård 2008), MAS can be permitted in an organic breeding programme, if marker screening is performed without enzymes originating from genetically-modified microorganisms and without radiation (Lammerts van Bueren et al. 2003, Lammerts van Bueren & Struik 2004, Backe & Østergård 2008).

3) State of development and future prospects of MAS

“Over the next decade, MAS technologies will become substantially cheaper and easier to apply at large scale, and knowledge from genomics research will become more readily translated from publications into breeding tools.”

Xu & Crouch 2008

“Despite the much proclaimed successes of agbiotech in manipulating a few simple input traits by transgenesis, it is almost certainly the case that the more significant, and normally quite unremarked, achievement of modern hi-tech breeding has been in the use of marker-assisted technologies.”

Murphy 2007

Given its great potential MAS has been greeted with much enthusiasm and expectation in public and private plant breeding, stimulating tremendous investments in the development of molecular marker maps and research to detect associations between phenotypes and markers (Cahill & Schmidt 2004, Ruane & Sonnino 2007, Eathington et al. 2007, Bernardo 2008, Hospital 2009, Utomo & Linscombe 2009). Molecular markers are now available for a wide range of traits and crop species.

There is no comprehensive documentation regarding the successful use of MAS for breeding new crop varieties or developing breeding material. Comprehensive figures on the number of released MAS-varieties and their adoption by farmers are not available. However, from the literature it is evident that MAS plays a prominent role in plant breeding, and a range of examples of successful, practical outcomes can be found in literature and variety registrations (Xu & Crouch 2008, Dwivedi et al. 2007). In the course of the present work 28 MAS-derived varieties released by public breeders were identified (Table 1). One of the reasons for the limited number of published reports is that breeders in the public sector do not publish their MAS results. For public plant breeders the final ‘product’ is a new variety and not a publication. Although new varieties are registered, explicit details regarding the use of MAS may not be supplied (Xu & Crouch 2008). Also, private sector plant breeders usually do not disclose information about breeding strategies (Williams et al. 2007, Foolad 2007, Xu & Crouch 2008, Hospital 2009, Utomo & Linscombe 2009). As private breeding companies do not accord the same sort of prestige to new varieties developed by MAS that is granted to new genetically-

engineered varieties, it remains largely unknown that there are, for example, hundreds of tomato hybrids on the market, which were developed using marker-assisted selection.

One of the most successful applications of MAS today has been that for introgressing and/or pyramiding major effect genes (Francia et al. 2005, Foolad 2007, Dwivedi et al. 2007, Xu & Crouch 2008). This approach has led to the commercial release of several disease-resistant cultivars in barley, bean, pearl millet, rice, soybean, wheat and tomato. Further successful applications resulted in the release of varieties with improved quality, such as a high-quality protein maize variety in India and low-amylose rice varieties in the USA. In addition, MAS breeding also achieved the improvement of complex traits such as drought-tolerance in an aerobic rice variety and yield in tomato (see Table 1).

Although the actual impact of MAS on the release of new crop varieties is difficult to assess, many scientists advance the view that MAS is still in its early phase, in particular for improving quantitative traits (Semagn et al. 2006, Foolad 2007, Reynolds & Tuberosa 2007, Collard & Mackill 2008, Xu & Crouch 2008, Collins et al. 2008, Walsh 2009, Heffner et al. 2009). To speed up its implementation, the application gap between research laboratories and plant breeding institutes should be closed (Collard & MacKill 2008). Marker technology has not often reached the breeders in the desired measure. Therefore, a more effective collaboration between molecular biologists and plant breeders needs to be worked out (Semagn et al. 2006, Dargie 2007, Reece & Haribabu 2007, Collard & Mackill 2008). In addition, DNA marker technology is still at an early stage (Collard & MacKill 2008), and the current costs of applying MAS are still a limiting factor for many breeding programmes (Xu & Crouch 2007, Collard & Mackill 2008).

There is optimism that MAS will realise its enormous potential and will therefore give rise to a much greater level of adoption in plant breeding in the near future (Collard & Mackill 2008). Among the several factors contributing to this optimism are new technological advances such as new marker technologies (Ganal et al. 2009, Gupta et al. 2008), association mapping (Zhu et al. 2008, Takeda & Matsuoka 2008, Nordborg & Weigel 2008), genomic selection (Bernardo & Yu, Walsh 2009, Heffner et al. 2009) and metabolomics-assisted breeding (Fernie & Schauer 2009). Over the next decade, MAS is projected to become cheaper and easier to apply at large scale and knowledge from genomic research should become more readily translated into breeding tools and thus more routinely used in breeding programmes (Xu & Crouch 2008). In the short term, it is expected that the greatest growth in MAS will be for qualitative traits that are difficult or expensive to select by conventional phenotypic methods (Dwivedi et al. 2007, Xu & Crouch 2008). In the medium term, it is envisaged that there will be a shift from MAS for qualitative traits to MAS for breeding quantitative traits (Dwivedi et al. 2007, Xu & Crouch 2008).

image Close-up shot of 'Nap-Hal' wheat, growing in Germany in 2003. The 'Nap-Hal' patent is owned by Monsanto.



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Table 1.0:
Commercially available MAS-varieties developed by public institutions

Plant	Cultivar / Breeding Line	Trait	Country	Year of Release	Breeder
Barley	Tango	Disease resistance	USA	2000	Oregon State University ¹⁾
	SloopSA	Disease resistance	Australia	2002	University of Adelaide ²⁾
	GairdnerPlus	Disease resistance	Australia	2006	University of Adelaide ³⁾
	Doria	Disease resistance	Italy	2006	Istituto Sperimentale per la Cerealicoltura ⁴⁾
Bean	USPT-ANT-1	Disease resistance	USA	2004	USDA-ARS ⁵⁾
	ABCP-8	Disease resistance	USA	2005	University of Nebraska / USDA-ARS ⁶⁾
	ABC-WeiHING	Disease resistance	USA	2006	University of Nebraska / USDA-ARS ⁷⁾
	USDK-CBB-15	Disease resistance	USA	2006	USDA-ARS ⁸⁾
Maize	Vivek QPM 9	High-Quality Protein	India	2008	Indian Council of Agricultural Research ⁹⁾
Pearl millet	HHB 67-2	Disease resistance	India	2005	Haryana Agricultural University / ICRISAT ¹⁰⁾
Rice	Cadet	Low-amylose	USA	2000	Texas Agricultural Experiment Station ¹¹⁾
	Jacinto	Low-amylose	USA	2000	Texas Agricultural Experiment Station ¹¹⁾
	XieYou 218	Disease resistance	China	2001	China National Rice Research Institute ¹²⁾
	Angke	Disease resistance	Indonesia	2002	n.k. ¹³⁾
	Conde	Disease resistance	Indonesia	2002	n.k. ¹³⁾
	Tubigan 7	Disease resistance	Philippines	2006	Philippine Rice Research Institute ¹⁴⁾
	Tubigan 11	Disease resistance	Philippines	2007	Philippine Rice Research Institute ¹⁵⁾
	MAS 946-1	Drought-tolerance	India	2007	University of Agricultural Sciences ¹⁶⁾
	Pusa 1460	Disease resistance	India	2007	Indian Agricultural Research Institute ¹⁷⁾
	RP Bio 226	Disease resistance	India	2007	Directorate of Rice Research ¹⁸⁾
Soybean	JTN-5303	Disease resistance	USA	2005	University of Tennessee and USDA-ARS ¹⁹⁾
Tomato	Ab2	High Yield	USA	2002	Hebrew University of Jerusalem ²⁰⁾
Wheat	Patwin	Disease resistance	USA	2006	University of California, Davis ²¹⁾
	Espresso	Disease resistance	USA	2006	University of California, Davis ²²⁾
	Lassik	Disease resistance	USA	2007	University of California, Davis ²²⁾
	Farnum	Disease resistance Protein content	USA	2008	Washington State University ²³⁾
	Westmore*	Disease resistance Protein content	USA	2007	University of California, Davis ²⁴⁾
	AGS2026	Disease resistance	USA	2007	University of Georgia ²⁵⁾

*: Westmore is a durum wheat variety.

Abbreviations: ICRISAT: International Crops Research Institute for the Semi-Arid tropics; n.K.: not known; USDA-ARS: United States Department of Agriculture - Agricultural Research Service.

References:

- 1):** Hayes et al. 2003, Rost 2001;
2): Langridge & Barr 2003, Eglinton et al. 2006;
3): Eglinton et al. 2006;
4): Kosova et al. 2008;
5): Mikas et al. 2003, Suszkiw 2004;
6): Mutlu et al. 2005;
7): Mutlu et al. 2008;
8): 9): Mudur 2008, Gupta et al. 2009; **10):** Dar et al. 2006; **11):** Dwivedi et al. 2007; **12):** Cheng et al. 2007; **13):** Toennissen et al. 2003; **14): 15): 16):** Chandrasheka 2007; Gandhi 2007; **17):** Gopalakrishnan et al. 2008; **18):** Sundaram et al. 2008; **19):** Core 2005, Arelli et al. 2007; **20):** Lipman et al. 2007;
21): Hospital 2009;
22): WheatCAP 2009;
23): Kidwell et al. 2008;
24): AOSCA 2008
25): WheatCAP 2009.

4) Feature I: MAS for superior rice

Rice is the world's most important food crop and a primary source of food for more than half the world's population (Khush 2005). In 2007, 645 million tonnes of rice were produced in 114 countries, with 90% of the harvest grown and consumed in Asia (Skamnioti & Gurr 2009).

Technological advances during the last 40 years led to an increase in rice production by 150%. However, according to various estimates, rice production will have to be increased further to satisfy growing demand (Khush 2005). To achieve the goal of increasing rice production in a sustainable manner, a number of challenges have to be met, including narrow genetic diversity in modern rice varieties, increase in severe occurrence of insects and diseases, decline in arable land, global water shortage, global climate change and increasing demand for high-quality rice (Zhang 2007, Jena & Mackill 2008, Collard et al. 2008, Peng et al. 2009).

Because of rice's global importance, small genome size, and genetic relatedness to other major cereal crops, an enormous amount of research activities have addressed rice genetics and genomics in the past decade (Collard et al. 2008). Sequence drafts of the entire genomes of two subspecies of rice – indica and japonica – were

completed in 2002 (Goff et al. 2002, Yu et al. 2002) and a high-quality version of the japonica species was publicised in 2005 (IRGSP 2005). Furthermore, hundreds of germplasm evaluations and genetic studies have identified a large variety of genes and QTLs. The database Gramene³ currently lists more than 8,000 entries for QTLs detected in rice. The wealth of these genomic data is now being applied to MAS-breeding programmes (Table 2), which aim at developing new improved rice varieties to meet the future challenges.

In the past two decades there has been much research into the genetic engineering of rice. Rice lines have been genetically engineered for resistance to diseases, resistance to insects, tolerance of herbicides, tolerance of abiotic stress, nutritional traits and photosynthetic traits. However, to date only three genetically-engineered herbicide-tolerant rice lines have passed regulatory approval for cultivation, whereas the approval is restricted to the USA (Abgios 2009). Despite the permission for cultivation, none of the three lines has yet been commercialised.

Whereas genetically-engineered rice lines have not yet received commercial approval, the first MAS-developed rice cultivars are commercially grown by farmers in the developing world, as will be shown in the following sections.

Table 2.0:
Examples of traits, for which marker-assisted breeding is being applied in rice.

Biotic Stresses	Abiotic Stresses	Grain Quality	Agronomic Traits
Blast Resistance	Submergence Tolerance	Cooking Quality	Yield
Bacterial Leaf Blight Resistance	Salt Tolerance	Eating Quality	
Tungro Virus Resistance	Drought Tolerance	Appearance Quality	
Gall Midge Resistance	Cold Tolerance		
Brown Planthopper Resistance			
Green Leafhopper Resistance			
Green Rice Leafhopper Resistance			

Information according to Jena & Mackill 2008, Collard et al. 2008, Leung 2008 and Zhang 2007.

image Conventional maize field in Germany. Wind spreads pollen and genetic information to other plants.



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4.1 MAS controls bacterial leaf blight

Bacterial leaf blight caused by the pathogen *Xanthomonas oryzae* pv *oryzae* (Xoo) is one of the most widely distributed and devastating rice diseases worldwide (Rao et al. 2002).

The most effective approach to combating bacterial blight is the use of resistant varieties in combination with agricultural management practices. Resistance genes have been detected in landraces (e.g. xa2 and xa5) and in wild rice species (e.g. xa21 in *O. longistaminata*) (Pha & Lang 2004). So far, 28 genes conferring resistance to different Xoo races have been identified (Dwivedi et al. 2007, Gopalakrishnan et al. 2008), and some of them have been incorporated into modern rice varieties by conventional breeding. As conventional backcrosses usually succeed in transferring only one gene at the same time, the large-scale and long-term cultivation of the conventionally-bred varieties resulted in evolution of newer races of the pathogen leading to breakdown of resistance in rice varieties across several Asian countries. One way to delay such a breakdown is to pyramid multiple resistance genes into a variety and to adjust agriculture management practices.

Gene pyramiding can be done by MAS or genetic engineering. Although several genetically-engineered rice lines containing genes for bacterial blight resistance have been tested in the laboratory or under field conditions (e.g. Tu et al. 2000, Zhai et al. 2001, Gandikota et al. 2001), none of these lines has yet been commercialised. Rather, the first biotechnology-derived bacterial blight resistant rice varieties commercially available resulted from pyramiding natural resistance genes via MAS.

Xieyou 218: first MAS-developed rice variety in China

For more than a decade, MAS has been extensively applied for the improvement of bacterial blight (BB) resistance in the rice breeding programmes in China. Breeding BB resistant rice, scientists from the China National Rice Research Institute (CNRRI) applied MAS, at each generation, to select individuals carrying the resistance gene. This resulted in hybrid rice Xieyou 218, which was released for cultivation in 2002. Xieyou 218 is believed to be the first MAS-developed rice variety commercially released in China (Cheng et al. 2007)

Angke and Conde fight bacterial blight in Indonesia

In January 2002, the government of Indonesia released two new rice varieties, Angke and Conde, which were derived by disease resistance breeding augmented with MAS to pyramid BB resistance genes into a commercially adapted variety (Toennissen et al. 2003). Angke and Conde carry the gene pyramids xa4 + xa5 and xa4 + xa7, respectively (Jena & Mackill 2008). Because both new varieties are derived from an existing popular variety, the MAS-improved products are well accepted by farmers and consumers.

Tubigan 7 controls bacterial blight in the Philippines

In 2006, the first MAS-developed rice variety was released to the market in the Philippines. The variety, called Tubigan 7 or NSIC Rc142 and developed by plant breeders at the Philippine Rice Research Institute (PRRI), contains the three resistance genes xa4, xa5 and xa21 integrated in the genetic background of IR64 (Toennissen et al. 2003), a highly popular rice variety in the Philippines. Tubigan 7 was released to the market after almost 10 years breeding work, which was supported by a research grant from the Rockefeller Foundation.

Pusa 1460: MAS results in first bacterial blight resistance in the aromatic germplasm

Basmati rice is highly susceptible to bacterial blight. As there is no known source of resistance in the available aromatic rice germplasm, breeders have to introgress resistance genes from non-Basmati varieties. However, this approach is challenged by the difficulties in recovering the aromatic Basmati qualities in the resulting variety. Now, thanks to MAS, researchers from the Indian Agricultural Research Institute have overcome these difficulties.

In crosses between a bacterial blight resistant non-Basmati rice variety and the popular high-yielding Basmati variety Pusa Basmati 1, they used molecular markers to select for both introgression of bacterial blight resistance genes x13 and xa21 and the recovery of the grain and cooking quality characteristics of Pusa Basmati 1 (Gopalakrishnan et al. 2008). The resulting variety, called Pusa 1460 or 'Improved Pusa Basmati 1', was released in India in 2007. Pusa 1460 is the only source of bacterial blight resistance now available in the aromatic germplasm and is being used as a donor for bacterial blight resistance in the Basmati improvement programme (Gopalakrishnan et al. 2008).

RP Bio 226: a triple pyramid against bacterial blight in India

Samba Mahsuri is a grain indica rice variety that is very popular with farmers and consumers across India because of its high yield and excellent cooking quality. However, the variety is susceptible to several diseases and pests, including bacterial blight. Using molecular marker-assisted backcross breeding, researchers from the Centre for Cellular and Molecular Biology (CCMB) and Indian Council of Agricultural Research (ICAR) introduced three genes for BB resistance (xa21, xa13 and xa5) into Samba Mahsuri (Sundaram et al. 2008). The resulting variety, RP Bio 226, was approved for commercialisation in 2007 (CSIR news 2007). Under conditions of bacterial blight infection, RP Bio 226 exhibits a significant yield advantage over Samba Mahsuri. Most importantly, RP Bio 226 retains the excellent grain and cooking qualities of Samba Mahsuri without compromising the yield. This work demonstrates the successful application of marker-assisted selection for targeted introgression of multiple resistance genes into a premium quality rice variety (Sundaram et al. 2008).

4.2 MAS for better quality rice

Rice quality traits encompass cooking, appearance and sensory properties (Zhang 2007, Fitzgerald et al. 2009). Historically, the primary target of most rice breeding programmes was enhancing yield potential. Consequently, many popular high-yielding rice cultivars have relatively poor quality. With the increased living standard in developing countries and changed consumers' preferences, the improvement of quality has become a priority (Zhang 2007). In countries where rice is consumed, traits of grain quality dictate market value and have a pivotal role in the adoption of new varieties (Fitzgerald et al. 2009).

Given the importance of rice quality for consumer acceptance, programmes have been initiated to improve quality with MAS. The first MAS-developed varieties with enhanced quality have already been released to the market.

Cadet & Jacinto with unique cooking quality

Amylose content in the grain is one of the factors, which determines cooking and eating qualities in rice. Studying non-commercial rice cultivars scientists of the USDA Agricultural Research Service discovered a molecular marker associated with a gene that influences amylose content in rice grain. In the breeding process this marker enabled the rapid identification of succession lines that had the desired gene and to discard those without. The outcome was the release of two new cultivars, Cadet and Jacinto, which have unique cooking and processing quality (Hardin 2000). Developing the two new cultivars took only five years rather than the usual seven to ten years with conventional breeding.

4.3 MAS tackles water shortage

Rice production consumes about 30% of all freshwater used worldwide (Peng et al. 2006). In Asia, more than 45% of total freshwater used is consumed by flood-irrigated rice (Peng et al. 2006). The demand for water-saving agriculture is increasing, as fresh-water resources are declining and the competition for this water between urban areas, the industrial sector and agriculture is increasing (Molden 2007). Tuong & Bouman (2003) estimate that, by 2025, 15 out of 75 million hectares of Asia's flood-irrigated rice crop will experience water shortage.

To tackle the problem of severe water shortage, production management methods to save water in rice cultivation must urgently be disseminated. In northeast China, for example, water-saving management practices are used that not only save almost 50% of water and reduce methane gas, but also take advantage of the fact that when 'drought stress' is applied at the right moment, there is a yield boost as the plant channels all its resources into the grain to ensure survival of the offspring.

Another approach to reducing water inputs in rice is to grow the crop in the same way as an irrigated upland crop, such as wheat or maize (Tuong & Bouman 2003). This approach, called 'aerobic rice system', makes use of varieties that combine the drought-resistant characteristics of traditional upland rice varieties with the high-yielding characteristics of lowland rice varieties (Lafitte et al. 2002, Atlin et al. 2006). Total water use of aerobic rice can be between 25% to 50% lower than that of flooded rice (Bouman et al. 2005).

Aerobic rice may replace irrigated rice and rain-fed lowland rice in some parts of the world, as is already occurring in northeast China (Bernier et al. 2008). However, at times there can be a large yield gap between aerobic and flooded rice and the yield losses of aerobic rice could outweigh the benefits of its water savings. Therefore, before aerobic rice technology can be adopted in large areas in the tropics, new aerobic rice varieties with minimum yield gaps compared to flooded varieties have to be developed (Peng et al. 2006, Tuong et al. 2004).

MAS 946-1 rice saves 60% of water.

"In today's scenario of global warming, water scarcity and changing temperatures, this new variety is a boon for the marginal farmers,"

Shailaja Hittalmani told F&B News (Chandrashekar 2007).

In 2007, MAS 946-1 became the first drought tolerant aerobic rice variety released in India. To develop the new variety, scientists at the University of Agricultural Sciences (UAS), Bangalore, crossed a deep-rooted upland japonica rice variety from the Philippines with a high-yielding indica variety. Bred with MAS, the new variety consumes up to 60% less water than traditional varieties. In addition, MAS 946-1 gives yields comparable with conventional varieties (Gandhi 2007). The new variety is the product of five years of research by a team lead by Shailaja Hittalmani at UAS, with funding from the International Rice Research Institute and the Rockefeller Foundation (Gandhi 2007).

4.4 MAS offers saline solution

"However, the fact remains that these transgenic plants showing improved performance towards salinity stress are yet to move from 'lab to the land'."

Singh et al. 2008

Rice varieties grown in saline environments are sensitive at both the vegetative and reproductive stages (Jena & Mackill 2008). Salinity is the second most widespread soil problem in rice-growing countries after drought (Mohammadi-Nejad et al. 2008). Of the 130 million hectares of land where rice is grown, about 30% contains levels of salt too high to allow normal rice yield (Naheed et al. 2007). The reduction in yields of rice under moderately salt-affected soils is estimated to be 68% (Naheed et al. 2007).



Salt tolerance is a complex and multigenic trait (Flowers 2004). Breeding salt tolerance rice varieties is, therefore, a challenging endeavour. The high variation for salt tolerance within rice landraces and varieties gives the opportunity to improve salt-stress tolerance through conventional breeding. Although conventional breeding programmes have developed some salt-tolerant rice varieties and several lines were released in the Philippines, Bangladesh and India (Ismail et al. 2007), the overall success of conventional breeding is low (Ismail et al. 2007, Singh et al. 2008). Therefore, there remains an urgent need to raise rice varieties that withstand high levels of salt and at the same time maintain optimum yield levels.

Although genetic engineering approaches for enhanced salt tolerance in rice have gained ground among scientists (e.g. Saijo et al. 2000, Hoshida et al. 2000, Ma et al. 2005, Su et al. 2006), progress has been slow (Yamaguchi & Blumwald 2005) and the achievements made so far are not astounding (Ashraf et al. 2008, Arzani 2008). Complex traits of abiotic stress phenomena in plants make genetic modification for efficient stress tolerance difficult to achieve (Wang et al. 2003). The question as to which are the best candidate genes related to salinity stress tolerance improvement is still baffling plant scientists (Singh et al. 2008). The complexity of salt tolerance is likely to mean that the road to engineering such tolerance into sensitive species will be long (Flowers 2004).

Whereas rice varieties genetically engineered for improved performance towards salinity stress are yet to move 'from lab to the land' (Singh et al. 2008), the first salt tolerant rice varieties developed by MAS are now moving forward to the market.

The development of these MAS varieties started in 1997, when scientists at the International Rice Research Institute (IRRI) initiated a mapping programme for salinity tolerance in rice landraces. Four years later, a team at the IRRI, the Philippine Rice Research Institute, the Bangladesh Rice Research Institute and the University of California discovered a major QTL for salt tolerance in the rice landrace Pokkali (Bonilla et al. 2002). This QTL, named *Saltol*, accounts for between 40 and 65% of the salt tolerance observed, and confers tolerance during the seedling stage. After the discovery, a precision marker-assisted backcrossing approach was used to incorporate *Saltol* into popular rice varieties in Bangladesh, Vietnam and India (Leung 2008, Ismail et al. 2007). In Bangladesh, for example, *Saltol* was transferred into two mega rice varieties, BR11 and BR28. It is expected that the newly salt-tolerant BR11 and BR28 will be ready to give to farmers by late 2009 and early 2010 respectively (Rahman et al. 2008).

4.5 MAS makes rice waterproof

In the south and southeast of Asia, rice cultivation is severely affected by submergence in fields because of heavy monsoon rains and poor drainage (Jena & Mackill 2008). Rice plants are sometimes submerged for several weeks, which can sharply reduce yields. The economic loss due to submergence is estimated to be up to USD 1 billion annually (Neeraja et al. 2007, Collins et al. 2008).

Conventional breeding efforts to improve submergence in rice varieties have been going on for more than three decades. Although submergence-tolerant varieties have been developed, they have never been widely adopted by farmers since they lack the desirable traits of the mega varieties, which are popular in the major rice-growing areas of Asia (Septiningsih et al. 2009, Neeraja et al. 2007). Scientists now apply a MAS strategy to overcome this constraint.

To ensure the adoption of the final approach by the farmers, researchers from the IRRI started a breeding programme to convert rice mega varieties by MAS. They work with *Sub1*, a major QTL that confers submergence tolerance. *Sub1* was originally mapped in a landrace from India (Xu et al. 2006), which can survive up to two weeks of complete submergence. One of the mega varieties used by IRRI scientists is *Swarna*, a rice cultivar widely grown in flood-prone regions in India. In collaboration with the Indian Directorate of Rice Research, IRRI scientists succeeded in converting submergence-susceptible *Swarna* into submergence-tolerant *Swarna-Sub1* in only three years (Neeraja et al. 2007). *Swarna-Sub1* shows a twofold or higher yield advantage over *Swarna* after submergence for 10 days or more during the vegetative stage (Septiningsih et al. 2009). Equally important, the introgression of *Sub1* was accomplished without affecting yield, plant height, harvest index or grain quality of *Swarna*, therefore ensuring high acceptance by farmers. Seeds of *Swarna-Sub1* are being produced now in India and Bangladesh for wide-scale distribution to farmers expecting market release in 2009 or 2010.

Swarna is not the only rice mega variety being utilised to breed submergence tolerance. IRRI scientists also use *Samba Mahsuri* and *CR1009* from India, *IR64* from the Philippines, *TDK1* from Laos and *BR11* from Bangladesh as recipient parents to introgress the QTL *Sub1* (Septiningsih et al. 2009). Field trials gave encouraging results. The *Sub1* lines were superior under stress and there were no significant differences under non-stress conditions (Septiningsih et al. 2009). It is expected that some of the *Sub1* varieties could be released to the market in 2010.

In summary, the work on *Sub1* shows that using a MAS approach to incorporate submergence tolerance can enhance mega varieties of rice. This approach has not only gained a lot of attention in scientific literature (Gad 2009, Normile 2008, Ronald 2008, Collins et al. 2008, Jena & Mackill 2008, Sasaki 2006), it also demonstrates that MAS can result in timely achievements given the increasing vulnerability of rice farming to flash floods provoked by the recent trends in climate change.

5) Feature II: Drought tolerance – breeding to fight against the ‘big dry’

“There are hundreds of patents that claim inventions that may improve drought tolerance. But it is hard to discern any of these likely to influence water productivity in the field.”

Passioura 2006

Drought is one of the stresses that threaten worldwide crop productivity most severely. Due to global climate change, droughts are predicted to occur more frequently.

Drought is a quantitative trait, in which numerous genes are involved, each of which having only small effects on the plant properties. The polygenic nature of drought resistance implies that several genes or chromosomal regions (QTLs) must be introgressed at the same time in order to obtain a significant impact. Breeding drought-tolerant varieties is therefore extremely difficult. This holds true not only for conventional breeding approaches, but also for MAS (Francia et al. 2005) and genetic engineering (Passioura 2006). Whereas conventional breeding and MAS have had at least some impact on the direct release of drought-tolerant varieties (for examples, see below), no genetically-engineered drought-tolerant crop has been commercialised to date. Even though some 50 genes have been reported to confer drought tolerance when over-expressed in genetically-engineered plants (Braidotti 2008), and a number of field trials have been conducted, there is scepticism that a panacea can be delivered in the form of a single ‘drought-tolerant’ gene. “To think gene transfer replaces conventional breeding for drought is unrealistic,” says Matthew Reynolds of CIMMYT, the International Maize and Wheat Improvement Centre (cited in Finkel 2009). John Passioura from CSIRO Plant Industry writes (Passioura 2006): “The hope that plants can be transformed by one or at most a few genes to grow well with very scarce water is in my view misplaced.” The complexity of the drought tolerance is likely to mean that the road to genetically engineering drought tolerance into sensitive varieties will be long. Meanwhile, conventionally-bred drought-tolerant crops, such as ZM521 maize and the wheat variety Drysdale and Rees, are grown on farmers’ fields.

ZM521 offers better livelihoods for poor farmers

The breeding of drought-tolerant varieties by conventional approaches is hampered mainly by the complex, polygenic nature of drought tolerance. However, there have been some successes in developing drought tolerance by conventional breeding. One of the examples is the open-pollinated maize variety ZM521 developed by the International Maize and Wheat Improvement Centre (CIMMYT). To develop the new variety, scientist from CIMMYT drew on thousands of native varieties of corn from seed banks, which were built up through decades of free exchange of landraces around the globe (Charles 2001). Through repeated cycles of inbreeding and selection, the scientists uncovered the previously hidden genetic traits that enable maize to withstand drought. Going further along the breeding process, CIMMYT scientist chose a participatory approach. They involved small farmers, NGOs and other stakeholders in the selection process of the best varieties. The result of this ‘mother-baby-trial’ approach is ZM521, a maize variety that not only exhibits remarkable vigour when afflicted by water shortage, but also yields between 30 and 50% more than traditional varieties under drought (CIMMYT 2001). Another of the pro-poor advantages of ZM521 is that it is open-pollinated. Because of cash constraints, smallholder farmers often resort to planting saved maize grain from the previous harvest. In contrast to hybrid and genetically-engineered maize varieties, such home-saved seeds can be used in open-pollinated forms. ZM521 seeds are now available free-of-charge to seed distributors around the world and in several African countries, including South Africa and Zimbabwe, ZM521 has been released for cultivation on farmers’ fields.

Drysdale and Rees give more crop per drop

The wheat varieties Drysdale and Rees are two further notable examples showing that conventional breeding can develop drought tolerance. By using the so-called DELTA technique, a gene selection approach based on carbon isotope discrimination, wheat-breeding scientists from Australia’s Commonwealth Scientific and Industrial Research Organisation (CSIRO) succeeded in increasing the water-use efficiency, which confers drought tolerance. Drysdale, for example, can outperform other varieties by up to 40% under very dry conditions (Richards 2006). The two drought-tolerant wheat varieties have been released for commercial cultivation in Australia by Graingene, a joint venture between AWB Limited, the Grains Research and Development Corporation (GRDC), Syngenta and CSIRO Plant Industry (Glover et al. 2005).

According to CIMMYT maize physiologist José Luis Araus, the DELTA technique developed by CSIRO has revolutionised breeding for drought tolerance. He is applying the technique now to breed drought tolerant maize in sub-Saharan Africa (Finkel 2009).

image Conventional maize suspected of being contaminated by genetically modified maize crops. Mexico.



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MAS for drought tolerance: outlook

Several recent publications reported the detection of QTLs with large effects for yield under drought. Most of the hitherto detected QTLs only had small effects and usually accounted for around 10% of the observed variation in drought tolerance. Now, major QTLs with large effects (accounting for more than 30% of the variation) have been found in maize (Landi et al. 2007), rice (Bernier et al. 2007, 2009, Kumar et al. 2007) and sorghum (Harris et al. 2007). They give promising opportunities for improving drought tolerance by MAS in these crops.

Another encouraging example is provided by the scientists who developed the wheat variety Drysdale (see above). To further improve Drysdale, the scientists now use DNA markers that track genes conferring drought tolerance. A variety developed by this MAS approach should be ready for commercialisation in 2010 (Finkel 2009).

6) Feature III: MAS harnesses biodiversity

“Although some scientists favour transgenic approaches, a ‘back to nature’ approach to genetic diversity may prove faster and more effective.”

Johal et al. 2008

“Perhaps most important, MAS will facilitate more efficient utilisation of new genetic variation from exotic sources, which will provide considerable added value.”

Xu & Crouch 2008

Plant breeding has led to increased productivity but, at the same time, it has narrowed the genetic basis of crop species. As a result, in most crops, the currently-cultured germplasm only represents a small section of the vast diversity available in the species. It is estimated that for most crop species, less than 5% of the biodiversity known to exist is being utilised in agriculture, particularly in the case of self-pollinated crops (Tanksley and McCouch, 1997).

Despite many studies that have demonstrated the value of genes or alleles originating from wild species and landraces (Tanksley and McCouch, 1997, Hajjar & Hodgkin 2007), breeders are traditionally reluctant about the use of non-cultivar germplasm in their breeding programmes due to complex, long-term and unpredictable outcomes; particularly in crops where quality traits are important market criteria (Peleman & van der Voort 2003b). Marker-assisted selection now enables the breeders to precisely introgress small sectors of wild species or landraces, thereby providing breeders with the tools to effectively unleash the vast resources held in wild genetic variation (Peleman & van der Voort 2003b, Xu & Crouch 2008, Zhu et al. 2008). This use of marker-assisted selection to ‘unlock’ wild genetic variation, already suggested 10 years ago (Tanksley & McCouch 1997), has gained renewed interest in recent years (Johal et al. 2008, Hospital 2009). There are several reasons for this: advances in quantitative genetics and genomic technologies have facilitated the exploration and utilisation of natural genetic variation (Hajjar & Hodgkin 2008, Johal et al. 2008); as it is feared that currently-cultivated germplasm is ill-equipped to withstand predicted changes in the environment due to global warming, the ability to map adaptive genes and QTLs in collections of landraces or samples from wild species has been recognised as having great potential for future trait improvement and food security (Johal et al. 2008); the realisation that genetic engineering may not boost plant yields and sustainability as quickly as hoped and that, in contrast, a ‘back to nature’ approach to genetic

diversity may prove faster and more effective than the genetic engineering approaches (Johal et al. 2008, Zamir 2001); the experimental demonstration of the potential of marker-assisted selection for unlocking natural variation (Hospital 2009); and finally, the successful development of commercially-available varieties such as AB2 tomato and Vivek QPM 9 maize (see below).

Taken together, the idea of using natural genetic variation found in wild species and early landrace varieties to revitalise modern crop varieties is both emotionally appealing and intellectually compelling (McCouch 2004). The utilisation of a marker-assisted approach provides a non-invasive road map to expedite the selective introgression of useful traits in the future (McCouch 2004). In the words of Dani Zamir from the Hebrew University of Jerusalem, "The time has come for the plant breeding community to move back to nature." (Zamir 2008)

AB2 tomato breaks agricultural yield barriers

One striking example of how MAS can be used to exploit natural variation is the development of the tomato variety AB2. To develop this variety, researchers from the Hebrew University of Jerusalem and the Max-Planck-Institut für Molekulare Pflanzenphysiologie in Golm introduced chromosome segments of the inedible wild species *Solanum pennellii* into the genetic background of an elite tomato variety via marker-assisted selection (Lippman et al. 2007, Fridman et al. 2004). This approach enabled the identification of a yield-associated QTL named Brix9-2-5. In collaboration with breeders of the Israeli company, AB Seeds, the processing tomato variety AB2 was developed, harbouring the QTL Brix9-2-5 and showing a high yield and increased sugar content. In 2008, AB2 was the leading tomato variety in California (PTAB 2009), which is the largest world producer of industrial processing tomatoes.

By breaking agricultural yield barriers, the AB2 tomato not only provides an example of the indispensable value of natural biodiversity for yield improvement, it also provides the rationale for implementing similar strategies for other agricultural organisms that are important for global food security.

Vivek QPM 9 speeds up quality protein improvement in maize

Although maize is widely used as both food and feed, normal maize kernels do not provide sufficient quantities of two essential amino acids, lysine and tryptophan (Babu et al. 2004). However, in a Peruvian maize landrace a naturally occurring gene, opaque-2, was discovered. This gene confers improved protein quality due to increased levels of lysine and tryptophan in the kernels (Dwivedi et al. 2007).

Utilising the opaque-2 gene, conventional breeding procedures have been successfully used to convert commercial maize lines into so-called Quality Protein Maize (QPM) (e.g. Krivanek et al. 2007). In countries where maize is an important component of the human diet, the release and adoption of QPM maize offers an opportunity to not only improve maize production but also to greatly improve the nutritional status and livelihood of the farmers, particularly the poor subsistence maize growers (Hussain et al. 2006).

Although conventional breeding procedures have been used successfully to convert commercial lines to QPM forms, the procedure is highly cumbersome and time-consuming (Babu et al. 2004, Semagn et al. 2006). Now, researchers from the Indian Council of Agricultural Research (ICAR) have developed a marker-assisted technique that enables a simple, rapid and efficient way to breed QPM maize (Gupta et al. 2009). Following the MAS method, the Indian scientists converted many promising inbred lines of maize into QPM. In 2008, the first of these lines, the variety Vivek QPM 9, was released for commercial cultivation in India (Gupta et al. 2009). The marker-assisted breeding technique sped up the creation of the new variety by several years. While conventional breeding techniques take nearly 10 years to develop a new variety of maize, with the help of marker-assisted technology the Indian scientists were able to develop Vivek QPM 9 in just three. Furthermore, Vivek QPM 9 has been found suitable for cultivation under organic farming (Gupta et al. 2009).

MAS programmes to convert locally-adapted maize germplasm to QPM have also been initiated in Vietnam, China and Indonesia (Srinivasan et al. 2004).

image One striking example of how MAS can be used to exploit natural variation is the development of the tomato variety AB2, which has a high yield and increased sugar content. In 2008, it became the leading variety in California, the world's largest producer of industrial processing tomatoes.



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7) Conventional and marker-assisted breeding for better nutrition

“Plant varieties identified by marker-assisted selection (MAS) are gaining prominence as a publicly acceptable alternative to transgenic crops, such as ‘Golden’ Rice.”

PH 2008

“The use of biotechnological tools, such as molecular marker-assisted selection, will significantly increase the pace and prospects of success for breeding to improve the nutritional value of staple food crops.”

Gregorio 2002

More than one-half of the world’s population suffers from micronutrient deficiencies caused largely by a dietary lack of iron, zinc, and vitamin A. This ‘hidden hunger’ can have devastating consequences for the life, health and well-being of affected individuals. Women and children in sub-Saharan Africa, south and southeast Asia, Latin America and the Caribbean are especially at risk for disease, premature death, and impaired cognitive abilities because of diets lacking iron, zinc and vitamin A.

To combat vitamin A deficiency, consumption of locally-available vegetables, fruits and other plant foods, such as algae products, is encouraged by several programmes and promoted with success in several regions of the world (Lorch, 2005). Programmes to combat vitamin A deficiency that target the root problem in a holistic manner have been very successful in the past decade in countries like the Philippines or Bangladesh.

It has been argued by some that an increase of the vitamin A contents in staple crops – so-called bio-fortification – may also contribute to combating vitamin A deficiencies and that bio-fortification may complement current approaches such as commercial fortification of food and diet supplementation.

Whereas genetic engineering approaches to bio-fortification have attracted much publicity in recent years, it has largely remained unnoticed by the general public that conventional breeding and MAS represent a viable alternative to genetic modification strategies for bio-fortification. HarvestPlus, for example, an interdisciplinary alliance of institutions and scientists working to breed bio-fortified crops, devotes 85% of its resources to conventional breeding, because of regulatory and political restrictions on the use of genetic engineering approaches and because significant progress can be made through conventional breeding (Nestel et al. 2006).

Given the genetic variation in concentrations of zinc, iron, and provitamin A among cultivars, conventional and marker-assisted breeding have the potential to increase the micronutrient density of staple crops (Gregorio 2002, Bouis, 2003, Nestel et al. 2006). By exploring the genetic variation in existing varieties and in germplasm collections, genes and QTLs affecting vitamin and mineral contents have recently been identified in staple crops, including rice, maize, wheat, cassava, sorghum and pearl millet. Efficient molecular marker techniques now allow the genes to be tagged, and thus markers can be used to facilitate crossing the genes into new breeding lines (see Table 3 for ongoing research activities).

Table 2.0:
Research projects for the nutritional improvement of varieties by marker-assisted selection.

Plant	Trait	Institution	Ref.
Bean	Iron	International Centre for Tropical Agriculture	1
	Zinc	International Centre for Tropical Agriculture	1
Cassava	Provitamin A	International Centre for Tropical Agriculture	2
Maize	Vitamin E	National Maize Improvement Centre of China	3
	Provitamin A	Cornell University, CIMMYT	4
Melon	Provitamin A	USDA Agricultural Research Service	5
Pearl Millet	Provitamin A	International Crops Research Institute for the Semi-Arid Tropics	6
Rice	Zinc	China Agricultural University	7
	Iron	International Centre for Tropical Agriculture	8
Sorghum	Provitamin A	Cornell University	9
Wheat	Zinc	Molecular Plant Breeding CRC	10

References:

- 1: Blair et al. 2009;
- 2: www.ciat.cgiar.org
- 3: Chander et al. 2008;
- 4: Harjes et al. 2008;
- 5: Cuevas et al. 2008;
- 6: ICRISAT 2009;
- 7: Garcia-Oliveira et al. 2009;
- 8: www.ciat.cgiar.org;
- 9: Salas-Fernandez et al. 2008;
- 10: Genc et al. 2009.

MAS enhances breeding of high provitamin A maize

Maize is a staple food for large groups of people in Africa. However, the traditional yellow maize varieties have low amounts of provitamin A, ranging from 0 to 1.5 micrograms (Davis et al. 2008, Harjes et al. 2008). As almost 50% of children in Africa under five are clinically or sub-clinically deficient in vitamin A (Stevens & Winter-Nelson 2008), the nutritional improvement of maize for provitamin A content would have a significant impact on the target populations.

There have been some genetic engineering approaches to increase provitamin A concentration in maize, with some success as reported recently (Naqvi, 2009). But CIMMYT, for example, currently focuses on non-transgenic approaches in its breeding programme, because provitamin A levels in conventionally-developed maize are higher than in genetically-engineered ones (Ortiz-Monasterio et al. 2007).

Given the great variation for provitamin A concentration in different maize varieties, there is considerable scope for conventionally bred maize with elevated provitamin A concentrations (Ortiz-Monasterio et al. 2007). Up to the present, conventional bio-fortification enhanced the provitamin A level to 15 micrograms (Tanumihardjo et al. 2008). This concentration is thought to be adequate to increase vitamin A in humans when maize is consumed as a staple crop (Tanumihardjo 2008).

In spite of the promising advances, conventional breeding approaches are currently hampered by the expense and time required to assess provitamin A concentration in maize kernels (Ortiz-Monasterio et al. 2007). But now, MAS offers a solution to overcome these limitations. Using state-of-the-art technology, a team of plant geneticists and crop scientists recently identified genetic markers that are associated with higher levels of provitamin A in maize (Harjes et al. 2008). With the help of these markers, provitamin A concentration in maize kernels can now be easily assessed. Scoring the target gene is not only cheaper than the previous methods used to assess provitamin A levels, it is also well within the capabilities of those developing countries that need bio-fortified maize. Moreover, the information needed for the marker-assisted selection is being made freely available for breeders worldwide in this specific project. This is in contrast to transgenic Yellow Rice, which is a proprietary technology that has been made (partially) available to those who have the expertise to make use of it. In summary, if maize varieties with increased vitamin A contents are considered a valuable tool to combat vitamin A deficiencies, they can easily be bred using MAS without the need for genetic engineering.

Provitamin A enhanced sweet potato

Sweet potato, a staple food for many people in Africa, has also been suggested as a candidate crop for bio-fortification approaches aimed at alleviating vitamin A deficiency. Traditionally, Africa's predominant sweet potato varieties are white- or yellow-fleshed varieties containing no or only small amounts of provitamin A. However, substantial levels of provitamin A can be found in many orange-fleshed varieties of sweet potato and data show that regular consumption of these varieties does improve vitamin A status (Tanumihardjo et al. 2008).

In 2001, the International Potato Centre (CIP), together with about 40 other organisations, launched the Vitamin A Partnership for Africa (VITAA)⁴. Since then, the VITAA programme coordinates efforts of local plant breeders to select orange-fleshed varieties with enhanced levels of provitamin A using conventional methods and to promote their increased adoption by farmers in Africa (Johns & Eyzaguirre 2007). Today, bio-fortified sweet potato varieties are being disseminated in Africa. The VITAA programme has been most effective in Uganda, Kenya, Mozambique and Tanzania. Although not a silver bullet for curing malnutrition, the introduction of orange-fleshed sweet potatoes can be considered a success story (Tanumihardjo et al. 2008).

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A close-up photograph of several golden wheat stalks, showing the detailed structure of the grain heads. The lighting is warm, highlighting the texture of the wheat.

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