

# **SMART BREEDING: THE NEXT GENERATION**

**MARKER ASSISTED SELECTION:  
A BIOTECHNOLOGY FOR PLANT BREEDING  
WITHOUT GENETIC ENGINEERING**

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**GREENPEACE**

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## Commonly used abbreviations

<b>BB</b>	bacterial leaf blight	<b>GE/GM</b>	genetically engineered/genetically modified
<b>BPH</b>	brown planthopper	<b>IRRI</b>	International Rice Research Institute
<b>CIMMYT</b>	International Maize and Wheat Improvement Center	<b>QTL</b>	quantitative trait loci
<b>CMD</b>	cassava mosaic disease	<b>QPM</b>	quality protein maize
<b>DTMA</b>	Drought Tolerant Maize for Africa project	<b>MAS</b>	marker-assisted selection
<b>ha</b>	hectare	<b>PPB</b>	participatory plant breeding
<b>GPC</b>	grain protein content		

## INNOVATION THAT LOOKS TO THE FUTURE, BUT DELIVERS TODAY: A SUMMARY BY GREENPEACE INTERNATIONAL



Marker assisted selection (MAS) is a plant breeding biotechnology that is delivering benefits without producing GM crops.

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Biotechnology is often equated with genetic engineering, and the support or opposition to genetically engineered (GE) crops is often distilled down to being for or against “science”. Yet biotechnology is about much more than genetic engineering, and science and innovation in biotechnology for plant breeding is about much more than cutting and pasting genes between organisms, as genetic engineering does.

The backdrop is the challenges we face in delivering tools for ecological farming<sup>1</sup> and sustainable food production. New technology and innovation is important in reducing inputs and enhancing outputs, but alone will not solve issues associated with infrastructure, land access and poverty. Probably the innovations that would have the biggest impact on equitable global food availability are very broad, including land tenure reform and property rights, different rural groupings having greater social equality, or movement towards lower meat consumption diets. Obstacles to these alternative forms of innovation are at least as much to do with commercial, institutional and cultural barriers as they are to do with technical limits.

New technology may also affect – among other things – power relationships, farmer debt levels, ownership rights and market access, so the stakes are high. This explains why a global study in 2009 – the International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD) – looked to distil global wisdom on what the best role of technology is, at least in developing countries. Its conclusion is that innovation across many dimensions of crop husbandry and food production and management have much to offer. But notably it had little to say on the potential contribution of GE crops.

Genetic engineering has an inability to deal with complex (multi-genetic) traits (often the ones most useful, such as increased yield) and, latterly, its delivery record – given the large amount of private and public sector money and political oxygen – is becoming increasingly difficult to justify. GE crops are very limited in sophistication, being almost completely dominated by herbicide tolerance and insect resistance traits. Could the numerous tools of biotechnology deliver better outcomes? This report tries to answer that question.

**A different application of biotechnology – marker assisted selection (MAS) – is quietly making a significant impact.** MAS uses a conventional breeding approach – it is not genetic engineering. Instead, MAS uses advanced genetic marker technology to assist the breeding of genes conferring the desired trait into new crop varieties, often with traits introduced from wild relatives or traditional varieties to boost genetic resilience. Consequently MAS has fewer safety concerns compared to GE crops, respects species barriers, is more acceptable to consumers, is faster to market and better tackles complex traits like drought resistance.



Given that knowledge of variety performance is critical, MAS lends itself to farmer participation and allows region-specific breeding. It's thus able to provide farmers with more suitable, locally adapted new varieties than solely centralised breeding. Such an approach ensures that local genetic, climatic and cultural diversities are taken into account as opposed to the standardised and homogenised model promoted by biotech companies with genetic engineering approaches. None of this is to say that MAS immediately solves all the problems of breeding or variety development, nor does it necessarily deliver technology control to those who need it. But it most certainly helps, and is delivering on the ground. The full benefits of MAS, however, will only materialise if it is an open source technology without associated patents on the techniques.

Examples of the success of MAS are tackling bacterial leaf blight, one of the most serious threats to rice in irrigated and rain-fed systems, across China, India and Indonesia. Rice blast is estimated to cause losses of 10-30% of annual rice harvest, but MAS has successfully tackled it through the breeding of resistant varieties in Thailand and Korea. MAS has extended the lifespan of a popular and effective pearl millet variety in Northern India by breeding in downy mildew resistance – the varieties are used across approximately 900,000 hectares. In Sudan, it has helped deal with striga (known locally as witchweed) in sorghum, and is expected to be used in other African countries soon. Cassava, a staple food for 200 million people in sub-Saharan Africa, has been bred with MAS to provide resistance to cassava mosaic disease – which can deliver yield losses of 20-90% at times – in Nigeria and Tanzania. MAS has brought fungal resistance to North American wheat farmers. Other successes include flood and drought tolerant rice.

MAS is delivering benefits on the ground across continents and in many different crops. Ten years ago, MAS was in its infancy, but is now so commonplace that it isn't always possible to determine whether a variety has been developed using MAS or traditional conventional breeding. This throws a sharp light on the GE crop controversy, which has been going on for over 20 years, but with little development beyond the limited range of herbicide tolerant and insect resistant traits in major commodity crops.

In 2009, Greenpeace produced the report *Smart Breeding* (authored, like this one, by Benno Vogel). Since then, the proliferation of MAS-bred traits in a number of crops has prompted this update of the original report. We hope that this new report demonstrates the valuable role that biological science and technology can have, and that Greenpeace is very happy to support new technology with the right risk profile and in the right context.

## OVERVIEW

### What is Marker Assisted Selection?

In traditional conventional 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 content or size can easily be measured, more complex traits such as disease resistance or drought tolerance are much more difficult to determine for the breeder. It is time consuming for breeders to identify those potatoes that are more drought tolerant than others in breeding programmes. 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 offspring 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 (usually 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.

## State of application of MAS

MAS has been expanding in recent years due to lowering costs, improved efficiency, and easiness of enhanced marker technologies. It is now effectively applied to a broad range of crop species, among them several crops that are important for food security such as barley, beans, cassava, chickpea, cowpea, groundnut, maize, potato, rice, sorghum, and wheat. As there is no comprehensive documentation regarding effective applications of MAS, precise figures on the number of MAS-varieties released and their actual adoption by farmers are not available. However, from the literature it is evident that MAS is a mainstay activity in private sector breeding companies and plays a prominent role in public breeding programmes. The latter is illustrated by the 136 publicly bred MAS-varieties identified in the course of the present work.

### Feature: MAS against biotic (biological) stresses

Biotic, or biological stresses such as viruses, fungi, bacteria, weeds and insects are a major constraint to agricultural productivity. The most economical and ecologically friendly way to control these biotic constraints is breeding of resistant crop varieties. Resistance breeding by MAS is highly efficient and precise compared to conventional approaches. It not only offers the opportunity to speed up variety development, but also provides a unique method for pyramiding (or combining traits) and thus to achieve durable resistance to both diseases and pests. In the last ten years, MAS has proven its potential to control biotic stresses in a range of crops, and current markers even tackle some of the biggest constraints worldwide such as bacterial blight and blast in rice, rusts in wheat, common bacterial blight in beans, striga in sorghum and cassava mosaic virus in cassava.

### Feature: MAS against abiotic (physical and chemical) stresses

Abiotic, or physical and chemical, stresses such as drought, salinity, or submergence are a major challenge for sustainable food production. Ongoing global climate change will further increase these challenges, making crops with abiotic stress tolerance a key for the future. MAS is seen as having potential to facilitate the development of crops tolerant to abiotic stresses. However, while the number of markers for abiotic stress tolerance genes has increased in recent years, so far only a few of them have been applied successfully in public breeding programmes. Nevertheless, recent releases of water submergence, drought and salt tolerant rice varieties confirm the potential of MAS. Furthermore, progresses in using MAS for breeding drought tolerance in maize, chickpea and sorghum, salt tolerance in durum wheat, or aluminium tolerance in barley, illustrate that the number of success stories will grow in future.

### Feature: MAS for quality traits

The breeding of crops with improved quality traits, e.g. enhanced protein content or optimised amino acid composition, is gaining importance in both developed and developing countries. Until recently the improvement of quality traits has almost always been a slow and expensive process, mainly due to the complex nature of these traits. The advent of molecular marker techniques now makes it possible to “tag” genes conferring desirable quality traits, and by that to speed up the breeding process. In crops such as barley, broccoli, maize, peanut, rice, soybean or wheat, MAS has already been used effectively to improve quality traits. Successful examples include bread wheat varieties with high grain protein content, and rice varieties with improved cooking quality. MAS can utilise the natural genetic variability of micronutrient levels and is currently being used to breed enhanced concentrations of provitamin A, iron and zinc into several crops.

## Feature: Harnessing biodiversity

Despite the value of genes (or alleles) originating from wild relatives and landraces (traditional varieties), breeders have traditionally been reluctant about the use of these resources in their breeding programmes. This is mainly due to problems associated with the co-transfer of undesirable genes linked with the desired trait which may cause reductions in yield, or “yield drag”. But today, the use of molecular markers enables to precisely introduce small sectors of the genomes of wild relatives or landraces into high yielding (e.g. elite) varieties. Thus, MAS provides breeders with the tools to effectively unleash the vast resources held in both wild relatives of crop plants and landrace genetic variation. In recent years, this genetic variation has successfully been unlocked by MAS for breeding better traits into a range of crops, such as high yield in rice and tomato, stripe rust resistance in wheat, planthopper resistance in rice or high-quality protein in maize.

## Feature: Harnessing farmer's knowledge

Participatory plant breeding (PPB) is a breeding approach that combines science-based methods and farmers' experience. As PPB offers a rapid, cost effective strategy for breeding region-specific, farmer-preferred, superior varieties, there is currently a growing interest to involve farmers in variety development. Although PPB is mainly focusing on conventional breeding techniques, some pioneering programmes have begun to combine farmers' knowledge with MAS. The first varieties bred through this merge of “bottom up” and “top down” approaches have already been released for cultivation. Among them are UMUCASS 33, a virus-resistant cassava variety, Birsa Vikas Dhan 111, a drought tolerant rice variety, and HHB 67-Improved, a disease resistant pearl millet variety.

## Outlook to the future: Harnessing science

Although MAS has become a valuable breeding tool, its application is still facing some drawbacks such as high costs or lack of breeder-friendly markers. However, as new tools and technologies such as next-generation sequencing, high-throughput genotyping and genome wide selection are making MAS more and more based on the whole genome, rather than small segments. The number of crop species with sequenced genomes is steadily growing, so the current drawbacks are expected to be overcome in the near future. Recent technological advances are increasingly making MAS a choice of public-sector plant breeding and this should lead to a wide adoption of MAS for more crop species in more countries.

MAS has already been used to breed a wide variety of desired traits into many crops. It's likely that MAS will continue to become more widely adopted. MAS offers several advantages over genetic engineering: MAS respects species barriers, raises less safety concerns, is accepted by the public and permitted in organic farming.



<sup>1</sup> See Greenpeace International's website for further information what Ecological Farming is and how it can be achieved. <http://www.greenpeace.org/international/en/campaigns/agriculture/solution-ecological-farming/>

## Glossary

**Abiotic stress** is a stress induced by non biological, non living chemical and physical parameters, e.g. drought or cold.

**Biotic stress** is a stress induced by living organisms (e.g. bacterial or fungi).

**Elite variety** is a modern variety with well-documented superior characteristics (e.g. high yield).

**Germplasm** is a collection of genetic resources for an organism. For plants, this can be a seed collection or a collection of cultivated varieties.

**Hybrid variety** is the offspring of a cross between two genetically different lines. Typically hybrid varieties have higher yields than open pollinated varieties, but their offspring loses this characteristic. Hybrid seeds are purchased by farmers each year, whilst seeds for growing the following season can be saved from open-pollinated varieties.

**Introgression** is the spreading of a gene through a gene pool. In plant breeding, it means the spreading of a particular gene of interest throughout a particular variety.

**Landrace** is a locally-adapted, traditional variety of a plant species. These have been developed over time and may have cultural value. They have generally been developed by breeding of open-pollinated varieties.

**Marker-assisted selection (MAS)** describes a breeding approach where a genetic sequence (identified by a molecular marker) that is linked to a particular trait (e.g. disease resistance) is bred into plants to create new varieties with that trait. MAS utilises conventional breeding rather than genetic engineering. It is also called “Smart Breeding”.

**Molecular markers** are short, detectable segments of DNA in the genome of an organism that represent the desired genes themselves, or are located near the DNA sequence of the desired genes. They can act as “signs” or “flags” for the presence of the desired genes during the breeding process.

**Open pollinated variety** distinguishes a plant variety of a hybrid variety. The seeds of open-pollinated plants will produce new generations of those plants that farmers can grow the next year. The seeds may vary in traits and this is often used to increase the diversity of traits, or to intensify a particular trait.

**Phenotype** refers to the overall characteristics of a plant, i.e. the collection of traits displayed by the plant.

**Polygenetic** refers to a trait that is affected by several or numerous interacting genes.

**Pyramiding** is a breeding strategy for taking genes or QTLs from different parents and stacking them into one variety. It has been successfully applied to develop varieties with durable resistance to stresses.

**Quality trait** is a non-argonomic characteristic of a plant such as cooking attributes, provitamin A levels or high protein content.

**Quantitative trait locus (QTL)** is a region of the plant genome (DNA) that effects the expression of a trait and is linked to, or contains, genes related to the trait.



# MARKER ASSISTED SELECTION

## A BIOTECHNOLOGY FOR PLANT BREEDING WITHOUT GENETIC ENGINEERING

**BENNO VOGEL**

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# 1. INTRODUCTION



While genetic engineering is in the public spotlight but has progressed little, a silent transformation in conventional plant breeding has taken place through the use of MAS.

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Despite increased investments in agriculture, global food insecurity remains a chronic problem. Recent estimates indicate that, globally, 842 million people are still suffering from hunger, regularly not getting enough food to conduct an active life (FAO 2013). In future, food insecurity may even increase, as efforts to mitigate the issue are faced by several challenges. The growing world population, projected to exceed nine billion by the year 2050 (UN DESA 2013), presents challenges. Rising wealth, accompanied by a higher consumption of processed food, meat and dairy, as well as the new demand for biofuels, add further pressure to the food supply system. At the same time agricultural production is hampered by declining freshwater resources, decreasing availability of arable land and the need to alleviate the negative effects of food production on the environment (IAASTD, 2009). Overarching all of these issues are the impacts of climate change, which substantially lessen the odds for attaining the unprecedented demand for increased food production.

Recently, seven priority actions to achieve food security in the face of climate change were identified (Beddington *et al.* 2012). One of these priority actions is to sustainably “intensify” agricultural production. However, the term “sustainable intensification” is somewhat controversial, as many modern agricultural systems are already viewed as over-intense in the sense that they cause environmental degradation (IAASTD 2009). Furthermore, there is recognition that action is needed throughout the whole food system including moderating demand, reducing waste and improving governance (Godfray & Garnett 2014). Nevertheless, it is generally recognised that it is desirable to grow crops with less fertilisers and pesticides while maintaining, if not increasing, yields. Growing of diverse “smart” crop varieties that are capable of producing “more with less” will be critical to achieve increases in crop yield within a framework of ecological agriculture. Thus, plant breeding and the cultivation of the resulting varieties constitute a major component of the interventions required to reorientate crop production (Mba *et al.* 2012).

Improved crop varieties, suited to a range of agroecosystems and farming practices, and resilient to climate change are key to “sustainable crop production intensification” (FAO 2011). The question is, how to breed them? Although conventional breeding offers a wide spectrum of methods today, many plant scientists assume that the answer has to be sought in the most advanced biotechnologies and thus in molecular breeding, including both genetic engineering and marker-assisted selection (MAS).

In the past, and still today, genetic engineering has captured most attention. Biotechnology companies, as well as some public sector scientists, have promoted genetic engineering as the best plant breeding approach to increase agricultural production in a sustainable way. However, previous achievements of commercial genetically engineered

(GE) crops are disappointing in breeding terms. Two simple traits, herbicide tolerance and insect resistance, solely or combined, introduced into four crops are consistently accounting for 99% of the global area grown with GE crops (James 2014).

While genetic engineering is in the public spotlight, it remains largely unnoticed that significant achievements of molecular breeding have been in the use of MAS. Therefore, it is time to change the focus and to put emphasis on the positive role that MAS can play in achieving both food security and sustainable development. To highlight the breeding area of MAS, this report first describes how MAS works, what advantages it offers compared to conventional breeding, and which varieties have been developed in private and public sector breeding programmes. Then, in features about biotic stress resistance, abiotic stress tolerance, quality traits and harnessing biodiversity the report pictures some of the success stories of MAS. After this, a feature about participatory plant breeding illustrates how MAS can be combined with farmers' knowledge to ensure adoption of newly developed varieties. Finally, the report takes a look to the future and confronts GE with MAS.



## 2: MARKER-ASSISTED SELECTION: FAST-TRACK PLANT BREEDING



With MAS, breeders can scan new varieties for the presence of specified molecular markers. If these are present, it indicates the variety possesses the desired trait.

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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. 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 (MAB) 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. Markers represent the target genes themselves or are located near the DNA sequence of the desired genes so they can act as “signs” or “flags”. 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 are present (see Fig. 1). Molecular markers can be employed to assist a wide range of components of modern plant breeding programmes (Collard & Mackill 2008). With respect to important breeding schemes, four main uses of molecular markers in plant breeding can currently be distinguished:

**Marker-assisted backcrossing (MABC)** is regarded as the simplest form of MAS, and actually it is the most widely and successfully used method in practical MAS (Jiang 2013a).

The backcross-based breeding approach is used to transfer one or few genes/quantitative trait loci (QTLs – stretch of DNA that is linked to, or contains, the genes of interest) from an agronomically inferior source (e.g. a landrace or crop wild relative) into a modern cultivar (e.g. an elite high yielding variety). In short, MABC can be efficiently used to improve an existing variety for a specific trait, because it reduces both the time needed to breed a variety and the risk of undesirable linkage drag with unwanted genes (e.g. those that reduce yield) from the trait donor.

“

The whole idea of genomics and marker-assisted selection is amazing. It's now more affordable, easier and faster — we can run hundreds of screenings. We will not be cutting and slicing genes to make GMOs, but the new technology will help us find gene combinations that naturally occur.

”

– Juskiw 2014

**Marker-assisted gene pyramiding (MAGP):** Pyramiding is a breeding strategy for taking genes or QTLs from different parents and stacking them in one progeny. MAGP is currently successfully applied to develop varieties with durable resistance to stresses. As MAGP substantially enhances the selection process and often offers the only effective method for accumulating multiple resistance genes, it is one of the most important applications of molecular markers to plant breeding (Jiang 2013a)

**Marker-assisted recurrent selection (MARS)** is a breeding approach by which individuals of a breeding population are selected based on their marker genotypes and intermated at random to produce the next generation. In this way, desirable genes or QTLs can be brought into the breeding scheme from many different sources (Xu *et al.* 2012). MARS has been proposed as an effective tool to breed complex traits (Xu *et al.* 2012) because it enables harnessing even those genes or QTLs experiencing minor effects of the phenotype.

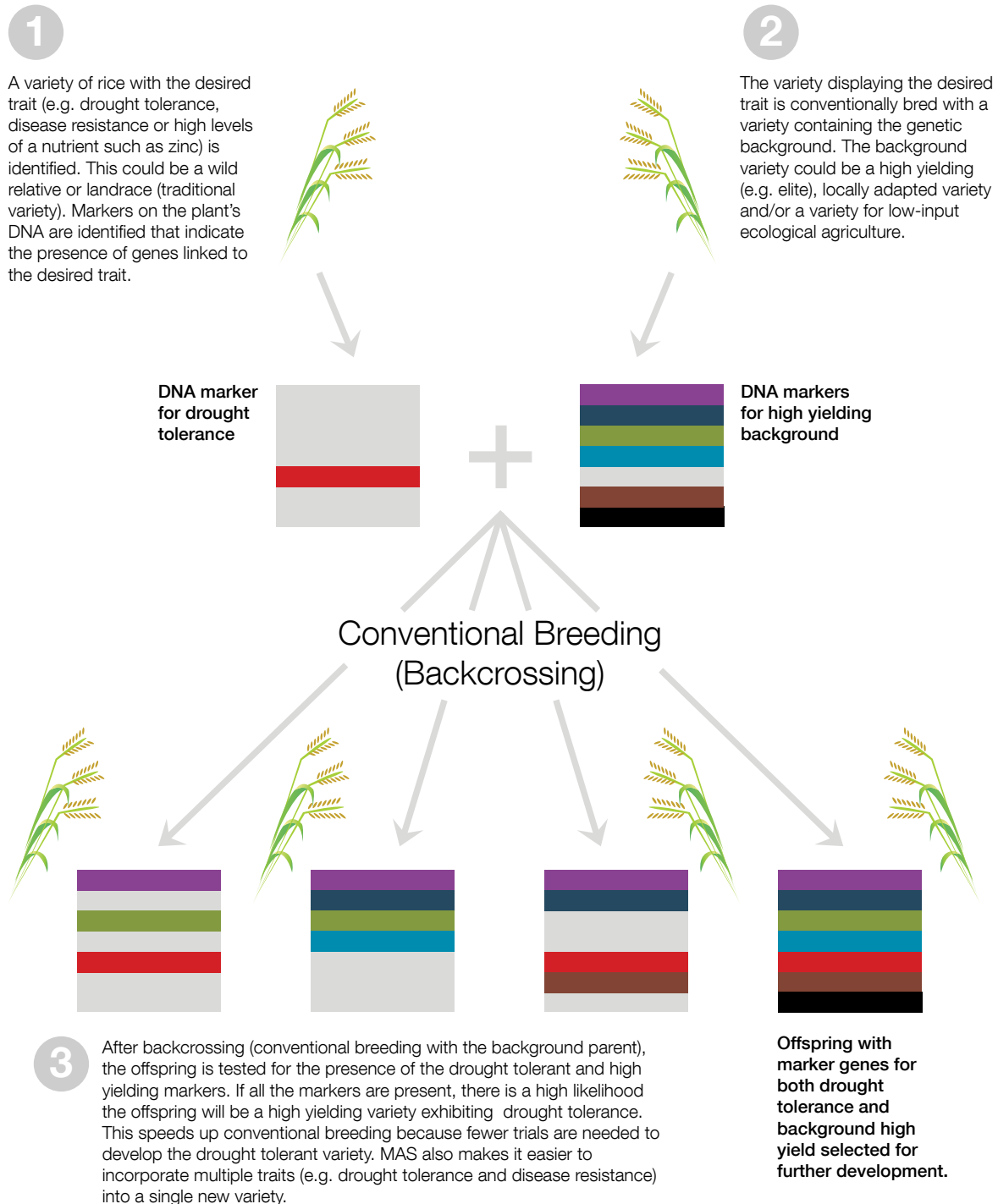
**Genomic selection (GS)** is a recent breeding approach that also relies on MAS (Tester & Langridge 2010). GS enables the simultaneous selection for many (tens or hundreds of thousands of) markers, which cover the entire genome. Hence, it is thought to provide the key in maximising the full potential of MAS, especially for breeding complex traits (Heffner *et al.* 2010, Jannink *et al.* 2010).

In recent years, integration of MAS-schemes into conventional breeding programmes has become a valuable strategy for crop improvement. Although MAS is not the silver bullet for all breeding problems, it can offer several advantages over conventional (phenotypic) selection (Xu & Crouch 2008, Collard & Mackill 2008, Jiang 2013a/b, Miah *et al.* 2013, Roychowdhury *et al.* 2014). Depending on several parameters, including type of crops species and kind of traits to be improved, MAS can be more efficient, effective and reliable than phenotypic selection and can shorten the development time of varieties significantly. Furthermore, in some cases it will be more cost effective than selection based on phenotypes. Finally, a major breakthrough brought to plant breeding by the use of molecular markers is that genotypic selection has become possible not only for simple (that are based on single genes) traits but also for complex traits that involve a broad range of genes, such as yield, abiotic stress tolerance, quality traits and some classes of disease resistance.

Although MAS has become a valuable breeding strategy, its application is still facing some drawbacks (Jiang 2013b). Not all markers can be applicable across populations due the lack of reliable marker-trait association. Also, not all markers are breeder-friendly. Furthermore, false selection may occur due to recombination between the markers and the genes of interest. Finally, although costs have decreased, they remain high. The high cost and technical or equipment demands of MAS continue to be a major obstacle for its large-scale use, especially in the developing countries (Jiang 2013b). However, as technology and application are further developed and improved (see Section 9), it can be expected that the drawbacks of MAS will be gradually overcome in near future (Jiang 2013b).

Taken together, although not a panacea MAS has become a very promising approach to conventional plant breeding as it can significantly reduce time, resources and efforts needed to develop plant varieties with desired traits.





**Fig 1. How marker assisted selection (MAS) works**

A simplified illustration of a MAS breeding scheme using the example of drought tolerant, high yielding rice.

### 3. STATE OF APPLICATION OF MAS



Today, molecular markers are effectively applied to a broad range of crop species, among them several food security crops.

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MAS has been greeted with much enthusiasm and expectation in public and private plant breeding because of its great potential. It is stimulating tremendous investments in the development of molecular marker maps and research to detect associations between phenotypes and markers (Ruane & Sonnino 2007). As a result, thousands of genes and quantitative trait loci (QTL) have been mapped across the major crop species, laying the foundation for MAS-techniques (Xu *et al.* 2012).

Initially, the use of molecular markers was restricted to a few economically important cereal crops. However, due to lowering costs, increased efficiency and ease of enhanced marker technologies, MAS has been expanding in recent years. Today, molecular markers are effectively applied to a broad range of crop species, among them several food security crops such as barley, beans, cassava, chickpea, cowpea, groundnut, maize, potato, rice, sorghum, and wheat (Van Damme *et al.* 2011).

There is no comprehensive documentation regarding effective applications of MAS so precise figures on the number of released MAS-varieties and their actual adoption by farmers are not available. However, from the peer-reviewed scientific literature it is evident that MAS plays a prominent role in plant breeding, and in the course of the present work numerous examples of successful, practical outcomes of MAS have been identified.

Crop-trait combinations found to have been realised by MAS are shown in Table 1. MAS-derived varieties tracked to have been released for cultivation by private and public sector breeding programmes are displayed in Table 2 and the Annex, respectively.

“

Molecular breeding, in particular marker assisted selection, backcrossing and recurrent selection are mainstay activities by private sector seed companies and agribusinesses.

”

– Anthony & Ferroni 2012

#### MAS-varieties released by the private sector

As MAS is seen as a comparative advantage, private-sector breeding companies have done major investments in MAS-infrastructure in the last decade (Koebner 2003, Cahill & Schmidt 2004, Dwivedi *et al.* 2007). Monsanto for example, one of the leading companies, has invested over \$175m US dollars in its MAS-platform (Dhawan 2013). The attraction of the private sector to MAS, especially for big companies, is reflected by the growing number of patent applications associated with molecular markers (Meyer *et al.* 2013). In 2009, about 2 900 patents related to MAS have been found in a patent database search; 890 patents were filed by Pioneer, 498 by Monsanto and 83 by Syngenta (Brumlop & Finckh 2011).

Although very limited details are available about the nature, scale, and scope of private MAS-programmes it is known that today MAS is used by small, medium-sized and big seed companies for the improvement of various crops such a maize, soybeans, sunflower, sorghum, sugar beets, oilseed rape, wheat, barley, rye, rice as well as broccoli, lettuce, tomato, pepper, melon, onion and cucumber (e.g. Eathington *et al.* 2007, Dwivedi *et al.* 2007, Brumlop & Finckh 2011, Seminis 2012, Syngenta 2012, Miedaner & Korzun 2012, Pioneer 2013a/b, Simko 2013).

Only very few examples of MAS-varieties released by seed companies are trackable (Table 2) because the private sector does not normally release details of its breeding methodologies to the public (Xu & Crouch 2008). Among

**Table 1: Crop-trait combinations identified to have been realised in variety development by MAS**

<b>Trait</b>	<b>Crops in which varieties has been developed by MAS</b>
<b>Biotic stress resistance</b>	
Insect resistance	maize, rice, wheat
Fungal resistance	barley, bean, chilli, lettuce, pearl millet, rice, soybean, tomato, wheat
Bacteria resistance	bean, lettuce, rice
Virus resistance	barley, bean, cassava, tomato, wheat, lettuce
Nematode resistance	barley, peanut, potato, soybean
Parasite resistance	sorghum
<b>Abiotic stress resistance</b>	
Acid soil tolerance	barley, rice
Drought tolerance	maize, rice
Salt tolerance	rice
Flood tolerance	rice
<b>Quality</b>	
High protein grain	wheat
High-quality protein	maize
Cooking quality	rice
High glucoraphanin	broccoli
Malting quality	barley
Oil quality	peanut, soybean
Low cadmium grain	wheat
<b>Yield</b>	
High yield	rice, soybean, tomato



them are drought tolerant maize varieties, high yielding soybeans and stripe rust resistant wheat cultivars released in the USA. However, many more successful MAS-applications probably exist but remain within the confidentiality restriction of private companies around the world. This is exemplified by the fact that as much as 35% of Monsanto's plant breeding programmes are based on MAS (Edwards 2010) and that Syngenta already declared nine years ago that it would use marker-assisted breeding technology in developing more than 120 new varieties for commercial launch every year across a range of crops (Syngenta 2005).

**Table 2: Examples of MAS-derived varieties released by the private sector**

Variety/ Trade Mark	Trait	Country	Year	Company
<b>Barley</b>				
Litmus	Acid soil tolerance	Australia	2013	Syngenta <sup>1</sup>
<b>Broccoli</b>				
Beneforte	High glucoraphanin	USA	2011	Seminis <sup>2</sup>
<b>Maize</b>				
Sunrise	Insect resistance	Germany	2010	Saaten Union <sup>3</sup>
Agrisure Artesian*	Drought tolerance	USA	2011	Syngenta <sup>4</sup>
Optimum AQUAmax*	Drought tolerance	USA	2011	Pioneer <sup>5</sup>
<b>Potato</b>				
Figaro	Disease resistance	Germany	2012	SaKa Pflanzenzucht <sup>3</sup>
<b>Soybean</b>				
Vistive*	Low-linolenic acid	USA	2005	Monsanto <sup>6</sup>
Y Series*, **	Yield	USA	2009	Pioneer <sup>7</sup>
T Series*, ***	Yield	USA	2013	Pioneer <sup>8</sup>
<b>Wheat</b>				
Expresso	Disease resistance	USA	2006	Westbred <sup>9</sup>
Blanca Grande 515	Disease resistance	USA	2010	Resource Seeds <sup>9</sup>
Summit 515	Disease resistance	USA	2011	Syngenta <sup>9</sup>
New Dirkin	Disease resistance	USA	2013	Baglietto Seed Company <sup>9</sup>
Westmore****	Grain protein	USA	2007	Arizona Plant Breeders <sup>9</sup>
SY Tyra	Insect resistance	USA	2011	Syngenta <sup>10</sup>

\*: Varieties are stacked with transgenes conferring herbicide tolerance and/or insect resistance; \*\*: Y Series includes 32 varieties; \*\*\*: T Series includes 39 varieties; \*\*\*\*: Westmore is a durum wheat variety.

References: 1: Paynter (2014); 2: Mithen (2012); 3: Brumlop & Finckh (2011); 4: Fithian & Martin (2012); 5: Warner (2012); 6: Monsanto (2004); 7: Pioneer (2008); 8: Pioneer (2013b); 9: Jackson (2011); 10: TCAP (2013).

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Already routinely applied in the private sector breeding companies, such as the multinational companies, Monsanto, Pioneer Hi-Bred and Syngenta, MAS is yet to take hold in public crop improvement programmes mostly on account of high set-up costs and intellectual property rights restrictions.

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– Mba et al. 2012



## **MAS-varieties released by the public sector**

Although MAS is already routinely applied by private seed companies, its wider use in the public sector, particularly in institutions in the developing world, is still constrained by several bottlenecks (Xu *et al.* 2012, Mba *et al.* 2012). The constraints include limited availability of cost-effective and high-throughput genotyping systems, poor infrastructure, inadequate capacity and operational support, lack of breeder-friendly markers and an application gap between research laboratories and public plant breeding institutes (Xu & Crouch 2008, Ribaut *et al.* 2010, Xu *et al.* 2012, Jiang 2013b). A further important limiting factor for a wider distribution and application of MAS is the competition of big seed producing companies amongst each other (Meyer *et al.* 2013). By not publishing successful MAS-applications and by putting intellectual property rights into effect, private companies usually prevent breeders from public institutions to have free access to the private-sector marker technologies.

The use of MAS in the public sector is generally viewed as still being limited and lagging behind the expectations (Van Damme *et al.* 2011, Mba *et al.* 2012, Brumlop *et al.* 2013, Meyer *et al.* 2013, Jiang 2013b). Nevertheless, in the course of the present work, 136 MAS-derived varieties released by public breeders in 22 countries have been identified, mostly in literature, but also on institutional websites or in online newspapers (see Annex). This search was necessarily restricted by budget and confined to information in English language and breeders in the public sector do not always publish their MAS results. Therefore, it is assumed, that there are more MAS-derived varieties released by public breeding programmes.

One of the most successful applications of MAS in the public sector has been that for introgressing and/or pyramiding of major effect genes. This approach has led to the commercial release of several biotic stress resistant varieties in cassava, barley, bean, chilli, pearl millet, peanut, rice, sorghum, soybean, tomato and wheat. About two-thirds of the identified varieties have been bred using MAS for biotic stress tolerance. Further successful MAS-applications resulted in the release of varieties with improved quality, such as a high-quality protein maize, low-amylose rice, as well as low-cadmium and high-protein wheat. In addition, public MAS-breeding also achieved the improvement of complex traits such as drought- and salt-tolerance in rice and yields in tomato and rice (see Annex).



## 4. FEATURE: MAS AGAINST BIOTIC STRESSES



The importance of MAS cannot be ignored. In the last 10 years, MAS has proven its potential to control biotic stresses by releasing several disease- and pest-resistant varieties in a range of crops.

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Biotic stresses such as viruses, fungi, bacteria, weeds, insects and other pests and pathogens are a major constraint to agricultural productivity reducing global crop yields by 20 to 40% every year (FAO 2012). The most economical and ecologically friendly way to control these biotic constraints is breeding of crop varieties that are resilient in the face of pests and diseases. Although conventional resistance breeding has been quite successful, the process is laborious, time-consuming and highly dependent on environmental conditions. In contrast, resistance breeding by MAS is relatively simple, highly efficient and precise and thus offers the opportunity to speed up the development of varieties resistant to biotic stresses (Miah *et al.* 2013). Furthermore, as gene pyramiding can usually only be accomplished by using MAS, the use of molecular markers provides a unique method for achieving durable disease and pest resistances (Tester & Langridge 2010). In the last 10 years, MAS has proven its potential to control biotic stresses by releasing several disease and pest resistant varieties in a range of crops (Table 1). Indeed, the importance of MAS cannot be ignored, as current markers tackle some of the biggest biotic constraints worldwide such as bacterial blight and blast in rice, rusts in wheat, common bacterial blight in beans, striga in sorghum and cassava mosaic virus in cassava (Van Damme *et al.* 2011). These and some other examples of successful MAS-applications in the public sector are specified below.

### Rice

#### MAS empowers farmers to fight blight

Bacterial leaf blight (BB) caused by the pathogen *Xanthomonas oryzae* is one of the most serious threats to rice production in irrigated and rain-fed areas of the world (Khan *et al.* 2014). While seed treatment and phytosanitation practices are recommended, breeding of rice varieties with introgressed disease resistance genes has been and will continue to be, the most eco-friendly and economical method of BB control (Verdier *et al.* 2012). MAS has been quite successful in this respect, and various resistance genes such as Xa4, Xa5, Xa7, Xa13, Xa21, Xa23 in single or in pyramided form have been introgressed in modern varieties (Varshney & Tuberosa 2013a, Khan *et al.* 2014).

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Marker-assisted selection has been proven as a highly efficient breeding method in improvement of cultivars/lines for various biotic stresses in crop breeding programs, because of its efficacy in selecting plants with appropriate gene combinations in segregating population.

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– Gouda *et al.*  
2013

In China, MAS has been extensively applied for the improvement of BB resistance in hybrid rice breeding for more than a decade and several hybrids such as Xieyou 218, Xieyou 527, Guodao 1, Guodao 3, Guodao 6 and Il-you 8006 have been released as registered varieties at province or national level (Tang *et al.* 2010, Rao *et al.* 2014). Generally, these hybrid varieties have 10-15% higher yield than common rice hybrids and some of them such as Guodao 1, Guodao 3 and Guodao 6 have been nominated as super rice varieties in China (Cheng *et al.* 2009).

In other Asian countries, MAS has been used to pyramid BB resistance genes into varieties that are popular among farmers and therefore are already widely grown. Several of these improved varieties have been released for commercial cultivation in recent years: Angke and Conde in Indonesia, Tubigan 7 and Tubigan 11 in the Philippines (Verdier *et al.* 2012), as well as improved versions of Pusa Basmati 1, Samba Mahsuri, Tapaswini, Lalat and Basmati 386 in India (Gopalakrishnan *et al.* 2008, Sundaram *et al.* 2008, Dokku *et al.* 2013a/b, Bhatia *et al.* 2011).

Given the hitherto success of MAS in breeding for BB resistance, further variety releases can be expected in the future. Examples of popular varieties currently improved by marker assisted introgression of BB resistance genes are MK-75 in Myanmar (Win *et al.* 2013), Mangeumbyeo in Korea (Suh *et al.* 2013) and Mahsuri in India (Guvvala *et al.* 2013).

### **MAS on the attack against blast**

Rice blast, caused by the fungus *Magnaporthe oryzae*, is one of the most widespread and devastating diseases of rice (Miah *et al.* 2013). It is estimated that 10-30% of the annual rice harvest is lost due to blast disease, which is enough to feed 60 million people (Pennisi 2010). Finding an effective way to control rice blast is therefore a high priority. To curtail the spread of the disease chemical and biological methods, disease forecasting and cultivation practices have been applied widely. However, these measures are not very effective and the use of pesticides is expensive as well as neither practical nor ecologically-friendly (Miah *et al.* 2013).

A powerful tool to reduce harvest losses due to rice blast is the cultivation of resistant rice varieties. Since molecular marker for blast resistant genes are available for breeders, MAS-strategies have become essential to develop varieties with durable resistance against different races of *M. oryzae* (Miah *et al.* 2013). Examples of successful MAS-applications are the releases of blast resistant varieties Thanyasirin and RD18 in Thailand (Wongsaprom *et al.* 2010, BIOTEC 2012, 2013b), Pusa Sugandh 6 in India (IARI 2013), and Hwaweon 5 and Saeilmi in South Korea (Kim *et al.* 2013, Sang-Nag Ahn, Chungnam National University, South Korea, personal communication).

Further breeding programmes for pyramiding blast resistant genes into rice hybrids or traditional/open-pollinated varieties by MAS are ongoing in several countries, including China (Jiang *et al.* 2012), India (Singh *et al.* 2013, Gouda *et al.* 2013) and Tanzania (Venuprasad *et al.* 2013).

### **Two-in-one rice: Combining blight and blast resistance**

Blast and BB are the two most prevalent diseases jeopardising rice production. Today, MAS has become a strategy to achieve durable dual-disease resistance in rice. In Chinese and Indian hybrid rice breeding programmes, several efforts have been made in recent years to pyramid BB and blast resistance genes into elite maintainer or restorer lines (i.e. parental lines) using MAS (Zhuang *et al.* 2010, Fu *et al.* 2012, Zhan *et al.* 2012, Hari *et al.* 2013). A successful outcome of these efforts is the dual resistant hybrid rice Zhongyou 161, which was released for cultivation in China in 2009 (Zhuang *et al.* 2010). In addition to the improvement of rice hybrids, MAS is currently also used to combine BB and blast resistance genes into popular rice varieties such as RD6 in Thailand (Pinta *et al.* 2013) or MTU1010 in India (Kumari 2014).

### **Anmi fights off a vampire insect in South Korea**

The brown planthopper (BPH) *Nilaparvata lugens* is one of the most destructive pests of rice, and can be found throughout the rice-growing areas of Asia (Ghaffar *et al.* 2011). By sucking phloem sap, BPH causes significant yield losses every year in susceptible varieties. Extensive chemical control of BPH can cause serious problems including toxicity to natural enemies, increased total production cost, and possible long-term agro-ecosystem and human health damage (Ghaffar *et al.* 2011). An opportunity to replace chemical control strategies is offered by MAS. Today, 10 BPH-resistance genes are known that are suitable for MAS (Fujita *et al.* 2013a). One of these genes is Bph18. Discovered in a wild rice, it has successfully been introduced into the elite variety Junambyeo by breeders from International Rice Research Institute (IRRI) (Suh *et al.* 2011). In 2010, the improved variety, named Anmi after the Korean word for safe and delicious rice, was released for commercial cultivation in South Korea, where it is now contributing to rice farmers who are engaged in ecological agriculture. Recently, a BPH resistant variety, designated Milyang265, was developed in Korea with the help of markers for resistance gene Bph1 (Sang-Nag Ahn, Chungnam National University, South Korea, personal communication).

MAS-breeding programmes for PHB resistance are also being pursued in China (Hu *et al.* 2012) and Thailand (Jairin *et al.* 2009)

### **Pearl millet: MAS extends the lifespan of a popular variety in India**

A widely cited example of the power of MAS is the development of a new version of the pearl millet hybrid HHB 67 in India. It shows how MAS can extend the economically useful lifespan of farmer-preferred variety and thus can improve rural livelihoods of resource-poor farmers (Dargie 2013, Yadav *et al.* 2013).

The original HHB 67 variety was released in 1990 and was rapidly and widely adopted by farmers. However, after 10 years of widespread cultivation, HHB 67 started to succumb to the devastating blight of downy mildew disease (DM). To rescue the highly popular variety breeding teams at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and Haryana Agricultural University started to transfer two major QTLs for DM-resistance from the donor parent into HHB 67 by MAS. The backcross transfer was completed in just over three years, and HHB 67-Improved was released to farmers in 2005 (Yadav *et al.* 2013). Adoption by both the seed industry and resource-poor farmers in northwest India was massive (Dargie 2013). By 2011, HHB 67-Improved had already been cultivated on almost 900 000 ha of land in northern India and thereby has brought greater food security to around two million people (Yadav *et al.* 2013).

### **Sorghum: MAS banishes a witch weed in Sudan**

In Africa, sorghum is a valuable food grain for 300 million of the world's most food-insecure people. In 2010, scientists from the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) reported a breakthrough in sorghum breeding that could enable these people to attain food security (ASARECA 2010). By using MAS the scientists had succeeded in breeding striga resistant sorghum lines, thereby providing a long-awaited tool to overcome the most limiting biotic factor in the production of sorghum. Striga, also nicknamed witchweed, infests about 17 million ha of sorghum in Africa, causing yield losses of six to seven million tonnes every year. In 2012, four of the first MAS-derived striga resistant lines were released as varieties to farmers, royalty-free, in Sudan (ASARECA 2012). Named ASARECA.T1, ASARECA.W2, ASARECA.AG3 and ASARECA.AG4, they had been developed in just three years by transferring striga-resistance QTLs from an Indian sorghum line into locally adapted, farmer-preferred varieties (Deshpande *et al.* 2013). Further, MAS-derived striga resistant varieties are expected to be released in other African countries in the near future, as striga-resistance QTLs have also been introgressed in popular varieties from Mali, Kenya, Eritrea and Rwanda (Deshpande *et al.* 2013, Tadesse 2013, Niyibigira *et al.* 2013, Kimani 2013).



## **Cassava: MAS delivers bug-busting, high-yielding varieties for Nigeria and Tanzania**

Cassava is staple food for more than 200 million people in sub-saharan Africa (Tadele & Assefa 2012). To meet food security and the new emergent role of cassava as a cash and industrial crop, African cassava breeders started to explore high-yielding germplasm from Latin America, cassava's centre of origin, in the 1990s. Until recently, the attempts to release Latin American genotypes as varieties in Africa have failed, mainly due to their susceptibility to cassava mosaic disease (CMD), one of the major viral diseases of cassava, causing reported yield loss ranging from 20 to 90% (Okogbenin *et al.* 2013a). But now, thanks to multi-institutional collaboration and with the help of MAS, breeders have become able to introgress CMD resistance genes into germplasms from Latin America and thus to fast track the use of the exotic genotypes in Africa. In 2010, UMUCASS33 was released in Nigeria, representing the first Latin American variety to be cultivated in Africa (Okogbenin *et al.* 2013a). Since then, five further varieties – likewise selected using CMD resistance markers – have been released, namely UMUCASS41 (CR36-5) in Nigeria, as well as Pwani, Mkumba, Dodoma and Makutupora in Tanzania (GCP 2012, Okogbenin *et al.* 2013a/b). These varieties are not only a hope for farmers, but also an eye-opener for breeders, and thus bode well for the future as markers get fully integrated into cassava breeding (GCP 2011).

## **Wheat: MAS brings resistance to North American fields**

In publicly financed wheat breeding programmes of the US, Australia, Canada and CIMMYT, about 50 genes have been suggested for MAS, including resistance genes to more than a dozen biotic stresses (Miedaner & Korzun 2012). As robust markers for most of these genes have been developed, breeders now are able to use them in practical breeding. In North America, MAS has already proven to be a straight-forward technique to implement resistance genes and several wheat and durum wheat varieties have been released with markers that tackle biotic constraints such as stripe rust, leaf rust, foot rot, wheat streak mosaic virus, Hessian fly and orange wheat blossom midge (Gupta *et al.* 2010, TCAP 2013, Randhawa *et al.* 2013).

The usefulness of using MAS in wheat breeding can be exemplified by its application in California. In 2000, new virulent races of stripe rust appeared in Californian wheat fields causing losses that reached up to 25% of the crop. Eight years later, the first MAS-derived resistant wheat variety (Patwin) was released by the University of California. Today, several resistant varieties developed by public and public-private partnership MAS-programmes are grown in California (e.g. Lassik, Espresso, Blanca Grande 515, Summit 515, Westmore, New Dirkwin). By cultivating these varieties, the new stripe rust epidemic is well controlled and thus the need of costly, eco-unfriendly fungicide applications is eliminated (Medgaarden 2012).

## 5. FEATURE: MAS AGAINST ABIOTIC STRESSES



Recent achievements in breeding of submergence-, drought- and salt-tolerant rice varieties reveal the potential impact of MAS on food security, especially for resource-limited farmers.

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Abiotic stresses such as drought, salinity, cold, flooding, submergence and mineral toxicity are the most common challenge for sustainable food production in large parts of the world, in particular in developing countries. Indeed, abiotic stresses represent the main cause of crop failure worldwide, reducing average yields of all major crops by more than 50% (Varshney & Tuberosa 2013b). Ongoing global climate change will further increase these challenges in many areas, making improved abiotic stress resistance of crops a key issue for the future.

Conventional breeding methods have a proven track record of improving tolerance of abiotic stresses. For example, over 100 drought tolerant hybrids and open pollinated varieties of maize have been released in African countries since 2007 under the framework of the Drought Tolerant Maize for Africa project (DTMA 2013, Gilbert 2014). However, progresses with conventional methods are generally slow, mainly due to the polygenic nature of the stress tolerance traits. As typically several genes or QTLs must be introgressed at the same time into an individual variety in order to obtain a significant impact against abiotic stresses, MAS is generally seen as a tool having the potential to facilitate and accelerate the development of abiotic tolerant crops. Indeed, the DTMA project has MAS materials in the breeding pipeline and anticipates seeing varieties developed from the MAS effort in the future (Michael Olsen, CIMMYT personal communication).

While the number of genes and QTLs identified to confer abiotic stress tolerance has increased steadily in recent years, very few have yet been applied successfully in public breeding programmes. The limited use of MAS for breeding abiotic stress tolerance is due to of various reasons, including QTLs being unreliable or specific to the local environment, and problems associated with linkage drag (Ashraf & Foolad 2013).

Although mapped genes and QTLs for abiotic stress tolerance have not yet been routinely exploited by the public sector, recent achievements in breeding of submergence, drought and salt tolerant rice varieties, as outlined below, reveal the potential impact of MAS on food security, especially for resource-limited farmers. Given this potential, the number of success stories will undoubtedly increase in future, not only in rice but also in other crops.

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The opportunity to apply molecular marker technologies as a means of combining multiple tolerance genes/QTLs into individual rice varieties provides an unprecedented opportunity for breeders to rapidly develop tolerant cultivars for targeted environments.

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– Septiningsih  
*et al.* 2013

The latter is exemplified by recent progress in using MAS for breeding drought tolerance into chickpea and sorghum (Varshney *et al.* 2013, Kahi *et al.* 2013), salt tolerance in durum wheat (James *et al.* 2012) or aluminium tolerance in barley (Soto-Cerda *et al.* 2013).

## Rice

### 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. The economic loss due to submergence is estimated to be up to \$1bn US dollars annually (Collard *et al.* 2013). In Bangladesh and India alone, four million tonnes of rice, enough to feed 30 million people, are lost to floods every year (Xu *et al.* 2014).

In 2003, scientists from IRRI started a large-scale MAS-breeding programme to convert popular rice varieties into flood tolerant ones (Collard *et al.* 2013). They work with Sub1, a major QTL that confers submergence tolerance. Sub1 was originally mapped in a landrace from India that can survive up to two weeks of complete submergence. From the start, IRRI's MAS-programme was guided by the needs of smallholder farmers and the scientists shared Sub1 genes and Sub1 line early on, prior to publication. This open science approach facilitated breeding collaborations and, to date, several mega-varieties, including Swarna, Samba Mahsuri, IR64, B11 and Ciherang, have been converted into Sub1-varieties (Ismail *et al.* 2013, Xu *et al.* 2014; see Annex). The Sub1-varieties released for cultivation have been spreading rapidly in several countries over the last few years, and are currently grown by more than four million farmers in Asia (Ismail *et al.* 2013). How much these farmers appreciate the MAS-improved varieties can be illustrated by their feedback to breeders: as cited by Ismail *et al.* (2013), farmers describe Sub1-varieties as "magic", "a wonder", "a miracle", and report that "Sub1 changed my life."

In future, the number of farmers appreciating Sub1-varieties is set to expand further, as the introgression of the Sub1 QTL into popular varieties is ongoing in Asian and African countries (Collard *et al.* 2013).

### MAS for acid soil adaptation in Vietnam

Vietnam is one of the largest rice producers in the world. Rice is described as "white gold", and is an important source of income for rural households. However, as in many other areas of the tropics, rice production in Vietnam can be severely hampered by soil acidity. In acid lands, rice yields are low because the metal aluminium becomes soluble under low pH, and reaches toxic concentrations in soil waters, thereby causing stunted root growth.

In order to provide Vietnamese farmers with rice varieties that can cope with aluminium stress, scientists from the Cuu Long Delta Rice Research Institute (CLRRI) took a local wild rice, *Oryza rufipogon*, which grows naturally in acid soils, and crossed it with IR64, one of the world's most popular rice varieties. The backcrossing programme, which was guided by molecular markers, led to the release of variety AS996, which resembles IR64 but gives higher yields under acid soils (Buu & Lang 2007, Reece 2007). This variety has become popular among rice farmers and is grown on 100,000 ha in Vietnam (Sanchez *et al.* 2013). Recently, CLRRI released three further acid soil adapted varieties, MNR 3 MNR 4 and OM6677, which have been derived from the AS996 genotype and selected for aluminium toxicity tolerance through molecular markers (Nguyen Thi Lang, Cuu Long Delta Rice Research Institute, Vietnam, personal communication).

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Plant breeding has a proven track record of improving tolerance of abiotic stresses, in particular, since new molecular tools such as marker-assisted backcrossing became available to speed up the introgression of tolerance genes.

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– Mohanty *et al.*  
2013

### **MAS tackles water shortage**

Drought is the most widespread and damaging abiotic stress in rice production. Each year, water scarcity affects some 23 million ha of rain-fed rice in south and southeast Asia alone. In parts of India, severe drought can cut rice yields by as much as 40%, equal to losses of \$800m annually (IRRI 2014). As water shortage is becoming an increasingly severe problem, there is an urgent need to breed drought tolerant varieties of rice.

MAS has become a tool that enables breeders to incorporate drought-relevant traits into varieties with more accuracy and speed. One successful example of using MAS for drought tolerance is the development of the variety Birsa Vikas Dhan 111. Released in 2009 in India, it was bred by introgressing four QTLs for root traits from Azucena, a rice variety from the Philippines, into the Indian upland rice variety Kalinga III (Steele *et al.* 2013). Two further examples showing the potential of MAS are the water-saving aerobic rice varieties MAS 946-1 and MAS-26. Both of these varieties, which yield 60% more than traditional varieties under experiment station conditions, have been released in India for cultivation in the southeastern dry zone of Karnataka in 2007 and 2008, respectively (Pray *et al.* 2011). Apart from the projects in India, MAS-approaches have also led to the release of drought tolerant rice varieties in Vietnam (Nguyen Thi Lang, Cuu Long Delta Rice Research Institute, Vietnam, personal communication).

In recent years, many QTLs for high yield under drought conditions have been identified in rice. With the availability of these QTLs, several MAS programmes are currently working towards pyramiding the QTLs into high-yielding popular varieties (Guo & Ye 2014, Dixit *et al.* 2014). These programmes offer bright prospects for MAS in the development of drought tolerant rice varieties in future.

### **MAS offers saline solution**

Salinity is the second most widespread soil problem in rice-growing countries after drought, causing yield losses of up to 50% when salinity sensitive varieties are grown. To overcome the losses, breeders have been continuously introgressing salinity tolerance from traditional varieties to modern high-yielding varieties. In the last few years, such conventional breeding programmes have been quite successful. In India, Bangladesh and the Philippines, for example, more than a dozen salt tolerant rice varieties have been released since 2007 (Gregorio *et al.* 2013). However, as conventional breeding for salt tolerance in crops is generally slow, breeders recently started to use the modern tools of MAS to accelerate the breeding process. Using available markers, they have already been successful in improving the popular rice variety Sin Thwe Latt in Myanmar (GCP 2014), as well as in developing the salt tolerant varieties OM4900, OM5629 and OM2009 in Vietnam (Nguyen Thi Lang, Cuu Long Delta Rice Research Institute, Vietnam, personal communication).

Further salt tolerant rice varieties developed by MAS could be released for cultivation in the coming years, as breeders have introgressed Saltol, a major QTL for salt tolerance, into several popular rice varieties in India, Vietnam, Bangladesh and Philippines (Gregorio *et al.* 2013).

### **Outlook: MAS for high yields with low phosphorus**

Cropland with low plant-available phosphorus poses a serious problem for rice-farming communities, especially for poor, remote communities that must manage without fertilisers. But soon there may be improved rice varieties available for these farmers, as MAS enables breeders to incorporate the Pup1 gene into locally adapted rice varieties (Chin *et al.* 2011, Heuer *et al.* 2013, EIARD 2013). Pup1, identified in the landrace Kasalath, confers tolerance of phosphorus deficiency in soil and can thus significantly enhance grain yield under field conditions. As about 50% of land used worldwide for rice cultivation is phosphorus-deficient, the potential benefits of Pup1-varieties are incalculable (EIARD 2013).



### **Outlook: MAS for multiple abiotic stress tolerance**

Abiotic stresses often occur in combination in farmers' fields, causing incremental crop losses. Hence, breeders have started to use MAS for combining multiple tolerance genes/QTLs into individual rice varieties. A large-scale QTL pyramiding programme is already in its final stages where breeding lines that have QTLs for drought tolerance with Sub1 in the background of the recipient variety TDK1 are being developed and tested (Dixit *et al.* 2014). Pyramiding of Sub1 and Saltol in popular varieties is at an advanced stage and pyramiding of Pup1 with major drought QTLs is in preparation (Heuer *et al.* 2013). Furthermore, the Indian Government Department of Biotechnology recently started a major MAS-programme to transfer five major QTLs for grain yield under drought, and one QTL each for submergence (Sub1) and salinity (Saltol) into a number of popular Indian rice varieties. Taken together, these pyramiding programmes illustrate that MAS offers today an unprecedented opportunity for breeders to develop more resilient varieties for wider adaptation to the multiple abiotic stresses commonly experienced in farmers' fields.



## 6. FEATURE: MAS FOR QUALITY TRAITS



While conventional breeding takes nearly 10 years to develop a new variety of maize, with the help of MAS, Indian scientists were able to develop a nutritious maize variety in just three years.

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The improvement of crop quality traits is gaining unprecedented importance in breeding programmes of both developed and developing countries. By enhancing nutritional value, meeting consumer preferences or complying industrial needs, the breeding of quality-improved varieties gives farmers added value and a competitive market advantage. As crops with improved quality traits can result in improved human welfare and increased farm income, they have the potential to alleviate problems caused by poverty and malnutrition through both direct (food quality) and indirect effects (income stability).

The improvement of quality traits is traditionally a slow and expensive process, mainly due to the complex nature of these traits, which are not only controlled by the action of several genes, but are also subjected to environmental influences. However, the advent of molecular marker techniques now makes it possible to “tag” genes/QTLs conferring desirable quality traits, and by so doing to speed up the breeding process. In crops such as barley, broccoli, maize, peanut, rice, soybean or wheat, MAS has already been used effectively to improve quality traits (Table 1). Some of these successful stories are specified below.

### Maize: MAS speeds up quality protein improvement

Quality Protein Maize (QPM) is a biofortified maize variety that contains twice as much of the amino acids lysine and tryptophan than normal maize varieties. As these two amino acids are essential for human nutrition, cultivation of QPM maize varieties helps to alleviate protein malnutrition and can become a boon for young children (Lodha 2014). Although conventional breeding has been used successfully to convert commercial lines to QPM forms, the procedure is highly cumbersome and time-consuming (Vikal & Chawla 2014). To speed up the conversion, breeders from the Indian Council of Agricultural Research (ICAR) have developed a marker-assisted technique that enables a simple and efficient way of breeding QPM maize. In 2008, the first of these lines, the variety Vivek QPM 9, was released for cultivation in India (Gupta *et al.* 2013). While conventional breeding takes nearly 10 years to develop a new variety of maize, with the help of MAS the Indian scientists were able to develop Vivek QPM 9 in just three years.

MAS-programmes to convert locally-adapted maize germplasms to QPM are ongoing in Thailand (Jompuk *et al.* 2011) and India (Gupta *et al.* 2013).

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Although phenotyping for nutritional quality has improved, traditional plant breeding alone cannot be expected to solve the problem of selection for an invisible trait such as seed nutritional quality in a cost-effective manner without the help of genomic studies and marker-assisted selection.

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– Blair 2013

## Rice: MAS improves cooking and eating quality

Breeding new rice varieties without considering grain quality can leave farmers with a low-value product, and consumers with rice that they don't like to cook or eat. Therefore, breeding teams in rice production countries are looking for strategies that enable the improvement of grain qualities without weakening the agronomic attributes of a variety. One of the strategies they have found is combining conventional field selection and MAS. In this way, several high yielding but low-quality rice varieties are currently improved to meet consumer expectations. MAS-derived varieties have already been released in some countries, for example Cadet and Jacinto in the US (Hardin 2000), Nanjing 46 in China (Wang *et al.* 2009), OM4495 and OM5239 in Vietnam (Lang & Buu 2010), Tainan 16 in Taiwan (Chen *et al.* 2012), and Improved Manawthukha in Myanmar (Yi *et al.* 2009).

## Wheat

### MAS enhances nutritional quality

Wheat grain protein content (GPC) is important for human nutrition and has a strong influence on the quality of pasta and bread (Kumar *et al.* 2011). In addition, GPC is one of the main targets for hard and durum wheat breeding, as it determines premium prices in many markets around the world. With the recent availability of molecular markers for a high GPC gene derived from a wild wheat from Israel, breeders started to use MAS for developing wheat genotypes with enhanced GPC (Balyan *et al.* 2013). In North America, these efforts have led to the release of several varieties with high GPC, namely Lillian, Somerset and Burnside in Canada (Depauw *et al.* 2011), as well as Lassik, Farnum and durum wheat Desert King HP in the US (Balyan *et al.* 2013). As the presence of the high GPC gene results in a more efficient nitrogen remobilisation from senescing leaves to the grain, these varieties require less nitrogen fertilisation to achieve similar levels of GPC.

MAS-programmes for high GPC are also running in Argentina (Tabbita *et al.* 2013) and India (Kumar *et al.* 2011).

### MAS lowers cadmium in durum grains

In North America, soils in wheat production areas naturally contain elevated amounts of the highly toxic metal cadmium. As concentrations of cadmium in the grain of many durum wheats grown in these soils often exceed international food trade standards, low grain cadmium content has become an important selection criterion in durum wheat breeding programmes in the US and Canada. Recently, molecular markers have successfully been applied for the selection of breeding lines with low grain cadmium levels leading to the commercial release of Miwok in the US (TCAP 2013), and Brigade, CDC Verona, CDC Desire, CDC Vivid, AAC Current and AAC Durafeld in Canada (Randhawa *et al.* 2013; Ron DePauw, Agriculture and Agri-Food Canada, personal communication). These MAS-derived varieties not only help to improve consumer safety but also avoid the exclusion of North American durum wheat varieties from international export markets.

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Uses of molecular markers in MAS, along with precision in phenotyping, hold promise for tangible benefits through rapid turnover of the improved varieties with biofortified grains.

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– Balyan *et al.*  
2013

**Table 3: Conventionally bred biofortified crops developed by HarvestPlus**

<b>Enhanced Nutrient</b>	<b>Countries of variety releases</b>	<b>Year of release</b>
<b>Bean</b>		
Iron	Democratic Republic of Congo	2012
	Rwanda	2012
<b>Cassava</b>		
Provitamin A	Brazil	2005
	Democratic Republic of Congo	2008/2011
	Nigeria	2011
<b>Maize</b>		
Provitamin A	Nigeria	2012
	Zambia	2012
<b>Pearl Millet</b>		
Iron	India	2012
<b>Sweet Potato</b>		
Provitamin A	Uganda	2007
	Mozambique	2007
<b>Rice</b>		
Zinc	Bangladesh	2013
	India	2013
<b>Wheat</b>		
Zinc	India	2013
	Pakistan	2013

Source: [www.harvestplus.org](http://www.harvestplus.org)



## Outlook: MAS for more micronutrients

Worldwide, at least 2 billion people, mostly women and children, suffer from micronutrient deficiencies caused largely by a dietary lack of micronutrients such as iron, zinc, and vitamin A (Andersson *et al.* 2014). This “hidden hunger” leads to impaired cognitive abilities of children and adolescents, and can result in stunted growth and blindness as well as increased risk of disease and premature death (Andersson *et al.* 2014).

Given the genetic variation in concentrations of zinc, iron, and provitamin A among varieties, breeding biofortified crops has become one of the strategies to combat hidden hunger. Whereas genetic engineering approaches to biofortification (for example, GE “Golden” rice) 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 biofortification. For example, HarvestPlus – an interdisciplinary alliance of research institutions – has released several conventionally bred biofortified varieties in recent years (Table 3). However, in the long term, access to a better diet, including fresh fruit and vegetables, is a sustainable strategy solution to micronutrient deficiencies (Thompson & Amoroso 2011)

As the natural genetic variability of micronutrients has become trackable with molecular markers, MAS is believed to enable a more efficient and rapid development of biofortified crops in the near future. In maize, for example, the use of MAS for enhancing provitamin A (beta-carotene) levels has become a reality. Screening maize germplasms, scientists found favourable genes, named *lcyE* and *CrtRB1*, and developed inexpensive molecular markers that are now being used by breeders in developing countries to produce maize with higher provitamin A levels (Harjes *et al.* 2008, Yan *et al.* 2010). At CIMMYT the use of these markers has already yielded a number of promising breeding lines (Andersson *et al.* 2014). Cassava is not far away from making similar progress. Scientists at the International Center for Tropical Agriculture (CIAT) found a version of a gene called *PSY* that increases the levels of provitamin A compounds and thereby provides breeders a tool to develop biofortified varieties with MAS (Welsch *et al.* 2010).



## 7. FEATURE: HARNESSING BIODIVERSITY



Tomato breeding is a striking example of how MAS provides breeders with the tools to unleash the vast resources held in wild relatives of crops.

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Greenpeace

An unintended consequence of the success of plant breeding is the narrowing of the genetic base of the plants cultivated for food (Mba *et al.* 2012). The lack of genetic diversity among present-day crop varieties raises concerns about the prospects for continued breeding successes. New varieties are necessary in an era of global climate change, and most crop geneticists agree that enrichment of the cultivated gene pool will be necessary to meet the challenges that lie ahead (Tester & Langridge 2010). The reservoir for this enrichment can be found in the remarkable diversity of landraces and crop wild relatives growing in nature or being stored in one of the 1,750 genebanks around the world (Mba *et al.* 2012).

Despite the value of genes or QTLs originating from wild relatives and landraces, breeders have traditionally been reluctant about the use of these genetic resources in their breeding programmes, mainly due to the problems associated with linkage drag – the co-transfer of undesirable genes linked with the desired trait that could, for example, decrease yields. But now, MAS provides breeders with the tools to effectively unleash the vast resources held in wild genetic variation. As the use of molecular markers enables the precise introgression of small genomic sectors of wild relatives or landraces into varieties, the linkage drag can be reduced (Mir *et al.* 2014).

Given the new opportunity to broaden the genetic base of modern varieties, numerous efforts have been made in recent years to introgress new genes or QTLs into the background of cultivated varieties (Mir *et al.* 2014). In rice for example, at least 20 genes/QTLs have been introgressed from wild relatives tagged with molecular markers (Brar & Singh 2011). One of these genes, named Xa21, has been extensively used and more than a dozen rice varieties carrying Xa21 have been already released through MAS programmes worldwide (Mir *et al.* 2014). As the three examples below show, natural variation can even be unlocked to break yield barriers, when MAS-breeding takes a walk on the wild side. Further examples of recent MAS efforts to expedite the incorporation of wild genes and QTLs are shown in Table 4. These successful examples show that MAS is a useful tool for exploiting native traits from landraces and wild relatives to achieve yield gain and nutritional advantages, as well to enrich the cultivated gene pool for greater resilience to pest, diseases and climate change.

“ 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

## Rice

### Wild QTLs enhance yield

How to make use of wild germplasm to improve yields by MAS is exemplified by the exploitation of yield-enhancing QTLs from the weedy rice relative *Oryza rufipogon*. In China, breeders from the National Hybrid Rice Research and Development Center (CNHRRDC) successfully developed the strong restorer line Yuanhui 611 and the hybrid Y Liangyou 7 by transferring two QTLs – yld1.1 and yld2.1 – from the wild rice, gaining a yield increase of 20%. In 2008, Y Liangyou 7 was released for commercial cultivation and it was ranked as a super rice variety in Hunan Province (Wu *et al.* 2010).

The breeding of yield-enhancing QTLs from *O. rufipogon* into cultivated rice varieties by MAS is also pursued in countries other than China. In India, for example, QTLs yld1.1 and yld2.1 have been introgressed into an elite restorer parent of hybrid rice KMR3R, and the resulting lines are currently under field evaluation (Sundaram *et al.* 2013). In the US, breeders used the wild rice relative to transfer QTLs yld2.1 and yld6.1 into the variety Jefferson. The most top-performing introgression line was recently released as a parent in rice breeding programmes (Imai *et al.* 2013).

### Outlook: Spike could boost yield

In 2013, scientists from Japan and IRRI reported the finding of a unique gene, named Spike, from an Indonesian landrace, which could boost rice yields up to 36%. Combining molecular identification of the Spike gene and conventional breeding, the scientists transferred Spike into popular rice varieties such as IR64, BR11, Swarna, Ciherang and PSBRc18. The resulting lines are currently being tested in multilocation trials and could contribute to food security in rice-growing regions such as south and southeast Asia in near future (Fujita *et al.* 2013b).

“

Genomics technology and MAS have opened up many new opportunities to utilise rare genes from wild species and landraces located in the world's gene banks, which could improve pest and disease resistance, abiotic stress tolerance, nutritional quality, or yield.

”

– McCouch & Crowell 2013

## Tomato: AB2 breaks agricultural yield barriers

A 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-Institute of Molecular Plant Physiology in Golm introduced chromosome segments of the inedible wild species *Solanum pennellii* into the genetic background of an elite tomato variety via MAS (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. Today AB2 is a leading variety in California, which is the largest world producer of industrial processing tomatoes (Sacco *et al.* 2013).

**Table 4: Examples of MAS-derived varieties and MAS-derived breeding lines with genes or QTLs originating from wild relatives or landraces**

<b>Variety / Breeding line</b>	<b>Trait</b>	<b>Gene / QLT</b>	<b>Source of gene / QTL</b>
<b>Barley</b>			
CDC Polarstar <sup>(1)</sup>	Malting quality	Lox-1-null	OUI003 (landrace)
<b>Broccoli</b>			
Beneforte <sup>(2)</sup>	High glucosinolate	QTL	Brassica villosa
<b>Maize</b>			
Vivek QPM 9 <sup>(3)</sup>	High-quality protein	Opaque-2	Landrace
<b>Rice</b>			
Anmi <sup>(4)</sup>	Planthopper resistance	Bph18	Oryza australiensis
Xieyou 218, Zhongyou 218, some more varieties <sup>(5)</sup>	Disease resistance	Xa21	Oryza longistaminata
Sub1-varieties <sup>(6)</sup>	Submergence tolerance	Sub1	Fr13a (landrace)
Y Liangyou 7 <sup>(7)</sup>	High yield	Yld1.1, Yld2.1	Oryza rufipogon
Saltol-lines <sup>(8)</sup>	Salt tolerance	Saltol	Pokkali (landrace)
Pup1-lines <sup>(9)</sup>	Improved phosphate uptake	Pup1	Kasalath (landrace)
Spike-lines <sup>(10)</sup>	High yield	Nal1	Landrace
<b>Soybean</b>			
LDX01-1-65-line <sup>(11)</sup>	SCN resistance	QTL	Glycine soja
<b>Tomato</b>			
Ab2 <sup>(12)</sup>	High yield	Brix9-2-5	Solanum pennellii
Mountain Magic <sup>(13)</sup>	Late blight resistance	Ph-3	Solanum pimpinellifolium
<b>Wheat</b>			
Lassik, Farnum <sup>(14)</sup>	Grain protein content	Gpc-B1	Triticum turgidum ssp. dicoccoides
Otto <sup>(15)</sup>	Foot rot resistance	Pch1	Triticum ventricosum
Patwin-515 <sup>(16)</sup>	Stripe rust resistance	Yr15	Triticum dicoccoides
Nax-lines <sup>(17)</sup>	Salt tolerance	Nax 1, Nax 2	Triticum monococcum

References: 1: Hoki *et al.* (2010); 2: Mithen (2012); 3: Gupta *et al.* (2013); 4: IRRI (2011); 5: Brar & Singh (2011); 6: Ismail *et al.* (2013); 7: Wu *et al.* (2010); 8: Gregorio *et al.* (2013); 9: ElARD (2013); 10: Fujita *et al.* (2013b); 11: Diers *et al.* (2005); 12: Lippman *et al.* (2007); 13: Varshney & Tuberosa (2013a); 14: Balyan *et al.* (2013); 15: Carter *et al.* (2013); 16: Jackson (2011); 17: James *et al.* (2012).



## 8. FEATURE: HARNESSING FARMERS' KNOWLEDGE



The first varieties bred using MAS as part of participatory breeding programmes have already been released for cultivation.

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High-yielding varieties developed through conventional plant breeding during the mid-20th century's "Green Revolution" led to a significant boost in crop production. However, as the modern varieties had been bred by a centralised, top-down approach focusing on favourable agricultural environments, little regard was paid to the conditions that farmers face in poorly productive or marginal lands. The contrast between the farmers' reality and conventional plant-breeding philosophies was particularly striking in developing countries and thus low-resource farmers – those cultivating unfavourable lands, for example – benefitted little from these high-yielding varieties. (IAASTD 2009)

Given the fact that some 700 million people did not benefit from the varieties developed by the Green Revolution (Murphy 2007), breeders started to look for new methods likely to succeed in poorly productive or marginal lands. One promising approach they found was to bring farmers back into breeding. Called participatory plant breeding (PPB), the approach combines science-based breeding methods and farmers' experience and thereby reconciliates past and present competences to find solutions for a better adoption of newly bred varieties in developing countries (Stamp & Visser 2012). PPB approach allows region-specific breeding, which lends itself to providing farmers with more suitable, locally adapted new varieties than solely centralised breeding. PPB is not restricted to varieties for low-grade agricultural land, it can be applied to prime agricultural land and different systems, e.g. low-input systems. Different forms of PPB, also called client-oriented breeding, have been developed. These vary in their mode of operation, from those where both scientists and farmers are involved in all stages of the breeding process, to mere participatory varietal selection (PVS), in which farmers are only engaged in the downstream evaluation and selection of new breeding lines (Vroom 2009).

Since pioneering breeders have shown that PPB offers a rapid, cost-effective strategy for breeding farmer-preferred, superior varieties (Ashby 2009), there is a growing interest to involve farmers in varietal development (Ceccarelli 2012, Ceccarelli *et al.* 2013, Badstue *et al.* 2013, Iwanaga 2013, Meyer *et al.* 2013). In the Consultative Group on International Agricultural Research (CGIAR) for example, PVS has gained ground and is practiced in a number of programmes and projects carried out by the International Center for Tropical Agriculture (CIAT), the International Potato Center (CIP), the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the International Rice Research Institute (IRRI) (López Noriega *et al.* 2013).

“

In general, participatory plant breeding facilitates the rapid and enthusiastic adoption of crop varieties.

”

– Mba *et al.* 2012

Although PPB has hitherto mainly focused on conventional breeding techniques, it should not be seen as a peripheral venture that is separated from modern science. On the contrary, it should be thought of as a more sophisticated, client-orientated approach to crop improvement, and as such there is no reason why PPB should not benefit from high-tech endeavours such as MAS (Murphy 2007). Indeed, there are several projects employing MAS as part of participatory breeding programmes, and the first varieties bred through the merger of high and low-tech have already been released for cultivation (Table 5). These pioneering examples not only show that there is no barrier to combining MAS with farmers' knowledge and experiences, but also pave the way how MAS approaches can make the "Gene Revolution" of interest to resource-poor farmers. Given that participatory breeding may be the most effective way to reach the people who missed out on the high-yielding varieties developed during the of the Green Revolution (Murphy 2007), it is certainly worth to keep on merging MAS with farmers' knowledge.

“

The incorporation of participatory approaches consistently enables breeding programs to “break through” adoption bottlenecks caused by low levels of acceptability of new varieties by poor farmers.

”

– Ceccarelli *et al.*  
2013



**Table 5: Examples of finished and ongoing breeding programmes combining the use of molecular markers with participatory approaches**

*Breeding institutions with short description*

#### **Haryana Agricultural University, India**

MAS was applied to improve disease resistance in the popular pearl millet hybrid HHB 67. Farmers tested the most promising improved lines to evaluate if they were at least as good as the original HHB 67 (Witcombe *et al.* 2005). Today, HHB 67-Improved brings greater food security to around two million people (Yadav *et al.* 2013). For details, see Section 4.

#### **Gramin Vikas Trust / Birsa Agricultural University, India**

To develop drought tolerant rice, MAS was used to introgress four QTLs for root traits into the variety Kalinga III. Following MAS, farmers compared the lines with different combinations of root QTLs in their fields and assessed them for traits including maturity, yield and grain shape. In 2009, the most successful line was released as variety Birsa Vikas Dhan 111 (Steele *et al.* 2013). For details, see Section 5.

#### **University of Agricultural Sciences (AUS), India**

MAS and participatory techniques were combined together to develop drought tolerant rice varieties specifically adapted to the farmers own conditions and needs (Kanbar & Shashidhar 2011).

#### **International Rice Research Institute (IRRI)**

With the help of MAS, several popular rice varieties have been equipped with flood tolerance by transferring the Sub1 gene. To ensure relevance and buy-in by farmers the improved varieties were evaluated with farmers through PVS prior to release. Today, Sub1-rice varieties are grown by more than four million farmers in Asia (Ismail *et al.* 2013). For details, see Section 5.

#### **National Root Crops Research Institute (NRCRI), Nigeria**

Breeding lines developed through the use of markers for cassava mosaic disease (CMD) resistance have been evaluated in farmer-participatory trials. The resulting variety, named UMUCASS 33, was selected by farmers for its culinary quality, resistance to CMD, and architecture well suited to the cropping systems used by smallholder farmers (Okogbenin *et al.* 2013b). For details, see Section 4.

#### **Agricultural Research Institute (ARI), Tanzania**

Four MAS-derived cassava varieties, namely Pwani, Mkumba, Dodoma and Makutupra, have been released for cultivation. Bred with the help of markers for resistance to CMD, the varieties had been field evaluated with farmers using PVS prior to release (Geoffrey Mkamilo, Agriculture Research Institute, Tanzania, personal communication). For details, see Section 4.

#### **Pan-Africa Bean Research Alliance (PABRA), Uganda**

A core guiding principle of PABRA's breeding strategy is PPB and PVS, of which the Alliance is a trailblazer. In addition, MAS is used in those cases where reliable markers for target biotic constraints have been identified and can be used routinely in the breeding programme (Buruchara *et al.* 2011).

#### **AfricaRice**

Using MAS a gene conferring resistance to rice yellow mottle virus has been transferred into elite rice varieties from Mali, Guinea, Burkina Faso and The Gambia. The resulting lines currently are field evaluated with farmers using PVS in national programmes (AfricaRice 2012).





Local rice farmers inspect seed varieties on display in Yunnan, China. MAS lends itself to participatory plant breeding, which combines science-based breeding with farmers' experience to provide more suitable, locally adapted new varieties than solely centralised breeding.

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## 9. OUTLOOK ON THE FUTURE: HARNESSING SCIENCE



The new wealth of genomic information will provide breeders with new tools and resources to enable more elaborate MAS strategies and accelerate the development of new crop varieties.

© Emma Stoner / Greenpeace

Over the past 10 years, significant advancements have been made in the area of plant genomics and phenomics (Bohra 2013, Fiorani & Schurr 2013). Genome sequences of many crop species have become available (Bolger *et al.* 2014) and next-generation sequencing, high-throughput genotyping and next-generation phenotyping, as well as genome wide selection, are getting tools and technologies for an increasing number of crop species. As breeding becomes more and more genomic-based, it is expected that the drawbacks (see Section 2) of current MAS-applications will be overcome, especially with respect to the improvement of complex traits. Furthermore, the recent advances are increasingly making MAS a choice for public-sector plant breeding and this should lead to a wide adoption of MAS for more crop species in more countries.

“

It is expected that genomic selection will revolutionise breeding in the next decade.

”

– Morell *et al.*  
2012

### Harnessing next-generation sequencing technologies

Much of the current progress in genomic-based breeding is driven by next-generation sequencing technologies (NGS). As NGS platforms enable accurate sequencing at high speed and low cost, the genomes of more and more crop species are being sequenced (Van *et al.* 2013, Bolger *et al.* 2014). By making genome sequencing accessible to regular laboratories, NGS further allows for whole-genome re-sequencing of hundreds of individual plants within breeding populations or germplasm collections. Thus, it will accelerate the assessment of allelic variations as well as the exploitation of the genetic diversity found in genebanks (Van *et al.* 2013). Moreover, NGS puts genotyping-by-sequencing to work, and facilitates genome-wide association studies for the dissection of complex traits as well as genomic selection of superior varieties (Poland & Rife 2012).

Taken together, NGS applications have the potential to revolutionise the practice of applied plant breeding (Poland & Rife 2012) and will make MAS more feasible as large numbers of breeder-friendly markers are becoming available (Varshney *et al.* 2009, Van *et al.* 2013).

## Harnessing genome data

Rice was the first crop species to be sequenced in 2002. Since then, more and more species have been translated from “genomic poor” to “genomic rich” crops, and today, genome sequences for at least 39 crop species are publicly available (Table 6). Noteworthy, the list of published genome sequences includes several important food security crops such as barley, cassava, chickpea, maize, potato, rice, sorghum and wheat. Genome sequencing projects for many other important crop species are still in progress. Furthermore, in 2012 Beijing Genomics Institute, the largest genomics centre in the world, initiated the sequencing of some 150 crop genomes using NGS-technology (BGI 2013). Recently, the African Orphan Crops Consortium (AOOC) announced its plan to sequence 100 traditional African crops, including species such as eggplant, okra, onion, coconut, taro, tamarind and bittergourd (UCDavis 2014). The resulting sequence data will be placed in the public domain. There, they will be a valuable resource for the 250 plant breeders, which AOOC intends to train in MAS to improve the crops crucial for African farm family livelihoods and nutrition.

Reference genome sequences are pivotal to crop improvement via MAS, particularly for complex traits. Therefore, the new wealth of genome data will not only make markers more accessible within the public sector breeding programmes but will also provide breeders with new tools and resources to enable more elaborate MAS-strategies and to accelerate the development of new crop varieties.

“

High-throughput phenotyping and genomic selection of complex traits promise to revolutionize the breeding process by accelerating generation-advance and improving the precision of selection.

”

– Cabrera-Bosquet *et al.*  
2012

**Table 6: Crops with published genome sequences**

Year	Crop
2002	rice (indica and japonica)
...	
2006	black cottonwood
2007	grape
2008	papaya
2009	cucumber, maize, sorghum
2010	apple, castor bean, jatropha, soybean
2011	barbados nut, cocoa, chinese cabbage, chinese plum, clementine mandarin, date palm, hemp, pigeon pea, potato, woodland strawberry
2012	banana, barley, cassava, flax, foxtail millet, melon, neem, tomato, watermelon, wheat
2013	chickpea, lupin, sweet orange, peach, pear, kiwifruit, norway spruce, rubber tree

Sources: Michael & Jackson (2013), Bevan & Uauy (2013), Van *et al.* (2013).

## **Harnessing genotyping-by-sequencing**

With the advent of the NGS technologies, genotyping-by-sequencing (GBS) has become a cost efficient genotyping method. According to Kumar *et al.* (2012), GBS has the potential to be a truly revolutionary technology, as it brings high-density genotyping to the vast majority of crop species. Until now, investment in genomic resources has been concentrated to very few staples such as rice, wheat and maize, with almost no investment in the genomic resources of the many other species. Uses of GBS include applications in marker discovery and QTL mapping. Furthermore, as it is a rapid and low-cost tool for genotyping, GBS will allow breeders to implement genomic selection on a large scale in their breeding programmes (Poland & Rife 2012).

## **Harnessing phenomics**

As many crop traits are not only monitored by molecular markers but also by their phenotype, adequate genotyping and phenotyping are both important for the success of modern plant breeding with MAS (Xu *et al.* 2012). Whereas genotyping is becoming faster, cheaper and more automatic, progress in modern breeding is hampered by a phenotyping bottleneck. To overcome this bottleneck much importance is currently being given to phenomics (Furbank & Tester 2011, Xu *et al.* 2012, Bohra 2013, Fiorani & Schurr 2013). By combining novel technologies such as non-invasive imaging, spectroscopy, image analysis and robotics, phenomics increasingly enables high-throughput phenotyping and will thus make field evaluation of plant performance as well as the elucidation of QTLs for complex traits, much faster.

## **Harnessing genomic selection for breeding complex traits**

Genomic selection (GS) or genome-wide selection is a new form of MAS that will facilitate the breeding of complex traits governed by many genes/QTLs (Heffner *et al.* 2010, Jannink *et al.* 2010). In MAS, the selection of favourable individuals is based on markers that track a certain trait. In contrast, selection in GS is based on the so-called genomic-estimated breeding values (GEBVs). These values are the sum of the effects of all QTLs across a genome, exploiting all the genetic variation for a particular trait. Traditional MAS breeding schemes demand prior QTL information for complex traits, but in GS schemes the need for information on marker-trait associations can be avoided by using GEBVs. By eliminating the need for any prior QTL information, GS schemes can save time, money, and energy that is required for finding significant gene-trait relationships (Bohra 2013). Furthermore, as GS exploits all the genetic variance of particular traits, it can address small effect genes/QTLs that cannot be captured by traditional MAS (Nakaya & Isobe 2012). Thus, GS becomes a powerful tool not only for pyramiding multiple QTLs for one particular complex trait but also for introgressing QTLs for more than one complex trait at once.





As this organic farmer in Spain knows, GE crops can cause contamination on neighbouring farms. There are no such concerns with MAS, as the technology uses conventional breeding.

© Greenpeace / Pedro Armeste



## 10. MOLECULAR BREEDING: MAS OUTPERFORMS GENETIC ENGINEERING



Conventional breeding, helped by MAS, outperforms genetic engineering in producing crops with traits that are of interest for farmers.

© Emile Loreaux / Greenpeace

Improved crop varieties – suited to different agronomic practices and farmers' needs in locally diverse agro-ecosystems, and resilient to climate change – are key to making food production sustainable without surrendering yield. Important traits of these crop varieties include greater tolerance to heat and drought, increased input-use efficiency, and enhanced pest and disease resistance (FAO 2011). To achieve these traits, breeders today can access a broad suite of technological options, including molecular breeding techniques. Molecular breeding refers to the development of new crop varieties by using the means of biotechnology, which involves both genetic engineering and marker-assisted selection (Rao *et al.* 2014).

In the past, and still today, the overwhelming focus was on a genetic engineering strategy of crop improvement, and many plant scientists, seed companies and politicians have placed a great deal of hope on the potential of GE crops to launch a new agricultural revolution. However, in light of the high promotion and the reconstitution of GE crops as a “moral imperative” for food security, previous achievements of commercial GE crops are disappointing in breeding terms. In 2013, two simple traits, herbicide tolerance and insect resistance, solely or combined, and implemented in the four commodity crops maize, cotton, rapeseed and soybean, accounted for 99% of the global area grown with GE crops (James 2014). The remaining 1% was planted with herbicide tolerant sugar beet and alfalfa, drought tolerant maize, and virus resistant papayas and squash.

While commercial genetic engineering has remained a restricted technology, delivering almost exactly the same narrow suite of traits that were around in the 1990s, MAS has gone through a silent revolution with a steadily expanding list of realised traits. Today, in breeding terms at least, conventional breeding helped by MAS outperforms genetic engineering in producing crops with traits that are of interest to farmers (Table 7). Taking biotic stress resistance as an example: Whereas genetic engineering has successfully realised insect and virus resistance in crop varieties, MAS has been effective in making varieties resistant not only to insects and viruses, but also to fungi, bacteria, nematodes and parasites. Regarding yield, the eternal theme pursued by breeders, MAS also performs better than genetic engineering, as it has already proven its potential by releasing high-yielding varieties in soybean, rice and tomato. Moreover, MAS has been successfully used to breed

“

In the past, and still today, there has been too much emphasis on GMOs and too little focus on the potential merits and benefits of non-GM biotechnologies and the positive role that they can play for food security and sustainable development in developing countries.

”

– Ruane & Sonnino 2011

abiotic stress resistance such as tolerances to salt, drought, submergence and acid soils. In contrast to MAS, genetic engineering to date has only been successful in moving drought-tolerance from “lab to the land”.

Further – and significant in terms of attaining food security – MAS outperforms genetic engineering for the realisation of traits in some of the most important staple food crops of the world (Table 8). For example, in rice, a staple food for three billion people worldwide, Asian farmers today can grow MAS-derived varieties that tackle major biotic constraints like bacterial blight and blast as well as major abiotic stresses such as submergence and drought. On the other hand, genetic engineering succeeded in the approval of rice varieties with herbicide tolerance and insect resistance, but none of these varieties are currently grown commercially. A similar picture is found in wheat; while no GE wheat varieties have been approved yet for cultivation, MAS has been effective in improving biotic stress resistance and quality traits of wheat. Moreover, by releasing varieties of pearl millet with resistance to downy mildew disease in India, sorghum with resistance to striga in Sudan and cassava with resistance to cassava mosaic disease in Nigeria and Tanzania, MAS has proven its potential to improve key subsistence crops in developing countries.

MAS has proven to be a molecular breeding approach in which genomic knowledge is paying off without employing genetic engineering. New varieties developed using MAS offer several major advantages over GE crops: MAS respects species barriers, raises less safety concerns, is accepted by the public and permitted in 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 – in contrast to genetic engineering – no ethical concerns regarding “naturalness” and “plant integrity”.

**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 varieties are generally considered as safe as their conventionally bred counterparts. By combining genetic elements not found in conventionally bred crops, genetic engineering approaches result in varieties with novel traits with novel hazards, and thus the long safety history of conventionally bred varieties cannot be translated to GE-derived varieties. Due to the novel hazards, the environmental and food/feed safety of GE crops and their associated food products has to undergo mandatory assessment before market release in most countries. Consequently, GE-derived products must not only pass variety registration tests, just as MAS-derived and conventionally bred varieties do, but they must also undergo a costly GE regulatory system.

**MAS is accepted by consumers:** The potential impacts of GE crops on health and the environment have attracted much attention worldwide. As the issue remains controversial there are prevalent public concerns about the safety of GE crops. Moreover, people raise ethical concerns regarding intellectual property issues on crops and genes; about scientists “playing god”, as crops are transformed in unnatural ways and about the implications for traditional beliefs and values. As a consequence of public perception, GE crops are faced with widespread consumer opposition in many countries. While GE-derived varieties have, so far, failed to gain broad acceptance, MAS has largely remained free of prolonged public debates. As a non-invasive biotechnology approach MAS does not raise public concerns and thus there is little problem with public acceptance, which encourages wider use of this technology. There are, however, concerns that intellectual property issues associated with MAS may restrict its use (Meyer *et al.* 2013).

“

In this context [use of plant genetic resources] MAS presents not only an alternative but may be on the long run superior to genetic engineering approaches whenever the primary, secondary or tertiary gene pool are the source of desired traits.

”

– Brumlop &  
Finckh 2011

**Table 7: Traits realised successfully in crops by genetic engineering and MAS**

<b>Traits realised by genetic engineering*</b>	<b>Traits realised by MAS**</b>
<b>Agronomic traits</b>	
Herbicide tolerance	Herbicide tolerance
Insect resistance	Insect resistance
Virus resistance	Virus resistance
Male sterility /fertility restoration	Bacteria resistance
Drought tolerance	Fungi resistance
	Nematode resistance
	Parasite resistance
	Drought tolerance
	Salt tolerance
	Flood tolerance
	Acid soil tolerance
	Yield
<b>Quality traits</b>	
<i>Extended shelf life</i>	High protein grain
Increased bioethanol production	High-quality protein
<i>Improved feed quality</i>	Improved cooking quality
Improved oil quality	Improved oil quality
<i>Modified starch for industry</i>	High glucoraphanin
<i>Nicotine reduction</i>	Improved malting quality
	Low cadmium grain

\*: Traits for which GE events have been approved for cultivation according to the comprehensive GE crop approval database of the International Service for the Acquisition of Agri-biotech Applications (ISAAA 2014). Traits marked in italic have not been under commercial cultivation in 2013 (James 2014, BIO 2013).

\*\* : Traits for which varieties have been released for cultivation according to the information gathered in the course of the present report. The list of the traits is considered to be not comprehensive, mainly because private sector breeding companies usually do not disclose their MAS-results.

**MAS is permitted in organic farming:** Although the use of molecular markers is often debated, standards of organic agriculture do not exclude MAS (Lammerts van Bueren *et al.* 2010). Therefore, MAS-derived varieties resulting from breeding programmes for conventional agriculture can be used and adapted in organic farming. Moreover, MAS can be used for organic plant breeding, which aims at improving the quantity and quality of crops that can be grown with minimal external inputs such as fertilisers or pesticides. While MAS-derived varieties are permitted, GE crops are not accepted in organic farming, as it contrasts with the concept of naturalness and integrity (Verhoog 2007).

Genetic engineering remains a controversial technology that, to date, has only addressed a few traits and delivered almost exclusively private-sector goods developed by multinational companies in industrialised countries. In contrast, MAS is a breeding tool free of public perception issues and regulatory issues. It has already addressed a broad suite of traits and delivered goods developed by both, private seed companies and public breeding programmes. For that reason, genetic engineering should no longer overshadow MAS, and policymakers should reinforce the role of MAS as a piece of the puzzle of approaches and technologies necessary to meet food security in an era of climate change. In a recent report for the European Parliament taking into account genetic engineering and MAS as technology options for feeding 10 billion people, Meyer *et al.* (2013) concluded, that the “main focus in public breeding research support should be on marker-assisted selection and SMART breeding as very promising breeding technologies”.





**Table 8: Traits realised in variety releases by genetic engineering and MAS in important staple food crops**

	Traits realised in varieties by genetic engineering*		Traits realised in varieties by MAS**	
	Private sector	Public sector	Private sector	Public sector
<b>Wheat</b>	-	-	FR, HGP, HT, IR	FR, HGP, IR, LC, VR
<b>Rice</b>	<i>HT</i>	<i>IR</i>	-	AST, BR, DT, FR, FT, IR, ST, Y
<b>Maize</b>	DT, EP, HT, IR	<i>IF</i>	DT, IR	QPM
<b>Soybean</b>	HT, IR, OQ	-	NT, OQ, Y	NT
<b>Barley</b>	-	-	AST, VR	FR, MQ, NR, VR
<b>Sorghum</b>	-	-	-	PR
<b>Millet</b>	-	-	-	FR
<b>Bean</b>	-	<i>VR</i>	-	BR, FR, VR
<b>Cassava</b>	-	-	-	VR
<b>Potato</b>	<i>HT, IR, VR</i>		NR	-

\*: Traits for which GE events have been approved for cultivation according to the comprehensive GE crop approval database of the International Service for the Acquisition of Agri-biotech Applications (ISAAA 2014). Traits marked in italic have not been under commercial cultivation in 2013 (James 2014, BIO 2013).

\*\* : Traits for which varieties have been released for cultivation according to the informations gathered in the course of the present report. The list of the traits is considered to be not comprehensive, mainly because private sector breeding companies usually do not disclose their MAS-results.

Abbreviations: AST: acid soil tolerance; BR: bacteria resistance; CQ: cooking quality; DT: drought tolerance; EP: increased ethanol production; FR: fungus resistance; FT: flood tolerance; HGP: High grain protein; HT: herbicide tolerance; IF: improved feed quality; IR: insect resistance; LC: low cadmium grain; MQ: malting quality; NT: nematode resistance; OQ: oil quality; PR: parasite resistance; QPM: quality protein maize; ST: salt tolerance; VR: virus resistance; Y: increased yield.

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## ANNEX: MAS VARIETIES DEVELOPED BY PUBLIC INSTITUTIONS

Variety	Trait	Country	Year	Breeding institution/released by
<b>Barley</b>				
Tango	Disease resistance	USA	2000	Oregon State University <sup>(1)</sup>
SloopSA	Disease resistance	Australia	2002	University of Adelaide <sup>(2)</sup>
Sloop-Vic	Disease resistance	Australia	2002	University of Adelaide <sup>(2)</sup>
Doria	Disease resistance	Italy	2006	Istituto Sperimentale per la Cerealicoltura <sup>(3)</sup>
CDC Carter	Disease resistance	Canada	2010	University of Saskatchewan <sup>(4)</sup>
CDC Polarstar	Malting quality	Canada	2010	University of Saskatchewan <sup>(5)</sup>
<b>Bean</b>				
USPT-ANT-1	Disease resistance	USA	2004	USDA-ARS <sup>(6)</sup>
ABCP-8	Disease resistance	USA	2005	University of Nebraska / USDA-ARS <sup>(7)</sup>
ABC-Weihsing	Disease resistance	USA	2006	University of Nebraska / USDA-ARS <sup>(8)</sup>
Verano	Disease resistance	USA	2008	University of Puerto Rico / USDA-ARS <sup>(9)</sup>
Coyne	Disease resistance	USA	2008	University of Nebraska <sup>(10)</sup>
Fuji	Disease resistance	USA	2009	Michigan State University <sup>(11)</sup>
Teebus-RCR 2	Disease resistance	South Africa	2008	Agricultural Research Council <sup>(12)</sup>
CDC WM-2	Disease resistance	Canada	2009	University of Saskatchewan <sup>(13)</sup>
Arka Anoop	Disease resistance	India	2013	Indian Institute of Horticultural Research <sup>(14)</sup>
<b>Cassava</b>				
UMUCASS33	Disease resistance	Nigeria	2010	National Root Crops Research Institute <sup>(15)</sup>
UMUCASS41	Disease resistance	Nigeria	2012	National Root Crops Research Institute <sup>(15)</sup>
Pwani	Disease resistance	Tanzania	2012	Agricultural Research Institute <sup>(15)</sup>
Mkumba	Disease resistance	Tanzania	2012	Agricultural Research Institute <sup>(15)</sup>
Makutupora	Disease resistance	Tanzania	2012	Agricultural Research Institute <sup>(15)</sup>
Dodoma	Disease resistance	Tanzania	2012	Agricultural Research Institute <sup>(15)</sup>
<b>Chilli</b>				
Arka Meghana	Disease resistance	India	2013	Indian Institute of Horticultural Research <sup>(14)</sup>
<b>Maize</b>				
Vivek QPM 9	High-Quality Protein	India	2008	Indian Council of Agricultural Research <sup>(16)</sup>
Sirdamaize 113	Drought tolerance	Zimbabwe	2009	SIRDC <sup>(17)</sup>
<b>Peanut</b>				
NemaTAM	Disease resistance High O/L	USA	2003	Texas Agriculture Experiment Station <sup>(18)</sup>
TifNV-High O/L	Disease resistance	USA	2013	University of Georgia , USDA-ARS <sup>(19)</sup>
<b>Pearl millet</b>				
HHB 67-2	Disease resistance	India	2005	Haryana Agricultural University/ICRISAT <sup>(20)</sup>
<b>Rice</b>				
Cadet	Cooking quality	USA	2000	Texas Agricultural Experiment Station <sup>(21)</sup>
Jacinto	Cooking quality	USA	2000	Texas Agricultural Experiment Station <sup>(21)</sup>
Nanjing 46	Cooking quality	China	2008	Jiangsu Academy of Agricultural Sciences <sup>(22)</sup>
OM4495	Cooking quality	Vietnam	2005	Cuu Long Delta Rice Research Institute <sup>(23)</sup>
OM5239	Cooking quality	Vietnam	2005	Cuu Long Delta Rice Research Institute <sup>(23)</sup>



**MARKER-ASSISTED SELECTION**  
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Variety	Trait	Country	Year	Breeding institution/released by
<b>Rice (cont'd)</b>				
Improved Manawthukha	Cooking quality	Myanmar	2010	Department of Agricultural Research <sup>(15)</sup>
Tainan 16	Cooking quality	Taiwan	2012	TDARES <sup>(24)</sup>
Zhongyou 161	Cooking quality Disease resistance	China	2009	China National Rice Research Institute <sup>(25)</sup>
Xieyou 218	Disease resistance	China	2002	China National Rice Research Institute <sup>(26)</sup>
Guodao 1	Disease resistance	China	2002	China National Rice Research Institute <sup>(26)</sup>
Zhongyou 218	Disease resistance	China	2002	China National Rice Research Institute <sup>(26)</sup>
Xieyou 527	Disease resistance	China	2004	Sichuan Agricultural University <sup>(27)</sup>
D-you 527	Disease resistance	China	2004	Sichuan Agricultural University <sup>(27)</sup>
Zhunliangyou 527	Disease resistance	China	2004	Sichuan Agricultural University <sup>(27)</sup>
Gangyou 527	Disease resistance	China	2004	Sichuan Agricultural University <sup>(27)</sup>
Guodao 3	Disease resistance	China	2004	China National Rice Research Institute <sup>(26)</sup>
Guodao 6	Disease resistance	China	2004	China National Rice Research Institute <sup>(28)</sup>
Neizyou	Disease resistance	China	2004	China National Rice Research Institute <sup>(26)</sup>
Zhongyou 6	Disease resistance	China	2004	China National Rice Research Institute <sup>(29)</sup>
Zhongyou 1176	Disease resistance	China	2004	China National Rice Research Institute <sup>(29)</sup>
Ilyou 8006	Disease resistance	China	2005	China National Rice Research Institute <sup>(26)</sup>
Ilyou 218	Disease resistance	China	2005	China National Rice Research Institute <sup>(26)</sup>
Tianyou 6	Disease resistance	China	2005	China National Rice Research Institute <sup>(28)</sup>
ZhongbaiYou 1	Disease resistance	China	2006	China National Rice Research Institute <sup>(26)</sup>
Angke	Disease resistance	Indonesia	2002	ICRR/ICABIOGRAD <sup>(30)</sup>
Conde	Disease resistance	Indonesia	2002	ICRR/ICABIOGRAD <sup>(30)</sup>
Tubigan 7	Disease resistance	Philippines	2006	Philippine Rice Research Institute <sup>(26)</sup>
Tubigan 11	Disease resistance	Philippines	2007	Philippine Rice Research Institute <sup>(26)</sup>
Improved Pusa Basmati 1	Disease resistance	India	2007	Indian Agricultural Research Institute <sup>(26)</sup>
Improved Samba Mahsuri	Disease resistance	India	2007	Directorate of Rice Research <sup>(26)</sup>
Improved Tapaswini	Disease resistance	India	2011	Central Rice Research Institute <sup>(31)</sup>
Improved Lalat	Disease resistance	India	2011	Central Rice Research Institute <sup>(32)</sup>
Pusa Sugandh 6	Disease resistance	India	2013	Indian Agricultural Research Institute <sup>(33)</sup>
Punjab Basmati 3	Disease resistance	India	2013	Punjab Agricultural University <sup>(34)</sup>
Thanyasirin	Disease resistance	Thailand	2011	Kasetsart University/RMUTL/BIOTEC <sup>(35)</sup>
RD18	Disease resistance	Thailand	2013	Kasetsart University/RMUTL/BIOTEC <sup>(36)</sup>
Hwaweon 5	Disease resistance	South Korea	2012	Chungnam National University <sup>(37)</sup>
Saeilmi	Disease resistance	South Korea	2012	National Institute of Crop Science <sup>(38)</sup>
Anmi	Insect resistance	South Korea	2010	International Rice Research Institute <sup>(39)</sup>
Y Liangyou 7	High Yield	China	2008	CNHRDC <sup>(40)</sup>
MAS 946-1	Drought-tolerance	India	2007	University of Agricultural Sciences <sup>(41)</sup>
MAS 26	Drought-tolerance	India	2008	University of Agricultural Sciences <sup>(41)</sup>
Birsa Vikas Dhan 111	Drought-tolerance	India	2009	Birsa Agricultural University <sup>(42)</sup>
OM6161	Drought-tolerance	Vietnam	2010	Cuu Long Delta Rice Research Institute <sup>(43)</sup>
OM6162	Drought-tolerance	Vietnam	2010	Cuu Long Delta Rice Research Institute <sup>(43)</sup>
Om7347	Drought-tolerance	Vietnam	2011	Cuu Long Delta Rice Research Institute <sup>(43)</sup>
AS996	Acid soil tolerance	Vietnam	2004	Cuu Long Delta Rice Research Institute <sup>(43)</sup>
MNR 3	Acid soil tolerance	Vietnam	2012	Cuu Long Delta Rice Research Institute <sup>(43)</sup>
MNR 4	Acid soil tolerance	Vietnam	2012	Cuu Long Delta Rice Research Institute <sup>(43)</sup>

**MARKER-ASSISTED SELECTION**  
A BIOTECHNOLOGY FOR PLANT BREEDING WITHOUT GENETIC ENGINEERING

Variety	Trait	Country	Year	Breeding institution/released by
<b>Rice (cont'd)</b>				
Swarna-Sub1	Flood-tolerance	India	2009	International Rice Research Institute <sup>(44)</sup>
		Indonesia	2009	
		Bangladesh	2010	
		Nepal	2011	
		Myanmar	2011	
Samba Mahsuri-Sub1	Flood-tolerance	Nepal	2011	International Rice Research Institute <sup>(44)</sup>
		Bangladesh	2013	
		India	2013	
IR64-Sub1	Flood-tolerance	Philippines	2009	International Rice Research Institute <sup>(44)</sup>
		Indonesia	2009	
B11-Sub1	Flood-tolerance	Bangladesh	2010	International Rice Research Institute <sup>(44)</sup>
Ciherang-Sub1	Flood-tolerance	Indonesia	2012	International Rice Research Institute <sup>(44)</sup>
		Bangladesh	2013	
CR1009-Sub1	Flood-tolerance	India	2013	Annamalai University <sup>(45)</sup>
Homcholasit	Flood-tolerance	Thailand	2011	BIOTEC <sup>(46)</sup>
RD51	Flood-tolerance	Thailand	2013	BIOTEC <sup>(47)</sup>
OM4900	Salt tolerance	Vietnam	2009	Cuu Long Delta Rice Research Institute <sup>(43)</sup>
OM5629	Salt tolerance	Vietnam	2011	Cuu Long Delta Rice Research Institute <sup>(43)</sup>
OM2009	Salt tolerance	Vietnam	2011	Cuu Long Delta Rice Research Institute <sup>(43)</sup>
Improved Sin Thwe Latt	Salt tolerance	Myanmar	2011	Department of Agricultural Research <sup>(15)</sup>
<b>Sorghum</b>				
ASARECA.T1	Striga resistance	Sudan	2012	ASARECA <sup>(48)</sup>
ASARECA.W2	Striga resistance	Sudan	2012	ASARECA <sup>(48)</sup>
ASARECA.AG3	Striga resistance	Sudan	2012	ASARECA <sup>(48)</sup>
ASARECA.AG4	Striga resistance	Sudan	2012	ASARECA <sup>(48)</sup>
<b>Soybean</b>				
JTN-5303	Disease resistance	USA	2005	University of Tennessee / USDA-ARS <sup>(49)</sup>
JTN-5503	Disease resistance	USA	2005	University of Tennessee / USDA-ARS <sup>(49)</sup>
JTN-5109	Disease resistance	USA	2009	University of Tennessee / USDA-ARS <sup>(49)</sup>
DS-880	Disease resistance	USA	2010	USDA-ARS <sup>(49)</sup>
<b>Tomato</b>				
Ab2	High Yield	USA	2002	Hebrew University of Jerusalem <sup>(50)</sup>
Mountain Magic	Disease resistance	USA	2008	North Carolina State University <sup>(51)</sup>
Mountain Merit	Disease resistance	USA	2010	North Carolina State University <sup>(52)</sup>
Plum Regal	Disease resistance	USA	2010	North Carolina State University <sup>(53)</sup>
Mountain Honey	Disease resistance	USA	2013	North Carolina State University <sup>(54)</sup>
Mountain Vineyard	Disease resistance	USA	2013	North Carolina State University <sup>(55)</sup>
BRS Tospodoro	Disease resistance	Brazil	2010	NCVCR <sup>(56)</sup>
Arka Samrat	Disease resistance	India	2013	Indian Institute of Horticultural Research <sup>(14)</sup>
Arka Rakshak	Disease resistance	India	2013	Indian Institute of Horticultural Research <sup>(14)</sup>
<b>Wheat</b>				
Patwin	Disease resistance	USA	2006	University of California, Davis <sup>(57)</sup>
Mace	Disease resistance	USA	2007	University of Minnesota <sup>(58)</sup>
Shirley	Disease resistance	USA	2008	Virginia Agricultural Experiment Station <sup>(59)</sup>
Sabin	Disease resistance	USA	2009	University of Minnesota <sup>(60)</sup>
Patwin 515	Disease resistance	USA	2012	University of California, Davis <sup>(61)</sup>

Variety	Trait	Country	Year	Breeding institution/released by
<b>Wheat (cont'd)</b>				
UI Stone	Disease resistance	USA	2012	University of Idaho <sup>(62)</sup>
Otto	Disease resistance	USA	2013	Washington State University <sup>(63)</sup>
Glee	Disease resistance	USA	2013	Washington State University <sup>(64)</sup>
Dayn	Disease resistance	USA	2013	Washington State University <sup>(64)</sup>
AAC Brandon	Disease resistance	Canada	2013	Agriculture and Agri-Food Canada <sup>(65)</sup>
AAC Elie	Disease resistance	Canada	2013	Agriculture and Agri-Food Canada <sup>(65)</sup>
Biointa 2004	Disease resistance	Argentina	2009	Marcos Juárez Experimental Station <sup>(66)</sup>
Shield	Disease resistance	Australia	2012	Australian Grain Technologies <sup>(67)</sup>
Lassik	Disease resistance High grain protein	USA	2007	University of California, Davis <sup>(68)</sup>
Farnum	Disease resistance High grain protein	USA	2008	Washington State University <sup>(68)</sup>
Cataldo	Insect resistance	USA	2007	Idaho Agricultural Experiment Station <sup>(69)</sup>
Goodeve	Insect resistance	Canada	2007	Agriculture and Agri-Food Canada <sup>(70)</sup>
Glencross	Insect resistance	Canada	2008	Agriculture and Agri-Food Canada <sup>(70)</sup>
AAC Raymore*	Insect resistance	Canada	2013	Agriculture and Agri-Food Canada <sup>(65)</sup>
AAC Marchwell*	Insect resistance	Canada	2014	Agriculture and Agri-Food Canada <sup>(65)</sup>
Desert King HP*	High grain protein	USA	2011	University of California, Davis <sup>(68)</sup>
Lillian	High grain protein	Canada	2003	Agriculture and Agri-Food Canada <sup>(70)</sup>
Somerset	High grain protein	Canada	2004	Agriculture and Agri-Food Canada <sup>(70)</sup>
Burnside	High grain protein	Canada	2004	Agriculture and Agri-Food Canada <sup>(70)</sup>
Miwok*	Low cadmium	USA	2013	University of California, Davis <sup>(64)</sup>
Brigade*	Low cadmium	Canada	2008	Agriculture and Agri-Food Canada <sup>(70)</sup>
CDC Verona*	Low cadmium	Canada	2008	University of Saskatchewan <sup>(70)</sup>
CDC Vivid*	Low cadmium	Canada	2012	University of Saskatchewan <sup>(70)</sup>
CDC Desire*	Low cadmium	Canada	2012	University of Saskatchewan <sup>(70)</sup>
AAC Current*	Low cadmium	Canada	2013	Agriculture and Agri-Food Canada <sup>(65)</sup>
AAC Durafeld*	Low cadmium	Canada	2014	Agriculture and Agri-Food Canada <sup>(65)</sup>

\* *durum wheat variety*

Abbreviations: ASARECA: Association for Strengthening Agricultural Research in Eastern and Central Africa; CNHRRDC: China National Hybrid Rice Research and Development Center; ICABIOGRAD: Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development; ICRISAT: International Crops Research Institute for the Semi-Arid Tropics; ICRR: Indonesian Center for Rice Research; NCVCR: National Center for Vegetable Crops Research; RMUTL: Rajamangala University of Technology Lanna; SIRDC: Scientific and Industrial Research and Development Centre; TDARES: Tainan District Agricultural Research and Extension Station; USDA-ARS: United States Department of Agriculture Agricultural Research Service (ARS).

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