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Applications and potentials of marker assisted selection (MAS) in plant breeding

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Final report of the F+E project
“Applications and Potentials of Smart Breeding”
(FKZ 350 889 0020)
On behalf of the Federal Agency for Nature Conservation

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Cover picture: A diverse wheat composite cross population in the F8. Such materials could be used for the development of modern land races using MAS to select for homogeneity in important traits while maintaining overall population diversity (M. Finckh).

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Contents

List of figures

List of tables

List of tables in Annex A

Abbreviations

AB-QTL	advanced backcross QTL
AFLP	amplified fragment length polymorphism
ARS-USDA	Agricultural Research Service of the U.S. Department of Agriculture
BaMMV	barley mild mosaic virus
BaYMV	barley yellow mosaic virus
BSA	bulked segregant analysis
CAPS	cleaved amplified polymorphic sequences
CBD	Convention on Biological Diversity
CC	composite cross
cDNA	complementary DNA
CGIAR	Consultative Group on International Agricultural Research
CIMMYT	International Maize and Wheat Improvement Center
CPVR	Community Plant Variety Right
CWR	crop wild relatives
DArT	diversity array technology
DUS	distinctness, uniformity, and stability
EC	European Commission
EDV	essentially derived varieties
EPB	evolutionary-participatory plant breeding
EST	expressed sequence tag
EU	European Union
FHB	*Fusarium* head blight
FOSS	free and open source software
GATT	General Agreement on Tariffs and Trades

GFP	Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V.
GM	genetically modified
GMO	genetically modified organism
GPL	general public license
GPLPG	general public license for plant germplasm
GVS	Gesellschaft für Erwerb und Verwertung von Schutzrechten mbh
GxE	genotype by environment interaction
IBPGR	International Board for Plant Genetic Resources
IL	introgression library
INIBAP	International Network for the Improvement of Banana and Plantain
IPGRI	International Plant Genetic Research Institute
IPK	Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung
ISF	International Seed Federation
ISSR	inter-simple sequence repeat
IT PGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
JKI	Julius Kühn-Institut
LD	linkage disequilibrium
MABC	marker-assisted backcrossing
MAGE	marker-assisted germplasm evaluation
MARS	marker-assisted recurrent selection
MAS	marker-assisted selection
MAYG	„Mapping As You Go“
MPI	Max Planck-Institut
MTA	materials transfer agreement
NIL	near isogenic line
OECD	Organisation for Economic Co-operation and Development

PBR	plant breeders' rights
PCR	polymerase chain reaction
PGR	plant genetic resources
PIA	Pflanzen Innovations Agentur
PPB	participatory plant breeding
PS	phenotypic selection
PVP	plant variety protection
QPM	quality protein maize
QTL	quantitative trait locus
RAPD	random-amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
SCAR	sequence characterized amplified regions
SCLB	Southern Corn Leaf Blight
SFP	single feature polymorphism
SNP	single nucleotide polymorphism
SSLP	simple sequence length polymorphism
SSR	simple sequence repeat
STMS	sequence-tagged microsatellite sites
STS	sequence tagged site
TILLING	Targeting Induced Local Lesions IN Genomes
TRIPs	Trade Related Aspects of Intellectual Property Right
UPOV	Union for the Protection of New Varieties of Plants
VCU	value for cultivation and use
WTO	World Trade Organization
XAM	eXtreme array mapping

‘The most wonderful mystery of life may well be the means by which it created so much diversity from so little physical matter.’

E. O. Wilson, The Diversity of Life

1 Introduction

To improve plant varieties, since the late 19th century plant breeders relied on phenotypic selection, achieving breeding progress through the assessment of external and internal traits such as plant habitus, disease resistances, yield, or quality traits. New, improved varieties were developed by solely selecting plants with desirable phenotypes. Plant breeding techniques became very sophisticated over the years but time demanding too. Developing a new, improved plant variety by means of phenotypic selection can easily exceed 10 years.

Only with the advent of molecular markers in the late 1970s, it became possible to select desirable traits more directly. Easily detectable DNA markers can now be used in plant breeding. Marker-assisted selection (MAS) has turned into a tool which is – to varying degrees – utilized in breeding companies and research institutes for the development of improved varieties, allowing for a breeding approach based on the genotype of plants rather than assessing the phenotype only. DNA markers are sections of the genome of the organisms in question which are used for recognition. They can be understood as naturally occurring tags attached to specific segments of a chromosome, which in turn are associated with specific phenotypes. A marker can either be located within the gene of interest or be linked to a gene determining a trait of interest, which is the most common case. Thus MAS can be defined as selection for a trait based on genotype using associated markers rather than the phenotype of the trait (FOOLAD & SHARMA 2005).

Sometimes the term “Smart Breeding”, an acronym for “Selection with Markers and Advanced Reproductive Technologies”, which was first used in animal breeding (DAVIS et al. 1997), is used to describe marker supported breeding strategies.

The idea of MAS or Smart Breeding was taken up with great enthusiasm, and several

breeders expressed the hope to "skip several breeding cycles and condense timelines" (MAZUR 1995) and to finally having found a tool, "to control all allelic variation for all genes of agronomic importance" (PELEMAN & VAN DER VOORT 2003). According to XU & CROUCH (2008) the greatest benefits of MAS are the possibilities to "achieve the same breeding progress in a much shorter time than through conventional breeding", "pyramid combinations of genes that could not be readily combined through other means" and to "assemble target traits more precisely, with less unintentional losses".

Other scientists judged the power and potential of MAS less optimistic, especially regarding quantitative traits. BEAVIS (1998) said that "because most agronomically important traits in crops are quantitative, it seems fair to say that molecular markers have had little impact on crop improvement despite hundreds of published QTL (quantitative trait locus) experiments during the last 10 years" and YOUNG stated in 1999 that "even though marker-assisted selection now plays a prominent role in the field of plant breeding, examples of successful practical outcomes are rare. It is clear that DNA markers hold great promise, but realizing that promise remains elusive." In 2001, GUPTA et al. stated that "the lack of cost-effectiveness and non-availability of high throughput approaches for handling large segregating populations have limited the use of molecular marker technology for plant breeding" and that it "has yet to find its rightful place in plant breeding programs". Three years later HOLLAND (2004) still articulated the opinion, that "it is not likely that markers will soon be generally useful for manipulating complex traits like yield".

Ten years on from YOUNG'S "cautious optimistic" estimation of the current mood concerning the application of DNA markers in breeding, this report is discussing the subject of marker-assisted breeding once more, especially focusing on the question whether MAS that "has been touted as a means to improve the efficiency and speed of plant selection programs" (HOLLAND 2004) can also aid to maintain biodiversity. After a short general introduction of the methodology of marker-assisted breeding the current applications of MAS will be reviewed. The current "state of the art" concerning marker applications in plant breeding programs will be presented and its potentials in terms of maintaining biodiversity will be discussed.

Since the early 20th century, when modern plant breeding started, the development has been towards an increased use of genetically uniform varieties in agriculture. Towards the end of the 20th century many scientists raised the concern that intense agronomic improvements have led to erosion of genetic diversity and will continue to do so if

breeding practices remain unchanged (HAMMER 2004). Molecular markers might play an increasingly important role in the evaluation, conservation and use of diversity in germplasm and varieties in the future. As they facilitate the purposeful utilization of plant genetic resources (PGR), they can support efforts to broaden the genetic base of crop plants and to ensure diversity at all levels.

This report is based on a literature survey, completed with guided expert interviews. Based on the literature survey a questionnaire containing routing questions was developed and served as a basis for telephone interviews with breeders and scientists working within the field of marker-assisted selection. Following the interviews a workshop was held with experts where results from both the survey and the interviews were further discussed (chapter 6).

Not all crops can be covered in this review, thus the focus is restricted to marker application in the main agricultural crops grown in temperate zones.

1.1 The history of MAS

Although the idea for marker-assisted selection dates back to 1923, it is a young field of science and breeding. SAX (1923) observed an association between seed color (monogenic trait) and seed weight (polygenic, quantitatively inherited trait) in beans (*Phaseolus vulgaris* L.) and drew the conclusion that the single gene controlling seed color must be linked to one or more of the polygenes controlling seed size. The concept of using linked genes to follow the inheritance of genes controlling other traits had come into being. It was put forth in 1961 by THODAY, who made a first attempt to map and characterize all polygenes affecting a trait with the help of monogenic markers. Working with morphological markers, the main practical limitation of his work was the fact that only few suitable markers were available.

By the early 1980s, allozyme markers were being employed as a tool for the discrimination of genotypes, replacing the previously used morphological markers. Allozyme markers are based on protein polymorphisms; they are allelic forms of enzymes and can be separated on electrophoretic gels and detected by staining the gels. Advantages of this method are the low costs, technical simplicity and the co-dominant nature of the marker. Co-dominance means that alleles of both parents can be detected in the F_1, thus homozygous and heterozygous genotypes can be distinguished. However, the limited number of suitable allozyme loci in the genome and the requirement of fresh tissue of the right developmental stage are clear

disadvantages (TANKSLEY 1993; WEISING et al. 2005).

With the advent of DNA markers the situation again changed fundamentally, because with DNA markers genetic variation is observed at the DNA level. Thus more polymorphisms can be revealed and breeders could for the first time identify large numbers of markers dispersed throughout the genome of any species of interest, using the markers to detect associations with traits of interest, independent of their stage specific expression. Finally, the idea of MAS became a reality (RUANE & SONNINO 2007).

The first DNA-based genetic markers were restriction fragment length polymorphisms (RFLPs) (BOTSTEIN et al. 1980). A first linkage map in a crop plant (tomato) based on RFLPs was constructed in 1986 (BERNATZKY & TANKSLEY 1986) and two years later PATERSON et al. (1988) were the first who used a complete RFLP linkage map to resolve quantitative traits into discrete Mendelian factors.

Following the hybridization-based RFLPs, numerous DNA marker systems based on the polymerase chain reaction (PCR) were developed, such as random-amplified polymorphic DNAs (RAPDs) (WILLIAMS et al. 1990), amplified fragment length polymorphisms (AFLPs) (VOS et al. 1995), microsatellites, also termed simple sequence repeats (SSRs), (POWELL et al. 1996) or single nucleotide polymorphisms (SNPs) (GUPTA et al. 2001) (for technical details see chapter 1.2.1 to 1.2.6).

By now a stage has been reached, where genomics research is focusing on generating functional markers that can help identifying genes that underlie certain traits, thus facilitating their exploitation in crop improvement programs. It is assumed that breeders soon will be able to design genotypes *in silico* and to practice whole genome selection, in this way gradually moving from marker-assisted breeding on to "genomics-assisted breeding" (VARSHNEY et al. 2005a) or genomic selection (HEFFNER et al. 2009).

1.2 Introduction of marker technologies

In this chapter an overview over the "classical" technologies for the development of molecular markers is given to briefly introduce the methodologies connected with MAS. The major publications related to each of the technologies introduced are given, thus permitting further reading.

Markers can be developed either with or without knowledge of DNA sequences, the

technologies not requiring sequence information are presented first (chapter 1.2.1 to 1.2.3).

1.2.1 Restriction fragment length polymorphism (RFLP) markers

With the help of RFLPs variation between different individuals or accessions can be made visible by comparing DNA sequences at the same loci in different individuals. RFLPs are detected by cutting genomic DNA with restriction enzymes. Each of these enzymes has a specific recognition sequence which is typically palindromic and which leads to restriction fragments of certain length when the DNA is digested. Changes within these sequences which can be caused by point mutations, insertions or deletions, result in DNA fragments of differing length and molecular weights. These fragments are size-separated with agarose gel electrophoresis and analyzed by Southern blots using either locus-specific or multilocus probes. The former recognize one or a few specific regions of the genomic DNA, the latter recognize tandemly repeated DNA motifs such as microsatellites (see 1.2.4). The two main advantages of RFLP markers are co-dominance and high reproducibility. Disadvantages are the requirement of relatively large amounts of pure and intact DNA and the tedious experimental procedure (BOTSTEIN et al. 1980; WEISING et al. 2005; EDWARDS & MCCOUCH 2007).

1.2.2 Randomly-amplified polymorphic DNA (RAPD) markers

RAPD markers are based on the PCR amplification of random DNA segments with single, typically short primers of arbitrary nucleotide sequence (WILLIAMS et al. 1990). The primers bind to complementary sample DNA sequences and where two primers bind to the sample DNA in close enough proximity for successful PCR, a stretch of the DNA is amplified. The DNA amplification products are visualized by gel electrophoresis. Because the primers are arbitrarily chosen, no prior knowledge of the DNA sequence is needed. The genome is expected to be sampled randomly and the technology is especially useful if loci across an entire genome are to be assayed. A disadvantage of RAPD markers is the fact that the polymorphisms are detected only as the presence or absence of a band of a certain molecular weight, with no information on heterozygosity. Besides being dominantly inherited, RAPDs also show some problems with reproducibility of data. Their major advantages are the technical simplicity and the independence of any prior DNA sequence information (WEISING et al. 2005; EDWARDS & MCCOUCH 2007).

1.2.3 Amplified fragment length polymorphism (AFLP®) markers

The AFLP® technique[1] combines elements of RFLP and RAPD. It is based on the selective PCR amplification of restriction fragments. In a first step genomic DNA is digested and oligonucleotide adapters (defined short oligonucleotide sequences) are ligated to both ends of the resulting restriction fragments. In a second step the fragments are selectively amplified, using the adapter and restriction site sequences as primer binding sites for subsequent PCR reactions. As the 3´ ends of the primers extend into the restriction fragments by 1 to 4 bp, only those fragments are amplified, whose ends are perfectly complementary to the 3´ends of the selective primers. Thus, only a certain portion of the restriction fragments is amplified. In the last step the amplified fragments are resolved by gel electrophoresis and visualized by either autoradiography, silver staining or fluorescence, resulting in a unique reproducible fingerprint for each individual.

Possible reasons for AFLP-Polymorphisms are (i) sequence variations in a restriction site (the same as in RFLPs), (ii) insertions or deletions within an amplified fragment (also the same as in RFLPs) and (iii) differences in the nucleotide sequence immediately adjoining the restriction site (not detected with RFLPs). Thus, the usage of AFLP technologies results in the detection of higher levels of polymorphisms compared with RFLPs. AFLPs also have a much higher multiplex ratio (more markers per experiment) and better reproducibility than RAPDs. However, AFLPs require greater technical skill and as they require the use of polyacrylamide gels for detection, also larger investments in equipment. In the whole, AFLP markers allow the rapid generation of highly replicable markers, thus permitting high-resolution genotyping of fingerprinting quality. A drawback can be that most AFLP markers are dominant rather than co-dominant, due to the complex banding patterns. In some cases the scoring of AFLP polymorphisms as co-dominant marker loci is possible, because, for a single character, diploid homozygous individuals cause a more intense peak than heterozygous individuals. Specialized algorithms and software packages that are capable of finding such markers and scoring them co-dominantly have been developed (MEUDT & CLARKE 2007). Besides the literature covering AFLP marker development since 1995 (VOS et al. 1995; MUELLER & WOLFENBARGER 1999; WEISING et al. 2005; EDWARDS & MCCOUCH 2007), a review by MEUDT & CLARKE (2007) also discusses

[1] KeyGene N.V., Wageningen/The Netherlands is holder of the trademark AFLP®, included in the trademark are the AFLP process/method and reagents.

new directions for the AFLP technique in the genomic era.

1.2.4 Microsatellite markers

Microsatellites are also known as simple sequence repeats (SSRs) and the resulting markers are variously called simple sequence length polymorphisms (SSLPs), sequence-tagged microsatellite sites (STMS), SSR markers or microsatellite markers. SSRs are DNA stretches, consisting of tandemly repeated short nucleotide units (1-5 bases per unit). Such repeats are distributed throughout the genomes of all eucaryotic species. In microsatellite analysis, sequence information of the regions flanking the repeats is used for creating locus specific PCR primer pairs. The resulting amplification products are separated on polyacrylamide gels and visualized. The differences in the numbers of repeated units cause differences in band size, which are locus-specific, co-dominantly inherited and highly polymorphic. The technique reveals allele size differences even of a single base pair. A further advantage is the fact, that microsatellite markers can easily be distributed between labs by sharing primer sequences (POWELL et al. 1996; NYBOM 2004; WEISING et al. 2005). For reviews of microsatellite marker techniques see ELLEGREN (2004), GUPTA & VARSHNEY (2000), LI et al.(2002), for reviews of microsatellite marker development see MCCOUCH et al. (1997), ZANE et al. (2002) and WEISING et al. (2005).

Since expressed sequence tag (EST) (for details on EST see chapter 2.1) sequencing projects have provided sequence data that is available in online databases and can be scanned for identification of SSRs, so called EST-SSRs or genic microsatellites have been developed. Genic SSRs are quickly obtained by electronic sorting and have an expected high transferability because the primers are derived from conserved coding regions of the genome. This makes genic microsatellites a useful tool in characterization of genetic variation within natural populations or between breeding lines. Especially because the variation in transcribed genes with known function can be assessed, genic microsatellites are expected to enhance the role of genetic markers in evaluating germplasm (VARSHNEY et al. 2005b).

1.2.5 Single nucleotide polymorphism (SNP) markers

SNP markers are based on sequence differences at single-base pair positions in genomes. Single nucleotide exchanges in genomes are numerous; therefore SNP markers provide a great marker density. Another important advantage of SNP is that it is not a gel-based technology. For the large-scale genotyping required in marker-

assisted breeding programs, technologies based on gel electrophoresis are often too labor intensive and time consuming. The high density of SNP markers increases the probability to find polymorphisms in a target gene (see also chapter 2), which provides a huge advantage over previous markers that are at best closely linked to a locus of interest and not within. In the case of linkage it can easily happen that a linkage is lost when a marker is applied to other populations with different recombination patterns (XU & CROUCH 2008).

Although the use of SNP markers in plants is still in its infancy, SNP markers are expected to become the marker system of choice in the near future, especially as the full sequences of more plant genomes will become available (GANAL et al. 2009).

There are several methods of SNP discovery, either following the database approach, where SNPs are detected by mining sequence databases, or following the experimental approach, where genome regions of interest are screened for SNPs with one of numerous techniques developed for the detection of SNPs. In principle, the SNP methods reveal differences between a probe of known sequence and a target DNA containing the SNP site. The target DNA sections are mostly PCR products and mismatches with the probe reveal SNPs within the amplified target DNA segment. The mismatching DNA segments can be sequenced then as the most direct way to identify SNP polymorphisms (GUPTA et al. 2001; RAFALSKI 2002; WEISING et al. 2005). For a detailed review of SNP genotyping methods see KWOK (2001), SNP identification in crop plants has recently been reviewed by GANAL et al.(2009).

1.2.6 Other marker systems

Other marker systems that are frequently used are (i) SCAR markers (sequence characterized amplified regions), which are derived from RAPD markers and can be converted into co-dominant markers (PARAN & MICHELMORE 1993), (ii) CAPS markers (cleaved amplified polymorphic sequences), which are created by digesting PCR products with restriction enzymes, (iii) ISSR (inter-simple sequence repeat) markers, based on the amplification of inter-repeat sequences using PCR primers specific to microsatellites, (iv) and PCR-based STS (sequence tagged site) markers, derived from RFLP markers. For a recent review on molecular marker types, covering all types listed here see JONES et al. (2009).

1.3 QTL-identification

A QTL (Quantitative Trait Locus) is a chromosomal region supposed to contain a gene or genes that contribute to a quantitative trait. In QTL mapping experiments the genetic basis of quantitative traits is dissected into their single components. Many traits of agricultural importance are quantitative, i.e. based on polygenes. As environmental influences can have a considerable effect on the expression of these traits, DNA markers can have a great impact in breeding for such traits, because selection for quantitative traits normally requires large scale testing in various environments.

A benchmark article by LANDER & BOTSTEIN (1989), describing a set of analytical methods for mapping QTLs with the help of molecular markers, led to increased interest in locating QTLs, and some authors recognize the dissection of quantitative genetic variation into genes at the molecular level as the greatest challenge geneticists are facing in the twenty-first century (LUO et al. 2002).

The approximate numbers and locations of the QTLs underlying polygenic phenotypes can be estimated by experimental mapping approaches. Numerous molecular markers, scattered throughout the genome, are required for such QTL mapping approaches. In order to map QTLs, two homozygous inbred lines, differing in many phenotypic characteristics, are crossed to produce an F_1 progeny. The uniformly heterozygous F_1 is backcrossed to one or the other parental line resulting in a segregating mapping population, in which it can be monitored whether certain markers tend to co-segregate with specific phenotypes of interest that distinguish the parental lines.

If co-segregation between a marker of known chromosomal location and a phenotype is observed, it is assumed that the genes contributing to the phenotype and the marker must be closely linked (AVISE 2004). In this way it is possible to construct a genetic map, showing the position of QTLs for a certain trait on the different chromosomes. After this first step, QTL analysis can be applied to plant breeding and knowledge of QTL map locations is utilized for selection of improved varieties.

Even if some analyses can locate the QTLs associated with certain phenotypic traits with high precision (LUO et al. 2002), many QTL map localizations are not satisfactory concerning accuracy and resolution of the mapping results (KEARSEY & FARQUHAR 1998). In addition, not every QTL is of the same importance for a polygenic trait, requiring the identification of the more important QTLs. The lack of ability to detect the right number of QTLs affecting a trait is considered a major shortcoming of QTL

studies (TUBEROSA & SALVI 2004). Thus, HOLLAND (2004) lists several hindrances to the adoption of MAS as a general breeding strategy for polygenic traits, including (i) problems in accurately localizing and estimating the effects of the QTL, (ii) the difficulty of improving the already substantial gains from selection when heritability is high, (iii) inability to infer QTL effects from one breeding cross to another, and (iv) difficulty in integrating QTL mapping procedures into efficient breeding methods.

Many QTL mapping approaches assume that the desirable QTL alleles, once identified, will remain relevant during many cycles of selection, without interactions with other genes or the environment occurring (PODLICH et al. 2004). However, disbelief in this has led BERNARDO (2001) to the statement that MAS has "limited potential for enhancing selection for quantitative traits in hybrid crops". This is a reasonable objection, considering that QTLs identified in one population do not necessarily show the same effects when introgressed into other genetic backgrounds (CONCIBIDO et al. 2003), and that even in constant genetic backgrounds and testing environments QTLs do not necessarily behave as expected (BOUCHEZ et al. 2002). Nevertheless, YOUSEF & JUVIK (2002) showed that QTLs identified in a mapping population can very well exert the same effects in different genetic backgrounds and across two environments. By introgressing three marker-QTL alleles associated with enhanced seedling emergence into elite lines utilizing marker-assisted backcrossing (MABC), this trait was successfully enhanced in sweet corn.

In recent years it has become more and more accepted that QTL mapping alone is not directly useful in MAS. However, QTL confirmation, validation, and/or fine mapping are inevitable (COLLARD & MACKILL 2008). In 2004, PODLICH et al. suggested the "Mapping As You Go" (MAYG) approach, to overcome the problem of inaccurate estimation of QTLs and their effects. MAYG is a mapping-MAS strategy that accounts for the presence of epistasis and genotype by environment (GxE) interactions. QTL allele effects are continuously revised by remapping new elite germplasm generated over cycles of selection. In this way QTL estimates remain relevant to the current set of germplasm in the breeding program. The effectiveness of the MAYG approach has been investigated through simulation.

An approach beyond QTL mapping is connecting genetic components of the QTL variations to genomic databases. The use of near isogenic lines (NILs), in which a single QTL region can be isolated, can transform the task of QTL cloning into one similar to that performed for simple Mendelian traits. PARAN & ZAMIR (2003) expect

that the sequencing of plant genomes, the increasing availability of markers, and improvements in genotyping and phenotyping technologies will enhance map-based cloning of QTLs in the future.

Association studies with candidate genes can also be used to isolate QTLs without necessarily requiring detailed linkage information (see also chapter 2.2).

1.3.1 Advanced backcross AB-QTL analysis

QTL studies using populations which carry alleles of both parents at relatively high frequency (e.g. F2, BC1) are well suited for QTL mapping, but have some drawbacks when it comes to detecting and transferring useful QTLs from unadapted germplasm into elite breeding lines. Undesirable QTL alleles from the unadapted parent occur in high frequency and epistatic interactions are likely to occur, because donor alleles are present at a high frequency. TANKSLEY & NELSON (1996) proposed a method for simultaneously discovering valuable QTLs from unadapted germplasm (e.g. land races, wild species) and transferring them into elite breeding lines. The method is named advanced backcross QTL analysis (AB-QTL) and delays QTL analysis until the BC_2 or BC_3 generation. In BC_1 negative selection is conducted to reduce deleterious donor alleles, while in BC_2 and BC_3 populations are evaluated for traits of interest and genotyped using molecular markers. In this way the identification of QTL happens while these QTLs are transferred into an adapted genetic background. The AB-QTL method can be employed to exploit unadapted germplasm for the quantitative trait improvement of crop plants and has been applied successfully in several crop species, e.g. barley (*Hordeum vulgare* L.) (YUN et al. 2006), maize (*Zea mays*) (HO et al. 2002; MANO & OMORI 2008), rice (*Oryza sativa*) (XIAO et al. 1998; MONCADA et al. 2001), tomato (*Lycopersicon esculentum*) (TANKSLEY et al. 1996; BERNACCHI et al. 1998a; BERNACCHI et al. 1998b) and wheat (*Triticum aestivum* L.) (NARASIMHAMOORTHY et al. 2006).

2 Genomic selection

In the last decade some scientific milestones, including genome sequencing projects, EST databases (for details see 2.1), and microarray technologies, have enhanced the understanding of plant genomes and allowed for the identification of genes responsible for a desired trait (BRADY & PROVART 2007). Besides using random markers derived from anonymous polymorphic sites in the genome, it has become possible to generate

'functional markers', derived from polymorphisms within the transcribed regions of the genome (ANDERSEN & LÜBBERSTEDT 2003). Such markers are completely linked to the desired trait allele and have also been termed 'perfect markers' (VARSHNEY et al. 2005a). The main limitation of applying random, 'non-perfect' DNA markers such as RFLPs, AFLPs or microsatellite markers is the limited number of detectable polymorphisms, low throughput and high costs of assaying each locus. The development of SNPs allows higher throughput but still marker development and PCR reactions are required (HAZEN & KAY 2003).

In 2001, MEUWISSEN et al. proposed genomic selection as a form of MAS which simultaneously estimates all locus, haplotype, or marker effects across the entire genome. Instead of using a subset of significant markers for selection, all markers of a population are analyzed in order to explain the total genetic variance with dense genome wide markers. Marker effects are summed up to predict breeding values of individuals. Therefore VARSHNEY et al. (2005) suggested that marker-assisted breeding and selection will gradually evolve into "genomics-assisted breeding". In other publications the term "genomic selection" is used (HEFFNER et al. 2009).

Up to now genomic selection has almost exclusively been tested through simulation, thus work remains to be done to validate it empirically and to incorporate it into breeding schemes (HEFFNER et al. 2009). Nevertheless, because of the inadequacy of MAS for improving polygenic traits controlled by many loci of small effects, HEFFNER et al. (2009) propose the application of genomic selection as a solution to the two most important limitations of MAS, which are (i) the biparental mapping populations used in most QTL studies which do not allow a direct translation of results for breeding purposes and (ii) the statistical methods used to identify target loci. Currently, array mapping, association mapping and ecotilling are often discussed methodologies within the context of genomics-assisted breeding. Because of their novelty they are described in this chapter in greater detail than the molecular markers mentioned before.

2.1 Array mapping

With the completion of the genomic sequence of *Arabidopsis thaliana* the first plant genome was deciphered and plant genomics moved on to the era of functional genomics. The mere sequence of a genome is of limited value in revealing the function of genes. Gene expression needs to be studied in the next step and DNA microarrays have become the main technological approach to expression studies. Microarrays (also

known as biochips, DNA chips, and gene chips) were developed by SCHENA (1995) and co-workers in the early 1990s.

There are several ways in which genes can be arrayed, the two most common technologies being cDNA arrays and oligonucleotide arrays. To conduct an oligonucleotide array, oligonucleotides are synthesized *in situ* for setting up the array, requiring knowledge of sequence data. cDNA arrays are also applicable to nonmodel organisms, as they only require a large cDNA library and the development of ESTs (expressed sequence tags). ESTs are end segments of sequences from cDNA clones that correspond to mRNA (ADAMS et al. 1991) i.e. parts of expressed genes. To conduct a cDNA array, several thousand ESTs are needed. A unique set of these ESTs is amplified by PCR and used to conduct the array (GIBSON 2002).

No matter if cDNA arrays or oligonucleotide arrays are used, the basic steps are the following: (i) mRNA from cells or tissues in a sample is extracted, (ii) transcribed into cDNA or cRNA and fluorescence labeled, (iii) hybridized with the array by robotically spotting the probe onto a planar surface (often glass microscope slide or filter). Labeled cDNA/cRNA pieces bind to their complementary counterpart on the array. (iv) A laser scanner is used to measure the fluorescent signal of the hybridized probes. As the intensity of the signals from the samples correlates with the original concentration of mRNA in the cell/tissue, it can be estimated whether the expression of a gene is up- or down regulated, absent or unchanged (BARRETT & KAWASAKI 2003).

Besides RNA expression profiling, microarrays offer opportunities for DNA polymorphism analysis and have been found useful in linkage mapping, the dissection of QTLs or assessment of population structure (HAZEN & KAY 2003).

WINZELER et al. (1998) developed a method to uncover the allelic variation between two strains of yeast (*Saccharomyces cerevisiae*) by hybridizing labeled genomic DNA to a high-density oligonucleotide array designed for measuring gene expression. Fragments matching the array feature sequence perfectly will hybridize with a higher affinity than a fragment mismatching the sequence and thus every array oligonucleotide has the potential to measure a polymorphism (HAZEN & KAY 2003). The sequence polymorphisms detected as a difference in hybridization intensity between two samples function as molecular markers and are referred to as **single feature polymorphisms (SFPs)** (BOREVITZ et al. 2003). Microarrays can detect high numbers of SFP markers and as several hundred thousand loci can be measured in a

single experiment, all markers can be scored simultaneously, thus allowing the mapping of quantitative or multigenic trait loci. No amplification steps, gels or enzymatic manipulation are required to carry out a microarray (WINZELER et al. 1998) which makes such high-density oligonucleotide arrays an effective platform for identifying allelic variation (HAZEN et al. 2005).

WOLYN et al. (2004) developed a method called **eXtreme array mapping (XAM)** that combines array hybridization with bulked segregant analysis in order to map QTLs, hoping for a way to reduce time and effort needed to genotype and map QTL loci. Bulked segregant analysis (BSA) is a method to identify markers in specific regions of the genome. From a segregating population originating from a single cross, two bulked DNA samples are generated. Within each bulk the individuals are identical for the trait/gene of interest but arbitrary for all other genes. Ideally, the two samples differ genetically only in the selected region and are expected to have equal mixtures of both parental genotypes at loci unlinked to the mutation. The chromosomal region linked to the gene causing the phenotype will be fixed for alternative alleles between the two pools (MICHELMORE et al. 1991; HAZEN et al. 2005; BRADY & PROVART 2007). BSA has the advantage of identifying markers associated with a trait without needing the construction of a full genetic map. BSA is widely used in many marker development programs (LANGRIDGE & CHALMERS 2005). One possibility in BSA is to hybridize DNA from each pool to a microarray. In this way SFPs can be identified, indicating a genomic region of interest containing alleles that can be tested before introgression into elite germplasm (BRADY & PROVART 2007).

Another application of the microarray technology to the analysis of DNA variation is the **Diversity Array Technology (DArT™).** Using DArT, the presence and amount of a specific DNA fragment can be assessed in the total genomic DNA of an organism or a population. DArT does not rely on DNA sequence information and potential applications include germplasm characterization, genetic mapping, gene tagging or MAS (JACCOUD et al. 2001). In terms of cost and speed of marker discovery/analysis DArT can be a good alternative to other marker techniques such as RFLP, AFLP, microsatellite markers or SNP (DWIVEDI et al. 2007).

The major advantage of microarrays is the fact that gene expression patterns for a large number of genes or even a whole genome can be obtained in one experiment. As the elements placed on the chip are only between 20-200 µm in diameter and only spaced 50 µm apart, a whole genome complement can be placed on one chip (BARRETT &

KAWASAKI 2003). If mRNA is hybridized to a microarray probe, only a measure of steady-state RNA can be achieved. As not all biological processes will be regulated at this level of gene expression (RICHMOND & SOMERVILLE 2000) this can be overcome by hybridizing labeled total genomic DNA to a microarray probe (HAZEN & KAY 2003).

Overall, the costs for performing array mapping can be high. Current arrays cost approximately $400 per sample (BOREVITZ & ECKER 2004; http://www.ohsu.edu/gmsr/amc/amc_price.html) [14 July 2009]. Considering that no other method can detect so many polymorphisms so quickly, costs per marker are comparably low, however (HAZEN & KAY 2003).

At present, complete DNA sequences of only a few plants are available. However, it is presumed that meaningful SFPs could be detected by hybridizing related species to one and the same array (HAZEN & KAY 2003).

2.2 Association mapping

The aim of association mapping is to find statistical associations between markers and quantitative traits. In contrast to QTL mapping, where mapping is performed in the context of pedigrees, association mapping refers to associations within populations of unrelated individuals, identifying population-wide marker-phenotype associations on the basis of previously unrecorded sources of disequilibrium (FLINT-GARCIA et al. 2003a). In association mapping the genetic markers usually lie within candidate genes and association mapping relies on linkage disequilibrium (LD) between the candidate gene markers and the causal polymorphisms in the gene. This means that association mapping has, besides allowing for the identification and mapping of QTLs, the potential to identify polymorphisms within genes that are responsible for phenotypic differences (GUPTA et al. 2005).

Because it refers to linkage disequilibrium, association mapping is also known as LD mapping. LD is the "nonrandom association of alleles at different loci" (FLINT-GARCIA et al. 2003a). This is not to be mistaken for linkage. While linkage describes the association of two or more loci on a chromosome with limited recombination, linkage disequilibrium describes that some combinations of alleles occur more or less frequently in a population than it would be expected if the association of alleles was random. This can be due to linkage, selection, migration, or drift.

Association mapping was first used to identify natural variation in genes responsible

for human diseases but seems to be a powerful tool for identifying QTLs in plants too (FLINT-GARCIA et al. 2003a). The current major uses of association mapping in plants are (i) the detection of marker-trait associations in natural populations and subsequent marker-assisted selection and (ii) studies of genetic diversity in natural populations and studies of population genetics (GUPTA et al. 2005). Association mapping was successfully applied in several plant association studies including growth habit and bolting in sea beet (*Beta vulgaris* ssp. *maritima*) (HANSEN et al. 2001), flowering time in maize (THORNSBERRY et al. 2001), carotenoid content in maize (PALAISA et al. 2003), kernel composition in maize (WILSON et al. 2004), flowering time and pathogen resistance genes in *Arabidopsis* (ARANZANA et al. 2005), and kernel size in wheat (*Triticum aestivum* L.) (BRESEGHELLO & SORRELLS 2006). For a comprehensive list see the review by GUPTA et al. (2005).

Association mapping provides the advantages that currently existing populations can be used instead of creating mapping populations, that a large number of alleles can be surveyed per locus simultaneously and that resolution can be increased (FLINT-GARCIA et al. 2005).

The similarity of the plant genomes can be measured using linkage to various molecular markers, either SFPs or more traditional markers such as AFLPs or microsatellite markers (BRADY & PROVART 2007). Difficulties can arise in association mapping due to population structure (PRITCHARD et al. 2000). Genetically heterogeneous populations can cause spurious associations, thus constraining the use of association studies. However, a mixed-model approach was developed by YU et al. (2006) allowing the separation of true signals of functional associations from false signals generated by population structure. According to XU & CROUCH (2008) "genome-wide LD-based association mapping may provide a shortcut to discovering functional alleles and allelic variations that contribute to agronomic traits of interest". MACKAY & POWELL (2007) express the expectation that the successful use of association mapping will lead to "more efficient marker-assisted selection, facilitate gene discovery and help to meet the challenge of connecting sequence diversity with heritable phenotypic differences".

2.3 EcoTILLING

EcoTILLING is based on the methodology of TILLING (Targeting Induced Local Lesions IN Genomes), which was developed as a strategy in reverse genetics

(MCCALLUM et al. 2000a; MCCALLUM et al. 2000b). TILLING is a methodology that identifies DNA polymorphisms regardless of phenotypic consequence (TILL et al. 2003), allows the identification of single-base-pair allelic variation in target genes and can be applied to any organism that can be chemically mutagenized (MCCALLUM et al. 2000b). It is, on the one hand, an attractive strategy for functional genomics and, on the other hand, also attractive for agricultural applications. TILLING requires relatively few individual plants and is therefore appropriate for small- and large-scale screening.

In TILLING, traditional chemical mutagenesis is followed by PCR-based screening to identify point mutations in regions of interest. First, the regions of interest are amplified by PCR. By denaturing and reannealing the PCR products, heteroduplex molecules between wild-type fragments and mutated fragments form (MCCALLUM et al. 2000a), provided that at least one plant in the pool includes a mutation in the amplified region. The resultant double-stranded products are digested by CEL I, an endonuclease that specifically targets and digests heteroduplexes at mismatch positions (TILL et al. 2004). The cleaved products are resolved on denaturing polyacrylamide gels, individuals carrying a mutation in the gene of interest are identified and the mutant PCR product is sequenced (GILCHRIST & HAUGHN 2005). The TILLING methodology has been adapted to the discovery of polymorphisms in natural populations, termed **EcoTILLING** by COMAI et al. (2004). The cutting with CEL I allows the display of multiple mismatches in a DNA duplex. If an unknown homologous DNA is heteroduplexed to a known sequence, the number and position of polymorphisms can be revealed and the approximate position of each SNP within a few nucleotides is recorded (HENIKOFF et al. 2004).

EcoTILLING is applicable to any species, including heterozygous and polyploid ones. It often compares favorably to full sequencing because it reduces the number of sequences that need to be determined in order to identify a point mutation in a gene of interest. According to GILCHRIST & HAUGHN (2005), TILLING/ecoTILLING remains at the moment “the technique of choice for medium-to-high throughput reverse genetics in many organisms”. It has been applied e.g. to identify point mutations and deletions in mutants of the powdery mildew resistance gene *mlo* of barley (MEJLHEDE et al. 2006).

EcoTILLING is gel based and thereby a low cost method. As a marker system it combines two advantages. Being based on the gene of interest itself, it has the

advantage of a functional marker, and it produces a high number of marker alleles because every SNP in the amplified sequence results in a change in the overall fragment pattern (MEJLHEDE et al. 2006).

Currently, ecoTILLING and microarrays as two methods for natural polymorphism discovery, seem to be two complementary tools (COMAI et al. 2004). While microarrays have their strength in the detection of global natural polymorphisms among a few genotypes, ecoTILLING is better suited for surveying diversity at specific loci among many genotypes.

In general, it can be expected that developments in marker technologies during the next few years will go along with the development of sequencing technologies. The new generation of sequencing technologies, called next-generation sequencing or pyrosequencing, that has become available during the last few years permits the rapid production of sequence information and it can be expected that sequence information of many different crop plants will become available soon. Already now the 454 sequencing technology allows the identification of hundreds of microsatellite markers in any species (SANTANA et al. 2009).

3 Advantages of marker-assisted breeding

The use of DNA markers for screening and selecting of plants in a breeding program provides several advantages and is therefore very attractive to plant breeders.

(i) As DNA marker based genotypes can be obtained from almost any plant tissue, plants can be screened already at the seedling stage or even as seeds, thus allowing early selection for traits which may be expressed in adult plants only (i.e. grain or fruit quality, male sterility, photoperiod sensitivity). With the availability of preflowering genotypic information MAS allows for controlled pollination, e.g. in marker-assisted recurrent selection.

(ii) Target alleles that are difficult, expensive and/or time consuming to score phenotypically can be selected with the assistance of markers (e.g. environmentally sensitive traits, as DNA markers are mostly neutral to environmental variation).

(iii) Selections can be made on a single plant basis where this would not be possible by phenotypic selection. Poor heritability does not pose a problem if selection is based on marker information.

(iv) For traits with complex inheritance every individual genetic component

contributing to the trait can be selected separately. Also, multiple characters that would normally be epistatic (i.e. they show a certain positive or negative effect only in combination with each other) can be maintained and ultimately fixed.

(v) Recessive genes can be maintained without the need for progeny tests in each generation, as homozygous and heterozygous plants can be distinguished with the aid of (co-dominant) markers. In backcrossing, DNA markers can help to minimize linkage drag around the target gene and reduce the generations required to recover a recurrent parent's genetic background (see also chapter 4.1.2).

(vi) In the choice of parents in crossing programs markers can be applied too. Here they can either help to maximize diversity, and in this way support the exploitation of heterosis, or they can minimize diversity, if gene complexes built up in elite inbred germplasm are to be preserved (YOUNG 1999; KOEBNER & SUMMERS 2003; KOEBNER 2004; EDWARDS & MCCOUCH 2007; JENA & MACKILL 2008).

3.1 MAS versus phenotypic selection

MAS will probably never replace phenotypic selection (PS) entirely. Especially for disease resistances a final testing of breeding lines is always required, regardless how tight a marker is linked to a gene or QTL (YU et al. 2000). For RIBAUT & RAGOT (2006), working with improvement of drought adaptation in maize, it is no doubt that the collection and use of very high quality phenotypic data are critical for the application of MAS. DEKKERS & HOSPITAL (2002) come to the conclusion that it is "risky to carry out selection solely on the basis of marker effects, without confirming the estimated effects by phenotypic evaluation" and KOEBNER & SUMMERS (2003) claim for wheat breeding "that 'laboratory-based breeding' should remain the servant of the field breeder and not its master". According to them, large-scale MAS application could lead to an approach in breeding in which major breeding targets are attained by a single gene approach, thus possibly loosing the holistic advances that have been achieved by the PS of minor genes. The resulting varieties could become vulnerable to future changes in production systems, climate, or end use. Overall, relying only on MAS and thus excluding other potentially useful genes will rarely be the recommended approach in molecular breeding programs, and most programs involve at least one or two cycles of phenotypic evaluation during the breeding process (DWIVEDI et al. 2007).

There is no general pattern by which it can be predicted whether MAS or PS will be

more useful. Empirical comparisons of MAS and PS for increasing gain from selection have been made in several studies. The outcomes of these studies are conflicting. In some studies MAS is reported to be more effective/efficient than PS (e.g. YOUSEF & JUVIK 2001; ABALO et al. 2009) while other studies considered the two methods equal (e.g. VAN BERLOO & STAM 1999; WILLCOX et al. 2002; HOECK et al. 2003; MOREAU et al. 2004). In a third group of studies PS proved to be more effective/efficient than MAS (e.g. DAVIES et al. 2006; WILDE et al. 2007) and in other comparisons the effectiveness/efficiency of MAS and PS varied within the same study, depending on the populations or on the trait selected for (e.g. FLINT-GARCIA et al. 2003b; ROBBINS & STAUB 2009).

Comparing gains from MAS and phenotypic selection for several quantitative traits in sweet corn, YOUSEF & JUVIK (2001) found that in 20 out of 52 (38 %) of the MAS versus PS comparisons, MAS led to significantly higher gains. Success of PS selection was significantly greater than that of MAS in only 4 % of the comparisons. On average, selection gain from MAS was 10.9 % and only 6.1 % from PS. In the study it is stated that MAS is most appropriate when traits are difficult and costly to measure. In such cases the higher gain from MAS compensates for the higher costs.

In selecting for resistance to southwestern corn borer (*Diatraea grandiosella*) in maize, PS and MAS were equally successful (WILLCOX et al. 2002); the same was true for selecting high grain protein content in spring wheat across six environments (DAVIES et al. 2006). Also, in comparing MAS and PS for *Fusarium* head blight (FHB) resistance and Deoxynivalenol content in spring wheat, both strategies were successful (WILDE et al. 2007) while it depended on the trait selected for if PS or MAS was more effective when MAS and PS were compared for yield components in cucumber (ROBBINS & STAUB 2009).

4 Application of markers in breeding programs

The two major applications of DNA markers in plant sciences are detailed chromosome mapping and selection and introgression of both simple and quantitative traits. In addition, DNA markers are also used for germplasm evaluation, genetic diagnostics, phylogenetic analysis, study of genome organization and screening of transformants (GUPTA et al. 1999). Molecular marker maps have been constructed for a wide range of crops (see www.ncbi.nlm.nih.gov/genomes/PLANTS/PlantList.html) [16 June 2009].

In this review chromosome mapping will not be covered; the focus is on the selection purposes of DNA markers. Also, not all crops can be covered in this review, thus the focus is restricted to marker application in the main agricultural crops grown in temperate zones, mainly wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare*), potato (*Solanum tuberosum*), maize (*Zea mays*), fruits and vegetables, particularly tomato (*Solanum lycopersicum*), complemented with some examples from rice (*Oryza sativa*) and soybean (*Glycine max*).

While marker application has become an important tool in some areas of plant breeding and crop improvement, e.g. hybrid breeding or pyramiding of resistances, it is not or hardly ever being applied for some other objectives, such as breeding of minor crops or yield improvement (see also chapter 4.4.2 and 6). The areas where marker-assisted selection is considered a tool which is applied at least to a few crop species will be discussed here in more detail and examples for MAS applied in the various crops are summarized in chapter 4.4.2.

4.1 Breeding strategies in marker-assisted breeding

The breeding strategies for which MAS is used most frequently, are selection of simple traits or QTLs from breeding lines/populations, introgression of genes from breeding lines or wild relatives, MABC, marker-assisted recurrent selection (MARS), and pyramiding of genes.

4.1.1 Selection from breeding lines/populations

Markers are used for selecting qualitative as well as quantitative traits. MAS can aid selecting for all target alleles that are difficult to assay phenotypically. Especially in early generations, where breeders usually restrict their selection activities to highly heritable traits because a visual selection for complex traits like yield is not possible with only few plants per plot being available, MAS is said to be effective, cost- and time-saving. To improve early generation selection, markers should decrease the number of plants retained due to their early generation performance, and at the same time they should ensure a high probability of retaining superior lines (EATHINGTON et al. 1997). An important prerequisite for successful early-generation selection with MAS are large populations and low heritability of the selected traits, as under individual selection, the relative efficiency of MAS is greatest for characters with low heritability (LANDE & THOMPSON 1990). Results from KUCHEL (2007) and BONNETT

et al. (2005) show that maximum gain can be achieved at lowest cost in marker-assisted wheat breeding when molecular markers, closely linked to target genes, are utilized to enrich target loci within segregating populations in early generations. In the South Australian Barley Improvement Program (SABIP), where every year over 15,000 new F_3 derived lines are sown, it is the aim to screen these for a wide range of traits in the future in order to reduce the number of lines. However, in 2000 BARR et al. stated that, "this is fantasy for public sector breeders, as (…) MAS can only be used in early generation screening for very important material", the main limitations being costs, availability of suitable markers, and staff resources for sample and data handling.

Markers are also frequently used to select parents with desirable genes and gene combinations, and MARS schemes involve several successive generations of crossing individuals based on their genotypes. The achievable genetic gain through MARS is probably higher than that achievable through MABC (RIBAUT & RAGOT 2006).

4.1.2 Marker-assisted backcrossing (MABC)

Backcrossing is used in plant breeding to transfer (introgress) favorable traits from a donor plant into an elite genotype (recurrent parent). In repeated crossings the original cross is backcrossed with the recurrent parent until most of the genes stemming from the donor are eliminated (BECKER 1993). However, the donor segments attached to the target allele can remain relatively large, even after many backcrossing generations. In order to minimize this linkage drag, marker assays can be of advantage (FRISCH et al. 1999). Markers can be used in the context of MABC to either control the target gene (foreground selection) or to accelerate the reconstruction of the recurrent parent genotype (background selection). According to TANKSLEY et al. (1989), in traditional backcross breeding the reconstruction of the recurrent parent genotype requires more than six generations, while this may be reduced to only three generations in MABC. These findings are confirmed by results of FRISCH et al. (1999), who showed in a computer simulation that MAS can reconstruct a level of recurrent parent genome in BC_3 which would only be reached in BC_7 without the use of markers. However, the authors also state that large numbers of marker data points are required to achieve such results.

Regarding maize MABC is certainly the form of MAS with the most immediate and obvious benefits (RAGOT & LEE 2007). However, it has to be taken into account that

backcrossing is a very conservative breeding strategy and should not become the prime focus of a breeding program (BARR et al. 2000), as it does hardly ever broaden the genetic basis of plants in a substantial way. At the moment MABC also is and will probably remain the preferred means of backcrossing transgenes into elite inbred lines, which is also considerably contributing to its popularity (RAGOT & LEE 2007).

MABC is especially efficient if a single allele is to be transferred into a different genetic background, for example, in order to improve an existing variety for a specific trait. However, if the performance of a plant is determined by a complex genotype it is unlikely that this ideal genotype will be attained through MABC only (RIBAUT & RAGOT 2006). To overcome the limitation of only being able to improve existing elite genotypes, other approaches like marker-assisted recurrent selection (MARS) have to be considered (see following chapter for details).

4.1.3 Marker-assisted recurrent selection (MARS)

The improvement of complex traits via phenotypic recurrent selection is generally possible, but the long selection cycles impose restrictions on the practicability of this breeding method.

With the use of markers, recurrent selection can be accelerated considerably. In continuous nursery programs preflowering genotypic information is used for marker-assisted selection and controlled pollination. Thus, several selection-cycles are possible within one year, accumulating favorable QTL alleles in the breeding population (EATHINGTON et al. 2007).

Additionally, it is possible today to define an ideal genotype as a pattern of QTLs, all QTLs carrying favorable alleles from various parents. If individuals are crossed based on their molecular marker genotypes, it might be possible to get close to the ideal genotype after several successive generations of crossings. It is likely that through such a MARS breeding scheme higher genetic gain will be achieved than through MABC (RIBAUT & RAGOT 2006). Concepts how to achieve the ideal genotype using multitrait selection indices have been developed (PELEMAN & VAN DER VOORT 2003). Applications in practical plant breeding are not known to the authors.

4.1.4 Pyramiding

Using MAS, several genes can be combined into a single genotype. Pyramiding is also possible through conventional breeding but phenotypically testing individual plants for

all traits can be time-consuming and sometimes very difficult. The most frequent strategy of pyramiding is combining multiple resistance genes. Different resistance genes can be combined in order to develop broad-spectrum resistance to, e.g., diseases and insects. Either qualitative resistance genes can be combined or quantitative resistances controlled by QTLs. An example for the combination of resistance QTLs is the pyramiding of a major stripe rust resistance gene and two QTLs in the same genotype (CASTRO et al. 2003). In order to pyramid disease or pest resistance genes that have similar phenotypic effects, and for which the matching races are often not available, MAS might even be the only practical method – especially where one gene masks the presence of other genes (SANCHEZ et al. 2000; WALKER et al. 2002).

The Barley Yellow Mosaic Virus (BaYMV) complex as an example is a major threat to winter barley cultivation in Europe. As the disease is caused by various strains of BaYMV and Barley Mild Mosaic Virus (BaMMV), pyramiding resistance genes seems an intelligent strategy. However, phenotypic selection can not be carried out due to the lack of differentiating virus strains. Thus, MAS offers promising opportunities. Suitable strategies have been developed for pyramiding genes against the BaYMV complex. What has to be taken into account when applying such strategies in practical breeding is the fact that the pyramiding has to be repeated after each crossing, because the pyramided resistance genes are segregating in the progeny (WERNER et al. 2005).

4.2 Application of markers in germplasm storage, evaluation, and use

Marker-assisted germplasm evaluation is an important tool in the acquisition, storage and use of plant genetic resources (PGR) (BRETTING & WIDRLECHNER 1995) and the evaluation of germplasm can be considerably improved with the assistance of markers. Markers can be used prior to crossing to evaluate the breeding material. Also, mixing of seed samples can be discovered using markers instead of growing plants to maturity and assessing morphological characteristics (YASHITOLA et al. 2002). In order to broaden the genetic base of core breeding material, germplasm of diverse genetic background for crossings with elite cultivars can be identified with the assistance of markers (XU et al. 2004) and markers are on the whole a valuable tool for characterizing genetic resources, delivering detailed information usable in selecting parents. According to XU et al. (2003), molecular markers can be used for (i) differentiating cultivars and creating, maintaining, and improving heterotic groups; (ii) assessing collections and identifying germplasm redundancy, underrepresented alleles, and genetic gaps; (iii) monitoring genetic shifts that can occur during medium- or

long-term storage, regeneration, domestication, and breeding; (iv) identifying unique germplasm; and (v) constructing core collections.

4.2.1 Application of markers in germplasm storage

Almost 100 years ago VAVILOV (published in 1952) called attention to the potential of crop relatives for improving agriculture because he saw the potential of PGR respectively crop wild relatives (CWR) as a source of genes with great importance for practical plant breeding.

With an increased awareness of the threat, rapid loss of crop biodiversity posed to agricultural production and food security, the International Board for Plant Genetic Resources (IBPGR) was established in 1974. In 1991, the focus of the organization changed from the emergency conservation of genetic resources by storing resources in gene banks to research on how to conserve crop biodiversity through the sustainable use of genetic resources and the IBPGR became the International Plant Genetic Research Institute (IPGRI). In 2006, IPGRI and the International Network for the Improvement of Banana and Plantain (INIBAP) united and changed their name to Bioversity International. Bioversity International oversees efforts in plant collection and preservation worldwide and maintains a number of databases with information on more that 5 million accessions from germplasm collections worldwide, belonging to more than 20 000 species[2].

Considering the large number of accessions stored in gene banks it is obvious that only a limited number of accessions can be sampled in order to find new and useful genes. DNA profiles can help to determine and quantify the genetic uniqueness of each accession in a gene bank.

4.2.2 Application of markers in germplasm evaluation/characterization

The big challenge is the utilization of the genetic diversity stored in gene banks. In 1997, TANKSLEY & MCCOUCH stated that although "there is a general belief that genes useful for improving crops are contained in these seedbanks (…) the vast majority of the accessions in gene banks make no contribution to modern varieties, particularly with respect to complex traits such as yield and nutritional quality". They suggested a departure from "the old paradigm" of looking for the phenotype for the

[2] www.bioversityinternational.org [16 October 2009].

benefit of a “new paradigm” of looking for the genes.

Thus, the traditional approach of screening accessions from a gene bank for a clearly defined phenotypic character is only successful when breeding for simply inherited traits while beneficial alleles for complex traits like yield or quality traits can not be found through phenotypic evaluation alone (TANKSLEY & MCCOUCH 1997; LAMMERTS VAN BUEREN et al. 2010). Only a small proportion of the genetic variation contained in PGR can ever be exploited for crop improvement using phenotypic selection. Germplasm that is judged unsuitable for the improvement of varieties based on phenotypic examination, may nevertheless contain superior alleles that “lie buried amid the thousands of accessions maintained in gene banks” (XU et al. 2003). Many superior alleles will only be found with the help of molecular maps and QTL analysis.

Results from the application of the advanced backcross method to both tomato and rice shows that PGR contain favorable genes for the enhancement of complex traits like yield (XIAO et al. 1998; BERNACCHI et al. 1998a; BERNACCHI et al. 1998b). It is very unlikely that this genetic potential would have been identified without applying molecular mapping techniques like the AB-QTL method.

The genotypic evaluation of germplasm based on molecular markers (marker-assisted germplasm evaluation, MAGE) and/or QTL analysis can be used to identify and extract superior alleles from inferior germplasm. This complements phenotypic selection (XU et al. 2003).

The application of various molecular marker technologies to analyze diversity in PGR was reviewed by HODGKIN et al. (2001). For the effective management and use of germplasm resources it is critical to understand the range of diversity and the genetic structure of gene pools. Some germplasm accessions available for cultivated plants represent duplicate samples while others include rare alleles or very unusual allele combinations. Molecular markers can be used to screen germplasm collections for such redundancies or underrepresented alleles, they can help to discover genetic gaps in current collections, to monitor genetic shifts that occur during germplasm storage, regeneration, domestication, and breeding, or they can be used to construct representative subsets or core collections (a core collection contains most of the entire collection’s diversity with minimal redundancy) (XU et al. 2003). Examples of use of molecular markers to identify redundancies in germplasm collections include perennial kales (ZEVEN et al. 1998), wheat (CAO et al. 1998), barley (LUND et al. 2003), and carrot (LE CLERC et al. 2005a).

4.2.3 Application of markers in germplasm use/introgression

Crop wild relatives, including the progenitors of crops and species related to them, provide plant breeders with a pool of potentially useful genetic resources. The use of wild germplasm in crop improvement gained in prominence by the 1970s and 1980s (HAJJAR & HODGKIN 2007), when wild relatives of crops were used for breeding in a growing number of crop species.

The advancements in the field of genomics have considerably contributed to increase the use of wild relative genes, as they allow for the isolation of beneficial genes, the selection for traits which are difficult to detect based on phenotype, or the screening of whole collections of wild relatives. MAS has increasingly been applied for the maintenance of recessive alleles in backcrossing pedigrees and for pyramiding resistance genes (HAJJAR & HODGKIN 2007). A survey of the introduction of genes from crop wild relatives into cultivars (HAJJAR & HODGKIN 2007), reports on the use of more than 60 wild species to improve 13 agricultural crops. The survey concentrates on the period of the mid-1980s to 2005. The most common use of wild relatives is as a source of pest and disease resistance, although other characteristics such as abiotic stress tolerance, yield increase, improved quality, and cytoplasmatic male sterility and fertility restoration also have been improved using crop wild relatives in individual cases (Tab. 1).

Another review on enhancing crop gene pools with beneficial traits using wild relatives was published in 2008 by DWIVEDI et al. The full potential of new molecular technologies to increase the number of new varieties carrying favorable genes from wild relatives has presumably not yet been fully exhausted (HAJJAR & HODGKIN 2007; LAMMERTS VAN BUEREN et al. 2010).

The process by which beneficial traits from PGR are transferred to crop varieties is called **introgression**. Introgression is the process of introducing desired traits (often single major genes) from exotic or unadapted germplasm into adapted breeding material by backcrossing (SIMMONDS 1993) (see also marker-assisted backcrossing in chapter 4.1.2). While the use of PGR in traditional breeding can be tedious, requiring several cycles of backcrossing, molecular markers can aid in transferring genes with minimal linkage drag, thus making the introgression from PGR one of the most common applications of MAS.

Tab. 1: Use of crop wild relatives in the past 20 years in released cultivars of seven agricultural crops[a] (adapted from HAJJAR & HODGKIN 2007).

Crop	Pest/ disease resistance	Abiotic stress	Yield	Quality	Male sterility or fertility restoration	Total number of contributed traits[b]
Wheat	11	-	1	1	-	9
Rice	7	3	1	-	1	12
Maize	1	-	-	-	-	2
Lettuce	3	-	-	-	-	2
Potato	6	-	-	-	-	12
Tomato	10	2	-	2	-	55
Barley	-	1	-	-	-	1

[a] Numbers indicate the number of wild relatives that have contributed beneficial traits to crop varieties in each category of traits. Minus signs indicate that wild relatives have not contributed beneficially in that category.

[b] Total numbers of individual traits obtained from wild species are indicated in the last column for each crop. Some species have contributed several traits, some traits were obtained from more than one species.

Introgression libraries (IL) allow for the identification of favorable alleles in exotic germplasm, which can be exploited for improving elite breeding material. ILs are obtained by a cross between the unadapted PGR and an elite recurrent parent. This is followed by several generations of recurrent backcrossing where the average proportion of the PGR is reduced by 50 % in each generation. A set of polymorphic markers that can distinguish between parental alleles is used to trace chromosome segments through the crosses. The backcrossing is followed by at least one generation of selfing which leads to plants homozygous at targeted introgressed segments (ZAMIR 2001). This systematic introgression of individual, short, marker-defined PGR chromosome segments into the elite background results in a library of introgressed lines. Each line carries a different part of the donor genome and the library can be used

to screen for favorable alleles obtained from the PGR. **Introgression lines** were first developed in tomato in 1994 and have since then been adopted for several grain crops, including barley, maize, wheat and rye (FALKE et al. 2009).

4.3 Application of markers in variety distinction and plant cultivar registration

Molecular markers, especially AFLPs and microsatellite markers, are reported as an appropriate tool for distinguishing plant varieties or lines (HECKENBERGER et al. 2003a). As an example, BONOW et al. (2009) successfully discriminated 37 rice varieties using microsatellite markers and TAMS et al. (2009) surveyed the applicability of molecular markers for the discrimination of hybrid varieties of winter oilseed rape. They used microsatellite markers and came to the conclusion that detailed harmonization of methods between laboratories is crucial to obtain results which are consistent among laboratories and unequivocal when discriminating varieties.

Unknown varieties can be identified relatively easily by comparing the molecular key characteristics with known variety descriptions from established reference collections.

Another potential application of AFLP markers or microsatellite markers is the identification of **essentially derived varieties (EDV)** in the context of variety registration. Registered plant varieties are protected against plagiarism. However, protected germplasm is available for the development of new varieties, which is fixed in the concept of "breeder's exemption" in the convention of the Union for the Protection of New Varieties of Plants (UPOV) (see also 8.4.2). Using modern molecular breeding methods such as genetic engineering or marker-assisted backcrossing the breeder's exemption can be misused by adding only a few new genes to an existing variety or by selecting for lines which are very similar to one of their parents. If plant variety protection is claimed for such a "new" variety, the breeder of the original variety is not compensated for his or her investments. The concept of EDVs was therefore implemented into the UPOV convention in 1991 and into several national plant variety protection acts in order to deal with the, at the time, new situation (HECKENBERGER et al. 2003b; KORZUN & HECKENBERGER 2005). Both AFLPs and microsatellite markers are suitable marker systems for EDV identification (HECKENBERGER et al. 2003a)

In cultivar registration, molecular markers could offer assistance in the evaluation of crop cultivars for **distinctness, uniformity, and stability (DUS**; for details see also

chapter 8.4.1), as molecular techniques can potentially allow for the discrimination of varieties based on small base-pair differences within the genome. Their use in cultivar registration has therefore been under consideration within UPOV. However, no systems for the use of molecular markers in the determination of distinctness of new cultivars have been developed yet (CAMLIN 2003).

More recently (2009), the International Seed Federation (ISF), representing the majority of the worldwide plant breeders' and seed traders' community, and serving as an international forum where issues of interest to the world seed industry are discussed, published a position paper on Intellectual Property[3]. Here a paragraph was adopted on 27 May 2009, concerning the case of DNA markers. There it is stated that the ISF supports the use of DNA markers for variety identification purposes, e.g. where the assertion of intellectual property rights is concerned, and that DNA markers are favored where they can improve the current DUS testing process. However, concerns are raised that "DNA-based markers may not be predictive of the expression of phenotypic characteristics used in DUS testing" and that "the use of molecular markers on their own, without a link to a phenotypic characteristic or without use of a distance threshold, could create a significant risk of decreasing the minimum distance between varieties to the extreme". In conclusion it is stated that "with the present state of the art, the use of DNA-based markers alone for establishing DUS, could significantly decrease the scope of protection and should therefore not be accepted".

4.4 Breeding objectives and examples for marker application in breeding

Breeding objectives for which MAS is regularly utilized at least in some crops are mainly disease and pest resistances and secondarily yield improvement, quality traits, and abiotic stress resistance (see 4.4.1). Examples on the application of MAS found in scientific literature mostly report marker applications for these objectives (see 4.4.2).

4.4.1 Breeding objectives

Application of markers for breeding disease resistant varieties is especially interesting when breeding for resistance traits which are difficult or expensive to assess phenotypically. A prominent example is the selection for resistance to nematodes. In

[3] http://www.worldseed.org/en-us/international_seed/on_intellectual_property.html (assessed 28 August, 2009).

wheat there is extensive use of DNA markers for cereal cyst nematode (*Heterodera avenae* Woll.) resistance (EAGLES et al. 2001); in soybean the most prominent example for MAS application in breeding is resistance to soybean cyst nematode (*H. glycines*) (YOUNG 1999). In both cases the disease is of economic importance, the resistance due to a single gene and the bioassay is expensive and unreliable (EAGLES et al. 2001), thus MAS is a clear advantage.

Yield improvement as a generally very important breeding goal is more difficult to achieve with marker-assisted selection due to its complexity. However, the AB-QTL method, which can be used for the simultaneous identification and transfer of favorable QTL alleles, has successfully been used to improve yield in elite maize lines (HO et al. 2002) and also BOUCHEZ et al. (2002) successfully introgressed favorable QTLs for grain yield into maize elite lines.

Abiotic stress resistance is an important breeding goal mainly for all agricultural systems that do not or can not rely on external inputs (e.g. mineral fertilizer or irrigation). With regard to the changing climate conditions it might in general become important to breed plants which can deal with Abiotic stresses. As abiotic stress resistance is also a complex trait, only few successful MAS applications in breeding for such traits have been published. An example are the results of a marker-assisted backcross (MABC) experiment conducted at CIMMYT to improve grain yield in tropical maize under water-limited conditions (RIBAUT & RAGOT 2006).

Quality traits, which often exhibit polygenic inheritance and can be strongly influenced by environmental conditions, also have only occasionally been improved with MAS yet. However, MAS is particularly efficient on traits with low heritability and various MAS strategies for improving quality traits have been proposed. One example is the MABC strategy pursued by LECOMTE et al. (2004), who introgressed five QTLs controlling fruit quality in tomato from a parental line into three improved lines.

4.4.2 Examples for marker application

As it was one aim of this study to show the potential of MAS, 83 publications from the past 10-15 years, reporting marker application in breeding programs or in research projects were analyzed. The analysis was meant to give an impression of what the main application areas for MAS are, which outcomes MAS has achieved over the past decade, and also, where the limitations are to be looked for.

The publications were analyzed according to breeding strategy, breeding objective, type of marker technology applied, type of breeding program (private, public, or university research) and country, and the marker-assisted product that was achieved in the breeding project.

A summary of the analysis is given here (Fig. 1 to Fig. 5); tables listing the analyzed publications and their outcomes in more detail can be found in Annex A.

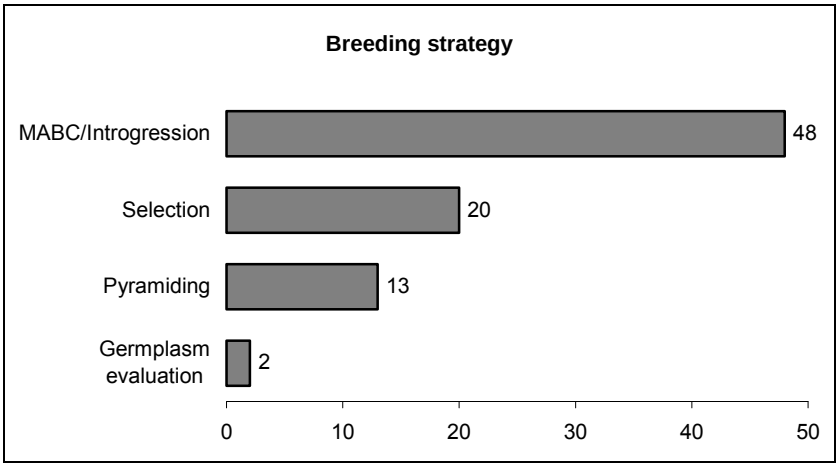

Fig. 1: Allocation of breeding strategies applied in breeding programs according to 83 publications (1995-2009) reporting marker application.

Regarding breeding strategies applied, MABC/Introgression is the main strategy with 48 publications out of 83 (Fig. 1). Regarding the breeding objective, breeding for disease/pest resistance is clearly dominating with 61 publications out of 83 (Fig. 2). Only few studies report the successful application of MAS for improved yield (8 studies), quality traits (6 studies), abiotic stress tolerance (5 studies), variety detection (2 studies), or growth character (1 study).

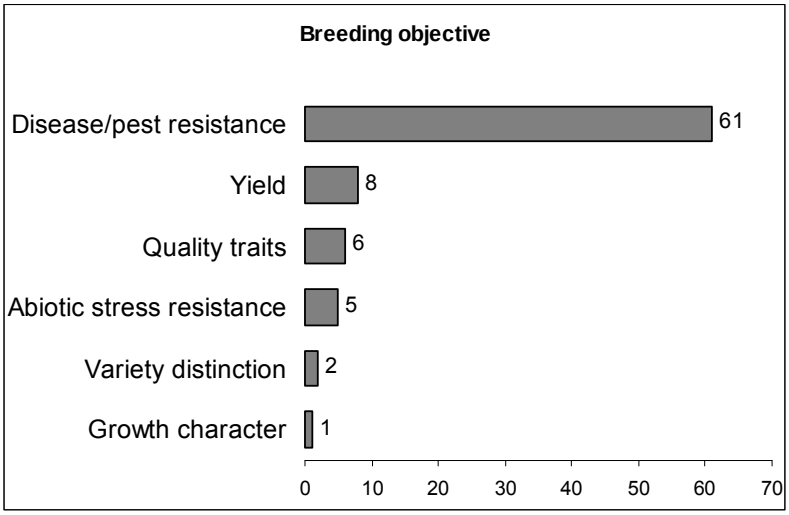

Fig. 2: Allocation of breeding objectives reported in 83 publications (1995-2009) on marker application in breeding programs.

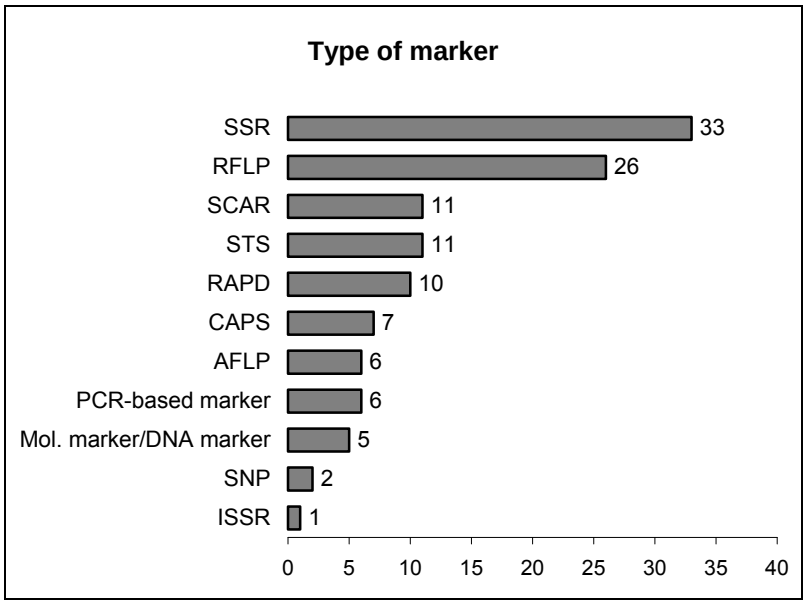

Fig. 3: Types of applied marker technologies reported in 83 publications (1995-2009) on marker application in breeding programs (in parts multiple entries).

The main marker technologies applied are microsatellite markers and RFLPs (Fig. 3).

The use of SNPs is only reported in two cases. It has, however, to be considered that the survey covers publications from 1995 to 2009. SNPs will very likely gain importance quickly and change the image significantly.

Regarding the type of breeding programs it can be clearly seen, that almost all publications result from public breeding programs (Fig. 4). Only eight publications out of 83 report the results of private breeding programs. Nevertheless, it would be incorrect to conclude that MAS is mainly conducted in public breeding programs. What has to be considered is that publishing is of little or no importance for private plant breeders, while it is one of the main aims in public research institutes and at universities. Therefore, literature based studies are inappropriate to measure the degree to which MAS is applied in private plant breeding.

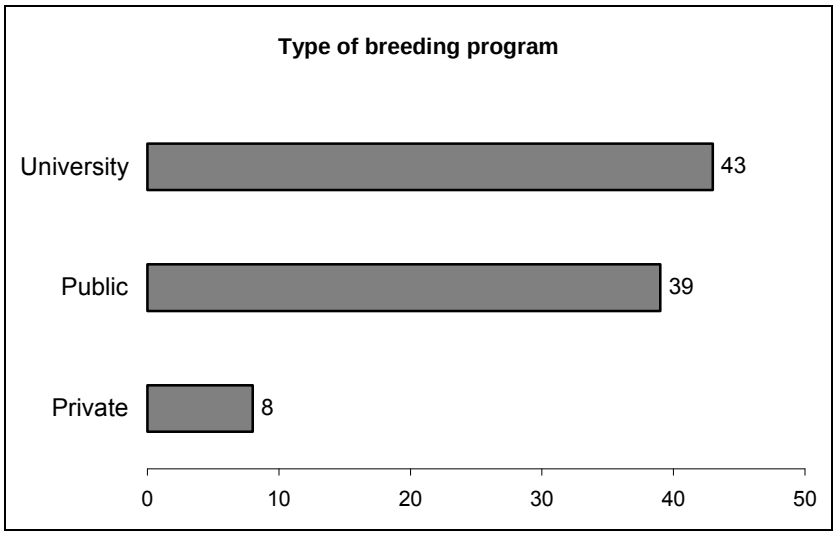

Fig. 4: Types of breeding program reported in 83 publications (1995-2009) on marker application (in parts multiple entries).

Reports on the development and validation of molecular markers and on QTL mapping are numerous in scientific publications. However, reports on the successful application of MAS in plant breeding programs are still limited. This fact is also stated in several papers (e.g. RIBAUT & RAGOT 2006; SEMAGN et al. 2006; EATHINGTON et al. 2007) or the reviews by DWIVEDI et al. (2007), XU & CROUCH (2008) or COLLARD & MACKILL (2008) who reviewed the applications of molecular markers in public and private

sector breeding programs, and also came to the conclusion that rate, scale and scope of uptake of genomics in crop breeding programs continually lag behind expectations.

In this survey we are focusing on publications related to marker applications in breeding programs rather than reporting marker development or validation. However, only few of the papers surveyed here have actually led to the release of germplasm lines or new commercial cultivars. Most germplasm lines (58 publications out of 83) have never been made available to the public and are summarized in "others" (Fig. 5).

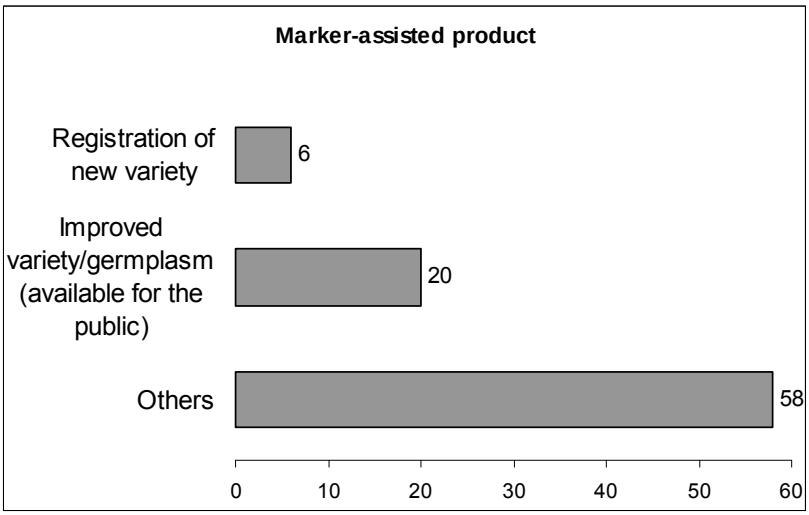

Fig. 5: Breeding outcome reported in 83 publications (1995-2009) on marker application in breeding programs (in parts multiple entries).

These findings correspond with YOUNGS findings from 1999, who claimed that from the more than 400 articles published between 1995 and 1999, which he surveyed and which all contained the keyword marker-assisted selection, only few if any led to the release of germplasm or varieties. Some information on new varieties that were bred with the assistance of markers can be found on web pages or in conference abstracts, but the information is often not very detailed. Overall, examples were found from 1995 on and publications from 1995 to 2009 are summarized here. Thus, current priorities and trends do not necessarily become visible.

From the 83 studies reviewed here, two thirds deal with simply inherited traits or the pyramiding of genes with major effects while about one third studies the usability of

MAS for improving quantitative traits (Fig. 2.).

The predominance of applications for resistance traits can most likely be ascribed to the fact that many resistances are monogenic, making MAS a beneficial option in all cases where phenotypic assays either are expensive or unreliable. However, yield or quality traits and abiotic stress tolerance are mostly polygenic traits, and although more than 1200 QTL mapping studies have been conducted in the major crop species (BERNARDO 2008), "the vast majority of the favorable alleles at these identified QTL reside in journals on library shelves rather than in cultivars that have been improved through the introgression or selection of these favorable QTL alleles" (BERNARDO 2008).

The few documented and published releases or registrations of varieties resulting from MAS breeding programs include two low-amylose rice varieties, 'Cadet' and 'Jacinto' (HARDIN 2000) and two Indonesian rice varieties, 'Angke' and 'Conde', with resistance to bacterial leaf blight (BUSTAMAM et al. 2002). A white bean variety resistant to BGYMV and common bacterial blight, 'Verano' (BEAVER et al. 2008), a leaf rust resistant wheat variety from Argentina, 'Biointa 2004' (BAINOTTI et al. 2009), and an Australian barley variety, 'SloopSA', resistant to cereal cyst nematode (BARR et al. 2000) have also been released. The soybean cultivar 'Sheyenne', tolerant to iron-deficiency chlorosis and resistant to lodging, was derived from another Pioneer variety. 'Sheyenne' was confirmed to be different from that variety with the help of markers (HELMS et al. 2008).

Other important examples for success in marker-assisted selection are a maize variety named "Sunrise", with high resistance against the western corn root worm (*Diabrotica virgifera*)[4] or a potato producing pure amylopectin, which is the "first product in Germany developed by Tilling that achieves market readiness"[5]. The maize variety was developed by the German Saaten-Union, the potato was developed by German Fraunhofer researchers and is processed by Emsland group, the largest German potato processor. As both examples originate from private breeding programs, they will most probably never appear in scientific journals. Nevertheless, press reports announcing MAS-breeding projects or releases of varieties that were bred with the assistance of markers are numerous. However, in this context an extensive overview over these

[4] http://www.saaten-union.de/index.cfm/nav/407/article/3201.html [9 March 2010].

[5] http://www.fraunhofer.de/en/press/research-news/2009/12/super-potato.jsp [9 March 2010].

reports would go beyond the scope of this study.

4.4.3 Wheat

Wheat is a naturally inbreeding species and most breeding programs are based on the pedigree breeding system. Although the volume of seed sale might be comparable to maize, the value of the wheat seed market is much lower, thus breeding activities are less rewarding than for hybrids, resulting in a much lower impact of MAS on breeding strategies (KOEBNER 2004). Because of the complexity of its genome, the hexaploid wheat is also a relatively difficult species for marker application (EAGLES et al. 2001).

The examples given in table A1 (Annex A) are from 2000 to 2009, all examples are from public breeding programs. While wheat breeding in many countries (especially the USA and Australia) is still mostly conducted in public breeding programs, this is not the case in most European countries. In these countries results from private breeding companies are rarely published. With the exception of one, all these examples concern the selection for disease or pest resistances, the majority of cases dealing with selection for monogenic disease resistances (leaf rust, stem rust), followed by selection for *Fusarium* resistance (QTLs). RFLP and microsatellite markers are the dominating technologies, mirroring more or less the technological state of the art during the past decade.

The tradition of collaborative projects in the wheat breeding sector in the USA was continued by the MAS wheat consortium, where researchers and breeders from 12 public programs across the USA were organized. The project started in 2001 and ended in 2005 and aimed at supporting breeders by facilitating the implementation of MAS capacities within the existing public wheat breeding programs. Molecular markers were used to transfer 22 resistance genes to fungi, viruses, and insects as well as genes related to bread, pasta and noodle quality which are backcrossed into different recurrent parents (DUBCOVSKY 2004). According to the project website the total number of backcrosses done during the project was approximately 3300 and 274 lines completed the backcrossing program, that is, they reached BC_5 or BC_6, encompassing all major US wheat market classes. Some lines were not finished by the end of the project, but breeders continue working on them (http://maswheat.ucdavis.edu/IFAFS/Achievements/Backcrosses.htm) [20 August 2009]. There is public access to the information created in the project through the project website (http://maswheat.ucdavis.edu) [26 June 2009], where detailed

information about marker sequences and research protocols is made available.

The project is followed by the Wheat CAP project, the "Coordinated Agricultural Project for Wheat", funded by the Cooperative State Research, Education, and Extension Service USDA/CSREES, where about 25 public wheat breeding programs in collaboration with 4 USDA-ARS genotyping labs work on the genetic improvement of US wheat (http://maswheat.ucdavis.edu) [26 June 2009].

A review article (WILLIAM et al. 2007) examining the extent to which molecular markers have been used at CIMMYT (International Maize and Wheat Improvement Center) in applied wheat breeding shows that about 45,000 MAS data points per year are generated between the two wheat growing seasons. In that article MAS is considered as a technology which will increasingly be adopted by wheat breeding programs in the private and public sectors. According to CIMMYT, the success of utilization of markers in wheat breeding depends to a high degree on the closeness of biotechnologists and breeders working together.

4.4.4 Barley

Barley is, like wheat, a self-pollinating species and in terms of the breeding system and the economic structure of its market it resembles wheat. However, MAS seems to have progressed further than in wheat, which is probably due to the simpler, diploid genome. The examples given in table A2 (Annex A) comprise the years 1998 to 2009, three examples are results from private breeding programs (TUVESSON et al. 1998; OKADA et al. 2003; KUCHEL et al. 2007), the other 9 examples result from public breeding programs. A main focus of marker selection in barley is breeding for barley yellow mosaic virus resistance; another important focus is rust resistance. The marker technologies used in the examples given are mainly RFLP and microsatellite markers as in wheat, again mirroring the technical state of the art during the past decade.

In contrast to wheat, barley varieties have been released that are based on MAS. In the USA the variety 'Tango', carrying two QTL for adult resistance to stripe rust, was released in 2000 (HAYES et al. 2003), claiming to be the first commercially released barley variety using MAS. However, 'Tango' yields less than its recurrent parent and is therefore primarily seen as a genetically characterized source of resistance to barley stripe rust rather than a variety of its own. As a result of the South Australian Barley Improvement Program the malting variety 'Sloop' was improved with cereal cyst nematode resistance introgressed from the variety 'Chebec' and released in 2002 as

'SloopSA' (BARR et al. 2000; EGLINTON et al. 2006).

4.4.5 Maize

For the cereals it is first of all maize to which MAS is being applied on a large scale. Maize varieties are predominantly F_1 hybrids and breeding in the developed countries is dominated by a small number of large private sector breeding companies (see also chapter 5.7). Hybrid breeding has the two advantages that the use of farm-saved seeds is impossible and that the inbred components of a successful hybrid are not available for competitors. The control over varieties has consequences on the financial returns of breeding and thus a major investment in MAS infrastructure has been made in large private sector breeding companies (Pioneer Hi-Bred, Syngenta, Monsanto in the USA, KWS and Limagrain in Europe) (KOEBNER 2003; KOEBNER 2004). These companies do not freely publish their procedures, for competition reasons, which also becomes visible in the examples given for maize in table A3 (Annex A). The examples comprise the years 1995 to 2006, all examples except one (RAGOT et al. 1995) are from public breeding programs, the majority focusing on backcrossing strategies. The improvement of quantitative traits prevails, reflecting the fact that in maize the advanced MAS technologies are increasingly utilized. Although the marker technologies used in the given examples are mostly RFLPs and microsatellite markers, in maize the whole process of marker genotyping is increasingly reliant on SNPs (KOEBNER 2004).

According to RAGOT AND LEE (2007) the most important use of MAS in maize by the private sector is backcrossing of transgenes into elite inbred lines (RAGOT et al. 1995). Other important examples for the successful application of MAS in maize are the use of microsatellite markers for the conversion of normal maize lines into Quality Protein Maize (QPM), containing more lysine and tryptophan than the native lines (BABU et al. 2004), or the introgression of favorable QTL for earliness and grain yield between maize elite lines (BOUCHEZ et al. 2002).

4.4.6 Rice

Rice, having the smallest genome of all cultivated cereals, being diploid and self-pollinating, is the most extensively studied species among cereals. This is on the one hand due to its global importance as a crop, on the other hand to its role as a model species. In rice, although it is a self-pollinating species, improvements in the management of male sterility have led to an expansion of F_1 hybrid breeding,

especially in China and in the USA. It is expected that MAS will grow in importance, mirroring the trend in maize (KOEBNER 2004).

The examples given in table A4 (Annex A) encompass the years 1996 to 2009, all examples are from public breeding programs. As in wheat, the main marker technologies used during the past decade were RFLP markers and microsatellite markers.

Up to now, MAS in rice breeding has mainly been utilized for the pyramiding of disease resistances, namely bacterial blight (e.g. CHEN et al. 2000; SINGH et al. 2001) and blast (NARAYANAN et al. 2004). In 2002, two cultivars resistant to bacterial leaf blight were released in Indonesia, which have been selected using MAS. The variety 'Angke' carries the resistance gene *xa5*, 'Conde' carries *Xa7* (BUSTAMAM et al. 2002). Several publications report introgressions from wild relatives (e.g. *O. glumeapatula*, *O. rufipogon*) in order to improve yield (BRONDANI et al. 2002; LIANG et al. 2004) and in 2006, two lines showing strong submergence tolerance were developed by introgressing a locus conferring submergence tolerance from cultivar 'FR13A' into the variety 'Swarma' (XU et al. 2006).

4.4.7 Potato

Potatoes are the world's most important vegetable and fourth most important food crop in terms of production (SIMKO 2004). Nevertheless, MAS has been practiced only little because the construction of classical genetic linkage maps was not practical for a long time, due to the tetraploidy of potatoes combined with tetrasomic inheritance. This complicates the detection of linkages and largely prevents the recovery of recessive phenotypes. Only with the technical developments that allowed to reduce ploidy from the tetraploid to the diploid level and with the advent of DNA-based markers it became possible to construct detailed genetic maps for potato and to carry out genome-wide studies on qualitative and quantitative disease resistance (GEBHARDT 2004).

Several linkage maps have been constructed with molecular markers over the past 20 years and the most important marker applications in potato breeding are marker-assisted resistance breeding and marker-assisted introgression (VREUGDENHIL et al. 2007). Although various markers have been developed (VREUGDENHIL et al. 2007), practical applications in breeding are still rare. Most marker applications are carried out in experimental populations of diploid potatoes (GEBHARDT 2007), which impedes the application of experimental results in practical breeding work.

However, the possibility of detecting marker-trait associations in populations of tetraploid varieties and breeding clones has been demonstrated (SIMKO et al. 2004). This is exciting as it "closes the gap between linkage mapping of quantitative and qualitative traits in experimental populations and DNA marker applications in breeding programs. It opens the possibility to develop PCR-based markers of general diagnostic value for parental screening and marker assisted selection" (GEBHARDT 2007).

One example elegantly demonstrating how MAS can be efficiently used in resistance breeding is the work by GEBHARDT et al. (2006). The authors applied molecular markers to develop breeding material that carries resistances to three different pathogens and the root cyst nematode (*Globodera rostochiensis*) (for details see table A5, Annex A).

4.4.8 Tomato

Tomato is one of the most important vegetable crop worldwide and according to FOOLAD & SHARMA (2005), MAS "is used routinely by many seed companies for the improvement of various simple traits (…). However, MAS is not yet a routine procedure for improving quantitative traits, although it has been employed to improve quantitative resistance to bacterial wilt, bacterial cancer and tomato yellow leaf curl virus as well as for improving fruit soluble solids."

The examples given in table A6 (Annex A) encompass the years 1997 to 2009, all examples are from public breeding programs. The main marker technology used in the cited publications was RFLP markers. MAS has mainly been utilized in resistance breeding, three papers report the introgression of QTLs.

In public tomato breeding programs, the use of MAS is less common than in seed companies, although MAS has been used to improve horizontal resistances to black mold (ROBERT et al. 2001) and for some complex fruit-quality characteristics (LECOMTE et al. 2004).

For a review of molecular markers in tomato, see FOOLAD & SHARMA (2005).

4.4.9 Soybean

Soybean is the world's leading oilseed crop with major uses in human foods, animal feed, as well as in many industrial products. It is a self-pollinating legume; the

majority of variety development is conducted by private soybean breeders, while basic research in breeding methodology and germplasm development is conducted by public breeders (SHANNON & SLEPER 2003).

The examples given in table A7 (Annex A) encompass the years 1996 to 2009, and except two all examples are from public breeding programs.

With the development of microsatellite markers the possibility of using MAS in soybean breeding increased substantially. This is reflected by the fact that in almost all examples listed in table A7, microsatellite markers were used. In soybean MAS has been especially useful in breeding for resistance to soybean cyst nematodes (*Heterodera glycines* Ichinohe) (CONCIBIDO et al. 1996; CAHILL & SCHMIDT 2004; ARELLI et al. 2006; ARELLI et al. 2007). This is due to the facts that phenotyping the resistance is expensive and that the resistance has a relatively simple inheritance.

As soybean has a narrow genetic base, also the use of exotic germplasm to broaden the genetic base is an important strategy where molecular markers are applied (CONCIBIDO et al. 2003).

An example for the successful application of MAS in breeding new varieties is the registration of the cultivar "Sheyenne' in 2008. 'Sheyenne' was derived from a Pioneer cultivar that was never released, and with the help of microsatellite markers it was shown that the variety is different from the Pioneer variety at certain loci (HELMS et al. 2008).

4.4.10 Beans and other vegetables

Additional reports on marker application in vegetable breeding programs comprise lettuce, pepper, cucumber, and several *Phaseolus* beans.

The examples given in table A8 (Annex A) encompass the years 1998 to 2008, all examples are from public breeding programs. RAPD and SCAR markers were used predominantly, main selection aims are – besides a report on yield improvement in cucumber (FAN et al. 2006) – disease resistances here as well.

Seven papers report the registration of improved bean germplasm lines (BEAVER et al. 1998; MIKLAS et al. 2003; MUTLU et al. 2005; BLAIR et al. 2006; MIKLAS et al. 2006a; MIKLAS et al. 2006b; MUTLU et al. 2008) and one white bean (*P. vulgaris* L.) variety resistant to bean golden yellow mosaic virus and carrying QTLs for common bacterial blight resistance was registered by BEAVER et al. (2008).

4.4.11 Fruits

Many fruit crops like the pome fruits have a large size, long juvenile phase, and a high level of heterozygosity which makes classical genetic analysis a difficult task. The cost-benefit relationships of MAS are more favorable for fruit crops than for annual crops because most fruit breeding programs consist of two stages. In the first stage, large populations of nonreplicated individuals are evaluated and only a small number of plants is selected for extensive asexual propagation to be tested in replicated trials in the second breeding stage. Especially during the first stage the large plant size and long life cycle have a negative impact. With the aid of MAS numbers of plants that have to be tested in the first breeding stage can be drastically reduced.

However, many important agronomic characters in fruit trees are related to major genes, e.g. several disease resistances, flower characteristics, and fruit or nut quality traits (DIRLEWANGER et al. 2004) and very few QTLs are available in fruit breeding.

The example given in table A9 (Annex A) from the year 2009 shows that markers can be applied successfully in fruit breeding now and more applications are to be expected, bearing in mind the tremendous efficiency gain which molecular markers provide in fruit breeding.

5 The discrepancy between marker development and marker application in breeding programs

There are far more publications on the development of markers than publications announcing the successful use of MAS in breeding. At first sight it is often difficult to distinguish whether a publication is actually reporting a MAS application or if only potential MAS applications of the actual research outputs are discussed. On closer inspection, most publications related to MAS deal with the detection/development of suitable markers and related research questions rather than reporting the application of MAS in breeding programs.

There is a tremendous amount of publications reporting the identification of new QTLs, however, very few of the QTLs reported have been used for MAS in breeding programs. XU & CROUCH stated in 2008: “It appears that the community is currently investing a large amount of time and money in generating an increasingly vast collection of publications with little impact on applied plant breeding, particularly in the public sector.” According to them the limited success in developing finished breeding products using MAS can be further illustrated by comparing the numbers of

publications on QTL mapping and on MAS that were generated since the development of the first molecular markers. The term "marker-assisted selection" was first used by BECKMANN & SOLLER in 1986, since then both the number of publications on MAS and on QTL mapping have increased dramatically. However, publications containing the term "marker-assisted selection" or "MAS" are continually lagging behind the number of articles containing the term "quantitative trait locus", "quantitative trait loci" or "QTL" (Fig. 6). New marker-trait associations are being published constantly and the amount of available genetic information seems to grow almost exponentially. The biggest challenge might therefore to be the "integration of this diverse and disparate information and interpretation in a specific biological context to convert it into knowledge" (RIBAUT & RAGOT 2006).

It has nevertheless to be kept in mind that plant breeders do not focus on publishing while for scientists marker development is by far easier to publish than marker application in breeding. This might lead to a distorted image. Interviews with breeders showed that molecular markers are applied internally; the applications are rarely published, however. Thus, the numbers on marker development and successful application might not be as far away form each other as Fig. 6 suggests.

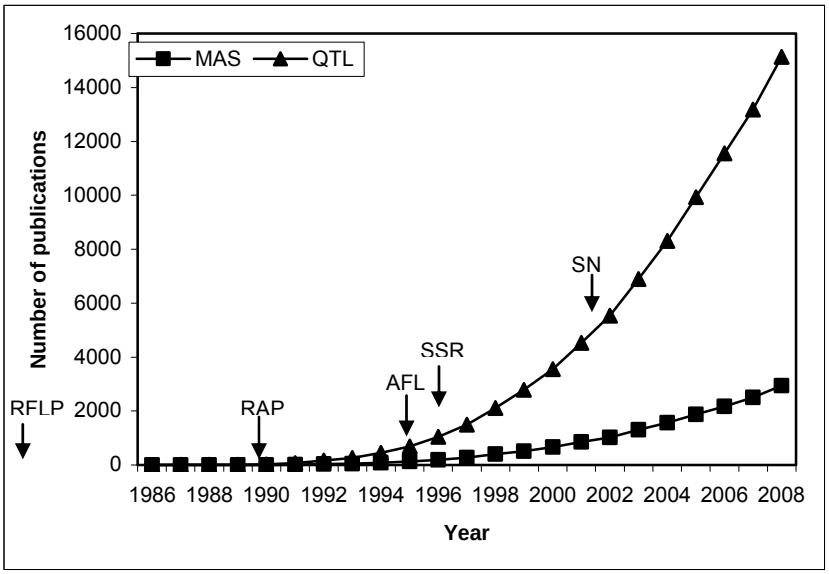

Fig. 6: Number of publications containing the term "quantitative trait locus/loci" and "marker-assisted selection" by years (1986-2008). Results from a search in the Web of Science database (2 October 2009) (adapted from Xu & Crouch, 2008).

Several other possible answers to the question, why reports on the successful application of MAS in plant breeding programs are still limited, are discussed below.

5.1 Marker technology development versus conversion into practical applications

Although DNA markers have been available since the late 1980s, PCR-based markers allowing high throughput (microsatellite markers) became only available in the mid-to late 1990s. Only during the last five to ten years these markers have been widely used (COLLARD & MACKILL 2008). The publication dates for the examples given in tables A1 to A9 in Annex A confirm this.

After the development of the first DNA-based markers (BOTSTEIN et al. 1980) and after the term "marker-assisted selection" was first used by BECKMANN & SOLLER in 1986, it took another ten years until the first substantial article on the application of MAS in plant breeding was published (CONCIBIDO et al. 1996). There seems to be a time lag of about ten years between the first application of new marker technologies and their widespread use in breeding programs. If this is the case, a notable increase in the number of publications describing MAS has to be expected in the next ten years and beyond (COLLARD & MACKILL 2008), especially if today's promises of SNP marker applications turn out to be true.

5.2 Publication aspects

QTL mapping – as a basic research process – regularly results in scientific publications. This explains the vast number of publications reporting the identification of new QTLs. However, scientists gain reputation mostly through carrying out innovative research and through publishing results within academic journals. Thus, there is little appeal to ensure that markers developed in research programs are also applied in breeding programs (COLLARD & MACKILL 2008). For plant breeding, in contrast, the aim is not to publish results but to release new varieties. Even if the new varieties are registered, details regarding the application of markers during the breeding process are not necessarily published.

In addition, in the private sector publication of results might even be discouraged due to competition reasons and the resulting unwillingness of researchers to share data and germplasm can cause serious limitation for the advancement of MAS applications (WILLIAM et al. 2007).

5.3 Lack of conversion of publications into practical applications

A high proportion of published markers fails the translation step from research to application (XU & CROUCH 2008). Converting promising publications into practical large-scale applications in breeding programs requires that different practical, economical, logistical, and genetical constraints are resolved.

Before MAS realizes its full potential in public sector breeding programs, (i) published markers need to be validated, (ii) simple, quick, and cheap technical protocols for tissue sampling need to be developed, (iii) high throughput precision phenotyping systems for QTL mapping are needed and, (iv) improved understanding of genotype by environment interaction and epistasis has to be gained (XU & CROUCH 2008).

5.4 GxE interactions and effects of genetic background

GxE interactions are complex phenomena which complicate the interpretation of genetical experiments and often make predictions difficult. They mostly cause difficulties in marker development and validation for quantitative traits.

RIBAUT & RAGOT (2006), having used a marker-assisted backcross approach to improve drought adaptation in maize, state that the success of marker-selection for complex traits will largely depend on two things: the accuracy of plant phenotyping on the one hand and the understanding of genetic phenomena such as gene networks, epistasis, and GxE interactions on the other hand. Because quantitative traits are influenced by the environment they often show varied degrees of GxE interactions. GxE interactions occur when two or more genotypes perform differently in different environments, and are thus described as differential genotypic sensitivities to environments (FALCONER & MACKAY 1996). Especially self-pollinating plants tend to show a high level of GxE interactions, which can be understood as a strategy to adapt to changing environments (LI et al. 2003).

If quantitative traits are to be improved with MAS it is essential to have information about the GxE interactions. GxE interactions impede the repeatability of QTL mapping results and consequently reduce the efficiency of selection (KOEBNER 2004). It particularly becomes a problem where genotypes have to be selected in one environment and used in a different one (KEARSEY & POONI 1996). Especially QTLs with small effects can vary in magnitude and direction of effects, depending on environmental conditions. The extent of GxE interactions is not always known after conducting a mapping study, because such studies are usually restricted to a few years

and/or locations (COLLARD & MACKILL 2008).

A number of sophisticated statistical approaches have been developed to provide more efficient characterization of GxE interactions and breeders have to choose the most suitable one for their needs (for a review see VAN EEUWIJK et al. 2005). Mostly modeling of the interactions is used to dissect the complex traits into manageable component traits and to describe the environmental effect on them. This supplies breeders with information containing less GxE interactions and therefore with QTLs that are more robust.

Some scientists go as far as distinguishing between interactions of genotype and environment (GxE) and genotype and management (GxM), thus speaking of GxExM interactions. Many researchers consider management as part of the environment, but as management is that part of the environment that is manageable and therefore predictable to some extent, this can be a useful approach (STRUIK & YIN 2009).

Epistasis is the phenomenon that genes sometimes show a certain positive or negative effect only in combination with each other. For QTLs this can lead to unpredictability of expression in genetic backgrounds other than the one in which they have been detected (KOEBNER 2004). Where GxE interactions or epistasis are important, it is necessary to regularly re-estimate QTL effects within the breeding program, as suggested by PODLICH et al. (2004) (see also chapter 1.3).

5.5 Economic aspects of marker application

Only few studies compare the economical aspects of conventional phenotypic selection and MAS. Landmark papers are the one by DREHER et al. (2003) and the companion paper by MORRIS et al. (2003). MORRIS et al. (2003) state in their paper that "as most plant breeders well know, the cost of using DNA markers can vary greatly depending on the crop, the breeding application, the trait(s) being targeted, the availability of suitable marker technology, and other factors. This application-specificity complicates economic analysis, but it does not invalidate it completely. Well-designed case studies can help plant breeders make better decisions about choice of breeding strategy by generating detailed empirical information about the costs and time requirements of alternative selection methods."

MORRIS et al. (2003) compared the costs for conventional and MAS methods for a particular breeding application. They introgressed an elite allele at a single dominant gene into an elite maize line and found that neither method shows clear superiority in

terms of both cost and speed. Phenotypic selection schemes were less expensive, but MAS required less time. Thus, the decision for or against MAS is dependent on the availability of operating capital in the breeding program. DREHER et al. (2003) compared the cost-effectiveness of conventional and marker-assisted maize breeding. They used the conversion of lines into quality protein maize (QPM) as an example and the results indicate that the use of microsatellite markers was cost-effective compared to phenotypic selection. The authors conclude that such detailed budget analyses – if used with detailed empirical data from actual breeding programs – can be very useful to improve the efficiency of existing protocols and to make decisions about future technology choices. For wheat, no economic analysis exists, and as late as 2003 MAS was considered non-competitive (KOEBNER & SUMMERS 2003). The authors "identify the need for a substantial reduction on assay price before MAS is likely to make more than a marginal impact on present (selection) practice". Even if the technology itself is no longer limiting, the affordability of large-scale MAS for breeding small grains is in most cases not given (KOEBNER & SUMMERS 2007). In discussions private wheat breeders also often mention that wheat breeding in general does not pay.

In general, the above studies show that no simple answer can be given to the question whether to apply MAS or phenotypic selection in a breeding program. But this type of analysis can nevertheless be very helpful if it is constantly updated as new genotyping systems become available (XU & CROUCH 2008).

5.6 Intellectual property rights (IPR) in marker application

In biotechnology there are many industrial applications with high economic value. Thus IPR – and especially patents – have become more and more important. The importance of molecular marker analyses for different applications was recognized very early, resulting in the filing of many patents in the last 10-15 years (JORASCH 2004). From the private sector, there are no reports of the cost-benefit ratio associated with commercializing MAS-derived cultivars. However, the growing number of patent applications associated with MAS shows that the use of such approaches is seen as a comparative advantage in commercial breeding programs (DWIVEDI et al. 2007).

If companies ensure their rights through the patenting of developed markers, no matter whether they are further utilized or not, they impede the use of these markers through others. Which leads to one of the central questions in this report, whether molecular markers can encourage the use of plant genetic resources or not.

Tab. 2: Examples for patent applications related to MAS technologies. The given examples are results from a patent investigation in the freepatentsonline database (www.freepatentsonline.com), [15 July 2009].

Title	Assignee	United States Patent No.	Publication Date
Identification and localization and introgression into plants of desired multigenic traits	Pioneer Hi-Bred International, Inc. (Des Moines, IA)	5,385,835	1995
Process for predicting the phenotypic trait of yield in maize	DeKalb Genetics Corp. (DeKalb, IL)	5,492,547	1996
Method to identify genetic markers that are linked to agronomically important genes	E. I. du Pont de Nemours and Company (Wilmington, DE)	5,746,023	1998
Cleaved amplified RFLP detection methods	The General Hospital Corporation (Boston, MA)	6,004,783	1999
Positional cloning of soybean cyst nematode resistance genes	Pioneer Hi-Bred International, Inc. (Johnston, IA)	6,162,967	2000
Method for identifying genetic marker loci associated with trait loci	E. I. du Pont de Nemours and Company (Wilmington, DE)	6,219,964	2001
Amplification of simple sequence repeats	Keygene, N. V. (Wageningen, NL)	6,218,119	2001
Marker-assisted identification of a gene associated with a phenotypic trait	Pioneer Hi-Bred International, Inc. (Des Moines, IA)	6,368,806	2002
Process predicting the value of a phenotypic trait in a plant breeding program	Dekalb Genetics Corporation (Dekalb, IL)	6,455,758	2002
QTL mapping in plant breeding populations	Pioneer Hi-Bred International, Inc. (Des Moines, IA)	6,399,855	2002
Detection of polymorphisms in AFLP fragments using primer extension techniques	Keygene N.V. (Wageningen, NL)	7,169,552	2007

A search in a patent database (www.freepatentsonline.com) using "marker-assisted selection" as search item resulted in 2894 patents related to MAS (including US patent applications), of which 890 patents (and patent applications) were filed by Pioneer,

498 by Monsanto and 83 by Syngenta. A few detailed examples for some MAS-related patent applications are given in Tab. 2.

It deserves to be discussed further whether IPR might affect the application of MAS, e.g. through licensing costs due to patents.

The continual assertion and protection of IPR is seen in different ways by different authors. In some opinions it has often inhibited knowledge dissemination, research and development (STAFFORD 2009), in other opinions the "patents describe the latest inventions made by innovative researchers and companies and the publication of these patents guarantees their public availability. This, in turn, allows the further development and improvement of these innovative techniques" (JORASCH 2004).

5.7 Influences of private and public sector breeding

Since the beginning of formalized plant breeding activities, most of these took place in public institutes or small family owned companies. However, since the 1960s large parts of plant breeding have shifted to and concentrated within the private sector. For the past decade a stagnation or even decline of public investments in agriculture and a rise of private investments has been reported. The increase in private investments has led to a focus on developing products with promising commercial applications and easily appropriable benefits (MORRIS et al. 2006).

In Western Europe it can clearly be seen that MAS is mainly applied in breeding programs for species where varieties are predominantly F_1 hybrids and direct economic gains for the private seed industry can be expected, such as maize (KOEBNER 2003). Worldwide, the large private maize breeding programs are investing heavily in the implementation of MAS (RAGOT & LEE 2007), while the uptake of MAS in wheat or barley breeding programs is slow (KOEBNER 2003). Wheat and barley as true breeding inbreds allow growers to save seed from the harvest for the next year, which reduces the profitability of wheat or barley breeding for the private sector. In contrast to Germany, where also the small-grain breeding programs are conducted in the private sector, small-grain breeding is a public activity in the United States. A census from 1996 showed that in the USA about 80 % of the total number of cereal breeders worked in the private sector while 20 % were in the public sector. For wheat about 60 % of the breeders were in the public sector, while only 7 % of the maize breeders worked in the public sector (FREY 1996). (Results from recent studies do not give numbers for the private sector anymore; therefore no newer data can be

shown.) However, there is currently a worrisome conjunction between public and private, trying to thwart farmers' seed production (KLOPPENBURG 2009).

About 60 % of the wheat varieties that were released in the USA during the 20th century were bred by state agricultural experiment stations, the Agricultural Research Service of the U.S. Department of Agriculture (ARS-USDA) or CIMMYT (DUBCOVSKY 2004). Between 2001 and 2003 public wheat varieties accounted for 78 % of wheat production in the USA[6]. As described in chapter 4.4.3, due to large-scale collaboration among public programs and the huge actions of CIMMYT, MAS varieties start appearing on the US market.

Private investments in wheat breeding in the USA concentrate on the soft red winter wheat varieties, which are grown in the eastern USA in crop rotations with maize and soybean. Even if wheat is not profitable, the breeding and distribution is done as a "service" to the customers (HEISEY et al. 2001). In the German private plant breeding sector, where breeders are much more specialized and often breed small grains only, this kind of cross-financing is only rarely possible.

6 Guided expert interviews and workshop with experts

In addition to the literature survey, guided expert interviews were carried out to complement the results obtained in the literature survey. Guided interviews are non standardized interviews which are used when the aim of an enquiry is to gather information about the opinions of a particular person (breeding and marker experts in this case). Guided interviews contain only open-ended questions, and the questionnaire is only used as a guideline for the interview, the conversation between interviewer and interviewee does not have to follow it strictly. Guided interviews generate qualitative data, which is why the number of interviews usually is limited, and quantitative conclusions can not be drawn (GLÄSER & LAUDEL 2006).

A questionnaire containing routing questions (see Annex D) was developed and emailed to 31 experts within the field of marker-assisted selection, 19 of those being breeders in medium-sized to big breeding companies, 12 being employed at universities or public research institutes (JKI, IPK). The questionnaire served as a basis for subsequent telephone interviews of about half an hour to one hour length

[6] http://maswheat.ucdavis.edu/AboutProject/index.htm [20 August 2009].

each. A total of 14 experts agreed to be interviewed, nine from private breeding companies, five from public institutions.

As private breeding companies generally keep their investments in technological developments secret, the interviews were made completely anonymous, thus hoping for as numerous and detailed answers as possible. Which actors from the private sector breeding companies precisely were involved in the interviews remains unrecorded. Nevertheless, due to breeders caution when talking about breeding details, a certain imprecision of the answers is to be assumed.

A summary of the interviews was presented at a workshop and further discussed with breeders and scientists to confirm, adjust, and complete the information gained through the interviews.

6.1 Results of expert interviews and workshop

Not all questions were answered by all participants. Due to the specific working areas of some respondents, certain questions were given more weight in some interviews, while other questions were not addressed. Multiple answers were possible in many cases. Also, not all participants of the workshop had been interviewed.

Altogether, the 14 respondents work with 18 different crop species. The main focus are cereals, including maize with 17 out of 35 responses, and canola with another 4 responses (Tab. 3).

When asked for the impact of MAS in breeding the above mentioned crops, all breeders consistently pointed out the big differences regarding the crop species. While MAS is not at all applied in some breeding programs, it is an important part of the breeding process in other programs. Up to now MAS is predominantly used in hybrid breeding, especially for maize, sugar beets, canola and hybrid rye. For the other (self-pollinating) cereal crops and the other crops listed in Tab. 3 MAS is applied considerably less frequent.

The decision for or against marker-assisted selection is solely based on economic considerations. Especially breeders working in small or medium-sized breeding companies point out that the application of molecular markers is still very limited, while breeders working in big companies and scientists do not make this statement as clearly. This underlines the fact that the application of molecular markers is mainly subject to economic decisions.

Tab. 3: Crop species bred by the 14 interviewed experts. (Whether molecular markers are used in the breeding process is not stated.)

Crop species	No. of responses
Barley	4
Canola	4
Maize	4
Wheat	4
Rye	3
Grasses	2
Potato	2
Vegetables	2
Apple	1
Grape	1
Lupine	1
Oil plants	1
Ornamentals	1
Protein plants	1
Small-seeded legumes	1
Sorghum	1
Sugar beet	1
Triticale	1
	35

The application of markers is seen as especially important where it can accelerate the breeding process (e.g. perennial crops) or where it allows to attain certain breeding aims which normally are very difficult or impossible to achieve (e.g. pyramiding resistance genes).

In almost all cases breeders expect the impact of molecular markers in plant breeding to increase in the future.

Application of markers in breeding companies today

The main breeding aims MAS is applied for by the respondents are

- resistances (14)
- establishment and classification of gene pools (5)
- quality assurance in seed production, variety identification and hybrid breeding (4)
- abiotic stress resistance (3)
- prediction of the general combining ability (3)
- nitrogen efficiency (2)
- pollen fertility restoration (2)

The number of responses is in each case given in parentheses, multiple responses were possible.

The respondents pointed out that MAS is still predominantly used to select for monogenic traits because markers can be developed and applied much easier for mono- or oligogenic traits with high heritability than for quantitative traits. However, in many cases (e.g. breeding for resistance) it would be far more important to have markers available for QTLs. Research on this is mainly done in public research institutes and universities.

Asked for the technological approaches used in their work, the respondents mentioned several different marker technologies which are currently applied in their breeding work (Fig. 7). This is congruent with the literature survey (Fig. 3). Microsatellite markers are still most important but the new generation of SNP markers is clearly catching up. The AFLP marker system is only used in a few breeding programs nowadays.

When asked for the application of markers in variety protection and distinction, the respondents stated that many breeders use markers to distinguish varieties or lines for internal purposes. Markers are applied to control purity of varieties and/or lines, to analyze pedigrees, and to test for membership to gene pools. Mostly, microsatellite markers are applied in such contexts.

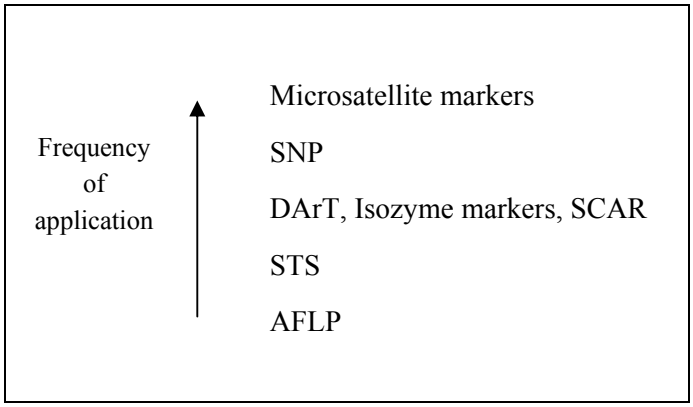

Fig. 7: Marker technologies which are currently applied in breeding or research programs (result of interviews with 14 experts).

The application of molecular markers in variety protection in the future was considered to be very unlikely; at least a routine-use of markers is not expected. To play a role in variety protection, molecular markers would have to be easier in application and cheaper than phenotypic characterization. It is not expected that this will be the case in the near future. Nevertheless, molecular markers will likely be used as an additional tool if problems occur regarding distinctness. Markers were also mentioned as a solution in disputes concerning EDV.

Breeding progress related to MAS

Regarding the breeding progress that was achieved through MAS during the past 10-15 years, the respondents expressed inconsistent opinions. For some crops they considered that MAS has not yet contributed to the breeding progress at all because markers gained importance in the selection work only recently. Others argue that the contribution of MAS itself is not identifiable at all because MAS is only one tool amongst many which jointly contribute to breeding progress.

However, some respondents gave examples from the past 10 years, showing that the application of markers significantly contributed to the achievement of some breeding aims. These are the following:

- pollen fertility restoration in rye
- leaf rust resistance in rye

- BaYMV resistance in barley
- BYDV resistance in barley
- barley scald (*Rhynchosporium*) resistance in barley
- nematode resistance in sugar beet

Examples for breeding progress achieved within the same time without applying markers are increase in yield levels and improvement of complex traits. Also, in some crops progress in resistance breeding was achieved without the help of molecular markers (e.g. wheat and hybrid rye, powdery mildew resistance of grape).

In the decision for or against MAS complexity of traits and cost-benefit analyses are of major importance. Concerning the improvement of complex traits MAS is still in its beginning and high costs of marker application were limiting for a long time. It is felt that these limits may slowly disappear. The more important a crop economically is, the more likely markers are applied in the breeding process. Cooperation between breeders and scientists can enhance the application of MAS considerably.

Developments promoted by MAS

During the workshop relevant topics were discussed that have been promoted by MAS. It was endorsed that markers have mainly led to improvements in resistance breeding, with BaYMV being the prime example. The importance of markers in resistance breeding is caused by difficulties with assessments in the field. Field assessments do not always deliver reliable results, as pathogen infestation may fluctuate from year to year. Another aspect of marker application is the valorization of plant genetic resources. The value of genetic resources being stored in gene banks could possibly rise through the application of markers as markers can help to reveal the presence of traits in PGR and may permit for their efficient use in the improvement of crop varieties. However, until now an increase in variability in agricultural crops through the utilization of MAS can not be observed.

A third and more critical aspect is the growing distance between the main cash crops and minor crops like, e.g., peas or faba beans. At the moment MAS is used to improve the main crops while minor crops are increasingly neglected. It remains open whether the minor crops will benefit from the progress made in the main crops in the future or if they will continue to loose importance. For grapes, however, for which sequence

information has been available since 2007, it was stated that all developments the main crops have gone through, now happen for grape at high speed. Nevertheless, in the case of minor crops such as peas or faba beans it is feared that they might be outdistanced completely because of their low economic value.

It remains open whether the widening gap between major and minor crops is mainly due to MAS or if it is caused by other technological progress in breeding (e.g. double haploids, DH) as well. However, DH lines can not be produced for all crops while e.g. tilling, the induction of mutations, is possible in most crops.

Future relevance of MAS

When asked what is projected for the next five years regarding the application of molecular markers, breeders did not point out precise aims. Altogether an increase in the application of markers is assumed, maybe also covering complex traits to a higher extent.

Regarding the impact of MAS on breeding in ten years time an increase in relevance and application is unanimously expected by all respondents. Especially the increased application of SNPs and improved technologies for sequencing will contribute to an increasing impact of MAS. Some respondents also expect that MABC strategies will gain importance and that field trials will not be replaced but complemented by the application of markers.

Again, the increase in importance of MAS is not expected to be the same for all crops. For high value crops it is expected that all developments in human genetics will be transferred with a time lag of three to five years. In contrast, for wheat and barley it is very unlikely that such developments will gain any relevance in the breeding process.

Limitations of marker application

Based on the different working areas of the respondents, potential limitations that might restrict the application of MAS in breeding were judged differently. The majority of the respondents considered high costs and availability of suitable markers as limiting or even very limiting. None of the respondents considered MAS less efficient compared to phenotypic selection. On the contrary, especially in breeding of bi- or perennial crops markers were expected to lead to a high efficiency gain.

Tab. 4: Assessment of potential limitations restricting the application of MAS.

Limitation	No. of responses[a)]				
	Rating[b)]				
	++	+	0	-	- -
High costs of MAS	4	1	2	1	1
Availability of suitable markers	2	2	5	0	0
No higher efficiency compared to classical (phenotypic) selection methods	0	0	3	2	4
High number of traits to select for	1	1	2	1	4
Genotype by environment interactions, epigenetics	0	0	1	3	5
Availability of suitable genome and/or marker databases	1	2	4	2	0
Availability of suitable software	0	1	1	3	4
Patent right, intellectual property rights	0	1	2	2	4

[a)] Not all 14 interview partners responded to all questions

[b)] ++ = applies fully, i.e. very limiting -- = does not apply, i.e. not limiting

Some breeders consider it a limitation if a high number of traits are to be selected for, others do not see this as a problem at all. While the limitations of GxE and epigenetic interactions were generally not seen as limiting in the interviews, participants at the workshop stated that such interactions can be extremely cumbersome in marker development because they impede the detection of marker-trait associations.

In addition, recombination can be limiting. Only when a marker system is well

established, GxE interactions do not pose problems and the application of robust molecular markers in the selection can be of great advantage to get around GxE.

In the interviews the respondents regarded the availability of suitable databases but not the availability of suitable software as limiting to some extent, while participants of the workshop remarked that the software available for marker development is not sufficient. Depending on the scope of application, shortcomings are possible. Also data management could also become a problem in the future, as the evaluation of data points for application in practical plant breeding has to happen within a narrow time frame between harvest and sowing.

In the interviews, only two respondents considered patent rights and IPR as a limitation. However, the ratings in Tab. 4 were questioned in the workshop because the matter is very complex. For plant breeders it is hardly possible to get into it in the required detailedness, thus advisory service is needed. Reacting to this, the "Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V." (GFP), an organization which among other things co-ordinates joint plant breeding research projects, has established the "GFP-Patentstelle" at the GVS (Gesellschaft für Erwerb und Verwertung von Schutzrechten mbh). The GVS conducts patent investigations for breeding companies and organizes utilization concepts for joint research projects organized by the GFP. In addition, the "Pflanzen Innovations Agentur" (PIA), an independent organization, supports all project partners involved in the German genome program GABI in working out and realizing IPR protection and utilization strategies.

Impact of genomic selection on marker application

The interview partners were asked to which degree plant breeding has benefited from the developments and findings in plant genome research of the past 10-15 years and what can be expected from the methods of genomic selection in the future.

The replies were unanimous that such approaches are very new and have not yet been applied to practical plant breeding. Some of the respondents expect that genomic selection will gain importance in the future at least in certain areas and hope to benefit from it considerably. Others clearly relate genomic selection to the "fine arts" and point out that such technologies would first have to prove their usefulness. Current examples of successful application are very few up to now and possibilities for application in practical plant breeding very limited.

However, several persons indicated that they have already benefited from the advances in sequencing.

Exploitation und utilization of plant genetic resources through MAS

In this paper the term plant genetic resources comprises unadapted gene bank material (PGR) and wild relatives (CWR).

Molecular markers are hardly used to exploit PGR/CWR. Respondents confirmed, however, that markers offer the opportunity to utilize PGR more effectively and to increases the utilization of PGR.

As markers facilitate the introduction of fragments from wild species, which can be especially important in resistance breeding, it is expected that markers will increase the utilization of PGR. One of the respondents pointed out that molecular markers are crucial when intercrossing PGR. Decent yield levels as required for European production systems can not be achieved without MAS. Besides, some PGR are very badly adapted, thus showing hardly any results when grown for phenotyping and recessive traits are very unlikely to be found when looking at the phenotype. In variety development PGR are currently used very little. This might change, however, as backcrossing programs are becoming easier through the application of markers.

Two of the respondents use introgression lines (IL) in their breeding work. Breeding companies investing in the exploitation of PGR normally work together with public research institutes. The participants of the interviews and of the workshop assured that pre breeding activities can not be carried out in small- and medium-sized companies. Such activities belong into the hands of public research institutes (universities, JKI, MPI, IPK). Despite the fact that pre breeding programs are crucial for plant breeders, pre breeding programs have been reduced worldwide during the past years, which is seen with great concern. The use of PGR poses higher risks to breeders and must therefore be carried out in public institutes – at least for the self-pollinating crops and minor crops with low economic value.

Even if the use of PGR in variety development is very limited, there are a few successful applications in resistance breeding, such as

- Apple – wild relatives (fire blight resistance)
- Barley – *Hordeum bulbosum* (various resistances)

- Maize – landraces
- Canola – secondary gene pool
- Rye – Iranian land population (restorer gene)
- Grape – wild relatives (resistances)
- Wheat – *Aegilops ventricosa* (*Pseudocersosporella* resistance)
- Sugar beet – wild relatives

Upon further enquiry, two of the respondents gave their view on the question whether MAS generally leads to an increase in diversity or whether a further narrowing of the variety spectrum is to be expected. It was stated that breeders decide themselves whether they want to maintain and increase diversity or not. A loss of diversity can be detected through monitoring of the breeding material; which conclusions are drawn from such monitoring is up to every breeder himself. Canola was mentioned as an example. Its gene pool was narrowed twice when varieties were selected for the absence of erucic acid and glucosinolate. Today, the genes for erucic acid and glucosinolate content are known and in the secondary gene pool material without the undesired traits can be selected. Thus there is the possibility to either establish a new gene pool or select in the existing one. It was also said that markers can be used to identify genetic diversity. If it is a breeding aim to increase genetic diversity, markers can be very helpful in doing so.

When asked, how the utilization of PGR could be improved, the respondents pointed out the importance of high quality phenotypic and genotypic characterization of material stored in gene banks. New and extended methods for phenotyping, allowing high throughput phenotyping are required. Moreover, the documentation of PGR would have to be improved considerably. Crop specific databases are needed, providing data relevant for breeders which are consistently comparable no matter who collected the data.

Last but not least, pre breeding needs a change of image. Its importance in ensuring genetic diversity needs to gain more public and political awareness as pointed out before.

MAS and genetic engineering

The respondents were asked which potential plants might have that were conventionally bred with the assistance of markers compared to transgenic plants. They were also asked if MAS plants could replace some transgenic approaches due to their higher public acceptance.

Again, the answers were relatively consistent. It was pointed out that MAS and genetic engineering can not be seen as competing methods. Decisions for or against one or the other are made as the case arises and it is decided for every breeding aim anew which method is more suitable in the particular case.

MAS can be applied where genes from the primary, secondary or tertiary gene pool are used. Provided that genetic variation for the desired trait can be found in the breeding material available, markers can be used for selection purposes. If this variation is not available, transgenic approaches can not be substituted.

It was pointed out that the potential for recombination is incomparably higher in plants that were bred using MAS than in transgenic plants. Transgenic plants carry the transgene normally isolated, without a homologous allele. When choosing the transgene, the breeder's decision is made once and for all and the transgenic characteristic can not be changed or modified during the breeding process or after release. In contrast, when selecting with the assistance of markers, breeders have the possibility to react on changing conditions during the breeding process by changing the selection criteria.

The situation is different for vegetatively propagated crops. Here MAS and genetic engineering have to be understood as entirely independent approaches. In this context MAS always includes new crossing and selection from the progeny while transgenic approaches allow for the improvement of an existing variety, e.g. in terms of resistance characteristics. Regarding the example of grape it was said that MAS indeed allows the purposeful breeding of a new and improved variety carrying new resistances. However, such a variety will get a new variety name requiring a tedious introduction on the market. In the case of grapes particular varieties are demanded and transgenic approaches may clearly be of advantage provided taste is not affected.

Breeding is seen by the experts as a "compromise in time and space". Time is an important factor in plant breeding. Certain aims, which can be achieved using MAS might be reached faster using transgenic approaches. However, in the case of minor

crops transgenic approaches can also be very time consuming. Altogether, transgenic approaches are mostly used in resistance breeding while they are less suitable for improving yield characteristics. It was pointed out that intensified research in the area of meiotic recombination is needed, if it is the wish to continue breeding work with MAS. Ideally one would be able to control meiotic recombination. However, for the next few decades this will probably remain a dream.

7 MAS and transgenic crops

Molecular marker technologies and the development of transgenic plants are the two major areas of today's molecular breeding strategies (GUPTA et al. 2001). When used as a strategy on its own, MAS relies on the primary and secondary gene pool and meiotic chromosome recombination (DUBCOVSKY 2004). All genes reside in their natural chromosomal location, thus minimizing the possibility of gene silencing or other epigenetic interactions. In addition, MAS traits are truly allelic.

In contrast to MAS, with genetic transformation genes and regulatory elements are removed from their genetic environment and mostly derive from totally unrelated species. The genetic elements can be manipulated and altered before they are newly combined and transferred into a new context to produce a so called genetically modified organism (GMO) or transgenic organism. The introduction of genes from unrelated species is a characteristic attribute of gene transformation and cannot be achieved with MAS (MÜLLER-RÖBER et al. 2007). However, only one or a few genes can be transferred by genetic transformation, and neither the number of copies nor the position of the transgenes introduced into the recipient genome can be regulated. Also, there are no different alleles of GMO traits.

Marker technology is an indispensable tool when producing GMOs as markers are needed to identify and locate the transgenes within the GMOs (BARR et al. 2000). Also, MAS and GM approaches can be combined by using MAS to identify recombinants with transgenic traits. Often, these are disease or pest resistances (WALKER et al. 2002; DATTA et al. 2002; NARAYANAN et al. 2004).

However, some of the main agricultural breeding goals like yield, stress resistance or long-term disease protection are quantitative traits that require the combination of several to many genes. The targeted recombination of a multitude of genes can be done efficiently with MAS. Computer based crossing schemes can greatly reduce the number of crosses needed (SØRENSEN 2009) and MAS can help in the early detection

of desirable recombinants which would go undetected based on the phenotype alone. Thus "MAS can accomplish what would be very difficult to achieve using a transgenic approach" (STAFFORD 2009).

Some goals that have been reached through a transgenic approach can also be achieved through MAS. An example is the so called "Golden Rice", a genetically modified rice variety containing high β-carotene levels as an approach to address dietary vitamin A deficiency in the developing world. Through association mapping YAN et al. (2010) identified favorable alleles in maize that lead to an increased β-carotene content in maize grains. These alleles were introgressed via MAS into tropical maize germplasm adapted to developing countries. Other examples are the non gm maize variety "Sunrise"[7] which is – similar to some of only recently authorized gm maize lines[8] – resistant to the coleopteran pest the western corn root worm *Diabrotica virgifera* and a non gm potato variety[9] that produces nearly pure amylopectin like the gm potato Amflora[10]. Thus, to increase the β-carotene content in cereal grains, to alter the starch composition in potatoe or to confer pest resistance in maize transgenic approaches are no longer mandatory.

GMOs are facing several legislative constraints due to biosafety concerns and in case of the EU also ethic considerations[11]. The introduction of varieties obtained through MAS is not subject to such restrictions. As MAS does not necessarily include genetic engineering the thought that it will not be subject to public distrust as are GMOs (MÜLLER-RÖBER et al. 2007) is justified. Organizations that criticize genetic engineering seem to accept MAS to a large extent (THEN 2005). In several publications the hope is expressed that by means of MAS products of modern biotechnology can be introduced into the market without experiencing the skepticism transgenic crops are facing worldwide (GUPTA et al. 2001; DUBCOVSKY 2004).

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[7] http://www.saaten-union.de/index.cfm/nav/407/article/3201.html [9 March 2010].

[8] E.g. lines 59122, MON88017, MON863 and MIR604.
http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

[9] http://www.fraunhofer.de/en/press/research-news/2009/12/super-potato.jsp [9 March 2010].

[10] Line EH92-527-1.
http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=39

[11] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:106:0001:0038:EN:PDF [7 January 2010].

However, breeders clearly refuse to separate MAS and GMO strategies as the decision for or against genetic engineering is often made as the case arises (see also chapter 6). Thus, some feel that the distinctions between MAS and GMOs are in some cases deliberately blurred by the big biotechnology companies (STAFFORD 2009). For instance the soybean cultivar 'Vistive', developed by Monsanto with low levels of linolenic fatty acid is being promoted as being "produced through conventional breeding", because the trait of reduced linolenic acid content was obtained by MAS. Nevertheless, the cultivar 'Vistive' also contains a transgene conferring resistance to Roundup (CUMMINS 2005).

8 Agricultural biodiversity, molecular markers, and legal matters

Biodiversity was defined by the 1992 Convention on Biological Diversity (CBD) as "…the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems."[12]. The term "agrobiodiversity" refers to "all crops and livestock, their wild relatives, and the species that interact with and support these species: pollinators, symbionts, pests, parasites, predators and competitors" (QUALSET et al. 1995). Under this term falls the diversity of organisms in agro-ecosystems and the diversity of such systems themselves. Biological diversity can be discussed at three different levels: genetic diversity (i.e. diversity within species), species diversity and diversity of ecosystems (HAMMER 2004).

Overall, there is great concern about the loss of agricultural biodiversity. Breeding has had major impacts on the diversity of agricultural crops. In this context it is therefore important to assess the potential impacts of marker technologies on genetic diversity. However, while the loss of agricultural biodiversity is of great concern, factors other than breeding are also important in this context. These include general agricultural developments such as mechanization and industrialization and also legal matters. In this chapter, we will briefly discuss the development of within-crop diversity over time, some of the consequences of genetic uniformity; strategies that have been developed to increase within-crop diversity and the potential use of markers in this context are also discussed. Along with agricultural developments since the early 20th

[12] www.cbd.int/biosafety/protocol.shtml [11 December, 2009].

century legal matters around the seed sector have evolved. Consumer protection, breeders' rights, and commercial considerations regarding intellectual property rights are issues there. While attending to these needs, seed legislations have had a major (maybe *the* major) impact on agricultural biodiversity. Therefore, seed legislations, their potential impact on biodiversity and potential alternatives to the current rules allowing for the maintenance and possibly increase of agricultural biodiversity are summarized in chapter 8.4. A more complete description of the legal issues can be found in Annex C.

8.1 Genetic uniformity in agricultural crops

Since the early 20th century, when modern plant breeding started, the trend has been towards an increased use of genetically uniform varieties in agriculture. The breeding for high quality and high yielding varieties that thrive under conditions, where high fertility of the soil is provided by mineral fertilizers and where diseases, pests and weeds are restricted, has narrowed the genetic base of many agricultural crops (PHILLIPS & WOLFE 2005). Consequently, a minimization of genetic diversity has taken place in the industrial agricultural landscapes of the 20th century (HAMMER 2004). The first beginnings of domestication caused a "bottleneck" and strongly limited genetic diversity. There is discussion, if this initial reduction in diversity was followed by a state of maximal genetic diversity, including large numbers of locally adapted landraces or not.

The concern that intense agronomic improvements lead to erosion of genetic diversity has been raised by many scientists (e.g. TANKSLEY & MCCOUCH, 1997) and indeed diversity was lost due to the spread of modern, commercial agriculture and changed management intensity (FOWLER & MOONEY 1990). For example, the use of short-strawed varieties led to an almost entire displacement of longer-strawed varieties within just a few years (HAMMER 2004). On the whole, substitution of local varieties through newly bred varieties can be seen as one of the main reasons for genetic biodiversity loss (FAO, 1996[13]). Many modern cultivars are derived from crosses between genetically related modern varieties while wild relatives are rarely included in crossings (FU 2006). By doing this over several generations, the genetic base of the breeding material will eventually narrow down (LABATE et al. 1999; DE KOEYER et al.

[13] http://www.fao.org/ag/AGP/AGPS/Pgrfa/pdf/swrshr_e.pdf [7 January 2010].

1999; YU & BERNARDO 2004; HINZE et al. 2005).

8.1.1 Consequences of genetic uniformity

The lack of diversity often results in a high vulnerability of crops to environmental stress, e.g. epidemic diseases and pest outbreaks. The Irish potato famine of 1845-1847 is an especially tragic example. All Irish potatoes were the progenies of just two separate South American introductions, which carried no late blight resistance (HAWKES 1983). Consequently, when the late blight pathogen (*Phytophthora infestans*) was introduced to Europe in the 1840s it caused almost complete crop losses (LARGE 1958). Another example is the more recent epidemic of southern corn leaf blight (SCLB) (*Helminthosporium maydis*) in the USA in 1970. About 85 % of the maize grown in the USA at that time was of hybrid origin and based on the Texas cytoplasmatic male sterile line. Unfortunately, the mitochondrial DNA of that line carried closely linked to the male sterility gene the susceptibility to *H. maydis* race T (HARLAN 1972). The year 1970 was unusually wet, providing favorable conditions for a rapid development of SCLB and the pathogen encountered susceptible hosts in virtually all maize fields. This led to a total loss in parts of the southern USA and in other regions yields were reduced by an average of 50 % (ULLSTRUP 1972).

8.1.2 Biodiversity as a buffer against catastrophic losses

Biodiversity which acts as a buffer against various biotic and abiotic stresses e.g. pests and diseases, weeds, cold, heat, drought, and soil variability is referred to as functional diversity (SCHMIDT 1978). The mechanisms by which functional diversity affects diseases, pests, weeds, and plant nutrition are numerous. Growing susceptible and resistant plants together increases the distance between susceptible plants. Also, resistant plants may serve as barriers to pathogen spread. In addition, induced systemic resistance or microbial interactions in the phyllosphere, rhizosphere, and endosphere may all contribute to disease reduction. Insect pests can be reduced by the enhancement of natural enemies, reduction of host density, or alteration of host quality. Plant diversity may also reduce bare soil due to layering of the canopy, thus leading to increased competition with weeds for light, water and nutrients and improving soil and water conservation. One of the main advantages of diversity within and among crops is yield stability, i.e. reliably high yields. Thus, variations in yield due to environmental variation, such as year, soils, pests, or disease are usually greatly reduced in diversified stands(FINCKH et al. 2000).

8.2 The use of markers for the assessment of diversity in modern crop varieties

A precise evaluation of the situation regarding agrobiodiversity is difficult because systematic comparison studies are missing (HAMMER 2004). Also, the debate over the extent of diversity loss in modern crop varieties has been befogged by a lack of reliable data (VELLVÉ 1993). There even exists an argument for the role of plant breeding in widening crop genetic diversity (WITCOMBE 1999). Molecular markers can provide new insights into questions regarding the loss of allelic variation through modern plant breeding practices.

Markers have been used in several studies to test the hypothesis that modern plant breeding has narrowed crop diversity over time, and to document uniformity and diversity in crops. To examine changes in diversity over time in an objective manner, AFLPs or microsatellite markers appear most useful (DONINI et al. 2000). As AFLP markers tend to be genetically clustered and thus not always provide the required genome coverage that is needed for diversity analyses, in most studies microsatellite markers are used with variable results (Tab 5). Some studies confirm the often stated loss of genetic diversity, others cannot find such tendencies. Of the 17 studies surveyed here, seven show a decrease in crop genetic diversity, five do not state any decrease, and four studies detected both decrease and increase, depending on the period of time studied, the loci or the subgroup of varieties studied. In one study, an increase in genetic diversity is stated.

Apparently, breeding sometimes results in qualitative rather than quantitative shifts in diversity over time. It often seems to be mostly the composition and occurrence of alleles that changes and not the number of alleles. As genetic improvement normally targets genes encoding specific traits that are located on specific chromosomal segments, it is possible that plant breeding only affects certain regions of the plant genome. Thus, allelic diversity changes at particular loci might be substantial at individual chromosomal segments (FU 2006). At the same time, on average the genome-wide reduction of genetic diversity due to breeding can be minor. It is important to be clear that while it might seem contradictory to be worrying about saving “old genes” in today’s world it is not so much the genes themselves we are in fear of loosing but rather the information they encode in all their combinatorial complexity (MCCOUCH 2004). In summary, assessment with molecular markers provides evidence that modern plant breeding often has a negative impact on crop genetic diversity.

8.2.1 Potential effects of MAS on the diversity of modern crop varieties

It is an often expressed concern that the application of MAS might reduce genetic diversity within crops (LAMMERTS VAN BUEREN et al. 2010). „The risk most frequently raised for MAS is the temptation to use only parents, for which either markers and/or polymorphic markers exist, thus further narrowing genetic diversity within breeding programs. In particular, this may concentrate the use of a few, well-characterized disease resistance genes to the exclusion of less well documented sources" (LANGRIDGE & CHALMERS 2005). Regarding breeding strategies there are also concerns, because a strong emphasis on markers in a breeding program might lead to focusing on the best breeding strategies based on the technology rather than the best strategy for a particular environment (BARR et al. 2000). Regarding maize it has been stated that MABC is certainly the type of MAS with the most immediate and obvious benefits (RAGOT & LEE 2007) and this statement can be extended to other crop species. Taking into account that backcrossing is a very conservative breeding strategy, it cannot be approved as the prime focus of a breeding program. A certain awareness of the breeders concerning these issues is essential in order to include sufficient diversity in MAS breeding programs (BARR et al. 2000). Overall, it cannot be said, however, that MAS per se influences crop diversity in one or the other direction (LAMMERTS VAN BUEREN et al. 2010).

8.3 Diversification strategies

Several strategies have been developed to make the benefits of functional diversity available. While cropping systems diversity is very important regarding overall resilience and stability, the focus in this report is on approaches to increase intra-varietal and intra-specific diversity.

8.3.1 Increasing inter-varietal diversity

Because of concerns about the narrowing of the genetic basis of many crops breeders are increasingly evaluating their breeding material using molecular markers (Tab. 5) and they may play an even more important role in broadening the genetic base and ensuring diversity at all levels in the future. For example, the narrow genetic base of US rice varieties poses problems in long-term yield improvements. Molecular markers were utilized to develop rational strategies to broaden the genetic basis of US rice. Markers were used to investigate the genetic diversity of 236 US rice accessions and to compare them with worldwide rice germplasm accessions. A diverse subset of 31

cultivars was identified, which can be used in the development of core collections and offers an efficient source of genetic diversity for future crop improvement (XU et al. 2004).

Programs like the Generation Challenge Program (GCP) of the Consultative Group on International Agricultural Research (CGIAR) aim at using marker technologies to explore the genetic diversity contained in PGR. In the case of the GCP the focus is on using "genetic diversity and advanced science to develop products for plant breeding programs to improve the livelihoods of resource-poor farmers in marginal, drought-prone environments"[14]

[14] www.generationcp.org [7 February, 2010].

Tab. 5: Impact of breeding on crop genetic diversity. Results of 17 studies (2000-2007) which investigated the impact of breeding on crop diversity using molecular markers.

Impact	Crop	No. of varieties/accessions analyzed	Marker	Reference
Decrease	Wheat	158 accessions of Italian durum wheat (<1915-1970 and beyond)	SSR	(FIGLIUOLO et al. 2007)
Decrease[1]	Wheat	75 Canadian hard red wheat cultivars released from 1845-2004	SSR	(FU et al. 2005)
Decrease	Wheat	253 CIMMYT and CIMMYT-related modern wheat cultivars, landraces, and *Triticum tauschii* accessions[7]	SSR	(REIF et al. 2005b)
Decrease[2]	Wheat	559 French bread wheat accessions (landraces and registered varieties) (1800-2000)	SSR	(ROUSSEL et al. 2004)
Decrease	Maize	133 maize varieties cultivated in France (1930-2001)	SSR	(LE CLERC et al. 2005b)
Decrease	Maize	85 German maize hybrids (1951-2001)	SSR	(REIF et al. 2005a)
Decrease	Oat	64 Nordic oat (*Avena sativa* L.) varieties and 17 landraces	SSR	(GRAU NERSTING et al. 2006)
No decrease	Wheat	55 UK winter wheat varieties from 1934-1994	AFLP SSR	(DONINI et al. 2000)
No decrease	Wheat	511 widely grown Central and Northern European varieties	SSR	(HUANG et al. 2007)
No decrease	Wheat	13 registered modern Canadian durum wheat varieties	AFLP	(SOLEIMANI et al. 2002)
No decrease	Barley	134 UK barley varieties (both winter and spring types)	AFLP SSR	(KOEBNER et al. 2003)

Impact	Crop	No. of varieties/accessions analyzed	Marker	Reference
No decrease	Maize	8 modern US elite inbreds and 32 historical inbreds	SSR	(LU & BERNARDO 2001)
Decrease & increase[3]	Wheat	75 Nordic spring wheat cultivars bred during the 20th century	SSR	(CHRISTIANSEN et al. 2002)
Decrease & increase[4]	Wheat	32 landraces and 166 bread wheat varieties of spring or winter type from the 19th to 21st century and Sweden, Norway, Denmark, Finland	S-SAP	(HYSING et al. 2008)
Decrease & increase[5]	Barley	49 two-rowed and 64 six-rowed winter barley cultivars	SSR	(ORDON et al. 2005)
Decrease & increase[6]	Oat	96 Canadian oat varieties released from 1886 to 2001	SSR	(FU et al. 2003)
Increase	Wheat	58 Italian and Mediterranean durum wheat accessions	SSR	(MACCAFERRI et al. 2003)

[1]About 19 % of alleles present in pre-1910 cultivars were undetected in post-1990 cultivars.

[2]Decrease of about 25 % in diversity between landraces and varieties. Comparing only registered varieties, changes in diversity were rather qualitative than quantitative, except at the end of the 1960s, when a bottleneck might have occurred.

[3]Genetic diversity increased from 1900 to 1940 and again from 1960 onwards. In between there was a loss of diversity.

[4]Diversity was reduced through selections from landraces during the early 20th century, gains in diversity from the late 1960s onwards through use of exotic germplasm.

[5]Slight decrease in the six-rowed cultivars; increase in the two-rowed cultivars.

[6]Different patterns of allelic change identified at different loci: random at three, shifting at one, increasing at two, and decreasing at five loci.

[7]Loss of genetic diversity from *T. tauschii* to landraces, and from landraces to elite germplasm. Genetic diversity narrowed from 1950 to 1989, broadened again from 1990-1997.

In this context MABC might contribute to the use of exotic germplasm. Even if wild relatives of modern crop varieties often are inferior to the modern cultivars with respect to quality or yield, they can be superior in terms of resistance. Molecular markers offer the possibility to utilize these resistances in plant breeding and have thus facilitated the large-scale cultivation of crops in disease or pest endemic regions as has been the case for bacterial blight in rice, maize, and potato or for nematodes in many crops (DWIVEDI et al. 2007).

8.3.2 Increasing intra-specific and intra-varietal diversity

Variety mixtures can increase diversity within species, while multilines and evolutionary breeding aim at diversifying varieties.

8.3.2.1 Variety mixtures

Variety mixtures are heterogeneous for resistances and homogenous for agronomically relevant traits (WOLFE 1985; MUNDT 2002). They are usually composed of two to five commercially available cultivars, each of which having inherent resistance to some of the occurring diseases. If several of the best available varieties are grown at the same time for compensation and complementation of yield and if genotypes are highly distinct, complementary effects can be observed (WOLFE 1985). As farmers cannot know in advance, which variety will yield best in a given season, mixtures are a safe bet.

Many trials have shown that in mixture yields are usually increased and generally diseases are reduced, which also can contribute to yield increases, compared to the performance of the pure stands of the varieties. As an example, FINCKH & MUNDT (1992) worked with mixtures of two to five varieties of winter wheat and found disease reductions of stripe rust between 13 and 97 % in mixtures compared to pure stands. Over 30 results of effects of mixtures on disease development have been reviewed by SMITHSON & LENNÉ (1996). From these findings it is obvious that diversity leads to disease reduction and yield increases over time in most cases.

8.3.2.2 Multilines

Multilines are mixtures of lines differing in single resistance genes. They are uniform in agronomically relevant traits (MUNDT 2002). Another definition, given by JENSEN (1965) is: “Multiline cultivars are re-constitutable composites of phenotypically

similar but genetically dissimilar lines." Two different approaches of multiline use have been described, the "clean crop" and the "dirty crop" approach. While in the clean crop approach all lines that are mixed are resistant against all occurring pathogen races, in the dirty crop approach the lines that are mixed are differential susceptible to the prevalent races. The idea behind the clean crop approach is to keep the disease out of the production system to the highest degree possible. With the dirty crop multiline approach the crop will be diseased to a certain degree under disease pressure but the disease epidemic will be slowed down (MARSHALL 1989).

8.3.2.3 Evolutionary breeding for diversity and participatory breeding

While cultivar mixtures and multilines have been very successful in increasing crop stability with respect to diseases, insect pests and abiotic stresses, they have to be remixed frequently and thus do not allow co-evolution to happen. Therefore, already in the early 20th century breeders have developed strategies that enhance and make use of evolutionary processes by creating composite crosses (CC). CC populations are populations of segregating individuals derived from intercrossing a number of parents from diverse evolutionary origins. This has been termed as "**evolutionary breeding**" (SUNESON 1956) (see Annex B for details).

Several studies have shown that CC populations adapt within a few generations to pedo-climatically and biologically diverse environments. PHILLIPS & WOLFE (2005) assume that 15 generations of natural selection are sufficient to develop CCs with a high agronomic value. Differentiation is mostly due to environmental pressures, plant-plant competition and plant-pathogen co-evolution (ENJALBERT et al. 1998).

In contrast to the more formalized approach of evolutionary breeding, **participatory plant breeding strategies (PPB)** may also contribute to an increase of inter- and intravarietal diversity. The notion of PPB is a relatively recent one, and most of the projects have started within the past 15 to 20 years, as well in public sector as in non-governmental crop improvement programs (SPERLING et al. 2001). PPB conserves and creates genetic resources in farmers' fields (WITCOMBE 1996), thus local systems in which farmers produce seeds and at the same time maintain and develop adapted varieties can be seen as the dynamic *in situ* conservation of various crops and varieties (ALMEKINDERS & ELINGS 2001) (see Annex B for details). Methods of working with farmers on the selection of segregating plant material have also proven successful (CECCARELLI 2006; GYAWALI et al. 2007).

The relevance of PPB to agricultural systems in developed countries, where external inputs such as fertilizer, irrigation and pesticides are not limited is sometimes questioned. However, more and more farmers choose to limit their inputs and rely on biological processes rather than external inputs for many reasons, including environmental and economic concerns. Under organic certification standards the options of using synthetic fertilizers and pesticides are generally not available. Varieties selected for conventional growing conditions are often not optimal for organic systems and often difficulties arise especially in high-quality seed production. European regulations require the use of organically produced seeds if the production is certified organic. However, access to appropriate seeds and varieties for organic and low-input growing conditions is hampered. Therefore, a growing number of farmers are interested in PPB approaches (DAWSON et al. 2008).

8.4 Seed systems, seed legislation and biodiversity

Two seed supply systems can be identified, the formal seed supply system and the farmers' seed supply system (LOUWAARS 2007). Formal seed supply systems are in most cases regulated by national legislation and international standardization of methodologies, operated by public or private sector specialists and involve cash transactions and large uniform quantities. Such systems are mainly organized in one direction and form a pyramid with small quantities of breeders' materials at the top and large quantities of commercial seed in the marketing channels. Formal seed systems work very effectively for most crops in industrialized countries. In contrast, farmers' seed systems are based on local seed selection alongside with crop production. In these traditional or informal seed supply systems, the farmers themselves are operating the system; the resulting diversity is based on the diversity of selection methods, selection objectives and ecosystems in which selection takes place.

The two systems have complementary roles in agricultural development and the management of plant genetic resources. It has been proposed that such different seed systems need to exist side by side, each serving the needs of different types of farmers and different types of crops. Seed systems need to be diversified by creating specific policies and supporting regulations for each, regarding each component in a diversified seed system as equally important.

In this chapter some of the most important seed certification and quality control regulations and their influence on breeding and diversity are presented in brief along

with some recent developments and alternative approaches to deal with patents and intellectual property rights. A more detailed description of the legal matters can be found in Annex C.

8.4.1 Requirements for variety registration

Variety registration and testing systems were developed in industrialized countries in the first half of the 19th century. Compulsory variety registration evolved in Europe in the first half of the 20th century under the influence of farmers associations and the seed industry itself. Europe and the USA created very different systems. The European system is mainly based on public institutions; in the USA, the market parties remained largely responsible for the voluntary variety registration. It seems inevitable for countries to have some kind of variety registration system that identifies varieties, in order to create transparent seed markets (LOUWAARS 2002a). However, it appears that current variety control systems tend to limit the number of varieties available on the market because these systems are not suited to identifying varieties appropriate for ecologically highly diverse conditions. Seed certification and quality control regulations put severe restrictions on initiatives that support farmers' seed systems as they tend to turn farmers' seed production and the exchange and sale of farm-saved seed into illegal activities (LOUWAARS 2007).

The two main requirements in the European variety registration and certification system are the "DUS" criteria (distinctness, uniformity, and stability) and the "VCU" (value for cultivation and use). **Distinctness** is usually based on a clearly visible phenotypic difference between the new variety and the most similar varieties that are commonly known. The variety has to be distinguishable by one or more characteristics. **Uniformity** means that all plants from the same batch of seeds have to be alike. It is, however, always assessed in relation to the way of propagation. For vegetatively propagated and self-pollinating plants uniformity requirements are highest. The uniformity requirements have met much criticism, mainly from developing countries, and increasingly from researchers working on broadening within-species diversity (FINCKH 2008). They fear that the broad adaptation of heterogeneous landraces might be endangered by the uniformity requirements and that the narrow genetic basis and uniformity will not allow rapid further adaptation. However, UPOV 1991 acknowledges the existence of not entirely uniform varieties in its variety definition. **Stability** of a variety is also very important in the system and required over several successive generations. If a variety or a seed sample changes

significantly in its expression of one or several traits with respect to the original description, it becomes a different variety and is not protected anymore (GHIJSEN 2002). VCU means that a variety has to offer a qualitative or technological advance when compared to other registered varieties (see Annex C for details on the EC directives).

Landraces or varieties developed by farmers through selection on-farm, or heterogeneous population varieties, which can provide better resistance to biotic and abiotic stresses, usually do not meet the DUS and VCU requirements and can therefore not be marketed within the EU. Thus, many small farmers, organic food producers and environmentalists see the current seed legislation with its original purposes, the quality and manageability of plant varieties, as obstacles in conserving and enhancing biodiversity. They criticize the EU legislation for not taking into account small farmers' needs and practices and for going against genetic diversity conservation principles in general (LOPEZ NORIEGA 2009). However, diversity is not only advantageous in low-input and organic systems. The vulnerability of highly homogeneous crops is a general problem, and diversity is generally needed as was shown, e.g., by the outbreak of southern corn leaf blight in the USA in 1970 (see also 8.1.1).

In recognition of the need to counteract genetic erosion in agricultural crops several EU directives were introduced in the past 10 years concerning field and vegetable crops legalizing to a certain extent the distribution of landraces or so-called conservation varieties (see Annex C for details on the directives and their evaluation by different stakeholders). However, seed of a conservation variety may only be produced in the region of origin, and marketing can only take place in the region of origin. Also, the quantity of seed marketed of a conservation variety is restricted to 0.3 % or 0.5 % respectively of the seed quantity used for all varieties of that species in the same year in the member state. These restrictions are a serious obstacle for the maintenance of conservation varieties. Thus, for varieties of some species the low amounts that can be produced would not be sufficient to build up the necessary market to prevent their extinction (MÜHLBAUER 2008). In addition, the spatial restrictions do not allow for the dynamic evolution and adaptation of germplasm where it is needed. Especially in times of climate change and strong environmental shifts these directives appear contraproductive and effectively prevent the targeted introduction and local adaptation of diverse materials. Whether newly bred modern landraces can be marketed under these directives too or if these directives are only helpful for the

maintenance of old landraces is not clear yet. The museum approach of supporting traditional landraces is, however, not sufficient.

8.4.2 Intellectual Property Rights, Plant Breeders' Rights, and Patents

In Europe, there is a strong relation between the seed catalogue system and intellectual property rights. In both cases, DUS testing is required and is often done by the same technical services. Most varieties registered for sale in a national catalogue are also protected by Plant Breeders' Rights (PBR). A system allowing intellectual property rights, valid throughout the Community, to be granted for plant varieties has been established by Community legislation. The Community Plant Variety Right (CPVR) is a form of intellectual property akin to a patent.

As knowledge and ideas become an increasingly important part of trade, the protection of such "intellectual property" through "intellectual property rights" (IPR) has gained importance during the past decades. IPR cover the areas of copyright, trademarks, geographical indications, industrial designs, and patents including the protection of new varieties of plants. The interest in IPR for the protection of germplasm in the seed industry is growing with the increasing investments in biotechnology (GHIJSEN 2002).

Protection of new varieties of plants happens by Plant Breeders' Rights (PBR), also known as Plant Variety Protection (PVP), and is granted to a plant variety if it is congruent with the DUS criteria (see 8.4.1 for details). International developments influence the national seed policy agendas increasingly and especially the economic agreements under the World Trade Organization (WTO) such as the TRIPs Agreement (agreement on Trade Related Aspects of Intellectual Property Right) lead to pressures to liberalize markets, including the seed market (LOUWAARS 2002b).

The TRIPs agreement has eliminated the principle of "national treatment", which means that the intellectual property of foreigners must be at least equally protected that of nationals.

The agreement explicitly allows WTO member nations to offer intellectual property rights in plants through an "effective *sui generis* system". Theoretically this provides nation states with an opportunity to shape legislation to protect the interests and needs of farmers and indigenous peoples by establishing IPR arrangements that respect and reward collective inventions. Nevertheless, in practice most nations simply adopt an existing PBR framework (KLOPPENBURG 2008). The most frequently adopted system in this context is the 1991 Convention of UPOV, the major *sui generis* system for

PVP. The original UPOV Convention from 1961 formulates two important principles: the **breeder's exemption** and the **farmer's privilege**. The breeders' exemption includes the right of anybody to use germplasm, including protected material, for further breeding. The farmer's privilege is the right of farmers to reproduce any materials, including those of protected varieties for their own and non-commercial use. Both principles reflect global practices in farmers' culture of sharing and exchanging germplasm. However, in the revised version of the UPOV convention of 1991 the breeder's exemption no longer applies in the case of so-called essentially derived varieties (EDV) and the farmer's privilege is restricted to provide a breeder sufficient possibilities to claim his rewards (see also 4.3). The farmers' privilege now requires a positive act by national authorities to permit it, on a crop-by-crop basis (LOUWAARS 2001). In some industrialized countries the right to re-use seeds or planting material on a farm has been restricted for particular crops (SALAZAR et al. 2007). Although UPOV is considered a well-balanced system for the protection of plant varieties, a solution must be found for the problem of the seed flow in informal seed systems or farmers' seed systems (GHIJSEN 2002). Strong IPRs like utility patents and PVP based on the 1991 Convention of UPOV are likely to have a direct and negative effect on the on-farm saving of seed and especially on the traditional exchange of seeds among farmers (LOUWAARS 2007).

As a reaction to the development of GM crops, Europe adopted a directive on patenting plants and animals (Directive 98/44/EC[15]) which permits the legal protection of biotechnological inventions. However, the need for patents on germplasm is debatable. Thus, a variety already covered by a PBR can not be patented. Nevertheless, a variety which includes a patented gene can be protected with a PBR too (KÄSTLER 2005). For a long time, utility patents, which are granted with respect to products or processes, were considered unsuitable for protecting living beings, however, the increasing importance of biotechnology in plant breeding has led to many patent applications and grants accompanied by some important court rulings (see Annex C).

The tightening of PBR systems affects farmers' seed practices while patents considerably affect farmers' access to seed. The patent system is not rooted in agriculture and includes neither breeder's exemption nor farmer's privilege principles

[15] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:213:0013:0021:EN:PDF [19 January 2010].

which are common in PBR legislation.

8.4.3 Legal aspects of evolutionary breeding and PPB approaches

The seed regulatory framework in most countries has been designed to serve the formal sector only, thus limiting the freedom that farmers have in handling their own seed (LOUWAARS 2007). According to ALMEKINDERS & LOUWAARS (2002) the major part of agricultural land in the world is still sown with seed produced informally in farmers' seed systems. Despite the efforts of large seed programs to replace farmers' seed systems, the aim of a formal seed sector supplying 100 % of the seed for planting is only realistic for a small number of crops and in few countries.

Cultivar mixtures and multilines aim at reducing diseases by diversifying resistance genes while keeping the crop homogeneous with regard to agronomically important traits. Evolutionary breeding approaches and PPB take one step further, aiming at diversified, adaptable populations with high resistance levels and good agronomical performance. Genetically variable materials are likely to be also useful in environments that differ from the original selection environment as their genetic diversity should buffer performance when exposed to other environments and in the case of outcrossing they can continue to evolve (DAWSON et al. 2008). Formally releasing diversified varieties or populations bred through PPB or by breeders could make such materials available to farmers outside the immediate area in which they were developed and their benefits could reach many more farmers. Unfortunately, the current variety release requirements, including the EU-Directives on conservation varieties (see Annex C), do not allow this.

Plants selected for diversified, organic or low-input agricultural systems, often fall outside the legal definition of "varieties" because they do not correspond, in legal terms, to varieties. Even if a farmers material responds to the strict criteria, it is often impossible to pay the registration costs of up to 5000 € for a vegetable variety and 15.000 € for a cereal variety (KÄSTLER 2005), as such varieties are only produced in small amounts for local farming. Finally, a registered variety is not allowed to adapt or evolve. If it does, re-registration as a different variety would be required.

8.4.4 Open-source seed systems a potential solution?

Through international agreements such as the Convention on Biological Diversity (CBD) and the International Treaty on Plant Genetic Resources for Food and

Agriculture (IT PGRFA), the global community has recognized the contribution of farmers to the maintenance of genetic resources. According to TRIPP (1997), the legal framework is seen as one of the most restricting factors that limit the access of farmers to genetic diversity. This applies to old landraces as well as to modern diversified materials. In addition, current IPR legislations are a threat to the development of diversified and/or farmers' varieties. Therefore, these legislations need re-interpretation and new forms of protection will have to be introduced to facilitate the continuing contribution of farmers to the maintenance of genetic diversity (SALAZAR et al. 2007) and the inclusion of diversity within varieties.

Recently, several researchers and persons concerned about the effects of the current legal situation on the accessibility of genetic resources have started to develop a conceptual framework based on the creative commons for genetic resources. In parallel to the software sector this has been termed the concept of open-source seeds which can play an important role in ensuring that innovations of the farmers are not enclosed and captured in proprietary right systems. The open-source concept has been a great success in software development.

A key principle in open source is that everyone is free to use a part of the commons but should not use intellectual property rights to privatize the commons. This is ensured through licenses that establish rights and obligations of the parties. Some licenses insist that persons who have improved or modified the software should make available the modified or improved version or the modifications themselves. Such a license is called a "copyleft" license. The "viral effect" of the original license is carried through to all future products that incorporate the original code (SRINIVAS 2006). A prominent example of the open-source software approach is the General Public License (GPL), promulgated by the Free Software Foundation.

The seed sector offers interesting potentials for open-source innovations. Like software developers, farmers find that their traditions of creativity and free seed exchange are being challenged by IPRs and have begun looking for ways to reassert their own norms of reciprocity and innovation (KLOPPENBURG 2008). Several scientists have started to look at the "free and open source software" (FOSS) movement as a model for development of "open source biology" practices and have suggested applying open source principles to the seed sector (SRINIVAS 2002; DOUTHWAITE 2002; KIPP 2005; VERZOLA 2005; KLOPPENBURG 2008; AOKI 2008). A BioLinux model to protect the biological commons in agricultural biodiversity, particularly germplasm and seeds has

been suggested. This model envisages that while innovators should be rewarded and are entitled to benefits, they are not entitled to enclose the commons or use intellectual property rights to block further innovations. In the BioLinux model, germplasm is akin to code, and everyone is free to use and develop varieties as long as no proprietary claims on the germplasm are made to prevent others from using it. It is expected that varieties are made available under a similar BioLinux license to others (SRINIVAS 2006).

Several efforts have been made to apply open source and copyleft principles to a variety of bioscience enterprises, e.g. mapping of the haplotypes of the human genome (International HapMap Project), drug development for neglected diseases in the global South (the Tropical Diseases Initiative), the standardization of the components of synthetic biology (BioBricks Foundation), and a database for grass genomics (Gramene) (KLOPPENBURG 2008).

In the plant breeding community itself the utility of open source methods for the seed sector has also been taken up. In 1999, Tom MICHAELS presented the idea of a new germplasm exchange agreement called General Public License for Plant Germplasm (GPLPG) as a mechanism to encourage the continued unencumbered and free exchange of plant germplasm. GPLPG can coexist with current release protocols, seed certification systems, and intellectual property protection mechanisms. Its fundamental feature is that any cultivar a breeder releases that incorporates any germplasm obtained under GPLPG, must likewise be made available to other breeders under GPLPG, and without further restriction, for use in their breeding program (MICHAELS 1999).

Through such a release mechanism he hopes to nourish the cooperative spirit in the public plant breeding community despite increasing opportunities and pressures for breeders to obtain proprietary protection. According to MICHAELS, GPLPG can be seen as an Material Transfer Agreement, which is familiar to the plant science community. As the MTA is now the standard mechanism for germplasm exchanges in universities, government agencies, and private companies, scientists and administrators are accustomed to its use. A possible role of markers here could be the prove that GPLPG material is involved.

Regulatory frameworks especially serving the informal sector of farmers' seed systems need to be developed. No all-encompassing solution is available yet, but the concept of open-source seeds and the use of molecular markers for characterizing diversified populations might present some building blocks to found new rules and standards on.

Properly deployed, the GPLPG could be an effective mechanism for creating a "protected commons" for those who are willing to freely share continuous access to a pool of plant germplasm, which is improved and distributed collectively (KLOPPENBURG 2008).

9 Concluding remarks

The central question of this survey was if molecular markers and smart breeding can contribute to the evaluation and use of plant genetic resources (PGR) and whether their application in breeding increases agrobiodiversity.

The potential of markers to better evaluate PGR is great while there is only little documentation on their actual use for PGR evaluation and use. Molecular mapping techniques like the AB-QTL method and marker-assisted backcrossing (MABC) in general can be applied for the maintenance of recessive alleles in backcrossing pedigrees and for pyramiding resistance (or other) genes. The most common use of wild relatives in plant breeding is as a source of pest and disease resistance, although other characteristics such as abiotic stress tolerance, yield increase, improved quality, and cytoplasmatic male sterility and fertility restoration also have been improved using crop wild relatives in individual cases.

The traditional approach of screening accessions from a gene bank for a clearly defined phenotypic characteristic is only successful when breeding for simply inherited traits. Many superior alleles can only be found with the help of molecular maps and QTL analysis and there MAS has in some cases been applied and certainly is superior. With MAS a much larger proportion of the genetic variation contained in PGR can be exploited for crop improvement in this way and markers facilitate the introduction of fragments from wild species. Thus, an increase in the utilization of PGR through molecular marker techniques is to be expected. This view was also expressed by plant breeders and scientists. In this context 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. Meiotic recombination approaches combined with MAS will always lead to the correct positioning of genes within the genome and allow for further crossing and selection with little risk of loosing the desired trait. In contrast, genetic transformation results in rather unpredictable integration sites, copy numbers and often spontaneous rearrangements and losses. The power of MAS would certainly be increased here through a better understanding of meiotic recombination, however. Where the desired trait for breeding is derived from non-related species, however, the limits of MAS are reached.

From the reviewed literature it is not possible to make final conclusions if and how much the application of marker technology has increased the use of PGR. The reason

for this is that scientific publications concentrate on the description of newly developed methods and application possibilities. As well for the evaluation of PGR as for the development of new varieties marker technology is only one of many tools that are being applied and will only rarely be mentioned explicitly. News releases pointing to the use of marker technology in variety development are often scant and superficial in the information they give and cannot be used as a serious addition to a scientific review. Also, they often do not differentiate between the use of marker technology and genetic transformation (e.g. in the case of soybean cultivar 'Vistive', chapter 7).

A major reason that at present molecular markers are hardly used to exploit PGR in practical plant breeding is that PGR evaluation is a pre breeding activity traditionally belonging into the hands of public research institutes (universities, JKI, MPI, IPK) in collaboration with breeding companies. They cannot be carried out in small and medium-sized companies. Resources for such activities by public institutions are constantly reduced. The PGR evaluation carried out by private companies concentrates on major cash crops such as maize or high value vegetables for which hybrid technology and patenting will allow high returns and in which these companies invest anyways. The consequence is that PGR for most species are neither being explored with markers nor with traditional methods due to a lack of public interest and thus resources. Thus, at this moment marker technology does not contribute to the evaluation and use of PGR in general and their potentials are not really being made use of. An important potential of marker technology for evaluation and use of PGR lies in their application in crops that might be developed in the future with characteristics that are interesting from an ecological or nutritional point of view such as specific amino acid production, N-fixation, stress tolerance, bioremediation properties, and, of course pest and disease resistance, among others. Clearly, such work would have to be supported by public research programs.

Plant breeding activities have had a major impact on agricultural biodiversity developments initially increasing diversity. More recently, hybrid technology, industrialization of agriculture and legal constraints rooted in plant varietal protection, among others, have contributed to a narrowing of the genetic base, however. MAS technology neither leads generally to an increase in diversity nor is a general further narrowing of the variety spectrum to be expected. Markers are first of all tools to identify genetic diversity. A loss of diversity can be detected through monitoring of the breeding material; additional variation can be introduced by MAS. Which conclusions are drawn from such monitoring is up to every breeder himself. If it is a breeding aim

to increase genetic diversity, markers can be very helpful in doing so. Thus, factors other than breeding technologies are equally important in this context. These include general agricultural developments such as mechanization and industrialization and very importantly legal matters.

The legal framework does not allow the use of diversified varieties and thus effectively impedes the maintenance of agricultural biodiversity and further evolution and adaptation of agricultural crops. Regulatory frameworks especially serving the informal sector of farmers' seed systems need to be developed. No all-encompassing solution is available yet, but the concept of open-source seeds and the use of molecular markers for characterizing diversified populations might present some building blocks to found new rules and standards on.

10 Summary

With the advent of molecular markers in the late 1970s marker-assisted selection (MAS), a breeding approach based on the genotype of plants rather than assessing the phenotype only, became possible. From the beginning, the expectations about the potentials of MAS have been huge. Nevertheless, there is little systematic knowledge about the degree to which MAS is being used in modern plant breeding programs and for what purposes.

In this report, the current "state of the art" concerning marker applications in plant breeding programs based on a literature review is presented. Potential risks of MAS are discussed along with the question, whether the technology of MAS could replace GMO approaches. The survey was complemented with guided expert interviews where breeders and scientists working within the field of marker-assisted selection were asked to which degree MAS plays a role in their breeding work. Additionally a workshop was held to discuss the findings.

While historically, domestication and breeding have greatly increased agricultural biodiversity, this diversity is eroding for the past half century with increasing speed. It has been argued that some modern breeding methods, the industrialization of agriculture and especially the current legal seed regulation framework are the main causes of this erosion.

Besides their usefulness in breeding, markers are also potent tools for the identification and study of biodiversity. Therefore, the questions arise, if and how marker technology is or could be useful in the conservation and use of biodiversity within the current legal framework. These questions are discussed in the last part of this study.

The two major applications of DNA markers in plant science today are detailed chromosome mapping and selection and introgression of both simple and quantitative traits. In addition, DNA markers are also used for germplasm evaluation, genetic diagnostics, phylogenetic analysis, study of genome organization and screening of transformants. In this review chromosome mapping is not covered; the focus is on the use of molecular markers for selection purposes.

While marker application has become an important tool in some breeding areas e.g. hybrid breeding, it is much less applied in breeding self-pollinating cereals as e.g. wheat or barley. The reason for this is that the decision for or against the application of molecular markers is solely based on economic reasons.

The breeding strategies MAS is used for most frequently are selection of simple traits or QTLs from breeding lines/populations, introgression of genes from breeding lines or wild relatives, marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), pyramiding of genes and variety distinction. Markers are also applied in germplasm storage, evaluation, and use. For broadening the genetic base of core breeding materials, diverse germplasm for crossings with elite cultivars can be identified with the assistance of markers. Current breeding objectives for which MAS is regularly utilized at least in some crops are mainly disease and pest resistances and secondarily yield improvement, quality traits, and abiotic stress resistance.

There are far more publications on the development of markers than publications announcing the successful use of MAS in breeding. At first sight it is often difficult to distinguish whether a publication is actually reporting a MAS application or if only potential MAS applications of the actual research outputs are discussed. On closer inspection, most of the studied publications related to MAS deal with the detection/development of suitable markers and related research questions rather than reporting the application of MAS in breeding programs. One could get the impression that MAS is an undying promise with very few successful applications. However, it has to be taken into account, that most applications of MAS in plant breeding programs are likely not published. In this respect interviews with experts adjusted the impression gained from a literature research.

The few documented and published releases or registrations of varieties resulting from MAS breeding programs include two low-amylose rice varieties, ‘Cadet’ and ‘Jacinto’, two Indonesian rice varieties, ‘Angke’ and ‘Conde’, with resistance to bacterial leaf blight, a white bean variety resistant to BGYMV and common bacterial blight, ‘Verano’, a leaf rust resistant wheat variety from Argentina, ‘Biointa 2004’, the soybean cultivar ‘Sheyenne’, derived from another Pioneer variety, which markers proved to be different from this variety, and an Australian barley variety, ‘SloopSA’, resistant to cereal cyst nematode. Other important examples for success in marker-assisted selection are a maize variety named “Sunrise”, with high resistance against the western corn root worm (Diabrotica virgifera) or a potato producing pure amylopectin, which is the “first product in Germany developed by Tilling that achieves market readiness”. The maize variety was developed by the German Saaten-Union, the potato was developed by German Fraunhofer researchers and is processed by Emsland group, the largest German potato manufacturer. As both examples originate from private breeding programs, they will most probably never appear in scientific journals.

Nevertheless, press reports announcing MAS-breeding projects or releases of varieties that were bred with the assistance of markers are numerous. However, in this context an extensive overview over these publications would go beyond the scope of the report.

In the expert interviews all breeders consistently pointed out the big differences regarding the crop species. While MAS is not at all applied in some breeding programs, it is an important part of the breeding process in other programs. Up to now MAS is predominantly used in hybrid breeding, especially for maize, sugar beets, canola and hybrid rye. For the other (self-pollinating) cereal crops and other minor crops MAS is applied considerably less frequent. The main reason for this is that the decision for or against marker-assisted selection is solely based on economic considerations. This growing distance between the main cash crops and minor crops like, e.g., peas or faba beans is seen as a very critical aspect.

The application of markers is seen as especially important where it can accelerate the breeding process (e.g. perennial crops) or where it allows to reach certain breeding aims which normally are very difficult or impossible to achieve (e.g. pyramiding resistance genes). It was endorsed that markers have mainly led to improvements in resistance breeding yet, with BaYMV being the prime example. The importance of markers in resistance breeding is caused by difficulties with assessments in the field.

The respondents pointed out that MAS is still predominantly used to select for monogenic traits because markers can be developed and applied much easier for mono- or oligogenic traits with high heritability than for quantitative traits. However, in many cases (e.g. breeding for resistance) it would be far more important to have markers available for QTLs. Research on this is mainly done in public research institutes and universities.

Asked for the technological approaches used in their work, the respondents mentioned several different marker technologies which are currently applied in their breeding work. Microsatellite markers are still most important but the new generation of SNP markers is clearly catching up. The AFLP marker system is only used in a few breeding programs nowadays.

Another aspect of marker application is the valorization of plant genetic resources. The value of genetic resources being stored in gene banks could possibly rise through the application of markers as markers can help to reveal the presence of traits in PGR and may permit for their efficient use in the improvement of crop varieties. However, some

respondents pointed out that until now an increase in variability in agricultural crops through the utilization of MAS can not be observed.

Although markers facilitate the introduction of fragments from wild species, which can be especially important in resistance breeding, molecular markers are currently hardly used to exploit plant genetic resources and/or crop wild relatives. Breeding companies investing in the exploitation of PGR normally work together with public research institutes. The participants of the interviews and of the workshop assured that pre breeding activities can not be carried out in small and medium-sized companies. Such activities belong into the hands of public research institutes (universities, JKI, MPI, IPK). The use of PGR poses higher risks to breeders and must therefore be carried out in public institutes – at least for the self-pollinating crops and minor crops with low economic value.

Also in variety development PGR are currently used very little. This might change, however, as backcrossing programs are becoming easier through the application of markers.

While genetically modified organisms are facing several legislative constraints due to biosafety concerns and bioethics questions, the introduction of varieties obtained through MAS is not meeting the same restrictions. Also the lack of public acceptance which GMOs frequently encounter is not observed with MAS. In several publications the hope is expressed that by means of MAS products of modern biotechnology can be introduced into the market without experiencing the skepticism transgenic crops are facing worldwide. However, there are also positions claiming that MAS, as a modern biotechnology method, has to be assessed for its biosafety in exactly the same way as GMOs. The opinion that MAS could be able to replace genetic engineering completely is strongly questioned, because gene transfer across species can not be achieved solely with MAS. Breeders clearly refuse to separate the two strategies of MAS and GMO approaches, as the decision for or against genetic engineering is often made as the case arises.

Further literature research on the topic of the loss of agricultural biodiversity showed that one of the main reasons for the loss of agricultural biodiversity lies in the legal requirement that variety registration is only possible if the variety is genetically as uniform as possible, distinguishable from others and – with the exception of hybrids – reproducible (DUS criteria). However, genetic uniformity within varieties makes these vulnerable to biotic and abiotic stress. In addition, in genetically uniform populations

evolutionary changes which are the basis of biodiversity development and maintenance are impossible. Evolutionary and participatory breeding approaches could help solve such problems by breeding for diversity. However, current European legislation does not allow the release of crops bred for diversity and thus hinders the maintenance of agricultural biodiversity on-farm and also participatory breeding approaches. With the help of molecular markers it might be possible to find methods for the definition and distinction of diversified varieties, e.g. based on frequencies rather than uniformity. In addition, issues about intellectual property rights might be tackled with some innovative schemes based on open-source biology.

11 Fazit

Diese Studie geht der zentralen Fragestellung nach, ob molekulare Marker zur Erhaltung und Erhöhung der Agrarbiodiversität und zur verstärkten Nutzung pflanzengenetischer Ressourcen (PGR) beitragen.

Wenn Genbankmaterial auf traditionellem Wege phänotypisch evaluiert wird, kann nur ein kleiner Anteil der genetischen Variation ausgenutzt werden. Viele günstige Allele können nur mit Hilfe von molekularen Markern und QTL-Analyse gefunden werden. Methoden wie die AB-QTL Methode oder markergestützte Rückkreuzung im Allgemeinen können eingesetzt werden, um rezessive Allele über Generationen zu verfolgen oder Resistenzgene zu pyramidisieren. So werden PGR in der Hauptsache auch als Resistenzquellen herangezogen. Daneben sind in einzelnen Fällen aber auch Merkmale wie abiotische Stresstoleranz, Ertrag, Qualität, cytoplasmatische männliche Sterilität und Fertilitätsrestauration durch Allele aus PGR verbessert worden.

Die traditionelle Screening Methode zur Evaluation von PGR ist nur bei der Suche nach relativ einfach vererbten Eigenschaften erfolgreich. Viele Allele von Interesse können jedoch nur mithilfe von Genkarten und QTL-Analysen identifiziert werden. Hier hat sich MAS in einigen Fällen als deutlich überlegen erwiesen. Mit MAS kann ein viel größerer Anteil der genetischen Variation erfasst werden. Ebenfalls erleichtern Marker die gezielte Einkreuzung von Eigenschaften aus Wildpflanzen. Somit ist durch die Nutzung von molekularen Markern eine vermehrte Nutzung von PGR zu erwarten. Diese Einschätzung wurde von Züchtern und Züchtungsforschern geteilt. Hier stellt bei der Nutzung des primären, sekundären oder tertiären Genpools MAS nicht nur eine Alternative zur Gentechnik dar sondern ist dieser wahrscheinlich auf Dauer überlegen. Durch meiotische Rekombination eingekreuzte und mithilfe von MAS selektierte Eigenschaften sind am richtigen Ort im Genom positioniert und werden meist stabil weiter vererbt. Im Gegensatz dazu sind die Orte der DNA-Integration und die Anzahl eingebauter Kopien bei der genetischen Transformation unvorhersagbar und gehen oft mit spontanen Neuanordnungen und Verlusten einher. Ein besseres Verständnis der meiotischen Rekombination könnte das Potenzial von MAS noch deutlich vergrößern. Wenn es allerdings um Eigenschaften geht, die von nicht-verwandten Arten eingebracht werden sollen, sind die Grenzen von MAS erreicht.

Die durchgesehene Literatur erlaubt keine endgültigen Schlussfolgerungen, ob und inwieweit Markertechnologien die Nutzung von PGR erhöht haben. Wissenschaftliche Veröffentlichungen dienen der Beschreibung von neuen Methoden und

Anwendungsmöglichkeiten. Markertechnologie ist nur eine von vielen Methoden, die eingesetzt werden, und diese werden deshalb auch nur selten explizit genannt. Pressemeldungen, die die Nutzung von Markertechnologie erwähnen, sind meist knapp und die Information ist oberflächlich. So können sie nicht ernsthaft zur Auswertung herangezogen werden. Hier wird auch oft nicht zwischen Markertechnologie und Gentechnologie unterschieden (siehe der Fall der Sojasorte ‚Vistive', Kapitel 7).

Einer der Hauptgründe, dass derzeit kaum molekulare Marker eingesetzt werden, um PGR zu nutzen ist, dass PGR Evaluation eine züchterische Vorarbeit ist, die traditionell von der öffentlichen Hand (Universitäten, JKI, MPI, IPK) in Zusammenarbeit mit Züchtungsunternehmen geleistet wird. Kleine und mittlere Unternehmen können diese Arbeiten nicht schultern, doch öffentliche Mittel für solche Arbeiten werden immer knapper. Die PGR Evalutionen der großen Unternehmen konzentrieren sich auf Cash-Crops wie Mais oder hochwertige Gemüse, in die die Unternehmen sowieso investieren und die durch Hybridtechnologie und Patente hohe Profite versprechen. Die Konsequenz ist, dass aus Mangel an öffentlichem Interesse und entsprechendem Mangel an Ressourcen die PGR der meisten Arten weder mit Markern noch mit traditionellen Methoden erforscht werden. Somit trägt MAS derzeit nicht zur Evalution von PGR bei. Markertechnologie birgt ein riesiges Potenzial bei der Evaluation von PGR von Pflanzen, die in Zukunft genutzt werden könnten für ökologische interessante Eigenschaften wie z.B. die Bildung spezieller Aminosäuren, N-Fixierung, Stresstoleranz, Bioremediation und Resistenzen gegen Schaderreger. Solche Arbeiten müssten allerdings von der öffentlichen Hand geleistet werden.

Die Pflanzenzüchtung hat großen Einfluss auf die Diversität landwirtschaftlich genutzter Kulturen gehabt. Während Selektion und Züchtung anfangs zur Erweiterung der genetischen Basis beitrugen, haben die Hybridtechnologie, die Industrialisierung der Landwirtschaft und legale Einschränkungen beim Sortenschutz die genetische Vielfalt eingeengt. MAS als eine Technologie in der modernen Pflanzenzüchtung führt per se weder zu einer Diversitätserhöhung, noch engt sie das Sortenspektrum und die genetische Basis von Arten weiter ein. Marker sind vor allem Werkzeug zur Identifizierung genetischer Eigenschaften. Ein Diversitätsverlust kann durch markergestütztes Monitoring des Zuchtmaterials entdeckt werden; welche Schlüsse die Züchter aus solchen Monitoring-Ergebnissen ziehen, liegt bei ihnen selbst. Wenn die Erhöhung der genetischen Diversität ein Selektionsziel ist, können molekulare Marker dies maßgeblich unterstützen. Das heißt, dass außer den Züchtungsmethoden andere Faktoren in diesem Zusammenhang wichtig sind. Dies sind unter anderem die

landwirtschaftlichen Entwicklungen allgemein, wie z.B. Mechanisierung und Industrialisierung, und mindestens so wichtig die gesetzlichen Rahmenbedingungen. Die Saatgut-Gesetzgebung erlaubt es nicht, diversifizierte Sorten kommerziell zu nutzen, und unterbindet damit die Erhaltung der Agrarbiodiversität und die weitere Evolution und Anpassung landwirtschaftlicher Nutzpflanzen. Ein gesetzlicher Rahmen, der den informellen Saatgutsektor der Landwirte unterstützt, ist dringend notwendig. Es gibt derzeit keine allumfassenden Lösungsansätze, aber das Konzept der open-source Sorten und die Nutzung molekularer Marker zur Charakterisierung diversifizierter Populationssorten könnten Bausteine sein, auf denen neue Regelungen und Standards beruhen könnten.

12 Deutsche Zusammenfassung

Durch die Entwicklung molekularer Marker wurde ab den späten 1970er Jahren die markergestützte Selektion (marker-assisted selection, MAS) möglich, ein Züchtungsansatz, der es erlaubt neben dem Phänotyp auch den Genotyp von Pflanzen zu betrachten. Grundsätzlich können molekulare Marker oder DNA-Marker als Kennungen verstanden werden, die mit einem bestimmten Chromosomabschnitt assoziiert sind, welcher wiederum mit einem bestimmten phänotypischen Merkmal verbunden ist. Ein Marker befindet sich entweder (i) direkt in dem Gen, das selektiert wird bzw. er ist dieses Gen selber, oder er ist (ii) mit dem Gen, das das interessierende Merkmal codiert, assoziiert. Das zweite ist der weitaus häufigste Fall. MAS kann daher als genotypbasierte Selektion bezeichnet werden, die anstatt des Phänotyps einer Pflanze Marker betrachtet, die mit diesem assoziiert sind (FOOLAD & SHARMA 2005). Oft wird für markergestützte Züchtungsansätze auch der Begriff „Smart Breeding“ verwendet, ein Akronym für „Selection with Markers and Advanced Reproductive Technologies”, das zuerst in der Tierzucht verwendet wurde.

Die Anwendung molekularer Marker hat für Züchter verschiedene Vorteile: Pflanzen können mit Markern bereits als Sämlinge analysiert werden, was eine frühe Selektion auch auf Merkmale erlaubt, die erst in adulten Pflanzen exprimiert werden. Da die genotypische Information schon vor der Blüte gewonnen werden kann, ermöglichen Marker bei rekurrenter Selektion kontrollierte Bestäubung. Merkmale, deren phänotypische Beurteilung unzuverlässig oder kostspielig ist, können mit molekularen Markern leichter beurteilt werden (z.B. Merkmale, die hohen Umwelteinflüssen unterliegen, Merkmale mit geringer Heritabilität oder einige Resistenzen). Marker ermöglichen außerdem Selektion auf der Basis einzelner Pflanzen statt ganzer Parzellen, rezessive Gene können in jeder Generation erkannt werden, homo- und heterozygote Pflanzen können unterschieden werden und bei Rückkreuzungen kann linkage drag möglichst gering gehalten werden (KOEBNER & SUMMERS 2003; EDWARDS & MCCOUCH 2007; JENA & MACKILL 2008).

Unter anderem aus diesen Gründen waren die Erwartungen an die Potenziale von MAS von Anfang an hoch. Dennoch gibt es bis heute wenige systematisch erfasste Daten darüber, wie häufig und für welche Züchtungsstrategien und -ziele MAS in der modernen Pflanzenzüchtung genutzt wird. In diesem Bericht wird – basierend auf einer Literaturstudie – der gegenwärtige Stand der Technik bezüglich Markeranwendung in Pflanzenzüchtungsprogrammen vorgestellt. Darüber hinaus

werden mögliche Auswirkungen der MAS diskutiert, wie auch die Frage, ob ein Einsatz der Markertechnologie gentechnische Ansätze möglicherweise ersetzen könnte. Die Literaturrecherche wurde ergänzt durch Interviews mit Züchtern und Züchtungsforschern aus Deutschland, die zur Bedeutung von MAS in ihrer Arbeit befragt wurden. Die Ergebnisse wurden weiterhin im Rahmen eines Expertenworkshops diskutiert.

Markeranwendungen in der Pflanzenzüchtung

Die Haupteinsatzgebiete von molekularen Markern in den Pflanzenwissenschaften sind einerseits detaillierte Genkartierung und andererseits Selektion und Introgression von sowohl monogenen als auch quantitativen Merkmalen. Daneben werden molekulare Marker aber auch zur Evaluierung genetischer Ressourcen, für phylogenetische Studien, genetische Diagnostik, zur Untersuchung von Genomaufbau oder für das Screening transgener Pflanzen eingesetzt. Dieser Bericht konzentriert sich auf den Einsatz von Markern für Selektionszwecke; auf die Genkartierung wird nicht detailliert eingegangen. Auch können nicht alle landwirtschaftlichen Kulturarten im Detail betrachtet werden. Der Fokus liegt auf den wichtigsten Kulturarten der gemäßigten Breiten, vor allem Weizen (*Triticum aestivum*), Gerste (*Hordeum vulgare*), Kartoffel (*Solanum tuberosum*), Mais (*Zea mays*), Obst und Gemüse, vor allem Tomate (*Solanum lycopersicum*), ergänzt um Reis (*Oryza sativa*) und Sojabohne (*Glycine max*).

Obwohl die Idee, markergestützt zu selektieren, schon im Jahr 1923 formuliert wurde, ist MAS ein relativ junges Feld der Züchtung und Züchtungsforschung. In den frühen 1980er Jahren wurden auf Protein-Polymorphismen basierende Allozym-Marker entwickelt, die morphologische Marker ersetzten und die Vorteile niedriger Kosten, einfacher technischer Anwendung und möglicher kodominanter Auswertung der Marker mit sich brachten. Eine grundlegende Veränderung der Selektionspraxis brachte aber erst die Einführung der molekularen Marker mit sich, da diese es ermöglichen, in einer beliebigen Pflanzenart eine hohe Anzahl an Polymorphismen über das ganze Genom verteilt zu identifizieren und deren Assoziation mit gewünschten Merkmalen für die Selektion zu nutzen, unabhängig davon, in welchem Stadium der Pflanzenentwicklung die pflanzlichen Merkmale exprimiert werden (RUANE & SONNINO 2007).

Auf die ersten DNA-basierten Marker wie restriction fragment length polymorphisms (RFLPs), PCR-basierte Marker wie random-amplified polymorphic DNAs (RAPDs),

amplified fragment length polymorphisms (AFLPs), Microsatelliten, auch simple sequence repeats (SSRs) genannt, oder single nucleotide polymorphisms (SNPs) (GUPTA et al. 2001) sind inzwischen Markertechnologien gefolgt, die das sogenannte genomics-assisted breeding (VARSHNEY et al. 2005a) oder genomic selection ermöglichen (HEFFNER et al. 2009). Genomic selection wird als eine Form der MAS beschrieben, die es ermöglicht, mittels dichter, im gesamten Genom lokalisierter Marker die gesamte genetische Variation gleichzeitig und an allen Loci zu analysieren (MEUWISSEN et al. 2001). Für die Selektion werden hier also keine ausgewählten signifikanten Marker herangezogen, sondern in einer Population alle Marker analysiert und die gesamte genetische Varianz ermittelt. Bisher ist genomic selection allerdings fast ausschließlich in Simulationsmodellen getestet worden. Im Zusammenhang mit genomic selction häufig genannte Methoden sind array mapping, association mapping und ecoTILLING.

Im Rahmen der Literaturrecherche wurden 83 Publikationen aus den vergangenen 10-15 Jahren ausgewertet, die den Einsatz von molekularen Markern in Züchtungsprogrammen darstellen. Es sollte auf diesem Wege ermittelt werden, was die Haupteinsatzgebiete von MAS sind, welche Ziele während des letzten Jahrzehnts damit erreicht wurden und auch, wo die Beschränkungen liegen. Dazu wurde die Literatur nach den Kriterien Züchtungsstrategien, Zuchtziele, Art der eingesetzten Markertechnologie, Art des Züchtungsprogramms (privatwirtschaftlich oder öffentlich), Land und züchterisches Endergebnis ausgewertet.

Von den 83 analysierten Publikationen berichten sechs von der Registrierung neuer, auf Markerselektion beruhender Sorten, 20 von der Verbesserung einer bestehenden Sorte oder der Entwicklung von Zuchtlinien. Zu den neuregistrierten Sorten gehören die zwei Reissorten ‘Cadet’ und ‘Jacinto’ mit niedrigem Amylosegehalt, die zwei indonesischen Reissorten ‘Angke’ und ‘Conde’, mit Resistenz gegen *Xanthomonas oryzae* pv. *Oryzae*, die Bohnensorte ‘Verano’ mit Resistenz gegen BGYMV und *Xanthomonas phaseoli*, die argentinische Weizensorte ‘Biointa 2004’ mit Braunrostresistenz, die Sojasorte ‘Sheyenne’, tolerant gegenüber Eisenmangel und standfest, die aus einer anderen Pioneer-Sorte gezüchtet wurde und mittels Marker von dieser unterschieden werden kann, und die australische Gerstensorte ‘SloopSA’ mit Nematodenresistenz.

Nur acht der 83 Publikationen stammen aus privatwirtschaftlichen Züchtungsprogrammen. Es wäre allerdings nicht korrekt, daraus zu schließen, MAS

würde nur in öffentlichen Züchtungsprogrammen eingesetzt. Die Veröffentlichung wissenschaftlicher Ergebnisse ist für private Züchtungsunternehmen von keiner oder nur geringer Bedeutung, während dies in öffentlichen Forschungsinstitutionen ein Hauptziel darstellt. Es lässt sich mit der Vorgehensweise einer Literaturrecherche daher nicht zuverlässig feststellen, bis zu welchem Grad MAS in privaten und öffentlichen Züchtungsprogrammen tatsächlich eingesetzt wird.

Die **Züchtungsstrategien**, für die MAS am häufigsten eingesetzt wird, sind die Selektion von monogenen Merkmalen oder QTLs aus Zuchtlinien und Populationen, die Introgression von Genen aus Zuchtlinien oder Wildarten, markergestützte Rückkreuzung, markergestützte rekurrente Selektion, das Pyramidisieren von Genen und Sortenunterscheidung. Auch bei der Lagerung, Evaluation und Nutzung pflanzengenetischer Ressourcen werden molekulare Marker eingesetzt. Um die genetische Basis von Zuchtmaterial zu erweitern, können Kreuzungen des Elitematerials mit nicht adaptiertem Material sinnvoll sein, wobei Marker die Auswahl geeigneter Kreuzungspartner unterstützen. Die **Hauptzuchtziele**, die zumindest in einigen wichtigen Kulturarten mittels Markertechnologie verfolgt werden, sind Krankheits- und Schädlingsresistenz, Ertrags- und Qualitätsverbesserung und abiotische Stresstoleranz. Dabei dominiert das Zuchtziel der Krankheits- und Schädlingsresistenzen mit 61 von 83 Publikationen deutlich. Um die genannten Züchtungsziele zu erreichen, werden mit 48 von 83 Fällen am häufigsten die Strategien der markergestützte Rückkreuzung bzw. Introgression eingesetzt.

Die hauptsächlich verwendeten **Markertechnologien** sind die Mikrosatelliten (33 von 83) und RFLPs (26 von 83). Der Einsatz von SNPs wird nur in zwei Fällen angegeben. Es muss hier aber berücksichtigt werden, dass Publikationen aus den Jahren 1995 bis 2009 ausgewertet wurden. Es ist davon auszugehen, dass SNPs stark an Bedeutung gewinnen werden. Auch die sehr große Dominanz der Krankheits- und Schädlingsresistenz bei den Zuchtzielen ist vor dem Hintergrund des Auswertungszeitraums eventuell zu relativieren.

Insgesamt ist die Zahl der Veröffentlichungen, die den erfolgreichen Einsatz von MAS in Züchtungsprogrammen darstellt, begrenzt, während wissenschaftliche Veröffentlichungen zur Entwicklung und Validierung von Markern und zum QTL mapping in großer Zahl vorhanden sind.

Diese Tatsache wird auch in der Literatur mehrfach konstatiert, z.B. SEMAGN et al. (2006), EATHINGTON et al. (2007) oder die Reviews von DWIVEDI et al. (2007) und

XU & CROUCH (2008) oder COLLARD & MACKILL (2008), die den Markereinsatz in öffentlichen und privaten Züchtungsprogrammen untersuchen und ebenfalls zu dem Ergebnis kommen, dass das tatsächliche Ausmaß der Markeranwendung in Züchtungsprogrammen noch immer den Erwartungen hinterher hinkt. Es könnte der Eindruck entstehen, MAS sei ein ewiges Versprechen mit sehr wenigen erfolgreichen Anwendungen. Es darf deshalb nicht vergessen werden, dass die allermeisten MAS-Anwendungen in Pflanzenzüchtungsprogrammen sehr wahrscheinlich nicht veröffentlicht werden.

Experteninterviews und -workshop

Neben der Literaturrecherche wurden anhand zuvor verschickter Fragebögen Telefoninterviews mit 14 ZüchtungsexpertInnen aus dem Bereich Wissenschaft und Praxis zur Bedeutung von MAS in ihrer Arbeit geführt. Die Befragten bearbeiten vorwiegend Getreide und Mais, daneben Raps und andere Kulturen. Die Bedeutung von MAS für die Züchtung hängt in hohem Maße von der Kulturart ab und reicht von nicht wichtig bis sehr wichtig. Am stärksten werden Marker bisher in der Hybridzüchtung eingesetzt. In der Mais- und Zuckerrübenzüchtung aber auch bei Raps und Hybridroggen wird ihnen bereits eine häufige Nutzung attestiert.

In der Entscheidung für oder gegen Markerselektion sind so gut wie ausschließlich ökonomische Gründe ausschlaggebend. Als besonders wichtig wird der Einsatz von Markern dort angesehen, wo er Züchtungsprozesse stark beschleunigen kann (z.B. mehrjährige Kulturen) oder wo er das Erreichen bestimmter Züchtungsziele überhaupt erst möglich macht (z.B. Pyramidisieren von Resistenzgenen).

Die **Züchtungsziele**, für die MAS von den befragten ZüchterInnen und WissenschaftlerInnen eingesetzt wird, sind – wie auch in der Literaturstudie festgestellt – vor allem Resistenzen, Pollenfertilitätsrestauration, Qualitätskontrolle in Saatgutproduktion, Sortenidentifizierung, Hybridzüchtung, Genpoolaufbau und –zuordnung, Vorhersagen zur allgemeinen Kombinationseignung, abiotische Stressresistenz und Stickstoffeffizienz. In der praktischen Züchtung werden noch immer überwiegend monogene Merkmale mittels MAS selektiert, da sich für hoch heritable, mono- oder oligogene Merkmale deutlich leichter Marker entwickeln und anwenden lassen als für quantitative Merkmale. Wichtiger wäre es aber oftmals, z.B. in der Resistenzzüchtung, Marker für QTLs zur Verfügung zu haben, woran in den universitären/öffentlichen Forschungseinrichtungen auch gearbeitet wird.

Als **Markertechnologie** werden derzeit am häufigsten Mikrosatelliten eingesetzt,

wobei SNPs aber merklich aufholen. Insgesamt werde die Bedeutung von MAS noch steigen, dies müsse aber differenziert betrachtet werden und wird vor allem für Cash-Crops erwartet, die mit einer Verzögerung von drei bis fünf Jahren alle Entwicklungen der Humangenetik übernehmen. Für Selbstbefruchter wie Weizen und Gerste werde MAS keine Bedeutung erlangen.

Dass molekulare Marker künftig eine Rolle im **Sortenschutz** spielen könnten, wird als sehr spekulativ angesehen. Um die phänotypische Beschreibung zu ersetzen, müssten Marker einfacher, kostengünstiger und ebenso verlässlich anwendbar sein, womit momentan nicht gerechnet wird. Sie könnten hingegen als zusätzliches Kriterium bei Problemfällen oder generell als Lösung für Streitfragen bei essentially derived varieties (EDV) einsetzbar sein.

Es wird angenommen, dass Marker die Möglichkeit bieten, **pflanzengenetische Ressourcen** (PGR) effektiver zu nutzen, da sie es sehr erleichtern, Fragmente aus Wildarten in Sorten einzukreuzen. Dies kann vor allem bei Resistenzzüchtungen wichtig sein. Allerdings werden Marker von den Züchtern nur wenig eingesetzt, um PGR zu erschließen. Übereinstimmend wurde betont, dass das Erschließen von PGR als pre-breeding Aktivität von mittelständischen Züchtungsunternehmen nicht leistbar ist und in die Hände öffentlicher Forschungsinstitute (Universitäten, JKI, MPI, IPK) gehört. Es wird kritisch angemerkt, dass dies nicht in ausreichendem Maße passiert und sogar eine weitere Abnahme zu befürchten ist.

Danach befragt, wie stark die Pflanzenzüchtung in den vergangenen 10-15 Jahren von den Erkenntnissen und Entwicklungen der pflanzlichen **Genomforschung** profitiert hat und was sich von den Methoden der genomic selection erhoffen lässt, antworteten die Befragten übereinstimmend, derartige Ansätze seien in der Entwicklung und noch sehr neu, bisher würden sie nicht praktisch eingesetzt. Ein Teil der Befragten geht davon aus, genomic selection werde zumindest punktuell bedeutsam werden, und hofft, stark davon profitieren zu können. Andere verweisen genomic selection in den Bereich der „schönen Künste“. Diese Techniken müssten ihre Nützlichkeit noch beweisen, bisherige Erfolgsbeispiele seien überschaubar und sie sähen nur beschränkte Anwendungsmöglichkeiten in der Praxis. Allerdings geben mehrere Befragte an, von den Fortschritten in der Sequenzierung bereits spürbar profitiert zu haben.

MAS und Gentechnik

Gentechnisch veränderte Organismen müssen aus Gründen der Biosicherheit vor einer Vermarktung auf ihre gesundheitlichen und Umweltauswirkungen überprüft und

zugelassen werden. Ethische Aspekte in Mitgliedsstaaten sollen vor einer Marktzulassung speziell berücksichtigt werden.

Sorten, die durch den Einsatz von MAS gezüchtet wurden, unterliegen diesen Einschränkungen nicht. Auch den Mangel an öffentlicher Akzeptanz, auf den gentechnisch veränderte Pflanzen in der Regel stoßen, kann man bei mittels Markertechnologie gezüchteten Pflanzen nicht feststellen. In der Literatur wird mehrfach der Hoffnung Ausdruck verliehen, durch MAS könnten Produkte der modernen Biotechnologie auf den Markt gebracht werden, die nicht die gleiche skeptische Ablehnung erfahren wie transgene Pflanzen (DUBCOVSKY 2004). Es gibt allerdings auch Stimmen, die verlangen, auf MAS zurückgehende Pflanzen müssten als Produkte der modernen Biotechnologie bezüglich Biosicherheit genauso gründlich geprüft werden wie transgene Pflanzen. Die Auffassung, MAS könnte es ermöglichen, gentechnische Ansätze komplett zu ersetzen, muss in Frage gestellt werden, da der Gentransfer zwischen Arten für einige Ziele unumgänglich erscheint. Die befragten Züchter lehnen es strikt ab, zwischen MAS und transgenen Ansätzen klar zu unterscheiden, da die Entscheidung für oder gegen einen transgenen Züchtungsansatz von Fall zu Fall getroffen wird und die beiden Methoden nicht als konkurrierend angesehen werden. Bei komplexeren und quantitativen Merkmalen könnte MAS allerdings aussichtsreicher sein als der transgene Ansatz.

Erhalt der Agrarbiodiversität, Diversitätszüchtung und molekulare Marker

Neben ihrem Nutzen für die Züchtung sind Markertechnologien auch interessante Werkzeuge für die Identifizierung und Untersuchung von Biodiversität. Während Domestikation und Züchtung von Kulturpflanzen die Agrarbiodiversität zunächst stark erweitert haben, wurde diese Diversität während des vergangenen Jahrhunderts mit zunehmender Geschwindigkeit wieder eingeschränkt.

Genetische Uniformität innerhalb von Sorten macht diese aber angreifbar gegenüber biotischem und abiotischem Stress. Darüber hinaus sind evolutionäre Anpassungen, die die Basis der Weiterentwicklung und Erhaltung von Biodiversität darstellen, in genetisch uniformen Populationen nicht möglich.

Verschiedene Strategien existieren, die die Vorteile funktionaler Diversität nutzbar machen sollen. Man kann dabei zwischen Strategien unterscheiden, die die Diversität innerhalb von Sorten erhöhen, und solchen, die die Diversität zwischen Sorten und innerhalb von Anbausystemen erhöhen. Diversität innerhalb von Anbausystemen ist notwendig, um Elastizität und Stabilität insgesamt zu gewährleisten. Bei der Diversität

innerhalb von Sorten (und Arten) geht es vor allem darum, deren Anpassungsfähigkeit zu verbessern. Marker werden heute zunehmend zur Evaluierung von Zuchtmaterial verwendet. Auf diese Weise kann eine zu enge genetische Basis von Sorten einer Kulturpflanzenart vermieden und eine Diversität auf allen Ebenen gewährleistet werden. Es ist gut möglich, dass diese Rolle der molekularen Marker in Zukunft noch wichtiger werden wird.

Um Diversität innerhalb von Anbausystemen und Sorten zu erreichen, können Sortenmischungen oder Multilinien angebaut werden, was zum Ziel hat, den Krankheitsdruck durch diversifizierte Resistenzen zu reduzieren, während bezüglich agronomisch wichtiger Merkmale Homogenität gewahrt bleibt. Ansätze der Evolutionszüchtung (SUNESON 1956) gehen noch einen Schritt weiter, indem sie heterogene, anpassungsfähige Populationen mit hohen Resistenzlevels und guten agronomischen Eigenschaften selektieren. Solche Populationen können sich – im Gegensatz zu Sortenmischungen oder Multilinien – über Generationen hinweg an die Anbaubedingungen anpassen. Es sind daher bereits in den frühen 1920er Jahren Strategien entwickelt worden, wie man sich diese evolutionären Prozesse zu nutze machen kann, indem man so genannte composite crosses (CCs), auch Evolutionsramsche genannt, herstellte. CC Populationen sind spaltende Populationen, die auf die Kreuzung mehrerer verschiedener Elternlinien unterschiedlicher Herkünfte und Eigenschaften zurückgehen und die anschließend als Ramsche weitergeführt werden.

Ein weiterer Weg zur Erhöhung der Diversität innerhalb von Sorten und zwischen Sorten können partizipative Züchtungsansätze sein. Die partizipative Züchtung kann sowohl innerhalb als auch außerhalb konventioneller Pflanzenzüchtungsansätze stattfinden und hat vor allem das Ziel, den Bedürfnissen von Landwirten in low-input Systemen wie dem Ökolandbau oder in Entwicklungsländern besser gerecht zu werden. Da Pflanzen meist unter den Bedingungen am besten gedeihen, unter denen sie selektiert wurden, sind Sorten, die für Hochertragsstandorte mit hohem externen Input entwickelt wurden, an weniger optimalen Standorten oft ungeeignet (ALMEKINDERS & ELINGS 2001; MURPHY et al. 2005). Das Konzept der partizipativen Züchtung ist relativ jung, die meisten Projekte in diesem Kontext sind in den vergangenen 15 bis 20 Jahren begonnen worden (SPERLING et al. 2001). Viel ist im Bereich der Sortenevaluierung durch Landwirte geforscht worden, ein Prozess, der im Englischen oft als „Participatory Varietal Selection“ bezeichnet wird (SPERLING et al. 2001). Aber auch Selektionen aus segregierendem Material sind erfolgreich mit

Landwirten zusammen durchgeführt worden (CECCARELLI 2006; GYAWALI et al. 2007). Solche Ansätze, die die Landwirte aktiv an der Züchtung beteiligen, werden meist als „Participatory Plant Breeding (PPB)“ bezeichnet.

Agrarbiodiversität, Sorten- und Saatgutgesetzgebung und alternative Ansätze

Einer der Hauptgründe für den Verlust von Agrarbiodiversität liegt in den gesetzlichen Anforderungen für die Sortenregistrierung. Eine Sortenregistrierung ist nur dann möglich, wenn eine Sorte so einheitlich wie möglich ist, unterscheidbar von anderen Sorten und – mit der Ausnahme von Hybridsorten – reproduzierbar (DUS-Kriterien). Die Europäische Sorten- und Saatgutgesetzgebung erlaubt daher das Inverkehrbringen heterogener Sorten und/oder Populationen nicht und verhindert damit die Erhaltung von Agrarbiodiversität on-farm oder in partizipativen Züchtungsansätzen.

Genetisch diverses Material, seien es Multilinien, Sortenmischungen, samenfeste Sorten oder Synthetics, das entweder in partizipativen Projekten oder von Züchtern entwickelt wurde, verspricht aber durch seine Diversität und Anpassungsfähigkeit auch in anderen Umwelten als der ursprünglichen Selektionsumgebung gute Anbaueignung (DAWSON et al. 2008). Würde solches Material also offiziell vermarktet werden, könnten die Vorteile der heterogenen Sorten auch von Landwirten in anderen Gegenden genutzt werden, die an der Selektion nicht selber beteiligt waren. Besonders in der ökologischen Landwirtschaft oder in anderen Anbausystemen, in denen biotische und abiotische Stressfaktoren wie Krankheitsdruck, Nährstoff- oder Wassermangel nicht oder nur zum Teil ausgeglichen werden können, wären genau solche Sorten erforderlich. Doch im Rahmen der gegenwärtigen Saatgutgesetzgebung ist es unmöglich, Sorten anzumelden, die auf Anpassungsfähigkeit selektiert wurden (DAWSON et al. 2008). Mit dem Einsatz molekularer Marker könnte eventuell ein Weg gefunden werden, wie heterogene Sorten beschrieben und unterschieden werden können, so dass Uniformität kein zwingendes Kriterium bei der Sortenregistrierung mehr darstellen würde.

Fragen des Sortenschutzes könnten allerdings auch mit innovativen Entwürfen, wie z.B. der open-source Biologie, angegangen werden. Dieser Begriff ist in Anlehnung an das open-source Konzept im Software-Bereich entstanden. Grundprinzip der open-source Idee ist, dass jeder berechtigt ist, die Software als Allgemeingut zu nutzen. Auch Veränderungen und Weiterentwicklungen sind jedem erlaubt, nicht aber die Privatisierung und kommerzielle Nutzung z.B. durch Patentanmeldung im Rahmen gewerblicher Schutz- und Urheberrechte. Dies wird gewährleistet, indem die Software

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Verschiedene Wissenschaftler sehen die FOSS-Bewegung (free and open source software movement) als Modell für die Entwicklung von open-source Biologie Praktiken, die sich auf den Saatgutsektor anwenden ließen (z.B. DOUTHWAITE 2002; KIPP 2005; KLOPPENBURG 2008; AOKI 2008). Bei SRINIVAS (2006) wird ein BioLinux-Modell vorgeschlagen, mit dem die Allgemeingüter Agrarbiodiversität, pflanzengenetische Ressourcen und Saatgut geschützt werden könnten. Dieses Modell sieht vor, dass Innovation zwar honoriert und entgolten wird, es aber nicht möglich ist, durch gewerbliche Schutz- und Urheberrechte weitere Innovationen zu blockieren. Im BioLinux-Modell entsprechen genetische Ressourcen dem Quellcode einer Software, und jedermann ist berechtigt, diese zu nutzen und Sorten zu entwickeln, solange keine Urheberrechte andere von der Nutzung ausschließen. Neu- und Weiterentwicklungen müssen immer unter einer ähnlichen BioLinux-Lizenz verfügbar gemacht werden. Die konkreten Möglichkeiten zur Umsetzung solcher Strategien, einschließlich ihrer Durchführbarkeit, Finanzierbarkeit und der rechtlichen Voraussetzungen, sollten unbedingt im Detail beleuchtet werden. Sie könnten neue Wege für die Diversitätszüchtung eröffnen und ermöglichen, dass sich Agrarbiodiversität in Reaktion auf sich ändernde Umweltbedingungen durch eine natürliche Selektion in möglichst vielen Umwelten entwickelt.

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14 Annex

Annex A. Examples for marker applications in agricultural crops

Table A1: Examples for marker-applications in wheat breeding. (Based on an evaluation of 89 publications from 1995 to 2009, all published in scientific journals).

Wheat						
BREEDING STRATEGY	**TYPE OF BREEDING OBJECTIVE**	**BREEDING OBJECTIVE**	**TYPE OF MARKER**	**TYPE OF BREEDING PROGRAM[a], COUNTRY**	**MARKER-ASSISTED PRODUCT**	**REFERENCE**
Introgression	Disease/ pest resistance	Stacking of QTL for *Fusarium* head blight (FHB) resistance from non-adapted sources in an elite spring wheat background	SSR	U/Pr, D/AT	All three donor-QTL alleles significantly reduced DON content and FHB severity.	(MIEDANER et al. 2006)
MABC	Disease/ pest resistance	Leaf rust resistance (*Puccinia triticina*), stripe rust resistance (*P. striiformis*), leaf, stripe, and stem rust resistance gene complex	Molecular markers	U, US	Registration of five wheat isogenic lines	(CHICAIZA et al. 2006)
MABC	Disease/ pest resistance	Development of isogenic hard red spring wheat lines with leaf rust resistance	CAPS RFLP	U, US	Isogenic lines for leaf rust resistance genes in 'Express', 'Kern', and 'UC1037'	(HELGUERA et al. 2005)

MABC	Disease/ pest resistance	Resistance against cereal cyst nematode (*H. avenae*)[1]	RFLP	Pu, AU	>4000 wheat lines including advanced breeding lines and DH populations screened	(OGBONNAYA et al. 2001)
MABC	Disease/ pest resistance	Introduction of six *Fusarium* head blight QTLs, orange blossom wheat midge resistance (*Sm1*), and leaf rust resistance (*Lr21*)	SSR	Pu, CA	Successful introduction of chromosome segments containing FHB QTLs, *Sm1* and *Lr21* res. genes	(SOMERS et al. 2005)
Pyramiding	Disease/ pest resistance	Powdery mildew (*Erysiphe graminis* f.sp. *tritici*) resistance genes in elite cultivar 'Yang 158'	RFLP	Pu, CN	Gene combinations integrated into 'Yang 158'	(LIU et al. 2000)
Selection	Disease/ pest resistance	Breeding for *Fusarium* head blight resistance by selecting major QTL, *Fhb1*, for FHB resistance	SSR	Pu, US	Successful implementation of MAS for selecting lines with *Fhb1* QTL	(ANDERSON et al. 2007)
Selection	Disease/ pest resistance	Tracking of leaf rust (*Puccinia triticina*) resistance gene *Lr47*	DNA markers	Pu, AR	Registration of cultivar 'BIOINTA 2004'	(BAINOTTI et al. 2009)
Selection	Abiotic stress resistance	Tolerance to high levels of boron [2]	DNA markers	U, AU	?	(EAGLES et al. 2001)
Selection	Disease/ pest resistance	Introduction of three *Fusarium* head blight QTL into an elite winter wheat breeding population	SSR	U/Pr, DE	Lines with two introduced QTLs showed improved FHB resistance by ~40 %	(WILDE et al. 2008)

[1] Although expensive, application was considered cost-effective because of the importance of the disease and high costs and unreliability of the bioassay.

[2] Many breeding lines carry marker alleles but not the boron tolerance genes. This limits the use of MAS for boron tolerance.

Selection	Disease/ pest resistance	Resistance against *Fusarium* head blight	SSR	U, US	Development of NILs for FHB resistance QTL	(ZHOU et al. 2003a)

[a] U = University, Pr = Private breeding program, Pu = Public breeding program. AR = Argentina; AT = Austria; AU = Australia; BR = Brazil; CA = Canada; CH = Switzerland; CN = China; DE = Germany; FR = France; GR = Greece; ID = Indonesia; IN = India; IT = Italy; JP = Japan; KR = Korea (South); LB = Lebanon; MM = Myanmar; MX = Mexico; NL = Netherlands; PH = Philippines; PR = Puerto Rico; TH = Thailand; US = United States.

Note:

The use of markers in wheat breeding at the International Maize and Wheat Improvement Center (CIMMYT) was reviewed by WILLIAM et al. in 2007, indicating the generation of about 45,000 MAS data points per year between the two wheat growing seasons.

In the USA, wheat researchers and breeders from 12 public programs have established a national wheat MAS consortium (MASwheat), funded by the USDA Initiative for the Future of Agriculture and Food Systems (2001-2004) (DUBCOVSKY 2004). Information on MASwheat projects, germplasm releases and protocols used in the project are available at the project website (http://maswheat.ucdavis.edu) [26 June 2009]. The project is followed by the Wheat CAP project, the "Coordinated Agricultural Project for Wheat", funded by the Cooperative State Research, Education, and Extension Service USDA/CSREES, where about 25 public wheat breeding programs in collaboration with 4 USDA-ARS genotyping labs work on the genetic improvement of US wheat. EAGLES (2001) describes the utilization of MAS in five Australian breeding programs. However, lack of data from the groups involved hampers the evaluation of progress in the different programs.

Table A2: Examples for marker-applications in barley breeding. (Based on an evaluation of 89 publications from 1995 to 2009, all published in scientific journals).

Barley						
Breeding Strategy	**Type of breeding objective**	**Breeding objective**	**Type of marker**	**Type of Breeding Program, Country**[a]	**Marker-assisted product**	**Reference**
Introgression	Disease/ pest resistance	Transfer of resistance to cereal cyst nematode from 'Chebec' to the malting variety 'Sloop'	RFLP	Pu, AU	Release of variety 'SloopSA' in 2002	(Barr et al. 2000; Eglinton et al. 2006)
Introgression	Disease/ pest resistance	Transfer of the gene for tolerance to BYDV from 'Franklin' into 'Sloop'	PCR-based marker	Pu, AU	Improved lines	(Barr et al. 2000)
Introgression; MABC	Abiotic stress resistance	Enhancement of tolerance to boron toxicity in two-rowed barley	SSR	Pu, AU	Modest improvements in grain yield of lines carrying B tolerance genes at some toxic environments[1]	(Emebiri et al. 2009)
Introgression; MABC	Disease/ pest resistance	Resistance to Barley yellow mosaic virus I-III from donor line 'Y4'	RFLP	Pr, JP	'Mokkei 01530' is completely resistant to BaYMV I and has an acceptable level of resistance to BaYMV III	(Okada et al. 2003)

[1] Lines carrying B tolerance genes showed reduced yield in environments without B toxicity.

Introgression	Disease/ pest resistance; quality	Improvement of the rust resistance and grain protein content of the elite parent 'Stylet' [2]	SSR	Pr, AU	Production of 40 DH lines with improved rust resistance and quality	(KUCHEL et al. 2007)
Introgression	Disease/ pest resistance	Introgression of resistance to BYDV from *Hordeum bulbosum* into cv. 'Igri'.	CAPS SSR STS	Pu, DE	Fully resistant offspring carrying a *H. bulbosum* introgression	(SCHOLZ et al. 2009)
MABC	Disease resistance	Resistance to spot form of net blotch (*Pyrenophores teres* f. *maculata*) and CCN	SSR	U, AU	Line WI3586-1747, targeted for commercialization in 2006	(EGLINTON et al. 2006)
MABC	Disease/ pest resistance	QTL determining quantitative resistance to barley stripe rust (*Puccinia striiformis* f.sp. *hordei*)	RFLP RAPD AFLP	U, US	Release of variety 'Tango' with resistance to stripe rust[3]	(HAYES et al. 2003)
MABC	Quality traits	Development of high yielding NILs with traditional malting quality characteristics by transferring QTL from high yielding cv. 'Baroness' to malting barley industry standard cv. 'Harrington'	RFLP	U, US	Identification of one NIL that produced yield equal to 'Baronesse' while maintaining Harrington-like malt quality profile[4]	(SCHMIERER et al. 2004)

[2] One aim of the study was to validate the results of a previous simulation study. According to the authors, scale and cost of the backcross introgression strategy would prohibit most breeding programs from adopting the approach across all breeding populations.

[3] 'Tango' yields less than its recurrent parent and is therefore primarily seen as a genetically characterized source of resistance to barley stripe rust rather than a variety of its own.

[4] Brewers are reluctant to change malting barley cultivars, fearing altered flavour and brewing procedures. MAS offers a possibility to enhance yield without changing other characteristics.

Pyramiding; AB-QTL	Disease/ pest resistance	Stripe rust (*Puccinia striiformis* f.sp. *hordei*) resistance gene *Rspx* and 3 QTLs	RFLP STS SSR	U, US	Indication that combining qualitative and quantitative resistance in the same genotype is feasible	(CASTRO et al. 2003)
Pyramiding	Disease/pest resistance	Resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2)	RAPD SSR STS	U, DE	DH-populations carrying the pyramided resistances	(WERNER et al. 2005)
Selection	Disease/pest resistance	Screening for gene encoding resistance to BaMMV and BaYMV	STS	Pr, SE	Development and validation of an efficient MAS scheme	(TUVESSON et al. 1998)

[a] U = University, Pr = Private breeding program, Pu = Public breeding program. AR = Argentina; AT = Austria; AU = Australia; BR = Brazil; CA = Canada; CH = Switzerland; CN = China; DE = Germany; FR = France; GR = Greece; ID = Indonesia; IN = India; IT = Italy; JP = Japan; KR = Korea (South); LB = Lebanon; MM = Myanmar; MX = Mexico; NL = Netherlands; PH = Philippines; PR = Puerto Rico; TH = Thailand; US = United States.

Table A3: Examples for marker-applications in maize breeding. (Based on an evaluation of 89 publications from 1995 to 2009, all published in scientific journals).

Maize						
Breeding Strategy	**Type of breeding objective**	**Breeding objective**	**Type of marker**	**Type of Breeding Program, Country[a]**	**Marker-assisted product**	**Reference**
AB-QTL	Yield	Improved hybrid performance for yield, grain moisture, plant height	AFLP SSR	U, US	Identification of favorable QTLs	(Ho et al. 2002)
Introgression	Yield	Favorable QTL from one inbred parent into the genomic background of the other parent	RFLP	Pu, FR	Introgression of favorable QTLs for earliness and grain yield	(Bouchez et al. 2002)
Introgression, MABC	Disease/ pest resistance	Introgression of transgene construct, containing insecticidal protein genes, from transformed parent into elite inbred line	RFLP	Pr, CH & Pu, FR	Recovery of individuals which appeared to be genotypically fully converted	(Ragot et al. 1995)
MABC	Quality traits	Conversion of normal maize lines into Quality Protein Maize	SSR	Pu, IN	Converted lines had twice the amount of lysine and tryptophane	(Babu et al. 2004)
MABC	Abiotic stress resistance	Improvement of drought adaptation	RFLP PCR-based markers	Pu, MX	Best hybrids yielded under severe water stress conditions on average 50 % more[1]	(Ribaut & Ragot 2006)

[1] Under mild water stress, no difference was observed between MABC-derived hybrids and control hybrids. This indicates that the genetic regulation for drought tolerance depends on the stress intensity.

MABC	Disease/ pest resistance	QTLs for resistance to southwestern corn borer (SWCB), *Diatraea grandiosella* Dyar [2]	RFLP morpho-logical marker	Pu, US	Lines with improved resistance to SWCB	(WILLCOX et al. 2002)
MABC	Growth character	Enhancement of seedling emergence	RFLP	U, US	Three sweet corn elite inbreds enhanced with QTL from a donor inbred line	(YOUSEF & JUVIK 2002)

[a] U = University, Pr = Private breeding program, Pu = Public breeding program. AR = Argentina; AT = Austria; AU = Australia; BR = Brazil; CA = Canada; CH = Switzerland; CN = China; DE = Germany; FR = France; GR = Greece; ID = Indonesia; IN = India; IT = Italy; JP = Japan; KR = Korea (South); LB = Lebanon; MM = Myanmar; MX = Mexico; NL = Netherlands; PH = Philippines; PR = Puerto Rico; TH = Thailand; US = United States.

[2] The study was conducted to compare conventional selection and MAS. No significant differences concerning SWCB resistance were found between lines selected by the two methods.

Table A4: Examples for marker-applications in rice breeding. (Based on an evaluation of 89 publications from 1995 to 2009, all published in scientific journals).

Rice						
BREEDING STRATEGY	**TYPE OF BREEDING OBJECTIVE**	**BREEDING OBJECTIVE**	**TYPE OF MARKER**	**TYPE OF BREEDING PROGRAM, COUNTRY[a]**	**MARKER-ASSISTED PRODUCT**	**REFERENCE**
Germplasm evaluation	Disease/ pest resistance	Resistance to bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*)	SSR STS	Pu, IN	Detection of lines containing both resistance genes	(DAVIERWALA et al. 2001)
Introgression	Yield	Introgression from *Oryza grandiglumis* into *O. sativa*. Aim: QTL detection for yield	SSR	Pu, KR	no	(AHN et al. 2003)
Introgression; AB-QTL	Yield	Identification of yield-improving QTLs from *O. rufipogon*	RFLP SSR	U, US	Experimental lines carrying yield-improving QTLs	(XIAO et al. 1998)
Introgression; germplasm evaluation	Yield	Introgression of yield-related traits from *Q. glumaepatula* into cultivated rice	SSR STS	Pu, BR	*O. glumaepatula* considered as a useful source of genes in rice breeding programs	(BRONDANI et al. 2002)
Introgression; MACB	Disease/ pest resistance	Bacterial blight (BB) resistance (*X. oryzae* pv. *oryzae*) from elite restorer line of hybrid rice ('Minghui 63')	RFLP PCR-based markers	U, CN	Improved version of Minghui 63; 'Minghui 63(*Xa21*)'	(CHEN et al. 2000)
Introgression; MACB	Disease/ pest resistance	Improvement of BB resistance in elite restorer line of hybrid rice ('6078')	AFLP	U, CN	Improved version of '6078'; '6078(*Xa21*)'	(CHEN et al. 2001)

Introgression; MABC	Yield	Enhancement of yield in '9311' (parental line in hybrid rice production) by introgression from *O. rufipogon*	SSR	Pu, CN	One stable improved '9311' line	(LIANG et al. 2004)
Introgression; MABC	Quality traits	Improvement of eating and cooking quality of 'Zhenshan 97' (female parent of a number hybrids in China)	RFLP SSR	U, CN	Two improved versions of 'Zhenshan 97'[1]	(ZHOU et al. 2003b)
MABC	Disease/ pest resistance	Two QTLs for resistance to *rice yellow mottle virus* (RYMV)	RFLP SSR	Pu, FR	Lines carrying both showed partial resistance to RYMV	(AHMADI et al. 2001)
MABC	Disease/ pest resistance	Introduction of bacterial leaf blight resistance genes *xa5* and *Xa7* into IR64 background	STS	Pu, ID	Release of 'Angke' (*xa5*) and 'Conde' (*Xa7*) by the Dept. of Agriculture of Indonesia in 2002	(BUSTAMAM et al. 2002)
MABC	Disease/ pest resistance	Bacterial blight resistance	RFLP STS	Pu, PH	Lines with improved resistance	(SANCHEZ et al. 2000)
MABC	Abiotic stress resistance	Four QTLs for improvement of root morphological traits, and thereby drought tolerance	RFLP SSR	U, UK	NILs with increased root length under irrigated and drought conditions	(STEELE et al. 2006)
MABC	Abiotic stress resistance	Introgression of locus conferring submergence tolerance from cultivar 'FR13A' into 'Swarna'	Molecu-lar markers	U, US & Pu, PH	Development of two 'Swarna-*Sub1*' lines showing strong submergence tolerance	(XU et al. 2006)

[1] One concern with modifying the *Waxy* region of 'Zhenshan 97' is that the genetic background from 'Minghui 63' may decrease the level of heterozygosity, leading to a reduced level of heterosis. A thorough cleaning of the genetic background is obligatory.

MABC	Quality traits	Improvement of fragrance and amylose content in the variety 'Manawthukha' by introgression from donor parent 'Basmati370'	SSR	Pu, MM/ U, TH	Improved 'Manawthukha'	(YI et al. 2009)
Pyramiding; introgression, transformation	Disease/ pest resistance	Resistance to bacterial blight and a *Bt* gene conferring resistance to lepidopteran insects [2]	STS	U, CN	Improved version of Minghui 63; 'Minghui 63' / *Bt & Xa21'*	(JIANG et al. 2004)
Pyramiding; transformation	Disease/ pest resistance	Resistance to blast (*Magnaporte grisea*) and bacterial blight	STS	Pu, PH	Lines showing combined resistance to blast and blight[3]	(NARAYANAN et al. 2002)
Pyramiding; MABC	Disease/ pest resistance	Bacterial blight resistance in cultivar PR106	STS	U, IN	PR106 lines with pyramided resistance genes	(SINGH et al. 2001)
Selection	Disease/ pest resistance	Resistance to soybean cyst nematode (*H. glycines*)	RFLP	U, US	MAS-selected lines comparable to phenotypic selection	(CONCIBIDO et al. 1996)
Selection	Variety distinction	Identification of 43 cultivars or breeding lines of rice	SSR	U, AU	no	(GARLAND et al. 1999)

[a] U = University, Pr = Private breeding program, Pu = Public breeding program. AR = Argentina; AT = Austria; AU = Australia; BR = Brazil; CA = Canada; CH = Switzerland; CN = China; DE = Germany; FR = France; GR = Greece; ID = Indonesia; IN = India; IT = Italy; JP = Japan; KR = Korea (South); LB = Lebanon; MM = Myanmar; MX = Mexico; NL = Netherlands; PH = Philippines; PR = Puerto Rico; TH = Thailand; US = United States.

[2] *Xa21* and *cry1Ab/cry1Ac* have been individually introduced into the genetic background of 'Minghui 63' by MAS and by genetic engineering respectively.

[3] First report documenting the stacking of two major genes in rice using a combination of MAS and transformation.

Table A5: Examples for marker-applications in potato breeding. (Based on an evaluation of 89 publications from 1995 to 2009, all published in scientific journals).

Potato						
BREEDING STRATEGY	**TYPE OF BREEDING OBJECTIVE**	**BREEDING OBJECTIVE**	**TYPE OF MARKER**	**TYPE OF BREEDING PROGRAM, COUNTRY**[a]	**MARKER-ASSISTED PRODUCT**	**REFERENCE**
Introgression; MABC	Disease/ pest resistance	Broad-spectrum potato late blight (*Phytophthora infestans*) resistance derived from *Solanum bulbocastanum*	PCR marker	U, US	Several marker-positive breeding lines showed late blight resistance in the greenhouse	(COLTON et al. 2006)
Pyramiding	Disease/ pest resistance	Combination of resistances to potato virus Y, root cyst nematode (*Globodera rostochiensis*), Potato virus X , and potato wart (*Synchytrium endobioticum*)	CAPS SCAR PCR-based markers	Pu, DE	Thirty tetraploid plants showing resistance to the four diseases[1]	(GEBHARDT et al. 2006)

[a] U = University, Pr = Private breeding program, Pu = Public breeding program. AR = Argentina; AT = Austria; AU = Australia; BR = Brazil; CA = Canada; CH = Switzerland; CN = China; DE = Germany; FR = France; GR = Greece; ID = Indonesia; IN = India; IT = Italy; JP = Japan; KR = Korea (South); LB = Lebanon; MM = Myanmar; MX = Mexico; NL = Netherlands; PH = Philippines; PR = Puerto Rico; TH = Thailand; US = United States.

[1] The selected plants can be used as sources of multiple resistance traits in pedigree breeding and are available from a potato germplasm genebank.

Table A6: Examples for marker-applications in tomato breeding. (Based on an evaluation of 89 publications from 1995 to 2009, all published in scientific journals).

Tomato

BREEDING STRATEGY	TYPE OF BREEDING OBJECTIVE	BREEDING OBJECTIVE	TYPE OF MARKER	TYPE OF BREEDING PROGRAM, COUNTRY[a]	MARKER-ASSISTED PRODUCT	REFERENCE
Introgression; MABC	Disease/ pest resistance	Acylsugar-mediated pest resistance from *Lycopersicon pennellii*	RFLP RAPD	U, US	Acylsugar accumulation successfully introgressed but at levels lower than the F_1 control	(LAWSON et al. 1997)
Introgression; MABC	Quality traits	Introgression of 5 QTLs controlling fruit quality traits [1]	RFLP RAPD	Pu, FR	Development of three improved lines	(LECOMTE et al. 2004)
Introgression	Disease/ pest resistance	Blackmold (*Alternaria alternata*) resistance QTLs from *Lycopersicon cheesmanii*	RFLP CAPS	U, US	Experimental lines carrying black mold resistance QTL regions	(ROBERT et al. 2001)
MABC; Introgression	Disease/ pest resistance	Identification of QTLs for early blight (*Alternaria solani*) resistance from *Lycopersicon hirsutum*	RFLP	U, US	Identification and validation of QTLs for early blight resistance	(FOOLAD et al. 2002)
MABC	Disease/ pest resistance	Resistance to Tomato Spotted Wilt Virus (TSWV)	CAPS	U, IT	Two tomato elite lines carrying TSWV resistance	(LANGELLA et al. 2004)

[1] Aim of the study was (i) to estimate the efficiency of MABC-improved lines when used as parents of hybrids and (ii) to analyze QTLs by genetic background interactions.

Pyramiding	Disease/ pest resistance	Resistances to Tomato Yellow Leaf Curl Virus (TYLCV), *Fusarium oxysporum* fsp. *lycopersici*, *Verticillium* witl	DNA markers	U, LB	F_4 or F_5 tomato lines carrying combined resistances to all three pathogens[2]	(ATAMIAN et al. 2009)
Pyramiding	Disease/ pest resistance	Resistance to Tomato Mosaic Virus, *Fusarium oxysporum* f.s. *lycopersici*, *Fusarium oxysporum* f.s. *radicis-lycopersici,* and the nematode *Meloidogyne incognita*	CAPS RFLP	U, IT	Genotypes carrying all four resistances	(BARONE et al. 2005)

[a] U = University, Pr = Private breeding program, Pu = Public breeding program. AR = Argentina; AT = Austria; AU = Australia; BR = Brazil; CA = Canada; CH = Switzerland; CN = China; DE = Germany; FR = France; GR = Greece; ID = Indonesia; IN = India; IT = Italy; JP = Japan; KR = Korea (South); LB = Lebanon; MM = Myanmar; MX = Mexico; NL = Netherlands; PH = Philippines; PR = Puerto Rico; TH = Thailand; US = United States.

[2] The desired large fruit characteristic of the Lebanese type tomato was not yet well fixed.

Table A7: Examples for marker-applications in soybean breeding. (Based on an evaluation of 89 publications from 1995 to 2009, all published in scientific journals).

Soybean						
Breeding Strategy	**Type of breeding objective**	**Breeding objective**	**Type of marker**	**Type of Breeding Program, Country[a]**	**Marker-assisted product**	**Reference**
Introgression	Yield	Introgression of yield-enhancing QTL from *Gycine soja* [1]	AFLP SSR	Pr, US	Some individuals showed ~ 9 % yield advantage	(Concibido et al. 2003)
Introgression	Disease/ pest resistance	QTLs conditioning single and multiple Soybean Insect Resistance	SSR	U, US	Registration of four lines containing resistance QTLs	(Zhu et al. 2007)
Pyramiding	Disease/ pest resistance	Resistance to soybean mosaic virus	SSR	U, US	Lines carrying a three gene pyramid, resistant to all strains[2]	(Saghai Maroof et al. 2008)
Pyramiding	Disease/ pest resistance	Three independent loci for resistance to Soybean mosaic virus (SMV)	PCR-based, SSR	U, US & U, CN	Lines homozygous for all three SMV resistance genes	(Shi et al. 2009)

[1] There was limited adaptability of the yield-enhancing QTLs across genetic backgrounds. The yield effect could only be observed in two of six genetic backgrounds.

[2] Reactions conditioned in the isolines used for pyramiding sometimes differed from the original donor parent of the resistance gene. Pyramided lines also displayed uncharacteristic symptoms to some virus strains that differed from the reactions of the parental lines.

Pyramiding	Disease/ pest resistance	QTL conditioning corn earworm (*Heliocoverpa zea*) resistance in soybean and a synthetic *Bacillus thuringiensis* transgene from the recurrent parent 'Jack-Bt' [3]	SSR	U, GR	'Jack-Bt'-lines enriched with a QTL conditioning corn earworm resistance	(WALKER et al. 2002)
Selection	Disease/ pest resistance	Resistance to multiple nematode populations (SCN, *H. glycines*) and resistance to fungal diseases	SSR	Pu, US	Germplasm Registration, no variety protection planned	(ARELLI et al. 2006)
Selection	Disease/ pest resistance	SCN resistance	SSR	Pu, US	Registration of germplasm JTN-5303	(ARELLI et al. 2007)
Selection	Disease/ pest resistance	SCN resistance [4]	SNP	Pr, US	?	(CAHILL & SCHMIDT 2004)
Selection	Disease/ pest resistance	SCN resistance	RFLP	U, US	13 lines classified as resistant	(CONCIBIDO et al. 1996)
Selection	Variety distinction	Marker showed that 'Sheyenne', which is derived from Pioneer 9071 (never released) is different from Pioneer 9071 at certain loci.	SSR	U, US	Registration of the cultivar 'Sheyenne'	(HELMS et al. 2008)
Selection	Disease/ pest resistance	Resistance to frogeye leaf spot (*Cercospora sojina*)	SNP SSR	U, US	Registration of germplasm line S99-2281	(SHANNON et al. 2009)

[3] In this approach MAS was applied to introgress a resistance allele from a QTL with an unknown mode of action into a transgenic line.

[4] MAS has been used in the company for 10 years, especially in early generation single plant selections, which reportedly has resulted in numerous successful commercial soybean varieties, but no precise statements are available from the article.

[a] U = University, Pr = Private breeding program, Pu = Public breeding program. AR = Argentina; AT = Austria; AU = Australia; BR = Brazil; CA = Canada; CH = Switzerland; CN = China; DE = Germany; FR = France; GR = Greece; ID = Indonesia; IN = India; IT = Italy; JP = Japan; KR = Korea (South); LB = Lebanon; MM = Myanmar; MX = Mexico; NL = Netherlands; PH = Philippines; PR = Puerto Rico; TH = Thailand; US = United States.

Table A8: Examples for marker-applications in vegetable breeding. (Based on an evaluation of 89 publications from 1995 to 2009, all published in scientific journals).

Beans and other vegetables

BREEDING STRATEGY	TYPE OF BREEDING OBJECTIVE	BREEDING OBJECTIVE	TYPE OF MARKER	TYPE OF BREEDING PROGRAM, COUNTRY[a]	MARKER-ASSISTED PRODUCT	REFERENCE
Introgression; MABC	Disease/ pest resistance	Introgression of wild chromosomal regions (*Lactuca saligna*) into cultivated lettuce (*L. sativa*) [1]	AFLP	U, NL	Development of a set of backcross inbred lines (BILs)	(JEUKEN & LINDHOUT 2004)
Introgression; MABC	Disease/ pest resistance	Introgression of 4 *Phytophthora capsici* resistance QTLs into bell pepper (*Capsicum anuum*)	RAPD SCAR CAPS RFLP AFLP ISSR	Pu, FR	Introgression successful	(THABUIS et al. 2004)
MABC	Disease/ pest resistance	Resistance to *Uromyces appendiculatus* and *Colletotrichum lindemuthianum* in *Phaseolus vulgaris*	RAPD	Pu, BR	Five lines resistant to rust and anthracnose	(FALEIRO et al. 2004)
MABC	Disease/ pest resistance	Resistance to *Colletotrichum lindemuthianum* in *P. vulgaris*	SCAR	Pu, US	Registration of line USPT-ANT-1	(MIKLAS et al. 2003)

[1] *L. saligna* is an interesting resource for resistance to lettuce downy mildew (*Bremia lactucae*) and the set of BILs could be useful for future genetic studies.

MABC	Disease/ pest resistance	Common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) resistance in *P. vulgaris* [2]	SCAR	Pu, US	Registration of resistant germplasm line	(MIKLAS et al. 2006b)
MABC	Disease/ pest resistance	Common bacterial blight (*X. axonopodis* pv. *phaseoli*) resistance in dark red kidney bean (*P. vulgaris* L.) [3]	SCAR	Pu, US	Registration of resistant germplasm line	(MIKLAS et al. 2006a)
MABC	Disease/ pest resistance	Enhanced resistance to common bacterial blight (*X. campestris* pv. *phaseoli*) in Pinto Bean (*P. vulgaris* L.)	SCAR	Pu, US	Registration of germplasm line ABCP-8	(MUTLU et al. 2005)
Pyramiding	Disease/ pest resistance	Resistance to *U. appendiculatus*, *C. lindemuthianum,* and *Phaeoisariopsis griseola* into cultivar 'Perola' (*P. vulgaris* L.)	RAPD SCAR SSR	U, BR	~ 60 F_5 families, no field tests yet for productivity	(RAGAGNIN et al. 2003)
Selection	Disease/ pest resistance	Resistance to rust (*U. appendiculatus*), bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) in *P. vulgaris*	RAPD	Pu, US	Registration of line PR9357-107	(BEAVER et al. 1998)
Selection	Disease/ pest resistance	Resistance to Bean golden yellow mosaic virus (BGYMV) and QTLs for common bacterial blight resistance in white bean (*P. vulgaris* L.)	SCAR	U, PR & Pu, US	Registration of cultivar 'Verano'	(BEAVER et al. 2008)

[2] The line will be most useful for incorporating resistance to common bacterial blight in the white kidney market class, but also other large-seeded market classes and green bean of Andean origin as well.

[3] The line will be most useful for incorporating resistance to common bacterial blight in the dark red kidney market class, but also other large-seeded Andean dry and garden bean.

Selection	Disease/ pest resistance	Resistance to BGYMV in Red-Mottled Dry Bean (*P. vulgaris* L.)	SCAR	U, PR	Registration of germplasm lines	(BLAIR et al. 2006)
Selection	Yield	Improvement of quantitatively inherited yield traits in *Cucumis sativus*[4]	RAPD SCAR SSR	U, CN	Development of improved lines	(FAN et al. 2006)
Selection	Disease/ pest resistance	Tracking of QTL for common bacterial blight resistance in great northern common bean (*P. vulgaris* L.) [5]	?	U, US & Pu, US	Registration of resistant germplasm line	(MUTLU et al. 2008)
Selection	Disease/ pest resistance	Resistance to common bacterial blight (CBB) in common beans (*Phaseolus vulgaris* L.) [6]	RAPD SCAR	Pu, CA	Comparably low number of lines resistant to CBB	(YU et al. 2000)

[a] U = University, Pr = Private breeding program, Pu = Public breeding program. AR = Argentina; AT = Austria; AU = Australia; BR = Brazil; CA = Canada; CH = Switzerland; CN = China; DE = Germany; FR = France; GR = Greece; ID = Indonesia; IN = India; IT = Italy; JP = Japan; KR = Korea (South); LB = Lebanon; MM = Myanmar; MX = Mexico; NL = Netherlands; PH = Philippines; PR = Puerto Rico; TH = Thailand; US = United States

[4] A base population was simultaneously subjected to MAS and phenotypic mass selection for a comparative analysis of gain from selection MAS prove effective for selection of architectural traits, yield increased only after phenotypic selection.

[5] MAS was conducted in addition to phenotypic selection.

[6] Main objective of the study was to determine whether the markers identified in one line could be used for MAS also in a different population.

Table A9: Examples for marker-applications in apple breeding. (Based on an evaluation of 89 publications from 1995 to 2009, all published in scientific journals).

Apple						
Breeding Strategy	**Type of breeding objective**	**Breeding objective**	**Type of marker**	**Type of Breeding Program, Country**[a]	**Marker-assisted product**	**Reference**
Pyramiding	Disease/ pest resistance	Resistance to apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*)	SSR	Pu, CH	18 pyramided plants carrying resistance genes	(Kellerhals et al. 2009)

[a] U = University, Pr = Private breeding program, Pu = Public breeding program. AR = Argentina; AT = Austria; AU = Australia; BR = Brazil; CA = Canada; CH = Switzerland; CN = China; DE = Germany; FR = France; GR = Greece; ID = Indonesia; IN = India; IT = Italy; JP = Japan; KR = Korea (South); LB = Lebanon; MM = Myanmar; MX = Mexico; NL = Netherlands; PH = Philippines; PR = Puerto Rico; TH = Thailand; US = United States.

Annex B. Evolutionary and participatory breeding approaches

In the early 20th century breeders have developed strategies that enhance and make use of evolutionary processes by creating composite crosses (CC). CC populations are populations of segregating individuals derived from intercrossing a number of parents from diverse evolutionary origins. This has been termed as "**evolutionary breeding**" (SUNESON 1956).

Bulk populations created in this way can be grown in different environments and kept under natural selection for many generations and can either be used as dynamically managed plant genetic reservoirs for future breeding or directly as "modern landraces" with within-crop genetic diversity in place (MURPHY et al. 2005) for low-input cropping systems. The composite cross approach was developed in California in the 1920s by (HARLAN & MARTINI 1929). Most CCs have been made for barley (ALLARD 1961; RASMUSSON et al. 1967; MUONA et al. 1982). For wheat (*Triticum aestivum* L.) there were only a few studies carried out. One experiment was started in 1984 in France and the populations are cultivated without conscious selection at various sites in France (PAILLARD et al. 2000; GOLDRINGER et al. 2006). Another wheat CC was started in 2001 in the UK. Under the supervision of the John Innes Centre, 20 European wheat varieties were used for intercrosses in a half diallel. Bulked progenies of the three populations were distributed to France, Hungary and Germany where the populations evolve under different environmental conditions (WOLFE et al. 2006).

Participatory plant breeding (PPB) has been developed as an alternative and complementary breeding approach to conventional plant breeding. It aims at more efficiently addressing the needs of farmers in marginal areas, especially in developing countries, building on their capacity to select what best fits their environment. The formal, conventional plant breeding system generally has concentrated on the increase of yield in favorable environments where irrigation and agro-chemical inputs are available. However, as varieties typically do not perform well in environments without the external inputs which they were selected in, conventional plant breeding has often been ineffective in addressing the needs of low-input farming systems in many regions and crops (ALMEKINDERS & ELINGS 2001; MURPHY et al. 2005). On the one hand, much research has been done in evaluating varieties with farmers, a process which is

often termed **Participatory Varietal Selection** (ALMEKINDERS & ELINGS 2001; SPERLING et al. 2001). On the other hand, methods of working with farmers on the selection of segregating plant material have also proven successful (CECCARELLI 2006; GYAWALI et al. 2007). Such approaches to involve farmers more actively in plant breeding are termed **participatory breeding**.

ALMEKINDERS & ELINGS (2001) give an overview of different experiences and practices in PPB. For methods and impacts of ongoing initiatives see ALMEKINDERS & HARDON (2006). An overview over the extensive literature on PPB can be found in CLEVELAND & SOLERI (2002) and MORRIS & BELLON (2004). PPB initiatives for organic farming in Europe, e.g. in the Netherlands and in France are described in CHABLE (2005), DESCLAUX (2005) and LAMMERTS VAN BUEREN et al. (2005).

Annex C. The European seed legislation

In the European Union (EU) twelve basic Council Directives developed in the 1960s and 70s form the community legislation on seed and plant propagating material and ensure the quality and manageability of plant varieties commercialized in Europe.

The twelve basic Council Directives are structured into one horizontal Directive on the Common Catalogue of varieties of agricultural plant species and 11 vertical Marketing Directives. These are six Seed Directives (for fodder plant seed, beet seed, cereal seed, seed potatoes, seed of oil and fiber plants and vegetable seed), four Plant Propagating Material Directives (vine and fruit propagating material, vegetable other than seed and ornamental plants) and one Forest Reproductive Material Directive.[1]

Council Directive 2002/53/EC[2] on the Common Catalogue of varieties of agricultural plant species and Council Directive 2002/55/EC[3] on the marketing of vegetable seeds might be the most important in the context of variety registration as they lay down the legal basis for the registration of agricultural crop varieties and vegetable varieties in the European and National catalogues.

The European Common Catalogue and the National Catalogues list those varieties whose seed may be marketed within the Community. Each member of the EU is required to provide a National Catalogue of officially recognized varieties which may be freely marketed in the country. The National Catalogues are collated together in the Common Catalogue of Varieties on Agricultural Plant species. Only plant varieties which are registered in a National or the Common Catalogue and which are certified for commercialization within the EU can be sold.

Conservation varieties: Directive 2008/62/EC and Directive 2009/145/EC

The need to introduce a legal framework allowing the use of varieties threatened with genetic erosion was stated for the first time in 1998 in the European Directive

[1] All Directives are available from the Internet, for links see http://ec.europa.eu/food/plant/propagation/evaluation/index_en.htm [8 December 2009].

[2] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:193:0001:0011:EN:PDF

[3] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:193:0033:0059:EN:PDF

98/95/EC[4], concerning field crops, which mentions that it is essential to ensure the conservation of genetic resources and the necessity to introduce a legal basis for this in consideration 17[5].

A legal framework covering the so called conservation varieties has been established in 2008 with the Directive 2008/62/EC[6], by "*providing for certain derogations for acceptance of agricultural landraces and varieties which are naturally adapted to the local and regional conditions and threatened by genetic erosion and for marketing of seed and seed potatoes of those landraces and varieties*". While this created a possibility to grow, maintain and market agricultural landraces and varieties, the Directive 2008/62/EC has been criticized for several reasons.

In Article 11 it is stated that seeds of a conservation variety may only be produced in the region of origin[7], and in Article 13 it says that marketing can only take place in the region of origin[8]. This spatial restriction is considered an inappropriate trade barrier which would be a serious obstacle for the maintenance of conservation varieties (MÜHLBAUER 2008). Especially in times of climate change and strong environmental shifts this directive appears contraproductive and effectively prevents the targeted introduction and local adaptation of diverse materials.

In addition, the quantity of seed marketed of a conservation variety is restricted to 0.3 % or 0.5 % respectively of the seed quantity used for all varieties of that species in the

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[4] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1999:025:0001:0026:EN:PDF [8 December 2009].

[5] "*Whereas it is essential to ensure that plant genetic resources are conserved; whereas a legal basis to that end should be introduced to permit, within the framework of legislation of the seed trade, the conservation, by use in situ, of varieties threatened with genetic erosion*."

[6] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:162:0013:0019:EN:PDF [14 January 2010].

[7] "*Member States shall ensure that seed of a conservation variety may only be produced in the region of origin*"

[8] *"Member States shall ensure that seed of a conservation variety may only be marketed subject to the following conditions:*

(a) it has been produced in its region of origin or in a region referred to in Article 11;

(b) marketing takes place in its region of origin."

same year in the member state (Article 14)[9].

For varieties of some species this very small amount would not be sufficient to build up a sustainable market position which would be necessary to prevent the extinction of these varieties, however (MÜHLBAUER 2008).

A study compiled in the project "Farm Seed Opportunities" deals with matches and mismatches between the directive on conservation varieties with current practice in the conservation and use of varieties and landraces that are not included in national variety lists. Based on a detailed analysis, the study comes to the overall conclusion that "the current procedures and standards for uniformity are well capable to capture the diversity of conservation varieties. Their application is however costly, and they are not able to capture the dynamic nature of conservation varieties."(LOUWAARS et al. 2009).

In November 2009 a similar directive came into force for vegetables (Commission Directive 2009/145/EC[10]). Thus it is possible that also vegetable varieties with no intrinsic value for commercial crop production but developed for growing under particular conditions are accepted as a variety.

Evaluation of the marketing legislation on seed and propagating material

Taking the changed agricultural production and its changed problems into account, an

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[9] "*Each Member State shall ensure that, for each conservation variety, the quantity of seed marketed does not exceed 0.5 % of the seed of the same species used in that Member State in one growing season, or a quantity necessary to sow 100 ha, whichever is the greater quantity. For the species Pisum sativum, Triticum spp., Hordeum vulgare, Zea mays, Solanum tuberosum, Brassica napus and Helianthus annuus, that percentage shall not exceed 0.3 %, or a quantity necessary to sow 100 ha, whichever is the greater quantity. However, the total quantity of seed of conservation varieties marketed in each Member State shall not exceed 10 % of the seed of the species concerned used yearly in the Member State.*"

[10] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:312:0044:0054:EN:PDF [15 January 2010].

"Providing for certain derogations, for acceptance of vegetable landraces and varieties which have been traditionally grown in particular localities and regions and are threatened by genetic erosion and of vegetable varieties with no intrinsic value for commercial crop production but developed for growing under particular conditions and for marketing of seed of those landraces and varieties"

external evaluation of the marketing legislation on seed and propagating material **(S&PM)** was conducted in 2007 to 2008. External consultants were commissioned by the European Commission (EC) to state "how effectively and efficiently the legislation has met its original objectives, and to identify its strengths and areas for improvement and its robustness with regard to potential new challenges affecting this field."[11]

The evaluation was conducted by the Food Chain Evaluation Consortium (FCEC), headed by "Arcadia International ®" which is a European Economic Interest Grouping, working according to their own statement for a broad range of international private sector clients and institutions[12], also including large multinational agro-food companies.

A range of stakeholders and experts were consulted via surveys and face-to-face interviews and the results of the evaluation were presented at the "Seed Availability in the 21st Century Conference", held in Brussels in March 2009[13]. Results and recommendations were discussed with approximately 130 people representing different sectors of agricultural production (LOPEZ NORIEGA 2009). Most stakeholders consider that the legislation had a positive impact on the preservation of plant genetic resources in the EU and "do not wish to see a change of the successful and well-established" DUS and VCU systems[14]. It should, however, be highlighted that the evaluators' methodology had some limitations that hindered the participation of e.g. small farmers in remote areas of Europe. The questionnaires were only available online and only in English. It is also not clear to what extent evaluators consulted research institutes and universities.

According to the evaluation results, the legislation has "served a good purpose in achieving its objectives of ensuring the free movement throughout the Community of

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[11] Evaluation of the Community acquis on the marketing of seed and plant propagating material – Final Report.
http://ec.europa.eu/food/plant/propagation/evaluation/s_pm_evaluation_finalreport_en.pdf
http://ec.europa.eu/food/plant/propagation/evaluation/docs/AP_council_2009_en. pdf [8 December 2009].

[12] http://www.arcadia-international.net/references.php [14 January 2010].

[13] http://ec.europa.eu/food/plant/seed_conf/ [8 March 2010].

[14] http://ec.europa.eu/food/plant/propagation/evaluation/s_pm_evaluation_finalreport_en.pdf [8 December 2009].

healthy and good quality S&PM in a sufficiently wide range of varieties and still remains useful. Internal free market in S&PM is perceived as being well established, but the following problems, which interfere with the principle of a level playing field, have been identified:

- non-harmonized implementation of the community legislation
- additional national implementing measures
- a non-harmonized framework for costs and responsibility sharing
- the lack of organized circuits for the exchange of information between member states
- complexity of community legislation."

Three scenarios with regard to the Community legislation – the 'status quo', 'suppress', and 'modify' scenario – were examined in the evaluation. The results from the stakeholders' consultation support the 'modify' scenario, thus supporting the Commission's intention to revise the legislation. An action plan with the overall objective of developing an EU Seed Law that would lead to a modernization and a significant simplification of the current legislation has been presented. The main aims are:

- to ensure the availability of qualitative and healthy S&PM, and by this way to promote plant health and to support agriculture, horticulture and forestry
- to ensure that S&PM meets the expectations of users
- **to contribute to halt the loss of biodiversity**
- to achieve a harmonized implementation of the legislation throughout the Community
- to improve economic competitiveness by ensuring free circulation of S&PM in the Community

More details are given in the action plan, available at
http://ec.europa.eu/food/plant/propagation/evaluation/docs/AP_council_2009_en.pdf
[8 December 2009].

TRIPS, UPOV, Patents, and other international treaties

TRIPs

The major recent event in the area of IPR has been the WTO's TRIPs Agreement (agreement on Trade Related Aspects of Intellectual Property Right)[15]. It was negotiated in the 1986-1994 Uruguay Round, came into effect in 1995 and was signed by over 140 countries as a part of the new General Agreement on Tariffs and Trades (GATT, 1994). The TRIPs agreement has eliminated the principle of "national treatment", which means that the intellectual property of foreigners must be at least equally protected than the intellectual property of nationals. TRIPs introduced intellectual property rules into the multilateral trading system and is to date the most comprehensive multilateral agreement on intellectual property. It requires countries to establish property rights systems for plant varieties under TRIPs Article 27.1: "patents shall be available for any inventions, whether products or processes in all fields of technology, provided that they are new, involve an inventive step and are capable of industrial application". TRIPs does not define the key term "technology" and provides very limited exemptions from patentability under Articles 27.2 and 27.3 (SRINIVAS 2006). Article 27.3(b) requires WTO member nations to offer some form of intellectual property rights in plants through patenting, PBR, or an "effective *sui generis* system".

"Members may also exclude from patentability… (b) plants and animals other than microorganisms, and essentially biological processes for the production of plants and animals other than non-biological and microbiological processes. However, Members shall provide for the protection of plant varieties either by patents or by an effective sui generis system or a combination thereof."

Theoretically this provides nation states with an opportunity to shape legislation to protect the interests and needs of farmers and indigenous peoples by establishing IPR arrangements that respect and reward collective inventions. Nothing in the relevant international agreements prevents countries from establishing other forms of IPR protection (SALAZAR et al. 2007). Nevertheless, in practice most nations simply adopt an existing PBR framework (KLOPPENBURG 2008).

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[15] http://www.wto.org/english/tratop_e/trips_e/t_agm0_e.htm [14 January 2010].

The 1991 Convention of UPOV

The most frequently adopted system in this context is the 1991 Convention of UPOV. UPOV (International Union for the Protection of New Varieties of Plants) is an intergovernmental body that establishes international rules under which countries grant intellectual property rights for plant breeders' rights. It is the major *sui generis* system for PVP. The original UPOV Convention from 1961 formulates two important principles: the **breeder's exemption** and the **farmer's privilege**. The breeders' exemption includes the right of anybody to use germplasm, including protected material, for further breeding, the farmer's privilege is the right of farmers to reproduce any materials, including those of protected varieties for their own and non-commercial use. Both principles reflect global practices in farmers' culture of sharing and exchanging germplasm. However, during the 1980s, pressure mounted to tighten the conditions under which the breeder's exemption and the farmer's privilege are valid, resulting in the revised version of the UPOV convention of 1991. There it is stated that the breeder's exemption no longer applies in the case of so-called essentially derived varieties (EDV) and that the farmer's privilege is restricted to provide a breeder sufficient possibilities to claim his rewards. The farmers' privilege now requires a positive act by national authorities to permit it, on a crop-by-crop basis (LOUWAARS 2001). In some industrialized countries the right to re-use seeds or planting material on a farm has been restricted for particular crops (SALAZAR et al. 2007). Although UPOV is considered a well-balanced system for the protection of plant varieties, a solution must be found for the problem of the seed flow in informal seed systems or farmers' seed systems (GHIJSEN 2002).

The room for manoeuvre in national policy was considerably narrowed by the TRIPs agreement which now obliges each member state to provide for patent protection of all types of plant material, except for plant varieties, which have to be protected by patents or a *sui generis* system (TRIPP 1997).

Strong IPRs like utility patents and PVP based on the 1991 Convention of UPOV are likely to have a direct and negative effect on the on-farm saving of seed and especially on the traditional exchange of seeds among farmers (LOUWAARS 2007).

Patents

As a reaction to the development of GM crops, Europe adopted a directive on

patenting plants and animals (Directive 98/44/EC[16]) which permits the legal protection of biotechnological inventions. However, the need for patents on germplasm is debatable. Thus, a variety already covered by a PBR can not be patented. Nevertheless, a variety which includes a patented gene can be protected with a PBR too (KÄSTLER 2005). For a long time, utility patents, which are granted with respect to products or processes, were considered unsuitable for protecting living matter. The main reason for the increase in patents on living organisms is the increasing importance of biotechnology in plant breeding. With the *Diamond v. Chakrabarty case* in the USA in 1980, where it was ruled that a new strain of bacteria produced artificially was a patentable invention, the patenting of life became easier. Since then, many utility patents have been granted in the field of biotechnology. The *Hibberd case* in 1985 extended the protective ability of the utility patent system to conventionally bred plant varieties (GHIJSEN 2002). In the USA a plant and its seed, or even a characteristic of the plant, can be patented, given that it is new, innovative, and in some way useful. As a result, entire groups of varieties could fall under a single patent (SALAZAR et al. 2007).

In Europe, patents have also been filed for non GM plants. By March 2007 35 patents had been granted by the European Patent Office on normal plants. According to the global platform "no patents on seeds" the number is rapidly increasing[17].

Currently, two cases pending at the Enlarged Board of Appeal are expected to write patent history in Europe. These are the „wrinkled tomato case" (EP 1211926 B1 - method for breeding tomatoes having reduced water content and product of the method), and the "broccoli case" (EP 1069819 B1 - method for selective increase of the anticarcinogenic glucosinolates in *Brassica* species).

On the one hand, the tightening of PBR systems will affect farmers' seed practices, on the other hand, the patent system will considerably affect farmers' access to seed. The patent system is not rooted in agriculture and includes neither breeder's exemption nor farmer's privilege principles which are common in PBR legislation.

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[16] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:213:0013:0021:EN:PDF [19 January 2010].

[17] http://www.no-patents-on-seeds.org/index.php?option=com_content&task=category&sectionid=2&id=13&Itemid=20 [19 January 2010].

Impacts of international agreements on the management of agricultural and horticultural seeds

International agreements such as the Convention on Biological Diversity (CBD) and the International Treaty on Plant Genetic Resources for Food and Agriculture (IT PGRFA) have a significant influence on the management of agricultural and horticultural seeds. Both agreements have been developed to support the conservation and sustainable use of biodiversity, introducing the concept of national sovereignty over genetic resources (CBD) and the concept of farmers' rights (IT PGRFA).

The **CBD**[18] came into force in 1993. It provides an international, legally binding framework for the conservation and sustainable use of biological diversity. Its three main objectives are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising from such use (THE CRUCIBLE II GROUP 2000). Countries that have ratified the CBD are bound to facilitate access to their genetic resources. This might require benefit sharing arrangements and Prior Informed Consent from the source country. These conditions apply mostly to cross-border exchanges of genetic resources (SALAZAR et al. 2007). It can be argued that the CBD is in conflict with TRIPs, as conservation and privatization are contradictory goals. As both treaties provide legally binding obligations for governments it remains open, which one will take precedence (THE CRUCIBLE II GROUP 2000).

After seven years of negotiations, the **International Treaty on Plant Genetic Resources for Food and Agriculture**[19] also called "IT" or "seed treaty", was adopted by FAO member states in 2001 and came into force in 2004. The goal of the treaty is to create a legally binding framework for the protection and sustainable use of plant genetic resources for food and agriculture. Its core provisions place the resources of 36 species and genera of crops and 29 genera of forages in the multilateral system and guarantee access to these resources for breeding and research (BRUSH 2007). It acknowledges the rights of farmers to "save, use, exchange and sell farm-saved seed and other propagating material". However, this privilege is "subject to national legislation", which means that those rights are subordinated to IPR legislation (KLOPPENBURG 2008).

[18] http://www.cbd.int/doc/legal/cartagena-protocol-en.pdf [19 January 2010].

[19] http://www.planttreaty.org/texts_en.htm [14 January 2010].

Any party requesting germplasm from the gene banks of the CGIAR/FAO is required to accept a **Materials Transfer Agreement** (MTA)[20] that prohibits claiming of IPR on such germplasm. However, this is only due for germplasm "in the form received". If the material is altered through genetic manipulation, it can be patented (KLOPPENBURG 2008). For benefits derived from the use of the material or materials derived from it, the MTA requires "the fair and equitable sharing of the benefits" which should flow primarily, directly and indirectly, to farmers. As the MTA does not specify a workable method for calculating the magnitude of such benefits, and does not specify any enforceable mechanisms for collecting those benefits, it seems unlikely that any of these provisions will be implemented in a meaningful way (KLOPPENBURG 2008).

The seed treaty is perceived very critically by organizations such as GRAIN: "Far from its roots in the struggle to assert farmers' rights as a counterforce to breeder' rights, the Treaty has ended up being mainly about granting new privileges to industry. It will give seed companies free access to most of the worlds' public gene banks without any obligation to share their own materials in return"[21]. GRAIN is a small international non-profit organization that works to support small farmers and social movements in their struggles for community-controlled and biodiversity-based food systems.

[20] http://www.planttreaty.org/smta_en.htm [14 January 2010].

[21] Seedling, October 2005, http://www.grain.org/seedling/?id=411 [19 January 2010].

Annex D. Interview questions

1) Zur Markeranwendung im Züchtungsunternehmen

- Welche Kulturen werden im Züchtungsunternehmen bearbeitet?
- Welche Bedeutung hat marker-assisted selection (MAS) in der Züchtung dieser Kulturen in Ihrem Unternehmen bzw. generell?
- Für welche Züchtungsziele wird MAS eingesetzt, für welche nicht?
- Werden monogene oder quantitative Merkmale oder beide mittels MAS selektiert?
- Welche Markertechnologien kommen zum Einsatz?
- Werden Marker zur Unterscheidung von Sorten/Linien eingesetzt?
- Sehen Sie zukünftige Anwendungsmöglichkeiten von molekularen Markern im Sortenschutz?

2) Zum Züchtungsfortschritt

- Welche Züchtungsfortschritte sind in den letzten 10-15 Jahren im Züchtungsunternehmen durch MAS erzielt worden?
- Welche Züchtungsfortschritte wurden in dieser Zeit ohne MAS erzielt?
- Was war Ihrer Ansicht nach dafür jeweils entscheidend?

3) Was ist bezüglich MAS-Einsatz für die kommenden 5 Jahre projektiert?

4) Welche Bedeutung wird MAS Ihrer Ansicht nach in 10 Jahren für Ihre Züchtungsarbeit bzw. generell haben?

5) Gibt es Restriktionen, die den Einsatz von MAS einschränken (evtl. bezogen auf konkrete Beispiele) und welche sind das?

Inwieweit treffen die unten angeführten möglichen Einschränkungen zu?

(1=trifft voll zu, d.h. extrem einschränkend, 5=trifft überhaupt nicht zu, d.h. überhaupt nicht einschränkend)

	1	2	3	4	5
– hohe Kosten der MAS	□	□	□	□	□

- Genotyp-Umwelt-Interaktionen ☐ ☐ ☐ ☐ ☐
- Epigenetik ☐ ☐ ☐ ☐ ☐
- Verfügbarkeit geeigneter Marker ☐ ☐ ☐ ☐ ☐
- hohe Anzahl von Merkmalen, für die selektiert werden muss ☐ ☐ ☐ ☐ ☐
- kein Effizienzvorteil gegenüber klassischen Züchtungsmethoden ☐ ☐ ☐ ☐ ☐
- Verfügbarkeit geeigneter Genom- und/oder Marker-Datenbanken, die Zusammenarbeit zwischen Molekular-biologen und Züchtern erleichtern ☐ ☐ ☐ ☐ ☐
- Verfügbarkeit geeigneter ☐ ☐ ☐ ☐ ☐
- Analysesoftware
- Patentrecht, intellectual property rights ☐ ☐ ☐ ☐ ☐
- andere Einschränkungen: ☐ ☐ ☐ ☐ ☐

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6) Wie stark hat Ihr Unternehmen von den Erkenntnissen und Entwicklungen der pflanzlichen Genomforschung in den vergangenen 10-15 Jahren profitiert?

Was lässt sich von den Methoden der „genomics-assisted selection“ (z.B. Microarray-Technologien, association mapping, tilling/eco-tilling usw.) erhoffen?

Welche weiteren Fortschritte sind denkbar?

7) Werden in der Züchtungsarbeit Marker eingesetzt um pflanzengenetische Ressourcen (PGR) zu erschließen?

Wenn ja, wird die Evaluierung von PGR im Unternehmen selbst durchgeführt oder werden Ergebnisse anderer Forschergruppen genutzt?

8) In welchem Ausmaß werden PGR genutzt für die Züchtung neuer Sorten?

9) Bieten Marker die Möglichkeit, PGR effektiver zu nutzen und erhöht der Einsatz von MAS die Nutzung von PGR?

Wodurch könnte die Nutzung von PGR anderweitig verbessert werden?

10) Welche Potenziale haben mittels MAS gezüchtete Pflanzen im Vergleich mit transgenen Pflanzen? Könnten mittels MAS gezüchtete Pflanzen aufgrund ihrer vermutlich höheren Akzeptanz manche transgenen Ansätze ersetzen? Wo ist Ersatz nicht möglich?