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INCREASING THE EFFICIENCY OF POTATO BREEDING THROUGH MARKER ASSISTED SELECTION – GENERAL THOUGHTS. MOLECULAR MARKERS FOR LATE BLIGHT RESISTANCE – WHEN APPLIED FOR BREEDERS?

ABSTRACT

Despite many breathtaking breakthroughs in the area of crop genetics and genomics, plant breeding still widely depends on the methods that had been worked out almost a century ago. This is not because commercial plant breeders are overly conservative but because the new knowledge lacks efficient and economical tools that would permit their application in practice. Breeders desire supporting technologies that would facilitate laborious and time–consuming screening in the field and laboratory. In particular, resistance screening often cannot be performed satisfactorily as the necessary disease pressure and appropriate pathogen populations may be unavailable. In potato breeding, specific and often complex resistances need to be developed, at the same time maintaining high levels of quality and culinary characteristics.

Therefore, it is worthwhile to revisit the facts that comprise the progress in genetics of disease resistance and to analyze current technologies of genotyping and marker assisted selection, with the objective to detect those parameters that limit the efficiency of methods for commercial application. Selection in potato for resistance to late blight will be highlighted as an example. Maps, genes and markers for resistance have been identified – how universal are they? Single genes and quantitative trait loci for race–specific and race non–specific resistance are known – how efficient is their use? Marker technologies based on polymerase chain reaction and DNA hybridization have been developed that are far more efficient than first–generation technologies – is their use in commercial breeding economical? By discussing these issues concepts will emerge that help to pave the way for marker assisted selection (MAS) in potato breeding.

The most important parameters required for economical MAS include to have a clear idea of the traits to be selected for, to use proven, reliable markers, to have in place a robust system for the collection and management of DNA samples, and to use technologies whose total cost is below or equal to the cost of the conventional methods. The most striking advantages of MAS are that a breeder will obtain more information than by conventional methodology, the information will be more precise, field labour can be saved and in that way the breeding process will be intensified. The implementation of the new technology could lead to even closer collaboration of breeders and scientists. Possible disadvantages include the relative increase of laboratory and computer work within the breeding program, and possibly higher costs during the implementation phase of the new technology.

Key words: marker assisted selection, potato breeding, potato late blight resistance

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INTRODUCTION

Marker assisted selection (MAS) was first proposed by Sax (1923), but the first technological tools would not be available until very recently. A very humble genetic map based on 57 RFLP (restriction fragment length polymorphism) loci was ready in 1986 (Bernatzky and Tanksley 1986). Most of the genetic markers available until present have been inadequate for successful MAS. They comprise anonymous DNA fragments that are often loosely associated with a trait to be selected for (Young 1999), and their application is laborious, time consuming and expensive. Only in recent years, the increasing availability of information from genetic maps and from gene cloning and genomics studies has had a tremendous impact on the real possibilities for MAS application in practice. First progress has been made in homozygous plants such as the tomato (Tanksley *et al.* 1992), soybean (Meksem *et al.* 2001), barley and rice (Dunford *et al.* 1995, Horvath *et al.* 1995), but also in allopolyploid wheat (Seah *et al.* 2001). Today, detailed information about the genome of heterozygous plants including the potato, which could be used to develop appropriate markers for MAS, is also available (see e.g. Gebhardt and Valkonen 2001). For potato, an extremely dense linkage map based on AFLP (amplifield fragment length polymorphism) is being developed, currently it has more than 10000 markers that virtually cover the entire genome at an average distance of 100 kb (Brugmans *et al.* 2003).

At present, many breeders face difficulties to accumulate sufficient testing of genetic materials they have under development, due to limitations in field and laboratory space, financial and labor resources. As an example, testing for resistance to late blight, caused by *Phytophthora infestans*, was largely reduced during the 2003 growing season in many European countries due to the extreme dry and warm weather. Those materials will have to be evaluated again provided the climatic conditions in the coming years make the screening possible. However, for the breeders it would be of great help if their selection decisions could be supported by less variable information, such as those provided by MAS based on the genotype instead of the phenotype. Therefore, some general preconditions for successful application of MAS in commercial plant breeding will be highlighted, with special emphasis on potato breeding.

PRECONDITIONS FOR APPLICATION OF MAS IN COMMERCIAL BREEDING PROGRAMS

Three complexes of preconditions important for successful MAS can be identified. They include (1) the traits to be chosen for genetic screening, (2) the properties of molecular markers to be used, and (3) technological aspects that are required to make the method economical. The ideal trait to select for should be clear–cut, such as the trait conferred by a major gene that has a distinct phenotype. It should be genetically well–investigated. Further, the efficiency of MAS will be increased by

combining the screening for several traits using several markers at a time. The amount of DNA obtained from a single sample is usually large enough to screen for many markers. A molecular marker to be used in MAS should be closely linked to the trait, to guarantee a high rate of precision; very few false–positives and false–negatives should be present among the selected individuals. Preferable will be markers that work in a wide range of genetic backgrounds and can be applied with little technical effort. In fact, MAS will be economical when applied through comparatively safe, robust and cheap technology.

Which traits to choose for MAS

The ideal trait to be considered for MAS should be clear–cut and well known, such as the resistance based on a single major gene. Within the trait complexes yield, quality, vegetative period and resistance, a number of specific characteristics and genes amenable to selection by molecular markers can already be proposed. In potato, for both yield and vegetative period, several QTL (quantitative trait loci) have been described (Ganal *et al.* 1991, van den Berg *et al.* 1996, Chen *et al.* 2001). For quality and resistance, single genes affecting distinct characteristics have been cloned or mapped to a narrow chromosomal region (De Jong and Burns 1993, El–Kharbotly *et al.* 1996a, Li *et al.* 1998, Ewing *et al.* 2000, Naess *et al.* 2000, Ballvora *et al.* 2002, van der Vossen *et al.* 2002, Song *et al.* 2003). The classical potato *R*–genes for resistance to late blight are an example of the trait conferred by major genes (Müller and Behr 1949). At present, several of these genes have been overcome by all strains of the late blight pathogen worldwide, but some, in particular *R5*, *R8*, and *R9*, can still ensure considerable protection. *R*–genes for resistance to late blight, even when broken down, contribute to an overall increased resistance to this disease (Stewart *et al.* 2003). Therefore, screening for the presence of these factors is beneficial, as it increases the value of the breeding product. Although it is technically feasible to screen the individuals of a segregating potato cross population for presence or absence of the resistance governed by most of these *R*–genes, such a screen is rarely performed in practice. This is because (i) breeders lack the resources for appropriate screenings including the facilities to maintain *P. infestans* isolates and to carry out assays, (ii) resistance to late blight is neglected due to limited labour resources and in favour of more intensive selection for several other of the 40–50 characters that need to be combined in a single modern cultivar (Bradshaw and Mackay 1994). In 1991, Coffey and Gees stated that "knowledge of the genetics and biochemical parameters involved in resistance to late blight is still negligible, and consequently control of *P. infestans* still relies heavily on empirical approaches, both in plant breeding and in screening of new fungicides". Since then, our knowledge has considerably increased, but still little is known about the mechanisms of this resistance. The genetics of a trait chosen for MAS should be known in

detail to increase the chance to find precise markers. However, blight resistance conferred by *R*–genes is likely to be more complex than it has been thought. For example, the functional, resistance–conferring allele of the *R1* gene, which has been isolated recently (Ballvora *et al.* 2002), belongs to a cluster of several highly similar sequences within the genetic locus. This gene and its analogous pseudogenes at the *R1* locus are members of the family of NBS–LRR (nucleotide binding site–leucine rich repeat) genes. An insight into this gene family has been gained using model pathosystems (Meyers *et al.* 1999, Pan *et al.* 2000). The NBS–LRR gene family members provide resistance to all kinds of pathogens (Boyes *et al.* 1996, Meyers *et al.* 1999, Pan *et al.* 2000). Despite intensive analyses by means of genomics, few direct interactions of an *R*–gene product with a pathogen's eliciting proteins have been detected. Therefore, the molecular mechanisms of signalling that trigger the resistance response may involve a great number of genes and their products, but their simultaneous actions in complex networks are only partly known. Thus, a single major gene resistance such as that conferred by a classical potato *R*–gene can be seen as the result of an entire cellular response machinery that in effect is quantitative, to some extent.

For quantitatively determined traits for which QTL have been detected on genetic maps, and whose effects have not been resolved into single genes, the chances to obtain efficient genetic markers are lesser than for major gene traits. The quantitative (field) blight resistance of potato is such an example. Several QTL have been detected in various mapping studies (summarized in Gebhardt and Valkonen 2001), every single QTL covers a large part of the potato genetic map. Therefore, a QTL needs to be resolved into Mendelian factors (Paterson *et al.* 1988, 1991). Until a QTL can be resolved into the underlying, single Mendelian loci, several molecular markers will be needed to characterize a single QTL region. The precision of such QTL markers to detect a specific genotype is low due to the inherent blurred, statistical nature of a QTL.

Characteristics of markers for MAS

Undoubtedly, it is important that a molecular marker should precisely detect the presence or absence of a specific allele. The adoption of MAS by commercial breeding programs as a complement to approved selection technologies will be facilitated by high levels of diagnosis precision. When most of the individuals selected by molecular markers readily present the phenotype as predicted by the genotype, a breeder will be satisfied with the new method. Likewise, if the presence of the desired allele within the genotype is guaranteed, even if the trait is not detected in subsequent field evaluations, MAS also will fulfill the expectations. Absence of the trait conferred by a specific allele can be due to additional, interfering genetic factors, such as suppressors of resistance

conferred by an *R*– gene (El–Kharbotli *et al.* 1996b, Ordoñez *et al.* 1997). The trait will however re–appear in future generations for which these genotypes will be used as crossing parents.

Ideally, a marker should directly detect an allele that is key to the trait expression. It may be sufficient to design one marker for the functional allele at a locus. However, a system of several codominant markers that detect all alternative alleles occurring at that locus will maximize the information to be gained and will make the results of MAS more reliable. Knowledge of the genetic sequence from the cloned gene can be used to design markers for detection of allele–specific single nucleotide polymorphisms, base insertions/deletions, inversion of short stretches of DNA, and other point mutations that characterize an allelic variant at a locus of interest. An example of SCAR (sequence characterized amplified region) markers that reliably detect alleles of the gene conferring resistance to *Potato virus Y* has been given by Vidal *et al.* (2002). For the gene *Y–1*, codominant markers have been designed that precisely detect different alleles at the *Y–1* locus within heterozygous genotypes.

Most of the markers should be universal, i.e. they should detect the allele or trait within a wide range of genetic backgrounds as a precondition to carry out selection from crosses among many parental genotypes and progenitors. Again, markers based on DNA sequence of a functional allele should be the most satisfactory in this respect, SCAR markers being an example (Paran and Michelmore 1993). For potato, a composite map of disease resistance and defense related genes and genomic regions has been published by Gebhardt and Valkonen (2001). This and other studies have provided a valuable base for choosing and developing markers for genes that have been used in many breeding programs and are therefore widely distributed among potato cultivars. Gebhardt *et al.* (2004) tested five markers for the *R1* gene conferring race–specific resistance to late blight and for vegetative period, a trait that appears closely linked or pleiotropic to the *R1* gene, on a large collection of potato cultivars. These authors showed that several markers occurred significantly more frequently within cultivars with increased blight resistance and a prolonged vegetative period. This experiment proved that it is possible to develop markers that can be applied on a wide range of genotypes sharing a common genetic background. These markers detected a chromosomal fragment that probably was introgressed into the common potato from *S. demissum*, a wild source of late blight resistance. This study exemplifies that the increased utilization of genetic resources, as well as introgression of novel resistances require the continuous development of markers that detect newly introgressed alleles and loci. New *R*–genes for resistance to late blight have been detected in the last decade by conventional and molecular investigations (Table 1). These new genes and factors, and thirteen classical potato *R*–genes (Black *et al.* 1953, Schick and Schick 1959, Malcolmson and Black 1966,

Niederhauser 1996, personal communication), provide evidence that late blight resistance genes are widespread among the *Solanum* genepool and are not confined to the species of Mexican origin, as it was suggested in the past (Niederhauser and Mills 1953, Umaerus 1970).

Table 1

New major genes for resistance to late blight, in addition to the classical *R*–genes

Source of R–gene and description	Origin	Reference
Clone XY9 (*S. tuberosum* ssp. *andigena*), carries RX	Bolivia, Peru	Ordoñez *et al.* (1997)
Clone TPS–67 (*S. tuberosum* ssp. andigena), segregates for an *R*–gene, RT	Bolivia, Peru	Trognitz (1998b)
Super Chola (*S. tuberosum* ssp. andigena), segregates for an unknown *R*–gene	Ecuador	Trognitz *et al.* (1999)
Single *R*–gene, segregating in a cross with *S. berthaultii* (PI 265858)	Argentina	Orrillo *et al.* (1999)
R–gene from *S. berthaultii*, mapped to potato chromosome X	Argentina	Ewing *et al.* (2000)
S. santolallae (CIP 761691), segregates for an unknown *R*–gene	Peru	Trognitz *et al.*, unpublished
S. caripense, segregates for two unknown *R*–genes	Peru, Costa–Rica	Trognitz (1998a)
R–gene from *S. microdontum*, mapped	Bolivia	Sandbrink *et al*. (2000)
R–gene from *S. bulbocastanum* (potato chromosome VIII), isolated	Mexico	Song *et al*. (2003)

To be successful, MAS programs require easy–to–apply markers that will be available in sufficient quantity and at any time they are needed. The results of selection using molecular markers should be highly re–producible. Moreover, it can be assumed that a breeder will need access to laboratory equipment, which would be possible via collaboration with scientific institutions.

Requirements to the MAS technology

Whatever technology will eventually reach the stage of commercial application, it must be fast enough and allow for sufficient throughput to screen all seedlings before they have to be multiplied and transferred to other evaluations. The cost of MAS should be equal or lower than that of screening with the use of conventional methods. However, MAS is likely to produce more information about the genotypic value of breed–ing materials than any conventional method. Therefore, comparison of actual costs does not seem to be feasible in a straightforward way. A MAS technology should also be safe and robust as its success will de–pend considerably on its low rate of initial failure.

A SCENARIO FOR MARKER ASSISTED SELECTION

We highlight a general scenario for MAS in potato breeding and discuss its feasibility. This general breeding scheme includes five steps:

1. To cross progenitors and to produce segregating progenies. The genetic value and specific characteristics inherited by the progenitors will be known.
2. To grow seedling plants.
3. To collect samples and to isolate DNA from each of the progenies. This can be done through:
   - 3.1. direct PCR (polymerase chain reaction) – that will be preferred as no additional isolation steps are required,
   - 3.2. one–step DNA isolation,
   - 3.3. multi–step DNA isolation, without liquid nitrogen,
   - 3.4. multi–step DNA isolation using liquid nitrogen.
4. To apply molecular markers to the DNA samples. This could be done by:
   - 4.1. one–step marker application (e.g. via real–time PCR), or
   - 4.2. multi–step, including amplification, separation of amplicons and visualization.

   To apply the markers, any procedure will be possible, but their intrinsic economy must be kept in mind. It is evident that at least one parent of the cross must possess the marker.
5. To select those seedlings that possess most of the specific alleles as detected by the markers.

Steps 3 (sample preparation) and 4 (marker application) constitute technological elements additional to those present in conventional breeding programs. Many current commercial breeding programs are performed within small companies that, mostly due to financial and economical limitations, cannot make themselves all the investments that are needed to establish the facilities for MAS technology. Therefore, it might be worthwhile to consider the implementation of an international pilot "experimental breeding program" based on MAS and related to innovative technology. Such a pilot program could be useful in stimulating the development of appropriate technology and its uptake by commercial breeders. The pilot program could be built on joint public and private funds and serve all partners involved. Besides applying MAS for commercial purposes, this program could be maintained to further develop appropriate technology and, at the same time, to serve as a training facility.

EXPECTATIONS TO MAS FOR THE NEAR FUTURE

Although many questions still need to be solved before MAS becomes a standard in potato breeding, a few statements can already be made concerning an initial implementation phase. Specific cross populations,

progenitors and markers may originate from studies and collaborations within breeding programs interested in the technology. MAS will be carried out for selected genes and alleles. Breeders can then assess whether and how much the breeding for several single traits will be facilitated using this procedure. In turn, as the technology will be improved and results and experience will accumulate, it will be possible to reduce the amount of labour resources required in the conventional breeding sector in favour of resources for MAS technology. The breeding programs can either reduce the size of field trials, thereby maintaining the overall size of output, or accommodate a larger number of cross combinations and utilize at a time a wider range of genetic resources to increase the output of the program in terms of enlarged diversity or a greater number of new, superior–performing cultivars.

The implementation of MAS can also intensify cooperation between breeders and researchers, particularly to detect genes and alleles that play key roles in building up desired traits, as well as to design a variety of markers and to develop methods for their efficient application. Such cooperation could facilitate the extended application of MAS for a large number of qualitatively inherited traits and to adapt efficient methods of screening for quantitative traits.

Extending from the many groundbreaking developments in molecular biology and genetics during the past thirty years it is plausible to expect for the coming decades considerable progress in the area of conventional plant breeding. Powerful genomics tools, for example cDNA (mRNA) microarrays, are being developed to analyze the expression of multiple genes in response to specific challenges (Schenk *et al.* 2000, Moore *et al.* 2002), such as infection by a pathogen. Upon increased information on the expression patterns of multiple genes, MAS for most qualitative traits will possibly become a standard in many breeding programs. Moreover, efficient large–scale introgression of novel genes from genetic resources, exceeding the levels achieved in the past, will be feasible. MAS will also be carried out for many complex quantitative traits. Plant breeding is already receiving substantial support from research carried out in the areas of genomics, bioinformatics, and proteomics. A largely automated „crop design" technology could liberate human capacity currently absorbed by mechanical labour, that can then be dedicated to education and more creative work.

GENERAL EXPECTATIONS FOR THE MAS TECHNOLOGY AND A PROPOSAL TO FACILITATE THE IMPLEMENTATION OF MAS IN COMMERCIAL POTATO BREEDING

Presently, MAS is applied in soybean breeding to select for resistance to a nematode (Meksem *et al.* 2001). How soon MAS can become routine at a large scale in a wide range of plant breeding programs is difficult to predict. Nevertheless, it is important to stress important intrinsic char–

acteristics that can be deduced from current developments of the MAS technology, to avoid unfounded expectations. The two main objectives of MAS are to directly and precisely screen the genotype of segregating progenies and varieties and in that way to reduce the number of individuals that must be screened by conventional selection methods. At present, reduction through MAS of the total time needed for the breeding of a new cultivar appears unlikely, particularly in potato breeding. The field performance of a potato clone comprising the total of all combined characteristics can only be done at several field locations and in the course of several years, as it is commonly practiced. In addition, selection for those traits that are not covered by MAS will have to be continued by conventional means. However, rather than to maintain and field–screen all offspring of a cross, the screening will only be carried out on those individuals that possess the desired alleles. For the breeder this means that some 50–95% of the plants which currently have to be planted and screened in the field will be able to be eliminated prior to field trials when MAS is available. Furthermore, almost 100% of the plants that pass MAS and enter the field screening stage will have a chance to become a new cultivar.

During the successive phases of MAS implementation, European potato breeders will depend in their work on intensified cooperation with scientists and on complementary funding by the public sector, including the governments and the European Union. To make MAS research and development efficient, establishment of a joint international experimental breeding program that would centralize all operations for MAS and provide technology and training to all stakeholders, will be advantageous.

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