Marker Techniques as Tools for Efficient Plant Breeding

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ABSTRACT

Today, in plant biology molecular genetics opens up an innovative spectrum even under the applied aspect of plant breeding. The most spectacular approach is surely the area of gene transfer which at the same time is under severe and controversial discussion. One reason for this conflict is the fact that presently no convincing economic or ecological successes due to a gene transfer have been applied e.g. in food or feed production. There is still a need of more physiological knowledge as one prerequisite for useful gene transformations. Here, the lack of cloned genes may soon be overcome by improved genome analysis. Such analysis demands molecular marker techniques. These are predominantly RFLP (restriction fragment lenght polymorphism), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment lenght polymorphism), and SSR (single sequence repeats or micro satellites). Such tools are not only useful for the identification of genes phenotypically difficult to characterise but also during marker assisted selection (MAS). By MAS it is possible to select in populations classically produced by combination breeding, those individuals which express desired characters even if they are very rare. Particularly for the complex quantitatively inherited traits - like e.g. aluminum tolerance of soybean - the use of such a technique will speed up the process to produce adapted genotypes. On the short and medium term basis marker techniques will contribute more to plant breeding than gene transfer. Like any other molecular technique it requires, however, a great deal of classical breeding work, thus molecular markers are not replacing classical approaches but are improving the breeding efficiency.

Key words: Marker techniques, plant breeding, genome

INTRODUCTION

The availability of recombinant DNA techniques together with advances in molecular biology and cell culture provides access to a refined understanding of genome. A systematic molecular analysis of the structure and function of plant genomes will be essential for future developments in plant science and its applied wing: breeding. The increasing amount of information about the DNA documented in molecular marker collections and in dense gene maps together with an excellent bioinformation system allows increasingly calculations about the structure and function of genes. Particularly under the aspect of synteny comparisons will be possible, probably elucidating common principles. The identification of gene function will result in the identification of candidate genes which can be used for the improvement of crop plants, e.g. in breeding for resistance to biotic and abiotic stress.

STRUCTURAL GENOMICS

Diversity at the phenotypic level is caused by corresponding differences in the DNA-sequence. The procedures used for the genome and gene identification include chromosome walking, megabase techniques as well as tagging and c-DNA approaches. The RFLP method advanced to the very powerful AFLP (amplified fragment lenght polymorphism) technique (Vos *et al.*, 1995; Wenzel and Mohler, 1998; Schwarz *et al.*, 1999), allowing much denser maps by identifying very small differences in the genome. Additionally, microsatellites, small conserved base sequence patterns distributed rather evenly over the genome can be incorporated in those instances, where no other polymorphisms are detected (Hearne *et al.*, 1992). While AFLP (and RFLP or RAPD) marker systems depend predominantly on anonymous DNA-sequences, the microsatellite technique uses defined sequence motives of 2 or 4 base pairs. In eucaryotic genomes microsatellites express a highly disperse distribution.

Since in gene identification the application of molecular markers demands the need to know the localization of the marker in the genome, genetic linkage maps are an additional prerequisite for the localization and the analysis of gene functions e.g. barley, (Jahoor *et al.*, 1993; Graner *et al.*, 1996). Often for securer gene identification the bulked segregant analysis (BSA), doubled haploids (DILs), nearly isogenic lines (NILs) or recombinant inbread lines (RILs) are used as mapping populations. As a first central step in the direction of identifying genes a rapidly increasing number of monogenic, race specific genes showing gene for gene interaction have been mapped in economically important species (Table 1). This demands the production of mapping populations and the skills for exact phenotypic bonitations.

Besides monogenic characters an increasing number of quantitatively inherited genes (QTLs) are localized (e.g. Backes *et al.*, 1995; 1996). A linkage between a QTL and a genetical marker is given, when the phenotypic means of a class of markers are significantly different. The most commonly used procedure for mapping QTLs is today the interval mapping according to Lander and Botstein (1989), where chromosome segments flanked by two markers are analyzed.

After gene mapping, for gene identification marker based chromosome walking techniques are applied predominantly. Additionally, tagging techniques using increasingly transposon induced mutant populations together with cDNA approaches are important. For the walking technique high resolution maps have been constructed, allowing the saturation of the relevant chromosomal region with very closely linked markers. The closest ones will be used to select homologous clones from large insert libraries which in turn allow the construction of physical maps around these genes. As consequence of such approaches common features like genes for enzymes rich in leucin (leucin-rich repeats, LRRs) or enzymes responsible for signal transductions have been detected (Table 2). The information available allows first speculations on the type of function of genes identified.

Table 1. Extract of crop plants and diseases for which DNA probes are available

Host	Disease	Number of alleles identified	Technique	Reference
Barley	*Erysiphe graminis*	19	RFLP	
	Puccinia graminis	2	RFLP	
	P. hordei	1	RAPD	
	P. striiformis	QTL		
	Rhynchosporium secalis	4	RFLP	Backes *et al.*, 1995
		QTL		
	Pyrenophora teres	1	RFLP	
	Cochliobolus sativus	QTL	RFLP	
	Typula incarnata	1	RFLP	
	Pyrenophora graminea	QTL		
	Barley yellow mosaic virus	7	RFLP, RAPD	Graner *et al.*, 1996
Maize	Maize dwarf mosaic virus	2	RFLP	Ming *et al.*, 1997
	Bipolaris maydis	1	RFLP	Zaitlin *et al.*, 1993
	Puccinia sorghi	1	RFLP	Hulbert and Benetzen, 1991
	Cercospora zeae-maydis	QTL	RFLP	Bubeck *et al.*, 1993
	Colleotricum graminicola	QTL	RFLP	Jung *et al.*, 1994
	Helminthosporium trucicum	1	RFLP	Bentolina *et al.*, 1991
Potato	*Phytophthora infestans*	2	RFLP, AFLP	El-Karbotly *et al.*, 1996
		QTL	RFLP	Leonards-Schippers *et al.*, 1994
	Potato virus X	2	RFLP	Ritter *et al.*, 1991
	Potato virus Y	1	RFLP	Hämäläinen *et al.*, 1997
Rice	*Orseolia oryzae*	1	RFLP, RAPD	Mohan *et al.*, 1994
	Pyricularia oryzae	3	RFLP	Miamoto *et al.*, 1996
	P. grisea	2	RFLP	Yu *et al.*, 1996
	Rice tungro virus	1	RFLP, RAPD	
	Xanthomonas oryzae	6	RFLP	
Soybean	*Heterodera glycines*	1	RFLP	Webb *et al.*, 1995
	P. megasperma	3	RFLP	Diers *et al.*, 1992
	Soybean mosaic virus	1	Microsatellite, RFLP	Yu *et al.*, 1996
Wheat	*P. recondita*	3	RFLP, RAPD, STS	Feuillet *et al.*, 1995
	P. graminis	1	RFLP	Paull *et al.*, 1994
	Erysiphe graminis	8	RFLP	Hartl *et al.*, 1999
	Wheat streak mosaic virus	1	STS, RAPD	Talbert *et al.*, 1996

FUNCTIONAL GENOMICS

The tools used in functional genomics are (a) bioinformatics, i.e. database comparisons of functional domains, of protein families, (b) expression analysis, i.e. analysis of the transcriptome including inventory of expressed transcripts (ESTs), global gene expression analyses using DNA array technologies, serial analysis of gene expression (SAGE), and straightforward identification of genes of interest by differential techniques, and (c) the use of mutant collections (knock out mutants up to complete gene machines).

Table 2. Common structural characteristics of proteins of cloned genes for resistance

Group	Protein	Host/pathogen	Structure	Reference
I	PTO	tomato/*Pseudomonas*	Intracellular serin/threonin kinase membrane bound	Martin *et al.*, 1993
	PTI1		Serin/threonin kinase phosphorylated by PTO, interacting with PTO	Zhou *et al.*, 1995
IIa	RPS2	*Arabidopsis*/*Pseudomonas syringae*	Intracellular protein with leucine zipper, nucleotide binding site, leucine rich repeat	Bent, 1996
	RPS5			Warren *et al.*, 1998
	RPM1			Grant *et al.*, 1995
	PRF			Salermon *et al.*, 1996
IIb	N	tobacco/ TMV	Intracellular protein	Whitham *et al.*, 1994
		flax/*Cocliobolus carbonum*	IL - IR homology, nucleotide binding site, leucine rich repeates	
	L2, L6			Lawrence *et al.*, 1995
	L10			Anderson *et al.*, 1997
	M			
	RPP5	*Arabidopsis*/*Peronospora Parasitica*		Parker *et al.*, 1996
	RPP14			Jones *et al.*, 1996
III	Cf-2	tomato/*Cladosporium fulvum*	Transmembrane proteins with extracelular leucine-rich repeats	Jones *et al.*, 1996
	Cf-4			
	Cf-5			
	Cf-9			
	I2	*Fusarium oxysporum* sugarbeet/*Heterodera*		Milligan *et al.*, 1998
	Mi			
	Hs1			Cai *et al.*, 1997
IV	Xa21	Rice/*Xanthomonas oryzae*	Transmembrane protein with intracellular kinase and extracellular LRR	Song *et al.*, 1995
V	Mlo	Barley/*Erysiphe graminis*	Transmembrane proteins nuclear localized	Büschges *et al.*, 1997

In plants, like in other eukaryotes, biological processes such as growth, adaption to physiological shifts, defense against biotic or abiotic stress are mediated by distinct programs of differential gene expression. The identification of the subset of genes differentially expressed under certain growth conditions will facilitate the breeding of crops, resistant e.g. against pathogens. To steadily identify low abundance transcripts, which often code for important regulatory proteins like receptor proteins, enzymes involved in signal transduction or transcription factors, an enrichment procedure for such transcripts has to be applied. A number of enrichment techniques have been described of which representational difference analysis (RDA) has the ability to efficiently reduce the number of constitutively and abundantly expressed genes (Hubank and Schatz, 1994). To gain comprehensive insight into gene function rapidly, subtracted cDNA libraries enriched for differentially and rarely expressed genes can be prepared by using suppression substraction hybridization (SSH). In addition, SSH has the potential to facilitate the identification of genes which may not be detected by high-density expression profiling.

An experimental approach to gain insight into gene function is the analysis of the gene expression patterns at a genome-wide scale, ideally of the total mRNA (transcript) complement expressed by the genome. From their spatial and temporal expression patterns clues of the biological function of the genes can be obtained. Comparative expression analysis is very potential to gain insights into the complexity of gene expression and allows a global view of changes in gene expression patterns in response to physiological shifts. This way cDNAs can be very helpful to analyze complex genomes for example the large crop genomes. The powerful tools for mRNA expression monitoring are expressed sequence tags (EST) sequencing, serial analysis of gene expression (SAGE) and massively parallel expression analyses using oligonucleotide chips or DNA microarrays. Using robotics equipment, thousands of sequences can be generated within a relative short time period.

The technology for expression monitoring which is currently receiving major public attention because of its wide and powerful applications for genome analyses is the DNA chip or microarray technology (Brown and Botstein, 1999). The immense interest in this method results from its potential to analyze a huge number of genes in parallel (Hauser *et al.*, 1998).

The method is based on hybridization and principally corresponds to the classical Southern or Northern-blot techniques with the difference that in an array hybridization "probe" and "target" are permuted (Southern *et al.*, 1999). In such a "reverse" dot-blot or Northern-blot the "probe" is immobilized - the free nucleic acid is the "target". Because of its potential, the microarray technology has already reached a very high level of commercialization.

DNA arrays are gridded "spots" of individual DNA molecules immobilized to nylon filters or glass slides. The spot densities can vary from a few hundred to up to 300.000 spots. For expression profiling, the arrays are hybridized to total mRNA pools which have been converted to cDNA in the presence of fluorescent or radioactive labeled nucleotides. Individual components of the labeled target form heteroduplexes with the complementary DNA (probe) on the chip surface localized at a known location on the array. By measuring the signal intensity at each position on the array the identity and quantity of the components in the labeled mixture, which are able to hybridize to the array, can be determined. The array technology has a major impact on agriculture and plant biotechnology by speeding up plant breeding.

Using SSH Thümmler *et al.* (2000) were able to detect genes whose expression is specifically induced by the infection of potato leaves with one of its most devastating pathogens, *Phytophthora infestans*. A plasmid library was constructed which represented about 100.000 of primary transformants and which was expected of being greatly enriched for clones carrying sequences of *Phytophthora* induced genes. Of 96 clones randomly picked and analyzed, 58% turned out to code for seven known pathogenesis related (PR) proteins, for which differential gene expression has been demonstrated also showing that our library exhibits a redundancy of only 5 to 10 fold of the differentially expressed genes of high abundance. One novel gene turned out to be

clearly differentially regulated and thus, per definition, represents a new PR-gene. The other 42% of the clones code for weakly expressed genes, half of them represent genes not presented in the public databases, the other half exhibit homology to known enzymes partly with relation to pathogen resistance. For example, two genes carry putative LRR domains and can be linked to the class of resistance genes which also carry LRRs (Table 2). Our experiments demonstrate that gene libraries generated by the SSH method represent excellent sources for the identification of genes involved in defense against pathogen attacks.

CONCLUSION

Uncovering genes and their structure and function relies also on good classical genetics and phenotypic characterizations. A fruitful cooperation between classical and molecular genetics is the way to go. All successful crop varieties are selected for specific traits, but up till now without knowing their exact molecular function. Since this strategy has already been quite successful, it can be expected that after understanding e.g. the gene function of resistance genes, man has for the first time the chance to be more efficient in plant protection than the concurring trial and error approach of pathogens.

Genomics and proteomics generate the essential information for any future breakthroughs in agriculture and bioinformatics enables the exploration of the universe of biological data. In general, the functional information gained will provide a framework and a starting point for further detailed analysis. The first phase of genome analysis was mainly characterized by joint efforts to construct comprehensive maps. During the next phase research activities focussed on the utilization of these maps for genetical localization of agronomic traits e.g. by MAS and for elucidating the function of the responsible genes.

A systematic molecular evaluation of the complete genetic information of plants and the resulting cellular activities, such as transcription and protein expression will be essential for future developments. In order to obtain this information, currently a hierarchic approach has been taken namely genetic mapping. YAC-mapping, BAC-mapping, genomic sequencing, EST-sequencing, transcriptional profiling, proteome analyses (e.g. for barley Michalek *et al.*, 1997). While successful in the past and presence, as demonstrated by the on going analyses on *Arabidopsis* and rice, the costs are prohibitively high. The sequencing of the (relatively small) 100 Mb genome of *Arabidopsis* alone will cost in the range of US$ 100 million. Due to these costs the development of the modern technologies will take place primarily in the private sector. In consequence, molecular botany is eventually industrialized. It is open whether the tremendous costs will be paid back via the licenses of new resistant cultivars. It is probably more the production of special chemicals or a horizontal network between breeding and selling of the final processed products which have to balance the initial investments.

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