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MINI-REVIEW

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Molecular plant breeding: achievements in green biotechnology and future perspectives

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Abstract Since one decade ago, transgenic crop plants are globally grown; in 2004, it was estimated to cover a total of 81 Mio ha in 17 countries. At present, four plant species (soybean, maize, cotton and rapeseed) dominate with two traits (herbicide tolerance and insect resistance). The traits on which research concentrates and the constructs which might come next onto the market are outlined. The procedure on how to clone such genes of interest, e.g. via map-based cloning, and some other helpful approaches of green biotechnology, like high throughput techniques and functional markers, are summarised, and a rough calculation about the market value of transgenic crops in US dollars is quoted.

Introduction

More food and feed have to be grown on less land. Fortunately, in the 1970s, new tools for more efficient plant breeding arose, one of which is biotechnology. As agrochemistry and mechanisation have reached a level where further progress is neutral in relation to plant production—scientific progress helps environmental needs—only breeding may close the yield gap by a combined application of classical and molecular techniques. There is as much hope as there is scepticism concerning the future contribution of biotechnology and its most advanced part: gene transfer. The production of the first transgenic cultivars during the 1980s was driven by available technical possibilities rather than by central needs of the market. The biggest bottleneck was the availability of cloned genes. Their structural and functional analysis was much faster in bacteria, as it was easier to identify genes in viruses and bacteria than those from higher plants. In consequence, transfer of viral or bacterial genes into higher plants was prominent in the beginning of gene transfer activities. This first generation of genetically modified crops has been commercialised globally just for one decade. In most cases, input traits useful for the farmer have been improved during this period. In 2004, the global area of approved transgenic crops continued to grow, reaching an estimated global area of 81 Mio ha (Fig. 1). Biotechnologically engineered crops were grown by approximately 8.25 million farmers in 17 countries (James 2005). The focus of research today is upon functional gene analysis of higher plants, allowing alteration of output traits, which will reach commercialization at the end of this decade.

Besides the area of gene transfer, increasingly large-scale genome analysis provides access to a refined understanding of the genome. In addition, molecular markers became a powerful biotechnological tool, allowing a more efficient selection during the classical breeding process by marker-assisted selection (MAS). Improvement of existing cultivars, especially considering traits of interest to consumers like quality and food safety, may decrease the reluctance towards gene technology presently visible in Europe and particularly in Germany. Genomics will ultimately accelerate and alter agricultural research and plant breeding, resulting in a chain from a single isolated gene, via cell research, single phenotypes and cultivars, processed crops and, finally, trade to the consumer, incorporating green biotechnology as a basic process of food and feed production.

Classical prerequisites and achievements

Plant improvement needs patience; it easily takes 12 to 15 years from the first cross to a registered variety. Even using the new tools, one has to think in decades and predictions about their final usefulness are premature; but, by asking which predictions one would have made 100 years ago, when recombination as the basis of classical plant breeding was discovered by Mendel, one can be optimistic. Classical and biotechnological breeding techniques rely on the production and use of genetic variation followed by selection of the most suitable plant type. The

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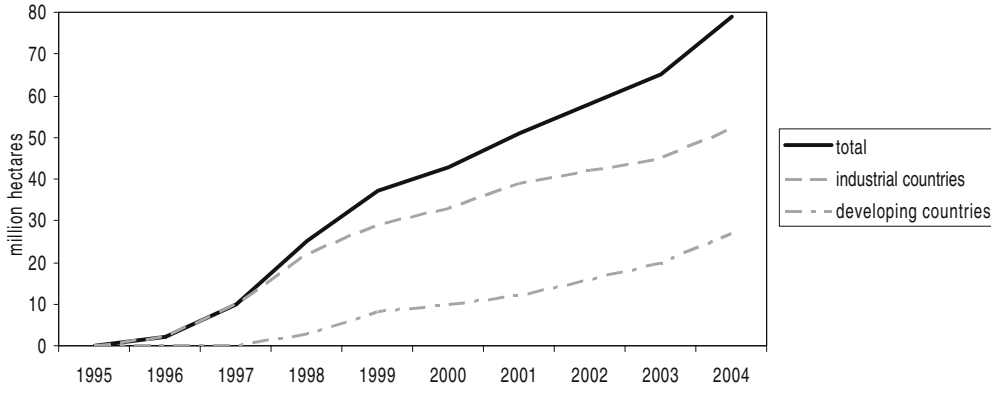

Fig. 1 Global area of transgenic crops (James 2005)

major challenge of the scientific century that just passed was the discovery and the programmed use of the Mendelian laws. Thus, it became possible to follow the process of science-based combination breeding. The yields increased dramatically (Table 1), a success causing today in parts of the world more criticism than applause.

As a higher plant contains 20,000 to 60,000 genes (*Arabidopsis* 25,000; rice 46,000; http://www.eugenes.org; Ware and Stein 2003), recombination of this huge amount of alleles by combination breeding is today and will be in the future the central process for the development of new varieties. Increasing knowledge about the specific function of genetic material will be helpful in parent selection and offers reliable tools for a more efficient selection within the new combinations of the $\sim n^{20,000}$ alleles (n=number of alleles per locus; Rommens and Kishore 2000). It should be stressed that even though there is a spectrum of new technologies, the present breeding progress documented by the annual registration of new cultivars all over the world is the result of classical breeding, and this will continue.

Biotechnological tools

Modern biotechnological research in plant breeding started with cell and tissue culture approaches, techniques which are still necessary, e.g., after the regeneration of a transformed cell. Biotechnological breeding processes like regeneration of somatic and generative cells into functional plants or even cell fusion to result in somatic hybrids, e.g., in rapeseed, have never been a topic of public discussion or political intervention. This changed when the first transgenic plants were subjected to field trials, the Petunia with altered flower color in Germany in 1991 (Meyer et al. 1992).

Table 1 Yield of wild species of cereals (dt/ha) in areas of origin and yield development of cereals in Germany (Geisler 1980; Anonymous 2005)

Year	Wheat	Barley	Rye	Oats
8000 BC (wild species)	3	3		
1300–1400	5	4	5	3
1500–1600	9	6	8	4
1800	10	8	9	6
1900	14	13	10	12
1950	26	24	22	22
1975	46	40	34	37
2004	82	71	61	52

Gene transfer

From a scientific point of view, the transfer of specific genes with a known function is very similar to classical breeding but, due to a stricter approach, the efficiency is increased. The prerequisites for gene transfer aiming at cultivar improvement are:

- Availability of the trait to be transferred as cloned DNA
- Availability of a powerful transfer system
- Availability of a reliable regeneration system predominantly from a single transformed cell

The last two points are solved in principle, although regeneration is still a problem. Regeneration is more an art than a science, particularly in cereals and grasses. However, recipes are available and, with sufficient trials and the use of a range of different genotypes, success should be achievable. Transfer works are predominantly via *Agrobacterium*, as such transformations are more stable than microbombardment techniques during subsequent meioses. Microbombardment has, however, the advantage that only the DNA of interest is transferred and a possible transfer of genetic information from the Ti plasmid is excluded. *Agrobacterium* mutants are available today, which also allow the transformation of monocotyledoneous plants. The most critical problem is still gene isolation. Most of these isolated genes are used in transformation experiments for basic research to elucidate biochemical pathways and add knowledge particularly in the field of metabolomics.

Transgenic plants

Besides gene transfer of model plants being helpful in basic research, an increasing transformation of crop plants aims at crop varieties improved in agronomic traits in food, feed or non-food sector characters. Nearly 100 species are ready

to be released or have already entered the market. This includes fruits, vegetables, agricultural crops, trees, and ornamentals, but most of these plants are prototypes only (Table 2). Growth in the open environment is legally controlled and substantially restricted. In Germany, e.g., only maize and carnations are allowed to be grown in the open environment.

In 2004, on 5% of the 1.5 billion ha of all global cultivable crop land, transgenic plants were grown. Under nutritional considerations, only cotton, maize, rapeseed and soybean are important today. Table 3 gives the growing area and the countries for these four forerunners. In 2004, commercial cultivation of these four transgenic crops increased similarly as in the previous years. Transgenic soybean reached coverage of 48.4 Mio ha, the biggest area, followed by maize on 19.3 Mio ha. Maize is projected to have the highest percentage growth rate for the near term as maize demand increases and as more beneficial traits become available and approved. Transgenic cotton is expected to continue to grow in 2005 and beyond, as India and China will increase their hectarage and new countries introduce the crop for the first time. Transgenic canola occupied 6% of global transgenic area (James 2005).

Table 3 Area of transgenic crops by country (James 2005)

Country	Area (Mio ha)	Main crops
USA	47.6	Soybean, maize, cotton, rapeseed
Argentina	16.2	Maize, soybean
Canada	5.4	Rapeseed, maize, soybean
Brazil	5.0	Soybean
China	3.7	Cotton
Paraguay	1.2	Soybean
South Africa	0.5	Maize, cotton, soybean
India	0.5	Cotton
Uruguay	0.3	Soybean, maize
Australia	0.2	Cotton
Mexico	0.1	Cotton, soybean
Spain	0.1	Maize
Philippines	0.1	Maize
Colombia	0.05	Cotton
Honduras	0.05	Maize
Germany	0 (300 ha)	Maize

Traits of interest

The two dominant transgenic crop/trait combinations in 2004 were herbicide tolerance by soybean occupying 48.4 Mio ha or 60% of the global 81 Mio ha grown with genetically modified crop plants and insect resistance by the *Bacillus thuringiensis* T-toxin protein (Bt)-containing maize occupying 11.2 Mio ha, equivalent to 14% of global area of transgenic crops. Stacked genes for herbicide tolerance and insect resistance deployed in both, cotton and maize continued to grow occupying 9% or 6.8 Mio ha (Table 4).

Close to release as cultivar is transgenic rice-producing pro-vitamin A. The group of I. Potrykus transferred two genes and one promoter gene into rice and could achieve initially in the "Golden Rice" 1.6 μg/g β-carotene production (Ye et al. 2000). In the meantime, R. Drake's group could increase the ß-carotene amount in the "Golden Rice 2" by a factor of 23 using a maize instead of the narcissus gene (Paine et al. 2005). This makes the amount of food necessary to compensate a vitamin A deficiency realistic. The maximum β-carotene yield reaches 37 μg/g. This rice variety will be commercially available in developing countries in 2009 (I. Potrykus, personal communication).

Besides the mentioned in- and output traits (separated by the dotted line in Fig. 2), huge numbers of crops with altered characteristics are ready to be tested under field conditions or will be tested in the near future. This includes the possibility to improve yield by F1 hybrid varieties using transgenic systems with male sterility, e.g., in rapeseed (Mariani et al. 1990). Table 5 gives a summary for traits ready for the market on the example potato.

Strong emphasis has been placed on the main storage products starch, oils, and proteins. In rapeseed, via gene transfer, substantial progress has been achieved, e.g. by transformation of ACP-thioestrases from *Umbellularia*, *Cuphea* and *Carthamus* as well as antisense desaturases altering the length of the fatty acids between C8 and C18 (Töpfer and Martini 1998; Kinney at al. 2002). For starch, its amylose and amylopectine composition could be altered in potato by genetic engineering (Kuipers et al. 1994;

Table 2 Transformed plant species

Alfalfa	Cauliflower	Lettuce	Petunia	Saintpaulia
Apple	Cherry	**Maize**	Plum	Sorghum
Aubergine	Chrysanthemum	Melon	Poplar	**Soybean**
Banana	Coffee	Oats	Potato	Sugar beet
Barley	**Cotton**	Olive	Radiccio	Sugarcane
Broccoli	Cucumber	Orange	**Rapeseed**	Sweet potato
Cabbage	Eucalyptus	Papaya	Raspberry	Strawberry
Calendula	Gerbera	Pea	Rice	Tobacco
Carnation	Grapes	Pear	Rose	Tomato
Carrot	Kiwi	Pelargonium	Rye	Wheat

In bold are the four crops grown globally (Kern 2005)

Table 4 Growing area and the transformed trait of the four commercially released crops in 2004 (James 2005)

Crop	Growing area		Transformed trait	Growing area	
	%	Mio ha		%	Mio ha
Maize	23	19.3	Herbicide tolerance	72	58.6
Soybean	60	48.4	Insect resistance	19	15.6
Cotton	11	9.0	Combination	9	6.8
Rapeseed	6	4.3			

Table 5 Selection of traits expressed in potato after gene transfer (for a review of literature, see Wenzel 2006)

Trait	
PVX resistance	Palatinose production
PVY resistance	Zeaxanthin production
PLRV resistance	*R1* resistance gene against *Phytophthora*
Bt Tolerance	Pectate lyase
Herbicide tolerance	*Erwinia* resistance
Inulin production	Starch synthase
Increased level of phytochrome B	Branching enzyme
Cyclodextrine production	Vaccine production

Börnke et al. 2002). For the processing industries, this saves an expensive separation step. Of immediate interest is the production of pharmaceutical compounds in plants, blocking the theoretical transfer of animal diseases to man (Fischer et al. 2004). Table 6 summarises some of the advanced experiments where clinical studies have been started.

Plant physiology and molecular–biological analyses contributed tremendously to elucidate plant response to abiotic stresses such as drought, cold and high salinity (Walia et al. 2005). However, promising reports on engineering tolerant plants by expressing, e.g. cold- or drought-regulated transcription factors (Yamaguchi-Shinozaki and Shinozaki 2001), have not yet generated improved crops. On the other hand, tolerant plants exist in nature like *Crambe* or *Thellungiella* species (Wong et al. 2005). The molecular and genetic analysis of such specialists represents promising approaches to identify key components and to confer enhanced stress tolerance. Plants tolerant to salt also usually reveal tolerance towards drought and, often, cold (Xiong et al. 2002). Common biotechnological adaptation strategies may consequently become possible.

In 2005, the complete sequence of the rice genome became available (Sasaki 2005), representing monocotyledons and one of the three most important crops. This enables understanding how the genome sequence comprehensively encodes developmental programs. Several functional genomic approaches are initiated to decode the linear sequence including full-length cDNA collections of genes, microarrays, natural variation (EcoTILLING), knockout collections, and comparative sequence analysis (Borevitz and Ecker 2004).

Search for the gene of interest

As already stated, examples exist where gene transfer, regeneration and field growth of transgenic plants are realised but, even though huge numbers of genes are isolated and transferred, it is still difficult to find a gene of

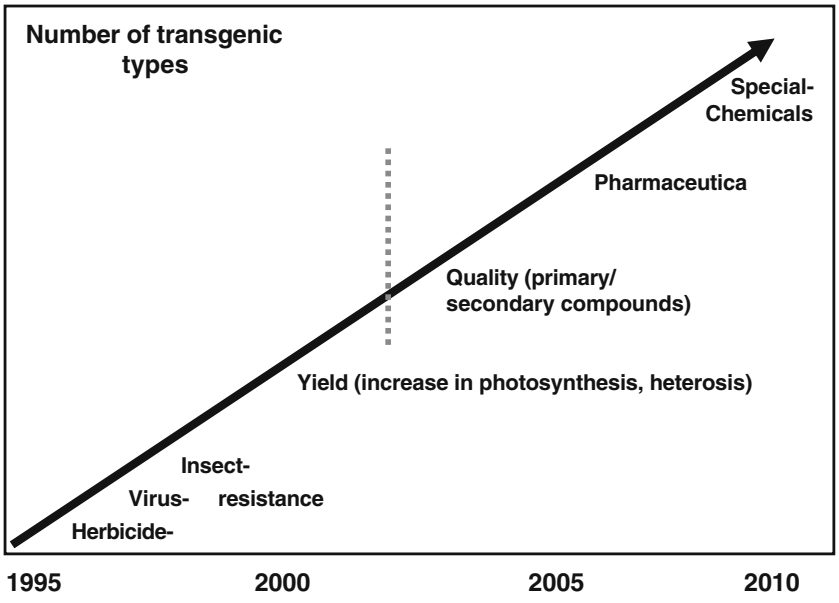

Fig. 2 Time scale of genetically modified characters in crop plants

Table 6 Some pharmaceutical proteins produced by transgenic plants (Dorfmüller 2005)

Protein	Therapy	Plant	Status
Antibodies			
Choriongoandropin	Immunotherapy	Tobacco	Preclinical status
Streptococcus	Caries prophylaxe	Tobacco	Clinical studies, phase II
Tumor marker	Immunotherapy	Tobacco	Clinical studies, phase I
Aprotinine	Clotting inhibitor	Maize	Clinical studies, phase II
Enterotoxin B	Vaccine	Tobacco	Clinical studies, phase I
Lipase		Tobacco	Clinical studies, phase II
Hepatitis B	Vaccine	Potato, maize, lettuce	Clinical studies, phase II

interest amongst the many thousand genes in the plant genome. Several procedures are working, the easiest of which is to go from the protein to the gene. If a function cannot be attributed to a specific protein, it becomes really difficult. The first plant gene for which no specific proteins could be assigned and still the gene could be cloned was a gene for *Pseudomonas* resistance in tomato by map-based cloning (Martin et al. 1993). BAC libraries covering the whole genome at least two to four times have been an essential tool in map-based cloning strategies. Many genes presently isolated are detected via map-based cloning. A resistance gene for *Cladosporium* resistance was subsequently isolated via transposon tagging (Jones 1996). The phenotypical analysis of mutants deficient for a functional gene allows the assigning of this gene to a certain function. The most efficient method to generate populations saturated with loss-of-function mutations is insertional mutagenesis. In plants, insertional elements with the ability to integrate randomly within chromosomes are transposons such as *Ac/Ds* or *En/Spm* (Martinssen 1998).

Molecular markers

Molecular markers and genetic maps of high marker densities are the way to go. They are, on the one hand, a tool for identification of genes; on the other hand, they are helpful using marker-assisted selection after recombination to find the desired genotype. Molecular markers are virtually unlimited in their number, detectable at all plant developmental stages, show no pleiotropic effects and, thus, are a powerful tool for: (a) higher efficiency in parental selection allowing a controlled combination of better combination of genotypes and, thus, heterotic parents and (b) pyramiding of single traits to result in more complex characters. Furthermore, they are presently the most efficient system to uncover a rare but desired genotype in large, segregating populations. Mapping of traits is a prerequisite for gene cloning. For map-based cloning, ultradense maps with distances of less than 0.1 cM (1,000 kb) between trait and marker are necessary. An increasing number of monogenic, race-specific genes showing a gene-for-gene interaction has been mapped, and agronomically important genes have been correlated to molecular markers, as demonstrated for potato in Table 7. For wheat, such validated markers are available for resistance genes against powdery mildew (*Pm1c, Pm17, Pm24*, *mlRD30*), the yellow dwarf virus, the cyst nematodes (*Cre1* and *Cre3*), and the rusts (*Lr9, Lr21, Lr24, Lr38, Lr47; Sr38, Yr5, Yr17)* and *Fusarium* head blight (Mohler and Singrün 2005). Presently, the most powerful application of such identified genes and molecular markers is opened up by MAS. It offers the opportunity of combining different genes for a given pathosystem in a single genotype (gene pyramiding). A prerequisite for gene pyramiding is that characters are not allelic. Furthermore, knowledge on the gene distances in genetic or better physical maps is very helpful. Using such information, it was possible to combine three race-specific powdery mildew genes (*Pm*) in a single line which is now under variety test, hoping that such a pyramided resistance will be rather durable (Fig. 3).

Table 7 Some important and mapped DNA markers on the example of potato (for a review of literature, see Wenzel 2006)

Trait	Gene	Chromosome
Potato virus Y	Ry_{adg}	XI
	Ny_{tbr}	IV
Potato virus X	*Rx1; Rx2*	XII, V
	Na	XI
	Nb	V
	Nx	IX
Potato leaf roll virus	*PLRV* QTL	XI
Globodera rostochiensis	*Gro*	III, VII, X, XI
	H1	V
Globodera pallida	*Gpa* QTL	IV;V;IX;XII
	Gpa2	XII
Phytophthora infestans	*RB*	X
	R1	V
	R2	IV
	R3; R6; R7	XI
	R_{blc}	VIII
	QTL	V
Synchytrium endobioticum	*Sen1*	XI
Erwinia carotovora	QTL	
Tuber starch, tuber yield	QTL	I–XII
Cold sweetening	QTL	V, IX
Skin colour	QTL	X
Tuber flesh color	QTL	III

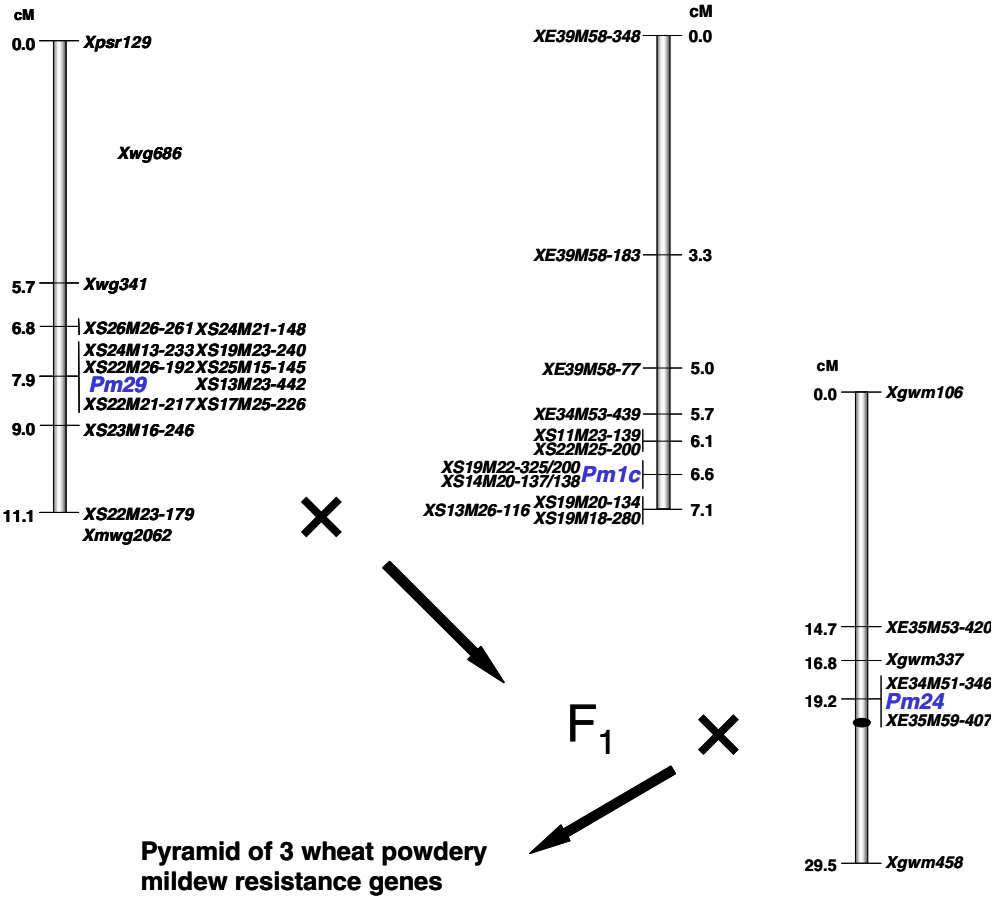

Fig. 3 Pyramiding of three powdery mildew genes by marker-assisted selection, resulting in an oligogenic resistance type which should be more durable (V. Mohler, personal communication)

Understanding the genetic basis of pathogen resistance has been broadened tremendously thanks to DNA-marker technologies. In this study, resistance gene analogs (RGAs) became interesting (Mohler and Wenzel 2004). RGAs are primarily located close to resistance genes and are often members of multigene families. Markers based on the use of RGA approaches often have a more general diagnostic value and, therefore, result in universal markers which the breeders need urgently.

Most characters relevant for crops show continuous phenotypic variation because they are controlled by multiple genes (quantitative trait loci, QTL) and by environmental factors. One of the key issues in food production is the difficult concept of quality, which is thought of as the sum total of physical characteristics embodied in the product. At present, the scientific community is not equipped to deal with the complex situation of understanding the nutritional needs, but first steps are done in the direction of functional foods, thus making breeding for quality a central goal. The final and most economical aim in crop production is yield, again a polygenic trait.

To select and produce cultivars combining several polygenic traits is the most difficult part of plant breeding. The strategy of hybrid production offers a way to combine at least complex characteristics from two parents. No strategies exist presently, however, to transfer QTLs via biotechnology.

Nevertheless, markers have entered the field of application successfully. Frisch (2005) calculated that selection for recombination between a target gene and flanking markers is highly effective even when the marker is rather distant from the target gene (Fig. 4). He expects a saving of three backcross generations even with a marker distance of 50 cM. Marker-assisted background selection can be used for such large distances as recombinants occur with increasing distance with a higher probability (Frisch et al. 1999). Hoisington and Melchinger (2005) elaborated factors on which a superior selection via markers depends: the heritability of the trait, the size of the mapping population employed for QTL mapping, the genetic architecture of the trait and the total budget of a breeding program. An alternative approach is association mapping based on linkage disequilibrium within a natural population, which is a promising but expensive tool (Remington et al. 2001). Thus, the molecular haplotypes of known candidate genes are analysed in populations (Li et al. 2002). This approach should enable the identification of candidate gene alleles associated with the QTL trait of interest. Even after molecular localization of a QTL, it is an unsolved question how to apply this knowledge in breeding programs. Several strategies for using QTL are being tested. The ideas encompass master or major genes,

regulatory elements, complete slicing of quantitatively inherited traits into single Mendelian factors and subsequent stepwise programmed pyramiding. At least some of the early predictions of the usefulness of molecular markers could be verified (Mohler and Singrün 2005).

Functional markers

To gain benefits from plant genomics, new knowledge must be "translated" into crop varieties with improved characteristics (Thro et al. 2004). Functional markers (FMs) are a good "translator" of gains from emerging technologies into improved crop cultivars. FMs are derived from polymorphic sites within genes causally involved in phenotypic trait variation. Once genetic effects have been assigned to functional sequence motifs, FMs derived from such motifs are used for fixation of gene alleles in a number of genetic backgrounds without additional calibration. FM development requires (1) functionally characterised genes, (2) allele sequences from such genes, (3) identification of polymorphic, functional motifs affecting plant phenotype within these genes and (4) validation of associations between DNA polymorphisms and trait variation (Chun et al. 2006).

Functional genomics

The number of genes is too large to be handled, and for understanding gene networking, really sophisticated analysis and bioinformatics are necessary. Clues of the biological function of the genes can be obtained from their spatial and temporal expression patterns. For expression profiling, DNA chips or microarrays are hybridised to total mRNA pools which have been converted to cDNA in the presence of labelled nucleotides (Brown and Bostein 1999). By measuring the signal intensity at each position on the array, the identity and quantity of the components in the labelled mixture can be determined. Serial analysis of gene expression (Velculescu et al. 1997) and arrays are tools for mRNA expression monitoring. On this way, cDNAs and expressed sequence tags (EST, Adams et al. 1991) can be very helpful to analyse complex genomes. Using robotics, thousands of sequences can be generated within a short time. EST databases turned out to be excellent resources for identifying genes by sequence comparison, for discovering new genes, and for assigning exons to genomic sequences.

For insight into gene function, subtracted cDNA libraries enriched for differentially and rarely expressed genes were prepared by using suppression substraction hybridization (SSH, Diatchenko et al. 1996). Ros et al. (2005) used, e.g., SSH in combination with cDNA array hybridization to search for genes, which are induced during the infection of potato with *Phytophthora infestans*. From libraries, more than 500 clones were randomly picked and sequenced. Of the nearly 300 unigenes found, 182 clones were selected for further analysis of the differential gene expression by cDNA array hybridization. Two subtractive cDNA libraries proved to be highly enriched for *P. infestans*-induced genes, like pathogenesis-related protein 1, lipid desaturase, peroxidase, chitinase and glucanase, with increased activity and others sowing depression like hexose transportes, leucin aminopeptidase or copper-amine oxidase. The differences in defense responses of the two cultivars could be clearly estimated.

A further strategy for the isolation of genes exploits the observation that many genes isolated in one plant species share similar sequences or represent members of comprehensive and widespread gene families, allowing an *in silico* cloning via bioinformatics.

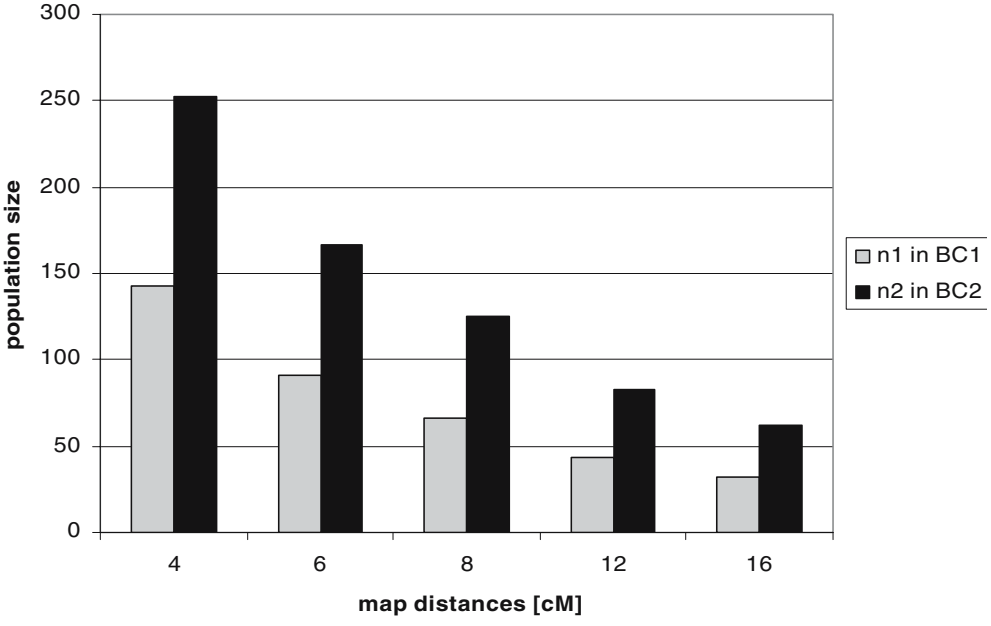

Fig. 4 Marker-assisted selection during a backcrossing program. The larger the distance between the marker and the gene, the smaller the population size can be chosen (Frisch 2005)

Conclusions

The experience of the first 9 years, 1996 to 2004, during which a cumulative total of over 385 million hectares of transgene crops were planted globally in 22 countries, has met the expectations of farmers. The global value of total crop production from biotech crops in 2003 was estimated at US$44 billion (James 2005). The net economic benefits to producers from transgenic crops in the USA in 2003 were estimated at US$1.9 billion whilst gains in Argentina for the 2001/2002 season were US$1.7 billion. China has projected potential gains of US*$5 billion in 2010, US*$ 1 billion from Bt-cotton and US$ 4 billion from Bt-rice, expected to be approved in the near term. A global study by Australian economists, on biotech grains, oil seeds, fruit and vegetables, projects a global potential gain of US*$ 210 billion by 2015 (James 2005). In the established industrial country markets of the USA and Canada, growth will continue with the introduction of new traits, for example, with maize MON 863 for corn rootworm control (James 2005). The EU Commission approved 17 maize varieties, with insect resistance conferred by MON 810, for planting in all 25 EU countries. The use of MON 810 maize opens up new opportunities for EU member countries to commercialise transgene maize, which Spain has successfully deployed since 1998. Taking all factors into account, the outlook for 2010 points to continued growth in the global hectarage of transgenic crops: up to 150 Mio ha in up to 30 countries (James 2005).

Speaking about the future, some additional topics should be mentioned where modern plant breeding might contribute to the improvement of abiotic resistances. The physiological and genetic control of day length as well as genes involved in the vernalisation process is increasingly understood. Getting possibilities to gear these processes, e.g. by increasing the efficiency of temperature dependent enzymes in countries with cold climates and doing the opposite in countries with hot climates, will surely result in tremendous increase in plant production area. A similar effect will be opened up by circumventing the day length dependence for many developmental processes of flowers or fruits. A further system for yield improvement will be the transfer of those genes responsible for the C3 or C4 carbohydrate circles. Particularly for the warmer climates of tropical and subtropical countries, such an alteration from the C3 to the C4 circle would pay with a striking yield increase. Of course, also other improvements of the photosynthetic activity will help. Work along this line is in progress by altering the photosensory perception and signal transduction via modified phytochrome B contents (Quail 2002).

It is expected that the incorporation of biotechnology and, in particular, of DNA technologies will be the most efficient way to combine economical and ecological aims. For producing a new variety, it would be ideal just to add one or a few missing traits to a superior cultivar. Although this can be achieved in principle by classical approaches, transfer procedures are more specific and, in consequence, superior. A big advantage of transfer procedures is that the existing optimised allele combination of a cultivar is not lost, e.g., by meiotic segregation during repeated recombinations necessary in a classical backcross program. A perhaps even more realistic tool presently is marker-assisted selection after a classical combination-breeding step. Due to a shortage of cloned genes with agronomic relevance, problems with QTLs and due to legal restrictions, MAS allows faster results in application than gene transfer.

Even the most intelligent approach may fail when regulations restrict this development; it will also fail, however, when light-minded strategies create problems which are difficult to overcome. Thus, it is necessary that worldwide-adopted regulations are accepted for the release of biotechnological modified plants. When making such regulations, one should keep in mind that classical breeding combines by chance two complete genomes while gene transfer works with identified DNA pieces. The reproduction of all thinkable ecological problems into biotechnological procedures is, however, not an intelligent way to go, in particular, as even the most superior genotype produced will not remain superior. The dynamics of evolution or factors like global warming will make breeding of new cultivars necessary, always demanding the most intelligent and goal-oriented strategies.

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