Rich cucumber harvest: Farm technicians (left to right) Wil Wilmer, Astrid Oispuu and John Geelen plan to extract the seeds from these vegetables raised in the Nunhems greenhouse in the Netherlands. The extra-long cucumbers are grown exclusively for seed production.

Masters of the plant tool box

On track to a greener future

Feeding the growing world population in a sustainable manner is one of the great challenges of our time. Yields from global agricultural production have to increase considerably. New technologies and modern plant-breeding methods can help to meet these challenges. So researchers from Bayer CropScience are putting plants through their paces: using the latest biotechnology and breeding methods, they can select plants more precisely and cross-breed them more effectively – creating hardier and higher-yielding varieties.

Searching for a hit: using fully automated genetic analyses and molecular markers, researchers Paul Buddiger (left) and Fiona Knubben-Schot examine samples from several thousand plants a day at the Nunhems biotechnology laboratory. The results aid in targeted vegetable variety development.

Precious progeny: a few days are all it takes for the cucumber plant to grow out of its seed coat, which still clings to the seedling. The emerging plant provides valuable benefits for vegetable farming around the world.

Biotechnology for spicy vegetables: Nunhems employee Peter Keunen harvests fresh chili peppers. The breeders know how spicy the vegetable will be even before the fruit take shape.

Cucumber art in the laboratory: biotechnology experts and plant breeders at Bayer CropScience sprout vegetable seeds in a Petri dish filled with a growing medium.

Space is getting scarce on our planet – and so is food: by 2025, one hectare of arable land, or an area about the size of a soccer field, will have to feed five people. In 1960, just two people lived off the same area. What's more, the expanding world population is just one of the challenges facing food production. Every year there are around 80 million more people living on our planet while the area of land under cultivation remains, at best, the same.

Growing world population: There will be nine billion people to be fed by 2050

In addition, the effects of climate change have an impact on farmers throughout the world: average global warming of just a few degrees can unleash dramatic weather phenomena. Even today, frost, hail, heatwaves or a sudden infestation of pests can destroy an entire year's harvest overnight. Other problems are drought and flooding, rising sea levels and the associated salination of groundwater. However well they farm their land, farmers in some parts of the world often lose between 30 and 70 percent of their harvest.

Modern agriculture thus faces what is probably the greatest challenge in its 10,000-year history: food, animal feed, fiber and alternative fuels must be produced in ever greater quantities with optimized resources, in challenging circumstances. Growing prosperity in emerging economies such as China, Russia, Brazil and India is increasing the consumption of protein-rich foods at a time when around 1 billion people throughout the world are still going hungry.

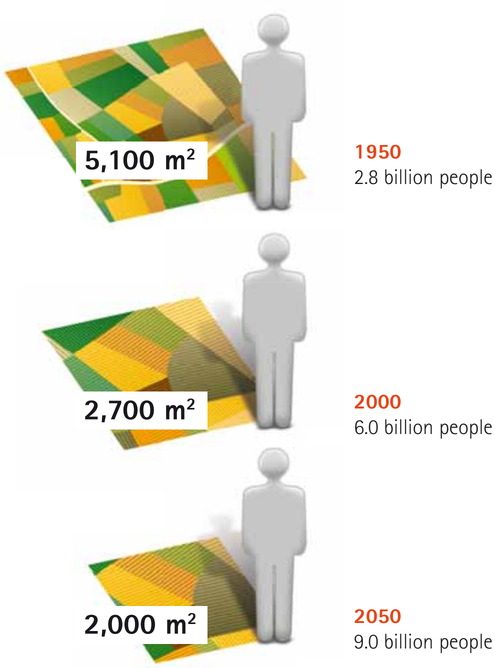
Plant biotechnology and modern breeding make crops tolerant to drought, frost and pests

In order to secure food supplies and meet today's global challenges, plant researchers are using biotechnology to accelerate advances in plant breeding techniques and develop plants with new properties. Biochemists and geneticists are already providing valuable new tools for the work of plant breeders: "Modern plant breeding incorporating biotechnology allows us to look deep inside the plant – through leaves, stems and roots into the innermost part of every cell, into the genetic material in the cell nucleus," explains Dr. Johan Botterman, Head of Product Research for BioScience, the Seeds and Traits business of Bayer CropScience, in Ghent, Belgium.

Multidisciplinary teams in Ghent and their colleagues from Nunhems – Bayer CropScience's vegetable seed experts – are putting plants such as rice, cotton, oilseed rape and tomatoes through their paces: improving the taste, shelf life and convenience of vegetables, making crops tolerant to stress factors such as drought, frost or nutrient deficiencies, and also helping them to withstand pests, diseases and herbicides. "We want to do more than just supply the growing world population with sufficient high-quality foods at affordable prices," says Botterman. The crops of the future must also be better suited to more environmentally friendly and climate-adapted agricultural methods, using less water and producing higher yields.

In order to do this, the Bayer researchers are combining biotechnology techniques with the plant breeder's years of experience of accessing germplasm, making crosses and selecting progeny from these crosses. While genetic engineering – the first wave of plant biotechnology – generally added individual genes (traits) to the "gene pool" of many crops, new selection techniques are intended to produce crops with more strongly enhanced traits – and more quickly as well: each new variety historically took around 10 years to develop using traditional breeding techniques alone, but adding biotechnology techniques today can shorten the selection process by about half that time. Genetic engineering and other biotechnology tools have already brought about fundamental changes in plant research. Plant genetic

Growing world population



The global population *is increasing by some 80 million people each year. The area of arable land available per person is continuing to shrink.*

Researching for the future: together with his interdisciplinary team, Dr. Johan Botterman (small photo), Head of Product Research at BioScience, wants to help secure the food supply for the world population. Laboratory technician Dimitri Paelinck (left) prepares various plant samples for freeze-drying in order to analyze new breeding successes.

engineering addresses issues such as pest control and weed management through insect resistance and herbicide tolerance traits. Now, using biotechnology, plant researchers and plant breeders are working even more intensively on traits such as tolerance to stress caused by drought and nutrient or light deficiency, towards increased yields and higher quality.

One of the modern plant-breeding methods which the researchers are using is called molecular breeding. This practice brings the work of plant breeders and plant scientists closer together. "Thanks to advances in molecular biology, it is possible today to describe plant characteristics in terms of their genes. And the more we learn about plants with the help of biotechnology, the better we can recognize the mechanisms and gene networks which determine specific traits," explains Botterman. By means of molecular analysis in the laboratory, BioScience researchers today can see more precisely – at a genetic level – what distinguishes individual plants in a breeding program. Molecular marker analysis creates a genetic fingerprint which identifies and characterizes the individual traits of a plant – just as a barcode identifies a product. Using this technique, the Bayer researchers can find out how crop yields are affected by certain environmental influences, for example.

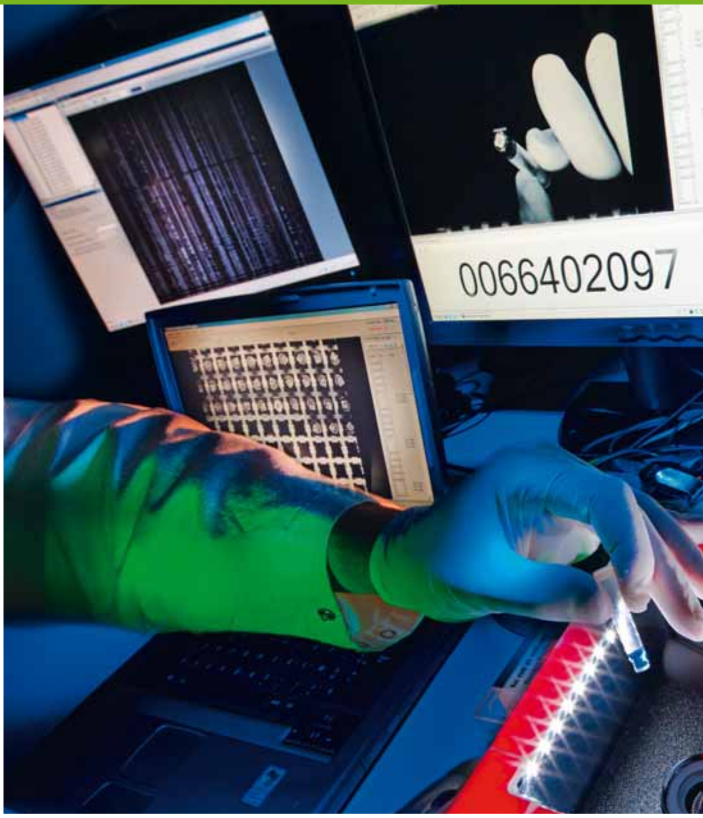
And there is another advantage. "Using molecular markers, we can search for a number of genes, and thus a number of traits, in a plant at the same time," says Benjamin Laga, one of the Group Leaders of the genetics teams at BioScience in Ghent. Traits such as improved photosynthetic efficiency, nutrient uptake and enhanced glucose transport are generally made up of a complex network of genetic components that can be easily identified using modern genetic techniques. When these characteristics have been identified, they can be seen in other plants in the future, giving useful indications for plant breeding.

Breeding 2.0: molecular biology saves time, money and space in the greenhouse

Some 10,000 years ago, humans were already deliberately trying to grow plants with particular traits in their fields, interbreed them and combine as many favorable characteristics as possible in one plant. However, genetic research has really taken plant breeding forward: in 1860, Gregor Mendel, an Augustinian monk and pioneer of genetics, had to wait and see whether the pea plants grown in his monastery garden would produce red flowers or white ones. Today there are many analytical techniques which – like looking through a magnifying glass – tell us much more about a plant than can be seen with the naked eye. For example, the experts at Nunhems know whether peppers will taste stronger or milder, long before they even take shape on the plant. "This targeted selection saves tremendous development time, space in the greenhouse and test fields, and thus money," explains Dr. Jan van den Berg,

“Snipping” genetic material

In order to analyze the characteristics of a plant more precisely, breeders today create genetic fingerprints for them. Certain combinations of markers are then used like a barcode to identify particular plant characteristics. In future, more and more cultivated plants will be analyzed using genetic markers, and this genetic information will be correlated with the identified characteristics. Although this technique is rather more superficial than precise genetic analysis, it has the advantage that several characteristics can be compared at the same time. A technique called “SNP analysis” currently produces particularly good markers. SNP – short for Single Nucleotide Polymorphism – identifies single base-pair mutations in a DNA strand. These SNPs (pronounced “snips”) are point mutations occurring at random throughout the genome, tiny genetic variations which are passed down from generation to generation in plants, animals and humans. Since many of them are correlated with particular traits, they are useful pointers around the genome – comparable with milestones on a highway.



Global Head of Molecular Breeding at Nunhems, referring to an economic aspect of plant breeding using molecular biology.

Van den Berg, together with a team of molecular geneticists, biochemists, and bioinformatics specialists, is working on traits related to fruit quality, disease resistance and yield in more than 10 vegetable crops. In order to efficiently use molecular breeding, the plants need to be properly characterized by a technology called phenomics. This technology deals with traits such as crop yield and stress tolerance. “We use this new measurement and analytical spectrum to record certain reactions in the plant at a very early stage, so that we can take prompt action to counter them,” says Dr. Michael Metzlaff, Research Liaison Manager with BioScience. For instance, abiotic stress, such as drought or cold, causes a kind of fever in the plant. “By regularly taking the temperature of every single plant in the field, we can very quickly see which ones have elevated stress levels,” explains Metzlaff.

In order to record these and other important parameters such as photosynthetic efficiency, nutrient uptake and growth rate and interpret them more clearly, a new facility has been constructed at the site in Ghent. The growth and performance of rice, oilseed and cotton seedlings will be monitored in a specially designed greenhouse. In controlled conditions of humidity and water supply, a fully automated 3D camera system will continually record data such as seedling height, growth form and length and breadth of leaves. Therefore the BioScience team in Ghent wants to interpret precise plant responses to their environment and go one step further than plant research has done in the past. Through their predictions, the BioScience molecular biologists aim to give the plant breeders useful tips for the development of new varieties, instead of associating certain traits with certain genes in retrospect. And, according to Metzlaff, this trend will be extended to modern agriculture. “Farmers need to monitor their plants even more regularly, more intensively and more precisely to determine whether the plants really are thriving,” says the biologist.

From breeder to plant designer: bioinformatics delivers higher yields

Continuing automation in the fields also plays its part. In Australia, for example, Metzlaff’s vision is already a reality. There, farmers are already driving around their fields in test tractors, with special probes linked to satellite navigation systems recording where it is too dry or too damp and which parts need more fertilizer. The farmers understand exactly how their young plants are doing, where the soil is low on nutrients and which seedlings are showing early signs of stress. “It’s not sufficient to integrate a single gene for stress tolerance into the plants – traits like drought or frost tolerance are far too complex for that. But in the future, gene combinations will definitely enable us to supply more stress-tolerant and thus better adapted plants,” says Botterman.

Plant biotechnology takes teamwork: Dimitri Paelinck (large photo) prepares the analysis robot for mass throughput tests in Ghent, Belgium. Meanwhile, Stefaan Verbouw (top) fills laboratory devices for DNA analysis and Dr. Michael Metzlaff (bottom left) interprets breeding models on the computer. Benjamin Laga (bottom center) discusses new approaches to oilseed rape research and Renée Dekkers (bottom right) stores fresh seedlings for the greenhouse in cold storage.

Equipped with a modern tool box, the plant breeder's work often comes in from the field to the laboratory – and the computer. "Today, if we conduct an experiment which takes a day, we spend a week analyzing the data," says Laga. When such enormous volumes of data are being collected, bioinformatics specialists and statisticians, with their modeling techniques, come into their own: in Ghent alone, the number of bioinformatics specialists among the 300 staff has risen from five to over 30 in a few years. The Bayer researchers now know very well how to obtain reliable data from their molecular markers. However, the real challenge is the statistical evaluation and interpretation of all the results obtained and recorded by plant breeders at Bayer CropScience over recent years. Says Laga, "The same trial can produce different results, particularly when we keep finding different environmental conditions in the field depending on the year and the region." For that reason, in the coming years the BioScience experts intend to stock their databases even more intensively and comprehensively with information about yields, pest infestations, length of exposure to sunshine, use of herbicides and much more.

When they have collected sufficient data for analysis, the biotechnologists will be able to recognize relevant markers even more clearly by means of statistical calculations. This will allow them to predict more precisely what happens in the plant when they modify a particular gene – or one of the many factors which control the gene

Quality check for seeds: laboratory technician Jan Bergs evaluates young melon plants after a vitality test in the climatic chamber.

network. By means of computer analysis, the researchers and breeders intend to produce crossing schemes for new plants: "Our computer models are now producing very reliable predictions – even without field testing." In this way, the plant breeders' wide-ranging work is complemented by innovative methods and becomes even more effective. "We now have completely new tools for plant breeding at our disposal," adds Botterman.

In order to meet its customers' needs as fully as possible, Nunhems operates 26 breeding facilities in 14 countries, and three research centers. "International collaboration is becoming increasingly important for Bayer CropScience," Botterman also confirms. The researchers at the Innovation Center in Ghent already collaborate with numerous companies, universities and other research institutes, both large and small, across the world. "In future, we want to integrate new technologies even more intensively," announces Botterman. The acquisition of Athenix Corp. in 2009 further expanded the company's R&D activities in the United States, particularly in corn and soybeans. This research group has developed new traits to help plants combat nematodes and insects and is part of the new Innovation Center established in Morrisville (North Carolina). "Since farmers, particularly in Western countries, more and more frequently complain of nematode damage, genes for resistance against nematodes are becoming increasingly important," says Botterman. Because the nematodes attack the plant roots, they generally hide in the soil and are very difficult to control with crop protection products. BioScience is also already investigating how to tap into the innovation power of Asia. "Especially for countries like China, with its rapidly increasing population, it is becoming more and more important to grow plants which use nutrients and water as efficiently as possible and obtain the highest possible yields even in less fertile soil conditions," says Botterman.

Epigenetics:
Finding the right gene switch for stress tolerance and growth

In order to design plants capable of higher yields and enhanced resistance, the biotechnologists in Ghent are also turning to the very new science of epigenetics. This field of research deals with environmental influences on genetic networks. For instance, the Bayer researchers were able to explain why some oilseed rape plants grew strongly while others, identical in their genetic make-up, remained stunted. The reason is that, when the plants are subjected to stress as a result of a deficiency in light or nutrients, short, single-strand ribonucleic acid (RNA) molecules can form in their cells, switching off individual genes and thus inhibiting the plant's growth. When cell biologists have learned to interpret these epigenetic mechanisms more effectively, they will be able to switch genes on or off more precisely.

Plant genetic engineering is important for research into pest control and weed management solutions: the bacterium Bacillus thuringiensis was the forerunner of the Bt concept, the first use of genetic engineering for pest control in maize and cotton. The bacterium produces proteins which change to a toxic form in the caterpillar's intestine and perforate it. The protein also paralyzes the pest's chewing mouthparts.

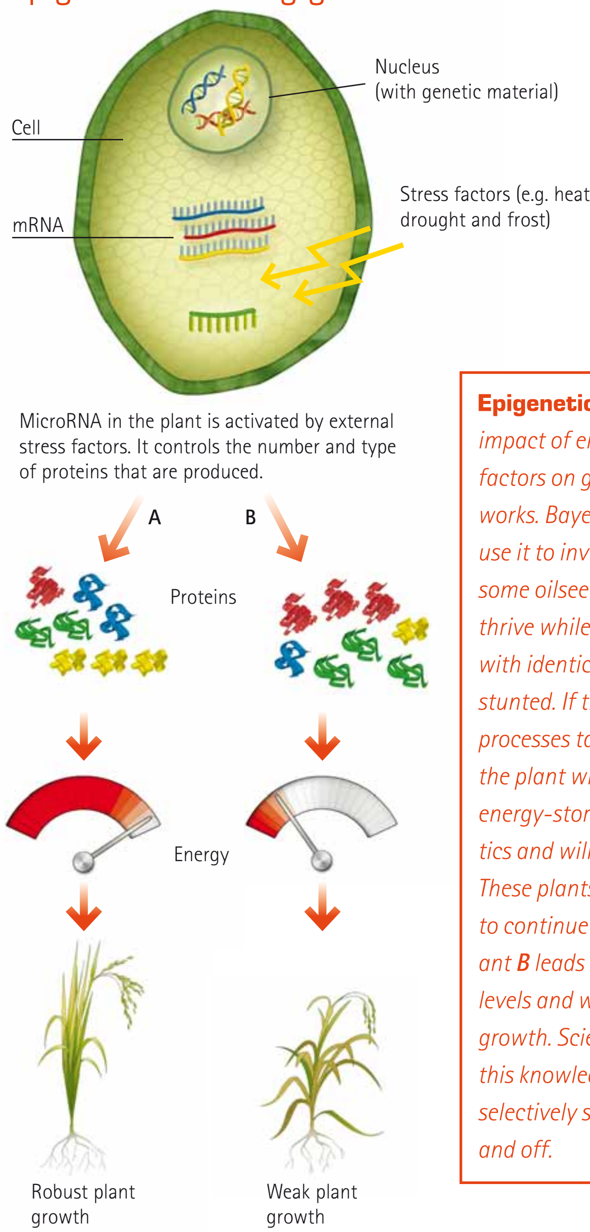
Genetic engineering helps to lower CO_2 emissions in agriculture

Following identification of the bacterial gene, it was a short step to thinking that plants could produce their own protection against insects if they carried the gene in their own genome. To achieve this, cell biologists isolated the Bt gene in the laboratory, multiplied it and transferred it to the crops with the help of another bacterium, Agrobacterium tumefaciens. Since the discovery of this naturally occurring superstar of genetic engineering, biotechnologists across the world have used the microorganism to insert foreign DNA containing the ingredients for new traits like insect resistance into plant cells and incorporate it into the plant genome. Plants with the Bt gene can thus protect themselves against caterpillars entirely by themselves. Protection concepts like these, using implanted natural substances, are very important for global food security: in Europe alone, up to 40 percent of harvests are destroyed by insects and other pests; in developing countries, the figure is as high as 80 percent.

In addition to pest control, genetic engineering has enabled more effective use of herbicides. The challenge is that broad-spectrum herbicides, such as the widely-used glufosinate, affect all the plants in a field equally – in other words, they do not distinguish between crops and weeds, broadleaf or grasses. However, plant researchers have developed a solution: a system made up of herbicide-tolerant plants and the appropriate complementary herbicides allows for simple and effective applications that preserve the crops. Herbicide-tolerant varieties are now almost universal in U.S. soybean cultivation. This has a number of advantages: many U.S. farmers have been able to employ no-till or reduced-till practices, so the soil remains undisturbed. If the soil is untilled, it is more resistant to erosion. Additionally, the field absorbs heavy rain, for instance, more quickly, and also stores water and nutrients more effectively and for longer than tilled soil. Another advantage is that lower machinery use reduces fuel usage and emissions of environmentally unfriendly CO_2 gas.

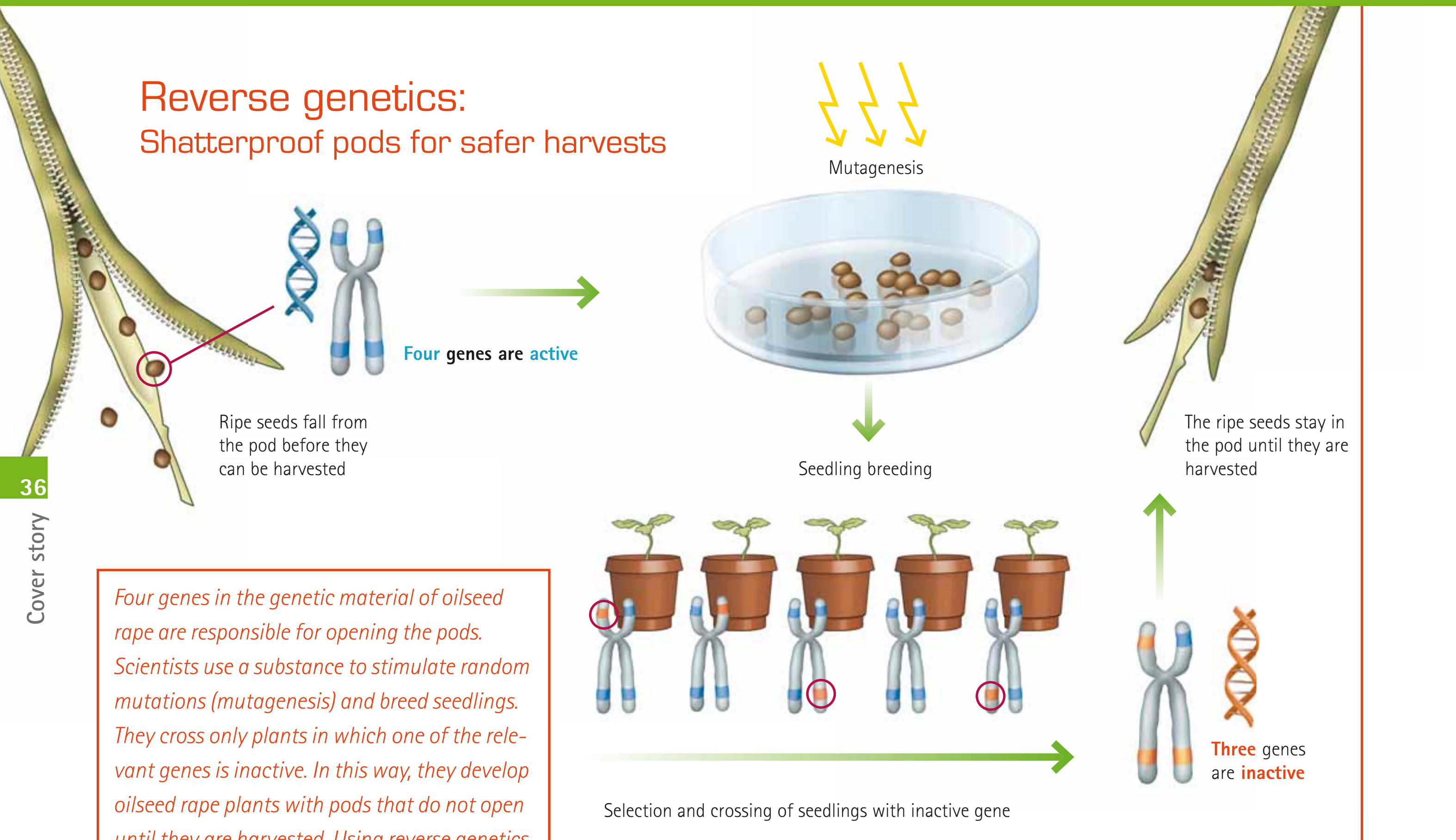
Genetic engineering techniques alone are not necessarily essential to make plants fit to cope with pest infestation. As an alternative to equipping plants with foreign genes, the Bayer researchers are also adopting new approaches: they have intensified their efforts to optimize the metabolism of plants to cope with particular environmental conditions. This process is based on the plant's genetic makeup. The Bayer researchers understand the basic makeup so well that they can see where and when a particular gene function makes a decisive difference.

Epigenetics: finding gene switches



Epigenetics *looks at the impact of environmental factors on genetic networks. Bayer scientists use it to investigate why some oilseed rape plants thrive while other plants with identical genes remain stunted. If the cellular processes take route* ***A****, the plant will have high energy-storing characteristics and will grow strongly. These plants are then used to continue breeding. Variant* ***B*** *leads to low energy levels and weak plant growth. Scientists can use this knowledge in future to selectively switch genes on and off.*

Reverse genetics:
Shatterproof pods for safer harvests



Four genes in the genetic material of oilseed rape are responsible for opening the pods. Scientists use a substance to stimulate random mutations (mutagenesis) and breed seedlings. They cross only plants in which one of the relevant genes is inactive. In this way, they develop oilseed rape plants with pods that do not open until they are harvested. Using reverse genetics, breeders can accelerate natural evolution.

However, information about key genes can be obtained not only from genetic research on bacteria, but also from model plants such as Thale cress (Arabidopsis thaliana) or directly from the genome of the crop itself. "We have advances in DNA sequencing to thank for this," says Metzlaff.

Reverse genetics:
Selectively accelerating the natural evolution of seeds

Plant research globally has reached a stage which would have been unimaginable 10 years ago. And Bayer CropScience has made its own contribution: with a number of other partners, a team led by Dr. Bart Lambert, Product Research Manager Oilseeds, has deciphered the oilseed rape genome. Working from 30,000 plant genes, Lambert then utilized a new approach called reverse genetics. The method is called "reverse" genetics because it creates a new phenotype from a combination of native genes which were modified by conventional mutagenesis and combined in a single plant or variety by molecular breeding. "We modify a gene or gene network to give the plant a new characteristic in its appearance," says Lambert.

In this reverse genetics process, the seed is dressed with a substance which causes mutations, distributed randomly throughout the entire plant genome. "Changes like these happen in nature too. But we accelerate this evolutionary process in a particular direction," says Lambert, citing the advantages of the method. From thousands of randomly mutated seed genomes, Lambert and his colleagues select the ones which show a promising mutation in the target gene. The BioScience researchers have created a very fast and reliable tracing method in order to do this, which multiplies and sequences the required gene segment. "To do this, of course, we need to know all about the gene and its function," says Lambert.

Changing gene networks and giving plants completely new traits

Through skillful selection, he and his team then combine individual mutated plants over several rounds of crossing and selection.

Bayer CropScience hopes to use reverse genetics to overcome a problem which plagues many oilseed rape farmers: seeds often fall from the grain-bearing fruits, called pods, of the ripe oilseed rape plant before harvesting and lie wasted on the fields. BioScience researchers are working to develop plants with pods that are more resistant to shattering. To do this, they modify the activity of a particular gene involved in the development of a tissue which holds the pod together. This tissue breaks down as the crop reaches maturity and under adverse conditions the pod opens, releasing the seeds prematurely.

Molecular markers for higher yields and healthy fatty acids in oilseed rape

In addition to the shatter-resistant pod, Lambert and his oilseed rape team have other plans with the reverse genetics method. The oilseed rape researchers have also worked specifically to influence fatty acid biosynthesis. This is because, although oilseed rape is one of the healthiest edible oils currently available, with low saturated fatty acids, processing by the food industry often creates trans-fatty acids which can cause cardiovascular disease. Researchers have modified the metabolism of the plant so that it now mainly produces fatty acids which do not create harmful byproducts during processing.

However, since the new type of oilseed rape with a high oleic acid / low linolenic acid profile was low yielding, the breeding process was continued to improve outputs. Here, too, the Bayer researchers' long experience of molecular breeding paid off: in a special process called marker-assisted backcrossing, the modified plants were crossed with high-yielding varieties and selections then made from the subsequent generations which showed as many traits as possible from the high-yield variety, plus the favorable oil profile characteristic. In the past, six rounds of crossing were necessary. "Using modern marker technology, we can now obtain the plants

The new age of better oils

From insignificant lamp oil to high-grade edible oil and climate-friendly biodiesel: the triumphant advance of oilseed rape is one of the most striking successes in the recent history of plant breeding. Since the 1990s, oilseed rape has held the second highest share of the world oil seed market, after soybeans. In 2009, around 54.1 million tons of oilseed rape were produced throughout the world. However, oilseed rape has only been used as a food and feedstuff since 1974. In that year, the first varieties suitable for use as edible oils were marketed under the name "zero rapeseed." The new varieties became established under the name "Canola," an abbreviation of Canadian oil, low acid, since the variety was originally developed in Canada, the largest oilseed rape producer in the world. In October 2009, Bayer researchers succeeded in deciphering the entire Canola genome, working in collaboration with two public research institutes, the Beijing Genomics Institute, Shenzen, China and the University of Queensland, Australia, as well as the biotech company Keygene in Wageningen, Netherlands.

Multiplying precious cucumber plants: Dr. Jan van den Berg, Global Head Molecular Breeding at Nunhems, in the tissue culture laboratory where scientists rapidly cut and multiply cucumber plantlets with valuable characteristics.

we want in three generations, with 100 percent reliability," explains Laga. With each generation occupying an average of four to six months, this saves around one year's development time.

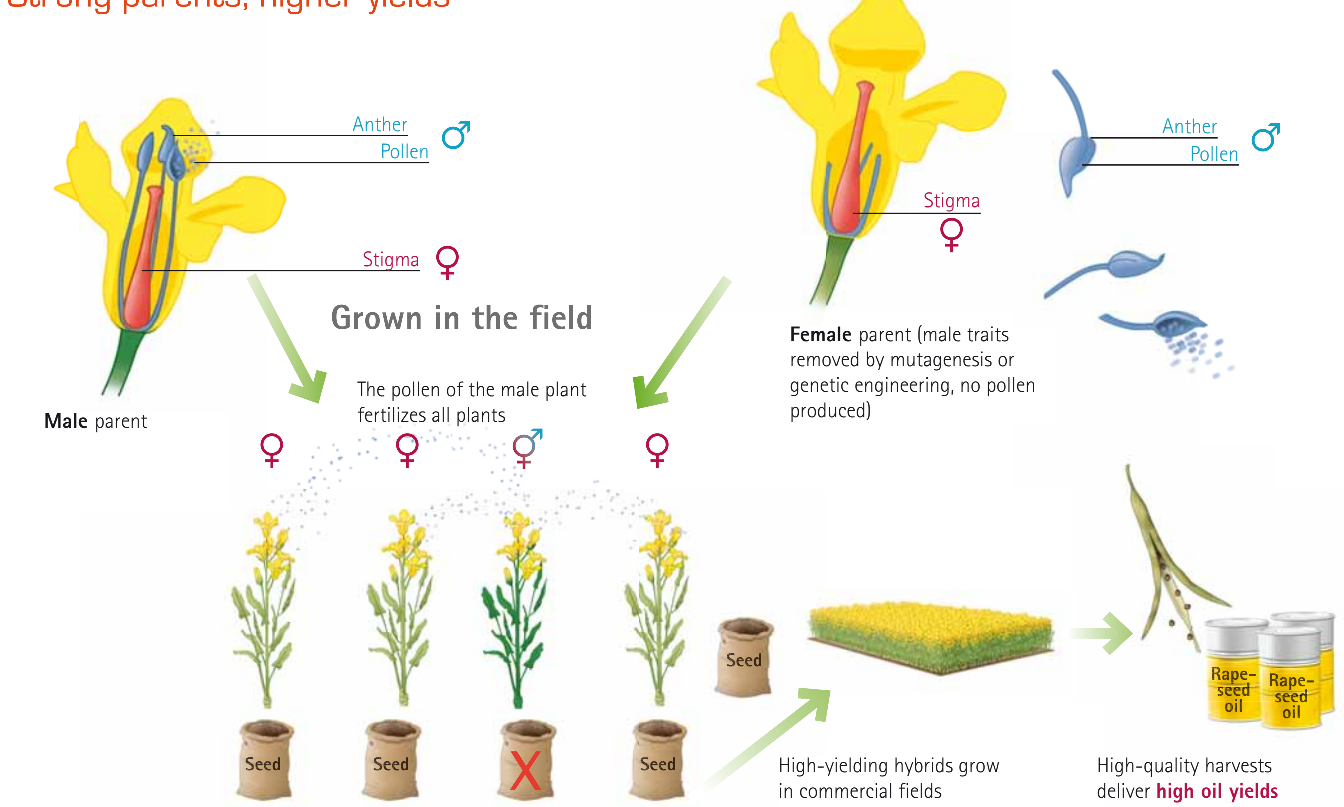
Breeding hybrid varieties:

Efficient crossing for higher yields and better stress tolerance in oilseed rape and rice

This is a valuable time saving, which is also increasingly being used in producing hybrids. The method is based on a fascinating phenomenon: if two pure-strain parent lines, genetically as far removed from one another as possible, are crossed, the progeny show better performance. The new hybrids are higher in yield and more tolerant to stress than traditional varieties. This effect is called "heterosis." Bayer CropScience is the market leader in hybrid oilseed rape, thanks to its high-yield InVigor® hybrids. Bayer CropScience is also one of the leading companies in rice with its Arize™ hybrid varieties.

"The hybridization procedure itself is no secret," says Paul Degreef, Global Head of Breeding at Nunhems. "The trick is to select the parent lines with the best possible traits, which are best suited to the prevailing climatic conditions." The Nunhems researchers' big advantage is their global infrastructure, which enables them to search for particular traits anywhere in the world. They collaborate with international colleagues and exchange germplasm – varieties of seed which already show one or two interesting properties. The molecular markers are important here, too. "They are intended to pick out the most promising seeds from the large pool of existing seeds, thus accelerating and improving control of the selection process," explains Dirk Decherf, an oilseed rape breeder with BioScience.

Strong parents, higher yields



Hybrids *currently dominate the seed market in most vegetables, maize, rice and, increasingly, oilseed rape around the world. In order to produce a hybrid which provides higher yields, breeders must first develop pure-strain male and female parent lines with the desired characteristics. The male plant supplies the pollen but only the seed from the female plant is used for growing the high-yielding commercial hybrid crop.*

Exploring cell biology: using state-of-the-art methods, Maria Hendricks, Joop Hillen and Miny Sillekens support the plant breeders in the laboratory.

For instance, in order to find out which oilseed rape plants carry a desirable trait, all you need to do is bring a piece of leaf into the laboratory and search for the required genes using molecular markers. In another case, a plant would no longer need to be infected with a particular disease in order to determine its resistance; a look through the molecular microscope is enough to identify whether the plant carries the markers known for resistance to that disease. Molecular markers make it possible to create an enormous range of new plants, which can now be varied in the tiniest detail according to the customer's requirements. Nunhems has developed over half of its 2,500 varieties of vegetable seeds in the last six years alone: "The demand for higher yields, as well as indicators of quality, such as new flavors and more aroma, for example, is increasing," says Degreef.

Genetic research is revolutionizing breeding – but field trials remain the most important experiment

Genome research has also revolutionized modern plant breeding: molecular markers and the enormous advances in the technology of genome sequencing make it possible to select plants and their traits more precisely than before and to hybridize them more effectively. And even if the breeder's work is more and more often done in the laboratory and on the computer rather than in the fields, it is still an art in its own right: "In nature, unlike the greenhouse, we still cannot dictate the exact temperature, light levels or moisture levels," says Botterman. Metzlaff agrees: "Many things that you see in the laboratory are not fully confirmed in the field." For this reason, field trials are still the definitive test for the Bayer CropScience researchers.

www.podcast.bayer.com
The Bayer podcast center provides a background film on this topic

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