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Genetically modified plants

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Abstract:

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Genetically modified (GM) plants are created by the process of genetic engineering that allows to move genetic material between organisms in order to improve their characteristics. In 2004, GM plants were grown on 81.0 million hectares by 8.25 million farmers in 17 countries (James, 2004). This year is 10th anniversary of their commercialization. The aim of this paper is to explain the technology of GM plants and potential benefits and risks it involves.

Key words: GMO, transgenic plants, plant trasformation

Introduction

Even before its scientific basis was uderstood, mankind took advantage of natural genetic variation and biotechnology. From the beginning, humans selectivelly breed wild plants, animals and even micro-organisms (yogurt cultures and yeasts) to produce domesticated variants better suited to own needs. Such selective breeding involves the transfer of unknown numbers and types of genes between individuals of the same species.

Application of biotechnology date back to 1800 B.C. when humans began to use yeast to leaven bread and ferment wine. By the 1860's, they started breeding plants through deliberate cross-polination. They transfered and selected genes to enhance the beneficial qualities of plants through cross-breeding without knowing the traits for which the genes coded. Over the past half-century, this so-called traditional or conventional breeding technology included techniques like polyploidisation and mutagenesis via x-rays. The revolution was made in 1972 with advent of genetic engineering. Scientists have been able to identify specific genes associated with desirable traits in one organisms and transfer those genes beyond boundaries of species into another organisms. While traditional plant breeding involves 10 to 12 years backcrossing hybrids with original plants to obtain desired few genes or traits, gene technology allows transfer of few selected genes between species drastically reducing both their random nature and time taken to produce an improvement.

The aim of this paper is to explain the principle of genetic modification of plants and to address to benefits and risks associated with this new technology.

What is genetic modification?

The term »genetically modified« (GM) is commonly used to described the application of recombinant deoxyribonucleic acid (rDNA) technology to the genetic alteration of microorganisms, plants and animals. Genetic modification, gene engineering, genetic

manipulation, gene thechnology or recombinant DNA technology involves »cutting-copying-pasting« approach to transfer genes from one organism to another. During this process bacterial enzymes (restriction endonucleases and DNA ligase) are used to recognise, cut and join DNA at specific sites acting as molecular »scissors-and-tape« (for a review see: Klug and Cummings, 2003). Since DNA does not always readily move from one organism to another, different »gene-delivery vehicals« are used (for a review see: Mitrović, 2003). The transferred gene, knowh as a transgene, carries instructions for making a protein which determines desirable trait. The modified cell is used to regenerate a new organism reffered as genetically modified organisms (GMO). Genetically modified plants are sometimes described as biotech, bioengineered or transgenic plants or crops. Process of genetic modification of plants will be disscussed further below.

Plant transformation

Stable incorporation and expression of foreign genes into plants is designated as plant transformation. This process is complex and involves following phases:

1. Phase 1 – selection and application of gene delivery vehicles by which transgene is transferred into plant cell.
2. Phase 2 – integration of transgene in plant genome and its expression in plant cell.
3. Phase 3 – recovery of a viable transgenic plant which can be time-consuming step involving tissue culture and plant regeneration.

Since the first reports of successful plant transformation in 1983, over 120 species in 35 different families embracing monocots and dicots, as well as algae, fungi and Hela cells have been transformed (van den Eede et al. 2004).

There are two main classes of delivery vehicles (vectors) used for plant transformation: biological and physical (Lorence, Verpoorte, 2004).

1. Biological delivery vehicles are based on two bacterial species Agrobacterium tumefaciens and Agrobacterium rhizogenes and their Ti (tumor-inducing) and Ri (root inducing) megaplasmids, respectively. These plasmids are normally transferred into plant cells, integrated in plant genom and involved in tumor formation (crown gall disease). Binary vector system from Ti plasmid is developed by genetic engineering and efficiently used to deliver transgene into many dicots (van den Eede et al. 2004). However, this system has still limited ability to transform monocots.
2. Physical delivery vehicle for plant transformation is mainly biolistic, microparticles bombardement or »gene gun«. This system is based on »shooting« of DNA-coated gold or tungsten microprojectiles into target plant tissue. The mechanism by which accelerated DNA-coated particles are able to deliver DNA into living plant cells without damage is still not clear (van den Eede et al. 2004).

However, currently available methods for gene trasfer are inneficient. Only small number of plant cells are successfully modified. Furthermore, regeneration of whole plant from culture cells may take months or years. Consequently, it is necessery to identify the modified cells in a culture mix using »marker genes« closely linked to genetic material to be transferred. Antibiotic resistance has often been used to »tag« genes so that they can be easily detected and cells conferring them selected.

The use of antibiotic resistance marker genes (ARMG) has, however, been a source of concern. Although the transfer of antibiotic resistance from a marker gene contained in a GM plant to microorganism normally present in the human gut has not been demonstrated experimentally, it has been suggested that potential risk, however small, of spreading resistance to therapeutic antibiotics could have serious health consequences and therefore should be avoided.

European Food Safety Authority (EFSA) proposed following classification for ARMGs:

1. Group 1 ARMGs contains antibiotic resistance genes which are widely distributed among soil and enteric bacteria and confer resistance to antibiotics which have no or only minor therapeutic relevance in human and veterinary medicine. Here belongs nptII gene conferring resistance to antibiotics kanamycin and neomycin and hph gene which encodes protein that inactivates hygromycin. No restriction are required with this class of marker genes.
2. Group 2 ARMGs contains antibiotic resistance genes which are widely distributed in microorganisms in the environment and confer resistance to antibiotics which are used for therapy in defined areas of human and veterinary medicine. This refers to following genes: cm^r, amp^r and aadA conferring resistance for chloramphenicol, ampicillin and streptomycin and spectinomycin, respectively. The use of these genes should be restricted to field trial purposes and not be present in GM plants placed on the market.

3. Group 3 ARMGs contains antibiotic resistance genes highly relevant for human therapy like nptIII gene conferring resistance to amikacin and tetA gene conferring resistance to tetracyclines. Irrespective of considerations about the realistic importance of the health threat, these genes should be avoided in the genome of transgenic plants to ensure the highest standard of preventive health care. Therefore these ARMGs should not be used for experimental field trials or present in GM plants placed on the market (EFSA, 2004).

Genetically modified plants

List of GM plants and genes involved in their alteration is given in Table 1 and 2, respectively (AGBIOS, 2004). GM plants are developed to express one or more of the following phenotypic characteristics: fatty acid composition, fertility restoration, herbicide tolerance, insect resistance, lepidopteran resistance, male sterility, modified color, nicotine reduced, ripening delayed, selectable marker and virus resistance.

In 2004, GM plants were grown on 81.0 million hectares by 8.25 million farmers in 17 countries (James, 2004). Herbicide tolerance, deployed in soybean, maize, canola and cotton (72% of the global GM crop acreage) is dominant trait followed by insect resistance in maize and cotton (19% of the global GM crop acreage) (James, 2004).

Benefits of GM plants

The World Health Organization (WHO) estimates that the global population will double by 2050 to more that 9 billion people. The most promising strategy for increasing global food production is GM technology. It allows development of plants with:

1. increased biological resistance to specific pests, diseases and stress reducing the need for chemical pesticides, decreasing the risk of crop failure and increasing yields,
2. adaptibility to harsh growing conditions, such as drought, soil with high salt content, temperature extremes, etc.,
3. tolerance to environmental safe herbicides,
4. desirable functional characteristics, such as reduced allergenicity or toxicity, delayed ripening, increased starch content or longer shelf life,
5. desirable nutritional characteristics, such as altered protein or fat content.

Besides, modern biotechnology ensures production of new therapeutic agents by »molecular farming« in plants. Through genetic engineering, plants can now be used to produce pharmacologically active proteins, including mammalian antibodies, blood products substitues, vaccines, hormones, cytokines, anticancer agents and variety of other therapeutic agents (Goldstein, Thomas, 2004).

Risks of GM plants

As we seen, GM plants can provide substantial benefits to humans, but they can also pose risks to ecosystems, nontarget species and even to humans (Wolfenbarger, Phifer 2000). Major concerns connected with GM plants are: risk of invasiveness, nontarget effects on beneficial and native organisms, indirect effects on species that depend on the targeted pest and risk of new viral diseases.

Each genetic modification, through traditional breeding or genetic engineering, can create changes that enhance an organism's ability to become an invasive species. The spread of transgenes (i.e. gene flow) through GM pollen is hard to control. Pollen can be carried on the wind for tens of kilometres and by bees up to 13.7 kilometres (Malone, 2002). There is potential risk of GM crops hybridizing (i.e. sharing their genes) with closely related wild species and subspecies and thereby creating herbicide resistant weeds (Jenczewski et al. 2003). This has certainly happened with conventional crops but there is no evidence of having occurred with GM crops. Also, there is potential of horizontal gene transfer from transgenic plants to soil microbes (Dunfield, Germida, 2004).

There are different ways of dealing with a problem of gene flow. The most interesting is chloroplast genetic engineering. It is well known fact that the chloroplast genome is absent from pollen (maternal inheritance). In this strategy transgene is put under control of chloroplast regulatory signals, so errant transgenes won't express in the nucleus. Since each cell contains 10,000 copies of the chloroplast genome, problem with low expression of transgene is solved (Malone, 2002).

Another ways of preventing gene flow are creation of GM plants: without pollen, without flower, without fertile seeds (the »Terminator« system) and with fertile seeds in which any foreign GM DNA is spliced out and destroyed (the »Exorcist« system) (Malone, 2002, Giovannetti, 2003).

Table 1. GM plants (AGBIOS, 2004)

Plant	Phenotypic trait
Argentine Canola	Oxynil herbicide tolerance, including bromoxynil and ioxynil.
Argentine Canola	Modified seed fatty acid content, specifically high laurate levels and myristic acid production.
Argentine Canola	Glyphosate herbicide tolerance.
Argentine Canola	Phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium.
Argentine Canola	Imidazolinone herbicide tolerance, specifically imazethapyr.
Argentine Canola	Glufosinate ammonium herbicide tolerance and fertility restored.
Argentine Canola	Modified seed fatty acid content, specifically high oleic acid, low linolenic acid content.
Carnation	Increased shelf-life due to reduced ethylene accumulation through introduction of truncated aminocyclopropane cyclase (ACC) synthase gene; Sulfonylurea herbicide tolerance, specifically triasulfuron and metsulfuron-methyl.
Carnation	Modified flower colour; Sulfonylurea herbicide tolerance, specifically triasulfuron and metsulfuron-methyl.
Chicory	Glufosinate ammonium herbicide tolerance and fertility restored.
Cotton	Resistance to lepidopteran pests including, but not limited to, cotton bollworm, pink bollworm, tobacco budworm.
Cotton	Oxynil herbicide tolerance, including bromoxynil and ioxynil.
Cotton	Resistance to lepidopteran insects; oxynil herbicide tolerance, including bromoxynil.
Cotton	Sulfonylurea herbicide tolerance, specifically triasulfuron and metsulfuron-methyl.
Cotton	Glyphosate herbicide tolerance.
Cotton	Phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium.
Cotton	Resistance to lepidopteran pests.
Creeping Bentgrass	Glyphosate herbicide tolerance.
Flax, Linseed	Sulfonylurea herbicide tolerance, specifically triasulfuron and metsulfuron-methyl.
Lentil	Imidazolinone herbicide tolerance, specifically imazethapyr.
Maize	Glyphosate herbicide tolerance.
Maize	Imidazolinone herbicide tolerance, specifically imazethapyr.
Maize	Resistance to European corn borer (*Ostrinia nubilalis*); glyphosate herbicide tolerance.
Maize	Phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium.
Maize	Resistance to European corn borer (*Ostrinia nubilalis*); phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium.
Maize	Resistance to European corn borer (*Ostrinia nubilalis*).
Maize	Glufosinate ammonium herbicide tolerance and male sterility.
Maize	Imidazolinone herbicide tolerance.
Maize	Cyclohexanone herbicide tolerance, specifically sethoxydim.
Maize	Glufosinate ammonium herbicide tolerance and fertility restored.
Maize	Resistance to European corn borer (*Ostrinia nubilalis*).
Maize	Resistance to corn root worm (*Coleopteran, Diabrotica sp.*)
Maize	Resistance to lepidopteran pests.
Melon	Delayed ripening by introduction of a gene that results in degradation of a precursor of the plant hormone ethylene.
Papaya	Resistance to viral infection, papaya ringspot virus (PRSV).
Polish Canola	Glyphosate herbicide tolerance.
Polish Canola	Phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium.
Potato	Resistance to Colorado potato beetle (*Leptinotarsa decemlineata*, Say).
Potato	Resistance to Colorado potato beetle (*Leptinotarsa decemlineata*, Say); resistance to potato leafroll luteovirus (PLRV).

Tab. 1. Part II

Plant	Phenotypic trait
Potato	Resistance to Colorado potato beetle (*Leptinotarsa decemlineata*, Say); resistance to potato virus Y (PVY).
Rice	Phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium.
Rice	Imidazolinone herbicide tolerance.
Rice	Imidazolinone herbicide tolerance, specifically imazethapyr.
Soybean	Glyphosate herbicide tolerance.
Soybean	Phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium.
Soybean	Modified seed fatty acid content, specifically high oleic acid expression.
Soybean	Modified seed fatty acid content, specifically low linolenic acid.
Squash	Resistance to viral infection, watermelon mosaic virus (WMV) 2, zucchini yellow mosaic virus (ZYMV).
Squash	Resistance to viral infection, cucumber mosaic virus (CMV), watermelon mosaic virus (WMV) 2, zucchini yellow mosaic virus (ZYMV).
Sugar Beet	Phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium.
Sugar Beet	Glyphosate herbicide tolerance.
Sugar Beet	Glyphosate herbicide tolerance.
Sunflower	Imidazolinone herbicide tolerance.
Tobacco	Oxynil herbicide tolerance, including bromoxynil and ioxynil.
Tobacco	Nicotine reduced.
Tomato	Increased shelf-life (delayed ripening) due to reduced ethylene accumulation through introduction of truncated aminocyclopropane cyclase (ACC) synthase gene.
Tomato	Resistance to lepidopteran pests including, but not limited to, cotton bollworm, pink bollworm, tobacco budworm.
Tomato	Delayed ripening by introduction of a gene that results in degradation of a precursor of the plant hormone ethylene.
Tomato	Delayed ripening by introduction of a gene that results in degradation of a precursor of the plant hormone ethylene.
Tomato	Delayed softening through suppression of polygalacturonase (PG) enzyme activity.
Wheat	Imidazolinone herbicide tolerance, specifically Cyanamid AC299 263 (imazamox, active ingredient).
Wheat	Glyphosate herbicide tolerance.
Wheat	Imidazolinone herbicide tolerance, specifically imazethapyr.

Concerning direct nontarget effects on beneficial and native organisms, the best example is effect of Bacillus thuringiensis (Bt) toxin. This bacterial toxin, called CryIA(b), is highly specific and efficient against certain insect pests, such as the European corn borer and the spruce budworm. When it was discovered, it was hailed as an ecologically friendly, natural pesticide free of the dangers posed by potent, but indiscriminate, organophosphate insecticides. Later, laboratory experiments revealed adverse effect on beautiful Monarch butterfly. Monarch larvae died after feeding with milkweed leaves dusted with pollen from Bt-modified corn (Wolfenbarger and Phifer 2000). The impacts of insecticide proteins released into soil by transformed plants on non-target microbial soil communities is still under evaluation (Dunfield, Germida, 2004, Motavalli et al. 2004).

Theoretically, viruses with new biological characteristics could potentially arise in transgenic viral-resistant plants through recombination and heteroencapsidation, but, empirically, there is no evidence of such event yet (Wolfenbarger, Phifer 2000).

British researcher Arpad Pusztai has indicated that rats fed on genetically modified potatoes expressing the lectin Galanthus nivalis agglutinin (GNA) for 110 days, the equivalent to 10 years in human terms, showed signs of stuned growth and increased vulnerability to disease (Ewen, Pusztai, 1999). Proliferation of gastric mucosa has been observed.

Area of the greatest concern is safety of GMO foods for human consumption. Possible effects of GM food on human nutrition, allergenic responses, potential effects of viral DNA in plants on human health, the fate of GM DNA in the digestive system

is under carefully evaluation (The Royal Society, 2002, Konig et al. 2004). The latest microarray-based technology is used in detection and traceability of GMO in food and feed (Miraglia et al. 2004).

Conclusions

Since the creation of the first GM plant in the early 1980s, neverending controversy about their potential benefits and safety begins. One side claims that GM plants, such as vitamin A-boosted »golden rice« or protein-enhanced potatoes can improve nutrition, that drought- and salt-resistant varieties can flourish in poor condition and end world hunger and insect-repelling plants can protect the environment by minimising pesticide use (Monastra, Rossi, 2003). The other side protests that the risks are still unclear, speaks of »frankenfood« with unforeseen, adverse effects on consumers, producing toxic proteins and allergens or transfering antibiotic-resistance and other genes to human gut bacteria. Some of them worns of »superpests«, since insect-repelling plants will speed the evolution of insecticide-resistant pests.

This has led to a de facto moratorium on GM plants and derived food in EU since 1998. These days The European Comission has published a list of 26 GM products (12 varieties of maize, 6 of oilseed rape, 5 of cotton, one of soybean, one biomass and one yeast cream) approved to be put on the EU market.

This is the 10th anniversary of GM plant's commertialization. There is still a great need for development of appropriate monitoring systems and methods to evaluate the environmental impact of GM plants. Further studies are necessary to increase the general knowledge about GMOs and their long-term effects on human health (Kuiper et al. 2004). Also, current legislation for GMO concerning environmental aspects and food and feed safety, procedures for commercialization and labeling provision should be harmonize worldwide.

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Summary

Genetički modifikovane biljke

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Odsek za biologiju sa ekologijom, Prirodno-matematički fakultet, Univerzitet u Nišu

Genetički modifikovane (GM) biljke su nastale tehnologijom genetičkog inženjerstva koja omogućava transfer genetičkog materijala između organizama s ciljem poboljšanja njihovih osobina. Prema podacima iz 2004, GM biljke su gajene na 81,0 miliona hektara od strane 8,25 milion farmera iz 17 zemalja (James, 2004). Ove godine je 10. godišnjica njihove komercijalizacije. Cilj ovog rada je da objasni tehnologiju GM biljaka i potencijalne koristi i opasnosti koje iz njene upotrebe proističu.