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**Genetic modifications as a future prospect
in the improvement of the major qualitative traits
of cereals. A review**

Modyfikacje genetyczne szansą na poprawę najważniejszych cech jakościowych
roślin zbożowych. Praca przeglądowa

Summary. Progress in cereals transformation which can be observed for last two decades has great importance in the development of plant science and agriculture. So far, non-vector techniques, particularly direct gene transfer using „gene gun”, have been often applied in cereals transformation. However, agrobiotechnology achievements enabled cereals transformation with the soil bacterium *Agrobacterium tumefaciens*. Initially, it was believed that this technique cannot be applied to cereals because monocotyledones are outside the host range of the crown gall disease. Nowadays, the top five cereals with the highest economic significance – rice (*Oryza sativa* L.), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and sorghum (*Sorghum bicolor* L.) are quite efficiently transformed by *A. tumefaciens*. By means of molecular genetic tools it is possible to obtain cereals with new, improved traits. The present paper is focused on agricultural development which can be observed by the application of GM cereals tolerant to biotic and abiotic stress factors. Moreover, we summarized the latest achievements in cereals transformation.

Key words: transformation, *Agrobacterium tumefaciens*, cereals, stress tolerance, GM crops

INTRODUCTION

The highest global importance in cereals production has quality and quantity of grain. The major limitations of these two factors are abiotic and biotic stresses that can lead to 30–60% yield loss [Dhlamini *et al.* 2005]. Drought, salinity, and temperature represent the major abiotic threats, whereas biotic stresses that include bacterial, viral and fungal pathogens, weeds, and pests have caused historically severe yield reductions. For this reason, designing of wide cereals genetic variation with higher grains production is big challenge to agricultural researchers and plant breeders. Thanks to conventional

breeding, the resistance/tolerance to both abiotic and biotic stresses has advanced greatly with a spectacular yield increase during the last century [Ji *et al.* 2013].

The cereals: wheat, barley, rice, maize and sorghum provide approximately 40% of the energy and protein component in human diet. Economic growth of developing countries will lead to higher demand for grain [Dunwell 2014]. However, current tendency to soil erosion, global warming and other environmental problems cause losing of farm lands on a global scale (Intergovernmental Panel on Climate Change¹). Environmental conditions caused plant response through numbers of physiological and biochemical processes. Identification of stress-protective and adaptation-related genes that are activated by stress factors is subject of many scientific researches. Overexpression of those genes is necessary in creating stress tolerant plant. It was proved that many active stress-responsive genes that are regulated by transcription factors, are useful in application in transgenic studies [Mrózowa *et al.* 2014].

Besides human consumption, some cereals are used as malt in brewing and distilling industry or as an additive for animal feeding. Moreover, last time cereals were successfully used in production of grains with modified quality. Transgenic cereals are able to produce modified proteins, carbohydrates, oils and other nutritional components [Morell 2012, Rawat *et al.* 2013]. Vaccine antigens, pharmaceuticals and other therapeutic proteins can be also produced in that expression systems [Dunwell 2014]. For that and many other reasons, application of biotechnology to crop improvement plays key role to sustain and elevate grain production. Developing of knowledge of the molecular mechanisms in plants, analysis of genes effects, transgenes regulation and commercialization of GM plants can bring numbers of beneficial economic effects.

Application of *Agrobacterium tumefaciens* is the most common technique for dicotyledonous species transformation. *A. tumefaciens* has ability to transfer the part of bacterial DNA segment from Ti plasmid (T-DNA) into plant cells. This T-DNA region contains genes encoding enzymes responsible for occurrence of plant tumors that are called crown gall. Developing biotechnology enabled the use of *A. tumefaciens* in generating transgenic plants without symptoms of disease. The first plant genetic modification with *A. tumefaciens* took place in 1983 and it was performed on tobacco plant [Hoekema *et al.* 1983]. At that time it was believed that this technique cannot be applied for monocotyledones, because these plants are outside the host range of the crown gall. For this reason, cereals were transformed mainly by gene gun method. The first successful transformation of cereal using *Agrobacterium* technique was applied for maize [Graves and Goldman 1986]. Now, after years of experiments and tests, *Agrobacterium* transformation is the most common and efficient transformation technique for major cereal crops: wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), [summarized in: Dunwell 2008] maize (*Zea mays* L.), rice (*Oryza sativa* L.) [Hiei and Komari 2008] and sorghum (*Sorghum bicolor* L.) [Wu *et al.* 2014].

In the summaries below the most significant achievements of crops transformation are presented. We shortly reviewed the first trails and the progress of cereals transformation with *Agrobacterium* with focus on agriculture development by application of modified cereals tolerant on biotic and abiotic stress factors.

THE FIRST TRIALS OF MONOCOTYLEDONES TRANSFORMATION WITH *Agrobacterium*

The main problem of monocotyledonous plant transformation by *Agrobacterium* was the fact that these plants are mostly outside the host range of crown gall disease, in contrast to dicotyledonous species. The first breakthrough was performed by Douglas *et al.* [1985] who tested bamboo plants. They found that *A. tumefaciens* is able to attach specifically to the bamboo cells in the same way as to the dicotyledonous plant cells. However, low level of compounds that induce *vir* genes limited transfer of bacterial T-DNA region [Usami *et al.* 1987]. Nevertheless, induction of *vir* genes can be obtained by artificially added chemicals with acetosyringone [Stachel *et al.* 1985]. Although numbers of tests were performed, some issues were still unsolved and the statement that monocotyledones could be transform by *Agrobacterium* was still controversial.

The most significant changes in results of cereal transformation were reported in the mid-1990s. The biggest breakthrough was performed by Chan *et al.* [1993] that have successfully transferred and expressed a reporter gene driven by an alpha-amylase promoter in a japonica type of rice (*Oryza sativa* L. cv. Tainung 62) using the *Agrobacterium*-mediated gene transfer system. One year later, Hiei *et al.* [1994] tested transformation of various tissues: shoot apices, segments of roots from young seedlings, scutella, immature embryos, calli induced from young roots and scutelia or cells in suspension cultures induced from scutella. Obtained results indicated that transgenic rice from calli co-cultivated with *A. tumefaciens* gave the highest frequency (23%) of tissues pieces that produced hygromycin resistant cells. Additionally, Ishida *et al.* [1996] obtained transgenic maize from immature embryos. The frequency of transformation was remarkably high, between 10 and 30%.

In 1982 it was proved that induction of cell division in response to wounding is crucial for transformation (Kahl 1982). For this fact, another approach consisted difficulties in monocotyledones transformation showed that cereals do not show wound response [Potrykus 1990]. Thus, lack of wound response in monocotyledonous plant can be a reason why transformation via leaf is so difficult. Hiei [1994] and Ishida's [1996] studies confirmed that hypothesis by using actively dividing cells not leaves. They showed that plant regeneration abilities were essential in cereal transformation.

TRANSGENIC CEREALS WITH ENHANCED TOLERANCE TO BIOTIC STRESS

For this moment, there are no commercial GM cereals that are tolerant to pathogens. However, many intense studies are performed mostly on wheat, that combine laboratory and field tests. In fact, wheat is attacked by the highest number of various fungal diseases. Among dangerous pathogens are *Puccinia graminis* (stem rust), *Mycosphaerella graminicola* (Septoria tritici blotch – STB) or the most common *Fusarium* (head blight or/and crown root). *Fusarium* infection leads to numbers of plant diseases and causes huge economical losses by decrease or total reduction of grain yield. Crops losses can reach even more than 40%. *Fusarium* growth and development is dependent on numbers of environmental factors, mostly on temperature (lower than for other fungal species) and

amount of rain. Agronomic factors e.g. soil cultivation, nitrogen fertilization, fungicides or the host genotype are very significant as well [Wiśniewska *et al.* 2014].

Additional risk of *Fusarium* infection is contamination of the grain with mycotoxins that are recognized as very dangerous for human and animals' organisms. Diseases can occur by indirect (with infected meat, milk or eggs) or direct (with infected food of plant origin e.g. grains) reaction. That can cause a number of digestive or immune system disorders. Consuming of infected food can lead to diarrhoea, kidney damage, cancer and eventually to death. Mycotoxins are also a huge danger for plants and cause disorders in plant development by induction of chromosomal aberration, necrosis and reduction activity of many enzymes [Wiśniewska *et al.* 2014]. Additionally, toxins cause inhibition of yeast growth during fermentation process, what disturbs bioethanol production.

Last studies showed that introduction of selected genes can significantly reduce the damages caused by *Fusarium* (about 53%) in GM cereals compared to control (non-GM). Among these genes are: bovine lactoferrin gene (all transgenic lines exhibited a significant level of resistance compared to untransformed) [Han *et al.* 2012], NPR1 gene (non-expressor of PR genes) from *Arabidopsis thaliana* [Gao *et al.* 2013], PvPGIP (polygalactouronase-inhibiting protein) gene from *Phaseolus vulgaris* [Ferrari *et al.* 2012] or the wheat lipid transfer protein gene TaLTP5 [Zhu *et al.* 2012].

Another approach consists the powdery mildew resistance. Many studies were performed on wheat and numbers of genes were tested. It was proved that mildew resistant wheat can be achieved by using of virus-induced gene silencing (VIGS) of *Mlo* genes [Várallyay *et al.* 2012] and alleles of the resistance locus *Pm3* in wheat, conferring race-specific resistance [Brunner *et al.* 2012]. However, next studies suggest that GM wheat which is resistant to one pest (powdery mildew) can be more susceptible to another pest (aphids) [von Burg *et al.* 2012, Zeller *et al.* 2013].

Similar studies, concerning powdery mildew, were performed on barley and showed positive effect of modification of the HvNAC6 transcription factor expression on plant resistance. Barley HvNAC6 is a member of the plant-specific NAC transcription factor family and it was shown that it acts as a positive regulator of basal resistance in barley against the biotrophic pathogen *Blumeria graminis* f. sp. *hordei* (*Bgh*) [Chen *et al.* 2013]. NAC transcription factor was originally derived from the names of three proteins, no apical meristem (NAM), ATAF1-2 (*Arabidopsis* transcription activation factor), and CUC2 (cup-shaped cotyledon) [<http://plantfdb.cbi.pku.edu.cn/family.php?fam=NAC>].

Rice blast caused by *Magnaporthe oryzae* was also examined. Chitin which is a component of fungal cell walls, acts as an elicitor in many plants. The CEBiP (plasma membrane glycoprotein) is a receptor for the chitin elicitor (CE) in rice. It was demonstrated that the perception of CE by CEBiP contributes to disease resistance against the rice blast fungus, *Magnaporthe oryzae*. Additionally, it was proved that knockdown of CEBiP expression allowed increased spread of the infection hyphae. To enhance defense response, chimeric genetic construct, composed of CEBiP and *Xa21* was constructed, which mediate resistance to rice bacterial leaf blight [Kishimoto *et al.* 2010]. Similar reaction was observed in lines with overexpression of the *WRKY30* gene [Peng *et al.* 2012]. Rice showed improvement of resistance to rice blast caused by *Magnaporthe grisea* but also to rice sheath blast that is caused by *Rhizoctonia solani*. Additionally, it

was found that JA (jasmonic acid) plays a crucial role in the WRKY30-mediated defense responses to fungal pathogens.

Kumar *et al.* [2003] produced *Rhizoctonia solani* tolerant rice by inserting rice chitinase gene – *chi11*. Moreover, Ignacimuthu and Ceasar [2012] showed increase resistance to leaf blast caused by *Pyricularia grisea* in finger millet by expression of the same rice gene encoding chitinase (*chi11*). Next to chitinase, the glucanase gene has been given the highest priority in development of resistant plants. Introduction of β -1,3 and 1,4-glucanase gene (*Gns1*) to rice indicated increase of resistance to blast infection [Nishizawa *et al.* 2003]. Similar effect was observed for another glucanase gene – OsGLN2 [Akiyama *et al.* 2004]. Moreover, combined introduction of chitinase and glucanase genes leads to higher fungal resistance. Zhu *et al.* [2007] designed Super Hybrid rice. They introduced two chitinase genes (*RCH10*, *RAC22*) from rice, glucanase gene (β -*Glu*) from alfalfa and a ribosome inactivating protein gene (B-RIP) from barley. Significant increase of resistance to rice blast disease was observed. Another study indicated that expression of rice chitinase (*chi11*) and tobacco β -1,3-glucanase genes caused resistance to sheath blight disease in rice [Sridevi *et al.* 2008].

Other dangerous plant pathogen is *Ustilago maydis* that causes huge losses in maize crops. Van der Linde *et al.* [2012] show that in maize, the resistance can be obtained by silencing of a gene encoding a putative cystatin (*CC9*), which is induced upon penetration by *U. maydis* wild type. Silencing of *cc9* resulted in a strongly induced maize defense gene expression and a hypersensitive response to *U. maydis* wild-type infection.

Another target for development of transgenic cereals is resistance to insects. This approach is based on toxin that was found in soil bacteria – *Bacillus thuringiensis*. Many proteins from this bacterium were recognized as toxic for insects and they were commonly used in agriculture and forestry as sprays. After years of studies, development of molecular biotechnology and microbiology led to expanding knowledge about these toxins and now genes responsible for their biosynthesis are widely isolated from *Bacillus thuringiensis* and introduced into the crops. The first research was performed on the corn borer (*Ostrinia nubilalis*), a lepidopteran pest of maize. Gradually, another *Bacillus* genes were identified and isolated that were useful to design plants tolerant to other pests (coleopteran species, corn root worm) [Narva *et al.* 2013]. On the USA market there are maize varieties with several *Bt* genes sometimes combined with herbicide tolerance genes [Edgerton *et al.* 2012]. Single variety can contain even eight transgenes [Tabashnik *et al.* 2013]. One of the most important tool for insect pest protection is MON810 maize carrying the *Bt* gene (*CryIAb*) developed by Monsanto. That maize line is highly resistant to European corn borer by production of delta endotoxin [Ostry *et al.* 2010]. Tests of insect resistance genes had additional positive side-effects in crops. In GM maize with expression of *Bt* gene encoding resistance to corn root worm, the enhanced nitrogen uptake and improvement of nitrogen use efficiency was observed [Haegele and Below 2013]. Those results can be useful in agronomical approaches. Next studies indicated that maize with *Bt* gene had higher microbial activity and nitrogen mineralization [Velasco *et al.* 2013]. Moreover, transgenic rice showed reduction of the methane emission flux. However, that study proved reduction of bacterial communities in paddy soil [Han *et al.* 2013].

One of the major tasks for scientists is to design a transgenic plant without resistance development in the target insects. Previous studies indicated that after prolonged application of any compound the resistance was developed. After marketed the first GMO products, researchers suggested a strategy of areas with non-transgenic plants called refugia. That could limit incidence of insects with mutant resistance gene. Unfortunately, some farmers did not adopt this strategy. It is presumed that now about five pests developed such resistance [Tabashnik *et al.* 2013].

The maize ribosome-inactivating protein (MRIP), which was found in mature kernels and cleaves part of the ribosome, provides resistance to maize pests as well. Expression of this protein in leaves of different species of plants has resulted in increased resistance to both insects and fungi. Another food crop protein that has antiinsect and antifungal activities is wheat germ agglutinin (WGA), which appears to affect insects by disrupting the peritrophic membrane. Dowd *et al.* [2012] investigated higher level of resistance to two major maize insect pests, the corn earworm (*Helicoverpa zea*) and fall armyworm (*Spodoptera frugiperda*) caused by these two proteins which were transgenically expressed in maize leaves. Additionally, resistance for fungus *Fusarium verticillioides*, which produces mycotoxins in maize was also tested.

Latest study concerns introduction of synthetic *avidin* gene into spring wheat (*Triticum aestivum* L.) cv. Giza 168 using a biolistic bombardment protocol [Abouseadaa *et al.* 2015]. Avidin as a glycoprotein that is able to bind biotin what is needed for insects to carboxylation reactions. For this fact, plants containing avidin are toxic to a wide range of insects. Therefore, transgenic wheat plants had improved resistance to *Sitophilus granarius*.

Biotic stress is also related with attacks by some bacteria or viruses. In this field of science, corresponding to GM cereals, some achievements are reported as well. In rice a silencing of the dominant allele *Xa13* leads to improve rice bacterial blast resistance [Li *et al.* 2012]. For virus resistance approach in wheat an expression of the artificial microRNA was performed. As a results wheat streak mosaic virus resistant plants were obtained [Fahim *et al.* 2012]. Expression of dsRNA- specific endoribonuclease gene provided resistance to maize rough dwarf disease [Cao *et al.* 2013]. In rice an expression of RNAi construct that contains CP (coat protein) gene and SP (specific proteins) gene from RSV (rice stripe virus) caused enhance resistance [Zhou *et al.* 2012]. Similar strategy was observed for RGDV (rice gall dwarf virus) [Shimizu *et al.* 2012] or for rice grassy stunt virus [Shimizu *et al.* 2013].

TRANSGENIC CEREALS WITH ENHANCED TOLERANCE TO ABIOTIC STRESS

Tolerance to abiotic stress is much more difficult approach for the researches. Nevertheless, the knowledge of plants response to changes of environmental condition is very important. Plants are intensively tested under different stress factors like drought, salt or nutrition deficiency and some results are presented below. Plant responds to environmental stresses through numbers of biological and biochemical processes. The biggest effort

has to be made for identification of stress protective genes or adaptation of genes that can be active during abiotic stress.

SALINITY

Both salinity and drought induce osmotic stress. According to reports GM plants showed 4–11% losses in yield. In contrast, non-transgenic control showed more than 56% losses [Dunwell 2014]. Study on wheat demonstrated that overexpression of *TaOPR1* gene cause increase tolerance on salinity [Dong *et al.* 2013]. That suggests this gene is a part of signaling pathway combined with regulation of the ABA-mediated signaling network. Studies with barley indicated that mitogen activated protein kinase HvMPK4 is involved in higher saline tolerance [Abass and Morris 2013]. It was proved that transgenic rice with overexpression of TaSIP gene (wheat gene encoding a salt tolerance protein) [Du *et al.* 2013] and *LcSain1* (sheepgrass) gene [Li *et al.* 2013] presented improved tolerance to salinity. Overexpression of *Arabidopsis CBF3* gene in GM oat enhanced salt stress tolerance. Additionally, it was noticed that even during salt stress condition, transgenic plants showed maintenance of leaf area, chlorophyll content, photosynthetic and transpiration rate as well as relative water content [Oraby and Ahmad 2012].

DROUGHT

It was proved that expression of molybdenum cofactor sulfurase from *Arabidopsis* in maize caused enhance of the drought tolerance [Lu *et al.* 2013]. Other study shows that *OspIL1* gene in GM rice promotes internode elongation and reduces plant height by cell-wall-related genes in response to drought. That data suggests plant growth can be improved under stress conditions [Todaka *et al.* 2012]. However, there are more reports consisted transgenic rice and drought tolerance. Overexpression of *OsbZIP16* [Chen *et al.* 2012a], *OsHsfA7* [Liu *et al.* 2013], γ -glutamylcysteine synthetase [Choe *et al.* 2013], MIOX [Duan *et al.* 2012] improved drought resistance as well. Zhang *et al.* [2012] suggested that overexpression of *Oshox22* gene leads to ABA biosynthesis which regulates drought response by ABA-mediated signal transduction pathways. Another approach indicates that transgenic maize with expression of *Bacillus subtilis* cold shock protein B revealed drought tolerance [Beazley *et al.* 2012].

COLD

In transgenic spring barley it was demonstrated that *TaCBF14* and *TaCBF15* wheat genes caused improvement of frost or other abiotic stress tolerance [Soltész *et al.* 2013]. Similar results were obtained for transgenic barley with overexpression of *Osmvb4* rice gene under the control of cold inducible AtCOR15 α promoter [Soltész *et al.* 2011] or TaDREB3 gene from wheat [Hackenberg *et al.* 2012, Kovalchuk *et al.* 2013] that

also survived low temperatures. Shou *et al.* (2004) showed that freezing tolerance in maize can be improved by constitutively expressing the active version of a tobacco MAPKKK gene, *NPK1*, which is an activator of the oxidative signaling pathway. Two *NPK1*-transgenic maize events were able to withstand up to 2°C lower freezing temperature compared with their nontransgenic siblings. The 2°C improvement in the freezing tolerance would dramatically minimize yield loss due to frost damage that often occurs in spring and fall seasons, thereby stabilizing the productivity of maize. In this study, the transgene used for engineering maize, *NPK1*, was involved in an H₂O₂ signaling pathway and modified maize plants expressing this gene displayed enhanced freezing tolerance. That results proved that the oxidative signaling pathway is one of the multiple pathways regulating plant response to stress. Additionally, that study showed that at least two genes (*GST* and *HSP17.8*), documented in the oxidative signaling pathway were up-regulated in both of the *NPK1*-transgenic events studied. Those results indicate that a dicotyledonous MAPKKK is able to activate the oxidative signaling pathway in a monocotyledonous plant. This activation, in turn, provided protection to plants from freezing damage.

NUTRIENT DEFICIENCY

Studies focused on improving crop under nutrient deficiency are mostly based on the maize overexpression of *Thellungiella halophila* H – pyrophosphatase gene [Pei *et al.* 2012]. In fact, under phosphate sufficient condition, transgenic plants indicate more vigorous growth of roots compared to non-GM control. However, stress of phosphate deficit caused development of more robust root systems. That result suggests transgenic plants subsequently accumulated more phosphorus. It was also proved that *Ph1* gene promotes phosphate uptake in rice [Sun *et al.* 2012]. Moreover, in wheat overexpression of *Ta-PHR1* gene leads to increase yield [Wang *et al.* 2013].

Another problem is presence of metals ions in soil. One of the most common ions is aluminum that causes inhibition of plant development, reduction of root growth and leads to decrease of water and nutrient uptake. For barley, potential candidate that can modulate Al tolerance is wheat *ALMT1* gene that encodes a membrane-bound protein which is responsible for Al-activated malate efflux [Ryan *et al.* 1997, Zhang 2001]. Overexpression of that gene in tobacco cells caused enhance tolerance to aluminum [Sasaki *et al.* 2004]. Studies on transgenic barley proved development of root growth, shoot biomass and grain yield [Delhaize *et al.* 2004]. Moreover, overexpression of *HvYSL* gene in barley indicated higher tolerance and improvement of crop in alkaline soil. Another study was based on this approach. GM rice with overexpression of this *HvYSL* gene showed enhance growth and yield compared to the wild- type [Gómez-Galera *et al.* 2012]. Other studies consisted transgenic rice as well, indicated that overexpression of the protein from thermophilic archaea improved tolerance to mercury [Chen *et al.* 2012b] and *OSHMA2* gene is involved in accumulation of cadmium [Takahashi *et al.* 2012].

HERBICIDE TOLERANCE

The main problem with using herbicides is the fact that some of them are non-selective, what means those herbicides are able to kill not only weed but also all crops. Transgenic researches are focused on identification, isolation and transfer resistance genes into crop. The first organic, systemic, selective herbicide was dichlorophenoxyacetate (2,4-D). Along with the green revolution, this herbicide has helped to increase the cereal production on the decades after 1950. Studies on soil bacteria have identified *rdpA* gene from *Sphingobium herbicidivorans* MH, which encodes the enzyme ariloxylkanoate-dioxygenase-1 (AAD-1); which is able to degrade 2,4-D and other herbicides. Corn plants containing the gene *rdpA* is tolerant to 2,4-D but also to the ariloxylphenoxypionate (AAPP) herbicides [Queiroz *et al.* 2014].

Another well known strategy is using glyphosate that is commonly applied by commercial companies. Identification of bacterial resistance genes and introduction into maize cells contributed to widely selling those plants by company. Second major trial of herbicide resistant was performed on glufosinate [Dunwell 2014].

Glyphosate is active ingredient of the herbicide Roundup produced by Monsanto. Roundup inhibits EPSP (5-enolpyruvylshikimate-3-phosphate) synthase, which is absolutely required for the survival of plant. Roundup Ready plants are genetically modified developed by Monsanto and carry the gene coding for a glyphosate-insensitive form, obtained from *Agrobacterium* sp. strain CD4. The gene product, CP4 EPSP synthase, contributed to crop resistance to glyphosate. Current Roundup Ready crops include soy, corn, canola, alfalfa, cotton or sorghum [Funke *et al.* 2006; <http://web.mit.edu/demoscience/Monsanto/about.html>].

CONCLUSION

Compared to dicotyledonous species, technology of cereal modification still faces significant hurdles. However, technology for transformation of major cereals is developed and well described. Current successful achievements are a base for next studies. That will enable to perform tests for basic and applied studies. The significant progress made in cereal transformation is promising for the future prospects and opportunities that can bring successful GM products. However, there are some issues, like regulatory aspects and especially public perception, which can limit scientific development of this approach. For this reason, it should be remembered that proper balance has to be maintained to make society feel safe.

REFERENCES

- Abass M., Morris P.C., 2013. The *Hordeum vulgare* signalling protein MAP kinase 4 is a regulator of biotic and abiotic stress responses. *J. Plant Physiol.* 170 (15), 1353–1359.

- Abouseadaa H.H., Osman G.H., Ramadan A.M., Hassanein S.E., Abdelsattar M.T., Morsy Y.B., Bahieldin A., 2015. Development of transgenic wheat (*Triticum aestivum* L.) expressing avirin gene conferring resistance to stored product insects. *BMC Plant Biol.* 15 (1), 183.
- Akiyama T., Pillai M.A., Sentoku N., 2004. Cloning, characterization and expression of OsGLN2, a rice endo-1,3-beta-glucanase gene regulated developmentally in flowers and hormonally in germinating seeds. *Planta*, 22, 129–139.
- Beazley K.A., Castiglioni P., Dizigan M.A., Kelly R.A., Korte J.A., Rock A. *et al.*, 2012. Corn plant event mon87460 and compositions and methods for detection thereof [US 20110138504].
- Brunner S., Stirnweis D., Diaz Quijano C., Buesing G., Herren G., Parlange F., Keller B., 2012. Transgenic Pm3 multilines of wheat show increased powdery mildew resistance in the field. *Plant Biotechnol. J.* 10 (4), 398–409.
- Von Burg S., Álvarez-Alfageme F., Romeis J., 2012. Indirect effect of a transgenic wheat on aphids through enhanced powdery mildew resistance. *PLoS One*, 7 (10), e46333.
- Cao X., Lu Y., Di D., Zhang Z., Liu H., Tian L., Li D., 2013. Enhanced virus resistance in transgenic maize expressing a dsRNA-specific endoribonuclease gene from *E. coli*. *PLoS One* 8 (4).
- Chan M.T., Chang H.H., Ho S.L., Tong W.F., Yu S.M., 1993. Agrobacterium-mediated production of transgenic rice plants expressing a chimeric alpha-amylase promoter/beta-glucuronidase gene. *Plant Mol. Biol.* 22 (3), 491–506.
- Chen H., Chen W., Zhou J., He H., Chen L., Chen H., Deng X.W., 2012a. Basic leucine zipper transcription factor OsbZIP16 positively regulates drought resistance in rice. *Plant Science: An International Journal of Experimental Plant Biology*, 193–194, 8–17.
- Chen Z., Pan Y., Wang S., Ding Y., Yang W., Zhu C., 2012b. Overexpression of a protein disulfide isomerase-like protein from *Methanothermobacter thermoautotrophicum* enhances mercury tolerance in transgenic rice. *Plant Sci. Int. J. Experiment. Plant Biol.* 197, 10–20.
- Chen Y.-J., Perera V., Christiansen M., Holme I.B., Gregersen P.L., Grant M.R., Lyngkjær M.F., 2013. The barley HvNAC6 transcription factor affects ABA accumulation and promotes basal resistance against powdery mildew. *Plant Mol. Biol.* 83 (6), 577–590.
- Choe Y.-H., Kim Y.-S., Kim I.-S., Bae M.-J., Lee E.-J., Kim Y.-H., Yoon H.-S., 2013. Homologous expression of γ -glutamylcysteine synthetase increases grain yield and tolerance of transgenic rice plants to environmental stresses. *J. Plant Physiol.* 170 (6), 610–618.
- Delhaize E., Ryan P.R., Hebb D.M., Yamamoto Y., Sasaki T., Matsumoto H., 2004. Engineering high-level aluminum tolerance in barley with the ALMT1 gene. *Proc. Natl. Acad. Sci. U.S.A.* 101 (42), 15249–15254.
- Dong W., Wang M., Xu F., Quan T., Peng K., Xiao L., Xia G., 2013. Wheat oxophytodienoate reductase gene TaOPR1 confers salinity tolerance via enhancement of abscisic acid signaling and reactive oxygen species scavenging. *Plant Physiol.* 161 (3), 1217–1228.
- Douglas C., Halperin W., Gordon M., Nester E., 1985. Specific attachment of *Agrobacterium tumefaciens* to bamboo cells in suspension cultures. *J. Bacteriol.* 161, 764–766.
- Dowd P.F., Johnson E.T., Price N.P., 2012. Enhanced pest resistance of maize leaves expressing monocot crop plant-derived ribosome-inactivating protein and agglutinin. *J. Agric. Food Chem.* 60 (43), 10768–10775.
- Du H.-Y., Shen Y.-Z., Huang Z.-J., 2013. Function of the wheat TaSIP gene in enhancing drought and salt tolerance in transgenic Arabidopsis and rice. *Plant Mol. Biol.* 81 (4–5), 417–429.
- Duan J., Zhang M., Zhang H., Xiong H., Liu P., Ali J., Li Z., 2012. OsMIOX, a myo-inositol oxygenase gene, improves drought tolerance through scavenging of reactive oxygen species in rice (*Oryza sativa* L.). *Plant Sci. Int. J. Experiment. Plant Biol.* 196, 143–151.

- Dunwell J.M., 2008. Transgenic wheat, barley and oats: future prospects. W: Jones H.D., Shewry P.R. (eds.), *Transgenic wheat, barley and oats: Production and characterisation, Methods in Molecular Biology* 478. Humana Press, 333–345.
- Dunwell J.M., 2014. Transgenic cereals: Current status and future prospects. *J. Cereal Sci.* 59 (3), 419–434.
- Edgerton M.D., Fridgen J., Anderson J.R., Ahlgrim J., Criswell M., Dhungana P., Stark S.B., 2012. Transgenic insect resistance traits increase corn yield and yield stability. *Nat. Biotechnol.* 30 (6), 493–496.
- Fahim M., Millar A.A., Wood C.C., Larkin P.J., 2012. Resistance to wheat streak mosaic virus generated by expression of an artificial polycistronic microRNA in wheat. *Plant Biotechnol. J.* 10 (2), 150–163.
- Ferrari S., Sella L., Janni M., De Lorenzo G., Favaron F., D'Ovidio R., 2012. Transgenic expression of polygalacturonase-inhibiting proteins in *Arabidopsis* and wheat increases resistance to the flower pathogen *Fusarium graminearum*. *Plant Biol.* 14 (1), 31–38.
- Funke T., Han H., Healy-Fried M.L., Fischer M., Schönbrunn E., 2006. Molecular basis for the herbicide resistance of Roundup Ready crops. *Proc. Natl. Acad. Sci. U.S.A.* 103 (35), 13010–13015.
- Gao C.-S., Kou X.-J., Li H.-P., Zhang J.-B., Saad A.S.I., Liao Y.-C., 2013. Inverse effects of *Arabidopsis* NPR1 gene on fusarium seedling blight and fusarium head blight in transgenic wheat. *Plant Pathol.* 62 (2), 383–392.
- Gómez-Galera S., Sudhaka D., Pelacho A.M., Capell T., Christou P., 2012. Constitutive expression of a barley Fe phytosiderophore transporter increases alkaline soil tolerance and results in iron partitioning between vegetative and storage tissues under stress. *Plant Physiol. Biochem. (Paris)* 53, 46–53.
- Graves A.F., Goldman S.L., 1986. The transformation of *Zea mays* seedlings with *Agrobacterium tumefaciens*. *Plant Mol. Biol.* 7, 43–50.
- Hackenberg M., Shi B.-J., Gustafson P., Langridge P., 2012. A transgenic transcription factor (TaDREB3) in barley affects the expression of microRNAs and other small non-coding RNAs. *PLoS One*, 7 (8), e42030.
- Haeghele J.W., Below F.E., 2013. Transgenic corn rootworm protection increases grain yield and nitrogen use of maize. *Crop Sci.* 53 (2), 585.
- Han J., Lakshman D.K., Galvez L.C., Mitra S., Baenziger P.S., Mitra A., 2012. Transgenic expression of lactoferrin imparts enhanced resistance to head blight of wheat caused by *Fusarium graminearum*. *BMC Plant Biol.* 12 (1), 33.
- Han C., Zhong W., Shen W., Cai Z., Liu B., 2013. Transgenic Bt rice has adverse impacts on CH₄ flux and rhizospheric methanogenic archaeal and methanotrophic bacterial communities. *Plant and Soil* 369 (1–2), 297–316.
- Hiei Y., Komari T., 2008. *Agrobacterium*-mediated transformation of rice using immature embryos or calli induced from mature seed. *Nat. Protoc.* 3, 824–834.
- Hiei Y., Ohta S., Komari T., Kumashiro T., 1994. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *Plant J.* 6 (2), 271–282.
- Hoekema A., Hirsch P.R., Hooykaas P.J.J., Schilperoort R.A., 1983. A binary plant vector strategy based on separation of vir- and T-region of the *Agrobacterium tumefaciens* Ti-plasmid. *Nature* 303, 179–180.
- <http://plantfdb.cbi.pku.edu.cn/family.php?fam=NAC>
- <http://web.mit.edu/demoscience/Monsanto/about.html>
- Ignacimuthu S., Ceasar S.A., 2012. Development of transgenic finger millet (*Eleusine coracana* (L.) Gaertn.) resistant to leaf blast disease. *J. Biosci.* 37, 135–147.

- Ishida Y., Saito H., Ohta S., Hiei Y., Komari T., Kumashiro T., 1996. High efficiency transformation of maize (*Zea mays* L.) mediated by *Agrobacterium tumefaciens*. *Nat. Biotechnol.* 14, 745–750.
- Kahl G., 1982. *Molecular biology of wound healing: the conditioning phenomenon*. W: Kahl G., Schell J. (eds), *Molecular biology of plant tumors*. Academic Press, New York, 211–267.
- Kishimoto K., Kouzai Y., Kaku H., Shibuya N., Minami E., Nishizawa Y., 2010. Perception of the chitin oligosaccharides contributes to disease resistance to blast fungus *Magnaporthe oryzae* in rice. *Plant J. Cell Mol. Biol.* 64 (2), 343–354.
- Kovalchuk N., Jia W., Eini O., Morran S., Pyvovarenko T., Fletcher S., Lopato S., 2013. Optimization of TaDREB3 gene expression in transgenic barley using cold-inducible promoters. *Plant Biotechnol. J.* 11 (6), 659–670.
- Kumar K.K., Poovannan K., Nandakumar R., Thamilarasi K., Geetha C., Jayashree N., Kokiladevi E., Raja J.A.J., Samiyappan R., Sudhakar D., Balasubramanian P., 2003. A high throughput functional expression assay system for a defense gene conferring transgenic resistance on rice against the sheath blight pathogen, *Rhizoctonia solani*. *Plant Sci.* 165, 969–976.
- Li X., Hou S., Gao Q., Zhao P., Chen S., Qi D., Lee B.H., Cheng L., Liu G., 2013. LcSAIN1, a novel salt-induced gene from sheepgrass, confers salt stress tolerance in transgenic *Arabidopsis* and rice. *Plant Cell Physiol.* 54, 1172–1185.
- Li C., Wei J., Lin Y., Chen H., 2012. Gene silencing using the recessive rice bacterial blight resistance gene *xa13* as a new paradigm in plant breeding. *Plant Cell Rep.* 31 (5), 851–862.
- Liu A.-L., Zou J., Liu C.-F., Zhou X.-Y., Zhang X.-W., Luo G.-Y., Chen X.-B., 2013. Overexpression of OsHsfA7 enhanced salt and drought tolerance in transgenic rice. *BMB Reports*, 46 (1), 31–36.
- Lu Y., Li Y., Zhang J., Xiao Y., Yue Y., Duan L., Li Z., 2013. Overexpression of *Arabidopsis* molybdenum cofactor sulfurase gene confers drought tolerance in maize (*Zea mays* L.). *PLoS One*, 8 (1), e52126.
- Morell M.K., 2012. New cereal value chain: from seed to sewage. *Cereal Foods World* 57, 44–49.
- Mrízová K., Holasková E., Öz M.T., Jiskrová E., Frébort I., Galuszka P., 2014. Transgenic barley: a prospective tool for biotechnology and agriculture. *Biotechnol. Adv.* 32 (1), 137–157.
- Narva K.E., Siegfried B.D., Storer N.P., 2013. Transgenic approaches to western corn rootworm control. *Adv. Biochem. Eng. Biotechnol.* 136, 135–162.
- Nishizawa Y., Saruta M., Nakazono K., Nishio Z., Soma M., Yoshida T., Nakajima E., Hibi T. 2003. Characterization of transgenic rice plants over-expressing the stress-inducible β -glucanase gene *Gns1*. *Plant Mol. Biol.* 51, 143–152.
- Oraby H., Ahmad R., 2012. Physiological and biochemical changes of CBF3 transgenic oat in response to salinity stress. *Plant Sci. Int. J. Exp. Plant Biol.* 185–186, 331–339.
- Ostry V., Ovesna J., Skarkova J., Pouchova V., Ruprich J., 2010. A review on comparative data concerning *Fusarium* mycotoxins in Bt maize and non-Bt isogenic maize. *Mycotoxin Res.* 26 (3), 141–145.
- Pei L., Wang J., Li K., Li Y., Li B., Gao F., Yang A., 2012. Overexpression of *Thellungiella halophila* H⁺-pyrophosphatase gene improves low phosphate tolerance in maize. *PLoS One* 7, e43501.
- Peng X., Hu Y., Tang X., Zhou P., Deng X., Wang H., Guo Z., 2012. Constitutive expression of rice WRKY30 gene increases the endogenous jasmonic acid accumulation, PR gene expression and resistance to fungal pathogens in rice. *Planta* 236 (5), 1485–1498.
- Potrykus I., 1990. Gene transfer to cereals: An assessment. *Bio/Technology* 8 (6), 535–542.
- Queiroz A.R.S., Vidal R.A., 2014. The development of dichlorophenoxyacetate herbicide tolerant crops: literature review. *Planta Daninha* 32 (3), 649–654.
- Rawat N., Neelam K., Tiwari V.K., Dhaliwal H.S., 2013. Biofortification of cereals to overcome hidden hunger. *Plant Breed.* 132, 437–444.

- Ryan P.R., Skerrett M., Findlay G.P., Delhaize E., Tyerman S.D., 1997. Aluminum activates an anion channel in the apical cells of wheat roots. *Proc. Natl. Acad. Sci.* 94 (12), 6547–6552.
- Sasaki T., Yamamoto Y., Ezaki B., Katsuhara M., Ahn S.J., Ryan P.R., Matsumoto H., 2004. A wheat gene encoding an aluminum-activated malate transporter. *Plant J.* 37 (5), 645–653.
- Shimizu T., Nakazono-Nagaoka E., Akita F., Wei T., Sasaya T., Omura T., Uehara-Ichiki T., 2012. Hairpin RNA derived from the gene for Pns9, a viroplasm matrix protein of Rice gall dwarf virus, confers strong resistance to virus infection in transgenic rice plants. *J. Biotechnol.* 157 (3), 421–427.
- Shimizu T., Ogamino T., Hiraguri A., Nakazono-Nagaoka E., Uehara-Ichiki T., Nakajima M., Sasaya T., 2013. Strong resistance against Rice grassy stunt virus is induced in transgenic rice plants expressing double-stranded RNA of the viral genes for nucleocapsid or movement proteins as targets for RNA interference. *Phytopathology* 103 (5), 513–519.
- Shou H., Bordallo P., Fan J.B., Yeakley J.M., Bibikova M., Sheen J., *et al.* 2004. Expression of an active tobacco mitogen-activated protein kinase kinase kinase enhances freezing tolerance in transgenic maize. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3298–3303.
- Soltész A., Vágújfalvi A., Rizza F., Kerepesi I., Galiba G., Cattivelli L., Coraggio I., Crosatti C., 2011. The rice Osmyb4 gene enhances tolerance to frost and improves germination under unfavourable conditions in transgenic barley plants. *J. Appl. Genet.* 53, 133–143.
- Sridevi G., Parameswari C., Sabapathi N., Raghupathy V., Veluthambi K., 2008. Combined expression of chitinase and b-1,3-glucanase genes in indica rice (*Oryza sativa* L.) enhances resistance against *Rhizoctonia solani*. *Plant Sci.* 175, 283–290.
- Sun S., Gu M., Cao Y., Huang X., Zhang X., Ai P., Xu G., 2012. A constitutive expressed phosphate transporter, OsPht1;1, modulates phosphate uptake and translocation in phosphate-replete rice. *Plant Physiol.* 159 (4), 1571–1581.
- Stachel S.E., Messens E., van Montagu M., Zambryski P., 1985. Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in *Agrobacterium tumefaciens*. *Nature* 318 (6047), 624–629.
- Takahashi R., Ishimaru Y., Shimo H., Ogo Y., Senoura T., Nishizawa N.K., Nakanishi H., 2012. The OsHMA2 transporter is involved in root-to-shoot translocation of Zn and Cd in rice. *Plant Cell Environ.* 35 (11), 1948–1957.
- Tabashnik B.E., Brévault T., Carrière Y., 2013. Insect resistance to Bt crops: lessons from the first billion acres. *Nat. Biotechnol.* 31 (6), 510–521.
- Todaka D., Nakashima K., Maruyama K., Kidokoro S., Osakabe Y., Ito Y., Yamaguchi-Shinozaki K., 2012. Rice phytochrome-interacting factor-like protein OsPIL1 functions as a key regulator of internode elongation and induces a morphological response to drought stress. *Proc. Natl. Acad. Sci. U.S.A.* 109 (39), 15947–15952.
- Usami S., Morikawa S., Takebe I., Machida Y., 1987. Absence in monocotyledonous plants of the diffusible plant factors inducing T-DNA circularization and vir gene expression in *Agrobacterium*. *Mol. Gen. Genet.* 209 (2), 221–226.
- van der Linde K., Hemetsberger C., Kastner C., Kaschani F., van der Hoorn R.A., Kumlehn J., Doehle G., 2012. A maize cystatin suppresses host immunity by inhibiting apoplastic cysteine proteases. *Plant Cell* 24, 1285–1300.
- Várallyay E., Giczey G., Burgyán J., 2012. Virus-induced gene silencing of Mlo genes induces powdery mildew resistance in *Triticum aestivum*. *Arch. Virol.* 157 (7), 1345–1350.
- Velasco A.G.-V., Kowalchuk G.A., Mañero F.J.G., Ramos B., Yergeau E., García J.A.L., 2013. Increased microbial activity and nitrogen mineralization coupled to changes in microbial community structure in the rhizosphere of Bt corn. *Appl. Soil Ecol.* 68, 46–56.
- Wang J., Sun J., Miao J., Guo J., Shi Z., He M., Chen Y., Zhao X., Li B., Han F., Tong Y., Li Z., 2013. A phosphate starvation response regulator Ta-PHR1 is involved in phosphate signaling and increases grain yield in wheat. *Ann. Bot.* 111, 1139–1153.

- Wiśniewska H., Stępień Ł., Waśkiewicz A., Beszterda M., Góral T., Belter J., 2014. Toxigenic *Fusarium* species infecting wheat heads in Poland. *Cent. Eur. J. Biol.* 9 (2) 163–172.
- Wu E., Lenderts B., Glassman K., Berezowska-Kaniewska M., Christensen H., Asmus T., *et al.*, 2014. Optimized *Agrobacterium*-mediated sorghum transformation protocol and molecular data of transgenic sorghum plants. *In Vitro Cell. Dev. Biol. Plant* 50, 9–18.
- Zeller S.L., Kalinina O., Schmid B., 2013. Costs of resistance to fungal pathogens in genetically modified wheat. *J. Plant Ecol.* 6, 92e100.
- Zhang W.-H., 2001. Malate-permeable channels and cation channels activated by aluminum in the apical cells of wheat roots. *Plant Physiol.* 125 (3), 1459–1472.
- Zhang S., Haider I., Kohlen W., Jiang L., Bouwmeester H., Meijer A.H., Ouwerkerk P.B.F., 2012. Function of the HD-Zip I gene *Oshox22* in ABA-mediated drought and salt tolerances in rice. *Plant Mol. Biol.* 80 (6), 571–585.
- Zhou Y., Yuan Y., Yuan F., Wang M., Zhong H., Gu M., Liang G., 2012. RNAi-directed down-regulation of RSV results in increased resistance in rice (*Oryza sativa* L.). *Biotechnol. Lett.* 34 (5), 965–972.
- Zhu X., Li Z., Xu H., Zhou M., Du L., Zhang Z., 2012. Overexpression of wheat lipid transfer protein gene *TaLTP5* increases resistances to *Cochliobolus sativus* and *Fusarium graminearum* in transgenic wheat. *Funct. Integr. Genomics* 12 (3), 481–488.
- Zhu H., Xu X., Xiao G., Yuan L., Li B., 2007. Enhancing disease resistances of super hybrid rice with four antifungal genes. *Sci. China C Life Sci.* 50, 31–39.

Streszczenie. Postęp w zakresie transformacji zbóż, jaki miał miejsce przez ostatnie dwie dekady, ma ogromne znaczenie dla nauki oraz rolnictwa. Dotychczas w transformacji zbóż posługiwano się głównie technikami bezwektorowymi, przede wszystkim bezpośrednią metodą transferu genów, tzw. strzelbą genową (gene gun). Osiągnięcia z zakresu agrobiotechnologii umożliwiły transformację zbóż przy użyciu bakterii glebowej *Agrobacterium tumefaciens*, która do niedawna nie była wykorzystywana w doświadczeniach na zbożach, gdyż rośliny jednoliścienne nie stanowią dla niej organizmu gospodarza i nie wykazują objawów choroby guzowatości korzenia przez nią powodowanej. Obecnie pięć gatunków zbóż o największym znaczeniu gospodarczym – ryż (*Oryza sativa* L.), kukurydza (*Zea mays* L.), pszenica (*Triticum aestivum* L.), jęczmień (*Hordeum vulgare* L.) i sorgo (*Sorghum bicolor* L.) – jest powszechnie poddawanych transformacji z udziałem *A. tumefaciens*. Wykorzystanie narzędzi genetyki molekularnej pozwala na uzyskanie zbóż o nowych, polepszonych cechach. W niniejszej pracy skupiono się na możliwości rozwoju rolnictwa poprzez wdrażanie genetycznie zmodyfikowanych zbóż odpornych na stresy biotyczne i abiotyczne oraz podsumowano najważniejsze osiągnięcia ostatnich lat z zakresu transformacji zbóż.

Słowa kluczowe: transformacje, *Agrobacterium tumefaciens*, zboża, tolerancja na stresy, genetycznie modyfikowane zboża