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TRANSGENIC PLANTS EXPRESSING INSECT RESISTANCE GENES

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SUMMARY

Since first transgenic plant resistant to insects was produced some 20 years ago, a number of novel resistance genes of different origin were discovered and used for plant transformation. First transgenic insect resistant plant contained *cry* (*Bt*) gene from bacterium *Bacillus thuringiensis*. In the middle of 1990's transgenic maize and, some time later, cotton having *cry* genes started to be produced commercially. In the European Union several maize events having *cry* genes and conferring resistance either to European corn borer (*Ostrinia nubilalis* Hübner) or western corn rootworm (*Diabrotica virgifera virgifera* LeConte) have been approved for food and feed, some for cultivation as well. Several other maize events having stacked two or more genes for insect resistance or genes for insect resistance and herbicide tolerance have also been approved. Very effective insecticidal genes named *vip*, also originating from *Bacillus* species *(B. thuringiensis* and *B. cereus*) are very close to commercial exploitation. Promising results were, also, obtained, when other genes like those for enzyme cholesterol oxidase (microbial origin), avidin (from chicken egg white), chitinase and neuropeptides (insect origin) were used for plant transformation to confer insect resistance. Plants naturally produce different secondary metabolites that, if the expression level of those antimetabolites is high enough, adversely affect insects. A pest insect, in order to be able to feed on some plant species, has to develop resistance to certain antimetabolites that specific plant produces. However, expression of plant resistance genes from other plant species driven by strong promoters enables development of plants also resistant to pest insects that earlier easily fed on certain plant species. Genes of three groups of plant protein antimetabolites were used for this purpose: (i) proteinase inhibitors, (ii) α-amylase inhibitors and (iii) lectins. While single proteinase inhibitors have quite a narrow spectrum of insecticidal activity, lectins show insecticidal activity to different orders of insects, even to sap-sucking insects belonging to the order *Homoptera*.

Breeding new cultivars resistant to insect pests should be continued, and special efforts should be made in production of insect resistant transgenic plants that will hinder the development of insect resistance to the recombinantly- expressed antimetabolites or toxins. Possible way to achieve this is the stacking of two or more insecticidal genes with different mode of action into the same plant.

Keywords: transgenic plant, insect resistance, Bt toxin, proteinase inhibitor, avidin

INTRODUCTION

Losses due to pests and diseases have been estimated at 37% of the agricultural production worldwide, with 13% due to insects (Gatehouse et al., 1992). All plants possess a certain degree of resistance to insects, so only limited number of herbivores is able to feed on each individual plant species. This inherent resistance is based on various defence mechanisms, including a wide range of noxious secondary metabolites produced by the plant. Gatehouse (1991) split plant secondary metabolites exhibiting insecticidal activity in two broad categories (i) non-protein antimetabolites like alkaloides, non-protein aminoacids, terpenoids, rotenoids (isoflavanoids), tannins, polysaccharides, glucosinolates and cyanogenic glycosides and (ii) protein antimetabolites like proteinase inhibitors, α-amylase inhibitors, lectins and arcelins. As the insect attacking some host plant is adapted to the antimetabolites of the latter in terms of both quality and quantity, plant breeders have been attempting to introduce novel resistance genes into crop plants, either from other resistant cultivars, or from related semi-domesticated or wild species. Breeding new cultivars for increased insect resistance by conventional methods is, however, a difficult task. With the advent of genetic engineering, it has become possible to clone and introduce into plants transgenes, whether of bacterial, plant, or other origin in order to increase the level of insect resistance (Schuler et al., 1998). Expression of these insecticidal genes driven by strong promoters enabled development of insect resistant plants. The aim of this work was to give the review of insect resistance genes used for plant transformation, regardless of their commercial exploitation or technological developmental stage.

CRY GENES FROM Bacillus thuringiensis

Transgenic crops modified by *cry* (*Bt*) genes (from bacterium *Bacillus thuringiensis*) are so far the only insect resistant transgenic crops grown commercially. *Cry* genes were also the firstly used insecticidal genes for plant transformation (Vaeck et al., 1987). *B. thuringiensis* (Bt) is a gram-positive bacterium producing highly insecticidal protein crystals also called Bt toxins during sporulation. Spores and protein crystals of several strains of this bacterium have been used as bioinsecticide for many

years. After ingestion by susceptible insects, toxins bind to specific receptors in the gut and are solubilized and activated by proteinases in the insect midgut epithelium. The activated toxins induce the formation of a lytic pore in the midgut epithelial membrane that results in cell lysis, cessation of feeding, and death of the larva (Daniel et al., 2000).

Separate strains of Bt produce a variety of crystal toxins. More than 400 genes encoding toxins from wide range of *B. thuringiensis* have been identified so far (Crickmore et al., 2007). Many of the identified *cry* genes (for example *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1Ba*, *cry1Ca*, *cry1H*, *cry2Aa*, *cry3A*, *cry6A*, *cry9C, cry1F)* have been engineered into plants. Most cry proteins, even within cry1A subfamily, have a distinctive insecticidal spectrum. While some crystal toxins are specific to, and affect, larvae of lepidopteran pests, some other are toxic to coleopteran, or dipteran pests. The level of expression of wild-type Bt toxins in transgenic plants is low compared to many other heterologous genes. When the coding sequence of the *cry* genes was modified, in order to fit the codon usage of the plant species undergoing transformation, expression level was significantly increased (Van der Salm et al., 1994) and become sufficient to cause high mortality of target pests in the field. The first results concerning the transfer of *cry* genes into tobacco and tomato were published in 1987 (Vaeck et.al., 1987). Since then, genes encoding different Bt toxins with constitutive or tissue-specific promoters have been introduced into a range of crop plants, including tobacco, maize, rice, potato, apple, cotton, tomato, rapeseed. In all these cases, plants produced Bt toxins and the resistance to some relevant insects has been demonstrated (Pierepoint and Hughes, 1996).

Depending on which Bt toxin is expressed, genetically modified maize is resistant to the European corn borer (*Ostrinia nubilalis* Hübner) or some other lepidopteran species, or to the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) or some other coleopteran species. In the European Union (EU), maize events MON810 and Bt11, having *cry* genes from B. *thuringiensis* ssp. *kurstaki* and being resistant to the European corn borer, have been approved for food and feed, MON810 also for cultivation. Maize event MON863, having a *cry* gene from *B. thuringiensis* ssp. *kumamotoensis* conferring resistance to western corn rootworm, has been approved for food and feed. Several other maize events having stacked two or more genes for insect resistance or genes for insect resistance and herbicide tolerance have been approved as well (Anonymous, 2008). Bt cotton is the second most important transgenic insect resistant crop. Several insect resistant cotton events, which confer resistance to lepidopteran pests of cotton, have been approved in the EU so far (Anonymous, 2008).

Plants encoding Bt toxins have no need of protection with other insecticides, which results in less damage to the environment and prevents other negative effects of insecticide application. Well-documented and very important fact is that Bt toxins have no or very few adverse effects on mammals (including humans) and birds (Goldberg and Tjaden, 1990). In human gut, Bt proteins behave as any other dietary proteins and pose no significant risk to human health (Mendelsohn et al., 2003).

Despite many advantages of Bt technology, some disadvantages have also been reported. Resistance to Bt toxins has been developed in some target pests in many parts

of the world where Bt toxins have been used intensively. The way of solving this problem is sowing Bt crops with alternating rows of regular non-Bt crops (refuge). As resistance to Bt toxins is controlled by mutant, recessive alleles, the insect that has developed resistance to Bt toxins has more chances of mating with an insect that has not developed resistance (insect grown in refuge) (Bates et al., 2005). By the laws of genetics, the progenies produced will be insects that are not resistant to Bt toxins. Planting refuges is obligatory but farmers sometimes neglect this regulation.

Alternative way to avoid or slow down development of insect resistance is steacking insecticidal genes with different mode of action against insects in transgenic plants. As it will be cleared hereinafter, many genes of a different origin are potential candidates for such stacking or for sole expression in order to confer resistance of transgenic plants (Kereša, 2002).

OTHER RESISTANCE GENES FROM MICROORGANISMS

None of the other insecticidal genes, but *cry*, have been commercially exploited so far, still, extensive investigations are being carried out on many of them.

The closest to the commercial use are the *vip* genes, again from *Bacillus* species *(B. thuringiensis* and *B. cereus)*. The products of the *vip* genes (Vip proteins) were discovered recently (Estruch et al., 1996). Unlike Bt toxins, whose expression is restricted to sporulation, Vip insecticidal proteins are expressed in the vegetative stage of growth starting at mid-log phase as well as during sporulation. More than 50 Vip proteins have been identified so far (Crickmore et al. 2007). It is known that ingestion of Vip proteins causes swelling and disruption of the midgut epithelial cells by osmotic lysis in the target insects (Anonymous, 2003). One group of Vip toxins consists of binary toxins that are made of two components, Vip1 and Vip2. The combination of Vip1 and Vip2 is highly insecticidal to an agriculturally important insect, the western corn rootworm (*Diabrotica virgifera*), but does not show any insecticidal activity to lepidopteran insects. The other group consists of Vip3 toxins, which share no sequence similarity to Vip1 or Vip2. The first-identified Vip3 toxin, Vip3Aa1, is highly insecticidal to several major lepidopteran pests of maize and cotton (Estruch et al., 1996; Fang et al., 2007). According to the Agricultural Biotechnology Annual Report for Australia (Crothers, 2006), GM VIP cotton has the licence for limited and controlled release.

A highly effective protein that killed the larvae of the boll weevil (*Anthonomus grandis*) was discovered in *Streptomyces* culture filtrate (Purcell et al., 1993). The protein was identified as a cholesterol-oxidase. Morphological changes induced by ingestion of cholesterol oxidase suggest that enzyme has a direct effect on the midgut tissue of boll weevil larvae. Cholesterol oxidase disrupted the midgut epithelium at low doses and lysed the midgut cells at higher doses (Purcell et al., 1993). Corbin et al. (2001) transformed tobacco (*Nicotiana tabacum* L.) plants with the cholesterol oxidase *choM* gene and expressed cytosolic and chloroplast-targeted versions of the ChoM protein. Transgenic leaf tissues expressing cholesterol oxidase exerted insecticidal

activity against boll weevil larvae. When produced cytosolically, cholesterol oxidase could metabolize phytosterols *in vivo*, so the transgenic plants exhibiting cytosolic expression of *choM* gene accumulated low levels of saturated sterols known as stanols, and displayed severe developmental aberrations. The transgenic plants expressing chloroplast-targeted cholesterol oxidase appeared phenotypically and developmentally normal.

Bowen et al. (1998) investigated a toxin secreted by bacterium *Photorhabdus luminescens*, which lives in the gut of entomophagous nematodes. In insects infected with the nematode, the bacteria are released into the insect hemocoel; the insect dies and the nematodes and bacteria replicate in the cadaver. The toxin consists of a series of four native complexes encoded by toxin complex loci *tca*, *tcb*, *tcc* and *tcd*. Both *tca* and *tcd* encode complexes with high toxicity to tobacco hornworm (*Manduca sexta*) and therefore they represent potential alternatives to *Bt* for transgenic deployment. Liu et al. (2003) introduced the *tcdA* gene of *Photorhabdus luminescens* encoding a 283-kDa protein, toxin A, into *Arabidopsis thaliana* L. Toxin A is highly toxic to a variety of insects, including some agriculturally important pests. In the insect feeding trials, plants with toxin A expression above 700 ng/mg of extractable protein showed highly toxic effect to tobacco hornworm. Toxin A isolated from transgenic plants also strongly inhibited growth of the southern corn rootworm (*Diabrotica undecimpunctata howardi*).

RESISTANCE GENES FROM INSECTS OR SOURCES OTHER THAN PLANTS

Chitinase expression normally occurs in insects during molting when insects shed their old exoskeleton and peritrophic membrane (both contain chitin as major component) and resynthesize new ones. Thus, insect feedings on plants that constitutively express an insect chitinase gene might be adversely affected, owing to an inappropriately timed exposure to chitinase. Results of Ding et al. (1998) showed that transgenic tobacco plants expressing insect (*Manduca sexta*) chitinase transgene have enhanced resistance to budworm (*Heliothis virescens*) larvae, even if the mechanism of *Manduca* chitinase-mediated resistance is unknown. This chitinase may be directly toxic to larvae or the perforation of the peritrophic membrane caused by chitinase may enhance sensitivity of larvae to existing microbes or dietary components. Therefore, plants expressing an insect chitinase gene may have agronomic potential for insect control.

Protein inhibitors of proteases are ubiquitary compounds. They are present in multiple forms in numerous tissues of animals and plants as well as in microorganisms (Laskowski and Kato, 1980). It is known that overexpression of protease inhibitors (PIs) can protect plants against some insect species (Wolfson and Murdock, 1987). Thomas et al. (1995) expressed insect encoded anti-trypsin, anti-chymotrypsin and anti-elastase protease inhibitor genes from *Manduca sexta* in transgenic tobacco. When transgenic plants were tested against sweet potato whitefly type B, *Bemisia tabaci*, insect reproduction was reduced by as much as 98% compared to controls. This result suggests that *M. sexta*-derived PI may be useful in protecting crop plants against insects.

Insect neuropeptides are neurotransmitters that are able to interfere with a variety of physiological processes in insects (development, metabolism, and reproduction). They are active at very low concentrations and are non-toxic to vertebrates, including humans (Rao et al., 1996). It has been demonstrated that orally administered peptides may penetrate the insect gut and that undegraded fractions target haemocoelic cells and enter the haemolymph intact (Raina et al., 1994). Proctolin is the first isolated and characterised insect neuropeptide. It is only 5 amino acids long and it functions as a visceral and a skeletal neuromuscular transmitter. It may also have a role in central nervous system and as circulating hormone. Rao et al. (1996) constructed a synthetic gene encoding a polyproctolin precursor and introduced this gene into tobacco where it was efficiently transcribed, but the amount of peptide produced (likely due to low translational efficiency) in the transformed plants was too low to perform further analyses. Because of great potential of neuropeptides in insect pest management, research in this area should be continued.

Avidin is a glycoprotein from chicken (*Gallus gallus*) egg white that binds its ligand, biotin, with very high affinity. Biotin is a coenzyme required for all forms of life, so feeding avidin to many insects causes a biotin deficiency that leads to a stunted growth and mortality. Morgan et al. (1993) reported that avidin is toxic to seven species of stored-product beetles (Coleoptera) and moths (Lepidoptera). It was shown that avidin is also toxic to housefly (*Musa domestica*) (Levinson and Bergmann, 1959) and olive fruit fly (*Dacus oleae*) (Tsiropoulus, 1985). Kramer et al. (2000) reported that when expressed in transgenic maize at levels of ≥100 p.p.m., avidin is toxic to and prevents development of insects that damage grains during storage (*Sitophilus zeamais*, *Sitotroga cerealella*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis*, *Tribolium castaneum*, *Tribolium confusum*, *Cryptolestes pusillus*, *Plodia interpunctella*, *Anagasta kühniella*). Avidin expressed in transgenic tobacco and apple conferred a high level of insect resistance to potato tuber moth, *Phthorimaea operculella* (Zeller) and lightbrown apple moth, *Epiphyas postvittana* (Walker), respectively (Markwick et al., 2003). Concerning a wider spectrum of insecticidal activity (for example than of single *cry* gene), and the fact that avidin is present in the human diet in doses several times higher than those showing insecticidal activities in transgenic plants, it could be expected that avidin-expressing transgenic plants will be produced commercially in the near future. In fact, avidin is already produced in transgenic maize, but only for extraction as biochemical reagent (Hood et al., 1997).

RESISTANCE GENES FROM HIGHER PLANTS

From a wide range of insecticidal plant secondary metabolites, genes for protein antimetabolites (i) proteinase inhibitors, (ii) α-amylase inhibitors and (iii) lectins have been extensively used for plant transformation to confer insect resistance.

PROTEINASE INHIBITORS

Proteinase inhibiotors (PIs) are ubiquitous proteins widely distributed in multiple forms in several tissues of animals, plants and in microorganisms (Laskowski and Kato, 1980). The relatively high levels of proteinase inhibitors in storage organs such as seeds and tubers (1-15% of total proteins) may suggest that PIs in plants might function as a depot of safe storage forms of proteins which are immune to digestion until required during germination or sprouting (Ryan, 1988). There is, however, a number of evidence that the major role of PIs in plants, together with other secondary metabolites, is protection against the attacks of animals, microorganisms and insects (Richardson, 1980). In response to mechanical wounding or insect attack, plants accumulate PIs specific to one of the four mechanistic classes of proteinases - serine, cysteine, aspartic and metallo proteinases (Ryan, 1990). Insect proteinases are essential digestive enzymes that catalyse the release of amino acids from dietary protein to provide the nutrients required for larval growth and development. Whereas serine proteinases (trypsin-, chymotrypsin- and elastase like proteinases) are predominant in lepidopteran midgut, midguts of coleopteran species are rich in cysteine and aspartic proteinases (Abdeen et al., 2005). The mode of PI action on insects is still under debate, and it remains to ascertain whether PIs' deleterious effects stem from an anti-digestive effect through proteolysis inhibition (Jongsama and Bolter, 1997), or from a toxic effect by inducing proteinases hyperproduction, leading to a shortage in amino acids (Broadway and Duffey, 1986). PIs can also affect the water balance, moulting and enzyme regulation of the insects. Consequences are reduced growth and development of insects but also death. PIs reduces proteolytic enzyme activity *in vitro* in a number of insect species (Wolfson and Murdock, 1987; Christeller et al., 1992; Oppert et al., 1993; McManus and Burgess, 1995; Marchetti et al., 1998; Wilhite et al., 2000; Tamayo et al., 2000). Reduced growth and development of insects following chronic ingestion of PIs incorporated in artificial diet or when present at high levels *in planta* were also frequently reported (Birk and Applebaum, 1960; Steffens et al., 1978; Wolfson and Murdock, 1987; McManus and Burgess 1995). A study conducted by Tran et al. (1997) showed that plant PIs were potential antimetabolic aphid compounds.

Because of a significant inhibitory activity of PIs against insect digestive enzymes, genes encoding plant PIs have become attractive targets for crop improvement through recombinant DNA technology. The first example of the use of a plant-derived PI gene was described by Hilder et al. (1987) who transformed tobacco plants with trypsin inhibitor gene (*CpTI*) from *Vigna unguiculata*. Regenerated plants expressing CpTI under the control of cauliflower mosaic virus 35S promoter had significantly enhanced resistance to *Heliothis virescens*. Johnson et al. (1989) expressed in tobacco genes for tomato inhibitor II and potato inhibitor II (both have two reactive sites and act as trypsin/chymotrypsin inhibitors). Inhibitors expressed in tobacco leaves at levels >100 μg/g of tissue severely retarded the growth of *Manduca sexta* larvae as compared

to larvae fed untransformed plants. Duan et al. (1996) introduced potato inhibitor II gene into rice. Bioassay for insect resistance with the fifth-generation of transgenic rice plants showed that transgenic plants had increased resistance to a major rice insect pest, *Sesamia inferens*. Altpeter et al. (1999) introduced barley trypsin inhibitor (BTI-CMe) gene into wheat. The significant reduction of survival rate of *Sitotroga cerealella* reared on transgenic wheat seeds expressing BTI-CMe, compared to untransformed control plants, confirmed the potential of BTI-CMe for the increase of insect resistance. However, only early-instar larvae were inhibited in transgenic seeds and expression of BTI-CMe protein in transgenic leaves did not have a significant protective effect against leaf-feeding insects. Marchetti et al. (2000) reported introduction of three soybean genes (*KTi*$_3$, *C-II* and *PI-IV*) coding for serine PIs in tobacco and potato plants. The level of insect resistance (tested with *Spodoptera littoralis* Boisduval) was particularly high in tobacco, where many plants caused the death of all larvae. In potatoes, larval mortality was much less frequently achieved but the results were still very promising, i.e. larval weight gain was reduced by 50% in presence of adequate amounts of inhibitor. De Leo et al. (2001) evaluated effects of mustard trypsin inhibitor MTI-2 expressed at different levels in transgenic tobacco, arabidopsis and oilseed rape against three lepidopteran insect pests (*Plutella xylostella, Mamestra brassicae* and *Spodoptera littoralis*). Effects were different for different insects and expression levels, ranging from very high mortality to delayed larval development. De Leo and Gallerani (2002) also studied long-term effect of feeding *S. littoralis* larvae on transgenic plants expressing MTI-2 at different levels. When second instar larvae of *S. littoralis* were fed on high MTI-2 expressing tobacco plants, significantly reduced fertility was detected. Kereša (2002) showed that even the shortest known PIs from squash family of serine proteinases, expressed in tobacco and potato plants, cause significant reduction of *S. littoralis* larval growth. Expression of the maize PI gene (*mpi*) in rice plants enhanced resistance against the most devastating pest of rice, striped stem borer (*Chilo suppressalis*) (Vila et al., 2005).

Results reviewed above indicate that plant PIs are very promising antimetabolites that can easily be expressed in plants and confer certain level of insect resistance. It was found, however, that insects can adapt to the plant-expressed PIs by producing other insensitive proteinases in their midgut (Jongsma et al., 1995; Broadway, 1997). Strategy for avoiding the development of insect resistance to PIs in transgenic plants could be stacking of the genes for PIs with different mode of action in the same plant.

α-AMYLASE INHIBITORS

Protein α-amylase inhibitors are widespread and have been isolated from a variety of plant species and microorganisms. The physiological role of α-amylase inhibitors in plants is uncertain, but there is some evidence that they may act as protein reserve in seeds (Sharma and Pattabiraman, 1980). α-amylase inhibitors function in a similar manner as proteinase inhibitors, interfering with insect nutrient utilization. When tested in artificial diet, purified α-amylase inhibitors from wheat showed insecticidal effect to

coleopteran storage pests *Callosbruchus maculatus* and *Tribolium confusum* (Gatehouse et al., 1986). Gutierrez et al. (1993) have shown that different types of α-amylase inhibitors present in wheat endosperm are also active against different lepidopteran pest species. The common bean contains two isoforms of α-amylase inhibitors encoded by two allelic variants. The first isoform named α-amylase inhibitor I (α-AI-1) was found in cultivated beans inhibiting amylases of the cowpea weevil (*Callosobruchus maculatus)* and azuki bean weevil (*C. chinensis)* (Moreno and Chrispeels, 1989). The second variant, α-AI-2, was found in some wild species and showed inhibitory activity against Mexican bean weevil (*Zabrotes subfasciatus*) (Suzuki et al., 1993). All these findings encouraged scientists to express amylase inhibitor genes, and especially α-AI-1 from common bean, in different crop species in order to obtain resistance to certain insects. Shade et al., (1994) transformed pea with α-AI-1 gene and obtained transgenic seeds resistant to bruchid beetles (*Callosobruchus maculatus* and *C. chinensis*). Also pea weevil (*Bruchus pisorum*) when fed on transgenic seeds expressing α-AI-1 showed only 2-7% survival (De Sousa-majer et al., 2007). Morton et al. (2000) found that pea expressing the same gene was protected from pea weevil in field conditions as well. Recently, Ignacimuthu and Arockiasamy (2006) introduced α-AI-1 gene into Basmati rice. Results of the bioassay study revealed significant reduction in survival rate of rice weevil (*Sitophilus oryzae*) reared on transgenic rice seeds.

LECTINS

Lectins are carbohydrate-binding proteins that bind glycans of glycoproteins, glycolipids, or polysaccharides with high affinity (Goldstein and Hayes, 1978). Most of the plant lectins are secretory proteins, meaning that they enter the secretory system and subsequently accumulate either in vacuoles or in the cell wall and intercellular spaces. Although many roles have been proposed for plant lectins (Etzler, 1986), the most likely function for vacuolar lectins is plant defence. As far as insects are concerned, toxic effects appear to be mediated through binding of the lectins to the midgut epithelial cells with consequent disruption of the cell function. The bound lectins may inhibit nutrient absorption or disrupt midgut cells by stimulating endocytosis of the lectins, and possibly other toxic metabolites present in the midgut (Czapla and Lang, 1990). Various lectins have been proved toxic towards members of the Coleoptera, Lepidoptera (Czalpa and Lang, 1990) and Diptera (Eisemann et al., 1994). Most importantly, lectins can be used to control sap-sucking insects belonging to the order Homoptera, which includes some of the most devastating pests worldwide. Crop damage caused by these insects is not only due to feeding, but also to their role as vectors of plant viruses (Hilder et al., 1995). Powell et al. (1993) demonstrated, by insect feeding trials, significant antimetabolic effects of the lectins *Galanthus nivalis* agglutinin (GNA) and wheat germ agglutinin (WGA) to sucking insect pest *Nilaparvata lugens* of rice. Studies of the antimetabolic effect of GNA were extended to the aphids *Myzus persicae* (Hilder et al., 1995), *Aulacorthum solani* (Down et al., 1996) and

Sitobion avenae (Stoger et al., 1999). Transgenic plants expressing GNA (tobacco, potato and wheat) caused significantly reduced parthenogenetic fecundity, but only marginal or none decrease in aphid survival. Lectins from species other than *Galanthus nivalis* also possess insecticidal activity. Melander et al. (2003) expressed pea lectin in oilseed rape and obtained a significant reduction in pollen beetle larval weight, and small but significant reduction of larval survival. Hossain et al. (2006) show that onion leaf lectin gene expressed in Indian mustard offers protection against aphid colonization. All these results show that lectins from different origins have a potential to be exploited in crop protection as transgenic resistance factors against various insect pests.

CONCLUSION

Partially as the consequence of the climatic changes, some old, but also more and more new insect pests are becoming devastating for many cultivated crops. Breeding cultivars resistant to those pests is the strategy of choice for solving this problem. Genetic engineering opened the possibility to use insecticidal genes from different origin for breeding resistant plants. So far, only *cry* genes from *Bacillus thuringiensis* have been commercially exploited for this purpose, however many other genes of bacterial, plant, or other origin are successfully used to confer insect resistance. In the future special attention should be paid to producing insect resistant transgenic plants that will hinder the development of insect resistance to the recombinantly- expressed antimetabolites or toxins. A possible way to achieve this is the stacking of two or more insecticidal genes with different mode of action into the same plant.

TRANSGENIČNE BILJKE S ISPOLJAVANJEM GENA ZA OTPORNOST NA KUKCE

SAŽETAK

Otkako su prije dvadesetak godina proizvedene prve transgenične biljke otporne na kukce, otkriven je i za genetičke transformacije korišten velik broj gena za rezistentnost na kukce iz različitih izvora. Prve transgenične biljke otporne na kukce sadržavale su *cry* (*Bt*) gen iz bakterije *Bacillus thuringiensis.* Sredinom devedesetih godina prošlog stoljeća započela je komercijalna proizvodnja transgeničnog kukuruza, nešto kasnije i pamuka koji su sadržavali *cry* gen. U Europskoj uniji odobrena je uporaba nekoliko transgeničnih linija kukuruza s *cry* genima otpornim na kukuruznog moljca (*Ostrinia nubilalis* Hübner) ili kukuruznu zlaticu (*Diabrotica virgifera virgifera* LeConte). Neke od tih linija koriste se za proizvodnju hibrida odobrenih za konzumaciju u ljudskoj ishrani i ishrani stoke, a za neke je dozvoljen i uzgoj. Odobrena je i uporaba nekoliko linija koje u sebi ujedinjuju dva ili više gena za otpornost na

kukce, ili kombinaciju gena za otpornost na kukce i tolerantnost na herbicid. Vrlo blizu komercijalne uporabe su i insekticidni *vip* geni, koji također potječu iz bakterija roda *Bacillus (B. thuringiensis* i *B. cereus*). U borbi protiv štetnih kukaca obećavajuće rezultate pokazali su, međutim, i drugi geni korišteni za genetičke transformacije biljaka, kao npr. gen za enzim kolesterol oksidazu (iz mikroorganizama), za avidin (iz bjelanjka jajeta), za enzim hitinazu i neuropeptide (iz kukaca). Biljke imaju sposobnost da i same proizvode različite sekundarne metabolite koji, ako im je koncentracija u tkivu dovoljno visoka, štetno utječu na kukce. Kukci, kako bi se mogli hraniti na određenoj vrsti, morali su razviti otpornost na antimetabolite koje ta biljna vrsta proizvodi. Međutim, ispoljavanje gena rezistentnosti iz drugih biljnih vrsta i pod utjecajem jakog promotora omogućuje da, prije neotporne biljke, postanu otporne na štetne kukce koji su se na njima prije transformacije nesmetano hranili. Geni za tri skupine biljnih proteinskih antimetabolita koriste se u tu svrhu: (1) proteinazni inhibitori, (2) α-amilazni inhibitori i (3) lektini. Dok pojedini proteinazni inhibitori imaju prilično uzak spektar insekticidnog djelovanja, lektini su pokazali insekticidnu aktivnost prema različitim redovima kukaca, čak prema kukcima reda *Homoptera* koji sišu. Oplemenjivanje novih kultivara otpornih na kukce mora se nastaviti, pri čemu posebno treba voditi računa o proizvodnji transgeničnih biljaka koje će spriječiti razvoj otpornosti kukca na određeni rekombinantni, u biljkama eksprimirani antimetabolit ili toksin. Ovo je moguće postići ispoljavanjem u istoj biljci dva ili više gena s insekticidnim, ali različitim mehanizmom djelovanja na kukce.

Ključne riječi: transgenične biljke, otpornost na kukce, Bt toksin, proteinazni inhibitor, avidin

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