

## Transgenic cucumber – a current state

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### ABSTRACT

Genetically manipulated varieties are cultivated in several crops and this review presents the situation in case of cucumber, in which the genetically modified organisms (GMOs) were not introduced into practice. A part of transgenic cucumber lines showed differences in stable expression and inheritance of the inserted construct. The following new properties were introduced, depending on the composition of the construct: an enhanced CMV resistance, an enhanced gray mold resistance, an elevated superoxide dismutase (SOD) level in fruits (might be useful as a functional cosmetic), an increased yield and a more intensive development, sweet taste of the fruits and the production of parthenocarpic fruits. These new properties were mostly evaluated under the greenhouse conditions. In three cases only the field trials were performed. In one case only the comprehensive evaluation of the transgenic according to the formal rules was performed.

## ABBREVIATIONS:

- ACB* – acylbinding protein gene from *A. thaliana*  
*pASO* – cucumber ascorbate oxidase promoter  
CBF – C-repeat binding factor  
*CHN* – a chitinase gene from petunia (acidic), tobacco (basic), or bean (basic)  
*CMV-C cp* – coat protein gene of cucumber mosaic virus C  
*CMV-O cp* – coat protein gene of cucumber mosaic virus O  
CMV – cucumber mosaic virus  
CMV<sup>R</sup> – CMV resistant  
CMV/ZYMV<sup>T</sup> – CMV/ZYMV tolerant  
*pDefH9* – *Antirrhinum majus* deficiens homologue 9 promoter  
*DHN10* – gene encoding a *Solanum sogarandium* dehydrin with 10kDa  
*DHN24* – gene encoding a *Solanum sogarandium* dehydrin with 24kDa  
GMO – genetically modified organisms  
*pGT* – *Solanum sogarandium* glucosyl transferase promoter  
GRAS – generally recognized as safe  
IAA – indole-3-acetic acid  
*iaaM* – tryptophan monooxygenase gene  
ITE – independent transformation event  
*mSOD1* – the CuZnSOD cDNA from cassava  
*pnos* – nopaline synthase promoter  
*nptII* – neomycin phosphotransferase II gene  
*pPG* – tomato fruit-specific polygalacturonase promoter  
PR – pathogenesis related  
*pPR-2d* – tobacco  $\beta$ -1,3-glucanase promoter  
*RCC2* – a rice chitinase cDNA  
R<sup>H</sup>/R<sup>I</sup> – high resistant/intermediated resistant  
*p35S* – cauliflower mosaic virus 35S promoter  
S – sensitive  
SOD – superoxide dismutase  
*UGT* – UDPG transferase gene from maize  
*uidA* –  $\beta$ -D-glucuronidase (GUS) gene  
*ZGMMV-cp* – zucchini green mottle mosaic virus coat protein gene  
ZYMV – zucchini yellow mosaic virus  
ZYMV<sup>S</sup> – ZYMV sensitive

## INTRODUCTION

Since the mid 1990s, when transgenic crops were commercially introduced, global acreage has increased to ca. 55 million hectares by the year 2002 and genetic engineering technology was mostly evaluated as a phenomenal adaptation by more than 5.5 million farmers (James 2002, McCown 2003, Persley et al. 2002). However, the lists of transgenic crops which are cultivated remained quite stable during the last 3 years and include 5 vegetables (tomato, chicorree, melon, squash, and zucchini). For each of them, with the exception of tomato, only single varieties were introduced.

Cucumber (*Cucumis sativus* L.) is one of the most popular vegetables worldwide. Its first transformation either through an *Agrobacterium*-mediated system (Sarmiento et al. 1989, Trulson et al. 1986) or direct gene transfer (Chee and Slightom 1992) was described two decades ago. In addition to the marker and reporter genes, various types of transgenes with agronomic potential have been introduced. The enhanced biotic resistance was observed after introduction of cucumber mosaic virus coat protein (CMV-cp) gene (Chee and Slightom 1991, Nishibayashi et al. 1996 a), zucchini green mottle mosaic virus coat protein (ZGMMV-cp) gene (Lee et al. 2002) and chitinase genes (Raharjo et al. 1996, Tabei et al. 1998). Whereas, the introduction of *DHN10* gene was associated with a slightly enhanced tolerance to abiotic stresses (Yin et al. 2004 b). The introduction of *thaumatin II cDNA* construct enhanced sweet taste in fruits (Szwacka et al. 1996, 2002 a), whereas *mSOD1* gene caused higher level of superoxide dismutase (SOD) and might be useful as a functional cosmetic material (Lee et al. 2003). The introduction of *UGT* and *ACB* genes resulted in an increased yield (Salyaev et al. 2002 a, b), and *iaaM* gene led to parthenocarpic fruit production (Yin et al. 2005 b). Some characteristics of the transgenic cucumber with agronomic potential are summarized in Table 1.

The number of field trials with transgenic cucumber is sparse, as compared to many other species. First of all, the effectiveness of coat protein-mediated protection was investigated under field conditions (Gonsalves et al. 1992). Tabei et al. (1999) carried out an environmental risk assessment of transgenic cucumber lines containing rice chitinase cDNA under the conditions of a closed and a semi-closed greenhouse. Gajc-Wolska et al. (2001, 2003) compared several lines harboring *thaumatin II cDNA* and Twardowska (2003) evaluated different food dishes prepared with the sweetest line. Additionally, the effects of these diets on the growth and health status of rats were comprehensively analysed (Kosieradzka et al. 2001, Twardowska 2003).

Table 1. Transgenic cucumber with agronomic potential

Analysed transgene	Number of ITE analysed	Transgene expression		Transgene related phenotype	References
		RNA	Protein		
		Expressed	Not expressed		
<i>p35S::CMV-C cp</i>	6 lines	leaf	leaf	CMV <sup>R</sup>	Gonsalves et al. 1992
<i>p35S::CMV-O cp</i>	4 T <sub>0</sub> plants 3 T <sub>1</sub> lines	leaf	leaf cotyledon	CMV-Y <sup>R</sup> CMV/ZYMV <sup>T</sup> ZYMV <sup>S</sup>	Nishibayashi et al. 1996 a, b
ZGMMV-cp	3 T <sub>0</sub> plants 8 T <sub>1</sub> lines				Lee et al. 2002
<i>p35S::CHN</i>	3 lines	leaf, callus			Punga and Raharjo 1996
<i>p35S::RCC2</i>	3 T <sub>1</sub> lines	epidermal cells of leaves			Tabei et al. 1998 Kishimoto et al. 2002
<i>pGT::DHN10</i>	3 T <sub>1</sub> lines	leaf cotyledon hypocotyls root			Yin et al. 2004 b
<i>pGT::DHN24</i>	17 ITE	leaf cotyledon hypocotyls root	leaf cotyledon hypocotyls		Yin et al. unpubl.
<i>p35S::thaumain II cDNA</i>	11 ITE	leaf fruit			Szwacka et al. 1996, 2000, 2002 a, and 2002 b
<i>pPG::thaumain II cDNA</i>	5 ITE				Szwacka et al. 1999 a, and unpubl.
<i>pASO::mSOD1</i>	3 T <sub>0</sub> plants	leaf fruit			Lee et al. 2003
<i>UGT, ACB</i>					Salyaev et al. 2002 a, and 2002 b
<i>pDefH9::iacaM</i>	8 ITE			parthenocarpic fruits	Yin et al. 2005 b

The introduction of genetically modified organisms (GMO) into agriculture requires, according to European Union rules, additional analyses, the most important of which concern their substantial equivalence and undesirable effects (Kuiper et al. 2001). Both of them need the comparison of a lot of chemical components and nutrition evaluation of transgenic versus non-transgenic crop.

In this paper, we demonstrate the present state of the transgenic cucumber, particularly regarding the fate of the introduced genes as well as a phenotypic evaluation, especially with respect to the intended effect. The analysis of transformation methods used was also performed (Yin et al. 2005 a).

## 1. TRANSGENE INHERITANCE

Regular transgene transmission as well as its appropriate expression is the main prerequisite for the production of GMO varieties in generatively propagated plants, such as cucumber. In a cucumber, the inheritance of marker and reporter genes was analyzed on different genetic backgrounds. Both Mendelian and non-Mendelian transmission was documented. Segregation of the *nptII* gene occurred at a 3:1 ratio, expected for a single locus in R<sub>1</sub> progeny (Sarmiento et al. 1989, Chee and Slightom 1992). In other cases, the same gene was segregated at the predicted ratio in 80% of the lines (Szwacka et al. 2002 a). Furthermore, segregation of kanamycin resistance was investigated in two groups of transgenic lines, one containing *pPR-2d::uidA-pnos::nptII* and the second with *p35S::thaumatinIIcDNA-pnos::nptII* construct, to the third and fifth generations respectively (Yin et al. 2004 c). In the case of the first construct, 78% of the progeny exhibited a segregation ratio consistent with Mendelian inheritance, whereas in the second group it was only in 46%. A segregation ratio for 2 or 3 independent loci occurred in both cases.

The transmission of the agronomically important transgene and its expression were rarely studied. Most often, the integration and transmission of these genes were stable through the few generations analyzed. In one case, a chromosomal location of certain transgenes was determined (Tagashira et al. 2005).

Tabei et al. (1998) demonstrated that the *RCC2* gene, a rice chitinase gene, was transmitted to the T<sub>1</sub> progeny together with the resistance against gray mold. The segregation of disease resistance among the progeny was in accordance with the predicted Mendelian ratio of 3 : 1 (resistant : susceptible). The integration of *RCC2* gene was also confirmed in 7 of 13 progeny of CR33 line, which exhibited resistance.

Szwacka et al. (2002 a) reported that the copy number of the *thaumatin II* gene in 10 T<sub>1</sub> independent lines was single in most cases, or two and five in four T<sub>1</sub> lines. No truncation or rearrangement of the *thaumatin II* expression cassette was

detected in three subsequent generations. The chromosome location of the *p35S::thaumatinIIcDNA-pnos::nptII* construct, was determined by the FISH method, showing it was preferentially inserted in the euchromatic region of chromosomes 1, 2, 3, and 4 (Tagashira et al. 2005).

The interline variability in transgene expression levels as well as transgene inheritance is a ubiquitous phenomenon in many species (Deroles and Gardner 1988, Matzke and Matzke 1995, Mayer 1995, Pawlowski and Somers 1996).

## 2. PRACTICAL EVALUATION

Various aspects of the practical value of the mentioned transgenic lines were discussed. Expression of the transgenes, either at RNA or protein level, may confer the expected phenotype. However, in some cases, such positive relationship did not exist. In addition to transgene-related phenotype, other agronomic traits, metabolic profiles, as well as an environmental risk were evaluated.

### 2.1. Breeding material for tolerance to biotic and abiotic stress

#### Pathogen protection

Breeding for disease resistance has long been one of the crucial objectives in cucumber cultivation. Transformation techniques make it possible to use isolated genes from a variety of sources. Such transgenic material might serve as a unique breeding material for producing cultivars with enhanced resistance to biotic and abiotic stress. To date, the pathogen-derived coat protein gene, *CMV-cp* gene (Chee and Slightom 1991, Nishibayashi et al. 1996 b) and zucchini green mottle mosaic virus coat protein (*ZGMMV-cp*) gene (Lee et al. 2002), as well as the plant-derived pathogenesis-related (PR) chitinase gene (Raharjo et al. 1996, Tabei et al. 1998) have been introduced into the cucumber genome. The expression of *CMV-cp* gene either on the RNA or protein level positively correlated with resistance to CMV infection. Expression of rice chitinase gene enhanced the resistance to gray mold, whereas expression of chitinase genes from petunia, tobacco or bean did not offer resistance to inoculation with fungal pathogens: *Colletotrichum lagenarium*, *Alternaria cucumerina*, *Botrytis cinerea*, and *Rhizoctonia solani* (Punja and Raharjo 1996). Environment risk assessment of transgenic cucumber lines containing *RCC2* gene was evaluated (Tabei et al. 1999).

### Virus coat protein gene

Accumulation of CMV-cp transcripts seems to be positively correlated with CMV resistance (Nishibayashi et al. 1996 b). However, coat protein was not detectable in the leaves and cotyledons of transgenic plants. The progeny from a cross between control and transgenic plants containing the *CMV-cp* gene displayed strong resistance to CMV inoculation, but not to zucchini yellow mosaic virus (ZYMV). However, transgenic plants showed a reduced degree of disease symptoms following a double inoculation with CMV and ZYMV. The authors suggest that the CMV resistance of the transgenics was due to the synergism of the slight CMV tolerance in the pure line "1021" and the protection against CMV afforded by the introduction of the *CMV-cp* gene.

The expression of CMV coat protein gene offers protection against CMV infection (Chee and Slightom 1991). The virus coat protein (24kDa) was found in R<sub>1</sub> plants in the amount of 14 ng/mg of total cellular protein (Chee and Slightom 1991). The coat protein gene increased the resistance to CMV infection in field trials (Gonsalves et al. 1992). Eight weeks after transplanting, the percentage of infection in transgenic 'Poinsett 76' and 'Marketmore 76' plants (resistant control cultivar) averaged less than 5%, in contrast to about 72% in non-transgenic plants. Thirteen weeks after field planting, the level of CMV infection in transgenic lines was 35%, compared to 62% in 'Marketmore 76' and 85% in the non-transgenic 'Poinsett 76'.

### Chitinase genes

Raharjo et al. (1996) reported that the level of petunia chitinase activity in transgenic cucumber plants was varied and transgenic plants expressed tobacco or bean chitinase protein. However, expression of these genes did not offer resistance to *A. cucumerina*, *B. cinerea*, *C. lagenarium*, and *R. solani* (Punja and Raharjo 1996).

The expression level of class I rice chitinase cDNA (*RCC2*) driven by the *35S* promoter was positively correlated with the degree of resistance to gray mold (Kishimoto et al. 2002, Tabei et al. 1998). The rice chitinase levels in the most resistant line CR32 and the intermediate-resistant CR3 were higher than those of the susceptible CR20. Rice chitinase was detected in the epidermal and mesophyll cells. The fungal growth within leaf tissue was suppressed in the resistant lines, but not in the non-transgenic or the susceptible line CR20. The authors suggest that the high expression level and intracellular localization of rice chitinase may be involved in enhancing the resistance of transgenic plants to gray mold.

Furthermore, Tabei et al. (1999) carried out the environment risk assessment of transgenic cucumber lines containing *RCC2* gene in a closed and a semi-closed greenhouse. The three lines selected for the assay showed the highest resistance against gray mold. The following parameters were compared: 1) morphological characteristics of pustules and fruits during their maturation period; 2) reproductive aspects, e.g. pollen form and fertility, longevity of the pollen, pollen dispersal by wind, seed fertilities and cross compatibility of melon with wild relatives; 3) possibility of harmful influences on environment due to the presence of detrimental substances, i.e. volatile compounds, allelochemical substances; 4) presence of remaining *Agrobacterium*, which was used as a vector for the transgenic production. Apart from the expression of rice chitinase and the resistance to gray mold no other differences were found.

#### Chilling tolerance

The constructs used for engineering transgenic cucumber to resist abiotic stress include *Solanum sogarandinum* dehydrin genes and *Arabidopsis* C-repeat binding factor (CBF) genes.

#### Dehydrin (DHN) genes

Some low temperature-responsive genes are predicted to encode proteins with the function of dehydrins (Close 1997). In order to study the potential role of dehydrin in chilling tolerance in cucumber, the transgenic plants with two constructs containing dehydrin genes were produced. One of them was composed of the promoter sequence of a *S. sogarandinum* glucosyl transferase (*GT*) gene and the coding sequence of the *DHN10* gene, and the others *DHN24* gene driven by *GT* promoter (Rorat et al. 2004, Rorat unpubl.). Accumulation of *DHN10* mRNA was identified in leaf, cotyledon, hypocotyl and root tissues of the transgenic seedlings of three T<sub>1</sub> transgenic cucumber lines analysed (Yin et al. 2004 b). However, the *DHN10* protein was not detectable in these plants. The accumulation of *DHN10* transcripts was positively correlated with the chilling tolerance of some transgenic lines, independently of the electrolyte leakage effects. The transgenic lines exhibited a slightly enhanced chilling tolerance and a freezing tolerance either comparable to or less than the non-transgenic control. With the *DHN24* gene, 17 independent transformation events were obtained and the T<sub>1</sub> progeny produced (Yin, unpubl.). A proper evaluation of the practical importance of dehydrin genes in the cucumber chilling/freezing tolerance needs additional studies.

### CBF gene

To obtain increased environmental stress tolerance, Arabidopsis C-repeat binding factor (CBF) gene was successfully introduced, as verified by PCR analysis (Grumet et al. 2000). Northern analysis of several transgenic individuals showed a range of expression of the p35S::CBF1 and p35S::CBF3 genes. However, no information concerning the stress tolerance of these transgenic cucumber plants was reported.

## 2.2. Production of new substances

### Plant sweet-tasting protein

Thaumatococcus daniellii Benth. is a sweet-tasting plant protein produced by the fruits of a West African perennial plant *Thaumatococcus daniellii* Benth, and it is generally recognized as safe (GRAS) with the number 3732, for 30 years. Thaumatococcus is nearly 100 000 times sweeter than sucrose on a molar basis (Iyengar et al. 1979). Transgenic cucumber plants expressing the *thaumatin II cDNA*, driven by a constitutive 35S promoter (Szwacka et al. 2002 a) or callus tissues and plants expressing *thaumatin II cDNA* under the transcriptional control of an organ-specific *PG* promoter (Szwacka et al. 1999 a) were produced (Table 1). Transgenic fruits generated by some lines with *p35S* driven construct accumulating thaumatin II protein exhibited a sweet phenotype. Their nutritional value, agronomic performance and the metabolic status of the plants were evaluated.

### Transgene expression

Expression of *p35S::thaumatinIIcDNA* gene was analysed on RNA and protein level (Szwacka et al. 2000, 2002 a, b). Variable levels of transgene expression and a lack of correlation between protein and mRNA accumulation levels were observed, suggesting that thaumatin II expression may be controlled on a transcriptional as well as translational level. The levels of thaumatin II mRNA varied in leaves of particular lines. There were a greater than 10-fold differences in levels of thaumatin II transcript accumulation, independently of copy number. High or moderate levels of thaumatin mRNA were detected in ripe fruits of two T<sub>1</sub> lines and similar transcript levels were observed in subsequent T<sub>2</sub> and T<sub>3</sub> generations. Western blot analysis detected thaumatin II protein in its processed form (22kDa) in 11 out of 16 examined T<sub>2</sub> plants. It was shown that the accumulation of thaumatin II protein varied both temporally and spatially in organs of independent T<sub>3</sub> lines (Szwacka et al. 2002 a).

### Whether the expression of the transgene confers the expected phenotype

For T<sub>2</sub> fruits, a positive correlation between sweet taste intensity and thaumatin II protein accumulation was found (Szwacka et al. 1999 a, b, 2000, 2002 a, b). The taste of 265 analysed fruits differed to some extent from commercially available thaumatin. Fruits of some lines exhibited a cucumber-like aroma while others appeared different. The fruits from one line only exhibit a clear sweetness degree instead of acidity or bitterness.

### Nutritional evaluation of the transgenic cucumber

Chemical composition of the non-transgenic and transgenic fruits was compared and the effects of feeding rats with the fruits in balanced diets were determined (Kosieradzka et al. 2001). The transgenics contained more protein (20.3 vs 17.9% DM) and less fibre (9.4 vs 11.4% DM), had lower Na, K, Ca, and Mg contents and higher levels of Fe and Cu in ash. Feeding male rats (initial body weight 150 g) for 5 weeks with isoprotein diets containing 0 or 15% lyophilized transgenic or non-transgenic cucumbers did not affect weight gain, apparent health status, or relative organ weights of animals. Protein digestibility was slightly lower (89.2 vs 90%), and that of crude fibre was higher (28.2 vs 15.0%) in diets containing transgenic versus non-transgenic fruits, while digestibility of fat and N-free extractives did not differ. Similar results were obtained by Twardowska (2003). Moreover, the author observed the higher hematocrit following feeding with transgenics, probably a result of the reduced iron and higher protein content of the transgenics.

Some aspects of food technology were addressed using the fruits of one transgenic line with a clear sweetness level (Twardowska 2003). Substantial equivalence of the fruits and a high sensitivity of thaumatin II to chymotrypsin and trypsin digestion were observed. Several combinations - including fresh fruits, biological or acetic acid processing, salads with kiwi, apple, orange, pineapple, tomato, and melon - were evaluated. In all of them, the high stability of thaumatin II was observed during 6 hours of storage as well after acetic acid conservation for 4 weeks. Sensory analysis, using the hedonic scale, revealed some differences between transgenics and non-transgenic. The transgenic lines had a lower acceptance as whole fresh fruits and much higher in other combinations, especially after processing. The reason for lower acceptance of whole fruits was a brown spot on the corolla end of the fruit. The higher acceptance of processed products was mainly a result of sweetening.

### Field experiments

Transgenic lines with *p35S::thaumatinIIcDNA-pnos::nptII* construct were characterized by similar yields, physical parameters and nutritional value of fruits compared to the control cultivar (Gajc-Wolska et al. 2001). High scores in sensory assessment of both fresh and processed fruits prove their high taste and consumption values. The following characteristics were similar between transgenics and non-transgenics: taste quality and chemical composition of fruits; dry matter content (on average 4.3%); vitamin C content (6.4 mg 100 g<sup>-1</sup> f.m.). Content of soluble parts in cell sap ranged from 2.8% to 3.6% and total protein content was 0.91%. The highest potassium content was found in non-transgenic control lines and the highest calcium level in transgenic lines. Under the field conditions, the transgenic fruits were characterized by a sweeter taste, higher firmness of flesh and a higher overall quality, as well as a less bitter taste and less off-taste (Gajc-Wolska et al. 2003).

### Metabolic profiles

The metabolic profiles of five independent transgenic lines were compared with a non-transformed control. They varied considerably with respect to the chromosomal position of the transgene (Tagashira et al. 2005). Each of the locations was characterized by a specific metabolic profile and a level of metabolite changes. The frequency of the changes in 68 compounds that were analyzed in detail was between 17-40% and was not higher as by the *in vitro* derived control.

### Anti-aging cosmetic substance - superoxide dismutase

In some regions of the world, cucumber fruits have been used as a skin massage pack material for a long time. Therefore, the transgenic fruits with elevated levels of superoxide dismutase (SOD) might be useful as a functional cosmetic material. SOD plays an important role in cellular defense against oxidative stress in aerobic organisms. The *CuZnSOD cDNA (mSOD1)* from cassava was introduced into the cucumber using a fruit-dominant ascorbate oxidase-expressing promoter (Lee et al. 2003). Analysis of three transgenic plants demonstrated high levels of *mSOD1* gene transcript in the fruits of all of them, but very low levels in leaves. SOD-specific activity (approximately 150 units per mg total cellular protein) in transgenic fruits was about three times higher than in non-transgenic control. However, it was much lower (15 units) in leaves, almost on the same level as in non-transgenic plants. Native gel analysis showed that in extracts from transgenic

fruits, seven SOD isoenzymes were present, including three novel bands of CuZnSOD. One of them was the introduced cassava CuZnSOD (*mSOD1*), which was not found in control. Thus, an elevated SOD activity in fruits was a consequence of the introduced *mSOD1* gene and the additional two induced *CuZnSOD* genes.

### 2.3. Yield improvement

To create plants with high growth intensity and enhanced productivity, Salyaev et al. (2002 a, b) introduced target genes *UGT* (*iaglu*) from *Zea mays* L. and *ACB* from *Arabidopsis thaliana* L. into the cucumber and other vegetables. *UGT* encodes a UDPG-transferase (IAA-glucose synthase) from maturing corn endosperm, thereby, the stored form of IAA accumulates in kernels. The *ACB* gene encodes an acyl-binding protein, a transporter for acyl CoAs. Transgenic cucumber gave a harvest of up to 46 kg per plant in a greenhouse, which was three times higher in comparison to the control plant.

### 2.4. Parthenocarpic fruits

In the case of plants grown for the commercial value of their fruits, there is a great demand for plants able to develop fruits in the absence of fertilization (Mapelli et al. 1978), especially for obtaining fruits under environmental conditions unfavorable for pollination – such as greenhouse cultivation, for example. Additionally, parthenocarpic fruits are seedless and more acceptable to the consumers. In cucumber some varieties with parthenocarpy are known, but the genetic background is unclear. However, there is a little agreement regarding the number and kind of gene action involved in parthenocarpic fruit development (Sun et al. 2004). An endogenous increase in auxin synthesis within the ovules during early phases of floral and fruit development is a method to support fruit setting and growth without pollination. Previously, transgenic tobacco and eggplants expressing the coding region of the *iaaM* gene from *Pseudomonas syringae* pv. *savastanoi*, under the control of the regulatory sequences of the ovule-specific *DefH9* gene from *Antirrhinum majus*, showed parthenocarpic fruit development (Rotino et al. 1997). In the Department of Plant Genetics, Breeding and Biotechnology, Warsaw Agricultural University, the *pDefH9::iaaM* construct was introduced into the cucumber genome. Eight independent transformation events were obtained and the plants were cultivated in the greenhouse to the maturity (Yin et al. 2004 a). The fruit set without pollination ranged between 60-100%, depending on the line.

## 2.5. Unintended effects

Unintended effects, referred to as the formation of either new metabolites or alerted levels of existing metabolites, resulted from random insertion of specific DNA sequences into the plant genome (Kuiper et al. 2001). Unintended effects may be identified by an analysis of the agronomical/morphological characteristics of the new plant and an extensive chemical analysis of key nutrients, anti-nutrients, allergenes and toxins typical for the plant. In the transgenic thaumatin-expressing cucumber, a decrease in fiber content was noted (Kosieradzka et al. 2001). Previously, Schulze et al. (1995) observed that some randomly chosen plantlets, which did not reach the maturity, gave a strong NPT-signal. The authors proposed that high expression levels of the foreign gene could be the reason for stationary and/or abnormal growth and a resulting death of these plantlets.

## CONCLUDING REMARKS

Various transgenes have been introduced into the cucumber genome to produce new traits. In most cases, the preliminary evaluation was conducted with a low number of independent transformants and in only one generation. Therefore, the final evaluation regarding the practical importance of a new trait was difficult. Only in few cases, the genetic stability and transgene inheritance were evaluated and the similarity with the other plant crops was shown. The transgenic lines varied in their new phenotype in both molecular and whole plant level. Variability in transgene expression levels, as well as in a transgene inheritance, is a common phenomenon in many species (Deroles and Gardner 1988, Matzke and Matzke 1995, Mayer 1995, Pawlowski and Somers 1996) and also takes place in the cucumber (Yin and Malepszy 2003, Yin et al. 2004 a, c, Yin et al. 2005 a). An enhanced CMV-tolerance and thaumatin producing cucumber were extensively evaluated in field conditions. The thaumatin-expressing cucumber may have a commercial importance, and was analysed in many details, showing a substantial equivalence with non-transgenic variety. Very promising preliminary results with the transgenics containing *CBF* gene, enhanced SOD activity in the fruits and parthenocarpy need further evaluation in the agronomic conditions.

Various cucumber genotypes, including non-hybrid and hybrid cultivars, pure and inbred lines of different origins, were successfully used for *Agrobacterium*-mediated transformation (Yin et al. 2005 a). All constructs introduced into the cucumber genome have contained the gene of interest and the marker gene. A set of 8 groups of transgenic lines according to the construct composition was produced on the same genetic background (Yin et al. 2005 a). Except for practical importance of some of them, a unique opportunity for extensive studies at the proteomic and metabolomic level, concerning relations between the transgene and plant value is possible.

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#### TRANSGENICZNE OGÓRKI – STAN OBECNY

Streszczenie: W uprawie wielu gatunków stosuje się odmiany genetycznie modyfikowane, a niniejszy artykuł pokazuje sytuację u ogórka, gdzie dotąd GMO nie zostało wprowadzone do praktyki. Ogórki transgeniczne, podobnie jak inne gatunki, wykazują różnice w stabilności ekspresji oraz dziedziczeniu wprowadzanej konstrukcji. Nowe właściwości, zależnie od użytej konstrukcji, były oceniane w większości przypadków w warunkach szklarniowych. Tylko w trzech przypadkach przeprowadzono doświadczenia polowe. W zależności od składu konstrukcji uzyskano następujące nowe właściwości: zwiększona tolerancja na wirusa CMV, zwiększona tolerancja na szarą pleśń, zwiększony poziom dysmutazy ponadtlenkowej (SOD) w owocach (może być wykorzystana w kosmetyce), słodki smak owoców oraz wytwarzanie owoców partenokarpicznych. Tylko w jednym przypadku przeprowadzono pełną ocenę rośliny transgenicznej, zgodną z wymogami formalnymi.

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