Recent Progress in Cucumber (Cucumis sativus) Transformation

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ABSTRACT

Genetic transformation is vital to the transfer of novel genes into vegetable plants as well as the emerging area of functional genomics. However, the successful genetic transformation of cucumber still remains time-consuming and genotype dependent. This paper updates the progress made in recent years toward developing a genetic transformation system for cucumbers. Agrobacterium-mediated cucumber transformation offers advantages, such as single copy gene insertion, minimal rearrangement of DNA, low cost and comparatively high efficiency. The recent developments in cucumber transformation could lead to an increased efficiency of a cucumber breeding program. The most exciting recent progress has been in the production of elevated levels of an anti-aging superoxide dismutase in transgenic cucumber fruits, since cucumber fruits were considered to be one of the most promising economical plant bioreactors which can produce edible pharmaceutical proteins.

Keywords: Agrobacterium tumefaciens, genetic transformation, particle bombardment

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; 6-BA, 6-benzylaminopurine; CFMMV, Cucumber fruit mosaic virus; KGMMV, Cucumber green mottle mosaic virus; IBA, indole-3-butyric acid; KGMMV, Kyuri green mottle mosaic virus; NAA, 1-naphthylacetic acid; PPT, phosphinothricin; SOD, superoxide dismutase; trp promoter, tryptophane promoter; ZGMMV, Zucchini green mottle mosaic virus

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INTRODUCTION

The cucumber (Cucumis sativus L.) is one kind of major cucurbitaceous fruit, bred all over the word especially in Southeast Asia (Esquinias-Alcazar et al. 1983). By the year 2002, the total planting area of cucumber was 1.253 million hm² and the total yield was 44.537 million tons (30% of the world total yield) in China. Moreover, cucumber is planted in almost every province except Tibet of China (Huang et al. 2007). The process of breeding a new variety and improvement of characters, referring directly to national basket shopping, has become very important. At present, abiotic factors, including low temperature and drought, and biotic factors, including Green mottle mosaic virus, angular leaf spot, fusarium wilt and cucurbit scab seriously influence crop yield and quality of cucumber. Anti-disease, anti-insect and anti-adversity genes that exist in nature are difficult to transform into cucumber through traditional means due to their interspecific affinity. It is very hard to breed a new cucumber cultivar with profitable properties from other species. Plant gene engineering technology conquers that limit and all kind of genes, cloned from either plants, animals or even microorganisms, can be transferred into cucumber, creating new properties. This is a new approach for the genetic improvement of Cucurbitaceae (Compton et al. 2004).

A stable and highly efficiency cucumber regeneration system offers a stable basis for genetic transformation. The time spent on cucumber transformation can be greatly shortened by direct regeneration from cotyledons, hypocotyls,
Table 1 Cucumber transformation studies.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Transgene construct</th>
<th>Method</th>
<th>TE</th>
<th>Explant</th>
<th>TRP</th>
<th>Expression Level</th>
<th>Agrobacterium um strain</th>
<th>Selection</th>
<th>Plasmid vector</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>cv. Poinsett 76</td>
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<td>A.T.</td>
<td>10%</td>
<td>cotyledon</td>
<td>ND</td>
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<td>CS8Z707.</td>
<td>Km</td>
<td>pGA482</td>
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<tr>
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<td>ND</td>
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<td>A.R.</td>
<td>A4</td>
<td>Km</td>
<td>pARC8</td>
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<td>cv. JinYan No. 4</td>
<td>p35S::cgh1</td>
<td>A.R.</td>
<td>-</td>
<td>cotyledon</td>
<td>Pr</td>
<td>A.R.</td>
<td>ATCC15834</td>
<td>Km</td>
<td>PrB121cgh.</td>
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</tr>
<tr>
<td>cv. Libelle</td>
<td>p35S::nptII</td>
<td>B.T.</td>
<td>4 P/B</td>
<td>EC</td>
<td>ND</td>
<td>DNA</td>
<td>-</td>
<td>Kp</td>
<td>Km</td>
<td>pRT99-GUS</td>
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<td>16%</td>
<td>EC</td>
<td>ND</td>
<td>Pr</td>
<td>-</td>
<td>Kp</td>
<td>Km</td>
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<td>pASO::mSODI</td>
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<td>4%</td>
<td>cotyledon</td>
<td>Pr</td>
<td>A.T.</td>
<td>LBA4404</td>
<td>bialaphos</td>
<td>pGPTV-Bar</td>
<td>Lee et al. 2003</td>
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<tr>
<td>pure line 1021</td>
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<td>hypocotyl</td>
<td>ND</td>
<td>Pr</td>
<td>A.T.</td>
<td>EHA105</td>
<td>KmV</td>
<td>pGI121-Hm</td>
</tr>
<tr>
<td>cv. Jinyan no. 7</td>
<td>p35S::l-gas</td>
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<td>23%</td>
<td>cotyledon</td>
<td>hypocotyl</td>
<td>ND</td>
<td>Pr</td>
<td>A.T.</td>
<td>Mannose</td>
<td>He et al. 2006</td>
</tr>
<tr>
<td>Hybrid Bambina</td>
<td>p35S::nptII</td>
<td>A.T.</td>
<td>-</td>
<td>leaf</td>
<td>ND</td>
<td>Pr</td>
<td>A.T.</td>
<td>LBA4404</td>
<td>Km</td>
<td>pAL4404</td>
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<td>pure line 1021</td>
<td>p35S::CMV-O cp</td>
<td>A.T.</td>
<td>-</td>
<td>hypocotyl</td>
<td>CMV&lt;sup&gt;α&lt;/sup&gt;</td>
<td>RNA</td>
<td>A.T.</td>
<td>EHA101</td>
<td>Htg</td>
<td>pGI121-HmCP</td>
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<td>cv. Shimoshirazu</td>
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<td>-</td>
<td>petiole</td>
<td>gray mold</td>
<td>Pr</td>
<td>——</td>
<td>Km</td>
<td>PrB121-RCC2</td>
<td>Kishimoto et al. 2004</td>
</tr>
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<td>p35S::nptII</td>
<td>A.T.</td>
<td>-</td>
<td>cotyledon</td>
<td>gray mold</td>
<td>Pr</td>
<td>A.T.</td>
<td>LBA4404</td>
<td>Km</td>
<td>PrB121-RCC2</td>
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<td>4%</td>
<td>chilling</td>
<td>Pr</td>
<td>RNA</td>
<td>A.T.</td>
<td>EHA105</td>
<td>Km</td>
<td>PrB121-1</td>
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<td>-</td>
<td>chilling</td>
<td>Pr</td>
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<td>A.T.</td>
<td>EHA105</td>
<td>Km</td>
<td>PrB121-2</td>
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<td>B.T.</td>
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<td>cotyledon</td>
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<td>Pr</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>PrB221</td>
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<td>9%</td>
<td>petiole</td>
<td>leaf</td>
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<td>A.T.</td>
<td>LBA4404</td>
<td>Km</td>
<td>pCGN783</td>
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<td>672,3676 (Gy-14)</td>
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<td>-</td>
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<td>A.T.</td>
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<td>pBI21-1</td>
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<td>pSVBV::54-kDa</td>
<td>A.T.</td>
<td>-</td>
<td>cotyledon</td>
<td>CFMMV</td>
<td>Pr</td>
<td>A. T.</td>
<td>EHA105</td>
<td>Km</td>
<td>pCAMSV54</td>
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<tr>
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<td>pnos::nptII</td>
<td>A.T.</td>
<td>-</td>
<td>leaves</td>
<td>sweet</td>
<td>Pr</td>
<td>A.T.</td>
<td>LBA4404</td>
<td>Km</td>
<td>pUR528</td>
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<td>tasting</td>
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<tr>
<td>cv. Poinsett 76</td>
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<td>A.T.</td>
<td>DNA</td>
<td>EHA105</td>
<td>PPT</td>
<td>pGA492GL</td>
</tr>
</tbody>
</table>

Abbreviations: A.R., Agrobacterium rhizogenes-mediated transformation; A.T., Agrobacterium rhizogenes-mediated transformation; bar, bialaphos-resistant gene; hyg, hygromycin phosphotransferase gene; B.T., biolistic transformation; CMV, Cucumber mosaic virus; CFMMV, Cucumber fruit mosaic mosaic tobamovirus; CFMV<sup>α</sup>, Cucumber mosaic virus; CMV<sup>α</sup>, CMV resistance; CMV-0 cp, CMV-0 coat protein gene; Dhn 10, Solanum lycopersicum dehydrin (10 kDa) gene; Dhn 25, Solanum lycopersicum dehydrin (25 kDa) gene; Ec, embryogenic callus; gus, β-D-glucuronidase (GUS) gene; Hyg, hygromycin; Km, kanamycin; Luc, firefly luciferase gene; MSSODI, cytosolic CuZnSOD cDNA from cassava; ND, not determined; nptII, neomycin phosphotransferase II gene; p35S, Cauliflower mosaic virus 35S promoter; PB, particle bombardment; pnos, nopaline synthase promoter; PPT, phosphinothricin; pPR-2d, tobacco β-1,3-glucanase promoter; Pr, protein level; RCC2, a rice chitinase cDNA; RNA, RNA level; rolA, rolB, rolC, ORF13, genes involved in hairy root induction from plasmid pBI1724; A. rhizogenes stain MAF 03-01724; TE, transformation efficiency confirmed by PCR and Southern hybridization; TRP, transgene related phenotype; uidA, β-D-glucuronidase (GUS) gene.
leaves or petioles. Transformation had first been achieved through either Agrobacterium-mediated or direct gene transfer. Root-inducing (Ri) plasmids were used earlier than tumor-inducing (Ti) plasmids, but were less effective. Microprojectile bombardment and pollen-tube pathway were also appropriate means for introducing transgenes into cucumber. In addition to selector, reporter and marker genes, various types of transgenes with agronomic potential have been introduced into cucumbers, such as fung-resistant genes, antifreezing genes and genes that result in improvement of quality. These were transferred into cucumbers, being expressed and stably inherited in progenies, as summarized in Table 1.

In this review we highlight the achievements to date in cucumber genetic transformation. Stable regeneration systems and methods of transgene integration are also discussed. We also attempt to evaluate the prospects and problems inherent to cucumber genetic transformation.

CUCUMBER GENETIC TRANSFORMATION SYSTEMS

Explants used for gene transformation

An efficient and stable plant regeneration system is the most important requirement for plant transformation. The hormone combination and concentration are the main factors effecting plant regeneration while the source (leaf microexplants, leaf petiole, cotyledon and hypocotyls) and size of explants can also influence regeneration. Incubation with bacteria can extend the period in which mature plants are obtained. Regeneration systems can be divided into two classes based on different pathways.

Explants for cucumber organogenesis

Adventitious buds, which can grow into plantlets, can be obtained when cotyledon or hypocotyl explants are stimulated by exogenous cytokinins such as 6-BA and IBA. Organogenesis is a good method to directly regenerate large number of buds without the differentiation from somatic embryos and takes less time. Thus, it is the most efficient method in cucumber genetic transformation (Burza et al. 1995). Cucumber regeneration through organogenesis has been extensively reported throughout the world. Gambley et al. (1990) obtained adventitious buds, 50 per callus at most, from cotyledon segments of cucumber seedlings. Colijn-Hooymans et al. (1994b) discovered that regeneration frequency from 3- to 5-days-old cotyledons was highest (up to 100%) but that over 7 days there was an obvious decrease (only about 0.9 shoots per explant or less). They also reported polyploidization or increase in the number of chromosomes in seedlings. Selvaraj et al. (2007b) claimed that it takes about 120 to 140 days to get regenerated plantlets when subculturing once every 20 days. Callus dedifferentiated from hypocotyls could also be stimulated by 8.88 μM 6-BA to redifferentiate into buds at frequency of 80.3% over a period of four months (Selvaraj et al. 2006a).

Cucumber protoplasts

Protoplasts are cells without a cell wall and can be induced to form regenerated plantlets under appropriate culture conditions. They appear to be better suited for genetic transformation since they can easily take up and integrate exogenous genes less than 50 kb can be transferred into the cucumber genome by a Ti plasmid in a single copy. The Ri plasmid causes “hairy roots” in plant sections (0.05-0.01 /g541M) of 2,4-D/6-BA or NAA/6-BA. Colijn-Hooymans et al. (1988a) discovered that only in the case of leaf protoplasts were morphologically normal plants obtained but with only 20% diploid (normal ploidy level) and tetraploid or even octaploid were detected. However, there was no evidence that this could influence plant regeneration negatively.

METHODS OF TRANSGENE INTRODUCTION

Agrobacterium-mediated transformation

The genus Agrobacterium is a kind of plant pathogen that has been divided into five species based on disease symptomology and plant host range (Otten et al. 1996; Gelvin et al. 2003). Thus far, only two of them, A. tumefaciens and A. rhizogenes, have been used for plant transformation. T-DNA, a specific DNA fragment from the Ti plasmid of A. tumefaciens, could integrate into the plant genome regulated by the Vir gene and express this gene (Xu 1990). Foreign genes less than 50 kb can be transferred into the cucumber genome by a Ti plasmid in a single copy.

The first report of successful cucumber (cv. ‘Poinsett 76’) genetic transformation mediated by A. tumefaciens was accomplished by Chee et al. (1990a). The neomycin phosphotransferase II (nptII) gene, in binary vector plasmid pGA482, was confirmed to have been integrated into the cucumber genome by kanamycin selection (100 mg/l) and Southern blot analysis, and positive R1 generations. This demonstrated that foreign genes mediated by A. tumefaciens can be transfer into cucumber and inherited stably. The nopaline synthase gene (nos) under the Escherichia coli trp promoter was transformed into cucumber in the same year (Gafni 1990). The nptII and phosphinothricin resistance (bar) genes harbored in strain EHA105 were simultaneously transferred into cv. ‘Greenlong’ cotyledons successfully and five different plants (i.e. independent lines) (1.1%) were demonstrated to be transgenic following Southern hybridization (Vengadesan et al. 2005).

The Ri plasmid causes “hairy roots” in plant sections incubated with A. rhizogenes and shoots can be regenerated from transgenically positive roots. However, it was so laborious to regenerate plants from hairy roots that this limited their use in cucumber transformation (Tang et al. 2001). Trulson et al. (1991) obtained the first transgenic cucumbers in this way. The nptII gene in vector pARC8 of A. rhizogenes induced hairy roots on hypocotyl sections. The transgenic plants were positive for the nptII gene. The Icl and Ms genes promoters (Ismael et al. 1997) in transgenic hairy roots were simply transcribed and isocitrate lyase (ICL) and malate synthase (MS) were regulated at the transcription level in both transgenic and control cucumbers. The expression of Icl and Ms increased in hairy roots of whole plants when was controlled in the presence and absence of 25 mM sucrose, respectively. Transgenic plants can potentially be used as a system for the large-scale production of recombinant proteins for industrial and pharmaceutical uses (Austin et al. 1994; Horn et al. 2004). The coding sequence for cardenolide 16-O-glucohydrolase was inserted into the cucumber genome by plant expression vector pBH121 Ngh of A. rhizogenes strain 15834 (Shi et al. 2006). Glycolytic activity of transgenic cucumber roots was detected by HPLC using lanatoside glycosides as substrate. It was a valuable model for biotransformation of natural compounds by recombinant enzymes.

Microprojectile bombardment

Microprojectile or particle bombardment is a direct gene transformation method not often used in cucumber gene
transfer since multiple copies are inserted into the cucumber genome. Transgenic cucumber (cv. ‘Poinsett 76’) harboring the nos-nptII (Chee et al. 1992b) gene and the nptII and uidA genes (Schulze et al. 1995) were obtained by bombarding embryogenic callus suspension cultures. Nos-nptII gene (Chee et al. 1992b) transformation frequency was about 16% analyzed by Southern blot but only 25% of these transcripts expressed the gene. The authors concluded that multiple copies of the inserted gene were inserted into the cucumber genome, as demonstrated by PCR and Southern blot hybridization analyses, was the main reason transgene silencing. Twenty-eight vigorously developed and rooted cucumbers (cv. ‘Libelle’), regenerated 9 months after bombardment from kanamycin-resistant callus, were obtained and 67% of them showed β-glucuronidase (GUS) activity (Schulze et al. 1995). Multicopy integration and rearrangement of the transgenes was also confirmed by Southern blot analysis with uidA and nptII probes. The uidA gene in the transgenics still had a high and stable activity about one year after bombardment providing a new route for non-selectable genetic transformation. Hairy roots from cotyledons were obtained after transformation of rolA, B and C genes which are located on the T-DNA of the Ri plasmid A4 (Kodama et al. 1993). The stable integration of rol genes into the cucumber (cv. ‘Shimoshirazugai’i’) genome was confirmed by Southern blot analysis.

Pollen tube pathway method

There has only been one report of cucumber transformation using the pollen tube pathway. Li et al. (2000) reported a genetic transformation system of cucumber (cv. ‘Jingyan No. 4’) with high efficiency by injecting exogenous DNA with concentration of 100 mg/kg into the ovary 12 hours after pollination, and transgenic plants, derived from seeds, were obtained. Using this approach authors could obtain the highest transformation frequency but the lowest fruit setting ratio and number of seeds per fruit. Three positive plants detected by GUS analysis and Southern blots hybridization were obtained and showed nptII and uidA gene integration.

Selective marker genes in transgenic cucumbers

The most and earliest chimeric gene studied in cucumber transformation was the nptII gene for kanamycin resistance when used as a selectable marker. The nptII gene inserted into A. tumefaciens binary vector plasmid pGA482 (Chee et al. 1990a) and into vector pARC8 of A. rhizogenes (Trulson et al. 1986) was transferred in cucumber (cv. ‘Straight Eight’) cotyledons and hypocotyls, respectively. Transformed R0 and R1 cucumber plants, proved by hybridization with a 2.0 kb (BanHI and HindIII restriction) nptII gene-containing fragment, appeared normal and were tested positive for NPTII enzyme activity (Chee et al. 1990a). Even DNA fragment from Ri plasmid was detected in transcripts (Trulson et al. 1986). Following the success of these two cases, the nptII gene was selected as the preferred method in the field of cucumber transgenic transformation. The uidA gene in pTR99-GUS (Schulze et al. 1995), bar in strain EHA105 (Vengadesan et al. 2005), gus and bph in pG121-Hm (Nishibayashi et al. 1996b) co-fused with the nptII gene were studied under different genetic backgrounds (Table 1).

Gus is another important and promising reporter gene for cucumber genetic transformation. The gus gene fused to the CaMV 35S promoter harbored in pG121-Hm of EHA105 strain of A. tumefaciens transferred the uidA gene to pure line 1021 of C. sativus cultivar (pure line 1021) and was highly expressed in the young leaves of 12 regenerated plantlets (Nishibayashi et al. 1996a). Eight GUS-positive R0 plants examined by Southern blot analysis had either a single or a few positive bands which showed multiple copies of the inserted transgene. R1 progeny, which inherited the gus gene from R0, also showed stable GUS activity in the cells of all tissues. The expression and segregation ratio for the CMV-O coat-protein gene (cp gene), which provides resistance to Cucumber mosaic virus (CMV), were obtained directly from GUS assays and PCR analysis (Nishibayashi et al. 1996a). GUS activity was low in the presence of 25 mM sucrose and high in transgenic roots when sucrose was absent, demonstrating that sugar supply could control the expression of Icl and Ms genes when fused to a GUS reporter gene in cucumber roots of whole plants (Ismanizan et al. 1997).

Five other reporter or selector genes including pmi (encoding phosphomannose-isomerase, He et al. 2006), hph (hygromycin phosphotransferase gene, Nishibayashi et al. 1996b), bar (phosphinotricin resistance genes conferring resistance to PPT (Vengadesan et al. 2005), uidA (ß-glucuronidase gene fused with 35S promoter in pRT99GUS transmitted via particle gun, Schulze et al. 1995), luc (the firefly luciferase gene in pAL4404 and pAQ2, Sapountza-kus et al. 1996) were introduced into different lines. These genes were controlled under a constitutive or tissue-specific promoter. Selection using PPT or hygromycin B rather than kanamycin or the presence of 50 μM acetosyringone was found to enhance transformation frequency as evidenced by transient expression of GUS or hygromycin phosphotransferase (Nishibayashi et al. 1996b; Vengadesan et al. 2005) (Table 1).

PRACTICAL EVALUATIONS

Disease resistance

Agricultural yield tends to be reduced constantly by various plant diseases which are generally caused by phytopathogenic fungi, bacteria and viruses. Thus, breeding for disease resistance has become one of the most crucial objectives in cucumber cultivation. Four kinds of chitinase genes including the rice class I chitinase gene exhibited resistance to Phytophthora rot (Kishimoto et al. 2003a), an acidic chitinase gene from petunia and basic chitinase genes from tobacco and bean respectively (Raharjo et al. 1996), a class III chitinase gene against Botrytis cinerea (Kishimoto et al. 2004b) and a rice chitinase cDNA for resistance against gray mold (Tabei et al. 1998) were isolated from different plants and transformed into several cultivated varieties of cucumbers using molecular biology. The transgenic cucumbers showed varying levels of disease resistance and high expression and intracellular localization of rice chitinase, which might be involved in enhancing the resistance of transgenic plants to gray mold (Kishimoto et al. 2002c). However, Fusarium oxysporum f. sp. cucumerinum located in petiole vessels could avoid antifungal substance and resulted in Fusarium wilt in transgenic cucumber (Kishimoto et al. 2003a). The highly resistant transgenic cucumber strains were inheritable with a segregation ratio of 3:1 (resistant: susceptible) and can serve as good breeding materials for disease resistance (Tabei et al. 1998).

The CMV irus coat protein gene, CMV-O cp (Nishibayashi et al. 1996b) and the putative 54-kDa replicase gene of CFMMV (Gal-On et al. 2005) were transformed into cucumber genome with the aim of producing cucumber plants resistant to CMV and CFMMV resistance. The RNA transcripts from the CaMV 35S-cp gene were detected in the leaves of R0 transgenic cucumber plants and in the epicotyls containing two cotyledons of transgenic progeny plants by Northern blot analysis but protein signals were absent following Western blot analysis. Nishibayashi et al. (1996a) also discovered that cucumber agronomic characteristics of CMV-resistant plants were transferred to the anti-CMV cucumbers, and the combination of cucumber genotypes and the CMV-O cp gene. Line 144 (Gal-On et al. 2005), homozygous for the putative 54-kDa replicase gene, was immune to CFMMV infection and a substantial delay of disease symptoms, CGMMV, ZGMMV and KGMMV, was obtained at 14, 14 and 20 days post inoculation respectively, compared with the control cucumbers at 2, 8 and 8 days post inoculation (Table 1).
Chilling tolerance

The pGT::Dhn10 (Yin et al. 2004b) and pGT::Dhn24 (Yin et al. 2006c) gene fusions, which encode dehydrin proteins and have a potential role in chilling tolerance, were transmitted into the cucumber genome. mRNA of the Dhn10 gene was detected in the leaves, cotyledons, hypocotyls and roots by RT-PCR, but no Dhn10 protein by alkaline phosphatase-conjugated antibody. The presence of Dhn10 gene was regulated cold tolerance in transgenic lines at the transcriptional level. SK3-type DHN4 dehydrin encoded by the Solanum solanum Dhn1 gene was detected in leaves, cotyledons, stems and roots of transgenic seedlings by RT-PCR and Western blot analysis, with particularly high expression in roots. Different expression levels of pGT::Dhn24 gene among transgenic lines had no correlation with the degree of chilling tolerance. The chilling tolerance of cold-sensitive species such as cucumber can be improved through genetic technology by transmitting pGT::Dhn10 and pGT::Dhn24 gene fusions. The Arabidopsis thaliana CBF3 gene (CRT/DRE binding factor), a kind of antifreeze gene, fused with CaMV 35S promoter to construct plasmid pBINP-35S-CBF3 was successfully induced into cucumber and confirmed by PCR analysis (Deng et al. 2004). However, the chilling tolerance of the transgenic line was not reported.

Production of new traits

Superoxide dismutase (SOD) has an anti-aging function in cellular defense against oxidative stress in aerobic organisms and has been added to many cosmetics products. The CuZnSOD cDNA (mSOD1) from cassava fused with the ascorbate oxidase promoter in plasmid ASOp::mSODI/pGPTV-Bar was introduced into cucumber cv. ‘Winter Long’ (Lee et al. 2003). The mSOD1 gene was integrated into the nuclear genomes of three cucumber plants confirmed by Southern blot, and was highly expressed in transgenic cucumber fruits three times more than the control, but levels were low in leaves since the ASO promoter was expressed more dominantly in cucumber fruits than in other tissues. The elevated SOD activity in transgenic fruits was a consequence of the introduced mSOD1 chimeric gene and two additional induced CuZnSOD genes.

Thaumatins, produced by the fruits of a West African perennial plant Thaumatococcus daniellii, are a unique class of the sweet-tasting plant proteins and are approximately 100,000 times sweeter than sucrose on a molar basis (van der Wel et al. 1980). Transgenic C. sativus cv. ‘Borczczagowski’ plants containing thaumatin II cDNA driven by the CaMV 35S promoter in the binary plasmid pRUR528 were produced (Szwacka et al. 2002). Thaumatins might be controlled at the levels of both transcription and translation demonstrated by the lack of correlation between protein and mRNA levels. The positive correlation between thaumatin accumulation levels and sweet taste intensity was noticed in transgenic fruits with a sweet phenotype. It was also reported that transgenic protein levels of thaumatin which belonged to the pathogenesis-related (PR) protein family had no relationship with the increased tolerance for pathogenic fungus Pseudoperonospora cubensis.

Transgenic cucumbers can also play an important role in the development of potential systems for large-scale production of recombinant proteins for industrial and pharmaceutical uses. The cghl gene, encoding the Digitalis lanata EHRH cardenolide 16’-O-glucohydrolase, which catalyzes the deglucosylation of lanatosides, inserted downstream of the 35S promoter in plant expression vector pBII121cghl, was transformed in cucumber plants using A. rhizogenes 15834 (Shi et al. 2006). The CGH I activity in cucumber hairy roots was detected by HPLC.

Inheritance of transgenese

The stable transmission of important agronomic traits expressed by a transgene in cucumber plants from R0 to progeny was documented following both Mendelian and non-Mendelian transmission. The CMV-O cp gene was induced into cucumber (pure line 1021) plants by Ti-Aerobacterium-mediated transformation and the segregation ratio for the presence and absence of the cp gene was 1:1 by PCR and GUS analysis (Nishibayashi et al. 1996a). Transgenic cucumber line CR32 with the rice chitinase cDNA (RCC2) driven by Dhn10 gene promoter was transformed by PCR, Southern analysis and ELISA analysis showed segregation of disease resistance following the predicted Mendelian ratio of 3:1 (resistant: susceptible), as tested by χ2 analysis (χ2 = 0.078, P < 0.01) (Tabei et al. 1998). A phenotypic segregation of kanamycin resistance in transgenic cucumber with the construct PR-2huidA-nosnplII and thaumatinl-nosnplII was demonstrated and 78% of the hemizygous progenies exhibited a segregation ratio consistent with Mendelian inheritance while 25% were non-Mendelian (Yin et al. 2004a). He et al. (2006) also discovered that the pmi gene had a 3:1 segregation ratio in six transgenic lines. A 3:1 Mendelian ratio (kanamycin-resistance: kanamycin-sensitivity) was documented in T1 progeny (Szwacka et al. 2002; Gal-On et al. 2005).

FUTURE PERSPECTIVES

Six kinds of agronomically important genes including cghl (the Digitalis lanata EHRH cardenolide 16’-O-glucohydrolase), mSODI (superoxide dismutase, Lee et al. 2003), CMV-O cp (CMV resistance), RCC2 (the rice chitinase cDNA; Tabei et al. 1998), Dhn (chilling tolerance; Yin et al. 2004a, 2006c), and the Dhn24 54-kDa replicase gene (CMV and CFMMV resistance; Nishibayashi et al. 1996a) were thus far successfully transmitted to cucumber. The following expression and inherence of these genes were reported in cucumber transformation. There were still several serious problems for biotechnological transformation.

Marker or selectable genes induced with the target gene in transformation will be problematical because in the European Union the registration of genetically modified organisms (GMO) containing the antibiotic or herbicide resistance genes will be banned from 2008 (Kuiper et al. 2001; http://www.gmo-compass.org/eng/regulation/regulatory_process/). Two strategies had been reported for transgenic plants regeneration without the marker or selectable genes. One way was taking the pmi gene (encoding for phosphomannose-isomerase that converts mannose-6-phosphate to fructose-6-phosphate) as selectable method (He et al. 2006). Only the regeneration plants with the pmi gene could take the mannose as a carbon source while the control can not. This gene was capable to replace the npt II gene with selectable function. Another approach was excise or segregate marker gene from the genome of transgenic plants. Hohn et al. (2001) and David et al. (2001) proved this method of removing marker genes by the site-specific expression of transgenic sequence.

Some problems containing significant low frequency of transformation, unable to generate directly from explants, some DNA sequence of plasmid harboring in host genome, chromosome doubling in progenies, multiple gene copy in host plant, were hampered the cucumber gene transformation. Proper transgenic methods as well as vectors with highly harboring and expression capability should be chosen for different cucumber lines.

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