Development of transgenic cucumber expressing TPSP gene and morphological alterations

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Abstract To develop transgenic cucumber tolerant to abiotic stress, a cotyledonary-node explants were co-cultivated with Agrobacterium tumefaciens (EHA101) carrying TPSP gene (pHC30-TPSP). After transfer to fresh medium every two week for eight weeks, putative transgenic plants were selected when shoots grown a length greater than 3 cm from the cotyledonary-node explants on selection medium supplemented with 5 mg l⁻¹ phosphinotricin as selectable agent. The confirmation of transgenic cucumber was based on the Northern blot analysis. Thirty four shoots (5.2%) with resistance to phosphinotricin were obtained from 660 explants inoculated. Of them, transformants were only confirmed from 11 plants (1.7%). Transgenic cucumber expressing TPSP gene was more synthesized at 3.8 times amounts of trehalose (0.014 mg g fresh wt⁻¹) than non-transformants (0.0037 mg g fresh wt⁻¹). However, all of transgenic plants showed abnormal morphology, including stunted growth (< height 15 cm), shrunk leaves, and sterility as compared with non-transgenic plants (> height 150 cm) under the same growth environment. These results lead us to speculate that the over-production of trehalose was toxic for cucumber, even though that had known for rice as non-toxic.

Keywords Agrobacterium, stunted growth, transgenic cucumber, trehalose

Introduction

Cucumber is one of the major vegetable crops in Asia and Europe. Recent studies in plant molecular breeding have been progressed by the introduction of specific genes into cells and tissues, regenerated to plant. Using Agrobacterium-mediated transformation technology, cultivars with superior agronomic characters such as virus resistance (Gaba et al. 2004) could be developed. Although the cucumber was known as a recalcitrant plant species, a stable transformation system has been improved without chimerical transgenic event (Kim et al. 2008). Abiotic factors such as low temperature, drought, and high salinity are common stress conditions that adversely affect the plant growth and yield. The development of the abiotic stress-tolerance could be bred into genotypes with increasing yield. Trehalose is a non-reducing disaccharide composed of two glucose that are found in bacteria, algae, fungi, yeast, and some plants (Elbein 1974). This component works as a protective molecule under a variety of abiotic stress conditions because of their high hydrophilicity and chemical inertness in many organisms (Eleutherio et al. 1993). Thus, trehalose allows plants to tolerate naturally occurring stress during the period of dehydration and rehydration (Drennan et al. 1993).

Transgenic tobacco plants expressing TPS or otsA gene were shown the enhancing of dehydration tolerance (Romero et al. 1997), and trehalose-producing tobacco plants showed enhanced tolerance to drought and salinity stresses (Jun et al. 2005). Similar results were also reported in transgenic rice plants increases trehalose accumulation and abiotic stresses tolerance (Jang et al. 2003; Garg et al. 2002). These plants also showed a specific physiological and morphological alterations, including increased water potential and stunted growth (Pilon-Smits et al. 1998). Recently, Jang et al. (2003) reported that transgenic rice plants produced by introduction of a gene encoding a bifunctional fusion (TPSP) of the trehalose-6-phosphate (T-6-P) synthase (TPS) and T-6-P
phosphatase (TPP) of Escherichia coli increased trehalose accumulation and tolerance to drought, salt, and cold without stunting growth, and that the trehalose levels in leaf and seed extracts of the transgenic rice plants were specially 200-fold higher than that of transgenic tobacco plants with TPS or TPP gene (Jang et al. 2003). Up to now, there have been no reports on the production of abiotic stress resistance transgenic cucumber and alterations of their morphological characteristics.

This paper described the development of abiotic stress-tolerant cucumber. We report here for a transgenic cucumber events expressing TPSP gene and the morphological alterations, including stunted growth.

Materials and Methods

Plant materials

Zygotically embryos of F1 hybrid cucumber (Cucumis sativus L., c.v. Eunchim) were used for the explants of transformation experiments. The embryos were dissected out of the mature seeds and the surface was disinfected with 70% ethanol for 1 min and 1% sodium hypochlorite for 15 min, and then rinsed three times with sterile deionized-distilled water. These seeds were germinated in the dark on MS medium (Murashige and Skoog 1962). The pH of all media was adjusted to 5.8 before autoclaving. Twenty-five ml of medium was dispensed into 90 × 15-mm plastic Petri dishes. Explants of 2 to 3 cm-long cotyledonary node were prepared from 7-10 day old seedlings by making a horizontal slice through the hypocotyls region, approximately 3-5 mm below the cotyledon. A subsequent vertical slice was made between the cotyledons, and the embryonic axis was removed.

Expression vector and preparation of Agrobacterium suspension

The transformation of cucumber was performed with the binary vectors pHC30-TPSP, which contained a bifunctional fusion (TPSP) of the trehalose-6-phosphate (T-6-P) synthase (TPS) and T-6-P phosphatase (TPP) of Escherichia coli, under the control of the maize ubiquitin promoter (Ubi1) and the herbicide resistance gene (bar) as selective marker, respectively (Fig. 1, provided by Dr. J.K. Kim). Disarmed Agrobacterium tumefaciens strains (EHA101) were used as helper strains in a binary vector system. The binary vector was introduced into A. tumefaciens strains EHA101 by the freeze-thaw method (An et al. 1987). The Agrobacteria were grown in YEP medium amended with the appropriate antibi-otics to an OD₆₀₀ = 0.6 to 0.8 at 27°C. The pellets after being centrifuged at 3,500 rpm for 10 min were resuspended to a final OD₆₀₀ = 0.6 to 0.8 in an 1/10 MS basal medium amended with 3.2 mg/l BA, 0.5 mg/l IBA, 200 μM acetosyringone (AS) and 3% sucrose. The medium was buffered with 20 mM MES, pH 5.4. All components including growth regulators, vitamins, components and AS filters were sterilized post autoclaving.

Production of transgenic cucumber

Cotyledonary-node explants were immersed in the Agrobacterium suspensions for 30 min and then incubated on cocultivation media (pH 5.4) supplemented with 20 mM MES, 100 mg/l cysteine, 3.2 mg/l BA, 0.5 mg/l IBA, 200 μM acetosyringone and 3% sucrose. Six explants were cultured per 90 × 15 mm Petri-dish and the explants were positioned with the adaxial side on a filter paper laid over the media. After co-cultivation the explants were washed three times by a sterilized distilled water and then were cultured on shoot induction medium (MS salt, B5 vitamin, 3% sucrose, 3.2 mg/l BA, 0.5 mg/l IBA, 5 mg/l phosphorothricin, 50 mg/l ticarcillin, 50 mg/l cefotaxime, 50 mg/l vancomycin, 3 mM MES, pH 5.6, SI). After 2 weeks of culture, the hypocotyl region was excised from each of the explants, and the remaining explant, cotyledon with differentiating node, was subsequently subcultured onto fresh SI medium. Following an additional 2 weeks of culture on SI medium, the cotyle-dons were removed from the differentiating node. The node explant was subcultured to shoot elongation medium (MS salt, 0.1 mg/l IBA, 0.5 mg/l GA₃, 3 mg/l phosphorothricin, 50 mg/l ticarcillin, 50 mg/l cefotaxime, 50 mg/l vancomycin, 3 mM MES, pH 5.6, SE) solidified with 0.8% agar. Subculture to fresh SE medium was done every two weeks until shoots reached a length greater than 3 cm. Elongated shoots were transferred to root initiation medium (RD) comprised of 1/2 MS salts, 3% sucrose, 3 mM MES, 50 mg/l cefotaxime, 0.8% agar, pH 5.6. The rooted plants were transferred to soil. Plantlets (R₀) were acclimatized and grown to maturity in the

Fig. 1 Plant transformation vector (pHC30-TPSP), which consists of the CaMV35S promoter (CaMV35S) linked to the TPSP coding region, and a gene expression cassette that contains the 35S promoter, the bar-coding region, and the 3′ region of nopaline synthase (nos). The TPSP construct was made by in-frame fusion of the E. coli adaA and aceB genes, which encode TPS and TPP, respectively.