Monitoring of Possible Horizontal Gene Transfer from Transgenic Potatoes to Soil Microorganisms in the Potato Fields and the Emergence of Variants in *Phytophthora infestans*

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To examine the possibility of horizontal gene transfer between transgenic potatoes and microorganisms in potato fields, the gene flow from transgenic potatoes containing the nucleoside diphosphate kinase 2 (NDPK2) gene to microorganisms in soils was investigated. The soil samples collected from the potato fields from March to October 2007 were examined by PCR, Southern hybridization, and AFLP fingerprinting. The NDPK2 gene from soil genomic DNAs was not detected by both PCR and Southern hybridization, indicating that gene transfer did not occur in the potato fields. In addition, no discrepancy was found in pathogenicity and noticeable changes for the appearance of variants of *Phytophthora infestans* in each generation when serial inoculations and the analysis of genomic DNAs by AFLP were conducted. Thus, these data suggest that transgenic potatoes do not give significant impacts on the communities of soil microorganisms and the emergence of variants, although continued research efforts may be necessary to make a decisive conclusion.

Keywords: AFLP, emerging variants, horizontal gene transfer, soil microorganisms, transgenic NDPK2 potatoes

Ever since the first transgenic plants developed using recombinant DNA technology appeared in the 1980s [11], numerous transgenic crops with novel and desired traits, such as herbicide-resistant soybean, canola, and sugar beet, and insect-resistant cotton, have been commercialized [8]. In addition, the successful development of golden rice containing vitamin A in 2000 [20] indicated that the number of transgenic crops with novel traits would rapidly increase worldwide. Despite an anxiety of causing harmful effects on the environment and human health, global areas and adoption rates of transgenic crops are assumed to continue to grow each year in the future [8].

Gene flow in the environment includes two types of gene transfer; horizontal gene transfer (HGT) and vertical gene transfer (VGT). HGT is the movement of genetic material between unrelated organisms, such as between plants and microbes, whereas VGT is the gene flow between closely related organisms, normally through sexual hybridization [13]. HGT is considered a significant cause in the variation of bacteria genomes [15] and may be a common phenomenon for the evolution of bacteria and possibly also eukaryotes. HGT from bacteria to plants and between bacteria has been reported [2, 3], whereas no HGT from transgenic potatoes to *Erwinia chrysanthemi* and *Acinetobacter calcoaceticus* was found in previous work [14, 17]. In addition, HGT from transgenic sugar beet to bacteria in the fields did not occur, although the DNA of transgenic plants persisted up to two years after release [6]. However, other workers showed that the horizontal gene transfer of antibiotic resistance genes from transgenic tobacco plants to bacteria occurred when *Acinetobacter* spp. BD413 with homologous sequences of tobacco genes and *Ralstonia solanacearum* co-infected a transgenic tobacco plant [9]. This result indicates that gene flow from transgenic plants to soil bacteria may occur in the field, although there is no information for the mechanism of HGT from transgenic plants to other organisms.

On the other hand, the release and cultivation of transgenic crops with disease or pest resistance have raised concerns about the emergence of new pathogens or super-disease, because numerous genes used to produce transgenic plants are derived from bacterial genomes, such as antibiotic, herbicide, and insect resistance genes [1, 2]. Moreover, it is
reported that the majority of vectors used to transfer foreign genetic materials to plants carry the 35S promoter gene derived from the cauliflower mosaic virus [7]. In previous reports, some researchers insisted that viruses with novel biological traits could potentially arise in transgenic plants conferring resistance to viruses through recombination and heteroencapsulation [19]. In addition, other workers investigated the change of pathogenicity in an endophytic fungus, 
*Beauveria bassiana*, using transgenic and non-transgenic maize containing the Bt toxin gene in the field and the greenhouse. The results showed that no significant differences were observed in the pathogenicity of 
*B. bassiana* recovered from non-transgenic and transgenic maize [12]. Although the evidence for the disturbances in pathogen community in transgenic plants is not reported as yet, the effects of transgenic plants on phytopathogens may persist in the field for a long time in the case of antibiotic resistance genes and viruses with novel biological traits.

Considering the fast-growing agricultural biotechnology and rapidly increasing global area of transgenic crops every year, the establishment of various techniques for the environmental risk assessment is necessary for the evaluation of transgenic plants. Thus, we have investigated the possibility of horizontal gene transfer from domestically developed transgenic NDPK2 potatoes (*Solanum tuberosum* L. cv. Atlantic) [18], with enhanced tolerance to oxidative stress, to soil microorganisms in potato fields. In addition, the possibility of promoting the emergence of variants during the cultivation of transgenic potatoes was examined by using *P. infestans*, a causative agent of potato late blight.

The non-transgenic (*Solanum tuberosum* L. cv. Atlantic) and transgenic NDPK2 potatoes were cultivated in confined potato fields for seven months. The NDPK2 potatoes containing a stress-inducible sweet potato peroxidase (SWPA2) promoter (Fig. 1) was grown from March 2007 to October 2007 in the potato fields (7 m × 23 m) of the Bioevaluation Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), which is located in Ochang, Chungcheongbuk Province. The transgenic NDPK2 potatoes with SWPA2 promoter was provided from KRIBB.

The roots of potato plants and residues from soil samples, which were collected at intervals of three weeks from the rhizosphere of the potato fields including transgenic NDPK2 and non-transgenic potatoes, were removed by using the sieve No. 14 (aperture 1.4 mm). The extraction and purification of soil genomic DNAs were conducted as described previously [10].

Genomic DNAs from the frozen and grounded fresh young leaves (1 g) of transgenic NDPK2 and non-transgenic potatoes were isolated by using a DNAase Plant Maxi kit (Qiagen, Valencia, CA, U.S.A.). PCR amplifications of the transgenic NDPK2 potatoes were carried out by using specific primers (P01: 5'-ATG TAC GGT CAC GCC AAC CT-3'; and P02: 5'-AAG ACG GAA CTG AGG ACG AAA-3') to the sequences flanking the SWPA2 promoter and NDPK2 gene. The PCR amplification reaction and Southern hybridization were performed as described previously [10], except for using the purified SW-NDPK product labeled with [α-32P]-dCTP as a probe.

Amplified fragment-length polymorphism (AFLP) analysis [4] was conducted by using genomic DNA samples extracted from soil samples of potato fields. Genomic DNAs were treated with EcoR1 and Tru91, and ligations of DNA fragments to adapters were performed with a ligation kit (Promega, Madison, WI, U.S.A.). Primers to amplify the DNA fragments ligated with adapters and PCR amplification reaction were used as described previously [10], except for the other modified primer sets (M13411: 5'-GAT GAG TCC TGA GTA AAG TAAC-3'; and E13411: 5'-GAC TGC GTA CCA ATT CAG TAAC-3'). 5'-End labeling with fluorescein was applied by using the primer M13411. DNA products from the PCR and similarity between transgenic NDPK2 and non-transgenic potatoes were analyzed by using the ABI 3730XL Capillary DNA Sequencer (SolGent, Daejon, Korea) and the NTSYSpc (version 2.1) software program [16], respectively. In addition, the AFLP peaks identified from amplification products were compiled and manually aligned (tolerance limit: ±0.5 bp) to produce large data matrices. The presence or absence of each peak was coded as 1 or 0. Principal components analysis (PCA) [5] was applied using covariance data matrices to reduce their dimensionality. PCA was performed with S-Plus 6 for Windows (Insightful Corp., U.S.A.).

*P. infestans* KACC40407, which was obtained from the Korean Agricultural Culture Collection (National Academy of Agricultural Science, Korea), was grown on V-8 juice agar (200 ml of V-8 juice, 2 g of CaCO3, 15 g of agar, and 11 of DW) plate at 24°C for 1 week in the dark. For spore production, the spores were harvested by washing the plates with double sterile water. Spore suspension was diluted to reach 1×107 spores/ml to use as inoculum. Transgenic and non-transgenic potatoes were vegetatively grown from seed tubers in vinyl pots (4.5 cm in diameter) in the greenhouse at 20°C for 6–7 weeks. For potato late blight assay, a spore suspension containing 0.5% (v/v) Tween 20 was inoculated on the surface of the leaflets at the sixth-

![Fig. 1. Schematic diagram of the constructed pSN-K vector and designed PCR primer to detect the inserted DNA of transgenic potato plant.](image-url)
leaf stage and incubated in the dark at 20°C and 90±5% relative humidity (RH) for 15 days. The pathogenicity of *P. infestans* was scored as the size of lesions occurred on the surface of the leaflets in transgenic and non-transgenic potatoes. The re-isolation of *P. infestans* from the leaflets of inoculated potatoes was performed on V-8 juice agar plates after washing the leaflets with the 10% sodium hypochloride, and the recovered *P. infestans* was used as inoculum of the next generation.

The AFLP analysis of genomic DNAs extracted from *P. infestans* isolated in each inoculation event for the seven generations was conducted as described above, except for primers used (MOO: 5'-GAT GAG TCC TGA GTAA-3'; and E00: 5'-GAC TGC GTA CCA ATT C-3').

The possibility of horizontal gene transfer released into the environment from transgenic NDPK2 potatoes to soil microorganisms was investigated by using genomic DNAs extracted from the soils collected at intervals of three weeks from both transgenic and non-transgenic potato fields cultivated for a 7-month period. PCR products of soil genomic DNAs were amplified using primers of the flanking regions specific to the SWPA2 promoter and NDPK2 gene (Fig. 1) and subjected to Southern blot analysis. Genomic DNAs amplified from the SW-NDPK fusion primer as positive control showed positive bands at the expected position by PCR and Southern hybridization. However, no homologies of genomic DNAs were detected by Southern blotting analysis, suggesting that gene flow from transgenic potatoes to soil microorganisms did not occur for the 7-month period in confined potato fields (Fig. 2). Specifically, any positive signals for the SW-NDPK probe of transgenic potatoes were not detected in the soils of potato fields collected for a 3-month period after harvest. These results indicate that the possibility of the horizontal gene flow from transgenic potatoes to soil microorganisms is very low in the field condition. However, considering the
reports of some workers on the persistence of plant DNAs in soils under field conditions up to 2 years [6], an extended observation for gene transfer is required in the future.

To examine any influence on the microbial community in transgenic potato fields before and after harvest, AFLP was conducted by using the soil genomic DNAs extracted from soil samples collected at intervals of 3 weeks from three different locations of transgenic and non-transgenic potato fields. The AFLP patterns of genomic DNAs obtained from the soil samples of transgenic and non-transgenic potato fields were investigated by dividing into three groups according to the growth stages, namely before seedling (March), during cultivation (May), and after harvest (July), after which the obtained AFLP profiles were calculated by PCA. As shown in Fig. 3, PCA explained 36.4% of the total variability with two principal component axes. Although the PCA plot did not show a significant difference between transgenic and non-transgenic potatoes, the microbial communities of soils derived from each growth stage showed quite different patterns depending on sampling time. Specifically, the microbial communities of soils before seedling were grouped against those of soils collected during cultivation and after harvest on the first principle component axis (PC1). These results suggest that the changes of microbial communities in the potato fields seem to be associated with the seasonal change for transgenic and non-transgenic potatoes.

On the other hand, to investigate the possibility that transgenic potatoes play an important role in promoting emerging variants, \textit{P. infestans} was inoculated to the leaflets of transgenic and non-transgenic potatoes. The fungus was re-isolated from the leaflets of inoculated potatoes and its pathogenicity was scored at every passage of inoculation during the seven generations. No difference in pathogenicity was found in fungi isolated from either transgenic or non-transgenic potatoes (Fig. 4A). Moreover, genomic DNAs extracted from the fungi isolated from each inoculation event grouped into two clusters showed over 78% similarities as compared with the control strain (\textit{P. infestans} KACC40707), although minor variations were detected among different generations in AFLP analysis (Fig. 4B). In addition, some fungi isolated from different generations exhibited more similarities than that of the same generation isolated from transgenic and non-transgenic potatoes. However, no significant changes for the appearance of variants of \textit{P. infestans} isolated from the leaflets of
transgenic and non-transgenic potatoes were found. These results indicate that transgenic potatoes as compared with non-transgenic potatoes accelerate neither the genomic variation nor the pathogenicity of the fungus, although continued research efforts through long-term observation should be performed to make a decisive conclusion.

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