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Transgenic sweetpotato plants expressing spike protein of porcine epidemic diarrhea virus

Kyoung-Sil Yang[1], Suk-Yoon Kwon[1], Sun-Hyung Kim[1], Jeong-Ju Kim[2], Joon-Seol Lee[2], Sang-Soo Kwak[1], Haeng-Soon Lee[1*]

[1]Environmental Biotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-806, Korea

[2]Mokpo Experiment Station, National Institute of Crop Science, Rural Development Administration, Muan, Jeonnam 534-833, Korea

Porcine epidemic diarrhea virus (PEDV) causes enteritis in swine of all ages, and is fatal in neonatal piglets. The spike protein of PEDV is a primary target antigen for developing an effective vaccine against coronaviruses, since it mediates essential biological functions. Sweetpotato [*Ipomoea batatas* (L.) Lam.] is one of the most important crops to secure a staple food supply in 21st century and is an attractive plant producing plant-based vaccine. To develop transgenic sweetpotato plants expressing antigen against PEDV, we constructed the transformation vectors using partial fragment of PEDV spike protein (SP1) under the control of a CaMV 35S promoter or sporamin promoter with high expression in the storage roots of sweetpotato (referred to as 35S::PEDV-SP1 and Spo::PEDV-SP1, respectively). Transgenic sweetpotato plants were successfully developed by *Agrobacterium*-mediated transformation. Kanamycin-resistant embryogenic calli were selected on MS medium containing 400 mg/L claforan and 100 mg/L kanamycin. Embryogenic calli transferred to hormone-free MS medium with kanamycin gave rise to somatic embryos and then converted into plantlets in the same medium. The putative transgenic plants were selected by PCR with nptII or SP1-specific primer. Southern blot analysis of PCR-positive regenerants confirmed that the SP1 gene was inserted into genome of sweetpotato plants. Northern blot analysis reveled that SP1 gene of PEDV was highly expressed in transgenic sweetpotato leaves. Transgenic plants are growing in the field of Mokpo Experiment Station, NICS, RDA for mass propagation. The further characterization of transgenic sweetpotato plants and activities of the plant-derived antigen are under study.

* corresponding author: Tel. 042-860-4439, e-mail: hslee@kribb.re.kr

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Enhanced tolerance to oxidative stress in transgenic potato plants expressing CuZnSOD, APX and NDPK2

Myoung Duck Kim, Suk-Yoon Kwon, Haeng-Soon Lee, Sang-Soo Kwak*

Environmental Biotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Yusong, Daejeon 305-806, Korea

Oxidative stress is one of the major factors causing injury to plants exposed to environmental stress. To develop transgenic plants with enhanced tolerance to multiple environmental stresses, we are trying to manipulate the antioxidative mechanism under the control of an oxidative stress-inducible *SWPA2* promoter. In a previous study, we developed SSA potato plants expressing genes of both superoxide dismutase (CuZnSOD) and ascorbate peroxidase (APX) in chloroplasts (referred to as SSA plants) or nucleoside diphosphate kinase 2 (NDPK2) in cytosols (SN plants) under the control of *SWPA2* promoter. Both SSA and SN plants

showed a strong tolerance to methyl viologen (MV)-mediated oxidative stresses and high temperature. In this study, NDPK2 gene was further introduced into SSA plants (SSAN plants) under the control of *SWPA2* promoter by an *Agrobacterium tumefaciens*-mediated transformation to expect the sysnergetic effect of SSA plants and SN plants. SSAN potato plants were generated from leaf explants on MS medium containing 500 mg/L cefotaxime and 100 mg/L kanamycin. The integration of foreign genes was confirmed by PCR and Southern blot analysis. When leaf discs of SSAN plants were subjected to 3 μM MV, they showed approximately 20%, 30% and 50% less membrane damage than SSA, SN, or non-transgenic plants, respectively. Further characterization of SSAN plants is under study in terms of multiple stresses including oxidative stress, high temperature and salt at the level of whole plant. We anticipate that SSAN potato plants would be useful for commercial cultivation in unfavorable growth conditions.

***corresponding author: Tel. 042-860-4432, e-mail: sskwak@kribb.re.kr**

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Enhanced Salt Tolerance by Overexpression of a *Arabidopsis* Na^+/H^+ Antiporter in Tobacco

So-Hyeon Baek*, Chun-Sun Seo, Heh-Ran Choi[1], Woon-Chul Shin, Hyeon-Jung Kang, Hyun-Soon Kim[2]

Honam Agricultural Research Institute, NICS, RDA
[1]Gochang Agricultural Development & Technology Center,
[2]Research & Development Bureau, RDA

Salinity stress is one of the most serious factors limiting the productivity of agricultural crops throughout the world. One of the best possibilities the plants could survive salt stress is that they compartmentalize sodium ions away from the cytosol. Na^+/H^+ antiporters provide an efficient mechanism to prevent the toxic effects of sodium ion in the cytosol and play a major role to maintain osmotic balance by using sodium accumulated in the vacuole. In order to study the role of the Na^+/H^+ antiporter (NHX) in plants, we isolated a NHX cDNA from *Arabidopsis*. The full-length cDNA of the NHX gene consists of 1,633 nucleotides and 583 amino acid residues. The AtNHX shares from 99% identity of deduced amino acid sequence with *Arabidopsis* AtNHX1. A recombinant binary vector containing *Arabidopsis* NHX gene was introduced into tobacco plants by *Agrobacterium*-mediated gene transfer. Presence of the transgene in T_0 plants was confirmed by PCR and Southern analyses. Southern analysis showed that the AtNHX gene was stably integrated into the genome of the transgenic tobacco plants. The seeds of wild-type plants and To transgenic plants overexpressing AtNHX were tested on MS medium supplemented with 200 mM NaCl, after 15 days the former showed a low germination and the inhibition of growth but the latter displayed an unaffected germination and a normal growth. Furthermore, the transgenic seeds were germinated and grown on MS medium containing 250 mM NaCl. These results demonstrate the possibility of engineering salt tolerance in crops.

* corresponding author: Tel. 063-840-2168, e-mail: shbaek@rda.go.kr