P 67

Recovery of BASTA ®- Resistant *Sedum erythrostichum* via *Agrobacterium*-mediated Transformation

YOON, Eui-Su[1] · JEONG, Jae-Hun[2] · CHOI, Yong Eui [2*]

[1]*Department of Biology, Kongju National University, Kongju, 314-701, Korea*

[2]*Korea Ginseng Institute, Chung-Ang University, Ansung-shi, 456-756, Kyunggi-do, Korea*

Objectives

Sedums are used as ground cover, rock gardens and flower borders. In this study, the genetic transformation of *Sedum erythrostichum* through *Agrobacterium*-mediated co-cultivation was studied by introducing a herbicide-resistant gene (PAT) and the reporter gene (GUS). This study marks the first time genetic transformation of succulent *Sedum*.

Materials and Methods

1. Plant materials: *Sedum erythrostichum* Miq.

2. Hormonal condition for shoot induction: MS + 0.5 mg/l NAA + 2.0 mg/l kinetin + 3% sucrose

3. *Agrobacterium* co-cultivation: We obtained multiful shoots from the leaf explants in the selection medium: MS basal salt, 25 mg/l kanamycin, 300 mg/l cefotaxime, 0.5 mg/l NAA and 2.0 mg/l Kinetin.

4. Analysis of transgenic plantlets: Histochemical GUS assay, PCR analysis, Southern blot and RNA gel blot analysis.

5. Plantlets transferred to soil and sprayed with herbicide: Plantlets with both shoots and roots were transferred to the soil. And then Bialaphos (Basta ®) at 200 mg/l PPT was sprayed five times on the transgenic and non-transgenic plants within a week. After three weeks, survival of plantlets was investigated.

Results and Discussion

1. Hormonal condition for adventitious bud induction: Fifty six percent of explants produced adventitious buds on medium with 0.5 mg/l NAA and 2.0 mg/l BA. About 7.1 ± 1.7 buds were produced in each explant.

2. Genetic transformation: To select transformed buds, leaf explants were transferred to the medium with 300 mg/l cefotaxim and 25 mg/l kanamycin for three weeks and transferred to the hormone-free selection medium and sub-cultured three times to the same medium. Twenty four (3.75%) of the total 640 infected leaf explants produced kanamycin-resistant adventitious shoots.

3. Analysis of transgenic plantlets: Sixteen explants among selected 24 explants that survived on selection medium were proven to be GUS-positive. PAT, NPT II and GUS genes were detected in transgenic plantlets using PCR. Southern hybridization analysis was carried out on the GUS positive transgenic plantlets. All these plantlets showed a single hybridization band with PAT probe. Expression of the PAT gene was confirmed using RNA blot analysis in the transgenic sample, but not in the non-transformed plants.

4. Soil transfer: Ninety-five percent of the plantlets survived after soil transfer for three months.

5. Herbicide spraying: All GUS positive transgenic *Sedum* plantlets resulted in strong resistance to 200 mg/l PPT of Basta ® spraying.

*Corresponding author. Tel 031-670-4681 E-mail yechoi@cau.ac.kr