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In vitro shoot regeneration from leaves of *Kalanchoe diagremontiana* Hamet et Perr

Mohammed Shafi Ullah Bhuiyan[1], Tae Reyung Kim[2] and Kwan Sam Choi[1]

[1]*Dept. of Applied Biology, Chungnam National university, Daejeon 305-764, Korea*
[2]*Dept. of Genetics, University of Georgia, Athens, Georgia 30602, USA*

Objectives

Kalanchoe diagremontiana Hamet et Perr. is a miracle plant species having the ability to produce next generation plantlets on their leaves. The aim of this study was to establish an efficient and reproducible method for regeneration, which is a prerequisite for genetic transformation

Materials and Methods

1. Plant materials: The fully expanded leaves from *ex vitro* grown *Kalanchoe diagremontiana* were used.

2. Methods: The leaves were surface-sterilized in 1% sodium hypochlorite containing 0.1% Tween 20 (v/v) for 10 min and rinsed several times with sterilized distilled water and then cut into small pieces (about 5 mm in diameter) by cork borer. Leaf explants were placed with the abaxial sidedown on MS medium supplemented with 100 g/l myoinositol, 3% sucrose, 0.8% agar and various combinations of plant growth regulators TDZ or BAP and IAA. The pH of all media was adjusted to 5.7 prior to addition of agar. Plant growth regulators were added after sterilization to media cooled to 55°C. Explants were subcultured at 25 days of cultures. The cultures were incubated at 25±1°C with longday (16/8 h and shortday (8/16 h) light/dark photoperiod regime provided by 30 W cool-white fluorescent lamps at photon fluence rate about 138 mol m-2 s-1. At 50 days of cultures, cluster of shoots transferred to the rooting media (MS medium supplemented with 0.1 mg/l NAA).

Results and Discussion

The first morphogenetic change observed after two week in culture, explants slightly expanded and callus formation began at the edge of the explant. Callus or shoot formation from *kalanchoe diagremontiana* leaf explants depended on not only the combination and concentration of plant growth regulators but also on photoperiod regime. In MS medium supplemented with BAP, the explants were failed to regenerate any shoots, they can only produce callus. TDZ was more effective at promoting regeneration than BAP. The highest shoot regeneration efficiency (about 67%) from all combinations was obtained on MS medium supplemented with 1.0 mg/l TDZ and 0.4 mg/l IAA under 8/16 h light/dark photoperiod. Clusters of shoots were developed from the explants. Excised shoots or clusters of shoots began to root after 10 days of transfer to rooting medium.

Corresponding author: K.S. Choi, TEL: 042-821-5766, E-mail: kschoi@cnu.ac.kr