

# **Somatic Embryogenesis from Vegetative parts and Agrobacterium Mediated Transformation of Zoysiagrass**

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## **Objectives**

We have established the optimized conditions for the efficient regeneration of stem internodes and immature flowers of vegetatively propagated zoysiagrass. We have also produced transgenic plants of zoysiagrass with herbicide resistance gene.

## **Materials and Methods**

### **1. Materials**

Plant - Zoysiagrass cultivar Konhee growing in the green house of Konkuk University.

Seeds - Mature seeds of zoysiagrass common

*Agrobacterium* strains - LBA 4404 (pTOK233), LBA 4404 (pSB-UTPSP)

### **2. Methods :**

Stem internodes and immature flowers were surface-sterilized and cultured onto the callus induction medium containing 1-2 mg/l 2,4-Dichlorophenoxyacetic acid (2,4-D), 0.01-0.1 mg/l 6-benzylaminopurine (BAP), and 0.01 mg/l Abscisic acid (ABA). After embryogenic callus formation, they were transferred to regeneration medium with 1-2.5 mg/l BAP and 0.5 mg/l Naphthalene acetic acid (NAA).

Embryogenic callus was induced from the mature seeds in the MS media supplemented with 2 mg/l 2,4-D, 1.15 g/l proline. Embryogenic callus was infected with *Agrobacterium* strains LBA4404 (pTOK233) and LBA 4404 (pSB-UTPSP).

## Results and Discussion

Vegetatively propagated zoysiagrass cultivar Konhee was regenerated from stem internodes and immature flowers via somatic embryogenesis. This is the first time of somatic embryogenesis from vegetative part in zoysiagrass. Immature flowers formed embryogenic callus in MS media supplemented with 2 mg/l 2,4-D and 0.01 mg/l BAP. The embryogenic callus regenerated on the MS media supplemented with 2.5 mg/l BAP. The regenerated plants formed roots in 1/2MS media.

Stem internodes formed embryogenic callus on MS media containing 2 mg/l 2,4-D, 0.01 mg/l BAP and 0.01 mg/l ABA. Callus was separated from internodes and cultured into the suspension media with above hormone combination. Somatic embryo cluster (SEC) formed on the callus in the suspension media. The SEC was regenerated into shoots in the media containing 1 mg/l 6-BAP, 0.5 mg/l NAA and 0.2 mg/l Gibberellic acid (GA<sub>3</sub>). The shoots rooted on 1/2 MS media.

The optimum conditions for the *Agrobacterium* mediated transformation of zoysiagrass common were standardized. The conditions included were embryogenic callus induction, hygromycin and PPT selection concentration, sonication and vacuum infiltration. 2 mg/l 2,4-D induced 1% embryogenic callus in the MS media. 50 mg/l hygromycin and 3 mg/l PPT were optimized callus selection. Two minutes sonication followed by 20 minutes vacuum infiltration was found to have more transient transformation efficiency. Transformation was confirmed by stable GUS expression in the regenerated shoots selected from the hygromycin and PPT containing media. The PPT resistant plants grew normally with 0.4% basta spray while the control plants became yellow and died eventually.