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Production of herbicide-resistant transgenic maize using *Agrobacterium tumefaciens*-mediated transformation

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Objectives

This project focuses on production of transgenic maize-resistant herbicide.

Materials and Methods

1. Material

Plant - Domestic inbred line of Maize (HW1, KL103, HW3, HW4, KW7)

Agrobacterium strain - C58C1 transformed with pCAMBIA2300) carring with Ubi1-EPSPS gene

2. Methods

Agrobacterium cocultivated immature embryos of 5 domestic inbred lines. Immature zygotic embryos of the domestic inbred lines (HW1, KL103, HW3, HW4, KW7) were infected with *A. tumefaciens* strain C58C1 containing the binary vector (pCAMBIA2300) carring with Ubiquitin promoter (Ubi1)-*EPSPS* as target gene and 35S promoter-*npt II* gene conferring resistance to paromomycin as selective agent and cocultivated according to Cho et al (2005) method.

Results and Discussion

The transformation of model cultivars of maize, through the use of immature embryos by *Agrobacterium*-mediated gene delivery, is well-established (Gordon-Kamm et al. 1999). However, it remains difficult to routinely use any of these target tissues to obtain fertile transgenic plant from elite, commercially important maize inbred lines. we have recently achieved routine transformation of maize (*Zea mays*) using an *Agrobacterium tumefaciens* binary vector system in the hybrid line Hi II maize (Cho et al. 2005), and screened for production of embryogenic callus such as type II in domestic inbred line (Cho et al. 2005). This study was carried out to produce herbicide-resistant transgenic maize from *Agrobacterium* cocultivated immature embryos of 5 domestic inbred lines. Immature zygotic embryos of the domestic inbred lines (HW1, KL103, HW3, HW4, KW7) were infected with *A. tumefaciens* strain C58C1 containing the binary vector (pCAMBIA2300) carring with Ubiquitin promoter (Ubi1)-*EPSPS* as target gene and 35S promoter-*npt II* gene conferring resistance to paromomycin as selective agent and cocultivated according to Cho et al (2005) method. One hundred twenty-eight callus lines transformed (28 clones for HW3, 89 lines for HW4, 11 lines for KW7) showed the resistance in paromomycin antibiotics among immature embryos of HW1 (1,189), KL103 (2,516), HW3 (2,098), HW4 (3,056), KW7 (1,960) cocultivated. The maize transformants were presently obtained from the KL103 inbred line (1 plant), HW3 (15 plants), HW4 (44 plants), KW7 (12 plants), respectively. PCR analysis revealed that the EPSPS gene was integrated into genomic DNA of maize. Further, we will be to develop more transgenic lines of maize per inbred line, and will be analysed with Southern blot and Glyphosate assay forthe transformants.

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