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Pengaruh L-Sistein terhadap Efisiensi Transformasi Genetik Jagung (Zea mays) Menggunakan Agrobacterium

The Effect of L-Cysteine on the Efficiency of Agrobacterium-mediated Transformation of Maize (Zea mays)

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ABSTRACT

An efficient procedure of genetic transformation ultimately can accelerate the process of cultivar development of maize. The objective of this study was to evaluate the effect of L-cysteine added to co-cultivation medium on the efficiency of Agrobacterium-mediated transformation of two genotypes of maize. Explants of immature embryos were isolated from immature ears genotypes Hi-II and Tom Thumb harvested 11-13 days after pollination. Then explants were inoculated with Agrobacterium strain C58C1 carrying pPTN345 vector and cultured in co-cultivation medium for 2 days then on delay medium for 14 days, on selection medium for 4 x 14 days, on regeneration medium, and finally on germination medium. Co-cultivation media contained either 0 or 100 mg/L L-cysteine. Based on assay at 2 days after inoculation,the transient expression of GUS at scutelar side of explants co-cultivated on medium containing 100 mg/L cysteine was higher than that of the control (0 mg/L cysteine). Transient expression of GUS on the explants of Tom Thumb was higher than that of Hi-II. However, transgenic plants in this study were only produced from Hi-II explants co-cultivated in a medium amended with 100 mg/L L-cysteine. No transgenic plants was produced from explants of Tom Thumb due to low efficiency of induction of embriogenic calli. The efficiency of transformation using explants of Hi-II cocultivated in a medium amended with 100 mg/L L-cysteine was 4 independent transformants or transgenic plants out of 70 explants inoculated or 5.7%.

Key words: Agrobacterium tumefaciens, corn, L-cysteine, Hi-II, Tom Thumb

PENDAHULUAN

Perakitan varietas unggul jagung melalui pendekatan biologi molekuler dapat dipermudah jika menggunakan protokol transformasi genetik yang efisien. Transformasi genetik jagung telah berhasil dilakukan mengunakan elektroporasi (D'Halluin et al., 1992), penembak biolistik (Gordon-Kamm et al., 1990; Frame et al., 2000; Sutrisno et al., 2000), dan Agrobacterium tumefaciens (Ishida et al., 1996; Negrotto et al., 2000; Zhao et al., 1998; Frame et al., 2002). Transformasi menggunakan Agrobacterium lebih disenangi daripada menggunakan penembak biolistik karena penggunaan Agrobacterium menghasilkan proporsi transgen terekspresi yang lebih besar dengan jumlah alel atau kopi yang lebih rendah (Ishida et al., 1996; Zhao et al., 1998). Walaupun dengan efisiensi rendah, tanaman jagung transgenik telah berhasil diperoleh melalui transformasi mengunakan Agrobacterium dari eksplan A188 dan Hi-II yang diinokulasi menggunakan strain C58C1 (Dr. Thomas E. Clemente, University of Nebraska-Lincoln, Amerika Serikat, data tidak dipublikasi; Utomo, 2004). Hi-II adalah hibrida dari A188 x B73 (Armstrong et al., 1991). Transformasi di University of Nebraska-Lincoln tersebut menggunakan eksplan embrio zigotik muda yang diinduksi untuk menghasilkan kalus embriogenik Tipe II (Armstrong dan Green, 1985).

Sel-sel kalus jagung yang diinfeksi Agrobacterium sel karena respon hipersensitif atau apoptosis (Hansen, 2000). Respon tersebut dimediasi oleh ledakan oksidatif berupa produksi oksigen reaktif dalam jumlah banyak dalam waktu singkat (Wojtaszek, 1997). Antioksidan dithiothreitol dan polyvinylpolypyrolidone (PVP) dilaporkan mengurangi pengaruh negatif tersebut dan meningkatkan efisiensi transformasi pada anggur (Vitis vinifera) (Perl et al., 1996). Pengaruh yang sama juga dilaporkan untuk antioksidan L-sistein pada kedelai

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