

Phenolic Content in Differentiated Tissue Cultures of Untransformed and *Agrobacterium*-Transformed Roots of Anise (*Pimpinella anisum* L.)

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Abstract

To investigate the role of differentiation of anise tissue cultures on total phenolic and anethole contents, benzylaminopurine- and thidiazuron-induced shoot cultures were generated from roots of the A-8 clonal line and its *Agrobacterium rhizogenes*-induced genetically transformed derivative JB-10. Embryogenic cultures were induced following 2,4-D treatment. Root cultures were multiplied on hormone-free medium. The effect of proline on differentiation and phenolic synthesis was also investigated. GC/MS studies indicate that anethole was not produced in root or other differentiated cultures. The predominant phenolic metabolite, however, was an anethole precursor, epoxypseudoisoeugenol-2-methylbutyrate (EPB). Total phenolics and EPB contents were highest in root cultures, which also correlated with higher proline content. Embryo and shoot cultures had reduced phenolic level and EPB and proline contents. Antioxidant activity in all differentiating cultures was high on day 60 compared to that on day 30, and there was no significant difference between differentiating tissues. This indicated that antioxidant protection might be linked not only to phenolics but to other nonphenolic metabolites as well.

Keywords: Differentiated tissue cultures; untransformed roots; *Agrobacterium*-transformed roots; *Pimpinella anisum*; phenolics; free proline; epoxypseudoisoeugenol-2-methylbutyrate (EPB)