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Article

Induction and Marker Selection of Embryogenic-like Callus from the Hypocotyl of *Pinus thunbergii*

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Abstract

To induce embryogenic callus from *Pinus thunbergii* vegetative organs, and deeply understand the internal mechanism of the process, an orthogonal experimental design of three factors and four levels and proteome analysis were adopted. The results showed that the optimal medium was douglas-fir cotyledon medium (DCR), containing 2.5 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 1.0 mg L^{-1} 6-benzylaminopurine (6-BA), 0.3 g L^{-1} polyvinylpyrrolidone (PVP), 0.5 g L^{-1} acid casein hydrolysate (CH), 0.5 g L^{-1} L-glutamine, 1.0 g L^{-1} inositol, 6.0 g L^{-1} agar and 20.0 g L^{-1} sucrose. Microscopic examinations revealed a distinct embryogenic-like callus (EC) structure, and these ECs finally achieved redifferentiation. Analysis of the interactions between factors detected that although the 6-BA concentration alone was not significant, it became a significant effect factor when interacted with 2,4-D ($p < 0.05$). Peroxidase (POD), superoxide dismutase (SOD) and soluble sugar (SS) of EC were significantly higher than those of non-embryogenic callus (NEC), and label-free quantitative proteomics analysis showed that different types of PODs including peroxidase 4, phospholipid hydroperoxide glutathione peroxidase, and cationic peroxidase 1 in EC were significantly up-regulated, and they were involved in antioxidant biological processes, located in the intercellular region, and performed molecular functions such as heme binding, so POD was a suitable and stable physiological marker for EC. Four up-regulated proteins in EC included glutathione S-transferase, chalcone flavanone isomerase, phosphoenolpyruvate carboxykinase and endoglucanase. Five EC-specific proteins included indole-3-acetic acid-amido synthetase GH3.1, indole-3-acetate O-methyltransferase 1-like, cytokinin dehydrogenase, MLP-like protein 423 and 2-methoxy-6-polyprenyl-1,4-benzoquinol methylase. These proteins are also potential EC molecular markers. Among these proteins, glutathione S-transferase is beneficial to prevent cell death in EC, while indole-3-acetate O-methyltransferase 1-like and cytokinin dehydrogenase are beneficial to promote EC redifferentiation.

Keywords: *Pinus thunbergii*; hypocotyl; embryogenic-like callus; peroxidase; proteomics



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1. Introduction

Pinus thunbergii is a famous species used for the greening of coastal areas as well as afforestation of barren mountains and landscaping in China. However, the emergence