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Molecular cloning of a monodehydroascorbate reductase gene from poplar and its expression in response to stress

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Objectives

This study was performed to elucidate the precise functions of monodehydroascorbate reductase in poplar.

Materials and Methods

1. Plant Material- *Populus alba* x *P. tremula* var. *glandulosa*

2. Methods: Poplar suspension cells were subcultured by transferring 0.4 g (fresh weight) of cells to 100 ml liquid MS medium containing 1 mg l^{-1} 2,4-D, 0.1 mg l^{-1} NAA and 0.01 mg l^{-1} BAP. The suspensions were maintained at 100 rpm on a gyratory shaker in the culture room at 221 C under 20 mol m^2s^{-1} with cool white fluorescent light. Four-day-old cell suspensions were supplemented with $CdCl_2$, $AlCl_2$, $CuSO_4$, $ZnSO_4$ or $Pb(NO_3)_2$. For wounding treatment, leaf discs prepared from the leaves of poplar plants were floated on Petri plates containing MS medium. Total RNA was isolated using TRI Reagent.

Results and Discussion

We cloned a cytosolic monodehydroascorbate reductase gene (*Pomdhar1*) from poplar (*Populus alba* x *P. tremula* var. *glandulosa*) suspension cells and tested its expression pattern in response to various stresses. Poplar MDHAR (*Pomdhar1*) cDNA encodes a polypeptide of 434 amino acids possessing domains characteristics of FAD- and NAD(P)H-binding proteins. The predicted amino acid sequence of the open reading frame shows a high level of identity to the cytosolic MDHAR of tomato and Chinese cabbage, and does not possess N-terminal leader sequence suggesting that it encodes a cytosolic form of MDHAR. DNA blot analysis indicated that a single nuclear gene encodes this enzyme. *Pomdhar1* is expressed strongly in suspension cells and flowers, and weakly in leaves, stems and roots. The gene is highly expressed after subculturing but the expression was diminished abruptly since the beginning of logarithmic phase. The transcription of *Pomdhar1* is significantly up-regulated by wounding but not affected by heavy metals including copper, iron and zinc.

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