Expression of Recombinant *Alcohol Dehydrogenase* in *Escherichia coli* Strain BL21 (DE3) and *In Planta Agrobacterium* Transformation of Tomato Seeds

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Abstract

Alcohol dehydrogenase is an enzyme that is involved in various roles in plant such as in plant development, growth and plant responses to abiotic and biotic stresses. A recombinant *alcohol dehydrogenase 1* (*Adh1*) cDNA (r-*msAdh1*) from *Metroxylon sagu* has been previously isolated, containing 20 nucleotides derived from *Elaeis guineensis* at the 5'-end, with a molecular weight of 1.14 kb. The objective of this study is to determine the function of r-m*sAdh1* via analyses in prokaryotic and eukaryotic hosts. For expression in prokaryotic system, pET-41a(+) with a 8x His tag at the C terminal was used for r-msAdh1 protein purification and expression was achieved using IPTG for four to six hours in *Escherichia coli* strain BL21 (DE3) incubated at low temperature. The induced BL21 strain produced a small amount of soluble r-msAdh1 protein while large amount was present as insoluble aggregates. Subsequently, the r-*msAdh1* cDNA was transformed into tomato seeds (*Solanum lycopersicum* cv. MT1) via *Agrobacterium*-mediated *in planta* transformation. The integration of r-*msAdh1* cDNA and the selectable marker were detected in transformed seedlings, T_0, using polymerase chain reaction technique. The transformation efficiency was determined to be 33% for r-*msAdh1* cDNA and 46% for the selectable marker. For stability analysis of the transgene, eleven T_1 generation randomly selected from the transgenic T_0 were analyzed for the presence of the cDNA, and all seedlings were found to contain the full length of r-*msAdh1* cDNA. However, out of eleven T_1 transgenic lines produced, only four seedlings were used for expression analysis using the reverse transcriptase PCR (RT-PCR). Two transgenic lines, $T_1$9 and $T_1$11, were determined to contain r-*msAdh1* cDNA and verified by nucleotide sequencing. Although only a small number of T_1 transgenic seedlings was obtained, this study shows that tomato seeds could be used as a target tissue for *Agrobacterium*-mediated *in planta* transformation primarily because the protocol is easy, rapid and cheaper compared to tissue culture-based method.

Keywords: *Alcohol dehydrogenase*, *Metroxylon sagu*, BL21 (DE3), *Agrobacterium tumefaciens*, *tomato seeds*
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