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Molecular evidence of sorbitol dehydrogenase in tomato, a non-Rosaceae plant

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Abstract

The enzyme NAD-dependent sorbitol dehydrogenase (SDH) is well characterized in the Rosaceae family of fruit trees, which synthesizes sorbitol as a translocatable photosynthate. Expressed sequence tags of *SDH*-like sequences have also been generated from various non-Rosaceae species that do not synthesize sorbitol as a primary photosynthetic product, but the physiological roles of the encoded proteins in non-Rosaceae plants are unknown. Therefore, we isolated an *SDH*-like cDNA (SDL) from tomato (*Lycopersicon esculentum* Mill.). Genomic Southern blot analysis suggested that *SDL* exists in the tomato genome as a single-copy gene. Northern blot analysis showed that SDL is ubiquitously expressed in tomato plants.

Recombinant SDL protein was produced and purified for enzymatic characterization. SDL catalyzed the interconversion of sorbitol and fructose with NAD (H). SDL showed highest activity for sorbitol among the several substrates tested. SDL showed no activity with $NADP^+$. Thus, SDL was identified as a SDH, although the K_m values and substrate specificity of SDL were significantly different from those of SDH purified from the Japanese pear (*Pyrus pyrifolia*), a Rosaceae fruit tree. In addition, tomato was transformed with antisense SDL to evaluate the contribution of SDL to SDH activity in tomato. The transformation decreased SDH activity to approximately 50% on average. Taken together, these results provide molecular evidence of SDH in tomato, and SDL was renamed LeSDH.

Keywords: *Lycopersicon esculentum* Mill.; Solanaceae; Tomato; cDNA cloning; Recombinant protein expression; Antisense transformation; Sorbitol dehydrogenase; Carbohydrate; Sorbitol; Fructose

1. Introduction

Sorbitol is important in translocating photosynthate in fruit trees of the Rosaceae family (Kanayama et al., 1992; Kanayama, 1998; Sakanishi et al., 1998). NAD-dependent sorbitol dehydrogenase (SDH) catalyzes the oxidation of sorbitol to fructose. SDH has been purified from Japanese pear fruit (Oura et al., 2000). The expression analysis of *SDH* cloned from apple cDNA demonstrated the importance of SDH in the metabolism of sorbitol that is translocated to fruit (Yamada et al., 1998, 1999; Park et al., 2002).

SDH has also been found in plants that are not in the Rosaceae and that synthesize sucrose for translocation of photosynthate. SDH activity was detected in a crude extract from germinating soybean seeds (Kuo et al., 1990), and SDH was partially purified from developing maize endosperm (Doehlert, 1987) and from the shoot axes of *Viscum album*, a parasitic plant (Wanek and Richter, 1993).

Recently, plant genome mapping projects have revealed that SDH-like sequences are widespread in the plant kingdom, and are present in the expressed sequence tag (EST) databases of several plant species (Fig. 1). Nevertheless,

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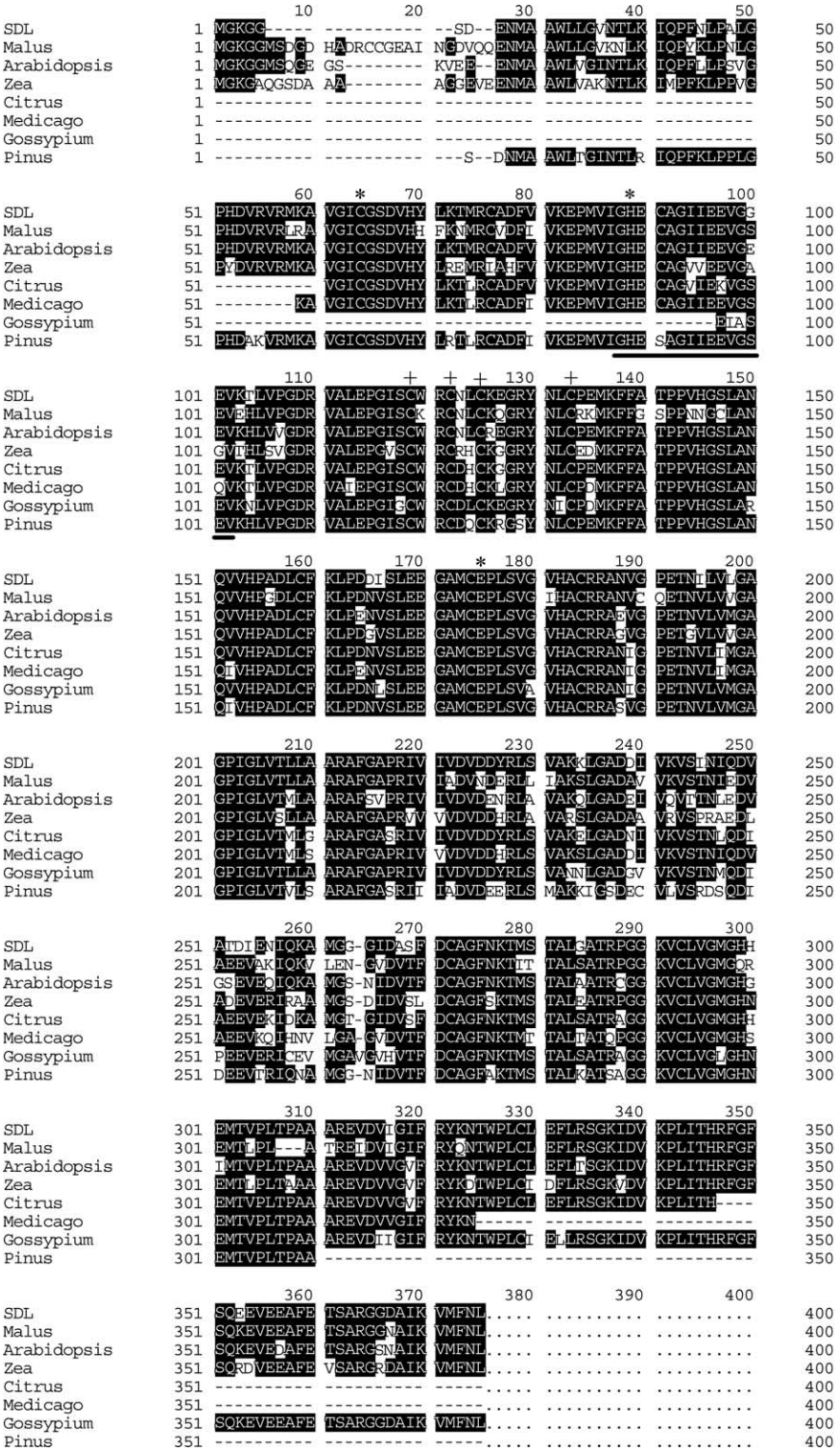

Fig. 1. Multiple alignment of the deduced tomato SDL amino acid sequence, the apple SDH sequence, and the SDH-like sequences in EST from species of different plant families. Black shading indicates identical amino acids. Asterisks and plus signs indicate conserved amino acid residues in the catalytic zinc binding site and in the structural zinc binding site, respectively. The zinc-containing alcohol dehydrogenase signature is underlined. SDL (accession number AB183015); *Malus domestica*, apple SDH (AB016256); *Arabidopsis thaliana* (AF370161); *Zea mays* (BT016754); *Citrus paradisi* × *Poncirus trifoliata* (CX668813); *Medicago sativa* (CB894631); *Gossypium raimondii* (CO082515); *Pinus taeda* (CO361351).

little information exists about the physiological roles of the proteins encoded by these genes. The ultimate goal of this research was to understand the significance of the wide-spread *SDH*-like genes. As a first step, we now provide molecular evidence of *SDH* genes in non-Rosaceae plants. This study is a molecular and biochemical characterization