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Gene expression and promoter analysis of a novel tomato aldo-keto reductase in response to environmental stresses

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ABSTRACT

The functional role of an uncharacterized tomato (*Solanum lycopersicum*) aldo-keto reductase 4B, denoted as SlAKR4B, was investigated. The gene expression of tomato SlAKR4B was detected at a high level in the senescent leaves and the ripening fruits of tomato. Although D-galacturonic acid reductase activities tended to be higher in tomato SlAKR4B-overexpressing transgenic tobacco BY-2 cell lines than those in control cell lines, SlAKR4B gene expression was not well correlated with L-ascorbic acid content among the cell lines. The analysis of the transgenic cell lines showed that tomato SlAKR4B has enzyme activities toward D-galacturonic acid as well as glyceraldehyde and glyoxal, suggesting that the SlAKR4B gene encodes a functional enzyme in tomato. Gene expression of SlAKR4B was induced by NaCl, H_2O_2, and plant hormones such as salicylic acid and jasmonic acid, suggesting that SlAKR4B is involved in the stress response. The transient expression assay using protoplasts showed the promoter activity of the SlAKR4B gene was as high as that of the cauliflower mosaic virus 35S promoter. Also, the promoter region of the SlAKR4B gene was suggested to contain *cis*-element(s) for abiotic stress-inducible expression.

1. Introduction

Aldo-keto reductases (AKRs; EC 1.1.1.21) comprise a diverse family of enzymes that catalyze the reduction of carbonyl compounds to the corresponding alcohols or the reverse oxidation. AKRs are widely present in bacteria, animals, and plants (Jez et al., 1997). The functional importance of AKRs in animals has been well characterized and extensively reviewed (Jez et al., 1997; Sengupta et al., 2015). In higher plants, the AKR family plays roles in diverse metabolic reactions and is classified into three groups, namely, AKR2, AKR4, and AKR6 according to their primary structures (Sengupta et al., 2015). The cDNA encoding AKR2 has been cloned from apple seedlings (Kanayama et al., 1992), and the overexpression of apple AKR2 in a transgenic tobacco plant increased the sorbitol content, suggesting that apple AKR2 plays an important role in sorbitol biosynthesis in apple (Tao et al., 1995). Genome analysis in *Arabidopsis* suggested that its genome contains at least 21 AKR homologs (Simpson et al., 2009). Some AKR4 isozymes are highly expressed in plants subjected to drought, salt, and cold stress (Simpson et al., 2009; Saito et al., 2013). An AKR6 isozyme from *Arabidopsis* could function as a potassium channel protein involved in ion transport across the membrane (Tang et al., 1995). In recent years, the AKR isozyme (Genebank accession number AF039182) from strawberry (*Fragaria* × *ananassa*), classified as AKR4B, has been reported to be involved in ascorbic acid (AsA) biosynthesis (Agius et al., 2003; Sengupta et al., 2015). The overexpression of the strawberry AKR4B, denoted as AKR2 in the previously conducted report, resulted in an increase of AsA content in *Arabidopsis* (Agius et al., 2003), potato (Hemavathi et al., 2009) and tomato (Cai et al., 2015). AsA is a highly abundant metabolite that scavenges reactive oxygen species generated during physiological processes (Conklin, 2001) and plays an important role in stress responses as well as in growth and development. In higher plants, AsA seems to be predominantly produced from D-fructose-6-phosphate through L-galactono-1,4-lactone (Wheeler et al., 1998). We also reported that AsA is biosynthesized through the pathway involving GDP-D-mannose (Tabata et al., 2002; Badejo et al., 2009; Kondo et al., 2015). However, some alternative AsA biosynthesis pathways have been proposed in higher plants (Loewus and Kelly, 1961; Agius et al., 2003; Lorence et al., 2004; Endres and Tenhaken, 2009). The galacturonate pathway, producing AsA from methylgalacturonic acid, has been proposed in plants (Loewus and Kelly, 1961; Agius et al., 2003). D-galacturonic acid reductase (GalUAR) catalyzes the reduction of D-galacturonic acid in the galacturonate pathway. Strawberry AKR4B is believed to function as GalUAR (Agius et al.,

Abbreviations: AKR, aldo-keto reductase; AsA, ascorbic acid; GalUAR, D-galacturonic acid reductase; GST, glutathione S-transferase; nt, nucleotide.

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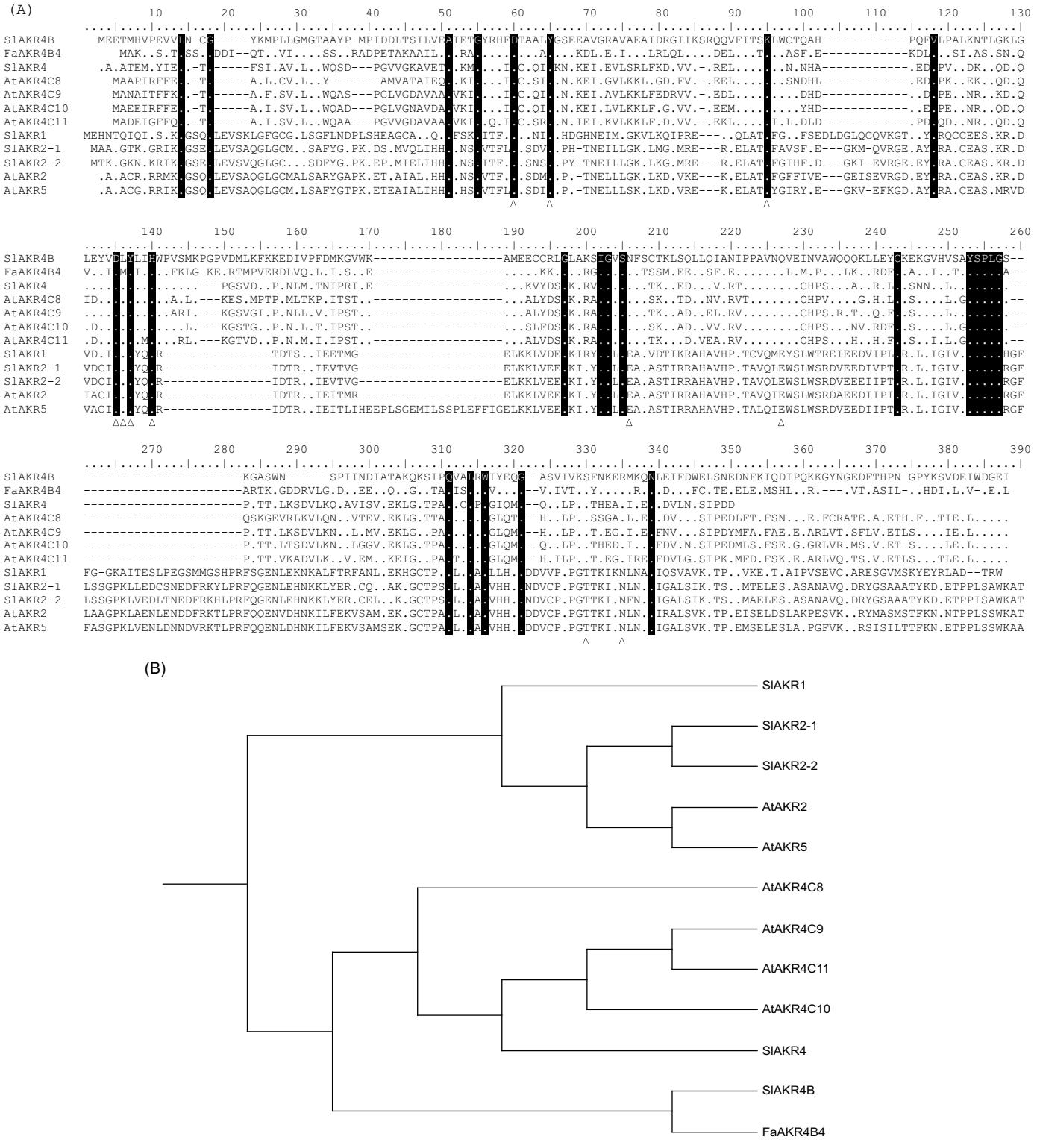

Fig. 1. Alignment and phylogenetic tree were showed the relationship of SlAKR4B to other members of the plant AKR family.
(A) Alignment of amino acid sequences of tomato SlAKR4B (Genebank accession number XM_004235326) with plant AKRs including tomato SlAKR1 (SGN unigene accession number SGN-U323295), -2-1 (SGN unigene accession number SGN-U571240), -2-2 (SGN unigene accession number SGN-U571241), and -4 (SGN unigene accession number SGN-U584148), strawberry FaAKR4B4 (Genebank accession number AF039182) and *Arabidopsis* AtAKR2 (Genebank accession number NP_176267), -4C8 (Genebank accession number ABH07514), -4C9 (Genebank accession number ABH07515), -4C10 (Genebank accession number ABH07516) -4C11 (Genebank accession number ABH07517) and -5 (Genebank accession number NP_00118527). Dots indicate the conserved amino acid residues identical to those of SlAKR4B. Amino acid residues conserved in every plant AKR are surrounded by black boxes. White triangles indicate the active sites and cofactor binding sites of plant AKRs. (B) Phylogenetic tree of analyzed plant AKRs. Multiple sequence alignment with CLUSTAL W (Thompson et al., 1994) was used to generate input files for phylogenetic tree view program, Treeview (Page, 1996). Full names and accession numbers for the sequences are described above.