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Cloning and characterization of 1-Hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate reductase (IDS) in *Rehmannia glutinosa* L.

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Objectives

Rehmannia glutinosa, belongs to the family Scrophulariaceae, is an important medicinal plant. Although there are seven species in the genus Rehmannia , only three are used for medicinal purposes: R. glutinosa L. in Korea, R. Glutinosa var. Purpurea Makino in Japan, and R. Glutinosa var. Hueichingensis. IDS was important enzymes of the iridoid biosynthesis in *Rehmannia glutinosa*. In this study, we have isolation of full-length IDS using RACE PCR with degenerated primers and check for expression pattern by stress.

Materials and Methods

○ Isolation of IDS(1-Hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase)
Total RNA was extracted from plant leaf and used for synthesis of first-strand cDNA using GeneRacer Kit (Invitrogen). And RACE-PCR was performed for cloning.

○ Growth of *Agrobacterium rhizogenes* and Establishment of hairy root cultures
A. rhizogenes strain R1000 cultures were grown to mid-log phase at 28C on a gyratory shaker in liquid Luria-Bertani (LB) medium. Excised leaves of Rehmannia glutinosa from in vitro grown seedlings were used as the material for transformation.

○ Treated with MJ & wounding
The hairy roots were cultured in 100ml volumetric flask containing 30ml 1/2MS medium and subcultured every 4weeks. And then Treated with 100uM MeJA at 0, 3, 6, 12, 24, 48, 72 hours. Wounding of hairy root was repeatedly cutting with a sharp scalpel. The root were collected at 0, 3, 6, 12, 24, 48 hours by vacuum filtration and stored at- 80. Eeach treatment were consisted of three flask and the experiment was repeated three times.

○ Real-time Quantitative RT-PCR
Total RNA was extracted from roots and hairy roots of *Rehmannia glutinosa* using RNase plant mini kit (Qiagen, USA) and was reverse-transcribed according to the manufacturer's protocol (Superscript II First strand synthesis kit, Invitrogen, USA) using an oligo(dT)20 primer. The resulting cDNA mixtures were used as templates for RT-PCR using the Qiagen Quantitect SYBR Green PCR system as performed.

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Results

○ Isolation IDS sequencing

IDS was cloned 1392bp ORF nucleotide sequences encoding 464 amino acid and it shows 84% identity to *P. kurrooa*, 80% *A. annua*, 77% *A. thaliana*, 74% *G. biloba* and 73% *P. taeda*.

Each genes isoelectric point (PI) and amino acid Mw (molecular weight) was calculated by the software Tool at http://www.expasy.org Proteins were predicted PI and MW IDS was 5.50 and 50.917 kDa.

○ Elicitor treatment

To analysis the effect of elicitors of defense responses on the expression of IDS, we treated 100uM MeJA in hairy root. Real time PCR was performed to study the expression of the genes in response to MeJA. When treated with 100 uM MeJA, the expression of gene increased gradually at 24 h. Wounding also two fold increased by control at 24h. It was demonstrated that the expression of IDS could be induced by MeJA and wounding.

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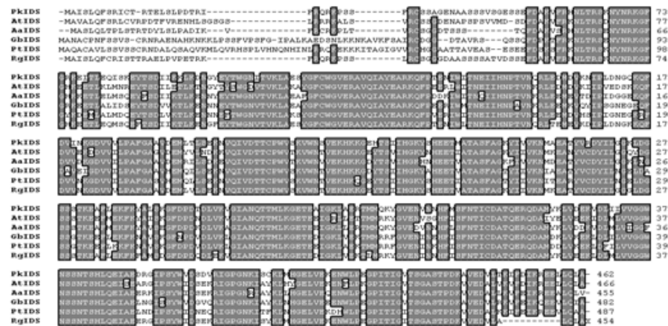

Fig 1. *R. glutinosa* amino acid sequencing Alignment with *P. kurrooa* (EF199770), *A. thaliana* (NM119600), *A. annua* (EU332141), *G. biloba* (DQ251632), *P. taeda* (EF095155)

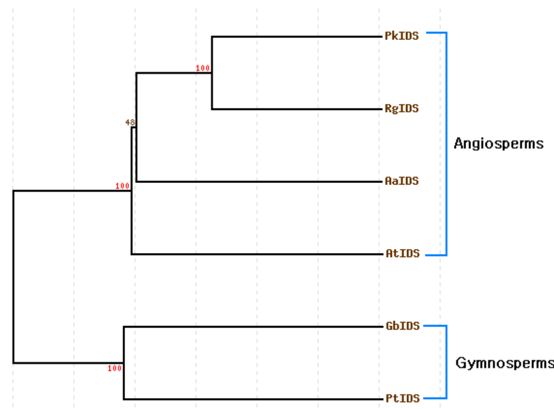

Fig 2. Phylogenetic tree analysis with *P. kurrooa* (EF199770), *A. thaliana* (NM119600), *A. annua* (EU332141), *G. biloba* (DQ251632), *P. taeda* (EF095155)