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Distinct expression patterns of two *Ginkgo biloba* 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate reductase/isopentenyl diphospahte synthase (HDR/IDS) promoters in Arabidopsis model

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ABSTRACT

1-Hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate reductase (HDR) or isopentenyl diphosphate synthase (IDS) is an enzyme at the final step of the MEP pathway. The multi-copy nature of IDS gene in a gymnosperm *Ginkgo biloba* is known. To evaluate the function of each isogene, the roles of the promoters were examined in Arabidopsis model. Among the promoters of *GbIDS* series, about 1.3 kb of *GbIDS1pro* and 1.5 kb of *GbIDS2pro* were cloned and fused with *GUS*. The *GbIDS1pro*::*GUS* was introduced into Arabidopsis to show GUS expression in most organs except for roots, petals, and stamina, whereas the *GbIDS2pro*::*GUS* was expressed only in the young leaves, internodes where the flower and shoot branched, and notably in primary root junction. This pattern of GUS expression correlated with high transcript level of *GbIDS2* compared to that of *GbIDS1* in Ginkgo roots. Methyl jasmonate (MeJA) treatment resulted in down-regulated *GbIDS1pro* activity in Arabidopsis leaves and upregulated *GbIDS2pro* activity in roots. The same pattern of gene regulation in roots was also seen upon treatments of gibberellins, abscisic acid, and indole butyric acid.

1. Introduction

Ginkgo biloba L., a 'living fossil' that survived from Jurassic era, is a perennial deciduous and diecious tree with broad range of uses. It contains valuable compounds such as ginkgolides and flavonoids with pronounced pharmacological and economic importance [1,2]. Ginkgolides, highly modified diterpene lactones, possess high anti-platelet antagonist factor activity [3] and insecticidal activity [4]. Although studies on the ginkgolide biosynthesis have been performed, exact site of the ginkgolides biosynthesis has been under debate [5–8]. However, recent studies give weight to the biosynthesis of ginkgolides in the roots and transportation to aerial parts [9,10].

Terpenoids are the most structurally diverse group of plant natural products. Although they have complicated structure and functions, terpenoids are biosynthetically polymers of simple five-carbon isoprene units, isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). The plants have two isoprenoid pathways, differing how the five-carbon units are generated: classical mevalonic acid (MVA) pathway and recently elucidated 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway [11]. Both pathways differ in not only site of operation and raw materials but also post-pathway end products; in general, MVA pathway operating in cytosol supplies IPP, which is subsequently converted into DMAPP by isopentenyl diphosphate isomerase (IDI) for sesqui- and triterpenes, whereas plastidic MEP pathway provides IPP and DMAPP for mono-, di-, and triterpenes [12].

The seven enzymes operating in the MEP pathway in *G biloba* were all characterized [10,13–18]. The last enzyme in the MEP pathway, 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate reductase/isopentenyl diphosphate synthase (HDR/IDS) produces IPP and DMAPP concomitantly [12] and was reported to have multi-copy gene in gymnosperms such as *G. biloba*, *Pinus taeda* [10], *Pinus densiflora* [19], and *Cycas revoluta* [10]. Kim et al. classified gymnosperm HDR/IDSs into two clades [10]: *IDS1* and *IDS2*. *Oryza sativa* also harbors two copies of *IDS* [20]. As expected from their multi-copy nature, *IDS* genes in gymnosperms exhibited tissue-specific transcription pattern and showed different responses upon MeJA treatment [10,19].

However, physiological role and regulatory mechanism of each *IDS* isogene are yet to be elucidated. Ginkgo harbors 3 copies of *IDS* gene, designated as *GbIDS1*, *GbIDS2* and *GbIDS2-1*. GbIDS1 and

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GbIDS2 were suggested to function in primary and secondary metabolisms, respectively, based on occurrence of isogene transcript among particular Ginkgo organs [10]. To provide decisive evidence of organ-specificity and to suggest physiological role of each isogene, we here report isolation of 1.3 kb of *GbIDS1* and 1.5 kb of *GbIDS2* from 5′-upstream regions and the promoter-driven GUS expression in Arabidopsis model, because reliable transformation of Ginkgo is yet not available. In addition, correlation of the promoter experiment in the model plant with *IDS* transcript levels in Ginkgo organs further substantiated putative role of each isogenic *HDR*/*IDS*.

2. Results

2.1. Isolation of GbIDS1 and GbIDS2 promoters

The promoter regions of *GbIDS1* (1269 bp, Genbank JX444962) and *GbIDS2* (1480 bp, JX444961) were isolated from the *G. biloba* genomic DNA (Supplementary Figs. 1 and 2). The predicted *cis*-acting regulatory elements from the *GbIDS* promoter sequences contained candidate sequences towards various biotic and abiotic stresses as listed in Supplementary Tables 1 and 2. Putative TATA box of *GbIDS1pro* and *GbIDS2pro*, placed at −108 bp and −73 bp, respectively, and other regulatory elements predicted by PlantCARE analysis are listed in Supplementary Figs. 1 and 2.

2.2. Histochemical analysis of GbIDSpro-driven GUS expression in Arabidopsis

To ascertain the developmental-spatial expression patterns of the *GbIDS1* and *GbIDS2*, each construct of *GbIDS1pro*::*GUS* and *GbIDS2pro*::*GUS* was introduced into Arabidopsis. In the case of *GbIDS1* promoter, GUS expression was found in almost all developmental stages and tissue types except for roots, stamina, and petals (Fig. 1A–D and Supplementary Fig. 3). In contrast, *GbIDS2* promoter-driven GUS expression was very organ-specific; the expression was shown only in young leaves including meristem, internodes where the flower and shoot branched, and particularly junction of primary root (Fig. 1E–H and Supplementary Fig. 3). Furthermore, expression in root junction was detected only at specific growth stages; 5.10 (the first flower bud visible) to right before 6.00 (the first flower open) (Fig. 1F).

The sectioning of *GbIDS1pro*::*GUS*-expressed leaf, inflorescence, and internode stem provided information on the tissue distribution of IDS. In leaf, strong GUS expression in parenchyma cells were detected, in contrast to the very weak expression in the epidermal cells, if any (Fig. 1I). Terminal inflorescence stem had little expression pattern. In penultimate internode (Fig. 1J), whole tissue except for pith and xylem was stained. In the case of *GbIDS2pro*::*GUS* Arabidopsis, duct system was the main site of expression: the central vasculature in the root junction and the vascular bundle of flowering stem (Fig. 1K–L). Hormone treatment did not cause additional organ or tissue staining (Data not shown).

2.3. Responsiveness of GbIDS promoters in Arabidopsis toward hormone treatments

GUS activity in Arabidopsis transformed with *IDSpro*::*GUS* was quantitatively assessed in each organ after hormone treatment. In particular, *GbIDS1pro*-driven GUS activity in the leaves and *GbIDS2pro*-driven GUS activity in roots are presented in Fig. 2A, because these two organs showed the most contrasting GUS staining patterns (Fig. 1 and Supplementary Fig. 3). The selection of the hormone was based on the putative hormone-related *cis*-acting elements in the *IDSpro* sequences (Supplementary Figs. 1 and 2 and Supplementary Tables 1 and 2). The activity of GUS in the leaves and roots after the hormone treatment was measured in 5.10 stage plants and flowers and buds in stage 6.50 plants (50% of flower opened) (Fig. 2). In the case of leaves, treatment of MeJA and GA resulted in pronounced decrease of GUS activity by 40 and 25%, respectively in *GbIDS1pro*-transformed Arabidopsis (Fig. 2A). However, in the roots of *GbIDS2pro*::*GUS* plant, MeJA and GA significantly stimulated GUS activity by 80 and 140%, respectively (Fig. 2A). GUS activity in ABA-treated plants also followed the same trend both in the leaves and root. However, SA treatments caused the opposite effect compared to MeJA and GA treatments by increasing the GbIDS1pro-related GUS activity in leaves by 10% and decreasing the GbIDS2pro-related GUS activity in roots by 20%. Therefore, behavior of *IDS1pro*-driven GUS activity and that of *IDS2pro*-driven activity upon MeJA and SA treatments in the roots and leaves were reciprocal. GUS activity in flowers and buds did not show such distinctive pattern observed in leaves and roots (Fig. 2B). In contrast to leaves and roots, GA and IBA treatments tended to increase GUS activity in flowers and buds, respectively, compared to MeJA and SA treatments (Fig. 2B). MeJA and SA had little effect on GUS activity in flowers and buds.

2.4. Transcript distribution of GbIDS1 and GbIDS2 in Ginkgo

The organ-specific transcription of *GbIDS*s in Ginkgo was examined in detail by RT-PCR (Fig. 3). In the case of *GbIDS1*, transcripts appeared in all organs examined except for root and cotyledon. However, *GbIDS2* transcripts were found in all organs including root and cotyledon, albeit in varying degree of transcript levels—the transcript level of *GbIDS2* in the leaves was relatively low compared to those of stem, root, as well as female and male flowers. In summary, *GbIDS2* level was lower than *GbIDS1* level in leaves. In contrast, *GbIDS2* levels were higher than those of GbIDS1 in male and female flowers. In root and cotyledon, only *GbIDS2* transcripts were seen.

2.5. GbIDS1 and GbIDS2 transcript level changes in Ginkgo by MeJA and SA treatments

To correlate the effects of MeJA and SA treatments in the transformed Arabidopsis with Ginkgo plant, *GbIDS* transcript levels in leaves and roots were measured after treating the elicitors to 2-month-old Ginkgo seedlings. Initially, the resting *GbIDS2* transcript level was higher than the resting *GbIDS1* level by 100% in the leaves (Fig. 2C), whereas in the roots *GbIDS1* transcript level was almost negligible compared to *GbIDS2* level (Fig. 2C). MeJA treatment decreased *GbIDS1* transcript level in the leaves by 50%, whereas SA caused little change (Fig. 2C). However, upon MeJA treatment, *GbIDS2* transcript level in the leaves and roots increased by 150 and 40%, respectively (Fig. 2C).

3. Discussion

Biosynthesis of isoprene unit in the classical MVA pathway is regulated in multiple levels. For example, 3-hydroxy-3-methylgluaryl-CoA reductase (HMGR), being the first committing enzyme in MVA pathway, is highly regulated from transcription to post-translation levels [21]. In the case of Arabidopsis, HMGR gene is encoded as two copies: *HMG1* and *HMG2*. Transcripts of *HMG1S*, one of the alternative splicing forms of *HMG1*, appear in whole plant [22], whereas transcripts of *HMG2* and *HMG1L* transcripts are present in seedling, root, and inflorescence [23]. This observation is interpreted as that HMG1S is involved in housekeeping process, whereas HMGR2 has a more restricted function [21]. Plants also harbor MEP pathway to synthesize isoprene units to build