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Molecular cloning, expression profiling and functional analysis of a *DXR* gene encoding 1-deoxy-D-xylulose 5-phosphate reductoisomerase from *Camptotheca acuminata*

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Summary

As the second enzyme of the non-mevalonate terpenoid pathway for isopentenyl diphosphate biosynthesis, DXP reductoisomerase (DXR, EC: 1.1.1.267) catalyzes a committed step of the MEP pathway for camptothecin (CPT) biosynthesis. In order to understand more about the role of DXR involved in the CPT biosynthesis at the molecular level, the full-length DXR cDNA sequence (designated as *CaDXR*) was isolated and characterized for the first time from a medicinal *Nyssaceae* plant species, *Camptotheca acuminata*. The full-length cDNA of *CaDXR* was 1823 bp containing a 1416 bp open reading frame (ORF) encoding a polypeptide of 472 amino acids. Comparative and bioinformatic analyses revealed that *CaDXR* showed extensive homology with DXRs from other plant species and contained a conserved

Abbreviations: CPT, camptothecin; CTAB, cetyltrimethylammonium bromide; DXP, deoxy-D-xylulose 5-phosphate; DXR, deoxy-D-xylulose 5-phosphate methylerythritol phosphate reductoisomerase; MeJA, methyl jasmonate; MEP, mevalonate-independent plastidial methylerythritol 4-phosphate; ORF, open reading frame; RACE, rapid amplification of cDNA ends

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transit peptide for plastids, an extended Pro-rich region and a highly conserved NADPH binding motif in its N-terminal region owned by all plant DXRs. Phylogenetic analysis indicated that CaDXR was more ancient than other plant DXRs. Tissue expression pattern analysis revealed that *CaDXR* expressed strongly in stem, weak in leaf and root. *CaDXR* was found to be an elicitor-responsive gene, which could be induced by exogenous elicitor of methyl jasmonate. The functional color complementation assay indicated that *CaDXR* could accelerate the biosynthesis of carotenoids in the *Escherichia coli* transformant, demonstrating that DXP reductoisomerase plays an influential step in isoprenoid biosynthesis.

Introduction

Camptotheca acuminata of the family *Nyssaceae* is a deciduous and medicinal tree from south China and has been listed as an endangered species in China since 1997 (Li et al., 2002). The main active ingredients of *C. acuminata* extracts are quinoline and indole alkaloids (Wall et al., 1966). The camptothecin (CPT), a terpenoid quinoline alkaloid, and its analogues are the potent topoisomerase I inhibitors, which have been used as anticancer and antiviral indole alkaloids to cure ovarian, lung and colorectal cancers (Li and Adair, 1994; Oberlies and Kroll, 2004). Because of the present and potential clinical uses of CPT and its derivatives, it is a necessity to develop sustainable and alternative production sources of these compounds (Lorence et al., 2004). Based on the reality of limited natural *Camptotheca* plant materials, to map the CPTs biosynthetic pathway at the level of molecular genetics may lead to means to enhance CPTs production (Lorence and Nessler, 2004).

All terpenoids such as CPTs are biosynthesized via recently discovered and mevalonate-independent plastidial methylerythritol 4-phosphate (MEP) pathway (Lichtenthaler et al., 1997a, b; Zeidler et al., 1997; Lichtenthaler, 1999; Rohmer, 1999). The MEP pathway starts with the formation of 1-deoxy-D-xylulose 5-phosphate (DXP) from D-glyceraldehyde 3-phosphate and pyruvate catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS, EC: 4.1.3.37) (Sprenger et al., 1997; Bouvier et al., 1998; Lange et al., 1998; Lois et al., 1998). Then DXP is converted into MEP and the reaction is catalyzed by a NADPH-dependent reductoisomerase (deoxy-D-xylulose 5-phosphate methylerythritol phosphate reductoisomerase (DXR), EC1.1.1.267) specified by the *DXR* gene. Because DXP is an intermediate not only for isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) biosynthesis, but also for the cofactors thiamine and pyridoxol (Julliard and Douce, 1991; Julliard, 1992; Himmeldirk et al., 1996), the reaction catalyzed by DXR is actually the first committed step of the MEP pathway (Kuzuyama et al., 1998). Moreover, earlier experimental results also showed that DXR participated in the control of isoprenoid accumulation in plants (Veau et al., 2000; Walter et al., 2000; Mahmoud and Croteau, 2001). Earlier investigations showed that overexpression of DXR in transgenic peppermint plants could lead to an increase of the essential oil monoterpenes in the MEP pathway (Mahmoud and Croteau, 2001). DXR generally plays a key regulatory role in the control of terpenoids on the pathway and even in the regulation of CPT biosynthesis just like that in peppermint (Mahmoud and Croteau, 2001). Unfortunately, until now there have been only a few reports on the cloning of genes involved in the MEP pathway from *C. acuminata* (Lu et al., 2005) and no reports on the cloning of *DXR* genes from any *Nyssaceae* species. In the present study, we report, for the first time, the cloning and characterization of the *DXR* gene from the *Nyssaceae* plant *C. acuminata* by rapid amplification of cDNA ends (RACE). The expression profiles of *CaDXR* in various tissues and under the induction by methyl jasmonate (MeJA) elicitor were also investigated, which will facilitate future work to map and regulate this important step involved in CPT biosynthetic pathway at the level of molecular genetics.

Materials and methods

Plant materials and RNA isolation

The cultured callus lines, initiated from young leaves of Chinese happy tree (*C. acuminata* Decne. var. *acuminata*), were maintained on solid Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) supplemented with 5.0 mg/L naphthaleneacetic acid (NAA), 0.5 mg/L 6-benzylaminopurine (6-BAP) and 0.3 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) at 25 °C in darkness for 4 weeks and used as the starting material for total RNA isolation. In the experiment of investigating induction by MeJA elicitor, 4-d-old callus