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ISOLATION AND BIOINFORMATICS ANALYSIS OF GLUTAMYL-TRNA REDUCTASE IN CHINESE JUJUBE

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Abstract

Tetrapyrroles, such as chlorophyll and heme, are integral to the metabolism of all living organisms. Glutamyl-tRNA reductase (GluTR) is the first unique enzyme in the tetrapyrrole biosynthetic pathway in plants. We firstly cloned the GluTR complete sequences (1733 bp) from Chinese jujube (Ziziphus jujuba Mill., belonging to the family Rhamnaceae), named as ZjGluTR (GenBank accession no. KF530842). ZjGluTR shares high similarity (90%) to those of Malus domestica and Prunus mume through BLASTX analysis. It contains a 1659-bp ORF and encodes a predicted polypeptide of 552 amino acids, with an estimated molecular mass of 60.0 kDa and a theoretical pI of 8.55. The speculated formula of ZjGluTR is $C_{2615}H_{4318}N_{756}O_{804}S_{25}$. ZjGluTR has a chloroplast transit peptide which contains 61 amino acids in the N terminal. The subcellular localization result showed that ZjGluTR protein exists in chloroplasts. ZjGluTR has three typical functional domains, i.e. GluTR N-terminal domain (101-259 aa), NAD(P)-binding Rossmann-fold domain (266-422 aa) and GluTR dimerization domain (425-534 aa). The molecular phylogenetic tree of GluTR indicated that the family Rhamnaceae has a close genetic relationship with the family Rosaceae. Our studies on the GluTR using molecular biology and bioinformatics approaches would play an important role in chlorophyll metabolic research of Chinese jujube.

Key words*: Chinese jujube, GluTR, isolation, bioinformatics analysis.*

INTRODUCTION

Tetrapyrroles, such as chlorophyll and heme, are integral to the metabolism of all living organisms. The first common precursor molecule of all tetrapyrroles is derived from 5-aminolevulinic acid (ALA). It is synthesized through two distinct biosynthetic routes: Firstly, in humans, animals, fungi and the α-group of the proteobacteria, the condensation of succinyl coenzyme A and glycine with the release of CO_2 is catalyzed by ALA-synthase (Kikuchi et al., 1958; Neuberger, 1968; Avissar et al., 1989; Ferreira, 1995). Secondly, the older pathway, utilizing the C_5-skeleton of glutamate, was first discovered in plants (Beale and Castelfranco, 1973). Subsequently, the C_5-pathway was found to be common to plants, green algae, archaea and most bacteria (Schön et al., 1986; Jahn et al., 1992). In the first dedicated step, the NADPH-dependent reduction of glutamyl-tRNA to glutamate-1-semialdehyde (GSA) is catalyzed by glutamyl-tRNA reductase (GluTR) (Mayer et al., 1987; Chen et al., 1990; Jahn et al., 1992, Verkamp et al., 1992; Vothknecht et al., 1996, 1998). In the subsequent reaction, GSA is transaminated by the pyridoxal/pyridoxamine 5'-phosphate-dependent glutamate-1-seminaldehyde-2, 1-aminomutase (GSAM) to form ALA (Grimm, 1990; Jahn et al., 1991; Smith et al., 1992; Ilag and Jahn, 1992). In plants, all of the components required for such a conversion are located in the chloroplasts.

Other pathways of ALA formation in plants have been reported (Ramaswamy and Nair, 1973; Meller and Gassman, 1982), but efforts to thoroughly characterize these 'alternative ALA-forming pathways' have been unsuccessful. The existence of the C_5 pathway is widely accepted. Furthermore, lethal effect produced in some lines by antisense HEMA1 (Kumar et al., 2000) and antisense GSA (Höfgen et al., 1994) favors the C_5 pathway as a sole source for ALA biosynthesis.

Currently, studies on ALA biosynthesis in Chinese jujube (*Ziziphus jujuba* Mill.)-have not been reported. We carried out relevant research

on the *GluTR* using molecular biology and bioinformatics approaches, which would provide a foundation for chlorophyll metabolic studies of Chinese jujube.

MATERIALS AND METHODS

Materials

Ziziphus jujuba Mill. 'Xingguang' was used as material to isolate total RNA. The fresh young leaves were collected, then frozen with liquid nitrogen rapidly and kept at -80 °C.

Methods

Total RNA isolation

Isolation of total RNA was carried out according to the instructions of improved CTAB method (Zhao et al., 2009). DNase I treatment was applied to remove contaminating genomic DNA. First-strand cDNA was synthesized as described by TaKaRa RNA PCR Kit (AMV) Ver.3.0 (TaKaRa).

Amplification of the full length cDNA of GluTR

Homology cloning method was used to obtain full-length cDNA of *GluTR* in Chinese jujube, the pair primers of 5' end-primer (5′-ATGGCCGTGTCGACCAGT T-3′) and 3' end-primer (5′-GAGGATGTTGCCTCTTATTC-3′) were used in this study. PCR was performed in a volume of 25 μL containing 15.5 μL of ddH_2O, 2 μL of the first strand cDNA, 2.5 μL of ExTaq DNA polymerase buffer, 2 μL of $MgCl_2$, 0.5 μL of dNTPs, 0.5 μL of ExTaq DNA polymerase, and 1 μL of each primer. PCR were optimized to consist with the following parameters, i.e. denaturation of 5 min at 95 °C; 35 cycles of 30 s at 94 °C, 60 s at 55 °C and 90 s at 72 °C; extension at 72 °C for 10 min. PCR products were separated on 1% agarose gels. Amplified fragment was cloned into pMD-19T vector and sequenced by Beijing ZhongKe XiLin Biotechnology CO., Ltd.

Analysis of cDNA and protein sequences

cDNA sequence was analyzed using BLAST (http://www. http://blast.ncbi.nlm. nih.gov/) and the ORF FINDER (http://www.ncbi.nlm.nih.gov/ gorf/gorf.html). The protein sequence was analyzed by Protparam (http://expasy.org/ tools/protparam.html), WoLF PSORT (http://wolfpsort.seq.cbrc.jp), ChloroP Server 1.1 (http://www.cbs.dtu.dk/services/ ChloroP/), Pfam (http://pfam.sanger.ac.uk) and SWISS-MODEL Workspace (http://www.expasy.ch/swissmod/ SWISS-MODEL.html). Homology tree was educed according to MEGA 6 using neighbor-joining method.

RESULTS AND DISCUSSIONS

Cloning of the full-length cDNA of *ZjGluTR*

Approximately 1750-bp fragment was identified by homology cloning method (Figure 1). The fragment was cloned into a cloning vector, and sequenced subsequently. A 1733-bp expressed sequence tags (EST) was obtained after removing vector and adapter sequences. We named the sequence as *ZjGluTR* (GenBank accession no. KF530842). BLASTX analysis showed that the 1733-bp sequence shared high similarity (90%) to those of *Malus domestica* (XP008378624.1) and *Prunus mume* (XP008219178.1).

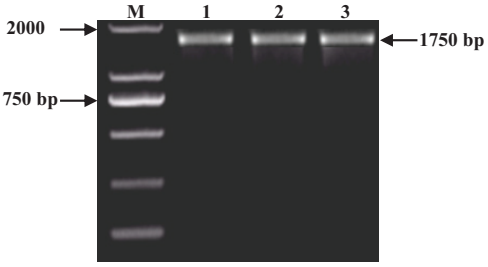

Figure 1. The PCR result of *ZjGluTR* by homologous cloning. Note: line 1, 2 and 3 were three replication

Protein sequence analysis and characterization of ZjGluTR

The full-length cDNA sequence of *ZjGluTR*, containing a 1659-bp ORF, encoded a predicted polypeptide of 552 amino acids, with an

estimated molecular mass of 60.0 kDa and a theoretical pI of 8.55. The speculated formula of protein was $C_{2615}H_{4318}N_{756}O_{804}S_{25}$.

The results predicted from Search Pfam showed that ZjGluTR contained three functional domains, including GluTR N-terminal domain, Shikimate/quinate 5-dehydrogenase domain and GluTR dimerisation domain. GluTR N-terminal domain and GluTR dimerisation domain were typical characteristics of GluTR.

Relevant literature reported that the precursor protein of Glutamyl-tRNA reductase has a transit peptide in the N terminal. Therefore, we carried out transit peptide prediction for the ZjGluTR. The results showed that the ZjGluTR has a chloroplast transit peptide which contains 61 amino acids in the N terminal. At the same time, WoLF PSORT was used to predict the ZjGluTR subcellular localization. The result showed that ZjGluTR protein exists in plant chloroplasts with the identity of 79%. This result was consistent with the ZjGluTR chloroplast transit peptide prediction.

Alignment result by homology modeling showed that ZjGluTR has three typical functional domains (Figure 2), such as GluTR N-terminal domain (101-259 aa), NAD(P)-binding Rossmann-fold domains (266-422 aa) and GluTR dimerization domain (425-534 aa). The results were consistent with Schubert's reports that each GluTR monomer consists of three distinct domains (Schubert et al., 2002). In addition, Shikimate/quinate 5-dehydrogenase domain (262-414 aa) was also predicted, which was not the typical characteristic of GluTR. Meanwhile, the tertiary structure of ZjGluTR was constructed by SWISS-MODEL Workspace (Figure 3b), which has the same tertiary structure as *Methanopyrus kandleri* GluTR (Figure 3a) (Schubert et al., 2002).

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IPR000343: Glutamyl-tRNA reductase, Family
  TIGR01035: 104 - 533  hemA: glutamyl-tRNA reductase

IPR000343: Glutamyl-tRNA reductase, Family
  SSF69742: 101 - 259   Glutamyl tRNA-reductase catalytic, N-terminal domain

IPR006151: Shikimate/quinate 5-dehydrogenase, Domain
  PF01488: 262 - 414    Shikimate_DH

noIPR: unintegrated, unintegrated
  SSF51735: 266 - 422   NAD(P)-binding Rossmann-fold domains

noIPR: unintegrated, unintegrated
  SSF69075: 425 - 534   Glutamyl tRNA-reductase dimerization domain
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Figure 2. Alignment results of *ZjGluTR* homology modeling

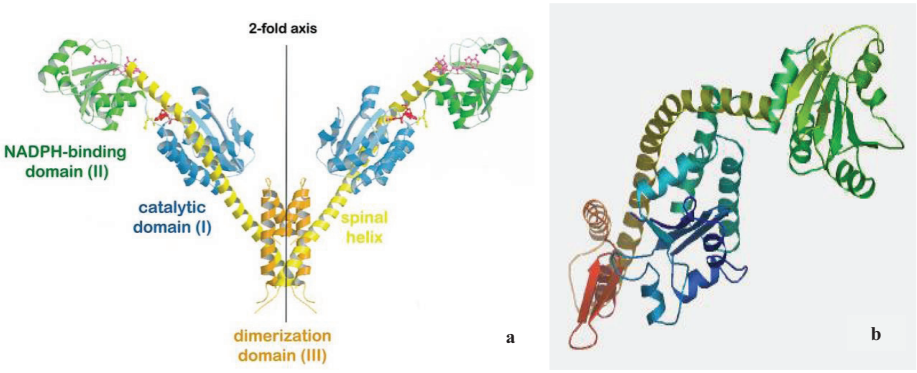

Figure 3. A schematic diagram of the *Methanopyrus kandleri GluTR* dimer viewed perpendicular (a) (Schubert et al., 2002) and the tertiary structure of *ZjGluTR* (b)

The phylogenetic tree of GluTR

The molecular phylogenetic tree of ZjGluTR and other 11 plant species was constructed by MEGA 6 (Figure 4). This tree showed that GluTR of *Z. jujuba* (belonging to the family Rhmnaceae) was firstly clustered with that of *Malus domestica* and *Prunus mume* (the family Rosaceae) which indicated that ZjGluTR should have a closely genetic relationship with that of Rosaceae.

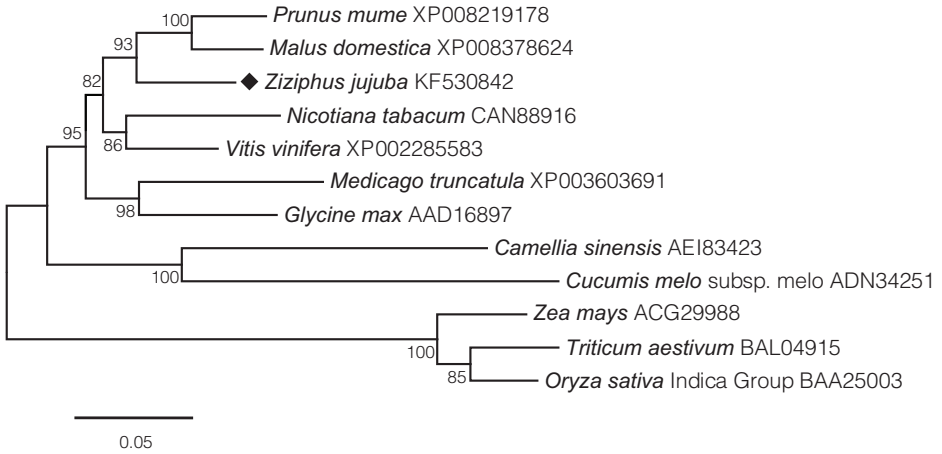

Figure 4. The phylogenetic tree of *ZjGluTR* and other 11 plant species

CONCLUSIONS

GluTR is the first committed enzyme in plant tetrapyrrole biosynthesis and is likely to be involved in the control of this metabolic pathway. The tetrapyrrole biosynthetic pathway provides the vital cofactors and pigments for photoautotrophic growth (chlorophyll) (Czarnecki et al., 2011). Some of the transgenic studies showed that plant chlorophyll deficiency, ranging from patchy yellow to total yellow. Moreover, the plants that completely lacked chlorophyll failed to survive under the growth conditions (Kumar et al., 2000; Höfgen et al., 1994). These observations suggest that suppression of the enzymes of the C_5 pathway affect the growth of the plant. Therefore, studies on the regulated synthesis of tetrapyrroles, including heme and chlorophyll, are important. Thus, cloning and bioinformatics analysis of *ZjGluTR* is very helpful for molecular biology research of chlorophyll synthesis in Chinese jujube.

REFERENCES

Vissar Y J, Ormerod J G, Beale S I, 1989. Distribution of δ-aminolevulinic acid biosynthetic pathways among phototrophic bacterial groups. Archives of microbiology, 151(6): 513-519.

Beale S I, Castelfranco P A, 1973. ^{14}C incorporation from exogenous compounds into δ-aminolevulinic acid by greening cucumber cotyledons. Biochemical and biophysical research communications, 52(1): 143-149.

Chen M W, Jahn D, O'Neill G P et al., 1990. Purification of the glutamyl-tRNA reductase from Chlamydomonas reinhardtii involved in delta-aminolevulinic acid formation during chlorophyll biosynthesis. Journal of Biological Chemistry, 265(7): 4058-4063.

Czarnecki O, Hedtke B, Melzer M et al., 2011. An Arabidopsis GluTR binding protein mediates spatial separation of 5-aminolevulinic acid synthesis in chloroplasts. The Plant Cell Online, 23(12): 4476-4491.

Ferreira G C, 1995. Heme biosynthesis: biochemistry, molecular biology, and relationship to disease. Journal of bioenergetics and biomembranes, 27(2): 147-150.

Grimm B, 1990. Primary structure of a key enzyme in plant tetrapyrrole synthesis: glutamate 1-semialdehyde aminotransferase. Proceedings of the National Academy of Sciences, 87(11): 4169-4173.

Höfgen R, Axelsen K B, Kannangara C G et al., 1994. A visible marker for antisense mRNA expression in plants: inhibition of chlorophyll synthesis with a glutamate-1-semialdehyde aminotransferase antisense gene. Proceedings of the National Academy of Sciences, 91(5): 1726-1730.

Ilag L L, Jahn D, 1992. Activity and spectroscopic properties of the *Escherichia coli* glutamate 1-semialdehyde aminotransferase and the putative active site mutant K265R. Biochemistry, 31(31): 7143-7151.

Jahn D, Chen M W, Söll D, 1991. Purification and functional characterization of glutamate-1-semialdehyde aminotransferase from Chlamydomonas reinhardtii. Journal of Biological Chemistry, 266(1): 161-167.

Jahn D, Verkamp E, 1992. Glutamyl-transfer RNA: a precursor of heme and chlorophyll biosynthesis. Trends in biochemical sciences, 17(6): 215-218.

Kikuchi G, Kumar A, Talmage P et al. The enzymatic synthesis of δ-aminolevulinic acid. Journal of Biological Chemistry, 1958, 233(5): 1214-1219.

Kumar A M, Söll D, 2000. Antisense HEMA1 RNA expression inhibits heme and chlorophyll biosynthesis in Arabidopsis. Plant physiology, 122(1): 49-56.

Mayer S M, Beale S I, Weinstein J D, 1987. Enzymatic conversion of glutamate to delta-aminolevulinic acid in soluble extracts of *Euglena gracilis*. Journal of Biological Chemistry, 262(26): 12541-12549.

Meller E, Gassman M L, 1982. Biosynthesis of 5-aminolevulinic acid: two pathways in higher plants. Plant Science Letters, 26(1): 23-29.

Neuberger A, 1968. Biochemical basis and regulatory mechanisms of porphyrin synthesis. Proceedings of the Royal Society of Medicine, 61(2): 191-193.

Ramaswamy N K, Nair P M, 1973. δ-Aminolevulinic acid synthetase from cold-stored potatoes.

Biochimica et Biophysica Acta (BBA)-Enzymology, 293(1): 269-277.

Schön A, Krupp G, Gough S, et al., 1986. The RNA required in the first step of chlorophyll biosynthesis is a chloroplast glutamate tRNA. Nature, 322: 281-284.

Schubert W D, Moser J, Schauer S et al., 2002. Structure and function of glutamyl-tRNA reductase, the first enzyme of tetrapyrrole biosynthesis in plants and prokaryotes. Photosynthesis research, 74(2): 205-215.

Smith M A, Kannangara C G, Grimm B, 1992. Glutamate 1-semialdehyde aminotransferase: anomalous enantiomeric reaction and enzyme mechanism. Biochemistry, 31(45): 11249-11254.

Verkamp E, Jahn M, Jahn D, et al., 1992. Glutamyl-tRNA reductase from *Escherichia coli* and *Synechocystis* 6803. Gene structure and expression. Journal of Biological Chemistry, 267(12): 8275-8280.

Vothknecht U C, Kannangara C G, Von Wettstein D, 1996. Expression of catalytically active barley glutamyl tRNAGlu reductase in *Escherichia coli* as a fusion protein with glutathione S-transferase. Proceedings of the National Academy of Sciences, 93(17): 9287-9291.

Vothknecht U C, Kannangara C G, von Wettstein D, 1998. Barley glutamyl $tRNA^{Glu}$ reductase: Mutations affecting haem inhibition and enzyme activity. Phytochemistry, 47(4): 513-519.

Zhao J, Liu Z C, Dai L et al., 2009. Isolation of Total RNA for Different Organs and Tissues of *Ziziphus jujuba* Mill. Journal of Plant Genetic Resources, 111-117.