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Isolation, Similarity and Subcellular Localisation of Transaldolase from Sugarcane (*Saccharum officinarum*)

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Abstract: This study focused on isolation, cloning of TAL from sugarcane. Transaldolase is one of the enzymes of the Pentose Phosphate Pathway (PPP). Transaldolase in non-oxidative phase of OPPP transfer a three carbon dihydroxyacetone moiety from sedoheptulose-7-phosphate and glyceraldehyde-3-phophate to produce Erythrose-4-Phophate (E4P) and fructose-6-phophate. E4P is the precursor for many secondary metabolic pathways including aromatic amino acids, lignin and flavonoid synthesis. Earlier studies revealed that OPPP is incomplete in the cytosol of plants as no genes encoding for a cytosolic TAL. Moreover, there is no study about the *TAL* genes from sugarcane until to date. Thus, the objective of this study is to isolate *TAL* gene from sugarcane, to compare its similarity with other plants, to determine its subcellular localization. A total of 1601 bp of TAL has been isolated by PCR. Similarity, studies by ClustalW revealed that TAL show highest similarity (75%) with *Zea mays*. Analysis of subcellular localization by using Target 1.1 revealed that of TAL from sugarcane was not located in the plastidic.

Key words: The oxidative pentose pathway, transaldolase, subcellular localisation, E4P, *TAL* gene

INTRODUCTION

The Oxidative Pentose Phosphate Pathway (OPPP) is involved in the metabolism of carbohydrates via the oxidation of glucose-6-phosphate. The pathway is composed of two functionally-connected phases. The first phase is the oxidative phase of the pentose phosphate pathway, is irreversible and consists of the oxidation of glucose-6-phosphate that leads to the production of ribulose-5-phosphate. The enzymes involved are successively Glucose-6-Phosphate Dehydrogenase (G6PDH), 6-Phosphogluconolactonase (6PG) and 6-Phosphogluconate Dehydrogenase (6PGDH). The second phase is the non-oxidative phase. It consists of a reversible series of interconversion between 3-, 4-, 5-, 6- and 7-carbons sugars catalyzed by the enzymes Ribose-5-Phosphate Isomerase (RPI), Ribulose-5-Phosphate-3-Epimerase (RPE) and Transketolase (TK) and Transaldolase (TAL). All enzymes of the OPPP apart from TAL are also part of the Reductive Pentose Phosphate Pathway (RPPP) or Calvin cycle (Debnam *et al*., 2004).

The first phase of the OPPP provides 50-60% of the required NADPH, a major reducing power for various anabolic pathways including the biosynthesis of fatty acids, the reduction of nitrite (Debnam *et al*., 2004) for cell protection against oxidative stress and synthesis of glutamate. The second phase provides ribose-5-phosphate and erythrose-4-phosphate. Ribose-5-phosphate is the main substrate for nucleic acid biosynthesis RNA, DNA and nucleotides coenzymes such as ATP, FAD, NADH and NADPH. The complete OPPP is located in cytosol of animal and prokaryotic system.

However, in plant the oxidative phase OPPP is located in cytosol but the non-oxidative phase of OPPP is unclear. Until to date, no study had been conducted on TAL from sugarcane. Thus, the objectives of study are isolate *TAL* gene from sugarcane, compare its similarities with other plant and determine its subcellular localization. According to Graeve *et al*. (1994), the oxidative pentose phosphate pathway are found in the cytosol and stroma of plant cells. Although, the non-oxidative enzymes of OPPP are located in plastid (Henkes *et al*., 2001). However, Eicks *et al*. (2002) shows TAL with other enzymes of non-oxidative part of pentose phosphate pathway in higher plants to be confined in storma because this kind of plant cells in the cytosol, cant utilize pentose phosphates into triose phosphate and hexose phosphate.

MATERIALS AND METHODS

Sugarcane plants were planted at Department of Biology in University Putra Malaysia. After 6-7 weeks, leaves samples are harvested to obtain DNA. DNA was extracted according to method by Nulit (2012). To amplify

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TAL, primers were designed as followed left primer: 5' TCC TTCTTTGTC AGC CGA GT-3' and right primer: 5'-CCC GAT TCA GAA AAT GGA GA-3'. PCR master mix was performed in a thermal cycler (Biometra). PCR products were separate on 1% agarose gel electrophoresis and purified by using Wizard® SV gel and PCR Clean-Up System kit (Promega, USA). Then, followed by cloning by using yT&A cloning vector (Yeastern Biotech Co., Ltd.). Then, the plasmid vector was digested with HindIII and BglI and purified and sequencing it. The DNA nucleotide sequences were first aligned with the Genbank database to search for any homology sequences using BLAST. ClustalW was conducted to study sequence similarity and determine the subcellular localization of TAL by using Target P Version 1.1.

RESULTS AND DISCUSSION

The length of TAL sugarcane had been isolated in the present study is 1601 bp. ClustalW analysis shows that the similarity of TAL sugarcane is high, >68% when compared with other plant species (Table 1). TAL sugarcane has higher similarity (72%) with TK *Zea mays*. Both of these plant species are closely related due to they in the same family Poaceae. It might be suggests that sugarcane and *Zea mays* may be have same condition of evolution and the alternative splicing TAL was nearly same. Analysis of subcellular localization by using Target P version 1.1 revealed that of TAL from sugarcane was not located in the plastidic (Fig. 1). Result of subcellular localization TAL from sugarcane is difference from TAL other plants which is located in the plastidic.

Table 1: Percentage of homology of sugarcane transaldolase amino acid sequences compared with other species from http://www.uniprot.org database

Plant species	Accession number	Identity (%)	E-value
Oryza sativa subsp. *indica*	A6MZFO-ORYSI	71	3.3E-4
Oryza sativa subsp. *japonica*	Q5JK10-ORYSJ	71	2.4E-4
Zea mays	B4FRC9-MAIZE	75	4.0E-5
Solanum tuberosum	Q7XJH9-SOLLC	72	0.051
Arabidopsis thaliana	Q9LYR4-ARATH	68	1.4

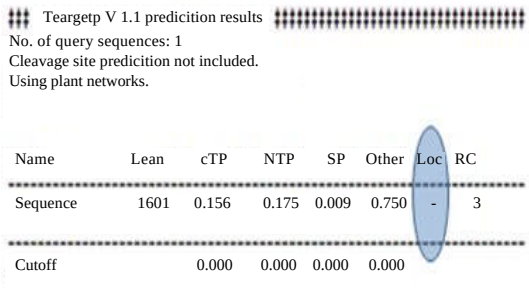

Fig. 1: Target P1.1 results of subcellular localization of TAL from sugarcane

CONCLUSION

TAL sugarcane has high similarity with other plant species and is not located in the plastid of sugarcane plant.

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