

Cloning, expression and characterization of sugarcane (*Saccharum officinarum* L.) transketolase

ABSTRACT

Pentose phosphate pathway (PPP) composed of two functionally-connected phases, the oxidative and non-oxidative phase. Both phases catalysed by a series of enzymes. Transketolase is one of key enzymes of non-oxidative phase in which transfer two carbon units from fructose-6-phosphate to erythrose-4-phosphate and convert glyceraldehyde-3-phosphate to xylulose-5-phosphate. In plant, erythrose-4-phosphate enters the shikimate pathway which produces many secondary metabolites such as aromatic amino acids, flavonoids, lignin. Although transketolase in plant system is important, study of this enzyme is still limited. Until to date, TKT genes had been isolated only from seven plants species, thus, the aim of present study to isolate, study the similarity and phylogeny of transketolase from sugarcane. Unlike bacteria, fungal and animal, PPP is complete in the cytosol and all enzymes are found cytosolic. However, in plant, the oxidative phase found localised in the cytosol but the sub localisation for non-oxidative phase might be restricted to plastid. Thus, this study was conducted to determine subcellular localization of sugarcane transketolase. The isolation of sugarcane TKT was done by reverse transcription polymerase chain reaction, followed by cloning into pJET1.2 vector and sequencing. This study has isolated 2,327 bp length of sugarcane TKT. The molecular phylogenetic tree analysis found that transketolase from sugarcane and *Zea mays* in one group. Classification analysis found that both plants showed closer relationship due to both plants in the same taxon i.e. family Poaceae. Target P 1.1 and Chloro P predicted that the compartmentation of sugarcane transketolase is localised in the chloroplast which is 85 amino acids are plant plastid target sequence. This led to conclusion that the PPP is incomplete in the cytosol of sugarcane. This study also found that the similarity sequence of sugarcane TKT closely related with the taxonomy plants.

Keyword: CDNA cloning; Pentose phosphate pathway; Phylogenetic; Protein expression; Subcellular localisation; Sugarcane; Transketolase.