ABSTRACT

Starch biosynthesis is still not fully understood. The current models of starch biosynthesis focus on four key enzymes, with limited understanding of how these key enzymes and their multiple isoforms are coordinated to synthesise starch granules. Apart from the currently known key enzymes, there are potentially other proteins that may be involved in starch biosynthesis and have not yet been discovered. A comparative proteomics analysis of EM653, a *sugary* rice mutant, and Taichung 65, its wild type, has been carried out to identify differentially expressed proteins (DEPs) with possible involvement in starch biosynthesis. The EM653 mutant is deficient in isoamylase (Isa), one of the key enzymes in starch biosynthesis. It exhibits a *sugary* phenotype, where phytoglycogen is synthesised instead of amylopectin. The pleiotropic effect of the Isa deficiency in *sugary* rice affects the expression of associated starch biosynthetic enzymes. Two complementary comparative proteomics approaches were used in this study to identify DEPs in *sugary* and normal rice endosperm. A gel-free shotgun approach, liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used to analyse proteins from the enriched amyloplastic fraction of *sugary* and normal rice endosperm. A total of 929 proteins were identified, of which 160 were differentially expressed. Meanwhile, a gel-based approach, two-dimensional gel electrophoresis coupled with LC-MS/MS (2DE-LC-MS/MS) was used to analyse endosperm proteins and 40 DEPs were identified. Next, the gene expression of seven target genes in *sugary* and normal rice was determined through quantitative polymerase chain reaction (qPCR) using a relative quantification approach. For three genes directly involved in starch biosynthesis (*Isa1*, *PPDK1* and *PPDK2*), mRNA expression was correlated with the protein expression. Next, *in silico* analysis of the DEPs was carried out to identify proteins with possible involvement in starch biosynthesis. Notably, this study had identified two poorly

characterised amylases, OsBam8b and plastidial Amy3, which may be directly involved in starch biosynthesis. A xylanase inhibitor protein (XIP) that may act as an amylase inhibitor with potential involvement in starch metabolism was also identified. This analysis also showed the importance of energy metabolism in starch biosynthesis with 15 DEPs found crucial in ensuring an adequate energy supply to sustain starch synthesis during endosperm development. Other than that, 11 DEPs were found to be involved in the partitioning of carbon flow between the various metabolic pathways which likely affects endosperm development. Lastly, eight DEPs were found to be involved in the endoplasmic reticulum (ER) protein processing which indirectly affects starch biosynthesis. This study has several potential applications. OsBam8b and plastidial Amy3 may have industrial applications such as in the baking, bio-alcohol, and animal feed industry. Meanwhile, based on its potential role as an amylase inhibitor, XIP has potential applications as a pesticide in agriculture and as a treatment for diabetes. In conclusion, this study has identified additional proteins with potential involvement in starch biosynthesis. Further studies to investigate the role of these proteins are needed as new findings will lead to new perspectives which may be vital for the elucidation of starch biosynthesis in plants. This will open up opportunities for crop improvement, which is essential for food security.

Keywords: Amyloplast, biosynthesis, isoamylase, proteomics, starch

Perbandingan Proteomik Endosperma Beras Untuk Penemuan Enzim Yang Berkaitan Dengan Biosintesis Kanji

ABSTRAK

Biosintesis kanji masih belum difahami sepenuhnya. Model semasa biosintesis kanji bertumpu kepada empat enzim utama, dengan pemahaman yang terhad tentang bagaimana enzim utama ini dan pelbagai isoforma mereka diselaras untuk mensintesis granul kanji. Selain enzim utama yang diketahui pada masa ini, terdapat potensi protein lain yang mungkin terlibat dalam biosintesis kanji dan masih belum ditemui. Analisis perbandingan proteomik EM653, sejenis mutan beras sugary, dan Taichung 65, jenis liarnya, telah dijalankan untuk mengenal pasti protein yang diekspreskan secara berbeza (DEP) yang mungkin terlibat dalam biosintesis kanji. Mutan EM653 kekurangan enzim isoamilase (Isa), salah satu enzim utama biosintesis kanji. Mutan ini mempamerkan fenotip sugary, di mana glikogen tumbuhan disintesis dan bukannya amilopektin. Kesan pleiotropik kekurangan Isa dalam beras sugary mempengaruhi ekspresi enzim biosintetik kanji yang berkait-rapat. Dua pendekatan perbandingan proteomik yang berpelengkap digunakan dalam kajian ini untuk mengenal pasti DEP dalam endosperma beras sugary dan beras biasa. Pendekatan senapang patah tanpa gel, kromatografi cecair dengan spektrometri jisim seiring, (LC-MS/MS) digunakan untuk menganalisis protein daripada pecahan amiloplastik yang diperkaya daripada endosperma beras sugary dan normal. Sejumlah 929 protein telah dikenal pasti, di mana 160 daripadanya diekspreskan secara berbeza. Sementara itu, pendekatan berasaskan gel, elektroforesis gel dua dimensi berpasang dengan LC-MS/MS (2DE-LC-MS/MS), digunakan untuk menganalisis protein endosperma dan 40 DEP telah dikenal pasti. Seterusnya, ekspresi gen tujuh gen sasaran dalam beras sugary dan beras biasa telah ditentukan melalui kaedah tindak balas rantai polimerase kuantitatif (qPCR)

menggunakan pendekatan kuantifikasi relatif. Didapati bahawa untuk tiga gen yang terlibat secara langsung dalam biosintesis kanji (Isa1, PPDK1 dan PPDK2), ekspresi mRNA dikaitkan dengan ekspresi protein. Seterusnya, analisis in silico DEP telah dijalankan untuk mengenal pasti protein yang mungkin terlibat dalam biosintesis kanji. Paling penting, kajian ini telah mengenal pasti dua amilase yang kurang diperincikan, OsBam8b dan plastidial Amy3, yang mungkin terlibat secara langsung dalam biosintesis kanji. Protein perencat xilanase (XIP) yang mungkin bertindak sebagai perencat amilase dengan potensi terlibat dalam metabolisme kanji turut dikenal pasti. Analisis ini juga menunjukkan kepentingan metabolisme tenaga dalam biosintesis kanji di mana 15 DEP didapati penting dalam memastikan bekalan tenaga yang mencukupi untuk mengekalkan sintesis kanji semasa perkembangan endosperma. Selain itu, 11 DEP didapati terlibat dalam pembahagian aliran karbon antara pelbagai laluan metabolik yang mungkin mempengaruhi perkembangan endosperma. Akhir sekali, lapan DEP didapati terlibat dalam pemprosesan protein oleh retikulum endosplasma (ER) yang secara tidak langsung menjejaskan biosintesis kanji. Kajian ini mempunyai beberapa aplikasi yang berpotensi. OsBam8b dan plastidial Amy3 mungkin mempunyai aplikasi industri seperti dalam industri pembuatan roti, bio-alkohol dan makanan haiwan. Sementara itu, berdasarkan peranan potensinya sebagai perencat amilase, XIP mempunyai aplikasi potensi sebagai racun perosak dalam pertanian dan sebagai rawatan untuk diabetes. Kesimpulannya, kajian ini telah mengenal pasti beberapa protein tambahan yang berpotensi terlibat dalam biosintesis kanji. Kajian lanjut mengenai peranan protein ini diperlukan kerana penemuan baharu akan membawa kepada perspektif baharu yang mungkin penting untuk menjelaskan biosintesis kanji dalam tumbuhan. Ini akan membuka peluang untuk penambahbaikan tanaman, yang penting untuk sekuriti makanan.

Kata kunci: *Amiloplast, biosintesis, isoamilase, kanji, proteomik*