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Targeting Analysis of Lumenal Proteins of Chloroplast of Wheat using Proteomic Techniques

Abu Hena Mostafa Kamal[1], Da-Eun Kim[1], Myoung-Won Oh[1], Keun-Yook Chung[2], Yong-Gu Cho[1], Hong-Sig Kim[1], Beom-Heon Song[1], Chul-Won Lee[1], Nobuyuki Uozumi[3], Jong-Soon Choi[4], Kun Cho[5] and Sun-Hee Woo[1]*

[1]Dept. of Crop Science,

[2]Dept. of Agricultural Chemistry, Chungbuk National University, Cheong-ju 361-763, Korea

[3]Dept. of Biomolecular Engineering, Graduate School of Engineering, Tohoku University, Sendai 980-8579 Japan

[4]Division of Life Science, Korea Basic Science Institute, Daejeon 305-333, Korea

[5]Mass Spectrometry Research Center, Korea Basic Science Institute, Ohchang, Chungbuk 863-883, Korea

*Corresponding author email: shwoo@chungbuk.ac.kr

Plastid proteomics are essential organelles present in virtually all cells in plants and green algae. Plastids are responsible for the synthesis and storage of key molecules required for the basic architecture and functions of plant cells. The proteome of plastid, and in particular of chloroplast, have received significant amounts of attention in recent years. Various fractionation and mass spectrometry (MS) techniques have been applied to catalogue the chloroplast proteome and its sub-organelles compartments. To better understanding the function of the lumenal sub-organelles within the thylakoid network, we have carried out a systematical analysis and identification of the lumenal proteins in the thylakoid of wheat by using Tricine-SDS-PAGE, and LTQ-ESI-FTICR mass spectrometry followed by SWISS-PROT database searching. We isolation and fractionation these membrane from fully developed wheat leaves using a combination of differential and gradient centrifugation couple to high speed ultra-centrifuge. After collecting all proteins to eliminate possible same proteins, we estimated that there are 407 different proteins including chloroplast, chloroplast stroma, lumenal, and thylakoid membrane proteins excluding 20 proteins, which were identified in nucleus, cytoplasm and mitochondria. A combination of these three programs (PSORT, TargetP, TMHMM, and TOPPRED) was found to provide a useful tool for evaluating chloroplast localization, transit peptide, transmembranes, and also could reveal possible alternative processing sites and dual targeting. Finally, we report also sub-cellular location specific protein interaction network using Cytoscape software, which provides further insight into the biochemical pathways of photosynthesis. The present work helps understanding photosynthesis process in wheat at the molecular level and provides a new overview of the biochemical machinery of the thylakoid in wheat.

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