HOKKAIDO UNIVERSITY

Title	Comprehensive analysis of the phosphoproteome dynamics responsible for carbon/nitrogen-nutrient availability and identification of a cell death related receptor-like kinase in Arabidopsis [an abstract of dissertation and a summary of dissertation review]
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Doctoral Dissertation Evaluation Review

Degree requested Doctor of Life Science, Applicant's name Xingwen Li

Examiner :

Chief examiner	Professor, Junji Yamaguchi
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Title of Doctoral Dissertation

Comprehensive analysis of the phosphoproteome dynamics responsible for carbon/nitrogen-nutrient availability and identification of a cell death related receptor-like kinase in *Arabidopsis*
(シロイヌナズナにおける炭素/窒素栄養バランスに応答したリン酸化タンパク質変動の網羅的解析と細胞死に関与する新規受容体型キナーゼの機能解析)

Results of Evaluation of the Doctoral Dissertation (Report)

Nutrient availability, in particular the availability of sugar (carbon (C)) and nitrogen (N), is important for the regulation of plant metabolism and development. In addition to independent utilization of C and N nutrients, plants sense and respond to the balance of C and N nutrients (C/N-nutrient) available to them. High C/low N-nutrient stress has been shown to arrest early post-germinative growth while promoting progression to senescence in Arabidopsis plants. Although several signaling components of the C/N-nutrient response have been identified, the inclusive molecular basis of plant C/N-nutrient response remains unclear. In this doctoral dissertation, I investigated the dynamic change of phosphorylation status of signaling proteins during plant C/N response. I Also identified a novel C/N responsible kinase, Leucine rich repeat Malectin Kinase 1 (LMK1), which has ability to induce programed cell death.

1. Functional networks of C/N response

To obtain a more comprehensive understanding on signaling pathway upon C/N response, I carried out phosphoproteome analysis to investigate the primary and global dynamics of C/N-nutrient related phosphorylation signals in Arabidopsis seedlings. Through the analysis, I identified 193 phospho-regulated proteins, which largely involve in various physiological progression. Based on this knowledge, I established the functional networks of the C/N-responsive phosphoproteins.

2. Identification of cell death related receptor-like kinase LMK1

Through my phosphoproteome analysis, I identified an LRR/Malectin RLK LMK1 as a novel C/N-nutrient related protein kinase. In the high C/ low N treatment, LMK1 can sense the unbalanced nutrient status and active autophosphorylation. LMK1 possess LRR and malectin domains at the extracellular domain (ECD) and categorized as LRR-RLKs family.

Interestingly, I also found the over-expression of LMK1 can induce kinase-dependent cell death in *N. Benthamiana*. ECD truncated forms of LMK1 showed remarkable suppression on cell death phenomenon, indicating that LRR domain and Malectin domain contribute in Cell death. The interaction in ECD with other regulator seems critical for LMK1 function. Interestingly, ECD also affect the stability of LMK1. Lacking ECD of LMK1 causes the protein accumulation in *N. Benthamiana,* indicating the degradation of LMK1 requires the other interactors.

Taken together, here I report through my phosphoproteome analysis, I identified phosphoproteins involves in plant C/N response and established the functional networks during C/N regulation, providing new insights of signaling pathway of carbon/nitrogen-nutrient availability.

In conclusion, the author has new findings of Plant signaling pathway of carbon/nitrogen-nutrient availability, and these will contribute to develop plant science.

Therefore, we acknowledge that the author is qualified to be granted a Doctorate of Life Science from Hokkaido University.