バイオサイエンス研究科　博士論文要旨

所属 （主指導教官）	遺伝子教育研究センター　植物細胞工学　（佐野　浩教授）		
氏名	Yap Yun Kiam (葉　婉　琴)	提出	平成　１４年　１月　８日
題目	Analyses of wound and pathogen signal transduction mediated by WIPK, a mitogen- activated protein kinase from tobacco. （WIPKを介したタバコ病傷害シグナル伝達機構の解析）		

要旨

Mitogen-activated protein (MAP) kinase cascade is a conserved pathway in all eukaryotes, which plays pivotal role in transducing extra-cellular signals to evoke various cellular and nuclear responses. It consists of 3 components, MAPKKK, MAPKK and MAPK; among which MAPK is the best characterized. Many members involved in this cascade have been isolated from Arabidopsis, tobacco, parsley, alfalfa and other plant species, and characterized in relation to cell regulation, various biotic and abiotic stresses.

The aim of this study is to investigate the regulation and function of WIPK, a wound-induced protein kinase from tobacco. It was first isolated by differential display from a cDNA library of tobacco leaves infected with tobacco mosaic virus (TMV), and reported to be transciptionally and/or post-translationally activated by mechanical wounding, elicitor treatments, and gene to gene specific pathogen response (Avr9/Cf-9). Analysis of WIPK over-expressing transgenic plants suggested that it played a role in transducing the extra-cellular signals through the jasmonic acid pathway, which subsequently activated the downstream defense genes. As *WIPK* is a multi-stress responsive gene, examination of its transcriptional regulation and protein function will serve as a good model to understand the complex stress signal pathways and the cross-talks within them.

In this study, I first conducted promoter analysis, which is described in chapter 1, to define cis-elements for wound- and pathogen responses and also to determine secondary signals that might be involved in the regulation of *WIPK* gene. In this chapter, I first described isolation of the promoter region, then construction of full-length and 5'-truncated promoter-GUS tobacco transgenic plants. Second, I analyzed the GUS expression of these plants to determine essential promoter regions.

Finally, I investigated effect of jasmonic acid and salicyclic acid on transcription regulation of *WIPK*. From these analyses, I determined a 430 bp promoter region which was sufficient to confer both wound- and pathogen responses to the reporter gene. In addition, I observed that WIPK was expressed exclusively around the wounded area and lesion area (leaves showing hypersensitive response to TMV infection), which supported its roles in these signal pathways. I also found that salicyclic acid (SA) was able to induce the expression of *WIPK*, which is the first report describing a secondary signal for *WIPK* gene induction.

In order to understand function of WIPK, I used a yeast two-hybrid screening to isolate proteins that interact with WIPK. In chapter 2, I described isolation and characterization of a positive clone that encoded a WIPK interacting protein, and designated *NtWAF* (*Nicotiana tabacum* WIPK-associated factor). *NtWAF* transcripts showed similar temporal induction profile as those of *WIPK* upon wounding and activation of hypersensitive response, which supported the roles of these proteins in both signal pathways. The N-terminal of NtWAF contained a putative B3 type DNA binding domain similar to those of Arabidopsis auxin responsive factor. Moreover, transient assay in BY2 cells also indicated that the middle region of NtWAF conferred trans-activation activity when fused with a GAL4 DNA binding domain. This implied it to be a putative transcription factor. Domain dissection analysis by yeast two-hybrid system indicated that the C-terminal of NtWAF interacted with WIPK and its own N-terminal, which suggested that the invivo interaction of these two proteins is a complex and highly regulated process. It was observed that the kinase deficient mutant of WIPK protein was unable to interact with NtWAF. Taken together these results, it is proposed that upon perception of extra-cellular signals, WIPK is phosphorylated, and interacts with NtWAF - a putative transcription factor, which then regulates other defense-related genes.

In conclusion, this study has provided new insights in the regulation and function of WIPK, which may play a critical role in defense signaling pathway. My finding will ultimately contribute to a better understanding of the regulation mechanisms of plant defense system.

論文審査結果の要旨

申請者氏名　Yap Yun Kiam (葉　婉　琴)

本研究では、タバコの病傷害初期応答遺伝子産物である WIPK の発現調節機構と標的タンパク質の同定をおこなった。WIPK は真核生物に広く保存される MAPK の一種で、タンパク質リン酸化経路の主要因子として、情報伝達系の要と言われる。

論文は2部構成になっている。第1章では WIPK プロモーターの解析をおこなった。WIPK 遺伝子のプロモーター領域を TAIL-PCR で単離し、全長（1122bp）および、3種類のディリーションクローンを作製した。LUC によるトランジエントアッセイと GUS によるトランスジェニック植物のアッセイを行った。その結果、転写開始点の上流 430bp 領域に、傷害と病原菌に応答するシスエレメントがあることをつきとめた。外見は異なる二つの情報系が、この領域に収斂し、WIPK を作動すること示唆された。第2章では、イースト two hybrid 系を用いて、WIPK に結合するタンパク質を同定した。NtWAF と名づけたこのタンパク質は 648 のアミノ酸からなり、明らかに異なる3つの領域からなる。C末端側には WIPK と結合する領域、中央には転写活性化領域、N末端側には DNA 結合領域が存在する。NtWAF はリン酸化された WIPK にのみ結合した。これらの解析を通して、WIPK は病傷害シグナルを受けて、リン酸化され、転写因子である NtWAF を活性化することが予想された。これらの研究を通して、MAPK の発現調節機構、転写因子との直接結合による転写活性調節機構などが明らかになった。

以上のように、本論文はこれまで知られていなかった、MAPK カスケードの下流に位置する調節因子の機能を明らかしたもので、学術上、応用上貢献するところが少なくない。よって審査委員一同は、本論文が博士（バイオサイエンス）の学位論文として価値あるものと認めた。