

# METHODS IN MOLECULAR BIOLOGY

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# Plant MAP Kinases

## Methods and Protocols

Edited by

**George Komis and Jozef Šamaj**

*Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research,  
Palacký University Olomouc, Olomouc, Czech Republic*

## Editors

George Komis  
Faculty of Science, Centre of the Region  
Haná for Biotechnological  
and Agricultural Research  
Palacký University Olomouc, Olomouc  
Czech Republic

Jozef Šamaj  
Faculty of Science, Centre of the Region  
Haná for Biotechnological  
and Agricultural Research  
Palacký University Olomouc, Olomouc  
Czech Republic

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*Cover illustration:* The cover image is a collage showing comparison of whole seedlings of *Arabidopsis thaliana* Columbia wild type (left image) and a mitogen activated protein kinase 6-2 mutant (*mpk6-2*; middle image). Contrasting of the roots with toluidine blue histochemical stain allows better visualization and documentation of differences in root patterning between wild type and mutant using binocular optics. The image on the right illustrates a part of living *Arabidopsis thaliana* root stably overexpressing the heterologous SIMKK of *Medicago sativa* tagged with yellow fluorescent protein (YFP; pseudocolored dark red). The root was counterstained with FM4-64 (pseudocolored blue) to delineate root cell borders and visualized with confocal laser scanning microscope. In this way two successive and juxtaposed lateral root primordia are visible, reflecting the impact of SIMKK-YFP overexpression on lateral root formation. All images were kindly provided by Dr. Miroslav Ovečka (Department of Cell Biology, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic).

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## Preface

The highly expanded family of mitogen-activated protein kinases (MAPKs) allows remarkable versatility in the adaptation and tolerance of plants to abiotic and biotic stresses when compared to other eukaryotes. Furthermore, MAPK signaling modules in model plants such as *Arabidopsis thaliana* are densely interconnected, often function redundantly, while different MAPK pathways may cross talk during a single signaling event. The above complexity as well as the central role of MAPK signaling in the reaction and adaptation to often unfavorable abiotic and biotic conditions have prompted the vigorous research necessary to uncover MAPK signaling networks, not only in the model plant *Arabidopsis* but also in important crops. Ever since the first description of plant MAPKs, the number of publications related to the topic increases in an exponential manner. For the above reasons, we believe that the time is ripe to provide a central source of proofread and exhaustively troubleshoot protocols that will encompass the entire array of experimental resources necessary for either the novice or the expert researcher.

The present book entitled “Plant MAP Kinases: Methods and Protocols” from the Methods in Molecular Biology™ series addresses the complexity of conditional and developmentally important plant MAPKs at many levels. Technically, the contents cover a wide array of techniques and methods used in MAPK research as these were contributed by experts of each method described.

The experimental survey of the plant MAPK world in the Part I is devoted to the collection of protocols aiming to interrogate MAPK function, and it starts with a robust transient expression system using *Arabidopsis* mesophyll protoplasts (Chapter 1). The following chapter (Chapter 2) addresses the MAPK transcriptional regulation during abiotic and biotic stresses with quantitative real-time PCR explained to thorough detail. Next three chapters (Chapters 3–5) are dedicated to the assessment of MAPK phosphorylation/activation and function by nonradioactive means. Chapter 3 demonstrates the efficient use of phospho-specific antibodies that were originally raised against mammalian MAPKs, in order to follow temporal and dose-dependent activation of MAPKs. The other two chapters employ two different electrophoretic approaches which allow the efficient discrimination of phosphorylated and non-phosphorylated protein forms in one dimension. This can be achieved by either one-dimensional isoelectric focusing (Chapter 4) or phospho-affinity-based denaturing SDS-PAGE (Chapter 5). Part 2 encompasses protocols discovering function of MAPK signaling by genetic tools including the engineering of constitutively active MAPKs (Chapter 6), the silencing of MAPKs by either virus-induced silencing (Chapter 7) or RNA interference (Chapter 8). In Part 3 effort is made to put MAPK signaling at the cellular context. Thus MAPKs are immunolocalized in either root whole-mount samples (Chapter 9) or Steedman wax sections (Chapter 10), while strategies for their in vivo imaging as well as for the subcellular visualization of their interactions are presented in following respective chapters (Chapters 11 and 12). Part IV surveys approaches to identify and study MAPK substrates. Thus, Chapter 13 shows the design of experimental work necessary to identify phosphorylation sites in putative MAPK substrates using as an example the microtubule-associated protein MAP65-1. Next, a strategy to generate phospho-specific

antibodies against verified substrates such as the WRKY transcription factors is presented (Chapter 14). Finally, a mutational approach towards the identification of MAPK substrates is aimed to uncover previously unknown targets of MAPK signaling (Chapter 15). The last part of the book tops up MAPK research and brings into light large-scale protocols. These will provide strategies for high-throughput screening of MAPK interactors by yeast two-hybrid technique (Chapter 16) or by protein microarrays (Chapter 17). Chapter 18 provides the protocol for tandem affinity purification of MAPK complexes. Finally, Chapter 19 describes iTRAQ for the enrichment of phosphoproteins which will allow the mass identification of MAPK targets.

On the side of the “Plant MAP Kinases: Methods and Protocols” the reader will find classical protocols that accompany MAPK research, including immunocomplex and in-gel kinase assays as well as redundant information of thoroughly described workflows including plant handling, work with transgenes and standard biochemical techniques such as co-immunoprecipitation, polyacrylamide gel electrophoresis, and western blotting to name a few. We trust therefore that all individual chapters are autonomous and can be used as a bench-side aid to researchers irrespective of the level of experience.

We are grateful to all 54 authors who contributed to the content of the present volume. Each author disclosed his/her experience in each respective chapter, but also provided critical troubleshooting—Notes—representing important sections for the novice reader. After all even the most ambitious experiment may fail due to the tiniest detail. We warmly acknowledge Professor John M. Walker, series editor, who apart from honoring us with the invitation to host the present volume also provided enthusiastic support throughout the entire editing procedure. Finally, we extend our thanks to the members of the Methods in Molecular Biology™ Springer editorial team for guiding us through the assembly of a useful book.

*Olomouc, Czech Republic*

*George Komis  
Jozef Šamaj*

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## Contributors

- HIROAKI ADACHI • *Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan*
- JEFFREY C. ANDERSON • *Department of Biochemistry, University of Missouri, Columbia, MO, USA; Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO, USA; Interdisciplinary Plant Group, University of Missouri, Columbia, MO, USA*
- TOMOYA ASANO • *Division of Functional Genomics, Advanced Science Research Center, Kanazawa University, Kanazawa, Japan*
- IAN T. BALDWIN • *Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Hans-Knoell, Germany*
- FRANTIŠEK BALUŠKA • *Department of Plant Cell Biology, Institute of Cellular and Molecular Botany, University of Bonn, Bonn, Germany*
- NICOLE BAUER • *Department of Stress and Developmental Biology, Leibniz Institute of Plant Biochemistry, Halle/Saale, Germany*
- SLÁVKA BEKEŠOVÁ • *Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- SOUHA BERRIRI • *Cell and Developmental Biology, Jones Innes Centre, Norwich Research Park, Norwich, UK*
- JEAN BIGEARD • *URGV Plant Genomics, UMR INRA-1165, CNRS-ERL8196, Université d'Evry Val d'Essonne, Evry Cedex, France*
- ELIZABETH K. BRAUER • *The Boyce Thompson Institute for Plant Research, Ithaca, NY, USA; Department of Plant Pathology and Plant Microbe Biology, Cornell University, Ithaca, NY, USA*
- JEAN COLCOMBET • *URGV Plant Genomics, UMR INRA-1165, CNRS-ERL8196, Université d'Evry Val d'Essonne, Evry Cedex, France*
- SARMINA DANGOL • *Department of Molecular Biology, College of Life Sciences, Sejong University, Seoul, Republic of Korea*
- ARMIN DJAMEI • *Gregor Mendel Institute of Molecular Plant Biology (GMI), Vienna, Austria*
- ANNA DOSKOČILOVÁ • *Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- LENNART ESCHEN-LIPPOLD • *Department of Stress and Developmental Biology, Leibniz Institute of Plant Biochemistry, Halle/Saale, Germany*
- LOÏC GÉRARD • *URGV Plant Genomics, UMR INRA-1165, CNRS-ERL8196, Université d'Evry Val d'Essonne, Evry Cedex, France*
- ANDREA A. GUST • *Department of Plant Biochemistry, ZMBP, University of Tübingen, Tübingen, Germany*
- DANIEL J. HAISCHER • *Department of Plant Biochemistry, ZMBP, University of Tübingen, Tübingen, Germany*
- CHRISTIAN HETTENHAUSEN • *Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China*

- HERIBERT HIRT • *URGV Plant Genomics, UMR INRA-1165, CNRS-ERL8196, Université d'Evry Val d'Essonne, Evry Cedex, France*
- ELODIE HUDIK • *Institut de Biologie des Plantes, UMR CNRS-8618, Plateau du Moulon de l'Université Paris Sud, Orsay Cedex, France*
- JONG HEE IM • *Department of Life Sciences, College of Life Sciences and Biotechnology, Korea University, Seoul, South Korea*
- NOBUAKI ISHIHAMA • *RIKEN Center for Sustainable Resource Science (CSRS), Tsurumi, Yokohama, Japan*
- NAM-SOO JWA • *Department of Molecular Biology, College of Life Sciences, Sejong University, Gunja-dong, Gwangjin-gu, Republic of Korea*
- VAIVA KAZANAVICIUTE • *Institute of Biotechnology (IBT), University of Vilnius, Vilnius, Lithuania*
- GEORGE KOMIS • *Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- PAVEL KŘENEK • *Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- JUSTIN LEE • *Department of Stress and Developmental Biology, Leibniz Institute of Plant Biochemistry, Halle/Saale, Germany*
- JULIA LÖHR • *Department of Stress and Developmental Biology, Leibniz Institute of Plant Biochemistry, Halle/Saale, Germany*
- IVAN LUPTOVČIAK • *Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- IRUTE MESKIENE • *Max F. Perutz Laboratories, University and Medical University of Vienna, Vienna, Austria; Institute of Biotechnology (IBT), University of Vilnius, Vilnius, Lithuania*
- HAKIM MIREAU • *Institut Jean-Pierre Bourgin, UMR1318 INRA-AgroParisTech, INRA Centre de Versailles-Grignon, Versailles Cedex, France*
- TAKUMI NISHIUCHI • *Division of Functional Genomics, Advanced Science Research Center, Kanazawa University, Kanazawa, Japan*
- MIROSLAV OVEČKA • *Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- MIEDER A.T. PALM-FORSTER • *Department of Stress and Developmental Biology, Leibniz Institute of Plant Biochemistry, Halle/Saale, Germany*
- SCOTT C. PECK • *Department of Biochemistry, University of Missouri, Columbia, MO, USA; Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO, USA; Interdisciplinary Plant Group University of Missouri, Columbia, MO, USA*
- DELPHINE PFLIEGER • *Laboratoire Analyse et Modélisation pour la Biologie et l'Environnement, CNRS UMR, Université d'Evry Val d'Essonne, Evry, France*
- GEORGE V. POPESCU • *The Boyce Thompson Institute for Plant Research, Ithaca, NY, USA; National Institute for Laser, Plasma and Radiation Physics, Bucharest, Romania*
- SORINA C. POPESCU • *The Boyce Thompson Institute for Plant Research, Ithaca, NY, USA*
- JOZEF ŠAMAJ • *Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- OLGA ŠAMAJOVÁ • *Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- ALOIS SCHWEIGHOFER • *Max F. Perutz Laboratories, University and Medical University of Vienna, Vienna, Austria; Institute of Biotechnology (IBT), University of Vilnius, Vilnius, Lithuania*

- VOLODYMYR SHUBCHYNSKY • *Max F. Perutz Laboratories, University and Medical University of Vienna, Vienna, Austria*
- RAKSHA SINGH • *Department of Molecular Biology, College of Life Sciences, Sejong University, Gunja-dong, Gwangjin-gu, Republic of Korea*
- VERONIKA SMÉKALOVÁ • *Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic*
- ANDREI SMERTENKO • *Institute of Biological Chemistry, Washington State University, Pullman, WA, USA*
- TOMÁŠ TAKÁČ • *Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- PAVOL VADOVIČ • *Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*
- ROLAND WILLMANN • *Department of Plant Biochemistry, ZMBP, University of Tübingen, Tübingen, Germany*
- JIANQIANG WU • *Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China*
- JUAN XU • *State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, China*
- SANG-DONG YOO • *Department of Life Sciences, College of Life Sciences and Biotechnology, Korea University, Seoul, South Korea*
- HIROFUMI YOSHIOKA • *Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan*
- MIKI YOSHIOKA • *Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan*
- SHUQUN ZHANG • *State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, China; Division of Biochemistry, Interdisciplinary Plant Group, and Bond Life Sciences Center, University of Missouri, Columbia, MO, USA*