Brassinosteroids are naturally occurring endogenous plant hormones indispensable for growth and development. Since their discovery, intense research based on genetic analysis of brassinosteroid-deficient and brassinosteroid-insensitive mutants, and on proteomics and genomics mainly in the model plant species *Arabidopsis thaliana* and *Oryza sativa* (rice) have yielded a comprehensive knowledge on the brassinosteroid signal transduction pathway, from receptor kinases to transcription factors and their targets, so that it has become one of the best characterized plant hormone pathways. Although originally described as linear, the brassinosteroid signal transduction pathway is highly interconnected with other signaling pathways, including auxin, gibberellic acid, abscisic acid, light, and sugar, thus supporting the notion that brassinosteroids are master regulators of plant growth. The brassinosteroid signal transduction pathway also shares components of and interacts with other receptor kinase pathways that regulate immunity and stomatal, root, and reproductive development. The prospective agronomical importance of the brassinosteroid hormones requires active research in plant species other than *Arabidopsis*, but preliminary results hint at a good level of transferability between dicotyledonous and monocotyledonous plant species, regarding the effects of brassinosteroids on plant architectural traits. Therefore, a potential exists to identify brassinosteroid mutants or transgenic plants with improved productivity in agronomically important crops, such as maize (*Zea mays*), wheat (*Triticum* sp.), sorghum (*Sorghum bicolor*), soybean (*Glycine max*), potato (*Solanum tuberosum*), poplar (*Populus* sp.), and tomato (*Solanum lycopersicum*).

Despite the important advances made in the brassinosteroid field, a number of essential questions remain to be answered. Research focused on resolving specific brassinosteroid signaling pathways for different cellular lineages has just started to develop. The advent of single-cell genomics technologies stands as a promise for the rapid identification of brassinosteroid pathways with single-cell resolution. Whereas the long-distance transport of brassinosteroid hormones has been ruled out, the mechanisms for brassinosteroid short-range movement remain a mystery. How brassinosteroid molecules are secreted from the cell where they are synthesized also needs to be clarified, because it is a prerequisite to elucidate the cell-to-cell signaling governed by the hormones.

In this book, we compiled state-of-the-art methodologies for the study of brassinosteroid hormones. In 16 chapters, recognized researchers in the brassinosteroid field bring together different experimental and theoretical biology methods for addressing the questions of how brassinosteroids function in *Arabidopsis* and other agriculturally valuable species, such as rice and sorghum. The topics cover a wide range of protocols to analyze brassinosteroid levels and synthesize fluorescently labeled brassinosteroid analogs for monitoring brassinosteroids in living cells (Chapters 1 and 2). We present detailed procedures for physiological analysis of brassinosteroid responses in *Arabidopsis* and in rice to study the crosstalk with other signaling pathways, such as light and immunity (Chapters 3, 4, 5, and 6). Chapters 7 and 8 describe genomic methods for the identification of brassinosteroid-regulated
genes and networks in the whole plant or in specific tissues. This book also contains mathematical modeling approaches to study the dynamics of brassinosteroid signaling components (Chapter 9) and quantitative microscopy methods to monitor the relative levels of the BRASSINOSTEROID INSENSITIVE1 (BRI1) protein in the plasma membrane (Chapter 10). We included biochemical and mass spectrometry protocols for purification and identification of brassinosteroid signaling components (Chapters 11 and 12) and for the analysis of the posttranslational modifications of the BRI1 receptor (Chapters 13 and 14). Furthermore, the potential of brassinosteroid molecules in agriculture is shown in Chapter 15, in which sorghum is proposed as a model crop species for the study of brassinosteroid signaling in root growth and development. Protocols to assess brassinosteroid effects on abiotic stress tolerance in Arabidopsis and Brassica napus (rapeseed) are presented in Chapter 16. We hope this book will not only serve as an ideal reference for researchers in the brassinosteroid field but also assess a wide range of appealing methodologies for researchers with a general interest in hormone signaling in plants. We thank all our colleagues who contributed to this book and Martine De Cock for help in editing the manuscripts.

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