

METHODS IN MOLECULAR BIOLOGY™

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Molecular Plant Taxonomy

Methods and Protocols

Edited by

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Preface

Plant taxonomy is an ancient discipline nowadays facing new challenges with the availability of a vast array of molecular approaches allowing reliable genealogy-based classifications. Although the primary focus of plant taxonomy is on the delimitation of species, molecular approaches also provide a better understanding of evolutionary processes, a particularly important issue for some taxonomic complex groups. This book describes laboratory protocols based on the use of nucleic acids and chromosomes for plant taxonomy, as well as guidelines for phylogenetic analysis of molecular data. It also provides introductory and application review chapters, which are of great importance to put the protocols into a broader perspective.

A thorough historical overview of plant taxonomy is provided in the introductory chapter by *Germinal Rouban* and *Myriam Gaudeuil* (Chapter 1). Strengths, limitations and the future of molecular techniques with regard to plant taxonomy are also explored, as compared to the use of classical morphological and anatomical data. Guidelines are then given by *Pascale Besse* (Chapter 2) to choose the best appropriate molecular technique depending on the plant taxonomic survey envisaged. Both chapters are prerequisite readings to understand the concepts underlying the “plant taxonomy” discipline and to fully appreciate the strengths and limits of each molecular technique presented in this book.

One of the advantages of molecular techniques for plant taxonomy is that analyses can be performed at early developmental stages, from living plant material as well as from voucher herbarium specimens. This allows an “integrative” approach combining modern molecular data with taxonomic description of reference species. Two chapters describe protocols for DNA extraction. *Kessa Semagn* (Chapter 3) provides various leaf tissue sampling methods particularly handy for field collection and a reliable DNA extraction protocol. *Lenka Závěská Drábková* (Chapter 4) proposes various DNA extraction protocols specifically designed for dried herbarium specimens.

Chapters 5–7 present protocols for classical sequencing of various genomic sequences commonly used in plant taxonomic and phylogenetic studies. Chloroplast DNA is one of the most commonly used DNA in plant taxonomy, *Berthold Heinze*, *Agnieszka Koziel-Monte* and *Daniela Jahm* (Chapter 5) describe primers and protocols used for the sequencing of chloroplast DNA as well as other useful methods (PCR-RFLP and dHPLC). Although mitochondrial DNA was given less attention as a target for plant taxonomy, *Jérôme Duménil* (Chapter 6) demonstrates how useful such DNA regions can be to resolve taxonomic issues and provide a detailed sequencing protocol. Finally, the internal transcribed spacer (ITS) of the nuclear ribosomal RNA genes is a region of choice to be sequenced in taxonomic studies. The advantages and possible drawbacks of using this region are presented, and a sequencing protocol is provided by *Pascale Besse* (Chapter 7). Recently, the availability of new DNA sequencing (NGS—next generation sequencing) and high-throughput genotyping methods

has opened a new era for plant molecular taxonomy. *David Edwards, Manuel Zander, Jessica Dalton-Morgan* and *Jacqueline Batley* (Chapter 8) provide detailed protocols for Single Nucleotide Polymorphisms (SNPs) discovery and genotyping.

Simple and reliable PCR-based methods other than direct sequencing can be utilized to provide useful molecular markers for resolving plant taxonomic issues. They rely on polymerase chain reaction (PCR) amplification of specific or anonymous regions in the genome and their analysis following electrophoretic size-fractioning to reveal size (length) variations. The isolation of microsatellite loci and their study (by PCR amplification) is a very important component of the molecular tool-kit available for plant taxonomy, particularly at low taxonomic levels (generally at the population level). *Hélène Vignes* and *Roman Rivallan* (Chapter 9) provide a protocol to isolate such regions through the construction of microsatellite-enriched libraries. Relatively high-throughput multi-locus genotyping methods are also available. Some of these methods target anonymous regions of the genome without the need for any prior knowledge on any of the taxon DNA sequences. *Kantipudi Nirmal Babu, Muliya Krishna Rajesh, Kukkumgai Samsudeen, Divakaran Mino, Erinjery Jose Suraby, Kallayan Anupama and Paul Ritto* (Chapter 10) describe protocols for the simplest of these methods: randomly amplified polymorphic DNA (RAPD) as well as more recent derived techniques. *Luis F. Goulao* and *Cristina M. Oliveira* (Chapter 11) provide detailed protocols for other methods such as amplified fragment length polymorphism (AFLP) and derived techniques based on simultaneous microsatellite amplification such as inter simple sequence repeats (ISSR) and selective amplification of microsatellite polymorphic loci (SAMPL). *Ruslan Kalendar* and *Alan Schulman* (Chapter 12) describe the usefulness and protocols for multi-locus tagging of LTR (long terminal repeats)-retrotransposons present in plant genomes such as inter-retrotransposon amplification polymorphism (IRAP), retrotransposon-microsatellite amplification polymorphism (REMAP) and inter-Primer Binding Site Polymorphism (iPBS).

Finally, *Alexandre De Bruyn, Darren P. Martin* and *Pierre Lefevre* (Chapter 13) provide detailed and step-by-step guidelines to the analyses of molecular data using phylogenetic reconstruction methods based on DNA sequences, using freely available computer programs.

As hybridization and polyploidization are very important components of plant speciation particularly for some plant groups, specific cytogenetic techniques may need to be developed in addition to molecular studies, as detailed in Chapters 14–16. *Jaume Pellicer* and *Ilia J Leitch* (Chapter 14) provide protocols for plant genome size estimation using flow cytometry, a technique that can be combined with direct chromosomal observations as in fluorochrome banding and FISH (fluorescent in situ hybridization), for which detailed protocols are given by *Sonja Siljak-Yakovlev, Fatima Pustahija, Vedrana Vicic* and *Odile Robin* (Chapter 15). These can help to determine ploidy level and assess genome organisation. Moreover, *Nathalie Piperidis* (Chapter 16) details the GISH (genomic in situ hybridisation) protocol, a powerful technique that can be used to resolve the parental origin of inter-specific and inter-generic hybrid plant species.

The final two chapters are examples of applications on the use of molecular data for the taxonomic revision of some plant groups: Malvaceae, by *Timothee Le Péchon* and *Luc Gigord* (Chapter 17) and Proteaceae, by *Peter H. Weston* (Chapter 18). These provide a clear illustration of the exciting contributions molecular approaches can make to plant taxonomy.

The reviews and protocols that appear as chapters in this book were selected to provide conceptual as well as technical guidelines to plant taxonomists and geneticists. Despite the present craze for “DNA barcoding”, it is now clear that using solely the two chloroplastic genes *matK* and *rbcL* for resolving plant taxonomy will not be sufficient, particularly in

some plant groups. We rather highly recommend that molecular techniques are used in an “integrative taxonomy” approach, combining nucleic acid and cytogenetic data together with other crucial information (taxonomy, morphology, anatomy, ecology, reproductive biology, biogeography, paleobotany), which will help not only to best circumvent species delimitation but as well to resolve the evolutionary processes in play. This is of great importance as speciation is indeed a dynamic process.

Ile de la Réunion, France

Pascale Besse

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