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Marker-Assisted Plant Breeding: Principles and Practices

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ISBN 978-81-322-2315-3 ISBN 978-81-322-2316-0 (eBook)
DOI 10.1007/978-81-322-2316-0

Library of Congress Control Number: 2015943502

Springer New Delhi Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer (India) Pvt. Ltd is part of Springer Science+Business Media (www.springer.com)

To
Prof. M.S. Swaminathan,
who reshaped agricultural research in India
and inspired a whole generation of plant scientists

Foreword

Plant breeding is the discipline that fashioned our crop plants out of the wild weedy species and continues its endeavor to modify their genotypes to enhance their performance and usefulness to the changing human needs and climate conditions. In the past, the new genotypes developed by plant breeders have been considerably successful in keeping pace with the growing global food needs and consumer preferences. For example, the evolution of hybrid varieties and semi-dwarf cereal genotypes has contributed to quantum jumps in crop productivity, and the latter was responsible for the 'green revolution' that made countries like India virtually self-sufficient in their food grain requirements within a short span of a few years.

The world population is increasing at a rapid rate and is expected to go past the nine billion mark by the year 2042. In addition, the nature and the relevance of both abiotic and biotic stresses are undergoing unrelenting changes in the wake of the environmental alterations engendered by climate change and global warming. In view of these, it is necessary not only to continue to evolve crop genotypes with higher yield potential and tolerance to the various prevailing stresses but also to develop them at a much faster pace. The plant breeders thus face unprecedented challenges of harnessing the reservoirs of genetic variability present in the unadapted germplasm with the minimum investment of time and in a highly precise and predictable manner.

Traditional plant breeding methods rely on phenotype-based selection, but phenotypic evaluation of many traits is problematic, unreliable or expensive. Also, the usefulness of trait phenotypes of individuals/lines in predicting the performance of their progeny is questionable. In addition, the conventional breeding methods do not allow the use of desirable genes from related species in an efficient manner, and there is always the risk of linkage drag. Plant breeders have always been trying to develop breeding strategies that would make their selections more effective and reliable and that would facilitate the utilization of unadapted germplasm with the minimum risk of linkage drag. One of the options that was pursued with some success was the use of simply inherited traits for an indirect selection for complex traits. This effort led to the discovery of protein-based markers and, eventually, the DNA-based markers.

Since the deployment of RFLPs in biological studies, several user-friendly DNA markers like SSRs and SNPs have been developed. The current

emphasis is on technologies that permit low-cost, high-throughput genotyping using molecular markers. Markers are being increasingly used for marker-assisted selection to facilitate gene introgression and for accelerated recurrent selection with the use of off-season nurseries and greenhouse facilities. In addition, markers have found applications in many other plant breeding activities like diversity analysis, germplasm characterization, hybrid seed lot genetic purity determination, elucidation of heterosis loci, etc. In view of the increasing integration of markers in plant breeding programs, many universities have introduced courses on marker-based plant breeding. There is, therefore, an urgent need for a book covering the various aspects relevant to the use of markers in plant breeding.

The book 'Marker-Assisted Plant Breeding' is designed to provide up-to-date information on molecular markers and their applications. The authors have attempted to provide sufficient basic information in an easily understandable narrative so that even the beginners have little difficulty in following the subject. This book will also be useful to teachers, breeders and research workers since it makes available at one place the current information on the various aspects of the subject. The development of different molecular markers and their various applications are described in a simple language, and in a clear and easily comprehendible manner. In the first chapter, the field of marker-assisted plant breeding is introduced and placed in the proper perspective in relation to plant breeding. The next three chapters describe the various molecular marker systems, while mapping populations and mapping procedures, including high-throughput genotyping and association mapping, are discussed in the subsequent five chapters. Four chapters are devoted to various applications of markers, while the last two chapters provide information about relevant bioinformatics tools and phenomics.

The authors deserve compliments for conceiving this book and for developing this concept into a useful and informative book. I am confident that the students, teachers and the professional plant breeders will find this book to be of considerable usefulness as it provides a wealth of information at one place. The book assumes contemporary relevance and importance, since varieties breed with the help of marker-assisted selection are eligible for certification under organic farming.

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Prof. M.S. Swaminathan

Preface

Improved genotypes developed by plant breeding remain pivotal to global food security. In the wake of ever-increasing human population, declining agricultural resources and the stresses generated by climate change, plant breeding is expected to make larger contributions in increasingly shorter time frames. Therefore, plant breeding methods and schemes would have to be made more efficient and capable of accelerated variety development, say, by making efficient use of off-season nurseries, greenhouse facilities and innovative breeding methods. One of the chief limitations of plant breeding is the low effectiveness of phenotypic selection for many traits, particularly the quantitative traits. Further, selection for many other traits is tedious, problematic, time consuming and/or poorly reliable due to threshold requirements, difficulties in assay procedures and phenotype measurement, etc. Breeders have long been searching for tools that would permit effective indirect selection for such traits. Oligogenic phenotypic traits were the first to be used for this purpose, followed by protein-based/isozyme markers. However, the chief limitation of the above marker systems was the limited availability of good informative markers closely linked to the traits of interest.

In 1980, Botstein and coworkers proposed the use of restriction fragment length polymorphism (RFLP) for linkage mapping in humans. RFLP soon emerged as the first DNA-based molecular marker system, and it was used for the preparation of marker linkage maps and for the mapping of several traits of interest in many crops. The greater abundance and other desirable features of RFLPs as compared to phenotypic and protein markers, prompted the development of other relatively more convenient DNA marker systems like random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), etc. Single nucleotide polymorphism (SNP) has emerged as the most abundant molecular marker that is amenable to high-throughput genotyping. Each of these marker systems offers some advantages and suffers from certain limitations.

Molecular markers provide a tool for identifying genomic regions involved in the control of traits of interest. They also facilitate selection for the target genomic regions on the basis of marker genotype rather than the phenotype of the concerned trait. The reliability of such indirect selection depends mainly on the strength of linkage between the marker and the genomic region of interest. Therefore, markers located within the genes of interest, particularly those associated with allelic differences with respect to

trait phenotype (the functional nucleotide polymorphism, FNP, being the ultimate), would be the most informative and useful. However, the practical usefulness of MAS will be primarily determined by the relative cost of marker development, identification of trait-linked markers and marker genotyping in the breeding populations as compared to the direct trait phenotype-based selection.

The first step in the use of markers for MAS is the identification of markers tightly linked to the traits of interest. Ordinarily, a suitable mapping population needs to be constructed to identify the linked markers by linkage mapping. Several different types of mapping populations, ranging from simple F_2 through recombinant inbred lines to multi-parent advanced generation intercross (MAGIC) and interconnected populations can be used for linkage analyses. Alternatively, a collection of germplasm lines/individuals from natural populations can be used for linkage disequilibrium-based association mapping. In addition, the rich genomic resources that are now becoming available for most crops of interest can be analysed for marker identification.

Molecular mapping of oligogenes is relatively simple, while that of quantitative trait loci (QTLs) poses many problems, and the results from mapping studies are affected by a variety of factors, including the genetic model and the statistical algorithm used for QTL analysis. Generally, different QTLs governing the same trait are identified from different studies and consensus QTLs need to be identified by QTL meta-analysis. In addition, for a reliable detection of association/linkage between markers and traits, the trait phenotypes have to be measured precisely and reliably; a discipline called phenomics devoted to large-scale precision phenotyping is currently the area of intensive research activity.

Molecular markers tightly linked to the desired traits can be used for MAS to select for the genes governing the concerned traits, recover the recurrent parent genotype in backcross programs as well as to eliminate linkage drag, wherever required. Innovative breeding schemes are being designed to facilitate an efficient utilization of resources and to maximize gains from the marker technology. For example, marker-assisted recurrent selection (MARS) is being used for the improvement of quantitative traits, including yield, and up to three generations can be raised in a single season using off-season nurseries or a phytotron. The comprehensive scheme of genomic selection has been proposed for the selection of all genomic regions influencing the traits of interest, whether or not they show significant association with the trait phenotype. An ambitious breeding scheme, breeding by design, has been proposed to accumulate all the positive alleles for all the relevant traits into a single genotype that may be expected to have an outstanding performance. Similarly, a reverse breeding scheme for isolation of complementing inbred pairs from any heterotic hybrid combination has been patented.

Molecular markers have found a variety of other applications, including genetic diversity analysis, phylogenetic studies and construction of heterotic pools. Markers enable unambiguous identification of lines/varieties and facilitate seed certification and PBR (plant breeder rights) implementation. Tightly linked markers provide the basis for fine mapping and positional cloning of genes, which enables generation of information on gene function and regulation, as well as production of transgenic lines expressing the traits of interest.

A successful integration of molecular marker technology in plant breeding would require a low-cost, user-friendly marker systems amenable to high-throughput marker genotyping. Considerable effort is currently focused on the development of low-cost marker identification and genotyping platforms, including genotyping strategies that reduce the volume of genotyping work and/or combine marker discovery with marker genotyping without greatly sacrificing the amount of information obtained. The exciting developments in the above areas are generating new information and concepts/ideas with concomitant creation of specialized terms and phrases that together constitute the discipline of 'marker-assisted plant breeding'.

The chief constraints that limit the integration of molecular markers as a common tool in plant breeding are relatively higher cost of marker genotyping, and the fact that marker technology may appear unfamiliar to those trained in conventional plant breeding. There is continuous generation of new information, concepts/ideas and, inevitably, terminology related to molecular markers and their applications for achieving plant breeding objectives. Further, the marker technology has triggered innovations in breeding strategies and methods and has necessitated the creation of statistical and bioinformatics tools for data processing to facilitate their use for timely decision making. Plant breeding students need to be exposed to the various concepts, procedures and techniques relevant to the field in order to be able to appreciate the opportunities and the limitations of various options offered by the marker technology. It is encouraging that most educational institutions are introducing courses devoted either fully or partly to molecular markers.

The book 'Marker-Assisted Plant Breeding, Principles to Practice' is designed for such students who have had little or no exposure to molecular markers, but have a basic knowledge of genetics and plant breeding, and some exposure to molecular biology. This book will also be useful for teachers, research workers and practicing plant breeders. We have attempted to explain the basic principles, procedures and techniques of marker technology and provide, in brief, the up-to-date information on various aspects in a clear and easily comprehendible manner. Figures and line drawings are provided to highlight the chief features of important procedures/schemes/concepts with a view to facilitate their understanding by the students. In the first chapter, the field of marker-assisted plant breeding is introduced and placed in the proper perspective in relation to plant breeding. The next three chapters describe the various molecular marker systems, while mapping populations and procedures, including high-throughput genotyping and phenotyping, are discussed in the following five chapters. Four chapters are devoted to various applications of molecular markers, including MAS, diversity analysis, positional cloning, etc. The last two chapters provide information about relevant bioinformatics tools and phenomics.

Varanasi, UP, India — Brahma Deo Singh
New Delhi, Delhi, India — Ashok Kumar Singh
November 25, 2014

Acknowledgements

We wish to acknowledge the valuable help received from several of our colleagues and many of our research scholars. Prof. Umesh Singh and Prof. A.M. Kayastha, Varanasi, Dr. Anjana Jajoo, Indore, Dr. Sanjeev Kumar and Dr. Kusum Yadav Lucknow, Dr. K.K. Vinod, Aduthurai, Dr. Vinay, Kumar, Hyderabad, Dr. J.K. Joy, Mohali, Dr. Gopala Krishnan, Dr. Shailesh Tripathi, Dr. Neelu Jain, Dr. M. Vignesh, Dr. Shailendra K. Jha, Dr. Ramya Kurian, Dr. Renu Singh and Ms. Prachi Yadav, New Delhi, and Dr R.K. Sharma, Palampur read parts of the manuscript and/or proof, and suggested very useful additions and alterations. Our research scholars Mallikarjuna, Haritha, Ranjith, Sateesh, Fiyaz, Niranjana, and Naresh, New Delhi, and Vinay K. Singh and Reena Deshmukh, Varanasi, assisted us in a variety of ways, including finding of a suitable flavour for the text, checking for format, style spelling, grammatical errors, etc.

Dr. Kusum Yadav, Lucknow, provided PC scatter plots and the gel image, Dr. Balram Marathi provided the LOD score curves for SIM and CIM, and Reena Deshmukh provided images of multiple sequence alignment and rooted and unrooted trees; these contributions have been suitably acknowledged at the appropriate places. The efforts of Ranjith deserve a special mention as he has meticulously generated and later corrected the line drawings for the entire book.

Several of our colleagues and numerous students suggested that we should develop a book on marker-assisted plant breeding; this was one of the reasons for our decision to undertake this effort. We appreciate that Prof. Kole encouraged us to contact Springer for the publication of this book. We would also like to record our happiness for Springer's decision to publish this book and are thankful to all those involved in this process.

In the end, we are highly appreciative of the affection, support and encouragement we have always received from our family members, including our wives, sons and daughters.

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