Chapter 1

Introductory Chapter: New Age Molecular Techniques in Plant Science

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Additional information is available at the end of the chapter

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1. Introduction

Plants are valuable research objects. Their life spans can reach up to 5000 years, and their survival potential under extreme conditions makes them more interesting. Knowledge about plants has maturated and deeper research areas have been generated.

After commercialization of first transgenic plant, agricultural revolution has been started. Biotechnological improvements have been rapidly integrated into agricultural technologies in response to the global needs.

Plant breeding is an application that changes the plant genetics to thousands of genes, crossing varieties and then selecting the new varieties (and genes) that are desired. In this intertwined event, the plant breeder crosses to ensure that the desired traits are gathered in sufficient numbers, taking into account the preferences for genetic backgrounds. Breeding studies are based on Mendelian genetics. There are several breeding objectives for each cultured plant species. These objectives are possible to alter suddenly. Therefore, new breeding programs should be adapted. At this point, the breeders must be in a close relation with the market and agricultural technologies. Breeding can be described as the continuous period of mating and selection. The only difference is the breeding methods preferred by the breeders.

In practice, biotechnology is often combined with plant breeding to develop plants. In this context, genetic markers mapped near genes responsible for important agricultural features are used to select the desired plant. New age molecular techniques can be easily adapted for plant system. Therefore, a new wide door opened to new possibilities for discoveries in plants.

Releasing the data of plant genomes, it is important to determine the relation between interconnected network of genes and gene products. The requirements of new approaches for analysis and interpretation of the results cannot be denied.

2. Molecular marker analysis

Molecular markers can be expressed as a DNA sequence or gene expression product that represents differences in genomic level in relation to a gene or a property. Molecular markers are markers that can be used to monitor differences at the DNA level and for a gene that is being investigated. DNA markers are also DNA regions in which polymorphism in individuals within a species can be determined [1, 2] .

Molecular markers are nontissue-specific DNA regions that are reliable, repeatable, standardizable, capable of identifying multiple regions in the genome, capable of identifying more than one region in the genome, independent of environmental conditions, dominant and codominant [2–4].

Molecular markers are classified as dominant and codominant markers. Heterozygous individuals cannot be distinguished from homozygous dominant individuals, since dominant markers are not suitable for identification of heterozygous individuals when related to dominance between alleles is dominated by dominant markers. Thus, three different individuals (AA, AA and AA) can be distinguished for any marker at any point [2, 4].

The use of molecular marker systems based on this meta-analysis has become more prevalent in genetic studies conducted by the discovery of the polymerase chain reaction (PCR). The rapid development of technology and the accompanying needs, the facilities of the laboratories where the applications will occur, the biological properties of the species and the abundance of markers in the genome have contributed to the development of DNA markers [5]. Restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA markers (RAPD), amplified fragment length polymorphism (AFLP), sequence labeled sequences (STS), microsatellites (SSR), cleaved polymorphic sequence (CAPS), single strand such as complementary polymorphism (SSCP), amplicon length polymorphism (ALP), interspecific sequence repeat polymorphism (ISSR), expressed sequence tags (EST) and single nucleotide polymorphism (SNP) [1, 6–11].

Molecular marker techniques have some advantages and disadvantages as compared to each other, but their reasons for preference vary according to the purpose of the study and the technical possibilities of the laboratory in which the study will be conducted. SSR and SNP markers are frequently preferred because of the high level of polymorphism nowadays [11, 12].

3. Marker-assisted selection (MAS)

Marker-assisted selection (MAS) accelerates the process of developing a new improved variety. Instead of choosing a character (which is a result of many genetic studies), the MAS is based on the genes that provide the desired character. This is called the quantitative feature locus (QTL) the choice of specific alleles in the marker locus is dependent [13, 14]. To summarize the theoretical advantages of MAS:

1. Avoid errors that are caused by environmental changes.
2. Applicability in a juvenile phase without leaving a character.
3. In order to be effective, a single plant may be applied here, while the phenotypic selection of some characters requires many seeds or tissues.
4. Phenotypic selection may be more economical. Although the MAS paternal choice does not take the place of sexual recombination and breeding strategies, it can greatly increase the selection effect of a superior genotype.

Therefore, MAS is considered to be an important technique for improving general plant regeneration. The advantages of MAS may not always be meaningful, and it is often discussed that phenotypic selection for many characters is faster and cheaper than MAS. Some of the factors that may affect the success of MAS in the negative are as follows:

1. Some breeding facilities are inadequate in terms of technical infrastructure and expertise required for the implementation of MAS.
2. Decreasing the influence of the MAS between the marker and the target QTL.
3. Marker must be polymorphic on parents.
4. MAS is only effective if the selected alleles are more important than the other alleles in the population. This last factor is the key to success or failure of every MAS application [13, 15, 16].

As can be seen clearly, MAS is based on the ability to predict the value of alleles. This prediction depends on a number of factors, but it is essentially an allele, the behavior of other alleles in existence and other physical environmental conditions that have not yet been tested. For example, a breeder may determine that the A1 allele at its locus is a positive effect on yield. However, this prediction is made in a limited environment and with a limited number of gene sources. A breeder who crosses a parent with allele A1 and a new parent with the allele A4 and selects the allele A1 as the bound marker will never know that the allele A4 is better than the allele A1, but not in the absence of the allele A1, plants may be susceptible to a disease. For these reasons, MAS should never be applied separately from phenotypic selection. The most successful applications of MAS arise in situations where it is used to improve it rather than applied to phenotype selection [5, 13–15].

4. Genetic linkage mapping

DNA marker technology is used in herbal organisms to study diversity and kinship levels, fingerprint studies, genomic and physical maps, genomic regions associated with various stress factors, and genomic information. Fingerprint analysis aims to identify similarities or differences among genetic materials. Based on the assumption that the variation in genetic markers represents a variation in genes, the use of markers in fingerprint analysis has been conceived. In the fingerprint analysis, markers are widely used to provide information on

many of the genomes at the same time. As a result, the proportion of loci that differs between genetic materials is determined. This type of analysis is used to select materials to be imported into the plant rehabilitation program, and with the use of lines with a high variation, the breeder has the choice of choosing what he wants from a wider variation. Fingerprint analysis is also used for various diagnoses. Fingerprint analysis based on genetic markers also has a widespread use in forensic envy. Genetic markers are used in genomic analysis, in evolutionary development, in the identification of structural changes in chromosomes, in genetic resources and in the protection of varieties and in genetic variation. DNA markers are the most trusted and preferred systems because they are not affected by any condition, and because they allow the whole of the genome to be narrated or done [17, 18].

Link maps can determine the position and genetic distance between the markers along the chromosome. A genetic linkage map is formed by determining how often the marker moves together. A good genetic map has many markers on the whole genome without big gaps. The rate of production of genetic maps increased as the rate of use of this information in plant breeding programs increased. Both simple and complex inherited genes can be easily identified by DNA markers [1]. Many characters (such as resistance to certain diseases) that are simply governed by a single gene have been transferred to different genotypes in a very short time, thanks to DNA markers provided that genetic maps are first made. The most effective use of molecular markers has been the refinement of quantitative characters possessing complex inheritance and governed by multiple genes. Many characters such as plant height, flowering time, brooding, yield and yield elements, quality, endurance against certain diseases and harms are being quantitatively controlled and have considerable prospects for plant breeding trials. Since quantitative characters are governed by multiple loci (QTLs), the degree of effect of each locus is different, and because they are highly affected by environmental conditions, it is difficult to determine and transfer in traditional breeding trials [19, 20]. However, due to detailed genetic maps made with molecular markers, the degree of effect of each locus can be determined by locating homozygous populations in different environmental conditions, and probable locations of these loci have been identified on chromosomes. The most important use of link maps is to identify chromosome regions that contain the locus of interest and the quantitative feature locus associated with the feature of interest. These types of maps are called QTL maps or genetic maps. The QTL mapping is based on the presence of markers and genes that open up through chromosomal recombination during meiosis and allow them to analyze this expansion in their offspring [12, 21–23].

Generally, the rate of polymorphism in plant species that can be tolerated is higher than that of self-fertilized plant species. For this reason, partly distantly related rootstocks/parents are selected in the mapping studies carried out on self-fertilized plants [19]. The choice of DNA markers to be used in a mapping study depends on the availability of the currently existing and characterized markers or the suitability of the specific markers for the organism being studied. When polymorphic markers are identified between parental/parent, these markers need to be screened in the entire mapping population. This process is called as marker genotyping [14, 20].

Link analysis can be done manually for several markers, but the use of computer programs is required to perform link analysis for a large number of markers. When genetic maps were constructed to cover a large number of plant species, researchers believed that the genes could be in similar order and in similar sequences in close-up car species. This observation called genetic and collinearity in terms of chromosome organization among species reveals the existence of hundreds or even thousands of common molecular markers that could be genetically mapped in different species. The use of co-markers in mapping studies allows genome-wide comparative analysis of different species [23].

Most DNA markers are selected from nonrepetitive regions in the genome. This means that repetitive DNA is included in the genetic markers as empty and large regions. Along with not being observed much in dicotyledonous plants, while high-order cholinergic activity is observed in monocotyledons, it is observed among some species of synthetic dicotyledonous plants as well as the reason. The strain between species reveals a number of meaningful results. Simply, the genetic information obtained for a species can be transferred to another species by eliminating experimental barriers [14, 24].

The rapid accumulation of sequence resources guarantees that genetic applications will progress with comparative genomics. The linkage of these genomic sources with close relatives and even farther relative species greatly facilitates the exploration of evolutionary narratives. This clarifies the exploration and exploration of important orthologous loci, the restructuring of phylogeny and other biological questions.

5. Omic technologies

The omic technologies makes the interactions understandable between the genes, proteins and the biochemical pathways by using several molecular and analytical methods such as bioinformatics and computational analysis methods. The main focus of omic technologies is the key traits of interest known as genomics, transcriptomics and proteomics. Improvements in instrumentation and analytical methods have driven the major data sources of omic technologies such as genomics, transcriptomics and proteomics forward [25, 26].

Technological improvements produce genome-scale data to use in breeding studies. In relation to the improvements in analytical methods, analysis of the metabolites becomes important. Profiling of the alterations of the metabolite accumulation provides an insight into the responses of the plants against several stress factors. A new omics research field “metabolomics” was born. Nontarget metabolome analysis is also useful to evaluate the tissue specific metabolites and secondary metabolites. It has been reported that significant progresses in metabolite quantitative trait locus (mQTL) analysis have been used in several plant species [27, 28].

The main issue in omic technologies is to combine the heterogeneous data sets. High-throughput quantitative omic data are the best option to describe the different levels of the information of a

biological system. Computational tools are effective to overcome this problem. An integrative analysis of the genome-scale data, comparative analysis of the genomes, phytochemicals, and biosynthetic pathways can be easily and successfully performed. Multi-omics-based systems are demonstrative to understand the pathways or molecules having role in certain plant functions [29, 30].

Epigenomics is one of the latest tools to understand a gene function regulation in an organism. The newest technologies have opportunity to enable the data to resolve the mechanisms. Epigenomics provides us ability to define phenotypic variations via DNA-protein interactions, chromatin modifications and RNA technologies. Also, usage of chromatin immunoprecipitation (ChIP) techniques with next generation sequencing (NGS) technologies can gain epigenomic data from plant species [31–33].

6. Next-generation sequencing (NGS)

The improvements in sequencing technologies, an important era in plant genomic researches have been started. In a short time, cost-effective sequencing technologies have been developed. Next-generation sequencing (NGS) platforms give opportunity to plant genomic studies for several breeding strategies [34–36]. It is available to work with the plant genome and the whole transcriptome by using NGS platforms without resequencing. HeliScope™, SMRT™, RNAP™ and Nanopore DNA sequencer are classified as 3rd generation sequencing technologies. Recent advances in DNA sequencing technologies produce new analyze methods to define the exact mechanisms of the traits. Genome-wide association studies known as GWAS are effective to discriminate the complex features in plants. GWAS can scan the molecular markers among the DNA, gene or genome rapidly, and it can be possible to find the genetic variation which is in relation to an agronomic trait. GWAS uses the NGS data to find genetic variations [30, 37, 38].

NGS technology is also effective for characterization of transgene constructs such as flanking regions and other element combinations [39]. NGS technology is more sensitive than qPCR GMO detection to find out the existence of unknown GMOs. Integration of NGS to other new age molecular methods such as DNA walking opens a new window in GMO screening [30, 39–42].

7. Bioinformatic analyses

Genomic information obtained by new-age molecular biology techniques is required to be stored, organized and analyzed. Bioinformatic methods have progressed rapidly and exchanged the status of the research. The use of bioinformatics tools is crucial for the processing of large-scale data in detail.

The important point is to process and analyze plant genomics data. NGS technology is the main challenge. In recent years, the increase in the number of sequenced plant genomes and the need for tools are obvious. The heterogeneous nature of the plants and innovative

bioinformatic tools have become mandatory. The German Federal ex situ Gene Bank of Agricultural and Horticultural Crops (GCBN), GIBS (Genebank information system), EURISCO, LAILAPS, PGP&e!DAL, PlantsDB, IPK blast server, Plabi PD are recent platforms for plant genomic resources [43].

The integrative improvements of multiple omic technologies and computational tools are helpful in plant biotechnology studies. Interdisciplinary collaborations are important to enable the network between different fields of life sciences. This must be the most important mission for the researchers working on plant biotechnology to provide new insights on agricultural problems. Otherwise, it will be a big challenge to solve the upcoming problems and to define the requirements of plant breeders.

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References

[1] Jiang G. Molecular markers A2 – Thomas, Brian. In: Murray BG, Murphy DJ, editors. Encyclopedia of Applied Plant Sciences. 2nd ed. Oxford: Academic Press; 2017. pp. 207-214

[2] Tripathy SK, Lenka D, Maharana M, Ithape DM. Biochemical analysis and validation of molecular markers for identification of quality protein maize. Plant Gene. 2018;**14**:69-73

[3] Bosmali I, Ordoudi SA, Tsimidou MZ, Madesis P. Greek PDO saffron authentication studies using species specific molecular markers. Food Research International. 2017;**100**:899-907

[4] Caixeta ET, Ferrão LFV, Maciel-Zambolim E, Zambolim L. Chapter 2 – Molecular Markers, Biotechnology and Plant Breeding. San Diego: Academic Press; 2014. pp. 19-45

[5] Skøt L, Grinberg NF. Genomic selection in crop plants A2 – Thomas, Brian. In: Murray BG, Murphy DJ, editors. Encyclopedia of Applied Plant Sciences. 2nd ed. Oxford: Academic Press; 2017. pp. 88-92

[6] Balázs E. Molecular markers in plant genetics and biotechnology. South African Journal of Botany. 2008;**74**:354

[7] Rocha CML, Vellicce GR, García MG, Pardo EM, Racedo J, Perera MF, de Lucía A, Gilli J, Bogado N, Bonnecarrère V, German S, Marcelino F, Ledesma F, Reznikov S, Ploper LD,

Welin B, Castagnaro AP. Use of AFLP markers to estimate molecular diversity of Phakopsora pachyrhizi. Electronic Journal of Biotechnology. 2015;**18**:439-444

[8] Saha D, Rana RS, Chakraborty S, Datta S, Kumar AA, Chakraborty AK, Karmakar PG. Development of a set of SSR markers for genetic polymorphism detection and interspecific hybrid jute breeding. The Crop Journal. 2017;**5**:416-429

[9] Shabir G, Aslam K, Khan AR, Shahid M, Manzoor H, Noreen S, Khan MA, Baber M, Sabar M, Shah SM, Arif M. Rice molecular markers and genetic mapping: Current status and prospects. Journal of Integrative Agriculture. 2017;**16**:1879-1891

[10] Xu J-Y, Zhu Y, Yi Z, Wu G, Xie G-Y, Qin M-J. Molecular diversity analysis of *Tetradium ruticarpum* (WuZhuYu) in China based on inter-primer binding site (iPBS) markers and inter-simple sequence repeat (ISSR) markers. Chinese Journal of Natural Medicines. 2018; **16**:1-9

[11] Zeinalabedini M, Dezhampour J, Majidian P, Khakzad M, Zanjani BM, Soleimani A, Farsi M. Molecular variability and genetic relationship and structure of Iranian Prunus rootstocks revealed by SSR and AFLP markers. Scientia Horticulturae. 2014;**172**:258-264

[12] Amanullah S, Liu S, Gao P, Zhu Z, Zhu Q, Fan C, Luan F. QTL mapping for melon (*Cucumis melo* L.) fruit traits by assembling and utilization of novel SNPs based CAPS markers. Scientia Horticulturae. 2018;**236**:18-29

[13] Devi EL, Devi CP, Kumar S, Sharma SK, Beemrote A, Chongtham SK, Singh CH, Tania C, Singh TB, Ningombam A, Akoijam R, Singh IM, Singh YR, Monteshori S, Omita Y, Prakash N, Ngachan SV. Marker assisted selection (MAS) towards generating stress tolerant crop plants. Plant Gene. 2017;**11**:205-218

[14] Nayak SN, Singh VK, Varshney RK. Marker-assisted selection A2 – Thomas, Brian. In: Murray BG, Murphy DJ, editors. Encyclopedia of Applied Plant Sciences. 2nd ed. Oxford: Academic Press; 2017. pp. 183-197

[15] Crossa J, Pérez-Rodríguez P, Cuevas J, Montesinos-López O, Jarquín D, de los Campos G, Burgueño J, González-Camacho JM, Pérez-Elizalde S, Beyene Y, Dreisigacker S, Singh R, Zhang X, Gowda M, Roorkiwal M, Rutkoski J, Varshney RK. Genomic selection in plant breeding: Methods, models, and perspectives. Trends in Plant Science. 2017;**22**:961-975

[16] Rashid B, Husnain T, Riazuddin S. Chapter 1 – Genomic approaches and abiotic stress tolerance in plants. In: Ahmad P, Rasool S, editors. Emerging Technologies and Management of Crop Stress Tolerance. San Diego: Academic Press; 2014. pp. 1-37

[17] Li J, Zou X, Zhang L, Cao L, Chen L. Linkage map construction using SSR markers and QTL analyses of stem expansion traits in Brassica juncea. Scientia Horticulturae. 2016;**209**: 67-72

[18] Zhang G, Zhang X, Ye H, Jiang S, Yu H, Li J, Shi Q, Chen G, Zhou Z, Luo J, You X. Construction of high-density genetic linkage maps and QTL mapping in the golden pompano. Aquaculture. 2018;**482**:90-95

[19] Li YH, Reif JC, Hong Hl, Li HH, Liu ZX, Ma YS, Li J, Tian Y, Li YF, Li WB, Qiu LJ. Genome-wide association mapping of QTL underlying seed oil and protein contents of a diverse panel of soybean accessions. Plant Science. 2018;**266**:95-101

[20] Yaobin Q, Peng C, Yichen C, Yue F, Derun H, Tingxu H, Xianjun S, Jiezheng Y. QTL-Seq identified a major QTL for grain length and weight in Rice using near isogenic F2 population. Rice Science. 2018;**25**:121-131

[21] El-Soda M, Malosetti M, Zwaan BJ, Koornneef M, Aarts MGM. Genotype × environment interaction QTL mapping in plants: Lessons from Arabidopsis. Trends in Plant Science. 2014;**19**:390-398

[22] Liu J, Liu B, Cheng F, Liang J, Wang X, Wu J. A high density linkage map facilitates QTL mapping of flowering time in Brassica rapa. Horticultural Plant Journal. 2016;**2**:217-223

[23] Sheng X, Qiu Y, Zhou Y, Zhu W. Joint parameter estimation in the QTL mapping of ordinal traits. Journal of Theoretical Biology. 2017;**432**:100-108

[24] Ganie SH, Upadhyay P, Das S, Prasad Sharma M. Authentication of medicinal plants by DNA markers. Plant Gene. 2015;**4**:83-99

[25] Lavarenne J, Guyomarc'h S, Sallaud C, Gantet P, Lucas M. The spring of systems biology driven breeding. Trends in Plant Science. 2018 (Article in Press)

[26] Poltronieri P. 1 – From Plant Genomics to Plant Biotechnology. Woodhead Publishing; 2013. pp. 3-13

[27] Brotman Y, Riewe D, Lisec J, Meyer RC, Willmitzer L, Altmann T. Identification of enzymatic and regulatory genes of plant metabolism through QTL analysis in Arabidopsis. Journal of Plant Physiology. 2011;**168**:1387-1394

[28] Tohge T, de Souza LP, Fernie AR. Genome-enabled plant metabolomics. Journal of Chromatography B. 2014;**966**:7-20

[29] Langridge P, Fleury D. Making the most of 'omics' for crop breeding. Trends in Biotechnology. 2011;**29**:33-40

[30] Mohanta TK, Bashir T, Hashem A, Abd_Allah EF. Systems biology approach in plant abiotic stresses. Plant Physiology and Biochemistry. 2017;**121**:58-73

[31] Ji L, Neumann DA, Schmitz RJ. Crop epigenomics: Identifying, unlocking, and harnessing cryptic variation in crop genomes. Molecular Plant. 2015;**8**:860-870

[32] Kalavacharla V, Subramani M, Ayyappan V, Dworkin MC, Hayford RK. Chapter 16 – Plant Epigenomics A2 – Tollefsbol, Trygve O, Handbook of Epigenetics. 2nd ed. Academic Press; 2017. pp. 245-258

[33] Tsaftaris A, Kapazoglou A, Darzentas N. 14 – From epigenetics to epigenomics and their implications in plant breeding A2 – Altman, Arie. In: Hasegawa PM, editor. Plant Biotechnology and Agriculture. San Diego: Academic Press; 2012. pp. 207-226

[34] Bhat JA, Ali S, Salgotra RK, Mir ZA, Dutta S, Jadon V, Tyagi A, Mushtaq M, Jain N, Singh PK, Singh GP, Prabhu KV. Genomic selection in the era of next generation sequencing for complex traits in plant breeding. Frontiers in Genetics. 2016;**7**:221

[35] Ray S, Satya P. Next generation sequencing technologies for next generation plant breeding. Frontiers in Plant Science. 2014;**5**:367

[36] Singh VK, Singh AK, Singh S, Singh BD. Next-generation sequencing (NGS) tools and impact in plant breeding. In: Al-Khayri JM, Jain SM, Johnson DV, editors. Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools. Cham: Springer International Publishing; 2015. pp. 563-612

[37] Hannon E, Weedon M, Bray N, O'Donovan M, Mill J. Pleiotropic effects of trait-associated genetic variation on DNA methylation: Utility for refining GWAS loci. The American Journal of Human Genetics. 2017;**100**:954-959

[38] Shi X, Ling H-Q. Current advances in genome sequencing of common wheat and its ancestral species. The Crop Journal. 2018;**6**:15-21

[39] Pauwels K, De Keersmaecker SCJ, De Schrijver A, du Jardin P, Roosens NHC, Herman P. Next-generation sequencing as a tool for the molecular characterisation and risk assessment of genetically modified plants: Added value or not? Trends in Food Science & Technology. 2015;**45**:319-326

[40] Fraiture M-A, Herman P, Papazova N, De Loose M, Deforce D, Ruttink T, Roosens NH. An integrated strategy combining DNA walking and NGS to detect GMOs. Food Chemistry. 2017;**232**:351-358

[41] Valdés A, Simó C, Ibáñez C, García-Cañas V. Chapter 13 – Profiling of genetically modified organisms using Omics technologies. In: García-Cañas V, Cifuentes A, Simó C, editors. Comprehensive Analytical Chemistry. Elsevier; 2014. pp. 349-373

[42] Willems S, Fraiture M-A, Deforce D, De Keersmaecker SCJ, De Loose M, Ruttink T, Herman P, Van Nieuwerburgh F, Roosens N. Statistical framework for detection of genetically modified organisms based on next generation sequencing. Food Chemistry. 2016; **192**:788-798

[43] Schmutzer T, Bolger ME, Rudd S, Chen J, Gundlach H, Arend D, Oppermann M, Weise S, Lange M, Spannagl M, Usadel B, Mayer KFX, Scholz U. Bioinformatics in the plant genomic and phenomic domain: The German contribution to resources, services and perspectives. Journal of Biotechnology. 2017;**261**:37-45