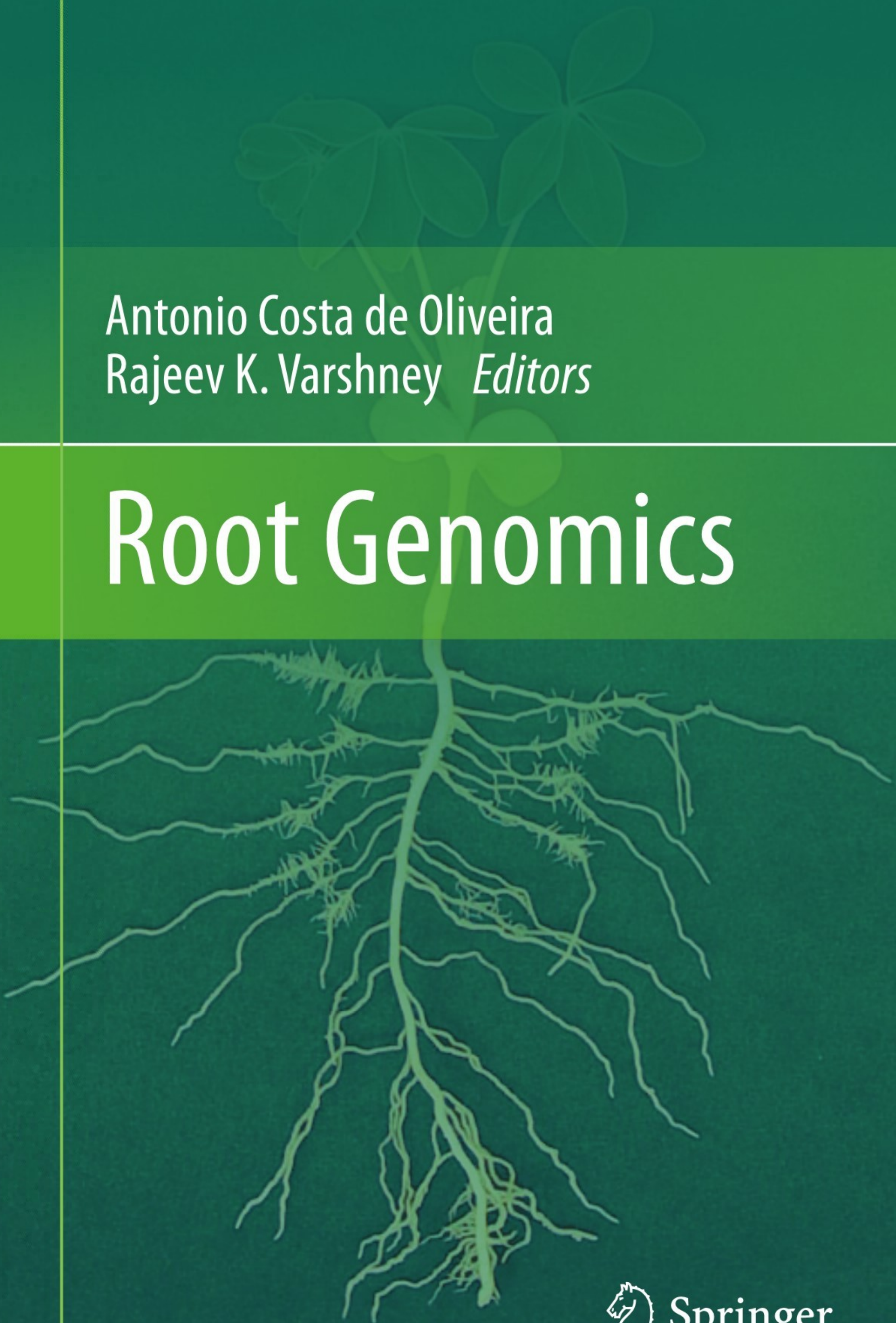
Antonio Costa de Oliveira
Rajeev K. Varshney *Editors*

Root Genomics



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Foreword

Root Biology: An Inconvenient Truth

The truth is that roots usually are as extensively underground as the aerial portions are above the ground. Crop plants would not live without roots. Roots absorb water and nutrients and anchor the plant in the soil. So why do not we know more about roots? It is likely due to the inconvenience of phenotyping root characteristics – and many of today's phenotyping methods are destructive. While we recognize the essentiality of roots and their relation to plant performance, the scientific community has not placed a sufficiently high priority on their analysis to make the needed major advances. Many of the factors that affect root health can result in a 50% yield loss when deficient. Given that the predicted human population increase is 50% by 2050, the improvement of root health in crop plants could play a major role in meeting the world's need for increased food.

The study of root biology involves extensive plant–soil–water interactions that are complicated by the microorganisms and insects in the rhizosphere that can alter root development. Each of the possible interactions has feedback effects in the plant; many effects are long-range effects within the plant. The soil environment relates to nutrient availability and uptake, which reflects the condition of the soil including acidity. Even alternation of dry and flooded conditions changes various ion states, which can change with the duration of flooding. Many climate change scenarios predict water shortages, making the understanding of root biology even more important in the future.

Much of today's phenotyping of roots is based on root architecture, such as root length, root diameter, root proliferation, root biomass, root mass density at different soil depths, diameter, and distribution of meta-xylem vessels, and root-to-shoot ratios. Early maturity, early shoot-growth vigor, and depth and rapidity of water absorption also are often assessed among other factors. New nondestructive approaches need to be encouraged such as X-ray imaging, light transmission imaging, and time-lapse recordings of root growth.

This book clearly documents that many new genetic/genomic technologies are rapidly being applied to the study of roots, including high-throughput genome sequencing, TILLING, use of molecular markers such as SSRs, DArTs, and SNPs for introgression of favorable genes, QTL analyses, marker assisted breeding, gene discovery, comparative mapping, transcription factor identification, transcriptional profiling, posttranscriptional events regulating microRNAs, and proteome profiling with complete roots. Some genetic approaches are constrained – such as genome-wide selection and gene cloning – by the difficulty in phenotyping.

Plants coordinate root growth with the soil environment. Many factors can inhibit root growth. In this book, aluminum, iron, and salt toxicity are extensively reviewed, providing a great deal of useful information. The root system is the primary site of interaction with the soil environment, which includes exudates of organic compounds from the plants and the microbes. Some of these exudates are known to represent signals that regulate microbe behaviors and even germination of seeds.

As illustrated in this book, it is amazing what we know about roots and their importance, but equally amazing is what we do not know – and we know even less about the complicated interactions and feedback mechanisms. The work reviewed in this book also shows the value of using model species such as *Arabidopsis*; e.g., 22 genes have been reported in *Arabidopsis* on lateral root development, 19 genes on primary root development, and 8 genes on root-hair formation.

One of the goals of this book was to show how root research relates to sustainable crop productivity. The chapters taken together represent an extensive review of the topic focusing primarily on highly productive crops under rainfed conditions. Crops are mostly rainfed in the most populated areas of the world; this suggests that it is imperative that root biology be a major research emphasis in the coming years – but will that be the case? Will the "inconvenient truth" be recognized?

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Preface

With the emerging recognition that agriculture needs to approach sustainability, the plant–soil–water interactions become of paramount importance in crop systems. In this scenario, roots arise from a minor to a major role in the understanding of plant growth and development. Novel technologies allow us to scan genomes in the fastest way ever, and there is not a day without further developments leading to cheaper and more precise genotyping techniques. However, the complexity of underground metabolism and the responses of root systems to a variety of stresses call for improvements in phenotyping as well as genotyping techniques.

The idea of organizing a book on Root Genomics dates as back as early 1990s in the graduate benches of Purdue University. The fascination with a system so important for the plant but yet so unknown served as both an incentive and a challenge to pursue this line of research. In 2002, an important opening for root biology occurred when the late Dr. Mike Gale, *FRS*, agreed to include a workshop in Root Genomics at the Plant and Animal Genome Meetings, held yearly at San Diego, CA. Since 2003, this workshop has generated fruitful discussions and created new paths for root research. Many speakers from different countries shared their experience in root genomics, regardless if they were working with model or crop species. One of the speakers, Rajeev Varshney, was very impressive in his enthusiasm and determination to target important aspects of drought stress. Sharing the same enthusiasm for studying roots and stress responses was crucial to put the idea of this book forward. Many of the authors have presented their work in the Root Genomics Workshop, but all were chosen by their significant contributions to agricultural and plant sciences and their common efforts for a better world. We are grateful to all the authors who not only provided a timely review of the published research work in their area of expertise but also shared their unpublished results to offer an updated view. We also appreciate their cooperation in meeting the deadlines, revising the manuscripts and in checking the galley-proofs.

We are thankful to Dr Jeff L. Bennetzen, who as a brilliant geneticist was a great role model and a friend (ACO) that has indirectly inspired this line of research. We thank Dr. Ronald Phillips, a major pioneer in the field of plant genetics and genomics and the father of many ideas that influenced modern plant sciences, for writing the foreword.

Both of us also recognize that the editorial work for this book took away precious time that we should have spent with our respective families. ACO acknowledges the efforts of his parents, Glauco and Izabel, for providing an atmosphere of learning and investigative thought during his young years, his wife Carla for her continuous encouragement, patience, and friendship, and his children Victoria (Vickie) and Eduardo (Dudu). Similarly, RKV acknowledges the help and support of his wife Monika and his children Prakhar (Kutkut) and Preksha (Nanu) who allowed their time to be taken away to fulfill RKV's editorial responsibilities in addition to research, managerial, and other administrative duties at ICRISAT and Generation Challenge Programme (GCP).

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Chapter 1
Introduction to Root Genomics

Antonio Costa de Oliveira and Rajeev K. Varshney

Contents

1.1 Introduction

The twenty first century has been marked by climate awareness and an overall increase in conscience towards environmentally friendly agriculture. Despite the natural phenomena playing hard against most crops, we need to gather all the possible information on the plant–soil–water interactions in order to breed for this century. Abiotic and biotic stresses will be targeted as most of the frontiers for agriculture lie in nonoptimal areas, and genetic improvements through science will play a major role in this conquer.

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Root development, one of the major processes essential to the development of flowering plants, remains poorly understood. Roots are a hidden part of plants for many aspects and have not been the main subject of interest of researchers. Nevertheless, roots play a major role in the plant–soil interactions, regarding biological and physical aspects. The understanding of the physiological, molecular, and developmental processes that roots undergo may represent a giant step on the achievement of a more sustainable and energy-efficient agriculture. This book may serve as a reference book in this context. Some concepts about root genomics together with an overview on different chapters presented in this volume are given in this article.

1.2 Root Genomics: An Overview

Root genomics research can be divided in the following four areas of research: (1) root growth and development; (2) functional analyses of abiotic stress responses; (3) functional analyses of biotic stress responses; and (4) quantitative trait loci (QTL) analysis and molecular breeding. The understanding of basic mechanisms involving root development and the interactions of roots and soils under various abiotic and biotic stresses will pave the way for the next decades. Also, mutations obtained in model species through the use of high throughput techniques such as TILLING (targeted induced local lesions in genome) are turning root genomics an exciting subject in plant molecular biology. An attempt has been made to cover all the above-mentioned four areas of root genomics research.

1.2.1 Root Growth and Development

The breakthrough depiction of root development has started with *Arabidopsis* roots (Dolan et al. 1993, 1994; Scheres et al. 1996). The events of division, enlargement, and differentiation of cells in the roots are spatially separated. At the root tip, there is a region of continuous cell division, the RAM (root apical meristem). The new cells formed enlarge by a factor of 100-fold through a process of cell elongation. After the cells reach a mature size, they differentiate into the various cell types of the root. Root growth is accompanied by the formation of a series of lateral roots, resulting in a branching pattern that covers higher volumes of soil space in every step of branching. A range of root systems can be found in different plants including from shallow patterns to very deep roots. Therefore, the identification of factors affecting the patterns of root development is the major point in decoding the genetic control of this organ.

In a paleontological context, the role of auxin in morphogenesis has allowed the identification of vascular patterns preserved in fossils as records of auxin gradients and growth dynamics (Boyce 2010). Roots evolved independently at least in lycophytes and euphyllophytes (Gensel et al. 2001). Root traces have been found in early Devonian soil horizons, contemporaneous with attached roots in lycophyte related fossils. The presence of root hairs, root cap, and endogenous initiation shared by roots has been proposed to have highly divergent origins (Boyce 2010). Shared regulation by similar helix-loop-helix transcription factors (Menand et al. 2007) suggests a homology between rhizoids and root hairs. The origin of root caps, on the other hand, is suggested to be a response to the need of having a protective tissue to the root apical meristem, a fast-growing region constantly in contact with a solid surface, i.e., the soil. The appearance of adventitious roots may date the evolution of endogenous initiation combined with reversed auxin transport, since the first appears to have occurred repeatedly through times and is suggested to have been required for the establishment of vascular continuity (Boyce 2005). Anatomical homogeneity/heterogeneity is suggested as a reflection of stable/unstable environments faced by land plants and epiphytes/swamp plants, respectively. Despite the environmental differences, auxin transport mechanisms are thought to limit the anatomical variations in roots (Boyce 2005; Raven and Edwards 2001).

Studying root development requires model species with simple root architecture. *Arabidopsis* and rice are model species that have been fully sequenced and therefore can provide good models for monocot and dicotyledoneous root development. *Arabidopsis* root is composed of 15 distinct cell types arranged as concentric cylinders around the radial axis (Iyer-Pascuzzi et al. 2009). MicroRNA-mediated signaling has been reported to be involved in plant root development (Meng et al. 2010). Several of these miRNAs are interestingly shared by *Arabidopsis* and rice despite their differences in root patterns and architecture. However, only a few genes governing root development have been described in cereals, and differences between monocots and dicots are quite remarkable when one regards at the root system. Therefore, both models are necessary for the better understanding of the branching patterns and functional specificities of roots. Two crown rootless mutants, *crown-rootless4* (*crl4*) and *OsGnom1*, affect the gene orthologous to *GNOM1* in *Arabidopsis* (Kitomi et al. 2008; Liu et al. 2009). *GNOM1* is a membrane-associated guanine-nucleotide exchange factor of the ADP-ribosylation factor G protein (ARF_GEF) that regulates the traffic of PIN1 (PINFORMED 1) auxin efflux carrier proteins that regulates auxin transport. *GNOM1* is thought to be required for the formation of the lateral primordium in Arabidopsis, by acting on the asymmetrical division of pericycle cells (Coudert et al. 2010). Recently, a new notion on root system architecture (RSA) has been described (Dorlodot et al. 2007). Root architecture importance for plants lies in the fact that soil nutrients are not evenly distributed and the ability to spatially deploy roots can constitute an advantage.

Developmental models could be an alternative to improve phenotyping in this very plastic organ. Mapping the dynamics of roots per se or after inducing root development under different stresses could bring better understanding and establish genotype differences. Shoot-borne-root formation characterizes the difference between cereals and the dicot model plant Arabidopsis. Several mutants that are impaired in shoot-borne-root formation (4), lateral roots (4), primary root (6), and root hairs (4) have been described in maize and rice (Hochholdinger et al. 2004).

Some of these genes controlling root development have been recently cloned and will shed light on the influence of distinct root functions and architecture on grain yield and performance in water-limited conditions (Hochholdinger and Tuberosa 2009). However, the overall trend is that single mutant standard analysis is shifting to genome-wide approaches, leading to a speeding up of the process of generating information. Proteomics- and metabolomics-generated datasets will need integration with bioinformatics tools in order to translate the overwhelming amount of data into biological meaningful phenomena.

1.2.2 Biotic Stress Tolerance

Biotic stress is caused by organism attacks to plants and can be caused by different pathogens (virus, bacteria, or fungi) or pests (insects). Pathogen infections trigger plant response mechanisms that are not restricted to the infection organ. The plant senses the pest attack and responds with a range of different expressions of genes regulating metabolites such as proteinase inhibitors, toxins, or volatiles that repel pests or attract natural enemies. Herbivores or pathogens can elicit different types of defense reaction. When vacuoles and trichomes are bursted as a consequence of a chewing herbivore attack, compounds such as organic isothiocyanates can be released (Bruce and Pickett 2007).

An interesting point of view is brought by on the cross-talk between shoot and root (Van Dam et al. 2004; Bezemer and van Dam 2005). Induced responses are complicated. The fact that hormone signaling pathways govern biotic and abiotic stress responses is characterized by the fact that ABA is involved in many abiotic responses and acts as a negative regulator of disease resistance (Fujita et al. 2006). Other phytohormones, such as Salycilic acid (SA), Jasmonic Acid (JA), and Ethylene (ET), play critical roles in biotic responses. Other responses are mediated by MAP-kinase cascades, which control many biotic and abiotic responses. Other evidence of this cross-talk is the presence of Reactive Oxygen Species (ROS) at converging points between biotic and abiotic response pathways. The integration of this network of responses is essential for the understanding of how roots participate in this process and the intricate process of cross-signaling that this may need.

1.2.3 Abiotic Stress Tolerance

Roots are subjected to a wide range of stresses such as drought, flooding, salinity, as well as nutrient starvation and metal toxicity such as Al, Cd, Fe, As, and Hg. Cadmiun is a nonessential element for plants, its toxicity resulting in chlorosis and stunting. Chlorosis seems to be an indirect effect on the uptake, transport, and use of other elements such as Ca, Mg, Fe, Mn, Cu, Zn, P, and K. Cd also interferes with hormones and disturbs plant water status, causing reduction of root hydraulic conductivity, decrease of transpiration, and increase of stomatal resistance (Prasad 1995; Das et al. 1997; Aina et al. 2007). A proteomics approach revealed the importance of two metabolic enzymes induced by 10 uM Cd that seems to play a key role in the response to several abiotic stresses: alanine aminotransferase (ALT) and Hexoquinase (HXK) suggest that these could be potential biomarkers for the study of Cd toxicity (Aina et al. 2007). The accumulation of NaCl at root peripheral regions limits growth by exerting osmotic and ionic stresses. Ionic stress is a consequence of Na^+ and Cl^- accumulation, disturbing the K^+/Na^+ ratio in the plant cell (Hasegawa et al. 2000). Time-dependent effect of NaCl on the activities of tonoplast proton pumps, showing distinct profiles for vacuolar proton transporting ATPase and vacuolar proton transporting pyrophosphatase were reported. Activity alterations were found to be due to posttranslational changes (Kabata and Ktobus 2008). The effects of salinity on *Arabidopsis* cells have been recently investigated (Dinnenny et al. 2008). Transcriptional changes in response to salinity seem to be highly constrained by developmental parameters. Iron deprivation and salt stress data sets were compared. The largest set of coregulated genes displayed concerted down-regulation in the epidermis and encoded genes important for protein biosynthesis. Epidermis cells seem to present the least conserved patterns when different stresses are applied (13–15%). A range of 244 genes are cell-type-specific and whose expression pattern does not substantially change with stress. Chloroplast accumulation was found to be a novel feature of the cortex in light-grown roots. Interestingly, rice roots under excess iron stress seem to accumulate Rubisco peptides, as revealed by proteomic studies (Costa de Oliveira, unpublished).

The responses of roots to abiotic stresses are though amenable to environmental influences as well as cell-type. The high plasticity observed in the developmental patterns plus the range of abiotic factors affecting root growth through the development of plants picture a complex scenario composed of many players as well as interactions among them.

1.2.4 QTL Analysis and Molecular Breeding

Root morphology is in most cases regulated by many genes with small effects and highly influenced by the environment. Therefore, the study of root system related genes will very often rely on QTLs analyses. A few examples on mapping and identification of QTLs explaining the variation for root traits have become available in some crop species (Price and Tomos 1997; Price et al. 2002; Giuliani et al. 2005). Adventitious rooting has been considered to improve phosphorus uptake and deep root growth to increase the ability to cope with drought (Ochoa et al. 2006; Macmillan et al. 2006; Steele et al. 2006). In some cases, QTLs associated with root traits have been cloned, e.g., root elongation in *Arabidopsis* (Sergeeva et al. 2006).

Although QTL analysis was developed to deal with environmental influence on target characters, the high degree of plasticity presented by roots can mislead studies and make it difficult to do a reliable phenotyping. However, at least in rice and maize, QTL by environment interactions have been found to be weak, and marker-assisted selection studies have been successful (Macmillan et al. 2006; Kamoshita et al. 2002; Steele et al. 2006, 2007; Giuliani et al. 2005; Landi et al. 2005).

1.3 About the Book

This book covers all the four areas of research mentioned above. Some highlights of the chapters included in this book are given below.

During the past decades, a considerable number of genes and gene networks have been well described in the model species *Arabidopsis thaliana*. This knowledge can be adapted for more complex plant systems as barley, rice, or maize. Despite their agronomic importance, only a little is known about molecular basis of root formation in crop species, and only few mutants together with corresponding genes have been well characterized. In this context, Orman and colleagues from Silesian University, Poland, have described the EST (expressed-sequence tag)-based approach, in Chap. 2, to search for potential orthologous genes involved in root morphogenesis between Arabidopsis, rice, and barley. The comprehensive gene list, developed by authors, should provide strong platform for molecular studies and gene identification in barley and related species.

Roots are exposed to a range of microbe, and there are several studies, as mentioned above, which deal with discussions on root–microbe interactions as well as impact of biotic stresses on the root architecture. The Chap. 3, authored by Mathesius and van Noorden from Australian National University, Australia, present the updates on genomics of root–microbe interactions. Microbes influence roots by producing signals, toxins, altering nutrient cycling, and by invading roots as endosymbionts or endoparasites. Genomic tools have helped to elucidate the molecular changes induced in roots by microbes. This chapter highlights some of the recent advances gained by genomic and postgenomic studies to enhance knowledge in the area of root–microbe interactions. Similarly, Deshpande and colleagues from Purdue University (USA), University of Georgia (USA), Michigan Technological University (USA), and Instituto Nacional de Tecnología Agropecuaria (INTA, Argentina), in Chap. 4, discuss the advances in the plant genetics for study of the roles of root exudates and microbes in the soil. In order to dissect the relationships between soil microbes, plant exudates, and plant function, authors planned to use host genetics to identify exudate::microbe correlates that segregate with specific plant genes. Their studies indicated the great potential for future investigations of the plant-determined chemical and organismal diversity in the soil.

Abiotic stresses are the major stresses for limiting crop productivity in several crop species, especially in developing countries. In majority of such cases, roots are the first plant organs to be exposed as well as to respond. Some of these abiotic stresses in the context of root genomics have been discussed in a few chapters. For instance, in Chap. 5, Gruber and colleagues from Institut des Sciences du Végétal (ISV) and Université Paris Diderot Paris 7 from France discuss the impact of abiotic stresses such as drought and salt on the action and number of root meristems to determine root architecture. In addition to Arabidopsis, authors have discussed recent results on model legumes able to interact symbiotically with soil rhizobia to form new meristems leading to the nitrogen-fixing nodule. Aluminum (Al) toxicity is another abiotic stress that limits agricultural productivity over much of the world's arable land by inhibiting root growth and development. Affected plants have difficulty in acquiring adequate water and nutrition from their soil environments and thus have stunted shoot development and diminished yield. Hoekenga from US Department of Agriculture (USDA) – Agricultural Research Station (ARS) (USA) and Magalhaes from EMBRAPA Maize and Sorghum (Brazil) discuss in Chap. 6 the Al-tolerance mechanisms. They propose and discuss the use of systems biology approaches to study the mechanisms of Al tolerance and apply this knowledge to crop improvement via marker-assisted breeding and translational genomics. Sousa and Costa de Oliveira from Eliseu Maciel School of Agronomy, Campus UFPel (Brazil) discuss, in Chap. 7, about root responses to other abiotic stresses such as soluble iron and short chain organic acids in flooded soils, especially in the context of rice. Authors review the progress on discovery of iron transporters as well as genetic variation present in rice genotypes for flooding tolerance.

A number of studies have described QTLs that provide access to valuable genetic diversity for the morphophysiological features that characterize root functionality. Although a number of major QTLs have been identified as mentioned above, none of these QTLs has been cloned so far in crop plants, mainly due to the difficulty to accurately phenotype the target traits in a sufficiently large number of plants. In this context, in Chap. 8, Tuberosa and colleagues present summary and discuss the strategies for QTL cloning, especially in the context of maize. QTL cloning should be facilitated by adoption of high-throughput phenomics platforms as well as by information made available through genome and the profiling of the transcriptome, proteome, and metabolome, all of which will contribute to the identification of plausible candidate genes. Sheshashayee and colleagues from University of Agricultural Sciences-Bangalore, India, in Chap. 9, have presented phenotyping methodology for root traits and biotechnological approaches to improve these roots traits with an objective of sustainable crop production. In Chap. 10, Varshney and colleagues from ICRISAT, India, and Hokkaido University, Japan, discuss the physiological and genomics approaches to dissect the root traits at genetic and molecular level in context of devising the strategies for breeding for root traits to enhance drought tolerance in chickpea. Authors have also discussed the use of next generation sequencing technologies towards gene discovery and marker development.

The last two chapters discuss the progress in the area of molecular breeding for root traits for crop improvement. For instance, Raman from Wagga Wagga Agricultural Institute, Australia, and Gustafson from University of Missouri, USA, in Chap. 11, review the progress made on various aspects of molecular breeding for Al resistance such as genetics, molecular mapping, comparative mapping, marker-assisted selection, candidate gene discovery and validation, and allele mining in key cereal crops including wheat, barley, rice, maize, oats, sorghum, and rye. Similarly, Ismail and

Thomson from International Rice Research Institute, Philippines, in Chap. 12, have summarized the progress made in unraveling molecular and physiological bases of tolerance of various abiotic stresses encountered in rice problem soils including salt stress and nutritional toxicities and deficiencies. Authors have also provided a brief account of the progress towards developing and using marker-assisted back crossing (MABC) for cultivar improvement in rice.

1.4 Concluding Remarks

The field of root genomics is an exciting and promising field of research. Some of these areas of research have been detailed in some chapters of the book. The technical advances in plant-*omics* are prone to generate enough data to push forward the science of root genomics. Candidate gene identification is a strategy that is getting stronger every year. The production of genomic sequences from many sequencing projects is making the availability of specific genes more frequent. Bioinformatic tools and reverse genetic approaches such as TILLING, gene knockout mutants, or RNAi are prone to increase the success in this strategy (Dorlodot et al. 2007). An ever neglected part of the plant, roots seem to hold the key for the next plant breeding revolution, leading to improved crop productivity in environmentally challenged situations.

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Chapter 2
EST-Based Approach for Dissecting Root Architecture in Barley Using Mutant Traits of Other Species

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Contents

2.1 Introduction

There are increasing evidences that root architecture is a fundamental aspect of plant growth. The role of root system includes acquisition of water and nutrients, anchorage of the plant in the soil, synthesis of hormones, and also storage functions. It was generally considered that root characteristics could be important for breeding, to obtain genotypes of a higher adaptability to unstable soil and climatic conditions (Gorny 1992; De Dorlodot et al. 2007) and higher productivity (Lynch 1995). Despite their importance, little is known about genetic basis of root system formation and architecture in major crop species. A great progress in understanding the molecular processes underlying root development has been achieved only in *Arabidopsis thaliana* (Scheres et al. 2002; Casimiro et al. 2003; Casson and

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Lindsey 2003; Ueda et al. 2005; Zhang et al. 2007; Busov et al. 2008). This progress was accomplished through detailed analysis of root mutants with the use of advanced molecular, genomic, and bioinformatic tools available for *Arabidopsis*. Recently, several root mutants have been reported in three cereal species, rice (Ma et al. 2001; Zimmer et al. 2003; Liu et al. 2005; Inukai et al. 2005; Jiang et al. 2005; Li et al. 2006a; Kim et al. 2007), maize (Lim et al. 2005; Woll et al. 2005; Wen et al. 2005; Hochholdinger et al. 2008), and wheat (Wang et al. 2006). Some of them have become the subject of studies similar to *Arabidopsis* that have led to the identification of homologous and novel genes controlling root system formation in monocotyledons (Morita and Kyozuka 2007). There is, however, a lack of similar knowledge in barley. These differences in progress of knowledge between monocotyledonous and dicotyledonous species could be considered as a result of the more extensive size of adult cereal root systems and lack of such efficient screening strategies like those developed for *Arabidopsis*. Based on this, we will focus on root development in monocotyledons, especially in barley, which is the fourth most important crop in the world after maize, wheat, and rice. Recently, it is becoming a novel cereal model plant because of its true diploidy (Sreenivasulu et al. 2008).

Root system of monocotyledonous plants is generally composed of two fundamental parts: seminal root system, which develops from initials present in embryo, and nodal (often called adventitious or shoot-borne) root system, which originates from shoot (Hackett 1968). The dicotyledonous species develop a taproot system with one primary root and lateral branches, which remain active during the whole life cycle. However, dicotyledonous plants can also form roots called "adventitious" under unusual circumstances such as wounding or hormone application, etc., at uncharacteristic sites on a plant. Following Hochholdinger and coworkers (2004), we also suggest not calling monocotyledonous stem-derived crown and brace roots "adventitious" because they belong to the normal developmental program of cereals. Despite having to fulfill the same fundamental functions, the root systems of monocotyledons and dicotyledons differ both in morphology and anatomy. In monocotyledons, the secondary root growth do not occur, and root vessels are relatively uniform cylinders (in the absence of environmental stimuli) (Gorny 1992). The adult crop plant exhibits an extensive shoot-born root system, which plays a major role in the postembryonic root architecture (Hochholdinger et al. 2004; Hochholdinger and Zimmermann 2008). Nevertheless, it has been reported that maize seminal roots have relatively high water uptake capacity compared to other root types, which makes them important throughout whole plant life (Osmont et al. 2007).

2.2 Root Mutants of *Arabidopsis* Published in Pubmed

Both forward and reverse genetic approaches have been used to increase knowledge about root architecture. As there are many mutagenesis methods, the use of chemical mutagenesis mostly by EMS and insertional mutagenesis using T-DNA insertion, followed by mutant screening, apparently dominates. Using EMS, 147 gene alleles were obtained, 140 alleles by insertional mutagenesis (e.g., 19 by transposable elements, 118 by T-DNA, 2 by promoter trap and 1 by activation tagging), whereas 22 alleles were obtained by physical approach (nine by fast neutrons, six by X-ray, seven by gamma rays). Reverse approach (e.g., RNAi, overexpression) were also commonly used to study influence of a gene of interest on root traits.

Using these strategies, it was possible to build the model pattern of root development in dicotyledons, based on data from reference *Arabidopsis*. Up to now, many genes have been shown to be involved in various aspects of *Arabidopsis* root development (Tables 2.1 and 2.2). Many of them have a pleiotropic effect not only on various stages of root development but also on whole plant per se. Nevertheless, we divided Arabidopisis genes controlling root system into formation of radial and longitudinal pattern, keeping in mind that assigning genes to only one chosen category could be misleading. The *Arabidopsis* radial pattern consists of a number of defined cell types organized in concentric layers, with the epidermis, ground tissue composed of cortex and endodermis, and the last main part called stele, which includes pericycle surrounding the central vascular cylinder (Scheres et al. 2002; Casson and Lindsey 2003). Based on this, we secondly divided genes responsible for root radial pattern into three groups, which assemble genes involved in epidermis, ground tissue, and stele development.

The first one (Table 2.1) includes genes involved in root hair development as a specific product of root epidermis. Both monocotyledonous and dicotyledonous root systems increase absorptive surface through the formation of root hairs. In *Arabidopsis*, root hairs always form on epidermal cells positioned over the radial cell wall between cortical cells (Dolan and Costa 2001). However, it is difficult to predict root hair-forming epidermal cells in cereals (Hochholdinger et al. 2004). In *Arabidopsis*, epidermis is composed of trichoblasts, which develop into root hair cells, and atrichoblasts, which remain hairless. The identity of these cells is regulated by positional information – hair-forming cells are located above two underlying cortical cells. The genetic analysis of root hair development has identified at least 39 genes that are required for the initiation and growth of the root hair. Some of them, such as *TRANSPARENT TESTA GLABRA1 (TTG1)*, *GLABRA3 (GL3)*, *ENHANCER OF GLABRA3 (EGL3)*, and *GLABRA2 (GL2)*, have been well described (Galway et al. 1994; Walker et al. 1999; Bernhardt et al. 2003). Both *TTG1* and *GL2* mutants have root hairs at nearly all root epidermal cells (Walker et al. 1999; Ohashi et al. 2003), whereas *GL3* and *EGL3* mutants have reduced numbers of atrichoblasts (Bernhardt et al. 2003). *TTG1* encodes a protein with WD40 repeats (Mendoza and Alvarez-Buylla 2000), which is localized in the nuclei of trichomes at all developmental stages (Zhao et al. 2008). It seems that *GL2* is a direct target of *GL3* and *EGL3*, whereas *TTG1* is directly regulated by *GL1* (Zhao et al. 2008).

The second group includes genes responsible for ground tissue pattering, composed of one cortex and one endodermis layer (Table 2.1), which originate from the common initial cell adjacent to the quiescent center (QC) (Scheres et al. 2002). Outside the endodermis, there are 4–6 layers in barley (Jackson 1922) and 8–15 in rice and corn (Hochholdinger et al. 2004) of bigger and thin-walled loosely packed

Table 2.1 Mutated genes responsible for *Arabidopsis* root radial pattern

Gene name (alias)	Accession number	Allele/mutation strategy/ reverse approach	Mutant phenotype	References
Root hairs				
TRANSPARENT TESTA GLABRA1 (TTG1)	AT5G24520	*ttg-1*/EMS	All cells with root hairs	Galway et al. (1994), Walker et al. (1999)
GLABRA2 (GL2)	AT1G79840	*p777*/T-DNA insertion	All cells with root hairs	Ohashi et al. (2003)
WEREWOLF (WER)	AT5G14750	*wer-1*/EMS	All cells with root hairs	Lee and Schiefelbein (1999)
CAPRICE (CPC)	AT2G46410	*cpc-1*/T-DNA insertion	All cells without root hairs	Wada et al. (1997)
GLABRA3 (GL3)	AT5G41315	*gl3-1*/EMS	Reduced number of atrichoblasts (much more root hairs)	Bernhardt et al. (2003)
ENHANCER OF GLABRA3 (EGL3)	AT1G63650	*egl3-77439*/T-DNA insertion	Reduced number of atrichoblasts (much more root hairs)	Bernhardt et al. (2003)
ENHANCER OF TRIPTICHON AND CAPRICE1 (ETC1)	AT1G01380	EMS	All cells without root hairs or root hairs are very sporadic	Kirk et al. (2004)
ETROPIC ROOT HAIR 1 (ERH1)	?	*erh1*/fast neutrons	Reduced number of atrichoblasts (much more root hairs)	Hauser et al. (1995), Schneider et al. (1997)
ETROPIC ROOT HAIR3 (ERH3)	AT1G80350	Gamma rays	Reduced number of atrichoblasts (much more root hairs)	Hauser et al. (1995), Schneider et al. (1997)
TORNADO1 (TRN1)	AT5G55540	*trn1-1*/T-DNA insertion	Radial pattern is unsettled, pattern of root hairs is twisted like DNA helix	Dolan (2000)
TORNADO2 (TRN2)	AT5G46700	*trn2-2*/EMS	Radial pattern is unsettled, pattern of root hairs is twisted like DNA helix	Dolan (2000)
ROOT HAIRLESS 1 (RHL1)	AT1G48380	*rhl1-1*/T-DNA insertion *rhl1-2*/unknown	Root hairs very sporadic, pattern of trichoblasts and atrichoblasts unsettled	Schneider et al. (1997, 1998)
ROOT HAIRLESS 2 (RHL2)	AT5G02820	T-DNA insertion	Root hairs very sporadic, pattern of trichoblasts and atrichoblasts unsettled	Schneider et al. (1997)
ROOT HAIRLESS 3 (RHL3)	AT3G20780	*rhl3-1*/EMS	Root hairs very sporadic, pattern of trichoblasts and atrichoblasts unsettled	Schneider et al. (1998)
CONSTITUTIVE TRIPLE RESPONSE (CTR1)	AT1G01380	ctrl-6/T-DNA insertion	Root hairs are formed on other place than usually	Kieber et al. (1993), Dolan et al. (1994)
	AT1G66340	*etr1-1*/EMS		Masucci and Schiefelbein (1996)

ETHYLENA RECEPTOR 1 (ETR1)			Root hairs are formed near to the basal part of cell	
ETHYLENE OVERPRODUCER 1 (ETO1)	AT3G51770	*eto1-1*/EMS	Root hairs are formed near to the apical part of cell	Masucci and Schiefelbein (1996), Yoshida et al. (2006)
ROOT HAIR DEFECTIVE 6 (RHD6)	?	EMS	Root hairs are very sporadic and formed near to the basal part of cell, more than one root hair on one cell	Masucci and Schiefelbein (1994), Dolan (2001)
SALT OVERLY SENSITIVE 4 (SOS4)	AT5G37850	*sos4-1*/EMS	Root hairs are very, very sporadic	Shi and Zhu (2002)
ROOT HAIR DEFECTIVE 1(RHD1)	AT1G64440	*rhd1-2*/EMS *rhd1-1*/EMS	Primordium is very big, root hairs with normal length	Schiefelbein and Somerville (1990)
TIP GROWTH DEFECTIVE 1 (TIP1)	AT5G20350	*tip1-1*/EMS	Primordium is bigger, root hairs are shorter and often branched, sometimes there are 2–4 root hairs on one cell	Ryan et al. (1998)
SUPERCENTIPEDE 1 (SCN1)	?	EMS	1–5 primordiums on one cell	Grierson et al. (2001)
TINY ROOT HAIR 1 (TRH1)	AT4G23640	*trh1*/EMS	Root hair growth stopped at primordium stage	Rigas et al. (2001), Vicente-Agullo et al. (2004)
HAIR DEFECTIVE 2 (RHD2)	AT5G51060	*rhd2-1*/EMS	Root hair growth stopped at primordium stage	Schiefelbein and Somerville (1990)
SHAVEN1,2,3 (SHV1,2,3)	?	EMS	Root hairs are shorter	Parker et al. (2000)
KOJAK (KJK)	AT3G03050	*csld3-1*/T-DNA insertion	Root hairs rupture at their tip soon after initiation	Favery et al. (2001)
MRH2	AT3G54870	*mrh2-1*/T-DNA insertion	Mutant exhibits wavy and branching root hair phenotype	Yang et al. (2007)
LRR/EXTENSIN 1 (LRX1)	AT1G12040	lrx1/En-1 transposition	Root hairs are shorter, often branched	Baumberger et al. (2001)
DEFORMED ROOT HAIRS 1 (DER1)	?	EMS	Root hairs are shorter, primordium is bigger, and sometimes there are 2 root hairs on one cell	Ringli et al. (2002)
	AT2G35630	EMS	Root hairs are wavy and branched	Whittington et al. (2001)

(*continued*)