Chapter 14
Genetic Mechanisms Involved in the Formation of Root System Architecture

Yuka Kitomi, Jun-Ichi Itoh, and Yusaku Uga

Abstract Root system is essential for absorbing water and nutrients as well as anchoring shoots to the ground. Understanding the genetic mechanisms related to the formation of root system architecture is necessary for improving rice productivity. Here, we first describe the potential of genetic improvement using quantitative trait locus (QTL) for root system architecture based on our field experiments using a genetic material of *DEEPER ROOTING 1*, which is a rice QTL controlling root growth angle. Next, we summarize the accumulated knowledge on the genetic mechanisms of root formation in rice including the development of the radicle, crown roots, lateral roots, and root hairs. We also overview the current status of the genetic dissection of root system architecture in rice, namely, the identification and characterization of natural and artificial alleles. Root traits are rarely chosen as breeding targets because their evaluation in a large number of plants under field conditions is more laborious and time-consuming than evaluation of aboveground traits. The genetic dissection of root system architecture would facilitate the breeding of root traits, eventually improving rice yield irrespective of soil and other environmental conditions.

Y. Kitomi (✉)
Breeding Material Development Unit, Division of Basic Research, Institute of Crop Science, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan
e-mail: ykitomi@affrc.go.jp

J.-I. Itoh
Laboratory of Plant Breeding & Genetics, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
e-mail: ajunito@mail.ecc.u-tokyo.ac.jp

Y. Uga
Breeding Material Development Unit, Division of Basic Research, Institute of Crop Science, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan

Department of International Agricultural Development, Graduate School of Agriculture, Tokyo University of Agriculture, Tokyo, Japan
e-mail: yuga@affrc.go.jp

T. Sasaki, M. Ashikari (eds.), *Rice Genomics, Genetics and Breeding*,
https://doi.org/10.1007/978-981-10-7461-5_14

Keywords Fibrous root system · Ideotype · Mutant · Phytohormone · QTL · Root formation · Root morphology

14.1 Introduction

14.1.1 What Is the Ideotype for the Second Green Revolution?

Half a century ago, ideotype breeding using a semidwarf gene, *sd1*, resulted in the Green Revolution in rice (Khush 2001), but breeders and researchers still have to increase rice yield because rice is a staple food for nearly half of the world's population. Thus, we need to consider what the ideotype for the second Green Revolution could be. Recent climate change is increasing the inequality of water and nutrient distribution in agricultural lands at a global level; global warming has caused serious drought damage in farmlands that rely on rainwater or that have limited access to irrigation (Scheffran and Battaglini 2011). What kinds of traits are needed to improve rice yield in this situation?

Root system traits should be such candidate traits to achieve the second Green Revolution. Root, which is the only organ absorbing water and nutrients from soil, is imperative for the survival of terrestrial plants, which cannot move around after germination. Optimal root development and distribution allow efficient acquisition of water and nutrients, which are heterogeneously distributed in soil (Gowda et al. 2011; Lynch 2013). For example, the topsoil tends to hold less water but more immobile nutrients such as phosphorus than does the subsoil. Nitrate, which is the main form of nitrogen under aerobic conditions, is leached by precipitation into subsoil. Therefore, root system architecture greatly affects the acquisition of water and nutrients from soil (Gewin 2010; Lynch 1995). Many wild species tend to have adequate root systems to adapt to severe stresses (Canadell et al. 1996); for example, drought-resistant plants tend to develop deeper root systems, which allow them to capture water from subsoil and thus avoid drought stress. Increased roots in subsoil would be also effective to avoid the negative impacts of drought on crop yield (de Dorlodot et al. 2007). Therefore, the genetic improvement of root system architecture has been regarded as an important approach to enhance crop production. However, it is more laborious and time-consuming to select root traits than aboveground traits. Therefore, many researchers are considering molecular breeding by using quantitative trait loci (QTLs) as one of the promising strategies for improving root system architecture (de Dorlodot et al. 2007; Yamamoto et al. 2014).

14.1.2 Prospects of Ideotype Breeding Using QTLs for Root System Architecture

In rice, a wide natural variation of root system architecture has been reported (Lafitte et al. 2001; O'Toole and Bland 1987; Uga et al. 2009). For example, typical upland rice has deeper, longer, and thicker roots than lowland rice (O'Toole and Bland 1987) and thus might be a useful breeding material to improve rice productivity. Root system architecture is a complex trait controlled by tens to hundreds of genes (Wachsman et al. 2015). Courtois et al. (2009) summarized 675 rice QTLs for 29 root parameters detected in 12 mapping populations reported in 24 published papers, but only one QTL associated with root system architecture has been isolated as a single gene in rice (Uga et al. 2013a).

The rice QTL *DEEPER ROOTING 1* (*DRO1*) has been identified on chromosome 9 in recombinant inbred lines derived from a cross between the lowland cultivar "IR64" and the upland cultivar "Kinandang Patong" (Uga et al. 2011) and has been cloned (Uga et al. 2013a). "Kinandang Patong," which has a functional allele of *DRO1*, has deep roots, whereas "IR64," which has a nonfunctional allele, has shallow roots. In a near-isogenic line (Dro1-NIL) that carries *DRO1* derived from "Kinandang Patong" in the genetic background of "IR64," *DRO1* increases root growth angle, resulting in deep rooting, but has a limited effect on other root and shoot traits. Field experiments using this unique line, which differs from "IR64" only in the increased root growth angle, have demonstrated that alteration of root system architecture improves rice productivity, as discussed in detail below (Fig. 14.1).

The yield performance of "IR64" and Dro1-NIL was compared under upland field conditions with no drought, moderate drought, or severe drought (Uga et al. 2013a). Under moderate drought in comparison with no drought, the grain weight of "IR64" decreased by nearly half, whereas that of Dro1-NIL was almost the same. Under severe drought, the grain weight of "IR64" was very low, whereas that of Dro1-NIL was more than 30% of that with no drought. This study suggests that deep rooting induced by *DRO1* enhances drought avoidance, resulting in higher grain yield (Fig. 14.1a).

Comparison among cultivars with different root and shoot morphologies has suggested that deep roots increase grain yield in paddy fields (Kawata et al. 1978; Morita et al. 1988), but the genetic aspects of this effect have not yet been clear in previous studies. In paddy fields, Dro1-NIL showed about 10% higher grain yield than did "IR64," irrespective of nitrogen treatment (Arai-Sanoh et al. 2014). There was no significant difference between "IR64" and Dro1-NIL in nitrogen content before heading, but nitrogen uptake was higher in Dro1-NIL than in "IR64" after heading. These results suggest that deep rooting induced by *DRO1* enhances nitrogen uptake from lower soil layers, resulting in better grain filling (Fig. 14.1b).

Because root growth angle influences the efficiency of nitrogen absorption, it might also affect the uptake of other minerals such as heavy metals. In Cd-contaminated soil, the grain and straw Cd concentrations were significantly

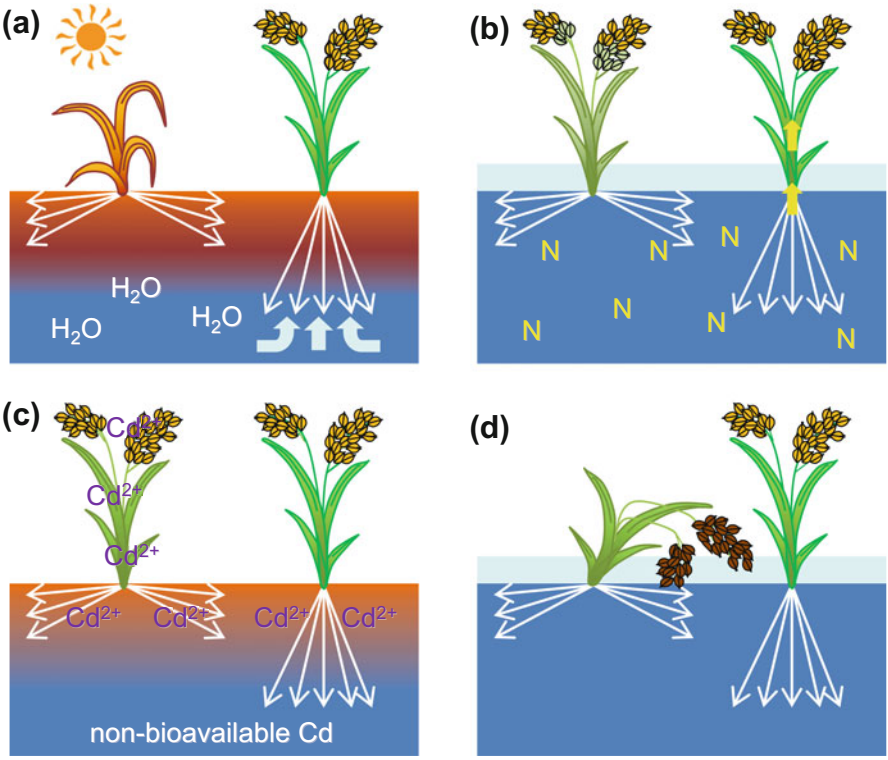

Fig. 14.1 Schematic models of the effect of *DRO1* on rice production and phytoremediation. (**a**) Drought avoidance (water uptake). In upland fields, where water is most abundant in subsoil, deep rooting caused by *DRO1* allows plants to efficiently capture water. (**b**) Yield performance (nitrogen uptake). Under irrigated conditions in paddy fields, deep rooting caused by *DRO1* allows plants to access nitrogen from the subsoil during reproductive stages. (**c**) Phytoremediation (Cd uptake). In a rain-fed paddy field after drainage, the bioavailable Cd concentration increases in the topsoil. Shallow rooting caused by *dro1* allows plants to accumulate bioavailable Cd from the topsoil. (**d**) Lodging resistance. In wet paddy fields, deep rooting caused by *DRO1* increases pushing resistance (an index of lodging resistance). Rice plants described in each part are IR64 on the left and Dro1-NIL on the right

higher in "IR64" than in Dro1-NIL (Uga et al. 2015a). These results were opposite to those in the case of nitrogen uptake because the bioavailable Cd concentration was increased in the oxidized topsoil layer by withholding irrigation water during the vegetative growth period. Therefore, shallow roots could capture Cd from topsoil, resulting in a high concentration of Cd in rice plants (Fig. 14.1c). This suggests that, for phytoremediation, the allele occurring shallow rooting is a potential genetic resource for developing plants with high Cd accumulation. From the viewpoint of food safety, the allele giving deep rooting could be a useful resource to avoid absorbing the bioavailable Cd from topsoil.

Comparisons of different cultivars and examination of the effects of root pruning suggest that deep roots influence lodging resistance (Sakata et al. 2004; Terashima 1997; Terashima et al. 1994, 1995), although the genetic aspects of this effect are still unknown. Under wet paddy field conditions, Dro1-NIL had stronger pushing resistance (an index of lodging resistance) than "IR64" (Arai-Sanoh et al. 2014), suggesting that deep rooting induced by *DRO1* improves lodging resistance (Fig. 14.1d).

These field experiments with Dro1-NIL confirm the potential of ideotype breeding for root system architecture, although further studies in other environments are needed. To design new root ideotypes that are adapted to diverse environmental stresses and to conduct ideotype breeding by using marker-assisted selection (Coudert et al. 2010), it will be necessary to update our understanding of the genetic mechanism associated with root system architecture. Information on gene networks involved in root formation has been accumulated for the model dicot plant *Arabidopsis thaliana*, but our knowledge of these aspects in rice is limited (Coudert et al. 2010; Rebouillat et al. 2009). In this chapter, we overview this knowledge and discuss the prospects of applying it to molecular breeding.

14.2 Root Formation

The first root of a rice plant, the radicle, is generated during embryogenesis. A radicle primordium originates endogenously from the embryo 4 days after pollination (DAP) (Itoh et al. 2005), whereas in *Arabidopsis* it is exogenously differentiated from the hypophysis (Dolan et al. 1993). After germination, the radicle is named the seminal root. Crown roots originate from the parenchyma cells adjacent to the peripheral cylinder of vascular bundles of the stem; therefore, crown roots are also named shoot-born roots (Fig. 14.2). Monocots develop a fibrous root system characterized by numerous crown roots, meanwhile dicots consist of only a main root system (Klepper 1992). A rice plant usually generates several hundreds of crown roots under field conditions (Klepper 1992). Lateral roots grow from seminal and crown roots (Fig. 14.2) and are responsible for taking up most water absorbed by the root system (Varney et al. 1993). In rice, two distinct types of lateral roots have been identified (Kawata and Shibayama 1965). L-type lateral roots are generally long and thick and are able to generate higher-order lateral branches, whereas S-type lateral roots are short, slender, and non-branching. Crown and lateral roots are classified as postembryonic roots because they are initiated after embryogenesis, whereas the seminal root (radicle) is generated during embryogenesis. Root hairs are tubular outgrowths of some root epidermal cells. Root hairs occupy most of the root surface area; they are thought to be important for water and nutrient uptake, anchorage, and interactions with soil microbes (Kim et al. 2007). Identification of genes associated with the formation of the different types of roots in rice has progressed together with the advances in molecular biology and DNA sequencing technology (Table 14.1).

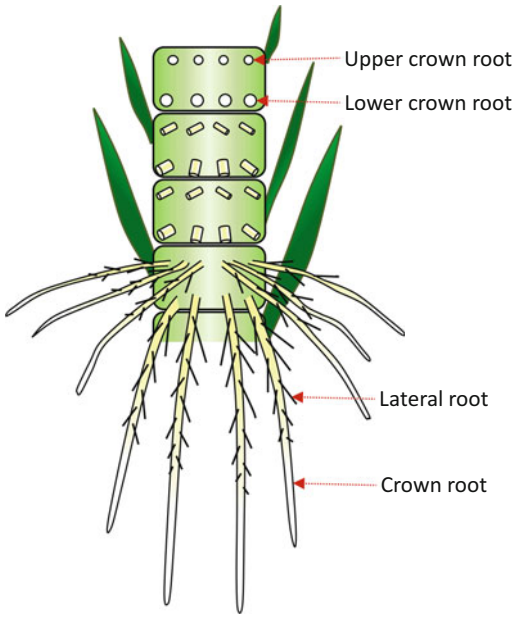

Fig. 14.2 Schematic view of the fibrous root system in rice

14.2.1 Root Apical Meristem

Except root hairs, each root type has a multicellular organization that can be described in terms of proximal-distal and radial polarity. All root cells are generated from stem cell daughters in the root apical meristem (RAM). Coordinated balance between cell division and differentiation is observed along the proximal-distal axis in the root tip, which can be divided into three zones according to cell division and elongation status: proximal division zone, transition zone, and distal elongation zone (Dolan et al. 1993). In rice, the root tip is formed by different types of cells arranged in concentric layers. The stele consists of the metaxylem, phloem, fibers, and pericycle and is surrounded by the endodermis, cortex, sclerenchyma, exodermis, and epidermis. In rice, these five cell layers are generated from single epidermis-endodermis structural initial cells by eight successive asymmetrical periclinal cell divisions following the first anticlinal division (Rebouillat et al. 2009).

The central region of RAM contains mitotically inactive cells, or the quiescent center (QC). The QC region in rice was examined by in situ hybridization with a probe for the rice *CYCLIN-DEPENDENT KINASE* (*CDK*) gene, a marker of cell division (Umeda et al. 1999). The analysis suggested that rice QC is large, unlike that of *Arabidopsis*, which consists of only four cells. In rice, the expression of a *WUSCHEL* (*WUS*)-type homeobox gene designated *QUIESCENT-CENTER-SPECIFIC HOMEOBOX* (*QHB*) was detected in the central cells of QC, similar to the

Table 14.1 Root development-related genes in rice

Phenotype	Gene	Chromosome	Locus ID		Encoded protein	References
			RAP	MSU		
Crown root number	*FIB*/*OsTAA1*	1	Os01g0169800	LOC_Os01g07500	Aminotransferase	Yoshikawa et al. (2014)
	OsRAA1	1	Os01g0257300	LOC_Os01g15340	GTP-binding protein	Ge et al. (2004)
	OsPIN3t (*OsPIN10a*)	1	Os01g0643300	LOC_Os01g45550	Auxin efflux carrier	Zhang et al. (2012a)
	OsYUC1	1	Os01g0645400	LOC_Os01g45760	Flavin monooxygenase	Yamamoto et al. (2007)
	ERF3	1	Os01g0797600	LOC_Os01g58420	AP2/ERF transcription factor	Zhao et al. (2015a)
	OsCKX4	1	Os01g0940000	LOC_Os01g71310	Cytokinin oxidase/dehydrogenase	Gao et al. (2014)
	OsCAND1	2	Os02g0167700	LOC_Os02g07120	SCF^{TIR1} ubiquitin ligase	Wang et al. (2011)
	OsRR2	2	Os02g0557800	LOC_Os02g35180	Type-A response regulator	Zhao et al. (2009)
	REH1/ *OsPIN1b*	2	Os02g0743400	LOC_Os02g50960	Auxin efflux carrier	Xu et al. (2005)
	CRL1/*ARL1*	3	Os03g0149100	LOC_Os03g05510	LOB/ASL transcription factor	Inukai et al. 2005), Liu et al. (2005)
	OsCOW1 (*OsYUC8*)	3	Os03g0162000	LOC_Os03g06654	Flavin monooxygenase	Woo et al. (2007)
	CRL4/ *OsGNOM1*	3	Os03g0666100	LOC_Os03g46330	Arf-GEF	Kitomi et al. (2008b), Liu et al. (2009)
	OsRR1	4	Os04g0442300	LOC_Os04g36070	Type-A response regulator	Kitomi et al. (2011)
	OsMT2b	5	Os05g0111300	LOC_Os05g02070	Metallothionein	Yuan et al. (2008)
	OsPIN2	6	Os06g0660200	LOC_Os06g44970	Auxin efflux carrier	Chen et al. (2012b)
	CRL5	7	Os07g0124700	LOC_Os07g03250	AP2/ERF transcription factor	Kitomi et al. (2011)
	CRL6	7	Os07g0497100	LOC_Os07g31450	CHD family protein	Wang et al. (2016)
	WOX11	7	Os07g0684900	LOC_Os07g48560	WUS-related homeobox protein	Zhao et al. (2009)
	OsIAA3 (*OsIAA31*)	12	Os12g0601400	LOC_Os12g40900	Aux/IAA protein	Nakamura et al. (2006)

(continued)

Table 14.1 (continued)

Phenotype	Gene	Chromosome	Locus ID		Encoded protein	References
			RAP	MSU		
	OsPID	12	Os12g0614600	LOC_Os12g42020	Serine/threonine kinase	Morita and Kyozuka 2007)
	OsmiR393a	1	n.a.	n.a.	MicroRNA	Bian et al. (2012)
	OsmiR393b	4	n.a.	n.a.	MicroRNA	Bian et al. (2012)
Lateral root number	*OsAUX1*	1	Os01g0856500	LOC_Os01g63770	Auxin influx carrier	Zhao et al. (2015b)
	LRL2/ *OsCYP2*	2	Os02g0121300	LOC_Os02g02890	Cyclophilin	Kang et al. (2013)
	OsIAA11	3	Os03g0633500	LOC_Os03g43400	Aux/IAA protein	Zhu et al. (2012)
	OsIAA13	3	Os03g0742900	LOC_Os03g53150	Aux/IAA protein	Kitomi et al. (2012)
	OsHO1	3	Os06g0603000	LOC_Os06g40080	Heme oxygenase	Chen et al. (2012a)
	OsORC3	10	Os10g0402200	LOC_Os10g26280	Origin recognition complex protein	Chen et al. (2013)
	OsWOX3A/ *OsNS* (*NAL2*)	11	Os11g0102100	LOC_Os11g01130	WUS-related homeobox protein	Cho et al. (2013)
	OsWOX3A/ *OsNS* (*NAL3*)	12	Os12g0101600	LOC_Os12g01120	WUS-related homeobox protein	Cho et al. (2013)
Root hair number	*OsFH1*	1	Os01g0897700	LOC_Os01g67240	Multidomain protein	Huang et al. (2013a)
	SRH2	3	Os03g0300000	LOC_Os03g18820	Xyloglucan 6-xylosytransferase	Wang et al. (2014a)
	OsEXPA17	6	Os06g0108600	LOC_Os06g01920	Expansin	Yu et al. (2011)
	OsRHL1	6	Os06g0184000	LOC_Os06g08500	bHLH transcription factor	Ding et al. (2009)
	RTH1/ *OsAPY1*	7	Os07g0682800	LOC_Os07g48430	ATP diphosphohydrolase	Yuo et al. (2009)
	OsSNDP1	10	Os10g0122600	LOC_Os10g03400	Phosphatidylinositol transfer protein	Huang et al. (2013b)
	OsEXPA30	10	Os10g0535900	LOC_Os10g39110	Expansin	Yu et al. (2011)
	RTH2/ *OsCSLD1*	10	Os10g0578200	LOC_Os10g42750	Cellulose synthase-like protein	Kim et al. (2007), Yuo et al. (2011)

Root meristem formation	*QHB*	1	Os01g0854500	LOC_Os01g63510	WUSCHEL-type homeobox protein	Kamiya et al. (2003b)
	DOCS1	2	Os02g0236100	LOC_Os02g14120	LRR-RLK	Huang et al. (2012)
	OsIAA23	6	Os06g0597000	LOC_Os06g39590	Aux/IAA protein	Ni et al. (2011)
	OsSHR1	7	Os07g0586900	LOC_Os07g39820	GRAS transcription factor	Kamiya et al. (2003a), Cui et al. (2007)
	OsSCR1	11	Os11g0124300	LOC_Os11g03110	GRAS transcription factor	Kamiya et al. (2003a), Cui et al. (2007)
Root length	*OsEXPA8*	1	Os01g0248900	LOC_Os01g14650	Expansin	Ma et al. (2013)
	OsSPR1	1	Os01g0898300	LOC_Os01g67290	Armadillo-like repeat domain protein	Jia et al. (2011)
	OsMOGS	1	Os01g0921200	LOC_Os01g69210	Mannosyl-oligosaccharide glucosidase	Wang et al. (2014b)
	OsCYT-INV1	2	Os02g0550600	LOC_Os02g34560	Alkaline/neutral invertase	Jia et al. (2008)
	OsCKI1	2	Os02g0622100	LOC_Os02g40860	Putative casein kinase I	Liu et al. (2003)
	RT/*OsGLU3*	4	Os04g0497200	LOC_Os04g41970	Endo-1,4-β-D-glucanase	Inukai et al. (2012), Zhang et al. (2012b)
	OsARF12	4	Os04g0671900	LOC_Os04g57610	Auxin response factor	Qi et al. (2012)
	OsRPK1	5	Os05g0486100	LOC_Os05g40770	LRR-RLK	Zou et al. (2014)
	OsDGL1	7	Os07g0209000	LOC_Os07g10830	Dolichyl-diphosphooligosaccharide–protein glycosyltransferase	Qin et al. (2013)
	OsGNA1	9	Os09g0488000	LOC_Os09g31310	Glucosamine-6-P acetyltransferase	Jiang et al. (2005)

n.a. not available

expression of its *Arabidopsis* ortholog *WUS-RELATED HOMEOBOX 5* (*WOX5*). *WOX5* is involved in QC maintenance, suggesting that *QHB* plays a similar role in rice (Kamiya et al. 2003b; Sarkar et al. 2007). The phytohormone auxin is crucial for QC maintenance (Friml et al. 2002; Sabatini et al. 1999). A mutation in a member of the *AUXIN* (*Aux*)/*INDOLE-3-ACETIC ACID* (*IAA*) gene family, *OsIAA23*, causes defects in postembryonic QC maintenance due to the disintegration of the root cap and termination of root growth, suggesting the importance of auxin in rice QC maintenance (Ni et al. 2011). The GRAS family genes *SCARECROW* (*SCR*) and *SHORT-ROOT* (*SHR*) are also the key regulators of QC maintenance and root radial patterning (Di Laurenzio et al. 1996; Helariutta et al. 2000; Sabatini et al. 2003). *OsSCR1* is specifically expressed in the endodermis, whereas *OsSHR1* is expressed in the stele, similar to the patterns of their expression in *Arabidopsis*; OsSCR1 and OsSHR1 interact with each other when produced in yeast, similar to SCR and SHR in *Arabidopsis* (Cui et al. 2007; Kamiya et al. 2003a). These data suggest that *OsSCR1* and *OsSHR1* control the division of the epidermis-endodermis initial cells in rice. Concerning the outer cell layers (epidermis, exodermis, and sclerenchyma) in rice, a mutation in *DEFECTIVE IN OUTER CELL LAYER SPECIFICATION 1* (*DOCS1*), which encodes a leucine-rich repeat receptor-like kinase (LRR-RLK), causes irregular epidermal cells with far fewer root hairs and transformation of some exodermal cells into additional sclerenchyma-like cells (Huang et al. 2012). The outer cell layers play an important role in protecting the inner root tissues from various stresses (Huang et al. 2009). Proper development of both inner and outer cell layers is essential for root development.

14.2.2 Radicle

A fertilized egg (zygote) undergoes iterative cell divisions to form a globular embryo with no apparent morphological differentiation until 3 DAP, and a radicle primordium is observed at 4 DAP together with a shoot apical meristem (Itoh et al. 2005). Therefore, radicle initiation is assumed to occur at the globular stage. The molecular mechanisms of radicle formation in rice and the key genes involved are presumed on the basis of experimental reports in *Arabidopsis*. Some rice mutants have defects in radicle formation; however, the causative genes have not yet been isolated.

Hong et al. (1995) reported three independent lines of *radicleless* (*ral*) mutants. One of them, *ral1*, is viable, although it has a reduced number of crown and lateral roots (Scarpella et al. 2003). The *ral1* plants also have narrower leaves with vascular patterning distortions associated with a defective response to auxin, indicating that *RAL1* has auxin-related functions. A mutant of *Oryza sativa CONNECTED EMBRYO* (*OsCEM*) produces multiple shoots and radicles (Yang and Hwa 2008). Endogenous indole-3-acetic acid (IAA) level in *oscem* embryos is lower than that in wild-type embryos despite no differences in vegetative stages.

This result also strongly suggests the relationship between auxin and radicle formation. Multiple radicles are sometimes observed in the *apical displacement 1* (*apd1*) mutant, in which the apical shoot region and scutellum are reduced and the basal region of the radicle is enlarged (Kinae et al. 2005). Formation of multiple radicles in *apd1* may be a secondary effect of the aberrant apical-basal patterning of the embryo (Kinae et al. 2005).

Our knowledge of radicle formation is limited compared to that of other types of rice roots because there are few reports of radicle-related genes. Recently, microarray analysis of spatiotemporal gene expression patterns during early embryogenesis was performed (Itoh et al. 2016). Based on the datasets from that study, we listed putative radicle-related genes and assessed their functions by performing in situ hybridization (Fig. 14.3). The list contained 23 genes (including 14 encoding transcription factors), which were classified into three groups according to their expression patterns (Fig. 14.3): genes with radicle-specific expression from radicle initiation to its development (Group I, 14 genes); genes with radicle-specific expression during radicle development (Group II, 4 genes); and genes not showing radicle-specific expression (Group III, 5 genes). We then focused on the transcription factor genes in Group I (Fig. 14.3a). Four genes for APETALA 2 (AP2)/ETHYLENE RESPONSIVE FACTOR (ERF) transcription factors with two AP2 repeats (Os06g0657500, Os02g0614300, Os04g0504500, and Os01g0899800) were expressed in the center of the 3-DAP embryo where a radicle will be formed (Fig. 14.3a). They share sequence similarities with *PLETHORA* (*PLT*) homologs, the key factors in root meristem formation and maintenance (Aida et al. 2004; Galinha et al. 2007). They are assumed to have roles in radicle formation, especially meristem initiation and development because PLT protein dosage in RAM, which is determined by auxin, is translated into distinct cellular responses: high levels of PLT promote stem cell identity and maintenance, whereas low levels enhance cell division and differentiation (Galinha et al. 2007). Two NAC transcription factors, Os06g0530400 (*OsNAC7*) and Os02g0745300, share sequence similarities with *SOMBRERO* (*SMB*) and *FEZ*, which are involved in root cap development in *Arabidopsis* (Willemsen et al. 2008). Both Os06g0530400 and Os02g0745300 showed a root cap-like pattern in the basal region of the embryo (Fig. 14.3a), suggesting that both genes may be involved in root cap development in radicle formation.

We also examined the expression patterns of Group I transcription factor genes in crown root primordia to check whether these patterns are similar to those in the radicle. Almost all of the genes examined had similar expression patterns, suggesting that they have the similar function during radicle and crown root formation (Fig. 14.4a). We also performed double-target in situ hybridization in crown root primordia, where the identities of cell layers are more easily distinguishable than in the radicle. First, we used the probe for *OsSCR1* and *AP2/ERF*s. *OsSCR1* mRNA was localized in the endodermis, including epidermis-endodermis initial cells, and in central QC cells (Fig. 14.4b). Os06g0657500 was expressed in the inner and outer layers of the endodermis without overlapping with the *OsSCR1* signal (Fig. 14.4b). Os02g0614300 was also expressed in the inner and

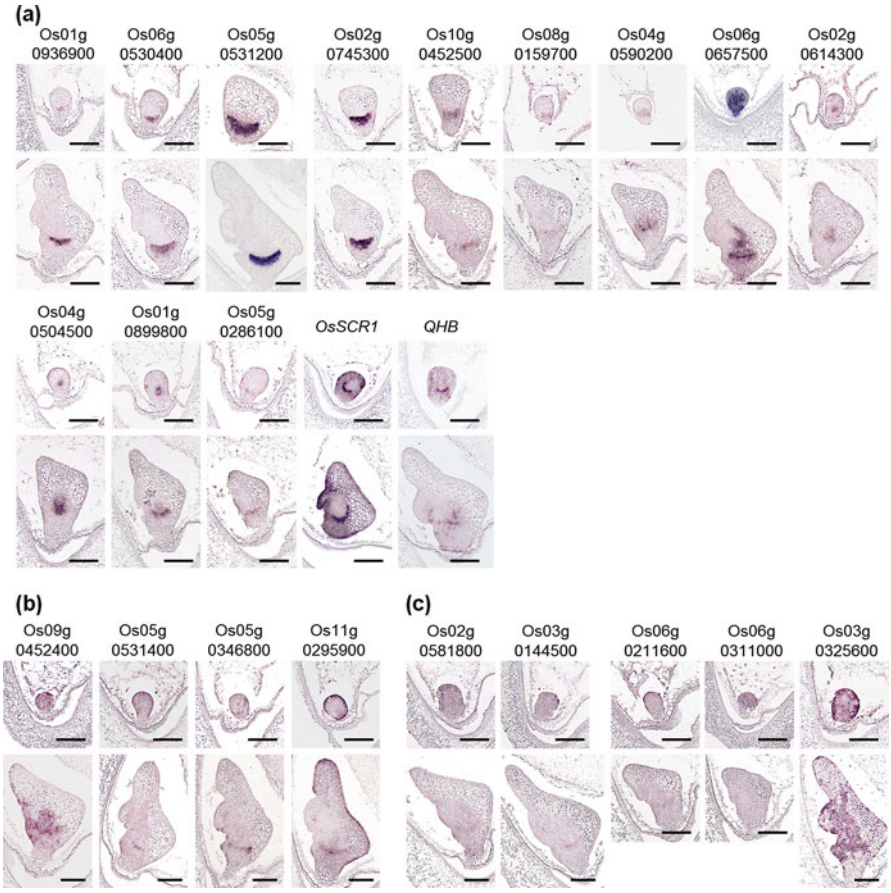

Fig. 14.3 Expression patterns of candidate genes essential for radicle formation in rice. (**a**) Group I: genes showing radicle-specific expression from radicle initiation to development. (**b**) Group II: genes showing radicle-specific expression during radicle development. (**c**) Group III: genes not showing radicle-specific expression. For each gene, a longitudinal section through a 3-DAP (days after pollination) to early 4-DAP embryo is shown in the top row and that through a late 4-DAP to 5-DAP embryo is shown in the *bottom row*. Signals were detected with DIG-NBT/BCIP. Bars = 100 μm. Of the 23 genes shown, 16 were selected as follows: 1st step, expression in the basal part of a 3-DAP embryo is >10 times that in the apical part according to microarray datasets in Itoh et al. (2016); 2nd step, high expression in the root of a 7-DAP embryo in Itoh's datasets; 3rd step, high expression in the root in a mature plant and embryo in a seed according to RiceXPro (Sato et al. 2011). Five transcription factor genes that are coexpressed with these 16 genes and are highly expressed in the radicle of a 7-DAP embryo were also chosen. Additionally, two genes (*OsSCR1* and *QHB*) previously reported to be expressed in the endodermis and central cells of QC were analyzed (Kamiya et al. 2003a, b)

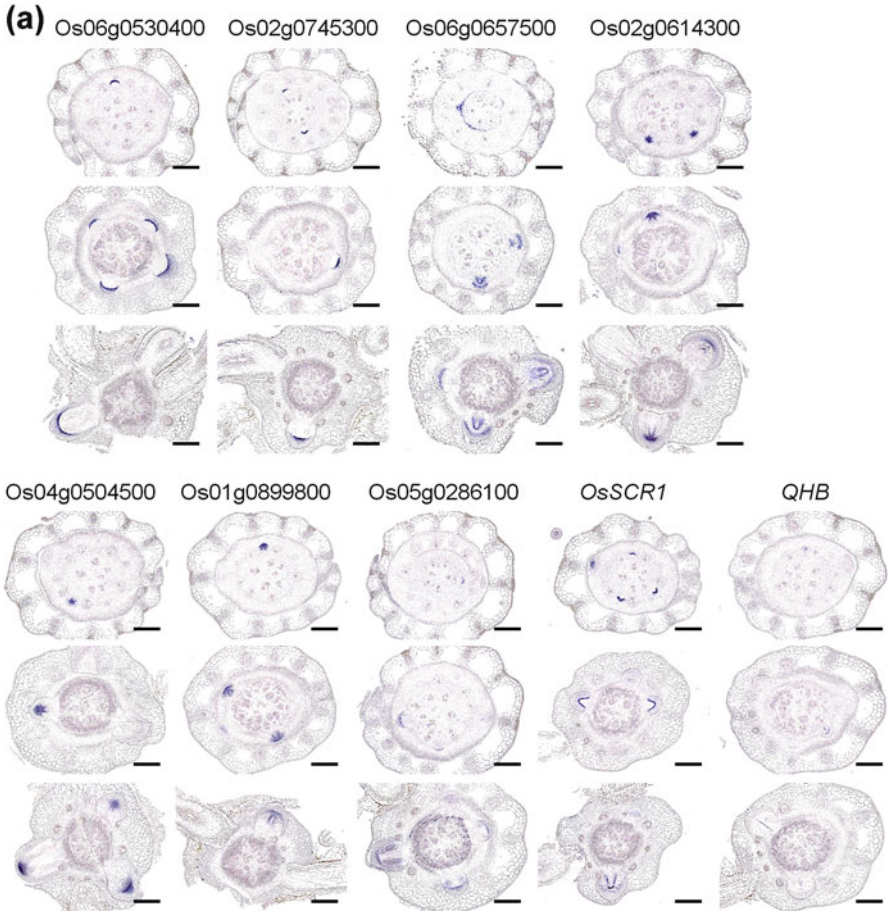

Fig. 14.4 Expression patterns of transcription factor genes in crown root primordia of rice. (**a**) Cross sections through the nodes of 7-day-old plants. Upper sections, initiation to early developing stage of crown root primordia. Middle sections, developing stage of crown root primordia. Lower sections, late developing to emergence stage. Signals were detected using DIG-NBT/BCIP. Bars = 200 μm. (**b**) Double-target in situ hybridization with probes for *OsSCR1* and *AP2/ERF* transcription factors. The *OsSCR1* signal was detected with biotin-TSA-fluorescein (green fluorescence), and Os06g0657500 and Os02g0614300 signals were detected with DIG-Fast Red (red fluorescence). Arrowheads indicate the central cells of QC. (**c**) Double-target in situ hybridization with the probes for NAC transcription factors. The Os02g0745300 signal was detected with biotin-TSA-fluorescein, and the Os06g0530400 signal was detected with DIG-Fast Red

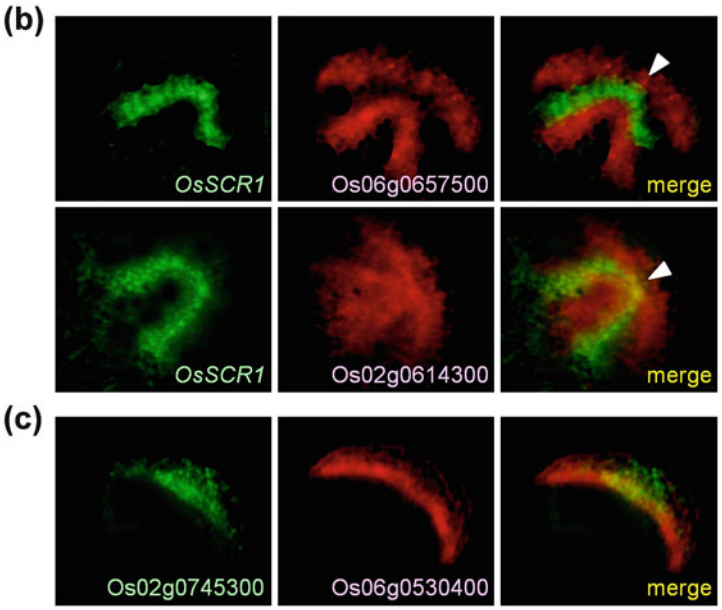

Fig. 14.4 (continued)

outer layers of the endodermis but overlapped with the *OsSCR1* signal in the stem cell region (Fig. 14.4b). The expression patterns of two *NAC*s were slightly different: the Os06g0530400 signal was observed in peripheral root cap cells and columella cells, whereas Os02g0745300 was expressed mainly in columella cells (Fig. 14.4c). Slight differences in the expression regions among the same gene family members might suggest the specialized function of each gene.

14.2.3 Crown Roots

Monocot plants produce numerous crown roots from nodes, which form a fibrous root system. During crown root morphogenesis, three developmental stages can be clearly distinguished: initiation, development, and growth (Coudert et al. 2010; Itoh et al. 2005; Kitomi et al. 2011; Zhao et al. 2009). The regulation of crown root formation in rice and that of lateral root formation in *Arabidopsis* share several common characteristics. The molecular mechanism of crown root formation is relatively well analyzed compared with that of other root types (Fig. 14.5).

Auxin is essential throughout root morphogenesis in these species, and auxin-related mutations lead to morphological abnormalities in rice crown roots and *Arabidopsis* lateral roots. Rice *YUCCA 1* (*OsYUC1*) and *CONSTITUTIVELY WILTED 1* (*OsCOW1*) encode flavin monooxygenases, the key enzymes in auxin

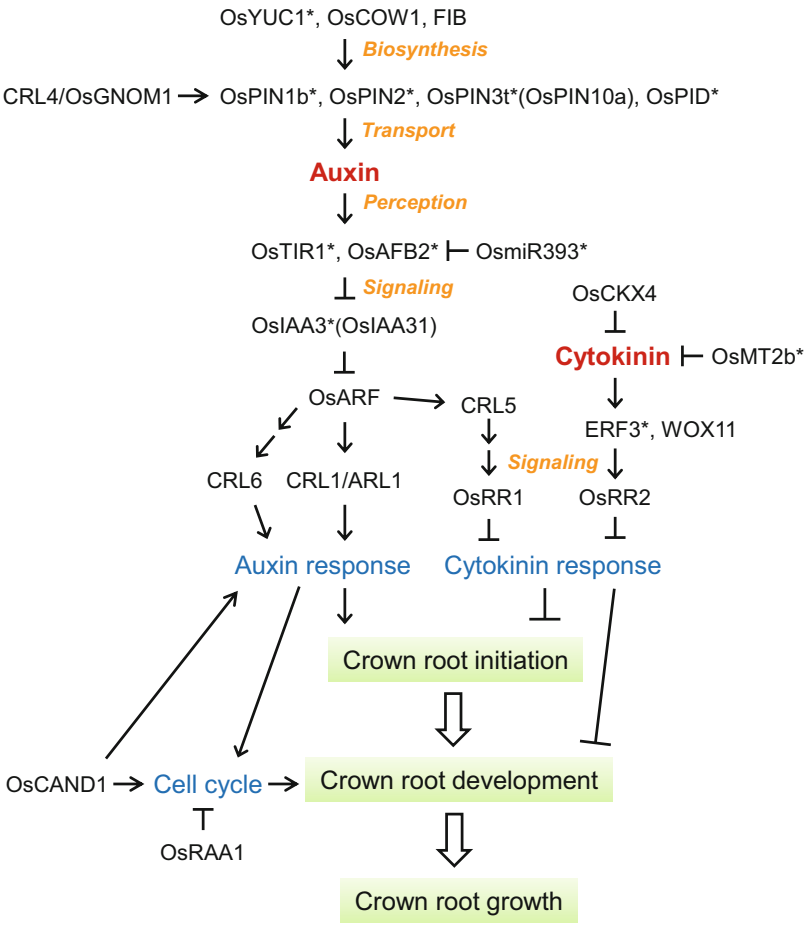

Fig. 14.5 Proposed gene regulatory pathways of crown root formation in rice. *Genes characterized by using reverse genetic approaches (overexpression, knockdown, or both). Genes without asterisks are cloned and characterized by using forward genetic approaches

biosynthesis (Woo et al. 2007; Yamamoto et al. 2007). Overexpression of *OsYUC1* enhances crown root formation, whereas *OsYUC1* antisense plants show severe growth retardation (Yamamoto et al. 2007). Alleles of *OsCOW1* with the insertion of the *Tos17* transposon or T-DNA decrease the root-to-shoot ratio by reducing crown and lateral root numbers (Woo et al. 2007). *OsCOW1* was also reported as *NARROW LEAF 7* (*NAL7*)/*OsYUC8*, which was identified in a mutant with narrow leaves (Fujino et al. 2008). The *fish bone* (*fib*) mutant defective in crown and lateral root formation has a mutation in the gene encoding TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1 (OsTAA1), an auxin biosynthetic enzyme (Yoshikawa et al. 2014). Several *PIN-FORMED* (*PIN*) genes, which encode auxin

efflux carriers mediating polar auxin transport, also play a pivotal role in crown root formation. Downregulation of *OsPIN1b* and *OsPIN3t* (termed *OsPIN10a* in Wang et al. 2009; *OsPIN3a* in Miyashita et al. 2010), and overexpression of *OsPIN2*, reduces crown root number (Chen et al. 2012b; Xu et al. 2005; Zhang et al. 2012a). Overexpression of *OsPINOID* (*OsPID*), which controls auxin distribution by controlling subcellular localization of PINs, also causes abnormal crown root development (Morita and Kyozuka 2007). Crown root initiation is impaired in *crown rootless4* (*crl4*)/*osgnom1* mutant (Kitomi et al. 2008b; Liu et al. 2009). GNOM is a membrane-associated guanine-nucleotide exchange factor for the G protein ADP-ribosylation factor (Arf-GEF) and plays an important role in polar auxin transport by establishing coordinated polar localization of PIN1 in *Arabidopsis* (Geldner et al. 2003; Steinmann et al. 1999). Distortion of polar auxin transport and altered expression patterns of *OsPIN*s were observed in *crl4*/*osgnom1* mutants, indicating that polar auxin transport is required for crown root initiation in rice.

Besides auxin biosynthesis and polar transport, auxin perception and signal transduction are essential for crown root formation. A rice microRNA, *miR393*, affects crown root initiation and seminal root development through negative regulation of the homologs of *Arabidopsis* auxin receptors *TRANSPORT INHIBITOR RESPONSE 1* (*TIR1*) and *AUXIN SIGNALING F-BOX 2* (*AFB2*), *OsTIR1*, and *OsAFB2* (Bian et al. 2012). Auxin signal is transmitted by a pathway mediated by Aux/IAA and AUXIN RESPONSE FACTOR (ARF) (Liscum and Reed 2002). Transgenic plants that produced constitutively active Aux/IAA, which was obtained by mutagenizing a conserved amino acid residue in the degradation-related domain (domain II) of OsIAA3 (OsIAA31 in Jain et al. 2006), have reduced crown root number (Nakamura et al. 2006). The *crl1*/*adventitious rootless1* (*arl1*) mutants develop few crown roots; *CRL1*/*ARL1* encodes a plant-specific LATERAL ORGAN BOUNDARIES DOMAIN (LBD)/ASYMMETRIC LEAVES2-LIKE (ASL) transcription factor, which acts downstream of the Aux/IAA and ARF-mediated auxin signaling pathway and whose expression is directly regulated by OsARF (Inukai et al. 2005; Liu et al. 2005). *CRL6*, which encodes a chromodomain helicase DNA-binding (CHD) family protein, is thought to influence crown root initiation and development through the Aux/IAA and ARF-mediated auxin signaling pathway because most of the 31 Aux/IAA genes are downregulated in the *crl6* mutant (Wang et al. 2016). The phenotype of the *crl2* mutant, impaired root gravitropism and crown root initiation, suggests that *CRL2* might also be involved in auxin signaling, although the causal gene has not yet been identified (Inukai et al. 2001; Yamamoto et al. 2010). Most of the auxin-related crown root mutants mentioned above also show defects in lateral root formation and root hair development, indicating the importance of auxin in overall root morphogenesis.

Cytokinin also plays an important role in the regulation of root morphogenesis and is widely known to act antagonistically to auxin: root formation is promoted by auxin but is suppressed by cytokinin. In *Arabidopsis*, root meristem size is controlled by the balance between cell differentiation and division, which results from antagonistic regulation by auxin and cytokinin (Dello Ioio et al. 2007, 2008). This

antagonistic regulation is also important in rice crown root formation. The phenotype of the dominant mutant *root enhancer1* (*ren1-D*), which has an increased crown root number, is caused by the activation of a *CYTOKININ OXIDASE/DEHYDROGENASE* (*CKX*) family gene, *OsCKX4* (Gao et al. 2014). CKXs are the only enzymes known to catalyze the irreversible degradation of cytokinin (Werner et al. 2003). Rice *METALLOTHIONEIN 2b* (*OsMT2b*) also has a role in the development of crown and lateral roots by influencing the endogenous cytokinin level (Yuan et al. 2008). Not only cytokinin content but also cytokinin signaling affects crown root formation. *CRL5* encodes an AP2/ERF transcription factor AINTEGUMENTA (ANT), and its expression is induced by OsARFs (Kitomi et al. 2011). Auxin-induced *CRL5* upregulates a type-A response regulator gene *OsRR1*, which suppresses cytokinin signaling and thus promotes crown root initiation. *WOX11* activates crown root development by directly repressing *OsRR2* (Zhao et al. 2009). Further analysis demonstrated that ERF3 interacts with WOX11 and promotes WOX11 binding to *OsRR2* (Zhao et al. 2015a).

Cell division is essential for crown root formation because it contributes to the development of crown root primordia. The *crl3* mutant produces a few crown root primordia consisting of vacuolated cells, whereas those in wild type consist of non-vacuolated cells (Kitomi et al. 2008a). Vacuolated cells divide in the early stage of crown root primordia development; however, cell division activity is gradually arrested, and primordia development is stopped in *crl3*. Overexpression of *O. sativa ROOT ARCHITECTURE ASSOCIATED 1* (*OsRAA1*) increases the number of crown and lateral roots compared with control plants (Ge et al. 2004). OsRAA1 is an anaphase-promoting complex/cyclosome (APC/C)-targeted protein to block the cell cycle at the transition from metaphase to anaphase (Han et al. 2008). A mutation in rice *CULLIN-ASSOCIATED AND NEDDYLATION-DISSOCIATED 1* (*OsCAND1*) causes a defect in the emergence of crown root primordia, although crown root initiation occurs normally (Wang et al. 2011). CAND1 is an SCF^{TIR1} E3 ubiquitin ligase involved in the degradation of Aux/IAA proteins in response to auxin in *Arabidopsis* (Chuang et al. 2004; Feng et al. 2004). *OsCAND1* is involved in auxin signaling to maintain the G2/M cell cycle transition in the crown root meristem and consequently the emergence of crown roots (Wang et al. 2011).

14.2.4 Lateral Roots

Molecular mechanisms of lateral root formation are similar to that of crown root formation; therefore, most of the crown rootless mutants show lateral rootless phenotype as well. However, the differences between crown root and lateral root obviously exist: the sites of their initiation, the number of inner cell layers, physiological functions, and plasticity in response to environmental stimuli (Luquet et al. 2005; Rebouillat et al. 2009; Suralta et al. 2008). Although most of the mutants lacking crown roots also lack lateral roots, some mutants have

abnormalities in lateral root formation without crown root defects. Analysis of such mutants might disclose lateral root-specific factors and schemes.

As mentioned above, auxin is a major player in lateral root formation. T-DNA-insertion mutants of the rice gene *AUXIN RESISTANT 1* (*OsAUX1*), which is evolutionarily close to the members of the auxin influx carrier gene family *AUX1/LIKE AUX 1* (*LAX*), have reduced lateral root number (Zhao et al. 2015b). The double mutant of *nal2* and *nal3* (*nal2/3*), which has mutations in two identical *OsWOX3A*/*OsNARROW SHEATH* (*OsNS*) genes located on chromosomes 11 and 12, respectively, produces fewer lateral roots than does the wild type (Cho et al. 2013). Reduced lateral root initiation in *nal2/3* seems to be attributable to compromised distribution of endogenous IAA caused by altered expression of *OsPIN1* and *OsPIN2*. Phenotypes of these mutants demonstrate that polar auxin transport mediated by auxin influx and efflux carrier proteins is important for lateral root formation. The phenotypes of some mutants also indicate the importance of auxin signaling mediated by Aux/IAA and ARF in lateral root formation. The gain-of-function mutants *osiaa11* and *osiaa13*, which have stabilizing mutations in domain II of Aux/IAA proteins, have dramatically reduced lateral root number (Kitomi et al. 2012; Zhu et al. 2012). The rice *cyclophilin 2* (*oscyp2*) mutant also shows impaired lateral root initiation (Kang et al. 2013). *OsCYP2* is involved in Aux/IAA degradation by stimulating the activity of the SCF^{TIR} ubiquitin E3 ligase complex. Auxin signaling is likely disturbed in these mutants because degradation of Aux/IAA proteins allows auxin-responsive transcription to be regulated by ARF proteins, which then act as transcriptional activators or repressors (Gray et al. 2001). A mutation in rice *HEME OXYGENASE* (*OsHO1*), which encodes an enzyme that catalyzes the degradation of heme into biliverdin IXa, Fe^{2+}, and carbon monoxide, also affects lateral root formation in a manner dependent on auxin and stress-related signals (Chen et al. 2012a). Some mutants with auxin-related abnormalities also have defects in lateral root formation; these include *lateral rootless 1* (*lrt1*), *lrt2*, *auxin-resistant mutant 1* (*arm1*), and *arm2* (Chhun et al. 2003; Faiyue et al. 2010; Hao and Ichii 1999; Wang et al. 2006b).

Cell cycle regulation is necessary for lateral root development. The mutant of *O. sativa ORIGIN RECOGNITION COMPLEX SUBUNIT 3* (*OsORC3*) has a temperature-dependent defect in lateral root development (Chen et al. 2013). In *OsORC3* knockdown plants, the emergence of lateral root primordia is blocked due to the perturbation of cell cycle-related gene expression in the primordia (Chen et al. 2013).

14.2.5 Root Hairs

Root hairs are long cylindrical outgrowths of individual root epidermal cells and are thus different from seminal, crown, and lateral roots. Root hair development includes three stages: cell fate determination, root hair initiation, and root hair elongation (Huang et al. 2013b). Vesicle trafficking, cytoskeleton reorganization,

and cell wall loosening and synthesis are major driving forces for root hair elongation that depend on gene expression promoted by signals such as auxin, cellular pH, calcium ions, extracellular reactive oxygen species (ROS), and phosphatidylinositols (Libault et al. 2010).

A mutation in *O. sativa SEC14-NODULIN DOMAIN PROTEIN* (*OsSNDP1*), which encodes a phosphatidylinositol transfer protein, leads to short-branched root hairs (Huang et al. 2013b). Similar defects were reported in *Arabidopsis* mutants with defects in phospholipid metabolism and signaling (Kusano et al. 2008; Vincent et al. 2005), indicating the critical roles of phospholipids in root hair elongation. *ROOT HAIRLESS 1* (*RTH1*)/*O. sativa APYRASE 1* (*OsAPY1*), which encodes an enzyme that hydrolyzes NTPs and/or diphosphates, also affects root hair elongation (Yuo et al. 2009). Apyrases control the concentration of extracellular ATP, which functions as a signal molecule for growth control and is localized in the regions of active growth and cell expansion such as root hair tips (Roux and Steinebrunner 2007; Wu et al. 2007). Rice *FORMIN HOMOLOGY 1* (*OsFH1*) is important for root hair elongation under submerged conditions (Huang et al. 2013a). Formins play critical roles in cytoskeleton organization by nucleating actin polymerization and elongation and bundling actin filaments, which drive tip growth (Paul and Pollard 2009). *OsFH1* is assumed to have similar functions, although no null mutant with a defective root hair phenotype has been reported in *Arabidopsis* or rice (Deeks et al. 2005; Yi et al. 2005).

Factors involved in cell wall modification are closely related to root hair development. Root hair length is reduced in the rice *short root hair 2* (*srh2*) mutant, which has a mutation in the *XYLOGLUCAN XYLOSYLTRANSFERASE 1* (*OsXXT1*) gene (Wang et al. 2014a). Xyloglucan is not considered to be an important component of cell wall in grasses, including rice, because its content is below 5% (Vogel 2008). However, the *srh2* mutant demonstrates the importance of xyloglucan in rice root hair development. Expansins, which are associated with cell wall loosening, permit turgor-driven cell elongation (Cosgrove 2000). Rice *EXPANSIN A17* (*OsEXPA17*) and *OsEXPA30* are also involved in root hair elongation because the *osexpA17* mutant shows defects in root hair elongation, and these defects are partially complemented by *OsEXPA30* (Yu et al. 2011). The promoters of *OsEXPA17* and *OsEXPA30* contain conserved root hair-specific *cis*-elements (RHEs), which are also found in root hair-specific genes and genes paralogous to *AtEXPA7* (Kim et al. 2006). These *EXPA*s with RHEs are expressed in a root hair-specific manner in *Arabidopsis* and rice (Kim et al. 2007; Yu et al. 2011). *OsEXPB5*, which is so far found in Gramineae family and is absent in dicots, also has RHEs in the promoter region, and its expression is strongly associated with root hair initiation and elongation (Won et al. 2010); however, its in vivo function in root hair development has not been demonstrated. *RTH2*/*O. sativa CELLULOSE SYNTHASE-LIKE D1* (*OsCSLD1*) is also required for root hair elongation (Kim et al. 2007; Yuo et al. 2011). Only *OsCSLD1* is specifically expressed in roots, similar to root hair-specific genes with RHEs, whereas other *OsCSLD* subfamily members are expressed in both roots and shoots.

The root epidermis comprises hair cells (trichoblasts) and non-hair cells (atrichoblasts). In each plant species, root hair patterning belongs to one of three types according to the way how the fate of each cell is determined (Kim et al. 2006). In Type 1, hair cells can differentiate from any epidermal cell. In Type 2, the root epidermis consists of cells of two sizes, and only the short cells differentiate into hair cells. In Type 3, hair cells produce rows along the longitudinal root axis, resulting in a striped pattern. Root hair patterning in rice is Type 2, and the differences in size between mature hair and non-hair cells result from differential cell expansion relatively late in the development, after initiation of root hair growth (Kawata and Ishihara 1959; Kim and Dolan 2011). A mutation in the *O. sativa ROOT HAIRLESS 1* (*OsRHL1*) gene, which encodes a basic helix-loop-helix (bHLH) transcription factor, results in very short root hairs (Ding et al. 2009). In the *osrhl1* mutant, clearly short and long epidermal cells characteristic of Type 2 species are not observed, suggesting that *OsRHL1* controls root hair elongation and epidermal cell patterning, similar to the bHLH gene *ROOT HAIR DEFECTIVE 6-LIKE 4* (*RSL4*) in *Arabidopsis* (Ding et al. 2009; Yi et al. 2010).

14.3 Formation of Root System Architecture

The outline of the rice root system is formed by multiple crown roots developed from several phytomers; a phytomer is a nodal unit consisting of a leaf, an axillary bud, and crown roots (Rebouillat et al. 2009). Crown roots developed from the upper and lower regions of each node are called the upper and lower crown roots, respectively (Fig. 14.2). The lower crown roots have a larger diameter than the upper crown roots (Abe and Morita 1994). Another feature of lower crown roots is downward elongation, whereas upper crown roots elongate randomly in directions ranging from lateral to vertical, suggesting that they respond to gravity more weakly than do the lower crown roots (Abe and Morita 1994). Overall, the growth angle of each upper and lower crown root determines vertical distribution of the whole root system in the soil. Shallow and steep root growth angles favor root distribution in the topsoil and subsoil, respectively. The maximum length of each crown root restricts range of access for absorption of water and nutrients from the soil. Short roots result in compact root systems, whereas long roots produce large root systems.

The genetic mechanism of root system development in rice has been dissected mainly on the basis of QTL analysis; the first such study was reported by Champoux et al. (1995). Hundreds of QTLs with small to intermediate genetic effects on many root parameters that affect root system architecture have been detected in rice; such parameters include the growth angle, length, volume, and thickness of the roots (Rebouillat et al. 2009). However, the genetic mechanisms underlying these QTLs are poorly understood. On the other hand, several genes for root development have been isolated in rice mutants showing abnormal root phenotypes (Rebouillat et al.

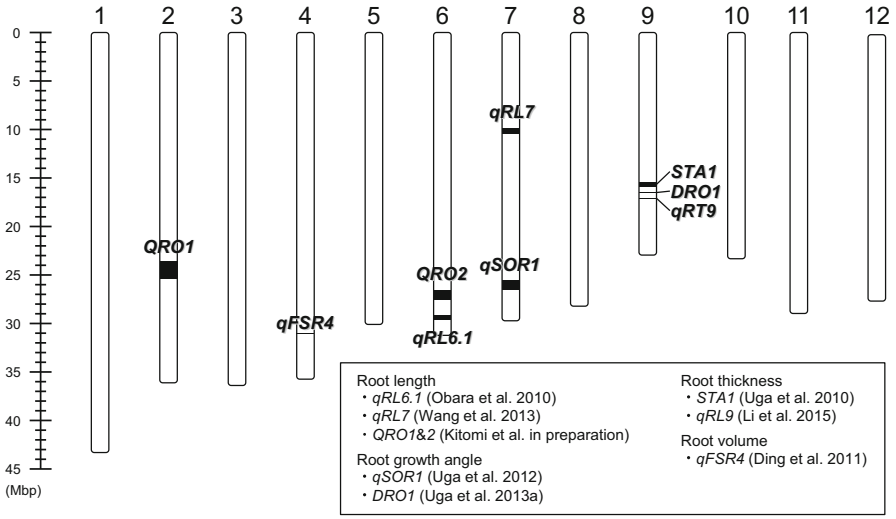

Fig. 14.6 Location of rice QTLs involved in root system architecture that are potentially useful for molecular breeding. Chromosome numbers are indicated above each linkage map. Black box indicates a region that contains a root QTL

2009; Wu and Cheng 2014). In this section, we discuss the genes and QTLs related to quantitative variation of root system architecture in rice (Table 14.1; Fig. 14.6).

14.3.1 Root Growth Angle

Root growth angle is controlled by several environmental factors such as gravity, light, and water potential (Oyanagi et al. 1993; Uga et al. 2015a). Root gravitropism has been well studied in *Arabidopsis* (Baldwin et al. 2013; Morita 2010), but not in monocot plants including rice. Only two QTLs for the root gravitropic response have been reported in rice (Norton and Price 2009), but the underlying genes have not yet been isolated. *DRO1*, which was reported originally as a major QTL responsible for root growth angle, is also involved in gravitropism (Uga et al. 2013a). *DRO1* is negatively regulated by auxin signaling downstream of Aux/IAA and ARF and is involved in cell elongation in the root tip, which causes gravitropic bending (Uga et al. 2013a). Under normal growth conditions, *DRO1* is expressed around the RAM in the root tip and crown root primordia. For the response to gravitropic stimuli (i.e., rotation of the roots from the normal vertical axis to the horizontal axis), *DRO1* transcripts in the outer cells of the distal elongation zone are repressed on the lower side than on the upper side of the roots by the redirected auxin flow to the lower side of the root, resulting in decreased cell elongation in the lower side relative to the upper side. This process contributes to asymmetric

growth, leading to root gravitropic bending. Thus, QTLs for gravitropism should affect root growth angle, resulting in a natural variation of root system architecture.

Genes with high sequence similarity to *DRO1* have been found in other monocots such as maize, sorghum, and barley, but their physiological and molecular functions are still unknown (Uga et al. 2013a). Recently, genes with low sequence similarity to DRO1 have been identified in dicots. The legume *Medicago truncatula* carrying mutations in *NEGATIVE GRAVITROPIC RESPONSE OF ROOTS* (*NGR*) shows a negative root gravitropic response (Ge and Chen 2016). Only triple mutants of three redundant *AtNGR* genes (At1g17400, At1g72490, At1g19115) in *Arabidopsis* also showed a similar negative root gravitropic response (Ge and Chen 2016). These *NGR* genes may be *DRO1* homologs in the IGT family, the members of which have relatively low sequence similarity to each other but have conserved amino acid motifs (Guseman et al. 2017). These findings suggest that the functions of *DRO1* and *DRO1* homologs in root gravitropism are conserved in monocots and dicots. The IGT family also includes *TILLER ANGLE CONTROL 1* (*TAC1*) and *LAZY1*, which control the branching angle of lateral shoot organs in both monocots and dicots (Guseman et al. 2017), suggesting that this gene family might be associated with the regulation of growth angle in shoot and root organs.

Many other QTLs for root growth angle have been reported in rice (Kitomi et al. 2015; Lou et al. 2015; Uga et al. 2012, 2013b, 2015b). *DRO2* (Uga et al. 2013b), *DRO3* (Uga et al. 2015b), *DRO4* (Kitomi et al. 2015), and *DRO5* (Kitomi et al. 2015) were detected in seven F_2 mapping populations derived from a cross between several rice accessions with different root growth angles and "Kinandang Patong" as a donor line with a large root growth angle. Therefore, QTLs associated with root growth angle distinct from *DRO1* exist in "Kinandang Patong." *qSOR1* (*quantitative trait locus for SOIL SURFACE ROOTING 1*) has been detected on chromosome 7 in recombinant inbred lines derived from a cross between "Gemdjah Beton," a lowland rice accession with a high proportion of crown roots that run along or near the soil surface, and "Sasanishiki," a lowland rice accession that does not form soil-surface roots (Uga et al. 2012). The "Gemdjah Beton" allele of *qSOR1* causes many thick crown roots to elongate near the soil surface from the seedling stage. This phenotype is very unique because thick crown roots generally elongate downward. *qSOR1* was fine-mapped to a 812-kb candidate region on chromosome 7 (Uga et al. 2012; Fig. 14.6). Lou et al. (2015) also reported QTLs for root growth angle on chromosomes 1, 2, 4, 7, and 10. Among them, three QTLs on chromosomes 2, 4, and 7 are located near the regions of *DRO4*, *DRO2*, and *qSOR1*, respectively. Cloning of these QTLs would deepen our understanding of the genetic mechanisms that determine root growth angle in rice.

14.3.2 Root Length

Maximal root length is determined by the rate and duration of root elongation. Root elongation is caused by cell division and elongation. Mutant analyses revealed that

genes related to cell wall growth, cell expansion, and auxin signaling are involved in the division and elongation of root cells. Cell wall development affects root elongation and the maintenance of root structure. *OsDGL1* encodes the dolichyl-diphosphooligosaccharide-protein glycosyltransferase 48-kDa subunit precursor (Qin et al. 2013). An ethyl methanesulfonate (EMS)-induced *osdgl1* mutant has a defect in *N*-glycosylation, an altered composition of matrix polysaccharides in the cell wall, and cell death in the root, resulting in a decrease in root elongation without a decrease in the numbers of crown roots, lateral roots, or root hairs. *OsMOGS* encodes a putative mannosyl-oligosaccharide glucosidase and acts downstream of *OsDGL1* during *N*-glycan processing in the endoplasmic reticulum (Wang et al. 2014b). An EMS-induced *osmogs* mutant has a decreased cell division and elongation in the root, resulting in short roots. OsMOGS is needed for cellulose biosynthesis and OsABCB-mediated auxin transport in rice (Wang et al. 2014b). Other genes associated with cell wall modification also control root elongation. *ROOT GROWTH INHIBITING* (*RT*)*/OsGLU3*, which encodes a membrane-anchored endo-1,4-β-D-glucanase, is involved in cell wall loosening necessary for root cell elongation (Inukai et al. 2012; Zhang et al. 2012b). The *rt./osglu3* mutants have short-root phenotypes due to a decrease in longitudinal cell elongation without changes in root differentiation, root cell division, or shoot development. Interestingly, cellulose content in roots is increased in an ethylene imine-induced *rt*. mutant (Inukai et al. 2012) but is decreased in EMS-induced *osglu3* mutants (Zhang et al. 2012b). To reconcile these contradictory findings, further studies are needed. *OsGNA1* encodes glucosamine-6-P acetyltransferase, which is involved in de novo UDP-*N*-acetylglucosamine biosynthesis (Jiang et al. 2005). A T-DNA insertion *osgna1* mutant has decreased root cell elongation caused by cell shrinkage, perhaps because of insufficient UDP-GlcNAc for protein *N*-glycosylation, which is necessary for plant development including cell wall synthesis (Lerouge et al. 1998; Lukowitz et al. 2001). Cell expansion occurs in turgor-driven cell elongation. Transgenic plants overexpressing *OsEXPA8*, which encodes a root-specific α-expansin (Shin et al. 2005), have increased seminal, crown, and lateral root length as well as plant height and increased leaf number and size caused by an increase in cell length in both shoot and root vascular bundles (Ma et al. 2013).

Auxin regulates cell fate determination and cell elongation (Tanaka et al. 2006). These effects are mostly mediated by ARFs. Loss-of-function *Tos17* and T-DNA insertion mutants of *osarf12*, which is a member of ARFs (Wang et al. 2007), have short-root phenotypes due to a smaller elongation zone in seminal roots compared to the wild type (Qi et al. 2012). The short elongation zone is likely caused by a low auxin concentration. *O. sativa SHORT POSTEMBRYONIC ROOTS 1* (*OsSPR1*) encodes a putative mitochondrial protein with an Armadillo-like repeat domain (Jia et al. 2011). EMS-induced *osspr1* mutants have short-root phenotypes (decreased lengths of seminal, crown, and lateral roots) due to reduced cell elongation, whereas lateral root initiation and lateral root number are similar to those in the wild type. *OsCYT-INV1*/*OsNIN8* encodes alkaline/neutral invertase and is homologous to *AtCYT-INV1* in *Arabidopsis* (Ji et al. 2005, Jia et al. 2008). An EMS-induced *Oscyt-inv1* mutant has a short-root phenotype due to a decreased cell length

probably caused by hexose deficiency, as hexoses play various roles in cell elongation.

Despite isolation of several genes, our knowledge of the genetic mechanism controlling root length in rice is still limited compared to that for *Arabidopsis*. Reverse genetics should be a valuable approach to gain a better understanding of the gene network that regulates root length. Antisense transgenic plants with downregulated *OsCKI1*, which encodes putative casein kinase I, have short seminal roots and a low number of crown and lateral roots caused by reduced cell elongation (Liu et al. 2003). Examination of transgenic rice plants over- and under-expressing *OsRPK1*, which encodes an LRR-RLK, revealed that this gene affects seminal root length and crown root number by negatively regulating polar auxin transport (Zou et al. 2014).

Several QTLs for root length have been fine-mapped in rice, although none of them have been cloned (Fig. 14.6). *qRL6.1*, a QTL controlling root length at the seedling stage under hydroponic conditions, was mapped to a 337-kb interval on chromosome 6 (Obara et al. 2010). *qRL7*, a QTL affecting root length at the heading stage under hydroponic conditions, was mapped to a 657-kb interval on chromosome 7 (Wang et al. 2013). Recently, *QUICK ROOTING 1* (*QRO1*) and *QRO2* have been fine-mapped on chromosomes 2 and 6, respectively, in chromosome segment substitution lines derived from a cross between "IR64" and "Kinandang Patong" and grown under hydroponic conditions (Kitomi et al. in press, Fig. 14.6).

14.3.3 Other Root Traits

The combination of growth angle and length in seminal and crown roots is the main determinant of root system architecture in cereals (Abe and Morita 1994; Araki et al. 2002), although other root traits such as volume and thickness are also important.

Root volume affects root surface area and thus absorption of water and nutrients from soil (Gowda et al. 2011; Wang et al. 2006a), but rice genes that control root volume have not yet been isolated. *qFSR4*, a QTL for root volume per tiller, has been fine-mapped on chromosome 4 (Ding et al. 2011). The 38-kb *qFSR4* candidate region has three open reading frames including *NAL1* (Qi et al. 2008). The *NAL1* gene is associated with polar auxin transport and controls leaf width. *qFSR4* also affects flag leaf width. *NAL1* may be the most promising candidate gene for *qFSR4* because polar auxin transport affects root development and shoot growth.

Root thickness affects uptake of water and nutrients as well as root penetration ability (Gowda et al. 2011; Wang et al. 2006a). *qRT9*, a QTL for root thickness, has been fine-mapped to an 11.5-kb candidate region on chromosome 9 (Li et al. 2015) with only one annotated open reading frame, Os09g0455300, which encodes a

putative bHLH transcription factor (OsbHLH120). Haplotype and expression analyses suggest that *OsbHLH120* is the candidate gene for *qRT9*. For water and nutrient translocation, stele and xylem structures should be more important than root thickness (Uga et al. 2008). *STELE TRANSVERSAL AREA 1* (*STA1*), a QTL controlling stele transversal area, has been fine-mapped to a 359-kb interval between SSR markers RM566 (14.70 Mb) and RM24334 (15.06 Mb) on chromosome 9 (Uga et al. 2008, 2010). *qRT9* (17.13 Mb) and *STA1* are located near *DRO1* (16.31 Mb). As mentioned above, upland rice tends to have deeper and thicker roots than those of lowland rice (O'Toole and Bland 1987). The tight linkage of these QTLs should be associated with the phenotypic relationship between these root traits.

14.4 Conclusions

Several rice-specific genes controlling the root system have been found. However, the many rice genes homologous to *Arabidopsis* genes associated with the formation of the main root system are also involved in the formation of the fibrous root system. Thus, many parts of the genetic mechanism related to the root system have features common between monocots and dicots. On the other hand, much remains to be clarified about the difference between monocots and dicots in the natural variation of root system architecture because most related genes have not been cloned. Because dicots have *DRO1* homologs, their genetic mechanism related to the natural variation in root system architecture might have many common features with that of monocots. Recent progress of forward and reverse genetic strategies, such as MutMap (Abe et al. 2012; Takagi et al. 2015) and TILLING (Suzuki et al. 2008; Till et al. 2007), and the CRISPR/Cas9-mediated genome editing system (Doudna and Charpentier 2014; Schaeffer and Nakata 2015) allow us to isolate genes and find new alleles easily. Using QTL cloning and these approaches, we would be able to elucidate the entire genetic network related to natural variation in rice root system architecture. Understanding of the genetic mechanism of root plasticity in response to environmental variation is also important for improving crop production under abiotic stresses, but it is difficult to obtain reliable phenotypic data and identify related genes or QTLs under field conditions. To resolve this issue, a reproducible root phenotyping platform with controlled soil water, nutrients, and temperature in which we can evaluate accurately a large number of plants is needed.

References

Abe J, Morita S (1994) Growth direction of nodal roots in rice: its variation and contribution to root system formation. Plant Soil 165:333–337

Abe A, Kosugi S, Yoshida K, Natsume S, Takagi H, Kanzaki H, Matsumura H, Yoshida K, Mitsuoka C, Tamiru M, Innan H, Cano L, Kamoun S, Terauchi R (2012) Genome sequencing reveals agronomically important loci in rice using MutMap. Nat Biotechnol 30:174–178

Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, Galinha C, Nussaume L, Noh YS, Amasino R, Scheres B (2004) The *PLETHORA* genes mediate patterning of the *Arabidopsis* root stem cell niche. Cell 119:109–120

Arai-Sanoh Y, Takai T, Yoshinaga S, Nakano H, Kojima M, Sakakibara H, Kondo M, Uga Y (2014) Deep rooting conferred by *DEEPER ROOTING 1* enhances rice yield in paddy fields. Sci Rep 4:5563

Araki H, Morita S, Tatsumi J, Iijima M (2002) Physio-morphological analysis on axile root growth in upland rice. Plant Prod Sci 5:286–293

Baldwin KL, Strohm AK, Masson PH (2013) Gravity sensing and signal transduction in vascular plant primary roots. Am J Bot 100:126–142

Bian H, Xie Y, Guo F, Han N, Ma S, Zeng Z, Wang J, Yang Y, Zhu M (2012) Distinctive expression patterns and roles of the miRNA393/TIR1 homolog module in regulating flag leaf inclination and primary and crown root growth in rice (*Oryza sativa*). New Phytol 196:149–161

Canadell J, Jackson RB, Ehleringer JB, Mooney HA, Sala OE, Schulze ED (1996) Maximum rooting depth of vegetation types at the global scale. Oecologia 108:583–595

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR (1995) Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor Appl Genet 90:969–981

Chen YH, Chao YY, Hsu YY, Hong CY, Kao CH (2012a) Heme oxygenase is involved in nitric oxide- and auxin-induced lateral root formation in rice. Plant Cell Rep 31:1085–1091

Chen Y, Fan X, Song W, Zhang Y, Xu G (2012b) Over-expression of *OsPIN2* leads to increased tiller numbers, angle and shorter plant height through suppression of *OsLAZY1*. Plant Biotechnol J 10:139–149

Chen X, Shi J, Hao X, Liu H, Shi J, Wu Y, Wu Z, Chen M, Wu P, Mao C (2013) *OsORC3* is required for lateral root development in rice. Plant J 74:339–350

Chhun T, Takeda S, Tsurumi S, Ichii M (2003) Interaction between two auxin-resistant mutants and their effects on lateral root formation in rice (*Oryza sativa* L.) J Exp Bot 54:2701–2708

Cho SH, Yoo SC, Zhang H, Pandeya D, Koh HJ, Hwang JY, Kim GT, Paek NC (2013) The rice *narrow leaf2* and *narrow leaf3* loci encode WUSCHEL-related homeobox 3A (OsWOX3A) and function in leaf, spikelet, tiller and lateral root development. New Phytol 198:1071–1084

Chuang HW, Zhang W, Gray WM (2004) Arabidopsis *ETA2*, an apparent ortholog of the human cullin-interacting protein CAND1, is required for auxin responses mediated by the SCF^{TIR1} ubiquitin ligase. Plant Cell 16:1883–1897

Cosgrove DJ (2000) Loosening of plant cell walls by expansins. Nature 407:321–326

Coudert Y, Périn C, Courtois B, Khong NG, Gantet P (2010) Genetic control of root development in rice, the model cereal. Trends Plant Sci 15:219–226

Courtois B, Ahmadi N, Khowaja F, Price AH, Rami JF, Frouin J, Hamelin C, Ruiz M (2009) Rice root genetic architecture: meta-analysis from a drought QTL database. Rice 2:115–128

Cui H, Levesque MP, Vernoux T, Jung JW, Paquette AJ, Gallagher KL, Wang JY, Blilou I, Scheres B, Benfey PN (2007) An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants. Science 316:421–425

de Dorlodot S, Forster B, Pagé L, Price A, Tuberosa R, Draye X (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. Trends Plant Sci 12:474–481

Deeks MJ, Cvrcková F, Machesky LM, Mikitová V, Ketelaar T, Zársky V, Davies B, Hussey PJ (2005) *Arabidopsis* group Ie formins localize to specific cell membrane domains, interact with

actin-binding proteins and cause defects in cell expansion upon aberrant expression. New Phytol 168:529–540

Dello Ioio R, Linhares FS, Scacchi E, Casamitjana-Martinez E, Heidstra R, Costantino P, Sabatini S (2007) Cytokinins determine *Arabidopsis* root-meristem size by controlling cell differentiation. Curr Biol 17:678–682

Dello Ioio R, Nakamura K, Moubayidin L, Perilli S, Taniguchi M, Morita MT, Aoyama T, Costantino P, Sabatini S (2008) A genetic framework for the control of cell division and differentiation in the root meristem. Science 322:1380–1384

Di Laurenzio L, Wysockadiller J, Malamy JE, Pysh L, Helariutta Y, Freshour G, Hahn MG, Feldmann KA, Benfey PN (1996) The *SCARECROW* gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root. Cell 86:423–433

Ding W, Yu Z, Tong Y, Huang W, Chen H, Wu P (2009) A transcription factor with a bHLH domain regulates root hair development in rice. Cell Res 19:1309–1311

Ding X, Li X, Xiong L (2011) Evaluation of near-isogenic lines for drought resistance QTL and fine mapping of a locus affecting flag leaf width, spikelet number, and root volume in rice. Theor Appl Genet 123:815–826

Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, Roberts K, Scheres B (1993) Cellular organization of the *Arabidopsis* root. Development 119:71–84

Doudna JA, Charpentier E (2014) The new frontier of genome engineering with CRISPR-Cas9. Science 346:1258096

Faiyue B, Vijayalakshmi C, Nawaz S, Nagato Y, Taketa S, Ichii M, Al-Azzawi MJ, Flowers TJ (2010) Studies on sodium bypass flow in lateral rootless mutants *lrt1* and *lrt2*, and crown rootless mutant *crl1* of rice (*Oryza sativa* L.) Plant Cell Environ 33:687–701

Feng S, Shen Y, Sullivan JA, Rubio V, Xiong Y, Sun TP, Deng XW (2004) Arabidopsis CAND1, an unmodified CUL1-interacting protein, is involved in multiple developmental pathways controlled by ubiquitin/proteasome-mediated protein degradation. Plant Cell 16:1870–1882

Friml J, Benková E, Blilou I, Wisniewska J, Hamann T, Ljung K, Woody S, Sandberg G, Scheres B, Jürgens G, Palme K (2002) AtPIN4 mediates sink-driven auxin gradients and root patterning in *Arabidopsis*. Cell 108:661–673

Fujino K, Matsuda Y, Ozawa K, Nishimura T, Koshiba T, Fraaije MW, Sekiguchi H (2008) *NARROW LEAF 7* controls leaf shape mediated by auxin in rice. Mol Gen Genomics 279:499–507

Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, Heidstra R, Scheres B (2007) PLETHORA proteins as dose-dependent master regulators of *Arabidopsis* root development. Nature 449:1053–1057

Gao S, Fang J, Xu F, Wang W, Sun X, Chu J, Cai B, Feng Y, Chu C (2014) CYTOKININ OXIDASE/DEHYDROGENASE4 integrates cytokinin and auxin signaling to control rice crown root formation. Plant Physiol 165:1035–1046

Ge L, Chen R (2016) Negative gravitropism in plant roots. Nat Plants 2:16155

Ge L, Chen H, Jiang JF, Zhao Y, Xu ML, Xu YY, Tan KH, Xu ZH, Chong K (2004) Overexpression of *OsRAA1* causes pleiotropic phenotypes in transgenic rice plants, including altered leaf, flower, and root development and root response to gravity. Plant Physiol 135:1502–1513

Geldner N, Anders N, Wolters H, Keicher J, Kornberger W, Muller P, Delbarre A, Ueda T, Nakano A, Jürgens G (2003) The *Arabidopsis* GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. Cell 112:219–230

Gewin V (2010) An underground revolution. Nature 466:552–553

Gowda VRP, Henry A, Yamauchi A, Shashidhar HE, Serraj R (2011) Root biology and genetic improvement for drought avoidance in rice. Field Crops Res 122:1–13

Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001) Auxin regulates the SCF^{TIR1}-dependent degradation of AUX/IAA proteins. Nature 414:271–276

Guseman JM, Webb K, Srinivasan C, Dardick C (2017) *DRO1* influences root system architecture in *Arabidopsis* and *Prunus* species. Plant J 89:1093–1105

Han Y, Cao H, Jiang J, Xu Y, Du J, Wang X, Yuan M, Wang Z, Xu Z, Chong K (2008) Rice ROOT ARCHITECTURE ASSOCIATED1 binds the proteasome subunit RPT4 and is degraded in a D-box and proteasome-dependent manner. Plant Physiol 148:843–855

Hao Z, Ichii M (1999) A mutant RM109 of rice (*Oryza sativa* L.) exhibiting altered lateral root initiation and gravitropism. Jpn J Crop Sci 68:245–252

Helariutta Y, Fukaki H, Wysocka-Diller J, Nakajima K, Jung J, Sena G, Hauser MT, Benfey PN (2000) The *SHORT-ROOT* gene controls radial patterning of the Arabidopsis root through radial signaling. Cell 101:555–567

Hong SK, Aoki T, Kitano H, Satoh H, Nagato Y (1995) Phenotypic diversity of 188 rice embryo mutants. Dev Genet 16:298–310

Huang CF, Yamaji N, Nishimura M, Tajima S, Ma JF (2009) A rice mutant sensitive to Al toxicity is defective in the specification of root outer cell layers. Plant Cell Physiol 50:976–985

Huang CF, Yamaji N, Ono K, Ma JF (2012) A leucine-rich repeat receptor-like kinase gene is involved in the specification of outer cell layers in rice roots. Plant J 69:565–576

Huang J, Kim CM, Xuan YH, Liu J, Kim TH, Kim BK, Han CD (2013a) Formin homology 1 (OsFH1) regulates root-hair elongation in rice (*Oryza sativa*). Planta 237:1227–1239

Huang J, Kim CM, Xuan YH, Park SJ, Piao HL, Je BI, Liu J, Kim TH, Kim BK, Han CD (2013b) *OsSNDP1*, a Sec14-nodulin domain-containing protein, plays a critical role in root hair elongation in rice. Plant Mol Biol 82:39–50

Inukai Y, Miwa M, Nagato Y, Kitano H, Yamauchi A (2001) Characterization of rice mutants deficient in formation of crown roots. Breed Sci 51:123–129

Inukai Y, Sakamoto T, Ueguchi-Tanaka M, Shibata Y, Gomi K, Umemura I, Hasegawa Y, Ashikari M, Kitano H, Matsuoka M (2005) *Crown Rootless1*, which is essential for crown root formation in rice, is a target of an AUXIN RESPONSE FACTOR in auxin signaling. Plant Cell 17:1387–1396

Inukai Y, Sakamoto T, Morinaka Y, Miwa M, Kojima M, Tanimoto E, Yamamoto H, Sato K, Katayama Y, Matsuoka M, Kitano H (2012) ROOT GROWTH INHIBITING, a rice endo-1,4-β-D-glucanase regulates cell wall loosening and is essential for root elongation. J Plant Growth Regul 31:373–381

Itoh J-I, Nonomura K-I, Ikeda K, Yamaki S, Inukai Y, Yamaguchi H, Kitano H, Nagato Y (2005) Rice plant development: from zygote to spikelet. Plant Cell Physiol 46:23–47

Itoh J-I, Sato Y, Sato Y, Hibara K-I, Shimizu-Sato S, Kobayashi H, Takehisa H, Sanguinet KA, Namiki N, Nagamura Y (2016) Genome-wide analysis of spatiotemporal gene expression patterns during early embryogenesis in rice. Development 143:1217–1227

Jain M, Kaur N, Garg R, Thakur JK, Tyagi AK, Khurana JP (2006) Structure and expression analysis of early auxin-responsive *Aux/IAA* gene family in rice (*Oryza sativa*). Funct Integr Genomics 6:47–59

Ji X, Van den Ende W, Van Laere A, Cheng S, Bennett J (2005) Structure, evolution, and expression of the two invertase gene families of rice. J Mol Evol 60:615–634

Jia L, Zhang B, Mao C, Li J, Wu Y, Wu P, Wu Z (2008) OsCYT-INV1 for alkaline/neutral invertase is involved in root cell development and reproductivity in rice (*Oryza sativa* L.) Planta 228:51–59

Jia L, Wu Z, Hao X, Carrie C, Zheng L, Whelan J, Wu Y, Wang S, Wu P, Mao C (2011) Identification of a novel mitochondrial protein, short postembryonic roots 1 (SPR1), involved in root development and iron homeostasis in *Oryza sativa*. New Phytol 189:843–855

Jiang H, Wang S, Dang L, Wang S, Chen H, Wu Y, Jiang X, Wu P (2005) A novel short-root gene encodes a glucosamine-6-phosphate acetyltransferase required for maintaining normal root cell shape in rice. Plant Physiol 138:232–242

Kamiya N, Itoh J-I, Morikami A, Nagato Y, Matsuoka M (2003a) The *SCARECROW* gene's role in asymmetric cell divisions in rice plants. Plant J 36:45–54

Kamiya N, Nagasaki H, Morikami A, Sato Y, Matsuoka M (2003b) Isolation and characterization of a rice *WUSCHEL*-type homeobox gene that is specifically expressed in the central cells of a quiescent center in the root apical meristem. Plant J 35:429–441

Kang B, Zhang Z, Wang L, Zheng L, Mao W, Li M, Wu Y, Wu P, Mo X (2013) OsCYP2, a chaperone involved in degradation of auxin-responsive proteins, plays crucial roles in rice lateral root initiation. Plant J 74:86–97

Kawata S, Ishihara K (1959) Studies on the root hair in rice plant (in Japanese). Jpn J Crop Sci 27:341–348

Kawata S, Shibayama H (1965) On the lateral root primordia formation in the crown roots of rice plants (in Japanese). Proc Crop Sci Soc Jpn 33:423–431

Kawata S, Soejima M, Yamazaki K (1978) The superficial root function and yield of hulled rice (in Japanese). Jpn J Crop Sci 47:617–628

Khush GS (2001) Green revolution: the way forward. Nat Rev Genet 2:815–822

Kim CM, Dolan L (2011) Root hair development involves asymmetric cell division in *Brachypodium distachyon* and symmetric division in *Oryza sativa*. New Phytol 192:601–610

Kim DW, Lee SH, Choi SB, Won SK, Heo YK, Cho M, Park YI, Cho HT (2006) Functional conservation of a root hair cell-specific *cis*-element in angiosperms with different root hair distribution patterns. Plant Cell 18:2958–2970

Kim CM, Park SH, Je BI, Park SH, Park SJ, Piao HL, Eun MY, Dolan L, Han CD (2007) *OsCSLD1*, a cellulose synthase-like D1 gene, is required for root hair morphogenesis in rice. Plant Physiol 143:1220–1230

Kinae T, Hong SK, Nagato Y (2005) *Apical displacement1* gene regulates apical-basal pattern formation in rice embryo. Plant Sci 168:1345–1351

Kitomi Y, Kitano H, Inukai Y (2008a) Mapping of the *CROWN ROOTLESS3* gene, *CRL3*, in rice. Rice Gen Newslett 24:31–33

Kitomi Y, Ogawa A, Kitano H, Inukai Y (2008b) *CRL4* regulates crown root formation through auxin transport in rice. Plant Root 2:19–28

Kitomi Y, Ito H, Hobo T, Aya K, Kitano H, Inukai Y (2011) The auxin responsive AP2/ERF transcription factor *CROWN ROOTLESS5* is involved in crown root initiation in rice through the induction of *OsRR1*, a type-A response regulator of cytokinin signaling. Plant J 67:472–484

Kitomi Y, Inahashi H, Inukai Y (2012) OsIAA13-mediated auxin signaling is involved in lateral root initiation in rice. Plant Sci 190:116–122

Kitomi Y, Kanno N, Kawai S, Mizubayashi T, Fukuoka S, Uga Y (2015) QTLs underlying natural variation of root growth angle among rice cultivars with the same functional allele of *DEEPER ROOTING 1*. Rice 8:16

Kitomi Y, Nakao E, Kawai S, Kanno N, Ando T, Fukuoka S, Irie K, Uga Y (in press) Fine mapping of *QUICK ROOTING 1* and *2*, quantitative trait loci increasing root length in rice. G3

Klepper B (1992) Development and growth of crop root systems. In: Hatfield JL, Stewart BA (eds) Limitations to plant root growth. Springer, New York, pp 1–25

Kusano H, Testerink C, Vermeer JE, Tsuge T, Shimada H, Oka A, Munnik T, Aoyama T (2008) The *Arabidopsis* phosphatidylinositol phosphate 5-kinase PIP5K3 is a key regulator of root hair tip growth. Plant Cell 20:367–380

Lafitte HR, Champoux MC, McLaren G, O'Toole JC (2001) Rice root morphological traits are related to isozyme group and adaptation. Field Crops Res 71:57–70

Lerouge P, Cabanes-Macheteau M, Rayon C, Fischette-Laine AC, Gomord V, Faye L (1998) N-glycoprotein biosynthesis in plants: recent developments and future trends. Plant Mol Biol 38:1–48

Li J, Han Y, Liu L, Chen Y, Du Y, Zhang J, Sun H, Zhao Q (2015) *qRT9*, a quantitative trait locus controlling root thickness and root length in upland rice. J Exp Bot 66:2723–2732

Libault M, Brechenmacher L, Cheng J, Xu D, Stacey G (2010) Root hair systems biology. Trends Plant Sci 15:641–650

Liscum E, Reed JW (2002) Genetics of AUX/IAA and ARF action in plant growth and development. Plant Mol Biol 49:387–400

Liu W, Xu ZH, Luo D, Xue HW (2003) Roles of *OsCKI1*, a rice casein kinase I, in root development and plant hormone sensitivity. Plant J 36:189–202

Liu H, Wang S, Yu X, Yu J, He X, Zhang S, Shou H, Wu P (2005) ARL1, a LOB domain protein required for adventitious root formation in rice. Plant J 43:47–56

Liu S, Wang J, Wang L, Wang X, Xue Y, Wu P, Shou H (2009) Adventitious root formation in rice requires OsGNOM1 and is mediated by the OsPINs family. Cell Res 19:1110–1119

Lou Q, Chen L, Mei H, Wei H, Feng F, Wang P, Xia H, Li T, Luo L (2015) Quantitative trait locus mapping of deep rooting by linkage and association analysis in rice. J Exp Bot 66:4749–4757

Lukowitz W, Nickle TC, Meinke DW, Last RL, Conklin PL, Somerville CR (2001) *Arabidopsis cyt1* mutants are deficient in a mannose-1-phosphate guanylyltransferase and point to a requirement of N-linked glycosylation for cellulose biosynthesis. Proc Natl Acad Sci U S A 98:2262–2267

Luquet D, Zhang BG, Dingkuhn M, Dexet A, Clément-Vidal A (2005) Phenotypic plasticity of rice seedlings: case of phosphorus deficiency. Plant Prod Sci 8:145–151

Lynch J (1995) Root architecture and plant productivity. Plant Physiol 109:7–13

Lynch J (2013) Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. Ann Bot 112:347–357

Ma N, Wang Y, Qiu S, Kang Z, Che S, Wang G, Huang J (2013) Overexpression of *OsEXPA8*, a root-specific gene, improves rice growth and root system architecture by facilitating cell extension. PLoS One 8:e75997

Miyashita Y, Takasugi T, Ito Y (2010) Identification and expression analysis of *PIN* genes in rice. Plant Sci 178:424–428

Morita MT (2010) Directional gravity sensing in gravitropism. Annu Rev Plant Biol 61:705–720

Morita Y, Kyozuka J (2007) Characterization of *OsPID*, the rice ortholog of *PINOID*, and its possible involvement in the control of polar auxin transport. Plant Cell Physiol 48:540–549

Morita S, Suga T, Yamazaki K (1988) The relationship between root length density and yield in rice plants (in Japanese). Jpn J Crop Sci 57:438–443

Nakamura A, Umemura I, Gomi K, Hasegawa Y, Kitano H, Sazuka T, Matsuoka M (2006) Production and characterization of auxin-insensitive rice by overexpression of a mutagenized rice IAA protein. Plant J 46:297–306

Ni J, Wang GH, Zhu ZX, Zhang HH, Wu YR, Wu P (2011) OsIAA23-mediated auxin signaling defines postembryonic maintenance of QC in rice. Plant J 68:433–442

Norton GJ, Price AH (2009) Mapping of quantitative trait loci for seminal root morphology and gravitropic response in rice. Euphytica 166:229–237

O'Toole JC, Bland WL (1987) Genotypic variation in crop plant root systems. Adv Agric 41:91–143

Obara M, Tamura W, Ebitani T, Yano M, Sato T, Yamaya T (2010) Fine-mapping of *qRL6.1*, a major QTL for root length of rice seedlings grown under a wide range of $NH4^+$ concentrations in hydroponic conditions. Theor Appl Genet 121:535–547

Oyanagi A, Nakamoto T, Morita S (1993) The gravitropic response of roots and the shaping of the root system in cereal plants. Environ Exp Bot 33:141–158

Paul AS, Pollard TD (2009) Review of the mechanism of processive actin filament elongation by formins. Cell Motil Cytoskel 66:606–617

Qi J, Qian Q, Bu Q, Li S, Chen Q, Sun J, Liang W, Zhou Y, Chu C, Li X, Ren F, Palme K, Zhao B, Chen J, Chen M, Li C (2008) Mutation of the rice *Narrow leaf1* gene, which encodes a novel protein, affects vein patterning and polar auxin transport. Plant Physiol 147:1947–1959

Qi Y, Wang S, Shen C, Zhang S, Chen Y, Xu Y, Liu Y, Wu Y, Jiang D (2012) OsARF12, a transcription activator on auxin response gene, regulates root elongation and affects iron accumulation in rice (*Oryza sativa*). New Phytol 193:109–120

Qin C, Li Y, Gan J, Wang W, Zhang H, Liu Y, Wu P (2013) OsDGL1, a homolog of an oligosaccharyltransferase complex subunit, is involved in N-glycosylation and root development in rice. Plant Cell Physiol 54:129–137

Rebouillat J, Dievart A, Verdeil JL, Escoute J, Giese G, Breitler JC, Gantet P, Espeout S, Guiderdoni E, Périn C (2009) Molecular genetics of rice root development. Rice 2:15–34

Roux SJ, Steinebrunner I (2007) Extracellular ATP: an unexpected role as a signaler in plants. Trends Plant Sci 12:522–527

Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, Scheres B (1999) An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell 99:463–472

Sabatini S, Heidstra R, Wildwater M, Scheres B (2003) SCARECROW is involved in positioning the stem cell niche in the *Arabidopsis* root meristem. Genes Dev 17:354–358

Sakata I, Kagiya T, Kawai Y, Oyanagi A (2004) Effects of pruning of roots growing at various angles on pushing resistance of rice cultivars (in Japanese). Jpn J Crop Sci 73:1–5

Sarkar AK, Luijten M, Miyashima S, Lenhard M, Hashimoto T, Nakajima K, Scheres B, Heidstra R, Laux T (2007) Conserved factors regulate signaling in *Arabidopsis thaliana* shoot and root stem cell organizers. Nature 446:811–814

Sato Y, Antonio BA, Namiki N, Takehisa H, Minami H, Kamatsuki K, Sugimoto K, Shimizu Y, Hirochika H, Nagamura Y (2011) RiceXPro: a platform for monitoring gene expression in *japonica* rice grown under natural field conditions. Nucleic Acids Res 39:D1141–D1148

Scarpella E, Rueb S, Meijer AH (2003) The *RADICLELESS1* gene is required for vascular pattern formation in rice. Development 130:645–658

Schaeffer SM, Nakata PA (2015) CRISPR/Cas9-mediated genome editing and gene replacement in plants: transitioning from lab to field. Plant Sci 240:130–142

Scheffran J, Battaglini A (2011) Climate and conflicts: the security risks of global warming. Reg Environ Chang 11(Suppl 1):27–39

Shin JH, Jeong DH, Park MC, An G (2005) Characterization and transcriptional expression of the α-expansin gene family in rice. Mol Cells 20:210–218

Steinmann T, Geldner N, Grebe M, Mangold S, Jackson CL, Paris S, Galweiler L, Palme K, Jürgens G (1999) Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. Science 286:316–318

Suralta RR, Inukai Y, Yamauchi A (2008) Utilizing chromosome segment substation lines (CSSLs) for evaluation of root responses to transient moisture stresses in rice. Plant Prod Sci 11:457–465

Suzuki T, Eiguchi M, Kumamaru T, Satoh H, Matsusaka H, Moriguchi K, Nagato Y, Kurata N (2008) MNU-induced mutant pools and high performance TILLING enable finding of any gene mutation in rice. Mol Gen Genomics 279:213–223

Takagi H, Tamiru M, Abe A, Yoshida K, Uemura A, Yaegashi H, Obara T, Oikawa K, Utsushi H, Kanzaki E, Mitsuoka C, Natsume S, Kosugi S, Kanzaki H, Matsumura H, Urasaki N, Kamoun S, Terauchi R (2015) MutMap accelerates breeding of a salt-tolerant rice cultivar. Nat Biotechnol 33:445–449

Tanaka H, Dhonukshe P, Brewer PB, Friml J (2006) Spatiotemporal asymmetric auxin distribution: a means to coordinate plant development. Cell Mol Life Sci 63:2738–2754

Terashima K (1997) Eco-physiological study of root lodging tolerance in direct-seeded rice cultivars. JARQ 31:155–162

Terashima K, Ogata T, Akita S (1994) Eco-physiological characteristics related with lodging tolerance of rice in direct sowing cultivation II. Root growth characteristics of tolerant cultivars to root lodging (in Japanese). Jpn J Crop Sci 63:34–41

Terashima K, Akita S, Sakai N (1995) Eco-physiological characteristics related with lodging tolerance of rice in direct sowing cultivation III. Relationship between the characteristics of root distribution in the soil and lodging tolerance (in Japanese). Jpn J Crop Sci 64:243–250

Till BJ, Cooper J, Tai TH, Colowit P, Greene EA, Henikoff S, Comai L (2007) Discovery of chemically induced mutations in rice by TILLING. BMC Plant Biol 7:19

Uga Y, Okuno K, Yano M (2008) QTL underlying natural variation in stele and xylem structures of rice root. Breed Sci 58:7–14

Uga Y, Ebana K, Abe J, Morita S, Okuno K, Yano M (2009) Variation in root morphology and anatomy among accessions of cultivated rice (*Oryza sativa* L.) with different genetic backgrounds. Breed Sci 59:87–93

Uga Y, Okuno K, Yano M (2010) Fine mapping of *Sta1*, a quantitative trait locus determining stele transversal area, on rice chromosome 9. Mol Breed 26:533–538

Uga Y, Okuno K, Yano M (2011) *Dro1*, a major QTL involved in deep rooting of rice under upland field conditions. J Exp Bot 62:2485–2494

Uga Y, Hanzawa E, Nagai S, Sasaki K, Yano M, Sato T (2012) Identification of *qSOR1*, a major rice QTL involved in soil-surface rooting in paddy fields. Theor Appl Genet 124:75–86

Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, Inoue H, Takehisa H, Motoyama R, Nagamura Y, Wu J, Matsumoto T, Takai T, Okuno K, Yano M (2013a) Control of root system architecture by *DEEPER ROOTING 1* increases rice yield under drought conditions. Nat Genet 45:1097–1102

Uga Y, Yamamoto E, Kanno N, Kawai S, Mizubayashi T, Fukuoka S (2013b) A major QTL controlling deep rooting on rice chromosome 4. Sci Rep 3:3040

Uga Y, Kitomi Y, Ishikawa S, Yano M (2015a) Genetic improvement for root growth angle to enhance crop production. Breed Sci 65:111–119

Uga Y, Kitomi Y, Yamamoto E, Kanno N, Kawai S, Mizubayashi T, Fukuoka S (2015b) A QTL for root growth angle on rice chromosome 7 is involved in the genetic pathway of *DEEPER ROOTING 1*. Rice 8:8

Umeda M, Umeda-Hara C, Yamaguchi M, Hashimoto J, Uchimiya H (1999) Differential expression of genes for cyclin-dependent protein kinases in rice plants. Plant Physiol 119:31–40

Varney GT, McCully ME, Canny MJ (1993) Sites of entry of water into the symplast of maize roots. New Phytol 125:733–741

Vincent P, Chua M, Nogue F, Fairbrother A, Mekeel H, Xu Y, Allen N, Bibikova TN, Gilroy S, Bankaitis VA (2005) A Sec14p-nodulin domain phosphatidylinositol transfer protein polarizes membrane growth of *Arabidopsis thaliana* root hairs. J Cell Biol 168:801–812

Vogel J (2008) Unique aspects of the grass cell wall. Curr Opin Plant Biol 11:301–307

Wachsman G, Sparks EE, Benfey PN (2015) Genes and networks regulating root anatomy and architecture. New Phytol 208:26–38

Wang H, Inukai Y, Yamauchi A (2006a) Root development and nutrient uptake. Crit Rev Plant Sci 25:279–301

Wang H, Taketa S, Miyao A, Hirochika H, Ichii M (2006b) Isolation of a novel lateral-rootless mutant in rice (*Oryza sativa* L.) with reduced sensitivity to auxin. Plant Sci 170:70–77

Wang D, Pei K, Fu Y, Sun Z, Li S, Liu H, Tang K, Han B, Tao Y (2007) Genome-wide analysis of the *auxin response factors* (ARF) gene family in rice (*Oryza sativa*). Gene 394:13–24

Wang JR, Hu H, Wang GH, Li J, Chen JY, Wu P (2009) Expression of *PIN* genes in rice (*Oryza sativa* L.): tissue specificity and regulation by hormones. Mol Plant 2:823–831

Wang XF, He FF, Ma XX, Mao CZ, Hodgman C, Lu CG, Wu P (2011) OsCAND1 is required for crown root emergence in rice. Mol Plant 4:289–299

Wang H, Xu X, Zhan X, Zhai R, Wu W, Shen X, Dai G, Cao L, Cheng S (2013) Identification of *qRL7*, a major quantitative trait locus associated with rice root length in hydroponic conditions. Breed Sci 63:267–274

Wang C, Li S, Ng S, Zhang B, Zhou Y, Whelan J, Wu P, Shou H (2014a) Mutation in xyloglucan 6-xylosytransferase results in abnormal root hair development in *Oryza sativa*. J Exp Bot 65:4149–4157

Wang S, Xu Y, Li Z, Zhang S, Lim JM, Lee KO, Li C, Qian Q, de Jiang A, Qi Y (2014b) OsMOGS is required for *N*-glycan formation and auxin-mediated root development in rice (*Oryza sativa* L.) Plant J 78:632–645

Wang Y, Wang D, Gan T, Liu L, Long W, Wang Y, Niu M, Li X, Zheng M, Jiang L, Wan J (2016) CRL6, a member of the CHD protein family, is required for crown root development in rice. Plant Physiol Biochem 105:185–194

Werner T, Motyka V, Laucou V, Smets R, Van Onckelen H, Schmülling T (2003) Cytokinin-deficient transgenic Arabidopsis plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. Plant Cell 15:2532–2550

Willemsen V, Bauch M, Bennett T, Campilho A, Wolkenfelt H, Xu J, Haseloff J, Scheres B (2008) The NAC domain transcription factors FEZ and SOMBRERO control the orientation of cell division plane in *Arabidopsis* root stem cells. Dev Cell 15:913–922

Won SK, Choi SB, Kumari S, Cho M, Lee SH, Cho HT (2010) Root hair-specific EXPANSIN B genes have been selected for Graminaceae root hairs. Mol Cells 30:369–376

Woo YM, Park HJ, Su'udi M, Yang JI, Park JJ, Back K, Park YM, An G (2007) Constitutively wilted 1, a member of the rice YUCCA gene family, is required for maintaining water homeostasis and an appropriate root to shoot ratio. Plant Mol Biol 65:125–136

Wu W, Cheng S (2014) Root genetic research, an opportunity and challenge to rice improvement. Field Crops Res 165:111–124

Wu J, Steinebrunner I, Sun Y, Butterfield T, Torres J, Arnold D, Gonzalez A, Jacob F, Reichler S, Roux SJ (2007) Apyrases (nucleoside triphosphate-diphosphohydrolases) play a key role in growth control in Arabidopsis. Plant Physiol 144:961–975

Xu M, Zhu L, Shou H, Wu P (2005) A *PIN1* family gene, *OsPIN1*, involved in auxin-dependent adventitious root emergence and tillering in rice. Plant Cell Physiol 46:1674–1681

Yamamoto Y, Kamiya N, Morinaka Y, Matsuoka M, Sazuka T (2007) Auxin biosynthesis by the *YUCCA* genes in rice. Plant Physiol 143:1362–1371

Yamamoto Y, Inukai Y, Kitano H, Sazuka T, Matsuoka M (2010) Characterization and mapping of the *CROWN ROOTLESS2* gene, *CRL2*, in rice. Rice Gen Newslett 25:25–26

Yamamoto T, Uga Y, Yano M (2014) Genomics-assisted allele mining and its integration into rice breeding. In: Tuberosa R, Graner A, Frison E (eds) Genomics of plant genetic resources. Springer, Heidelberg, pp 251–265

Yang XC, Hwa CM (2008) Genetic and physiological characterization of the *OsCem* mutant in rice: formation of connected embryos with multiple plumules or multiple radicles. Heredity 101:239–246

Yi K, Guo C, Chen D, Zhao B, Yang B, Ren H (2005) Cloning and functional characterization of a formin-like protein (AtFH8) from Arabidopsis. Plant Physiol 138:1071–1082

Yi K, Menand B, Bell E, Dolan L (2010) A basic helix-loop-helix transcription factor controls cell growth and size in root hairs. Nat Genet 42:264–267

Yoshikawa T, Ito M, Sumikura T, Nakayama A, Nishimura T, Kitano H, Yamaguchi I, Koshiba T, Hibara K, Nagato Y, Itoh J-I (2014) The rice *FISH BONE* gene encodes a tryptophan aminotransferase, which affects pleiotropic auxin-related processes. Plant J 78:927–936

Yu ZM, Kang B, He XW, Lü SL, Bai YH, Ding WN, Chen M, Cho HT, Wu P (2011) Root hair-specific expansins modulate root hair elongation in rice. Plant J 66:725–734

Yuan J, Chen D, Ren Y, Zhang X, Zhao J (2008) Characteristic and expression analysis of a metallothionein gene, *OsMT2b*, down-regulated by cytokinin suggests functions in root development and seed embryo germination of rice. Plant Physiol 146:1637–1650

Yuo T, Toyota M, Ichii M, Taketa S (2009) Molecular cloning of a root hairless gene rth1 in rice. Breed Sci 59:13–20

Yuo T, Shiotani K, Shitsukawa MA, Hirochika H, Ichii M, Taketa S (2011) *Root hairless 2* (*rth2*) mutant represents a loss-of-function allele of the cellulose synthase-like gene *OsCSLD1* in rice (*Oryza sativa* L.) Breed Sci 61:225–233

Zhang Q, Li J, Zhang W, Yan S, Wang R, Zhao J, Li Y, Qi Z, Sun Z, Zhu Z (2012a) The putative auxin efflux carrier *OsPIN3t* is involved in the drought stress response and drought tolerance. Plant J 72:805–816

Zhang JW, Xu L, Wu YR, Chen XA, Liu Y, Zhu SH, Ding WN, Wu P, Yi KK (2012b) OsGLU3, a putative membrane-bound endo-1,4-beta-glucanase, is required for root cell elongation and division in rice (*Oryza sativa* L.) Mol Plant 5:176–186

Zhao Y, Hu Y, Dai M, Huang L, Zhou DX (2009) The WUSCHEL-related homeobox gene *WOX11* is required to activate shoot-borne crown root development in rice. Plant Cell 21:736–748

Zhao Y, Cheng S, Song Y, Huang Y, Zhou S, Liu X, Zhou DX (2015a) The interaction between rice ERF3 and WOX11 promotes crown root development by regulating gene expression involved in cytokinin signaling. Plant Cell 27:2469–2483

Zhao H, Ma T, Wang X, Deng Y, Ma H, Zhang R, Zhao J (2015b) OsAUX1 controls lateral root initiation in rice (*Oryza sativa* L.) Plant Cell Environ 38:2208–2222

Zhu ZX, Liu Y, Liu SJ, Mao CZ, Wu YR, Wu P (2012) A gain-of-function mutation in *OsIAA11* affects lateral root development in rice. Mol Plant 5:154–161

Zou Y, Liu X, Wang Q, Chen Y, Liu C, Qiu Y, Zhang W (2014) OsRPK1, a novel leucine-rich repeat receptor-like kinase, negatively regulates polar auxin transport and root development in rice. Biochim Biophys Acta 1840:1676–1685