Chapter 2
Small RNAs in Rice: Molecular Species and Their Functions

Yutaka Sato, Misuzu Nosaka-Takahashi, Toshiya Suzuki, and Sae Shimizu-Sato

Abstract Small RNAs are major components of gene regulatory pathways conserved among eukaryotes. In basic and applied sciences, RNA interference (RNAi) and artificial microRNAs (amiRNAs) are often used to modulate gene expression. The molecular mechanisms of RNAi are mainly studied in nematode or insect cells as models. Functional analyses of endogenous small RNAs, including studies of rice as a model, have greatly contributed to our understanding of plant biology. In plants, small RNA-based gene regulation has unique characteristics not found in animals, and many small RNAs regulate biological phenomena specific to plants. Recently, small RNA profiling using next-generation sequencers became possible, and various small RNA species were identified in plants including rice; their functional analyses are underway. This chapter summarizes the components of small RNA pathways, the molecular species of small RNAs, and the unique function of small RNAs in rice. It also considers the functions of small RNAs in relation to agriculturally important traits.

Keywords *Oryza sativa* · Rice · Small RNA · siRNA · miRNA · DICER · AGO

2.1 Introduction

Plant endogenous small RNA species (20–30 nucleotides (nt)) are classified mostly into microRNAs (miRNAs) and small interfering RNAs (siRNAs) on the basis of their precursor structures and processing (Table 2.1). miRNAs are produced from single-stranded RNAs with a stem–loop structure, whereas siRNAs are produced from double-stranded RNAs (dsRNAs) with nearly perfect complementarity. On

Y. Sato (✉) · M. Nosaka-Takahashi · T. Suzuki · S. Shimizu-Sato
National Institute of Genetics, Mishima, Shizuoka, Japan
e-mail: yusato@nig.ac.jp

T. Sasaki, M. Ashikari (eds.), *Rice Genomics, Genetics and Breeding*,
https://doi.org/10.1007/978-981-10-7461-5_2

Table 2.1 Classification of small RNAs in rice

	miRNAs		ta-siRNAs (phasiRNAs)		nat-siRNAs	hc-siRNAs
Precursor	Single-stranded RNAs		Double-stranded RNAs		Double-stranded RNAs	Double-stranded RNAs
Processing	DCL1	DCL3a	DCL4	DCL3b	DCL2	DCL3a
Length	21 nt	24 nt	21 nt	24 nt	24 nt	24 nt
Function	mRNA cleavage	DNA methylation	mRNA cleavage	DNA methylation	mRNA cleavage	DNA methylation
			DNA methylation			Chromatin remodeling

the basis of the differences in their production pathways, siRNAs are further classified into trans-acting siRNAs (ta-siRNAs), natural-antisense transcript-derived siRNAs (nat-siRNAs), and heterochromatic siRNAs (hc-siRNAs); the former group is sometimes described as a subset of phased-secondary siRNAs (phasiRNAs) based on the similarities in biogenesis pathways (Table 2.1) (Vaucheret 2006; Komiya 2017).

Since next-generation sequencers became available, small RNA profiles have been analyzed in various plant species allowing us to capture the landscape of small RNAs. Some endogenous small RNAs, such as a fraction of miRNAs (and their regulatory targets), are widely conserved among land plants, suggesting that small RNA-based gene regulation is essential for plant survival (see later).

Small RNA profiling in rice (Nobuta et al. 2007; Xue et al. 2009; Chen et al. 2011) has revealed that siRNAs are abundant, whereas miRNA content is low, although the ratio between the two types of small RNA differs among tissues analyzed. For example, of total small RNAs in developing rice seeds, 87.5% are siRNAs and only 0.5% are miRNAs (Fig. 2.1) (Xue et al. 2009). In these large-scale small RNA profiles, the same miRNA sequences are usually retrieved multiple times, whereas the same siRNA sequences are rarely retrieved. This means that each miRNA is expressed at a much higher level than each siRNA and that a tremendous number of different siRNAs are expressed in rice cells. Most rice siRNAs (71.9%) are derived from intergenic regions or repetitive sequences such as transposons. Because most repetitive regions are heterochromatic, siRNAs derived from these regions are hc-siRNAs and are believed to be involved in heterochromatin formation or maintenance. The proportion of nat-siRNAs among total siRNAs is very small (0.9%), whereas phasiRNAs become very abundant at the reproductive stage of rice development (Fig. 2.1).

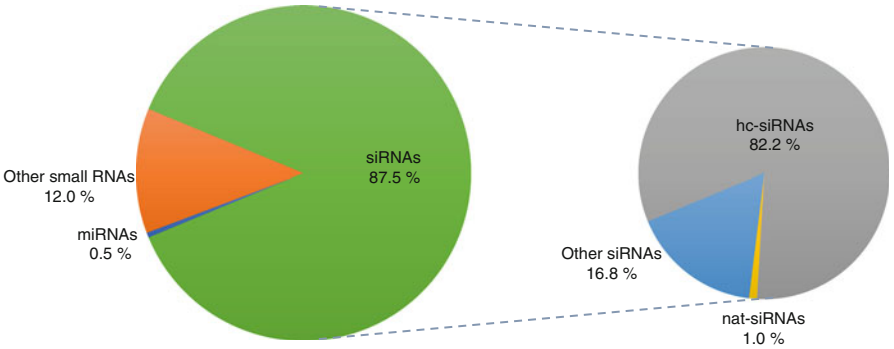

Fig. 2.1 Small RNA profiles in rice (Modified from Wu et al. 2010; Csorba et al. 2015; Borges and Martienssen 2015; Fang and Qi 2016; Komiya 2017). In developing rice seeds, 87.5% are siRNAs and only 0.5% are miRNAs. Among siRNAs, hc-siRNAs, nat-siRNAs, and other siRNAs compose 82.2%, 1.0%, and 16.8%, respectively

2.2 Protein Components of Small RNA Pathways in Rice

In plants, including rice, genes involved in miRNA or siRNA biogenesis or their targets often form families. For example, DICER, an enzyme required for generating small RNAs from fold-back structures of single- or double-stranded RNA, is encoded by four genes in *Arabidopsis* and five genes in rice (Margis et al. 2006; Kapoor et al. 2008). Rice DCL1 and DCL4/SHOOT ORGANIZATION1 (SHO1) produce 21-nt small RNAs, whereas DCL2, DCL3a, and DCL3b produce 22- or 24-nt small RNAs (Table 2.1). DCL1 is mainly engaged in miRNA biogenesis (Fig. 2.2a). Reduced DCL1 level results in developmental abnormalities possibly through decreased miRNA production (Liu et al. 2005). DCL2 produces nat-siRNA (Fig. 2.2d). DCL3a mainly produces hc-siRNA (Fig. 2.2e), and DCL3b produces phasiRNA (Fig. 2.2c) (Song et al. 2012). In rice, DCL3b is referred to as DCL5, a grass-specific DICER (Fei et al. 2013). In addition, DCL3a is involved in long miRNA (lmiRNA) processing (Fig. 2.2a); lmiRNAs are 24-nt miRNAs derived from potential stem–loop structures and act in a similar way to hc-siRNAs (Wu et al. 2010). DCL4 (SHO1) is required for generating ta-siRNA and phasiRNA (Fig. 2.2b, c) (Nagasaki et al. 2007; Liu et al. 2007; Song et al. 2012). Loss-of-function of DCL4 (SHO1) results in defective embryo development due to abnormal shoot organization (Nagasaki et al. 2007). DCL4 knockdown results in abnormal spikelets (Liu et al. 2007).

Small RNAs produced by DICERs are incorporated into Argonaute (AGO) proteins, which are a major component of the RNA-induced silencing complex (RISC); AGOs work as effectors of gene regulation. The rice genome contains 19 AGO copies, and the *Arabidopsis* genome has 10 copies (Kapoor et al. 2008).

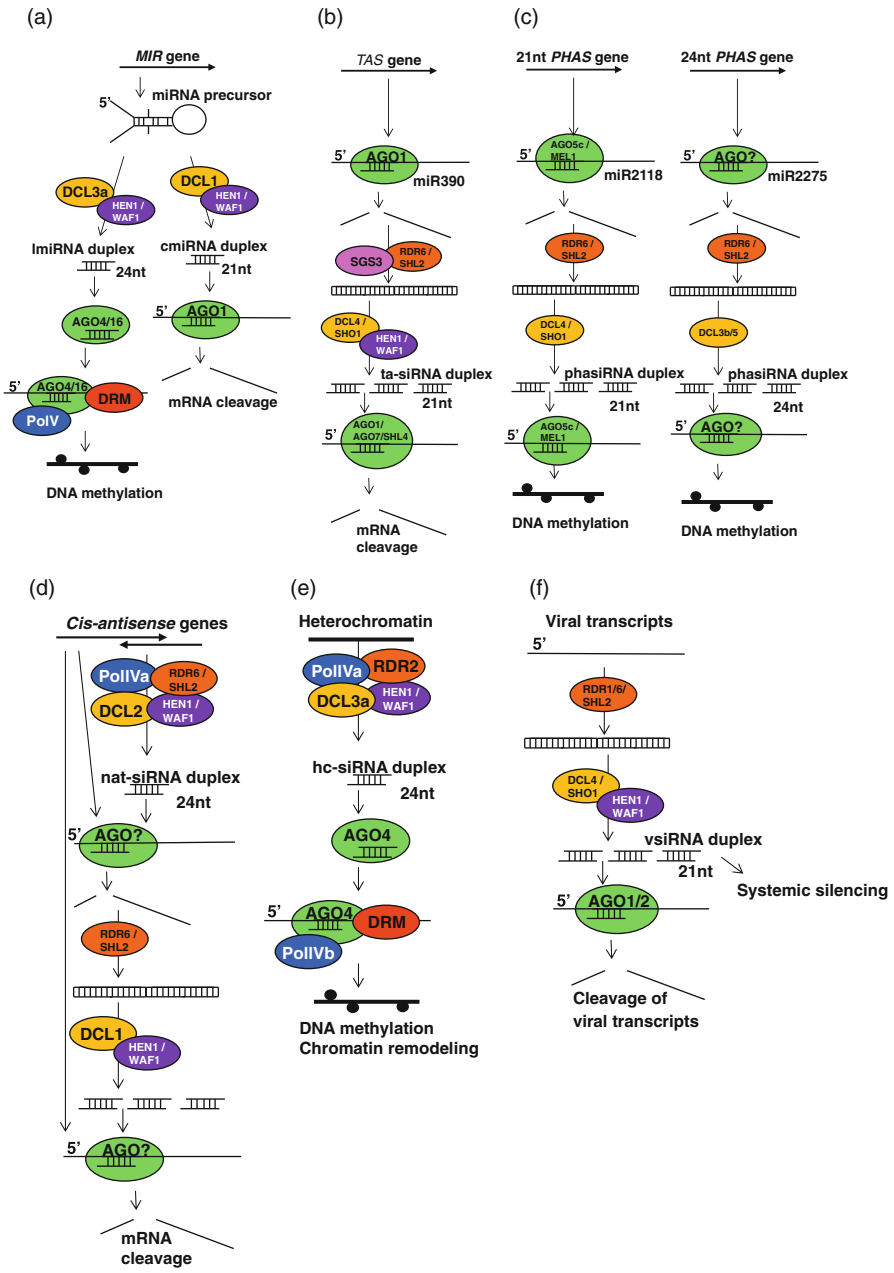

Fig. 2.2 Small RNA pathways in rice (Modified from Nobuta et al. 2007; Xue et al. 2009; Chen et al. 2011). (**a**) microRNA (miRNA) pathway. (**b**) Trans-acting siRNA (ta-siRNA) pathway. (**c**) Phased-secondary siRNA (phasiRNA) pathway. (**d**) Natural-antisense transcript-derived siRNA (nat-siRNA) pathway. (**e**) Heterochromatic siRNA (hc-siRNA) pathway. (**f**) Viral siRNA (vsiRNA) pathway

The base at the 5′ terminus of a small RNA determines AGO selectivity (Mourrain et al. 2000; Takeda et al. 2008), but selective incorporation of small RNAs alone does not explain the functional diversification of rice AGOs. For example, rice *SHOOTLESS4* (SHL4) encodes an ortholog of *Arabidopsis AGO7* (Nagasaki et al. 2007). Analysis of a *shl4* mutation revealed that *SHL4* is required for the regulation of *ETTIN* transcription factor genes by ta-siARF, one of the specific ta-siRNAs. *MEIOSIS ARRESTED AT LEPTOTENE1* (*MEL1*) in rice also encodes an AGO and is expressed in a cell type-specific manner; the phenotype of a mutation in this gene is observed in meiosis (Nonomura et al. 2007). Thus, the functional diversification of these AGOs is not related to biogenesis of small RNAs or the 5′ base of small RNAs but rather relies on their cell type-specific or developmentally regulated expression.

RNA-DEPENDENT RNA POLYMERASEs (RDRs) are required for dsRNA production. dsRNAs are substrates of DICER, which cleaves them into 21- or 24-nt small RNAs. The rice genome encodes six RDRs (RDR1–RDR6/SHOOTLESS2 (SHL2)); their precise roles are not well documented except for RDR2 and RDR6/SHL2. RDR2 produces hc-siRNA, whereas RDR6/SHL2 seems to have a broader function. RDR6/SHL2 generates viral double-stranded RNAs and is important for defense against RNA viruses (Jiang et al. 2012) and is also essential for rice development (Nagasaki et al. 2007; Toriba et al. 2010). Its loss-of-function *shootless2* (*shl2*) mutation leads to a severe phenotype during embryogenesis (the lack of the shoot apical meristem region). Weak alleles of *RDR6*/*SHL2* show abnormal spikelet development, which is mostly explained by the loss of ta-siARF. Thus, *RDR6*/*SHL2* is required for producing double-stranded RNA for ta-siRNA.

Arabidopsis HUA ENHANCER1 (HEN1) transfers the methyl group to the 3′-end hydroxyl group of small RNAs and stabilizes them. Rice *WAVY LEAF1* (*WAF1*) is an ortholog of *HEN1*; the *waf1* mutation causes severe developmental abnormalities (Abe et al. 2010). *Arabidopsis* small RNA pathways also include the SUPPRESSOR OF GENE SILENCING3 (SGS3), HASTY, HYPONASTIC LEAF1 (HYL1), and SERRATE proteins, and their loss-of-function mutants revealed their functions (Han et al. 2004; Borges and Martienssen 2015), but there is no clear description of the function of these components in rice. Considering the conservation of function and components of small RNA pathways in plants, these proteins probably function similarly in rice (Fig. 2.2). Overall, most genes working in small RNA pathways in rice were originally found from mutations with developmental abnormality such as embryogenesis-defective mutants. In addition, heterochronic mutation that affects juvenile to adult transition in *Arabidopsis*, *Maize*, and rice also helped to realize small RNA pathways and the function of several miRNAs, such as miR156 and miR172 (Auckerman and Sakai 2003; Hunter et al. 2003; Peragine et al. 2004; Lauter et al. 2005; Wu and Poething 2006; Smith et al. 2009; Chuck et al. 2007; Tanaka et al. 2011; Yoshikawa et al. 2013; Hibara et al. 2016). Thus, further analyses of these mutations would enforce our understanding on small RNA pathways in rice.

2.3 miRNA Gene Annotations in Rice

Sequences of mature plant miRNAs and their precursors are deposited in miRBase (http://www.mirbase.org). Each miRNA is numbered, and rice miRNAs are marked by letters representing the species name, for example, osa-miR156. The use of the same identifying number in different plant species means that the miRNA is conserved. In plants, the same miRNA is often produced from multiple genetic loci that form gene families. The primary transcript of each family member is marked by a lowercase letter, for example, osa-miR156a.

Among rice miRNA sequences deposited to miRBase, osa-miR156, 319/159, 160, 166, 171, 390, and 408 are the most conserved across land plants. They are followed by osa-miR162, 164, 167–169, 172, 393–395, 397–399, and 827, which are conserved in gymnosperms and angiosperms. The rest of rice miRNAs are specific to monocots, grasses, or rice. Usually, conserved miRNAs share the same target genes, which are also conserved (Cuperus et al. 2011).

In miRBase release 21, there are 575 entries of genomic loci for rice miRNA genes. Not all rice miRNA gene loci in the database produce mature miRNA, but their precursor transcripts potentially form stem–loop structures, and corresponding mature miRNA sequences have been present in several RNA profiles obtained by next-generation sequencing. The miRbase entries of rice miRNA genes could potentially contain 330 different mature miRNAs. In rice, miR395 forms the largest family with 25 members, followed by osa-miR812 (19 members), osa-miR169 (18), osa-miR2118 (18), osa-miR1861 (14), osa-miR166 (13), osa-miR156 (12), osa-miR399 (11), and osa-miR167 (10); some other families contain fewer than 10 members. Most of the conserved miRNAs form gene families; among conserved miRNAs listed above, only osa-miR390, 408, 394, and 827 are encoded by single-copy genes.

Unfortunately, the annotations of miRNA genes in the MSU database (http://rice.plantbiology.msu.edu) and RAP-DB (http://rapdb.dna.affrc.go.jp) are incomplete. For example, only 134 (MSU) and 170 (RAP-DB) gene IDs are assigned to the stem–loop regions of the 575 rice miRBase entries. The conserved miRNAs listed above are most likely functional and could be produced from 136 gene families, but only 14 (MSU) and 44 (RAP-DB) gene annotation IDs are assigned. Recent mRNA-seq analysis using next-generation sequencers clearly faces difficulties in detecting changes in the expression of most miRNA genes.

2.4 Small RNAs and Abiotic Stress Responses in Rice

Rice miRNAs and siRNAs play important roles in responses and tolerance to abiotic stresses (Jeong and Green 2013). Deficiency in inorganic phosphate (Pi) frequently limits plant growth and development. The relationship between rice miRNAs and phosphorus (Pi) deprivation response is well studied. Expression

of rice osa-miR399 is strongly induced by Pi starvation (Bari et al. 2006). Its target gene, *LEAF TIP NECROSIS1* (*LTN1*), was originally isolated from rice plants with mutations in this gene (Hu et al. 2011). *LTN1* is a putative ortholog of *Arabidopsis PHO2*, which encodes a ubiquitin-conjugating E2 enzyme (UBC24) and is important in Pi starvation signaling (Aung et al. 2006). Overexpressed osa-miR399 downregulates *LTN1* by degrading its transcript and increases Pi accumulation. Whereas osa-miR399 signaling is conserved between rice and *Arabidopsis*, osa-miR827 seems to regulate the Pi starvation response differently in these species. In *Arabidopsis*, the target gene of miR827 is *NLA* (*Nitrogen Limitation Adaptation*), which encodes a RING-type ubiquitin ligase with an SPX domain (SYG1/Pho81/XPR1); proteins containing this domain participate in Pi transport or Pi sensing. In *Arabidopsis*, the expression of miR827 increases in response to both nitrogen and Pi deficiency (Peng et al. 2007). In rice, osa-miR827 is upregulated by Pi starvation only (Lin et al. 2010). Analysis of transgenic plants and knockout lines indicates that rice osa-miR827 downregulates *OsSPX-MFS1* and *OsSPX-MFS2*, which encode proteins containing both an SPX domain and a major facilitator superfamily (MFS) domain; the MFS domain is found in membrane proteins involved in small solute transport. The level of *OsSPX-MFS1* mRNA is reduced by Pi starvation possibly because of the upregulation of osa-miR827, but that of *OsSPX-MFS2* mRNA is increased, suggesting a complex regulation of *OsSPX-MFS1* and *OsSPX-MFS2* in response to Pi starvation.

Comprehensive large-scale analyses by deep sequencing have revealed miRNAs related to Pi homeostasis in rice (Secco et al. 2013). Under Pi starvation, both osa-miR399 and osa-miR827 are upregulated. Twenty other miRNAs have been newly identified as Pi starvation responsive; 80% of these have been found only in rice. Among the latter miRNAs, the expression level of osa-miR3979 was dramatically reduced by Pi starvation, although osa-miR3979 was also downregulated by nitrogen starvation (Jeong et al. 2011). osa-miR444 was also induced not only by Pi starvation but also by nitrogen starvation (Yan et al. 2014). These results suggest that the network of rice miRNAs cooperatively signal in response to nutrient deprivation, promoting survival under severe stress conditions.

Drought and salt stresses are the most severe abiotic stresses and are also a major agricultural problem. osa-miR393 is induced by drought stress (Zhao et al. 2007) and targets two auxin receptor genes, *OsTIR1* (*Transport Inhibitor Response Protein 1*) and *OsAFB2* (*Auxin Signaling F-box Protein 2*) (Xia et al. 2012). Transgenic rice overexpressing osa-miR393 shows a reduced auxin response and reduced tolerance to drought and salt. The function of osa-miR156 is related to abiotic stress and rice development (Cui et al. 2014). osa-miR156 is strongly induced by drought and salt stresses (Jeong et al. 2011), and its overexpression increases stress tolerance. This miRNA also influences the metabolism of anthocyanin, which is accumulated in response to various stresses including drought and salt, through *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9* (*SPL9*) and its downstream gene, *DIHYDROFLAVONOL-4-REDUCTASE* (*DFR*).

Rice miRNAs are also induced by cold, heat, and oxidative stresses and by nonessential heavy metals. osa-miR319 is downregulated by cold stress, and its

overexpression increases cold tolerance by downregulating its target genes, *Os PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR 6* (*OsPCF6*) and *Os TEOSINTE BRANCHED/CYCLOIDEA/PCF* (*OsTCP21*), which are the plant-specific transcription factors containing bHLH motifs that allow DNA binding and protein–protein interaction (Wang et al. 2014). Overexpression of osa-miR529 increases rice tolerance to high levels of H_2O_2 (Yue et al. 2017). Overexpression of osa-miR390 increases Cd accumulation and reduces Cd tolerance (Ding et al. 2016). Transgenic rice plants overexpressing these miRNAs have an increased or decreased tolerance to abiotic stresses and nutrient deprivation. Further analysis of the molecular mechanisms of small RNA-mediated gene regulation in response to environmental stresses is expected to contribute to increasing yield and quality in crop production.

2.5 Small RNAs and Immune Response in Rice

Plants, including rice, are attacked by pathogenic bacteria, fungi, and viruses. As the first step of the immune response, conserved pathogen-associated molecular patterns (PAMPs) are recognized by the host and elicit PAMP-triggered immunity (PTI) to limit pathogen propagation. However, some pathogens produce effector proteins that suppress PTI. To counteract such pathogens, plants recognize the effector proteins with their resistance (R) proteins and activate the second step of the immune response, called effector-triggered immunity (ETI). In rice, both PTI and ETI respond to bacterial and fungal pathogens and lead to resistance. Small RNAs have an important role in rice immune responses, as outlined below (Seo et al. 2013; Baldrich and San Segundo 2016).

2.5.1 Small RNAs in Rice–Bacterium Interactions

The main bacterial disease of rice worldwide is bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). In cultivated rice, the major disease resistance gene, *Xa3*/*Xa26*, encodes a leucine-rich repeat kinase-like protein (Sun et al. 2004). Because *Xa3*/*Xa26* alleles in wild rice species (BB and BBCC genomes) also confer resistance to *Xoo* (Li et al. 2012), *Xa3*/*Xa26*-mediated resistance is durable. To investigate the role of small RNAs in *Xa3*/*Xa26*-mediated resistance, simultaneous genome-wide analyses of the expression of small RNAs and genes of rice inoculated with *Xoo* was carried out (Hong et al. 2015a). A number of miRNAs, some siRNAs, and a large number of genes were differentially expressed in plants with *Xa3*/*Xa26*-mediated resistance. Genes encoding the receptor-like kinase OsWAK and a UDP-glucosyltransferase domain-containing protein are likely regulated by small RNAs and involved in resistance.

2.5.2 Small RNAs Involved in Rice Immunity Against Blast Fungus

Rice blast is the most devastating disease affecting rice production. Rice blast fungus, *Magnaporthe oryzae*, can infect rice plants at any developmental stage. Overexpressed osa-miR7695 promotes rice resistance to *M. oryzae* by suppressing a metal transporter gene (Campo et al. 2013). Overexpressed osa-miR160a or osa-miR398 also enhances rice resistance to *M. oryzae*, which is associated with increased H_2O_2 accumulation at the infection site and upregulation of the expression of defense-related genes (Li et al. 2014). osa-miR398 is likely involved in PTI signaling, and osa-miR160 may be involved in ETI signaling.

Deep sequencing of small RNA libraries for global identification of rice miRNAs that are regulated by an elicitor produced by *M. oryzae* revealed that some of these miRNAs regulate genes which act in a small RNA pathway (miR393 regulates SGS3, miR168 regulates AGO1), hormone signaling, and a cross talk among various hormone pathways (Baldrich et al. 2015). The expression of miRNA target genes classified as "signaling" increases and that of genes classified as "development" decreases in elicitor-treated rice. Thus, it seems that miRNAs regulate the growth–defense balance. Of interest, the rice-specific osa-miR5819, osa-miR5075, and osa-miR2101 target the conserved peptide upstream open reading frame (CPuORF)-containing genes encoding CPuORF3-OsbZIP38, CPuORF4-OsbZIP27, and CPuORF7-SAM decarboxylase, respectively. CPuORF3-OsbZIP38 and CPuORF4-OsbZIP27 contain a short version of the sucrose control-upstream open reading frame (SC-uORF). This observation suggests a connection between small RNAs and sucrose-regulated translational control of the expression of these two rice bZIP transcription factors. This regulatory network integrates two of miRNA functions; one in metabolic regulation of gene expression and the other in rice immune responses.

2.5.3 Small RNAs in Rice–Virus Interactions

One of the major functions of the small RNA pathway is the defense against genomic parasites such as RNA viruses or transposons (Nosaka et al. 2012; Csorba et al. 2015). Rice virus diseases are generally restricted to specific rice-growing areas. *Rice stripe virus* (RSV) and *Rice dwarf virus* (RDV) are found in Asia. RSV is a negative-sense single-stranded RNA virus, and RDV is a double-stranded RNA virus. *Rice black-streaked dwarf virus* (RBSDV), a double-stranded RNA virus, infects various cereal species, including rice and maize. Small RNA profiling of rice plants infected by RDV or RSV demonstrated distinct effects of these viruses on the small RNA population (Du et al. 2011). RSV but not RDV infection interferes with the miRNA pathway and enhances the accumulation of some rice miRNA*s which are the opposite strands of miRNAs and are usually less stable than miRNAs. The

asterisk after miRNA denotes the opposite strand of miRNA. Rice RDR6 functions in the defense against RSV, whereas RDV infection downregulates RDR6 expression. Silencing of RDR6 enhances rice susceptibility to both RSV and RDV infections (Jiang et al. 2012; Hong et al. 2015b). However, overexpressed RDR6 does not improve resistance to RDV infection (Hong et al. 2015b). RDR6 protein accumulation in RDR6-overexpressing lines clearly decreases after RDV infection, possibly because of translational suppression of RDR6 and/or destabilization of the protein by RDV. Thus, there is a cross talk between the host and viruses.

2.5.4 Application of miRNA-Based Strategies to Produce Disease-Resistant Rice

Several disease-resistant transgenic rice lines have been produced by overexpressing either osa-miR160, osa-miR398, or osa-miR7695 against fungi and by overexpressing osa-miR444 or knocking out osa-miR528 to protect against viruses (Baldrich and San Segundo 2016; Wang et al. 2016; Wu et al. 2017). Artificial microRNAs, miRNA target mimicry, TALEN, and CRISPR/Cas9 technologies are also applicable for the improvement of disease resistance. Therefore, it is important to know the functions of small RNAs and small RNA pathways, as this knowledge could be applied to developing new methods of disease control in rice.

2.6 Rice Small RNAs and Crop Production

High-throughput sequencing revealed that diverse small RNAs including more than 300 miRNAs and various numbers of 24-nt siRNAs are expressed in rice grains and spikelets (Peng et al. 2011, 2013). They are predicted to be involved in various metabolic and developmental pathways, and they and their target genes could be used for increasing rice crop yield.

Several rice miRNAs affect grain productivity. Two groups independently identified a QTL named *IPA1*/*WFP*, which increases the number of panicle branches (Jiao et al. 2010; Miura et al. 2010). The *IPA1*/*WFP* locus encodes the plant-specific transcription factor SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14 (OsSPL14), which is regulated by osa-miR156 and osa-miR529. Increased expression of *OsSPL14* in panicles caused by osa-miR156 increases grain production (Jiao et al. 2010; Miura et al. 2010). Introduction of the $OsSPL14^{WFP}$ allele into the standard *Japonica* cultivar Nipponbare increased the number of panicle branches and grain production (Miura et al. 2010). Another *Japonica* cultivar, Aikawa 1, has a single nucleotide change at the osa-miR156-

targeted site in *OsSPL14*. Both the expression level of *OsSPL14* in young panicles and the number of panicle branches are higher in Aikawa 1 than in Nipponbare (Miura et al. 2010). Although the number of panicle branches was reduced in transgenic plants overexpressing osa-miR156 and osa-miR529 or in *OsSPL14* RNAi lines, overexpression of *OsSPL14* had a similar effect (Xie et al. 2006; Wang et al. 2015). This indicates that fine-tuning of *OsSPL14* expression is important for increasing rice grain yield as exemplified by $OsSPL14^{WFP}$ allele introduction (Miura et al. 2010). osa-miR172 targets five AP2-like transcription factors (*SNB*, *OsIDS1*, *SHAT1*, *OsTOE1*, and *OSGL15*) and is involved in panicle branching. Inhibition of osa-miR172 activity or overexpression of *SUPERNUMERARY BRACT* (*SNB)* and *OsTARGET OF EARLY ACTIVATION TAGGED1* (*OsTOE1*) causes dense panicles with a significantly larger number of branches and spikelets than in the wild type (Wang et al. 2015). The fact that OsSPL14 directly regulates osa-miR172 expression indicates the cooperative function of osa-miR156, osa-miR529, and osa-miR172 in reproductive branching (Wang et al. 2015). Overexpressed osa-miR397 enlarges grains and promotes panicle branching, thus increasing grain yield by up to 25% (Zang et al. 2013). This increase is due to downregulation of its target *OsLAC*, which encodes a laccase-like protein involved in sensitivity to brassinosteroids. Blocking osa-miR396 activity greatly increases grain yield by increasing the number of panicle branches and spikelets through a direct induction of *GROWTH-REGULATING FACTOR 6* (*OsGRF6*) (Gao et al. 2015). Another target of osa-miR396 is *OsGRF4*, which controls grain size and weight (Duan et al. 2015). Therefore, osa-miR396 controls both panicle branching and grain size. osa-miR398 targets Cu/Zn-superoxide dismutase genes and is also a promising target for improvement of rice grain yield (Zhang et al. 2017).

Grain productivity in rice is also affected by siRNAs. The RNAi lines of rice *DCL3a*, which produce 24-nt siRNAs associated with miniature inverted-repeat transposable elements, have fewer panicle branches and show other phenotypic defects compared with wild type (Wei et al. 2014). These siRNAs target many gibberellin and brassinosteroid homeostasis-related genes, indicating that the siRNAs are involved in panicle branching, which is regulated by these hormones.

Heterosis, the superior performance of hybrids in comparison with their parents, is an important phenomenon that helps to improve crop production. Some groups found differential expression of rice small RNAs between hybrids and their parents by using high-throughput sequencing (He et al. 2010; Chen et al. 2010; Zhang et al. 2014); this suggests that small RNAs have a potential role in rice hybrid vigor. Target genes of some differentially expressed small RNAs encode auxin response factors (ARFs) and some transcription factors (NAC1, PHAVOLUTA, and REVOLUTA) involved in the auxin signaling pathway (Zhang et al. 2014).

2.7 Conclusion

Recent progress in small RNA sequencing in plant cells has revealed a higher diversity of the molecular species of small RNAs than that of protein-coding mRNAs and opened a new paradigm in gene regulation. Only a limited number of small RNA functions in rice have been described so far. hc-siRNAs function in epigenetic modifications of DNA; the details are described in another chapter of this book (Chap. 24). Although the functions of most small RNAs are not yet elucidated and new modes of gene regulation by small RNAs may be found in the future, small RNAs and their regulation in rice are potentially useful for improving crop production.

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