A role of *OsROS1* in aleurone development and nutrient improvement in rice

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DNA methylation is a conserved epigenetic mark in eukaryotes involved in many important biological processes, such as genome integrity, gene imprinting, and gene regulation (1). In genomes, DNA methylation marks can be added through the DNA methylation pathway and can be removed through the DNA demethylation pathway (1). The REPRESSOR OF SILENCING 1 (ROS1)/DEMETER (DME; DEMETER-like, DML) family of DNA demethylases were first reported in plants (2, 3) and now are known to be involved in DNA demethylation in all eukaryotes (1). The bifunctional glycosylase/lyase activities of the ROS1 family of enzymes could initiate DNA demethylation through removal of methylcytosine from the DNA backbone, leaving a single-nucleotide gap that is filled with an unmethylated cytosine through a base excision repair (BER) mechanism (1). In both plants and mammals, the active DNA demethylation pathway depends on the BER mechanism, although different enzymes initiate the pathway in the two systems (1). In recent years, several additional enzymes and regulators involved in the active DNA demethylation pathway have been identified and studied in plants (1). In contrast to the extensive mechanistic understanding of DNA demethylation pathways, however, the developmental function of DNA demethylation factors is largely unknown in both model plants and crops. This knowledge gap is now bridged by the findings reported in PNAS by Liu et al. (4), who show that weak mutations of the *OsROS1* gene in rice lead to an increased number of aleurone cell layers through DNA hypermethylation and repression of two putative transcription factor genes, *RISBZ1* and *RPBF*, that increase aleurone layer cells when their expression levels are reduced (5). The *OsROS1* mutant contains more lipids, proteins, vitamins, minerals, and dietary fibers than the wild type and could be useful for nutrient improvement in rice breeding (Fig. 1).

DNA demethylation in mammals is required for many developmental processes, including embryo development and cell differentiation (6). However, in plants, only a few developmental functions have been reported for DNA demethylases before this study. In *Arabidopsis*, *AtDME* is highly expressed in central cells of the embryo sac and contributes to gene imprinting (2, 7), whereas *AtROS1* is expressed in somatic cells and controls stomatal development through demethylation of *EPIDERMAL PATTERNING FACTOR 2* promoter (8). Other roles of DNA demethylases include nodule differentiation and nitrogen fixation in *Medicago* (9), pericarp ripening and global loss of DNA demethylation (10, 11) due to *SlDML2* in tomato, DNA demethylation and bud break (12) due to PtaDML10 in poplar, and, in rice, involvement of *OsROS1* in male and female gametogenesis (13).

The study of Liu et al. (4) involved a large-scale screening of rice mutants for aleurone defects using a half-seed assay (5) and it led to the discovery of mutants with a thickened aleurone (*ta*) phenotype. This study focused primarily on the *ta2-1* allele (5) and the finding that the phenotype was caused by a dominant negative effect of altered *OsROS1* transcripts (*mOsROS1*). Consistent with this interpretation, the overexpression of *mOsROS1* in wild-type rice was sufficient to increase aleurone layers. Additional *OsROS1* weak alleles (named *ta2-2* to *ta2-6*) from a TILLING (5) screen have mutations in the conserved glycosylase domain (*ta2-2* and *ta2-3*) or in nonconserved regions of *OsROS1* (*ta2-4* to *ta2-6*). Interestingly, mutant alleles of *ta2-4* to *ta2-6* showed no visible defects in yield-related traits, but the dominant negative aleurone effect was observed for *ta2-3* and weakly for *ta2-4*, *ta2-5*, and *ta2-6* alleles. The mechanism underlying the dominant negative effect in *ta2* mutants is still not known.

To investigate how OsROS1 contributes to aleurone formation, the authors compared DNA methylomes of the wild-type and *ta2-1* endosperms. Their study suggests that OsROS1 may regulate DNA demethylation at the promoter regions of *RISBZ1* and *RPBF*, two previously known regulators of aleurone layer

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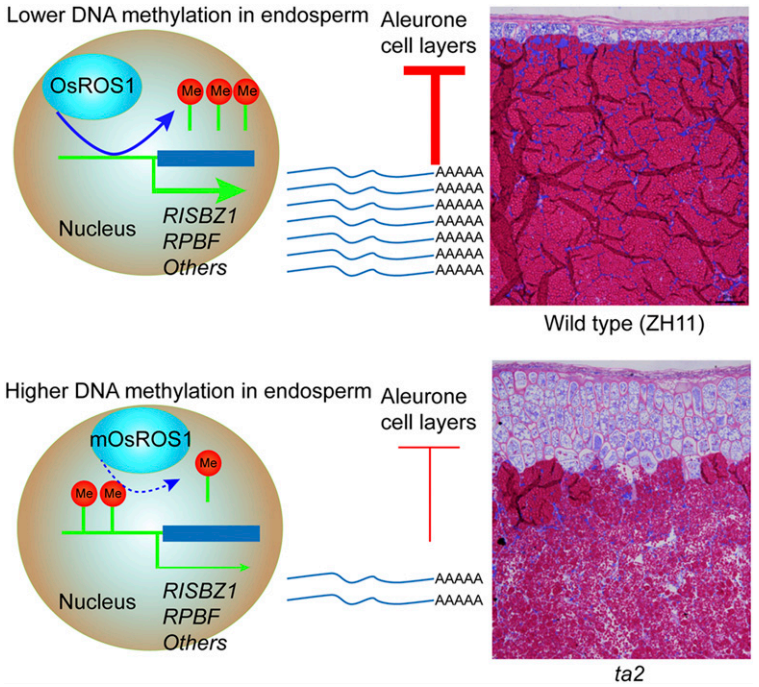

Fig. 1. The proposed model for OsROS1 in mediating aleurone development in rice. OsROS1 modulates the expression of genes such as *RISBZ1* and *RPBF* by DNA demethylation in the endosperm of rice. In the wild type, the higher expression of *RISBZ1* and *RPBF* genes is maintained by OsROS1 to ensure the formation of one aleurone cell layer. In *ta2* mutants, the attenuated function of mOsROS1 led to increased DNA methylation in the promoter regions, thus repressing the expression of genes such as *RISBZ1* and *RPBF*, which releases their inhibition on the aleurone cell-layer formation. Thus, the *ta2* mutants have more aleurone cell layers and contain more nutrients than the wild type (ZH11).

formation (5). Compared with the wild type, there was increased DNA methylation and decreased gene expression in *ta2-1* for these two genes. They also identified 15,147 hypermethylated genomic regions, and there is a possibility that many other genes could be targeted by OsROS1 and contribute to aleurone formation in rice (Fig. 1). In the future, it will be interesting to investigate additional genes that are hypermethylated and, thus, potentially regulated by OsROS1 in *ta2-1* mutants.

In summary, this study reveals a function of DNA demethylase in aleurone formation in rice grains and broadens our understanding of the DNA demethylation pathway in plant development, especially in cereal crops. It also provides a strategy for enhancing the nutritional value of rice, because it is the aleurone layer that stores proteins, lipids, vitamins, and minerals and is the most nutritious part of cereal grains (14). *OsROS1* could be modified in different rice varieties using the CRISPR-Cas9 technology (15), especially brown rice varieties in which, unlike in white rice, the aleurone, together with the pericarp and seed coat, is not removed during processing. Gene editing could also be used to find out whether the DNA demethylase-mediated aleurone formation is conserved in maize and other cereals, most of which have one single-cell layered aleurone.

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