Abstract: The heading date and grain size are two essential traits affecting rice yield. Here, we found that OsMOS1 promotes rice heading and affects its grain size. Knocking out OsMOS1 delayed heading, while the overexpression of OsMOS1 promoted heading in rice under long-day conditions. The transcriptions of the heading activators Ehd1, Hd3a, and RFT1 were decreased and the heading repressor Hd1 was increased in the osmos1 mutant. Conversely, the overexpression of OsMOS1 promoted the expressions of Ehd1, Hd3a, and RFT1, but inhibited the expression of Hd1. This suggests that OsMOS1 may control heading in rice by modulating the transcriptions of Ehd1, Hd3a, RFT1, and Hd1. In addition, knocking out OsMOS1 led to larger grains with longer grain lengths and higher grain weights. The seed cell size measurement showed that the cell lengths and cell widths of the outer glume epidermal cells of the osmos1 mutant were greater than those of the wild type. Furthermore, we also found that the overexpression of OsMOS1 in the Arabidopsis mos1 mutant background could suppress its phenotypes of late flowering and increased seed size. Thus, our study shows a conserved function of MOS1 in rice and Arabidopsis, and these findings shed light on the heading and seed size regulation in rice and suggest that OsMOS1 is a promising target for rice yield improvement.

Keywords: rice; heading date; florigen; MOS1; gene expression

1. Introduction

As one of the most important cereal crops, rice (Oryza sativa L.) provides stable food for more than half the world’s population [1]. The heading date (Flowering time) and grain size are two major agronomic traits that affect yield in rice [2]. Early heading during the growing season without enough vegetative growth stage may lead to insufficient nutrient accumulation in rice [3]. Additionally, if the heading is too late during the growing season, the later reproductive growth will be affected by lower temperatures and lead to a decreased final yield in rice [4]. The grain size includes the grain length, width, and thickness, which are associated with grain weight [2]. In general, the final grain size of rice is coordinately regulated by cell proliferation and cell expansion in the spikelet hull [5]. Increasing the grain yield has become an urgent necessity in rice breeding and it is worthwhile to explore new genes that control grain size and heading date in rice for yield improvement.

The rice heading date is controlled by multiple factors, among which the photoperiod is a major factor [6]. Photoperiodic flowering consists of a complicated network that converges into the generation of a mobile flowering signal called florigen [7]. Florigen is synthesized in the leaves and then moved to the shoot apex to induce flowering [7]. As a short-day (SD) plant, the rice heading date is drastically promoted under SD conditions and inhibited under long-day (LD) conditions [8]. There are two florigen genes, namely HEADING DATE 3a (Hd3a) and RICE FLOWERING LOCUS T1 (RFT1) in rice, which...
function as florigen in SD and LD, respectively [9,10]. The transcriptions of Hd3a and RFT1 are mainly controlled by a B-type response regulator EARLY HEADING DATE 1 (Ehd1) [11] and Hd1, which is an ortholog of the Arabidopsis floral activator CONSTANS (CO) [12,13]. Hd1 promotes floral transition under SDs by up-regulating Hd3a and RFT1 expression, while it strongly inhibits Hd3a and RFT1 expression by directly repressing Ehd1 transcription to restrain floral transition under LDs [14–16]. Hd1 is controlled by GI/GIGANTEA (GI), which inhibits flowering in rice by repressing the expression of Hd3a [11,12,17]. The floral signal transduction cascade mediated by GI-Hd1-Hd3a, in which Hd1 receives signals from GI and then affects the expression of Hd3a to regulate the flowering time, has been found to be conserved between Arabidopsis and rice [15]. Ehd1 integrates different upstream signals and promotes the expressions of Hd3a and RFT1 under both LD and SD conditions [8,15]. The expression of Ehd1 is up-regulated by several upstream positive regulators, including Ehd2, Ehd3, Ehd4 [18–20], Hd17 [21], OsMADS50, and OsMADS51 [22,23]. Additionally, several negative regulators, including Ghd7, Ghd8 [24,25], and DELAYED HEADING DATE1 (Dhd1) [26], prevent Ehd1 expression under non-inductive photoperiods to repress heading.

An evolutionarily conserved BAT2 (HLA-B ASSOCIATED TRANSCRIPT2) domain containing protein MODIFIER OF snc1 (MOS1) was first identified as a suppressor of the autoimmune mutants snc1 and bonzai1 (bon1) in Arabidopsis [27,28]. Autoimmunity in bon1 and snc1 is caused by the constitutive activation of an NLR gene SUPPRESSOR OF npr1-1 and CONSTITUTIVE 1 (SNC1) [29,30]. MOS1 interacts with TEOSINTE BRANCHED 1, CYCLOIDEA, and PCF 15 (TCP15) transcription factors, and might regulate the expression of SNC1 by directly binding to its promoter [31]. MOS1 has also been found to regulate the flowering time and endoreduplication [27]. MOS1 interacts with the spindle assembly checkpoint (SAC) component MAD2 and promotes flowering and inhibits endopolyploidization, but this function could be antagonized by another SAC component, MAD1 [27]. MOS1 interacts with the zinc-finger-containing transcription factor SUPPRESSOR OF FRIGIDA 4 (SUF4) to inhibit the expression of the central flowering time gene FLOWERING LOCUS C (FLC) and thus promote flowering [27]. The defection of endopolyploidization in the mos1 mutant can be suppressed by the mutations of SUF4, but not FLC, indicating that MOS1 modulates the endopolyploidization and flowering time through different genetic pathways [27]. In addition, MOS1 negatively regulates sugar responses and anthocyanin biosynthesis in Arabidopsis, possibly at the transcriptional level [32]. Thus, the existing finding indicates that MOS1 functions as a key coordinator in the regulation of growth and biotic and abiotic stress responses in Arabidopsis.

The functions of MOS1 in Arabidopsis have been studied; however, the roles of MOS1 in other plants, such as rice, remain unknown. In this study, OsMOS1, a rice gene homologous to Arabidopsis MOS1, was characterized. We assessed how OsMOS1 controlled heading and grain size by generating its knock-out mutant and overexpression transgenic plants. In addition, we investigated the functional conservation of MOS1, which controls flowering time and grain size in rice and Arabidopsis, by introducing OsMOS1 into atmos1 mutants. Here, we demonstrate a new function of MOS1 proteins in seed size control, in addition to their known roles in the flowering time of plants.

2. Results

2.1. Isolation and Expression of MOS1 in Rice

To gain a better understanding of MOS1 proteins in plants using information from Arabidopsis MOS1 (At4g24680), we selected a total of 12 plant species for MOS1 gene identification based on their evolutionary distance and availability of full-genome sequences. The genome databases of these organisms were searched with BLASTP (2 March 2019, NCBI, https://www.ncbi.nlm.nih.gov/) using the Arabidopsis AtMOS1 protein sequence. The full-length protein sequences of 17 candidate MOS1 proteins were identified from 12 plant species. There were one or two MOS1 proteins in each of the 12 selected plant species. A phylogenetic tree was constructed using the full-length protein sequences of the 17 candidate MOS1 proteins. Phylogenetic analysis revealed that 12 plant species had...
two clades of MOS1 (Figure 1a). The rice protein (accession numbers LOC_Os12g37860) was identified as a MOS1 protein in rice by confirming that it contained the conserved BAT2 domain (Figure 1b). We then named this gene OsMOS1. Sequence analyses revealed that OsMOS1 encodes a protein consisting of 1460 amino acids (Supplemental Figure S1). The deduced amino sequence of OsMOS1 shared 60% similarity with AtMOS1 (Supplemental Figure S1). The protein domain analyzed by the SMART website tool (8 March 2019, http://smart.embl-heidelberg.de/) showed that OsMOS1 contained a conserved BAT2 domain at the N-terminal region. In addition to the BAT2 domain, OsMOS1 also contained a PRK10263 (DNA Translocase FtsK) domain, which was different from other MOS1 proteins, including AtMOS1 in plants (Figure 1b).

Figure 1. Homology analysis of MOS1. (a) Phylogenetic tree of MOS1 proteins from 12 plant species. The tree was constructed by using MEGA6.06 software based on the MOS1 protein sequences from the National Coalition Building Institute (2 March 2019, NCBI, https://www.ncbi.nlm.nih.gov/). The scale bar indicates the branch length. (b) Protein structures of AtMOS1 and OsMOS1. PRK10263 (DNA Translocase FtsK) domain.

To gain insights into the possible functions of the OsMOS1 gene in rice, the expression patterns of the OsMOS1 gene in different rice tissues were examined by quantitative reverse transcription PCR (qPCR). The qPCR results revealed that the OsMOS1 gene was expressed over a wide range of tissues in the wild-type rice cultivar Nipponbare (NIP) (Figure 2). OsMOS1 transcripts were detectable in all of the selected organs, including the root (three-leaf stage), stem (three-leaf stage), leaf (three-leaf stage), and panicle (young panicles at P2 stages) of the wild-type rice cultivar NIP and with the highest transcript levels in the young panicles (Figure 2). The expression patterns of the AtMOS1 gene were also examined by taking advantage of the online, publicly available microarray data. The tissue expression patterns analyses using the BAR website (15 October 2022, http://www.bar.utoronto.ca/) revealed that the AtMOS1 gene was also expressed over a wide range of tissues in the wild-type Col-0 (Supplemental Figure S2). AtMOS1 transcripts were detectable in all of the selected organs, including the roots (four-rosette leaf stage), stems (four-rosette leaf stage, second internode), leaves (rosette leaf 2), and siliques (stage 8 siliques), with the highest transcript levels in the siliques (Supplemental Figure S2). These results reveal that OsMOS1 and AtMOS1 had similar tissue expression patterns, suggesting the functional conservation of MOS1 in rice and Arabidopsis.
were either weak reductions of functional mutants or had no compromised function in OsMOS1 (Nanjing, China) conditions (Figure 4a,b). Therefore, the osmos1-1 mutants in the NIP or R7954 (O. sativa L. ssp. indica) background using CRISPR/Cas9 gene-editing technology [33]. Homozygous mutants were identified in the T0 generation by sequencing the PCR product containing the CRISPR/Cas9 target sites. Four OsMOS1 mutants (osmos1-1, osmos1-2, osmos1-3, and osmos1-4) were obtained, and all but osmos1-1 and osmos1-2 were predicted to be loss-of-function (LOF) mutants (Figure 3). The osmos1-1 mutant contained a three-base-pair (bp) deletion at the position of 468–470 (relative to the translation initiation site), leading to a 1-amino acid deletion (amino acid 75) in its predicted coding protein. The osmos1-2 mutant contained a 6 bp deletion at the position of 468–473, leading to a 2-amino acid deletion (amino acids 75–76) in its predicted coding protein. The osmos1-3 mutant contained a 13 bp deletion at the position of 457–469, leading to reading frame shifts in OsMOS1 and a stop codon at amino acid 73. The osmos1-4 mutant was generated by targeting sequences different from those of osmos1-1, osmos1-2, and osmos1-3, so they were independent from osmos1-1, osmos1-2, and osmos1-3 and unlikely to have had the same off-target mutations, if any. The osmos1-4 mutant contained a 58 bp deletion at positions 495–552, leading to reading frame shifts in OsMOS1 and a stop codon at amino acid 123.

Heading date analysis found that osmos1-3, but not osmos1-1 or osmos1-2, headed approximately 11 days (d) later than the wild-type (WT) NIP under natural long-day (NLD) (Nanjing, China) conditions (Figure 4a,b). Therefore, the osmos1-1 and osmos1-2 mutants were either weak reductions of functional mutants or had no compromised function in OsMOS1. Similar to osmos1-3, osmos1-4 exhibited a significantly late heading phenotype compared with the control plant (Supplemental Figure S3).
Figure 3. Mutants of OsMOS1 generated by CRISPR/Cas9 technology. Diagram of the genomic region and mutations of OsMOS1. The top diagram is the gene structure of the OsMOS1 genomic sequence. Exons and introns are indicated by black boxes and lines between boxes, respectively. The bottom panel shows the sequence alignments between the mutants and wild type with the genomic sequences above and the protein sequence below. The dotted line indicates the deletion of a base pair or amino acid sequence, green capital letters indicate amino acid missense mutations, * sign indicates a generated stop codon, and PAM indicates the protospacer adjacent motif.

Figure 4. OsMOS1 promotes rice heading date. (a) Heading phenotype of Nip and osmos1 mutants under NLD conditions. NIP, Nipponbare. Bars = 20 cm. Red arrows indicate panicles. (b) Statistical analysis for the heading date of Nip and osmos1 mutants under NLD conditions. Error bars indicate the standard deviation, n = 15. Asterisks indicate a significant difference between NIP and osmos1 mutants (** p < 0.01, Student’s t-test). (c) Early heading date phenotypes of OsMOS1-overexpressing lines. NIP, Nipponbare. Bars = 20 cm. Red arrows indicate panicles. (d) The relative expression levels of OsMOS1 in Nip and osmos1 mutants. (e) The average heading dates of Nip and osmos1 mutants.
lines under NLD conditions. The numbers 17#, 18#, and 21# represent three independent transgenic lines. The red arrows indicate panicles. Bars = 20 cm. (d) Relative transcript levels of OsMOS1 in OsMOS1 transgenic lines. The rice ACTIN gene was used as the internal control. Error bars indicate the standard deviation, \( n = 3 \). Asterisks indicate a significant difference between NIP and OsMOS1-overexpressing lines \( (** \ p < 0.01, \text{Student’s } t\text{-test}) \). (e) Statistical analysis for the heading date of Nip and OsMOS1-overexpressing lines under NLD conditions. Error bars indicate the standard deviation, \( n = 15 \). Asterisks indicate a significant difference between NIP and OsMOS1-overexpressing lines \( (** \ p < 0.01, \text{Student’s } t\text{-test}) \).

To further evaluate the function of OsMOS1 in the heading date, an OsMOS1 overexpression vector was constructed and transformed into NIP. After the confirmation of the presence of the transgene by the PCR, we obtained 21 transgenic plants. The transcript levels of OsMOS1 were significantly increased in the overexpressing transgenic lines, and three representative OsMOS1-overexpressing transgenic lines, i.e., #17, #18, and #21, were used for further analyses (Figure 4d). Compared with the WT, the OsMOS1-overexpressing plants headed approximately 7 d earlier under NLD conditions (Figure 4c,e).

2.3. OsMOS1 Regulates the Expression of the Heading Date Genes in Rice

To assess the molecular mechanisms by which OsMOS1 regulates the heading date in rice, we examined the transcription levels of floral regulator genes in NIP, osmos1-3, and OsMOS1-overexpressing plants under LD growth conditions in a greenhouse. Florigen genes Hd3a and RFT1, heading repressor gene Hd1, and heading enhancer gene Ehd1 were selected for further gene expression analysis. Considering the diurnal rhythmic expression patterns of the above floral regulator genes, the expression of Hd1 was monitored at zeitgeber time (ZT) 14 h and Ehd1, Hd3a, and RFT1 were monitored at ZT 2 h, as previously reported [17]. Our results showed that the transcripts of the two florigen genes Hd3a and RFT1, and the LD heading enhancer Ehd1 were less abundant in the osmos1-3 (Figure 5a–c), but more abundant in OsMOS1-overexpressing plants (Figure 5e–g). The transcript levels of the LD heading repressor Hd1 were significantly increased in the osmos1-3 mutant (Figure 5d), but reduced in the OsMOS1-overexpressing plants (Figure 5h). Taken together, these results suggest that OsMOS1 may regulate heading by modulating the transcriptions of Hd1, Ehd1, Hd3a, and RFT1 in rice under LD conditions.

**Figure 5.** Expressions of the floral regulator genes in Nip, osmos1-3, and OsMOS1-overexpressing lines under LD conditions. (a–d) Expressions of the RFT1 (a), Hd3a (b), Ehd1 (c), and Hd1 (d) in Nip
and osmos1-3 under LD conditions. (e–h) Expressions of RFT1 (e), Hd3a (f), Ehd1 (g), and Hd1 (h) in NIP and OsMOS1-overexpressing lines under LD conditions. The fully emerged leaf blades from Nip, osmos1-3, and OsMOS1-overexpressing plants under LD growth conditions were sampled at 45 days after germination. The expression of Hd1 was observed at ZT 14 h and other floral regulator genes were monitored at ZT 2 h. The rice ACTIN gene was used as the internal control. Error bars indicate the standard deviation, n = 3. Asterisks indicate a significant difference between NIP and osmos1-3 (** p < 0.01, Student’s t-test).

2.4. MOS1 Negatively Regulates Seed Size in Rice and Arabidopsis

Grain phenotypic analysis indicated that OsMOS1 also affected grain size. The grain size of osmos1-3 was significantly larger than that of the wild-type NIP (Figure 6). The grain lengths, but not widths, of osmos1-3 increased by 8.3% compared with those of NIP (Figure 6a–c). As a result, the osmos1-3 mutant produced heavier grains (+8.2% in 1000-grain weight) than NIP (Figure 6d). However, there was no significant difference in the grain size between the wild-type NIP and OsMOS1-overexpressing plants (Supplemental Figure S4). To investigate whether the difference between osmos1-3 and NIP in grain size was caused by seed cell expansion, the outer glume epidermal cells were observed using a scanning electron microscope. As shown in Figure 6, the cell lengths and cell widths of osmos1-3 were greater than those of NIP (Figure 6e,f). Taken together, these results show that OsMOS1 negatively regulated grain size mainly by controlling the seed cell expansion.

Figure 6. Analyses of the grain and cell sizes of NIP and the osmos1-3 mutant. (a) Grain morphology of NIP and the osmos1-3 mutant. Scale bars correspond to 1 cm. (b,c) Statistical analyses of the grain length and width. Error bars indicate the standard deviation, n = 3 × 50. Asterisks indicate a significant difference compared with NIP (** p < 0.05, Student’s t-test). (d) Statistical analysis of the 1000-grain weight. Error bars indicate the standard deviation, n = 3 × 500. Asterisks indicate a significant difference compared with NIP (** p < 0.01, Student’s t-test). (e,f) Quantification of a single cell’s length and width per mm² in (d). Asterisks indicate a significant difference compared with NIP (* p < 0.05, Student’s t-test).

2.5. Overexpression of OsMOS1 Affects the Late Flowering and Large Seed Size Phenotypes of the Arabidopsis mos1 Mutant

In Arabidopsis, MOS1 functions as a positive regulator of the flowering time [27]. To investigate the functional conservation in the flowering time between rice and Arabidopsis MOS1 homologs, we introduced the Ubi:OsMOS1 transgene into the atmos1-6 mutant. The atmos1-6 mutant was a LOF mutant of AtMOS1 with an 80 bp deletion in the fourth exon of AtMOS1 [27]. After the confirmation of the presence of the transgene by PCR, we obtained five independent transgenic plants and RT-PCR analysis showed that OsMOS1 was successfully expressed in the transgenic Arabidopsis lines (Supplemental Figure S5). Two representative transgenic lines, #1 and #2, were used for further analyses. The atmos1-6 mutant was a LOF mutant of AtMOS1 with an 80 bp deletion in the fourth exon of AtMOS1 [27]. After the confirmation of the presence of the transgene by PCR, we obtained five independent transgenic plants and RT-PCR analysis showed that OsMOS1 was successfully expressed in the transgenic Arabidopsis lines (Supplemental Figure S5). Two representative transgenic lines, #1 and #2, were used for further analyses. The atmos1-6 mutant was a LOF mutant of AtMOS1 with an 80 bp deletion in the fourth exon of AtMOS1 [27]. After the confirmation of the presence of the transgene by PCR, we obtained five independent transgenic plants and RT-PCR analysis showed that OsMOS1 was successfully expressed in the transgenic Arabidopsis lines (Supplemental Figure S5). Two representative transgenic lines, #1 and #2, were used for further analyses.
length (b) and grain width (c) between NIP and the osmos1-3 mutant. Error bars indicate the standard deviation, $n = 3 \times 50$. Asterisks indicate a significant difference compared with NIP (\textbf{*} $p < 0.05$, Student’s $t$ test). (d) Statistical analysis of the 1000-grain weight between NIP and the osmos1-3 mutant. Error bars indicate the standard deviation, $n = 3 \times 500$. Asterisks indicate a significant difference compared with NIP (\textbf{**} $p < 0.01$, Student’s $t$ test). (e) Electron microscopy scan of the outer glume surface of a mature grain from NIP and osmos1-3 mutants. Scale bars correspond to 65 $\mu$m. (f) Quantification of a single cell’s length and width per mm$^2$ in (d). Asterisks indicate a significant difference compared with NIP (\textbf{*} $p < 0.05$, Student’s $t$ test).

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As with the osmos1-3 mutant, we found that the seed size of the atmos1-6 mutant was significantly larger than that of the wild-type Col-0 (Figure 8a). Compared with Col-0, the seed lengths and widths of atmos1 mutants increased by 26.3% and 20.6%, respectively. Thus, these results suggest that MOS1 has a conserved function in the plant seed size. Furthermore, we found that the overexpression of OsMOS1 also affected the seed size in Arabidopsis. The seed lengths of the two Ubi:OsMOS1/atmos1-6 transgenic lines were 488.76 $\mu$m and 472.93 $\mu$m respectively, which were significantly smaller than those of the atmos1-6 (532.15 $\mu$m) mutant but still larger than those of the WT (421.46 $\mu$m) (Figure 8b). In addition, the grain widths of the two Ubi:OsMOS1/atmos1-6 transgenic lines were 279.06 $\mu$m and 274.15 $\mu$m, respectively, which were significantly smaller than those of the atmos1-6 mutant (310.63 $\mu$m), but still larger than those of the WT (257.56 $\mu$m) (Figure 8c). Thus, these results suggest that the overexpression of OsMOS1 partially rescued the seed size phenotype of the Arabidopsis mos1-6 mutant.
mos1-6 mutant exhibited a late-flowering phenotype, whereas the Ubi:OsMOS1/atmos1-6 transgenic lines restored the late-flowering phenotype of atmos1-6 to the wild type (Figure 7a–c). As AtMOS1 promoted flowering by inhibiting the expression of the FLC gene [27], we later checked the expression of the FLC gene in Ubi:OsMOS1/atmos1-6 transgenic plants under LD conditions. We found that the upregulation of the FLC gene in the atmos1-6 mutant was restored to the WT level in Ubi:OsMOS1/atmos1-6 transgenic plants (Figure 7d). These results suggest that the overexpression of OsMOS1 rescued the late flowering phenotype of the atmos1-6 mutant by down-regulating the expression of FLC.

Figure 7. Analyses of flowering time and FLC gene expression in OsMOS1-overexpressing transgenic plants in Arabidopsis. (a) Flowering phenotypes of Col-0, atmos1-6, OE:OsMOS1/atmos1-6 #1, and OE:OsMOS1/atmos1-6 #2 plants under LD conditions. Scale bars = 5 cm. (b) Statistical analysis for flowering time of Col-0, atmos1-6, OE:OsMOS1/atmos1-6 #1, and OE:OsMOS1/atmos1-6 #2 plants under LD conditions. Asterisks indicate a significant difference compared with Col-0 (** \( p < 0.01 \), Student’s t-test). Error bars indicate the standard deviation, \( n = 15 \). (c) Statistical analysis for the rosette leaf numbers at the bolting stage of Col-0, atmos1-6, OE:OsMOS1/atmos1-6 #1, and OE:OsMOS1/atmos1-6 #2 plants under LD conditions. Asterisks indicate a significant difference compared with Col-0 (* \( p < 0.05 \), Student’s t-test). Error bars indicate the standard deviation, \( n = 15 \). (d) Expression of the FLC in Col-0, atmos1-6, OE:OsMOS1/atmos1-6 #1, and OE:OsMOS1/atmos1-6 #2 plants under LD conditions. The Arabidopsis UFP gene was used as the internal control. Error bars indicate the standard deviation, \( n = 3 \). Asterisks indicate a significant difference compared with Col-0 (* \( p < 0.05 \), Student’s t-test).
cannot fully rescue the seed size phenotype of the vegetative growth period usually results in more accumulation of nutrients, the larger mos1-6 results for three replicates (each replicate consisted of at least 30 cells). 3 × 500. Different letters indicate significant differences based on Duncan’s multiple range test among the means via ANOVA (p < 0.05). The cell length and width were determined by averaging the results for three replicates (each replicate consisted of at least 30 cells).

3. Discussion

MOS1 performs various regulatory activities in multiple aspects of plant growth, including the flowering time, cell cycle, and stress responses in Arabidopsis [27,28,31,32]. Here, we focused on the functional characterization of a rice MOS1, OsMOS1, which is a close homolog of Arabidopsis MOS1 as it contains the conserved BAT2 domain. Our results show that OsMOS1 is involved in the regulation of the heading date and seed size in rice. It controls rice heading by regulating the expressions of several floral-regulation genes and seed size in both rice and Arabidopsis.

OsMOS1 regulates both the heading date and grain size in rice. It is possible that the primary function of OsMOS1 is to promote the heading date in rice. As a longer vegetative growth period usually results in more accumulation of nutrients, the larger grain size in the osmos1-3 mutant was likely to be promoted by the increased accumulation of nutrients. Furthermore, we found that the role of MOS1 in regulating the flowering time and seed size appeared to be largely conserved between rice and Arabidopsis. The heterologous overexpression of OsMOS1 in the Arabidopsis atmos1 mutant could rescue or partially rescue its late flowering and large seed phenotypes. Meanwhile, it is worth noting that the loss of function of OsMOS1 or AtMOS1 will increase the seed size, but OsMOS1 cannot fully rescue the seed size phenotype of the atmos1 mutant, suggesting that there are other decisive factors involved. Therefore, despite the functional conservation in seed size between OsMOS1 and AtMOS1, the specific regulation mechanism may still have some differences.

There are two photoperiod heading pathways that are mediated by GI-Hd1-Hd3a/RFT and GI-Ehd1-Hd3a/RFT in rice. Here, we found that the expressions of Ehd1 and both the
florigens, RFT1 and Hd3a, were down-regulated in the osmos1-3 mutant but up-regulated in the OsMOS1-overexpressing transgenic lines under LD conditions. While the expression of Hd1, a repressor of heading under LD conditions, was up-regulated in the osmos1-3 mutant, it was down-regulated in OsMOS1-overexpressing transgenic lines. Taken together, our results suggest that OsMOS1 may regulate heading through both the Hd1- and Ehd1-mediated pathways in rice. Hd1 can also inhibit the heading date by directly repressing Ehd1 transcription to indirectly regulate the expressions of Hd3a and RFT1 under LD conditions [34]. Thus, this raises the possibility of the coordinated functioning of OsMOS1 in the above two heading regulation pathways in rice, which needs further exploration.

In the osmos1-3 mutant, the expressions of the two florigens RFT1 and Hd3a were significantly repressed, leading to heading being delayed. The expression of the florigen AtFT, the homolog of RFT1 in Arabidopsis, was also down-regulated in the atmos1 mutant [27]. In addition, the up-regulation of another flowering-related gene, AtFLC, in the atmos1 mutant could be recovered by OsMOS1 overexpression in Arabidopsis. Thus, these results indicate that MOS1 regulates the flowering time in rice and Arabidopsis by promoting florigen gene expression. Taken together, our study shows that OsMOS1 has important functions in regulating the rice heading date and grain size. Consequently, it is a promising gene target for breeding new rice varieties with enhanced yield.

4. Materials and Methods

4.1. Plant Materials and Growth Conditions

The wild-type rice in this study were NIP and R7954. The seeds of WT, osmos1 mutants, and transgenic plants were soaked in water at 28 °C in the dark for 3 d, then germinated for 2 d at 37 °C. Rice seedlings were planted in soil and grown under a 14/10 h light/dark cycle in a chamber at 28 °C or in a natural field during the summer in Nanjing, China. The seeds of Arabidopsis wild-type Col-0, atmos1-6 mutant [27], and Ubi:OsMOS1/atmos1-6 transgenic plants were sown in soil and grown in a chamber with 16 h light/8 h dark, 100 μmol/s/m², and 60% humidity at 22 °C after stratifying at 4 °C for 4 d.

4.2. Generation of the CRISPR/Cas9 Mutants

To generate the CRISPR/Cas9 mutants, the specific guide RNA spacer sequences of the OsMOS1 gene were selected using the CRISPR-PLANT website (20 March 2019, http://www.genome.arizona.edu/crispr/CRISPRsearch.html). Three guide RNA targets in the exon OsMOS1 were cloned into the pHUE411 construct [33] and then introduced into the calli of the rice cultivar NIP or R7954 via EHA105-mediated methods [35]. The genomic DNA of Ubi:OsMOS1 transgenic lines and the osmos1 mutants was extracted, the genomic regions surrounding the CRISPR/Cas9 target sites for OsMOS1 were amplified by PCR, and the segment was sequenced to screen for mutants.

4.3. Generation of Transgenic Plants

The full-length coding sequence (CDS) of OsMOS1 was amplified from the total RNA of wild-type NIP by the reverse transcription (RT)-polymerase chain reaction (PCR) using gene-specific primer sets (Supplementary Table S1). The amplicons were digested with restriction enzymes BamHI and KpnI and the resulting restriction fragments were subcloned into pCUbi1390, a plant binary vector harboring the Ubi promoter.

For the generation of OsMOS1 transgenic plants in rice, the Ubi:OsMOS1 construct was transformed into Agrobacterium tumefaciens EHA105 and then introduced into the calli of the rice cultivar Nip by EHA105-mediated methods [35]. The transgenic plants were screened with a hygromycin-resistant culture medium and the overexpression of OsMOS1 in independent T2 plants was confirmed by qPCR analysis. The heading date was defined as the number of days of seed-soaking until the appearance of the first (main) panicle. The heading date was determined by counting the heading date from 15 T2 generation plants.

For the generation of OsMOS1 transgenic plants in Arabidopsis, the Ubi:OsMOS1 recombinant construct was introduced into the atmos1-6 mutant using the floral dip method [36].
The transgenic plants were screened with hygromycin-resistant culture medium and the overexpression of OsMOS1 in independent T2 plants was confirmed by qPCR analysis. The flowering time was determined by counting the total number of rosette leaves from 15 T2 generation plants.

4.4. RNA Extraction and qPCR

The total RNA was extracted using TRIzol reagent (RP1001, Biotek, Beijing, China) according to the manufacturer’s instructions. The complementary DNA (cDNA) was synthesized by using the Transcript 1st strand cDNA synthesis kit (R323-01, Vazyme, Nanjing, China). qPCR was performed by using an SYBR Green Supermix with gene-specific primers in a Bio-Rad CFX96 real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA). Rice ACTIN and Arabidopsis UFP were used as the internal control genes for normalization. The expression levels were calculated by the comparative cycle threshold method as previously reported [37].

For the floral regulator gene expression in rice, fully emerged leaf blades were sampled 45 days after germination (DAG). The expression of Hd1 was observed at ZT 14 h; other floral regulator genes were monitored at ZT 2 h. The transcript levels of OsMOS1 in Ubi:OsMOS1 transgenic rice were detected by qPCR in the 30-day leaves under LD (14 day/10 night) chamber growth conditions. The transcript levels of OsMOS1 in Ubi:OsMOS1/atmos1-6 transgenic Arabidopsis were detected by RT-PCR in the 14-day leaves under LD (14 day/10 night) growth conditions. The transcript levels of AtFLC were detected by qPCR in rosette leaves of 3-week-old plants under LD growth conditions. The primer sequences used for the PCR are listed in Supplementary Table S1.

4.5. Seed Traits Measurement

The seed size and 1000-grain weight were measured when the rice and Arabidopsis plants were completely matured. The grain length, grain width, and 1000-grain weight were measured after the grains had been harvested and treated at 37 °C for at least 5 days. For the rice grain length and width measurement, 50 grains were measured once and the measurement was repeated 3 times. For the Arabidopsis seed length and width measurement, 500 grains were measured once and the measurement was repeated 3 times. For rice 1000-grain weight measurement, 500 grains were weighed once and the measurement was repeated 3 times. For rice 1000-grain weight measurement, 500 grains were weighed once and the measurement was repeated 3 times. The observation of the outer epidermal cells of NIP and osmos1-3 mutant lemmas using a scanning electron microscope was performed as described previously [38]. The cell length and width were measured with the software Image J. The seed cell length and width were determined by averaging the values of three replicates (each replicate consisted of at least 30 cells).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/10.3390/ijms232113448/s1, Table S1: Primers used in this study. Supplementary Figure S1. Protein sequence alignment of AtMOS1 and OsMOS1. The sequence alignment of full-length MOS1 proteins from rice and Arabidopsis was carried out using the MEGA5.02 program (2 October 2022, http://www.megasoftware.net/history.php) with default parameters and then displayed using GeneDoc (2 October 2022, http://www.softpedia.com/get/Science-CAD/GeneDoc.shtml). Supplementary Figure S2. Expression profiles of AtMOS1. (a) Expression level of AtMOS1 in different Arabidopsis tissues. The data were obtained from the BAR website (15 October 2022, http://www.bar.utoronto.ca/). The development datasets corresponded to microarray data derived from organs at various stages of development under LD conditions. Error bars indicate the standard deviation of three replicates. Supplementary Figure S3. Late heading phenotypes of osmos1-4 mutants under NLD conditions. Late heading phenotypes of osmos1-4 mutants under NLD conditions. Bars = 10 cm. Supplementary Figure S4. Analyses of the grain sizes of NIP and OsMOS1-overexpressing lines. (a) Grain morphology of NIP and OsMOS1-overexpressing lines. Scale bars correspond to 1 cm. (b) Statistical analysis of grain length and grain width between NIP and OsMOS1-overexpressing lines. Error bars indicate the standard deviation, n = 50. Supplementary...
tal Figure S5. RT-PCR analyses of the expression of the OsMOS1 genes in wild-type Arabidopsis (ecotype Col-0) and OE:OsMOS1/atmos1-6 (lines 1, 2, 3, 4, and 5) plants.

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Data Availability Statement: Genes from Arabidopsis in this study can be accessed on TAIR (2 March 2019, www.arabidopsis.org) under the following accession numbers: AtMOS1 (AT4G24680), AtIIP (AT4G01000), and AtFLC (AT5G10140). Genes from rice in this study can be accessed on RGAP (2 March 2019, http://rice.uga.edu/) under the following accession numbers: OsMOS1 (LOC_Os12g37860), OsHd3a (LOC_Os06g06320), OsRFT1 (LOC_Os06g06300), OsEhd1 (LOC_Os10g32600), and OsHd1 (LOC_Os06g16370).

Conflicts of Interest: The authors have no conflict of interest to declare.

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