

**Figure S1. Generation of *osa-MIR171f*-overexpressing (OE) rice lines.**

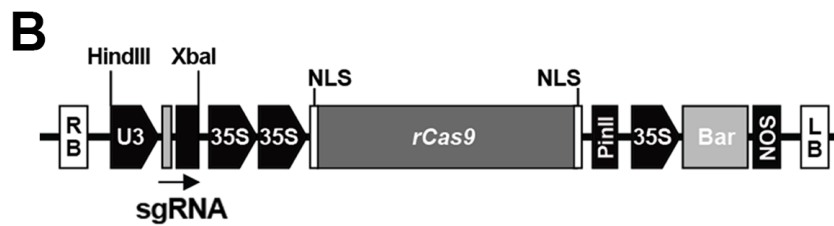
(A), Schematic visualization of the *GOS2::MIR171f* construct used to make the *osa-MIR171f*-OE rice plants. (B) *Osa*-pre-miR171f expression in non-transgenic plants (NT) or *osa-MIR171f*-OE rice. The expression was analyzed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). *OsTubA2* and *OsUbi1* were used as internal controls and relative expression levels are shown as fold values. Total RNA was prepared from 10-day-old plants. (C) Polyethylene glycol (PEG)-induced drought treatment. Plants were grown on Murashige and Skoog (MS) agar medium for 7 days and transferred into Yoshida solution for 3 weeks of growth, the seedlings were treated with 22.5% PEG-8000 (m/v) and incubated at 28°C under long-day conditions (16 light/8 dark). NT indicates non-transgenic plants and the line numbers indicate *osa-MIR171f*-OE plants. Lines 2, 3, 5 and 8 were selected for further study.

**A**

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AACCATTTCTACCTATCATTTTTCTTCTTGGGTTTGC GTT
CGCCATGTCGTTGCTGCCGGCTATTAATTCAGCTCGATG
TCTGCATATTTCCATGAACATTTTCAGCCATCTCTTGAT
CAGTTTACACTTGCGGAGCTGTTGGGCAAACCATTTATA
TGAGAGGATATGATCTGGTTGCATCCAAGCGTTTTTCTT
GGGCTGGGAGAGTCGGATGTTGGCATGGTTCAATCAAAC
CGGGCAAACTATGCACTAGCTAAGCAAGATGCAGGGAT
ATGCAGTATGGTTTTGTTTGGTCTGATTGAGCCGTGCCA
ATATCACAAGCTTGCCTGGCTTAATTACATGTAGTAGTG
TGAGGATATTGGATTCTTCCACAAGGTATGAGAATTGTC
ATGTTGTGTATTTGTGCTCCTTTATGCGTGTTATTTTAC
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ATGCAAAGCTTTTCAGAAACTATTTTGTCTGACGGTTCAA
GCTTTACAGATTTAAGTTTT

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**C**

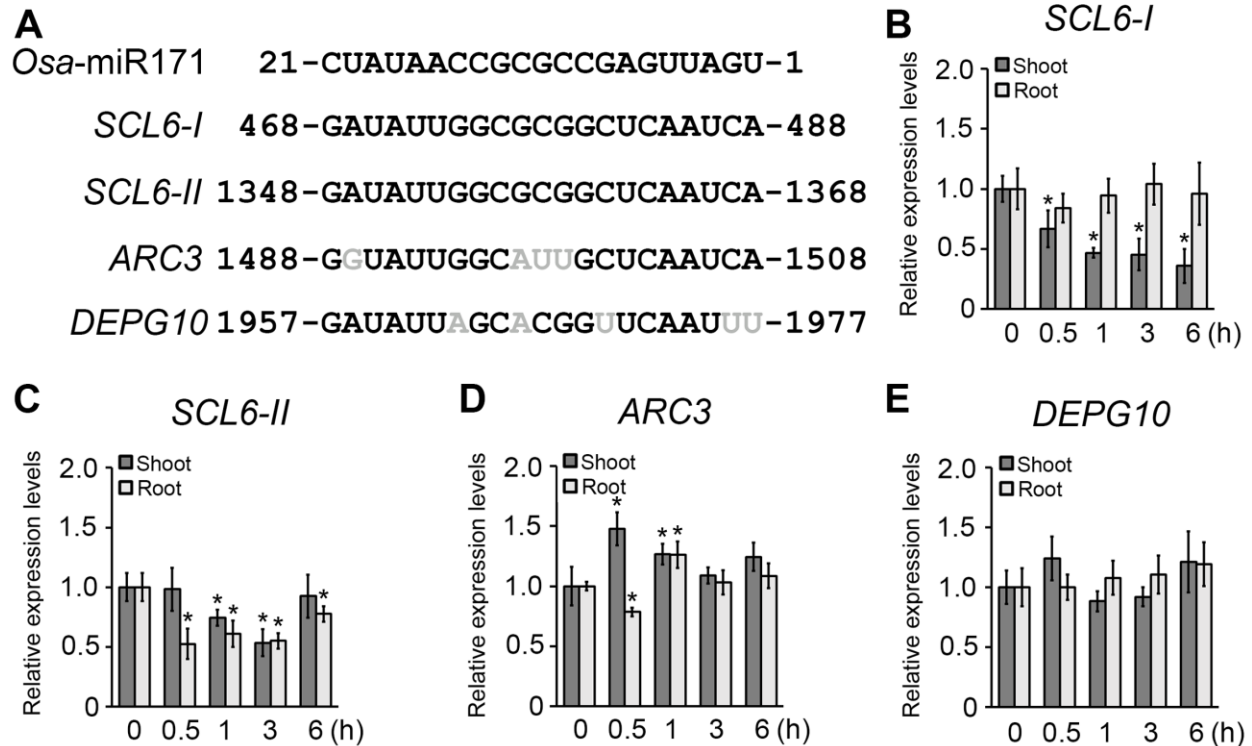
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NT : GGGAGAGTGCGATGTTGGCATGGTTCAATCAAA
Ko-2: GGGAGAGTGCGATGTTGGCATGGTTCAATCAAA
Ko-4: GGGAGAGTGCGATGTGGCATGGTTCAATCAAA
Ko-7: GGGAGAGTGCATGTTGGCATGGTTCAATCAAA
Ko-16: GGGAGAGTGCATGGCATGGTTCAATCAAA

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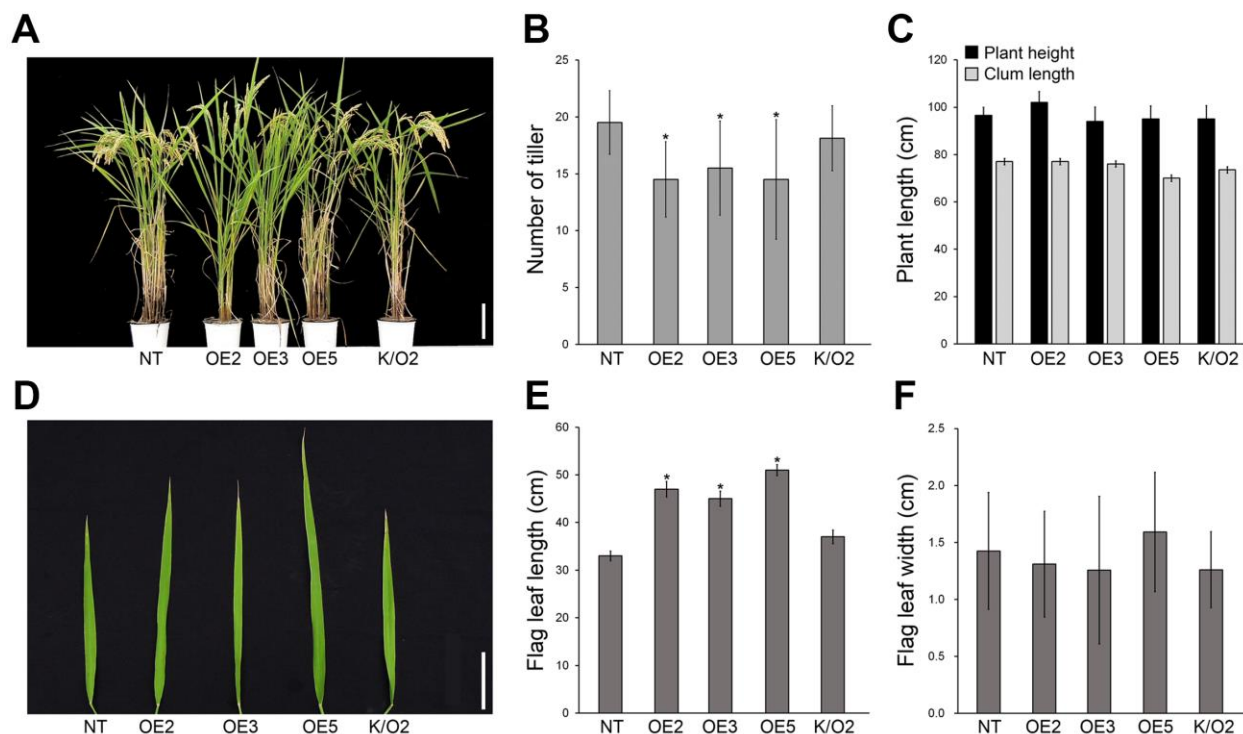
**Figure S2. Generation of *osa-miR171f*-knockout (K/O) rice using the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system.**

(A) *osa*-pri-*miR171f* sequence for primer design to create a single guide RNA (sgRNA) to be used in the CRISPR-Cas9 system. Bold and red sequences indicate *osa*-pre- and mature-*miR171f*. Underlined sequence indicates the single guide RNA (sgRNA) sequence, and the protospacer adjacent motif (PAM) is highlighted in blue. (B) Schematic representation of the recombinant CRISPR/Cas9 construct. A rice codon-optimized *Streptococcus pyogenes* Cas9 expression vector, including N-terminal and C-terminal nuclear localization signals (NLS), self-cleaving 2A peptide (P2A), and green fluorescent protein (GFP), driven by two cauliflower mosaic virus 35S (CaMV 35S) promoters. Expression of the single guide RNA (sgRNA) was driven from an U3 promoter. (C) DNA sequencing results showing that the *osa*-pre-*miR171f* is mutated in mutants. Gray sequences indicate nucleotide deletions. Red box indicates Line 2, which was selected for this study.



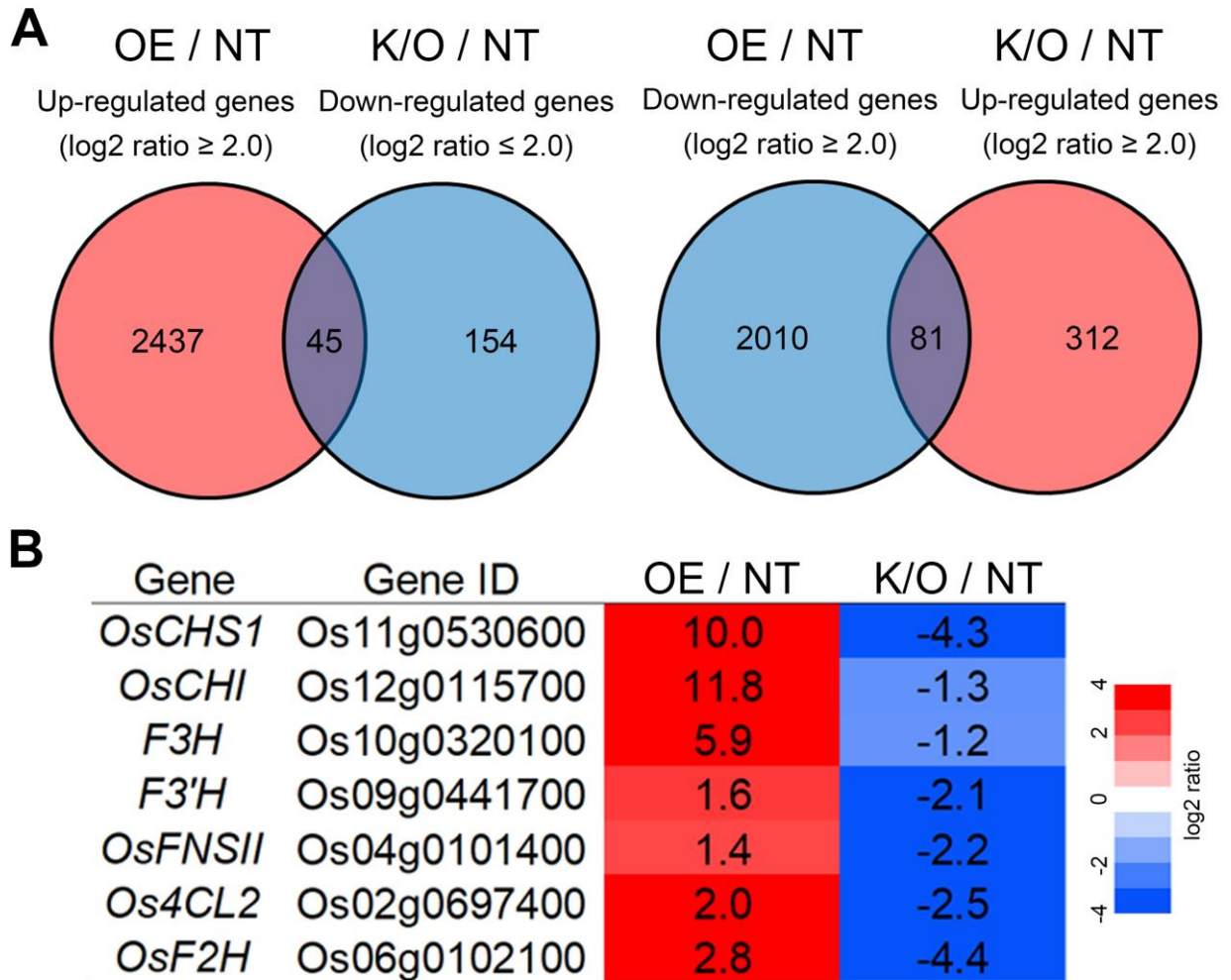
**Figure S3. Analysis of candidate *osa-miR171* targets in response to desiccation stress.**

(A) Sequence homology of *osa-miR171* and its putative target sequences in the *SCL6-I*, *SCL6-II*, *ARC3* and *DEPG10* transcripts. Gray sequences indicate mismatches between *osa-miR171* and its putative target sequences. (B-E) Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis shows changes in *SCL6-I* (B), *SCL6-II* (C), *ARC3* (D) and *DEPG10* (E) expression levels in response to desiccation stress in shoots and roots. Total RNA was prepared from 10-day-old plants exposed to stress for the indicated time periods. Control plants were not treated with desiccation stress (0hour time). Error bars indicate SD of triplicate measurements. *OsTubA2* and *OsUbi1* were used as internal controls and relative expression levels are shown as fold values. Asterisks indicate statistically significant differences between the corresponding samples and the controls ( $p$  value < 0.01, Student's *t*-test).



**Figure S4. Analysis of *osa-MIR171f*-overexpressing (OE) and -knockout (K/O) plant growth.**

(A) Morphology of 20-week-old non-transgenic (NT), *osa-MIR171f*-OE and K/O plants grown in the field. Scale bars = 15 cm. (B-E) Quantification of the number of tillers and plant height in *osa-MIR171f*-OE and *osa-mir171f*-K/O plants grown for 10 weeks (B and C) and 25 weeks (D and E). Error bars indicate SD of triplicate measurements. Asterisks indicate statistically significant differences between the samples and their corresponding control (p value < 0.01, Student's t-test).



**Figure S5. Transcriptome analysis of *osa-MIR171f*-overexpressing (OE) and -knockout (K/O) plants.**

(A) Venn diagrams of *osa-MIR171f*-regulated genes. Venn diagrams of up- and down-regulated genes in four-week-old *osa-MIR171f*-OE and -K/O plants compared to non-transgenic (NT) plants. (B) Heatmap displaying transcripts that are differentially expressed between NT and *osa-MIR171f*-OE and -K/O plants. Red and blue color represents up- and down-regulated genes, respectively.

**A**

**MI0001137(*Osa-pre-miR171f*)**

AACCAATTTCTACCTATCATTCTTCTTGGGTTTGCCTTGCATGTCGTTGC  
TGCCGGCTATAATTCAGCTCGATGTCGCATATTTCCATGAACATTTTCAGC  
CATCTCTTGATCAGTTTACACTTGGCGAGCTGTTGGGCAAACATTTATATGA  
GAGGATATGATCTGGTTGCATCCAAGCGTTTTCTTGGGCT GGGAGAGTGCGA  
TGTTGGCATGGTTCAATCAAACCGGGCAAACCTATGCACTAGCTAAGCAAGAT  
GCAGGGATATGCAGTATGGTTTTGTTGGTCT **TGATTGAGCCGTGCCAATATCA**  
CAAGCTTGCCCTGGCTTAATTACATGTAGTAGTGTGAGGATATTGGATTCTTCC  
ACAAGGTATGAGAATTGTCATGTTGTGATTTGTGCTCCTTTATGCGTGTTAT  
TTCACATGCCGCTATTAGATTTAAGTTTCTTTATGTTGTTTCATGCAAAGC  
TTTCAGAAACTATTTTGTCTGACGGTTCAAGCTTTACAGATTTAAGTTTT

**Osa-pre-miR171f for RT-qPCR :**

Forward: TGGGAGAGTGCGATGTTGGC  
Reverse: CAGGCAAGCTTGTGATATTGGC

Mature miR171f probe for Northern blot: TGATTGAGCCGTGCCAATATC

**B**

**Stem loop RT PCR**

TGTTGGCATGGTTCAATCAAACCGGGCAAACCTATGCACTAG  
CTAAGCAAGATGCAGGGATATGCAGTATGGTTTTGTTGGTCT  
**TGATTGAGCCGTGCCAATATC**GTCGTATCCAGTGC\_AGGG  
TCC  
G  
AGG  
TTATAGCAGCATAGGTCAGG TTAT

**Osa-miR171f- stem-loop RT primer:**

GTCGTATCCAGTGCAGGGTCCGAGGATTCGCACTGGATAC  
GACGATATT

Stem-loop RT Forward: TGGGAGAGTGCGATGTTGGC

Stem-loop RT Reverse: CAGGCAAGCTTGTGATATTGGC

**Figure S6. The positions of primers for RT-qPCR and Stem loop RT PCR**

(A) *osa-pre-miR171f* sequence for primer design and probe to create a RT-qPCR and northern blot to be used in the expression analysis. Red sequences and yellow color (including under line) indicate *osa*-mature-miR171f probe and *osa-pre-miR171f* RT-qPCR primers. (B) Schematic representation of the stem loop RT PCR for analysis of *osa*-mature-miR171f expression. Under line sequences, red and blue sequences indicate *osa*-miR171f stem loop RT primer, *osa*-mature-miR171f and *osa-pre-miR171f* stem loop RT PCR primers.

**Table S1. Agronomic traits of in T<sub>4</sub> generation of *osa-MIR171f* overexpressing plants and of knockout mutants grown under normal field conditions (2020).** Each parameter value represents the mean  $\pm$  SD (n = 30). OE2, OE3 OE5 and K/O2 indicate independent lines of T<sub>4</sub> generation of *osa-MIR171f* overexpressing (OE) and knockout (K/O) plants. The percentage differences (% $\Delta$ ) show increase or decrease in the parameters compared with their corresponding controls.

	Culm length (cm)	Panicle length (cm)	No.of panicles (/hill)	No.of total spikelets (/hill)	No.of spikelets (/panicle)	Filling rate (%)	Total grain weight (g)	1000 GW (g)
NT	<b>77.00 <math>\pm</math> 2.23</b>	<b>19.50 <math>\pm</math> 1.35</b>	<b>17.57 <math>\pm</math> 3.76</b>	<b>1093.21 <math>\pm</math> 298.99</b>	<b>89.25 <math>\pm</math> 17.87</b>	<b>88.80 <math>\pm</math> 5.14</b>	<b>22.35 <math>\pm</math> 6.57</b>	<b>23.06 <math>\pm</math> 1.83</b>
OE2	<b>77.20 <math>\pm</math> 3.81</b>	<b>25.40 <math>\pm</math> 1.32</b>	<b>14.20 <math>\pm</math> 2.68</b>	<b>1152.20 <math>\pm</math> 129.32</b>	<b>117.71 <math>\pm</math> 24.26</b>	<b>80.20 <math>\pm</math> 3.11</b>	<b>20.44 <math>\pm</math> 2.42</b>	<b>22.15 <math>\pm</math> 1.27</b>
% $\Delta$	0.26	30.26	-19.19	5.40	31.90	-9.68	-8.57	-3.94
<i>p-val</i>	0.47	0.61	0.02	0.64	0.01	0.68	0.48	0.27
OE3	<b>76.50 <math>\pm</math> 2.06</b>	<b>18.30 <math>\pm</math> 2.35</b>	<b>15.50 <math>\pm</math> 2.52</b>	<b>983.00 <math>\pm</math> 196.58</b>	<b>119.76 <math>\pm</math> 21.16</b>	<b>82.95 <math>\pm</math> 0.12</b>	<b>20.04 <math>\pm</math> 0.43</b>	<b>24.73 <math>\pm</math> 0.07</b>
% $\Delta$	-0.65	-6.15	-11.79	-10.08	34.19	-6.58	-10.33	7.27
<i>p-val</i>	0.52	0.08	0.04	0.42	0.01	0.12	0.43	0.07
OE5	<b>74.30 <math>\pm</math> 3.04</b>	<b>25.50 <math>\pm</math> 1.95</b>	<b>14.60 <math>\pm</math> 4.00</b>	<b>1151.50 <math>\pm</math> 194.35</b>	<b>92.52 <math>\pm</math> 17.73</b>	<b>80.54 <math>\pm</math> 5.28</b>	<b>21.19 <math>\pm</math> 4.76</b>	<b>22.67 <math>\pm</math> 1.45</b>
% $\Delta$	-3.51	30.77	-16.91	5.33	3.67	-9.30	-5.22	-1.68
<i>p-val</i>	0.78	0.72	0.04	0.62	0.71	0.52	0.64	0.62
K/O2	<b>75.50 <math>\pm</math> 1.86</b>	<b>21.50 <math>\pm</math> 3.32</b>	<b>16.67 <math>\pm</math> 2.73</b>	<b>896.83 <math>\pm</math> 195.97</b>	<b>77.39 <math>\pm</math> 9.90</b>	<b>80.17 <math>\pm</math> 2.72</b>	<b>15.81 <math>\pm</math> 3.31</b>	<b>22.08 <math>\pm</math> 1.43</b>
% $\Delta$	-1.95	10.26	-5.15	-17.96	-13.28	-9.71	-29.26	-4.22
<i>p-val</i>	0.48	0.62	0.59	0.10	0.19	< 0.01	0.01	0.21

**Table S2. Agronomic traits of in T<sub>5</sub> generation of *osa-MIR171f* overexpressing plants and T<sub>4</sub> generation of knockout mutants grown under drought conditions (2020).** Each parameter value represents the mean  $\pm$  SD (n = 30). OE2, OE3 OE5 and K/O2 indicate independent lines of T<sub>4</sub> generation of *osa-MIR171f* overexpressing (OE) and knockout (K/O) plants. The percentage differences (% $\Delta$ ) show increase or decrease in the parameters compared with their corresponding controls.

	Culm length (cm)	Panicle length (cm)	No.of panicles (/hill)	No.of total spikelets (/hill)	No.of spikelets (/panicle)	Filling rate (%)	Total grain weight (g)	1000 GW (g)
NT	<b>69.74</b> $\pm$ 8.17	<b>16.76</b> $\pm$ 2.37	<b>19.00</b> $\pm$ 5.45	<b>1335.53</b> $\pm$ 454.17	<b>72.26</b> $\pm$ 12.95	<b>40.44</b> $\pm$ 11.21	<b>11.14</b> $\pm$ 4.66	<b>21.08</b> $\pm$ 2.37
OE2	<b>69.88</b> $\pm$ 2.81	<b>16.63</b> $\pm$ 5.32	<b>16.25</b> $\pm$ 2.84	<b>937.25</b> $\pm$ 314.63	<b>57.67</b> $\pm$ 2.28	<b>64.17</b> $\pm$ 12.90	<b>11.55</b> $\pm$ 1.68	<b>20.47</b> $\pm$ 1.58
% $\Delta$	0.20	-0.83	-14.47	-29.82	-20.18	58.66	3.65	-2.90448
<i>p-val</i>	0.29	0.92	0.39	0.08	< 0.01	0.02	0.07	0.80
OE3	<b>71.50</b> $\pm$ 4.31	<b>17.13</b> $\pm$ 2.27	<b>16.75</b> $\pm$ 2.32	<b>1147.25</b> $\pm$ 514.62	<b>68.49</b> $\pm$ 5.85	<b>58.54</b> $\pm$ 8.23	<b>14.25</b> $\pm$ 3.24	<b>21.85</b> $\pm$ 1.38
% $\Delta$	2.53	2.14	-11.84	-14.09	-5.21	44.74	27.91	3.628413
<i>p-val</i>	0.63	0.78	0.48	0.41	< 0.01	0.05	0.02	0.75
OE5	<b>71.43</b> $\pm$ 4.73	<b>16.64</b> $\pm$ 3.82	<b>19.43</b> $\pm$ 1.84	<b>1311.57</b> $\pm$ 290.40	<b>67.50</b> $\pm$ 3.60	<b>49.45</b> $\pm$ 12.20	<b>14.93</b> $\pm$ 5.20	<b>22.60</b> $\pm$ 0.65
% $\Delta$	2.42	-0.72	2.25	-1.79	-6.58	22.26	34.02	7.169028
<i>p-val</i>	0.57	0.91	0.87	0.90	< 0.01	< 0.01	0.04	0.43
K/O2	<b>60.91</b> $\pm$ 4.30	<b>15.08</b> $\pm$ 4.59	<b>15.00</b> $\pm$ 2.13	<b>471.50</b> $\pm$ 270.24	<b>31.43</b> $\pm$ 8.46	<b>14.49</b> $\pm$ 8.76	<b>2.03</b> $\pm$ 1.64	<b>19.79</b> $\pm$ 9.82
% $\Delta$	-12.65	-10.02	-21.05	-64.69	-56.49	-64.17	-81.77	-6.15535
<i>p-val</i>	0.01	0.14	0.15	< 0.01	0.06	< 0.01	< 0.01	0.52



**Table S3. The sequences of oligonucleotides used in this study.**

Gene	Primer Sequence	
	Forward (5' to 3')	Reverse (5' to 3')
<b>For overexpressing transgenic plants</b>		
<i>Osa-MIR171f</i>	TCTAGAGTTTGC GTTCGCCATGTC	CCCGGGGGCAGGCATGTGAAATAACACG
<b>For RT-qPCR</b>		
Osa-pre-miR171f	TGGGAGAGTGCGATGTTGGC	CAGGCAAGCTTGTGATATTGGC
<i>ARC3</i>	CAGTTG CTA ATC GTG CTG CG	CACCATTAGCCCTCCCCTTC
<i>DEPG10</i>	AGATGCGGCTATCAACCCAG	AGGCACACCGTCAAACCTCAA
<i>SCL6-I</i>	CCA AAGCTGCTTGTGATATGTTAGC	TCACAAGGTTGGGGGCTTGC
<i>SCL6-II</i>	TGGAGCTGCACCTTACCCAG	CCAACCGAAGAATCGCTGGC
<i>OsCHS1</i>	GCCGGTGACCTGGTGAATTA	ACATGTTGGGGTTCCTCTGC
<i>OsCHI</i>	CTTGCTCCAAAGCAGAGGA	ACTGAAGACTGCACACCAAAC
<i>OsF3H</i>	GCGTCGTGGCAAAGATGAAG	GTGCTCGATGTCGTATCCGT
<i>OsF3'H</i>	AGCGACTGCATAGCGTACAT	CACAGACGTATCCTCACGGG
<i>Os4CL2</i>	GCGTGCTGACCATGTCAATG	GTCGTATTCCCCAACTGGCA
<i>OsF2H</i>	CCGTTCTTGTGCTTGCCTGCTC	GATGCTGGCCTCGAACTGA
<b>For stem-loop RT-PCR</b>		
<i>Osa-miR171f</i> -stem-loop RT primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGATATT	
<i>Osa-miR171f</i>	GCGGCGGTGTTGGCATGGTTCAATC	GTGCAGGGTCCGAGGT