

# **Mechanism of Delayed Seed Germination Caused by High Temperature During Grain Filling in Rice (*Oryza sativa* L.)**

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**Supplemental Table 1**

**Supplemental Figure 1**

**Supplemental Figure 2**

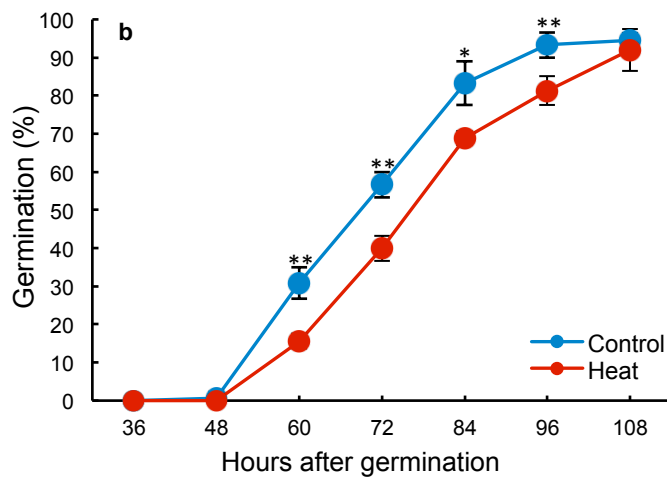
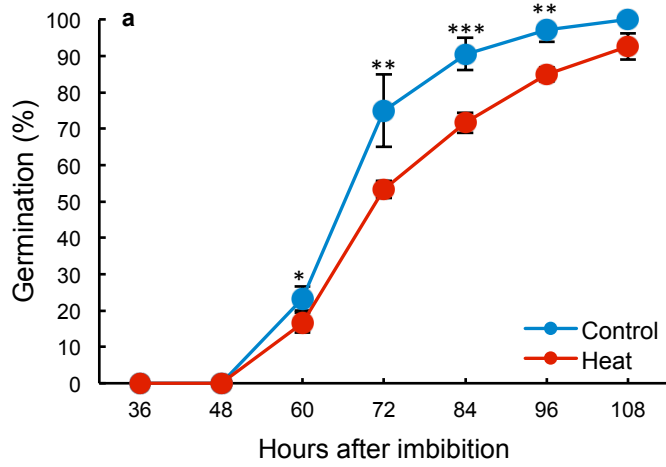
**Supplemental Figure 3**

**Supplemental Figure 4**

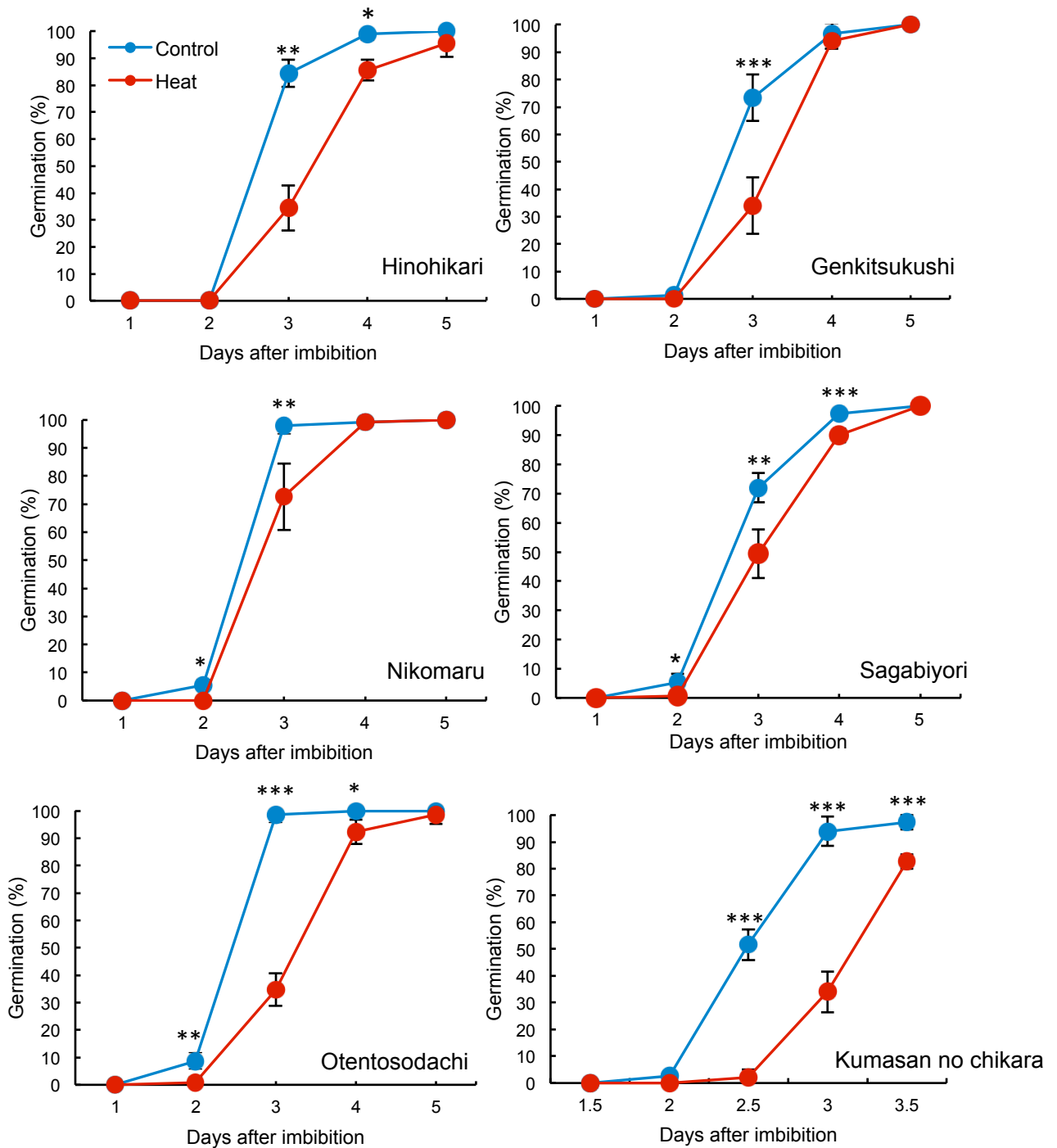
**Supplemental Table 1** Primer sequences used for qRT-PCR and Chop-qPCR

qRT-PCR primers	Forward (5'-3')	Reverse (5'-3')
<i>OsActin</i>	GACTCTGGTGATGGTGTGTCAGC	GGCTGGAAGAGGACCTCAGG
<i>OsGA3ox1</i>	GGAGAGCAAGGCCGTGTATCAGG	CTCTCCTTGTCTCTTCCTTCGCTAC
<i>OsGA20ox1</i>	CCACTACTTCCGGCGATTCTTCCAGCG	GACGTGGTCTCTGGTGGAGGATGGTG
<i>OsGA20ox2</i>	CGCCGACTACTTCTCCAGCACC	GCTGTCCGCGAAGAACTCCCT
<i>OsNCED1</i>	CTGGAGCACATGGAGCTAGTGCACTCC	CCGACGCCGAAGTAGCCGTACCTG
<i>OsNCED2</i>	TCATTCCAAAACACCTTCCA	TCCGGGGACCTCCTATGTAT
<i>OsNCED3</i>	CCCCTCCCAAACCATCCAAACCGA	TGTGAGCATATCCTGGCGTCGTGA
<i>OsNCED4</i>	TCCATCTCCTTCTCCCTCCTCCC	CCTCGCACCTGCTTGATCTTGCC
<i>OsNCED5</i>	CACATCCGAGCTCCTCGTCGTGAAC	GAATCAAGAATCCATGGATGGCCGGTG
<i>OsABA8'OH1</i>	AAGTACAGGTGGTCCACGTCCAA	CCAGCTTAGCTGATGCTAGTATTC
<i>OsABA8'OH2</i>	GACGAGGTGGAGTACAGCCCCTTC	GGACACATCAGCCACCATCAGCAGTAG
<i>OsABA8'OH3</i>	AGTACAGCCCATTCCCTGTG	ACGCCTAATCAAACCATTGC
<i>OsGAMYB</i>	CAGTGGCAATTCATTCACTGAATC	TCCAGATCCATTGAAGTGCTTTG
<i>OsAmy1A</i>	GATACGACGTGCAACACCTC	CGGATCGGATACAGCTCGTTG
<i>OsAmy1C</i>	TATCATGGAGGCTGACAGCG	GCTAATTGTGCCTCTCCACC
<i>OsAmy3B</i>	GATTGGGACACGGTATGACG	CTGCAGGAACCTCTGAGACCG
<i>OsAmy3E</i>	GAGAAGGAAGGCCTCAGGGTTCCTG	GAAGCTCGCTCGTACACATCTCGCAG
<i>OsDOG1L1</i>	GAGTCGTTGGCGACGGTGAAG	ATGAGGAAGTGACCGGCCTG
<i>OsDOG1L2</i>	TCTTAGTGAGTGGTGTGCTGGGA	GTGAATCATCAGCTTTCACAGC
<i>OsDOG1L3</i>	CAGTAGCAATAGTACCTCATAAT	AGCGAATCATGATTGTGACCTG
<i>OsDOG1L5-1</i>	GACAATCCGACAAGCTGCC	GTTGTTACTCCCGTGGCCTA
<i>OsDOG1L5-3</i>	TCCTGAGAGAGCGTAACTGCG	TACAGGCCCAAGAGCCCAATCTA

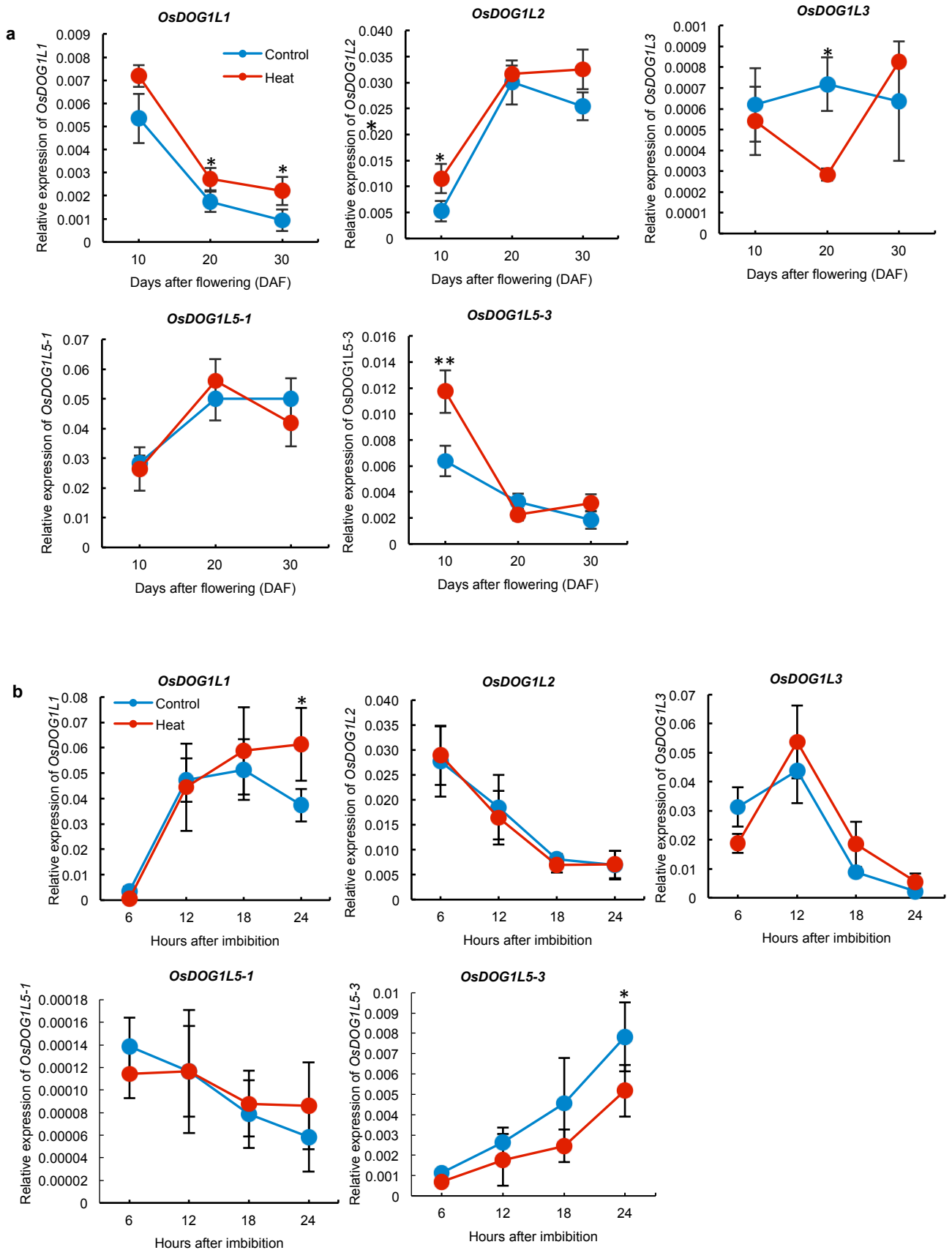
Chop-qPCR primers	Forward (5'-3')	Reverse (5'-3')
<i>OsABA8'OH1</i> : proP1	GAGCTGCATGCGCACGACCATTTC	CGGTATCACTGACCGCGGCTAGC
<i>OsABA8'OH1</i> : proP2	AGTACGTAGTACGTACGAGAGCGC	TACAGTGATAGCTAAATGGGCCTC
<i>OsABA8'OH1</i> : proP3	ATTGATTGCGCAATTATTCGGCAG	TACTAGTAGCTAGCACCACCACTA
<i>OsABA8'OH1</i> : proP4	GTGCTCGCGCCTTCTTGCTTGT	GGTCTTGACGAGTACAGCTGGA
<i>OsABA8'OH3</i> : proP1	CCTAGCTCGCGACACCATATGAG	GATCTTTCGAATGGTGGTGGCGT
<i>OsNCED5</i> : proP1	TGTCTCCTTCTCCCCAAACTC	AGGAGAGGTGAGAGCTGGAGTTGG
<i>OsAmy1C</i> : proP1	GTACATGAACAGAGGTGCAATGC	AACAGATTTTGCTAGTCGGTAGTC
<i>OsAmy3B</i> : proP1	CGCTGGATGCGGTGCTCGTTGAAG	GCACCGCCGAACCTGACGACCAGC
<i>OsAmy3B</i> : proP2	GCTGCATAGCACGACGCATACATA	ACTTGCCCAATGGATGGATCGTTG
<i>OsAmy3E</i> : proP1	GCGCTTGATCCGTACACCCGATGA	ACGCATGACGGAAACGGCGATACG



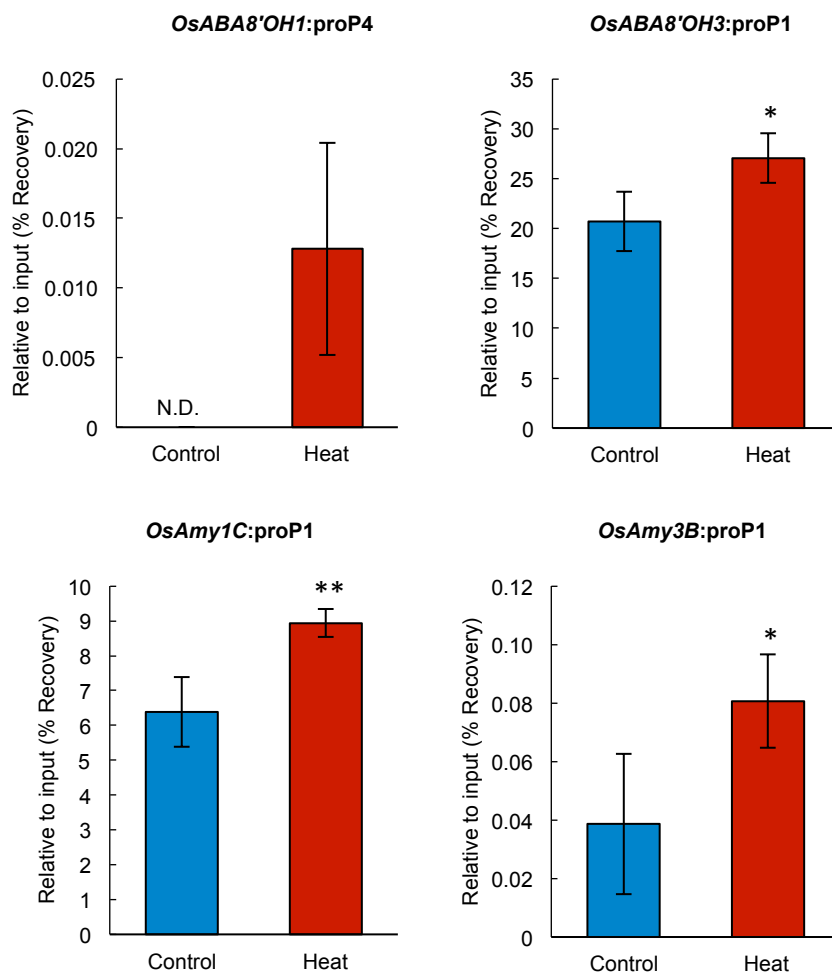
**Supplemental Figure 1.** Delay in germination caused by heat stress during grain filling in **a**, 2016 ( $n = 4$ ) and **b**, 2017 ( $n = 3$ ) with 30 seeds per replicate. Seeds were germinated at 27°C in the dark. Significant differences are shown as  $P < 0.05^*$ ,  $P < 0.01^{**}$ , and  $P < 0.001^{***}$  according to Student's  $t$ -test. Error bars, SD.



**Supplemental Figure 2.** Delay in germination of heat-stressed seeds of the heat stress-sensitive rice cultivar 'Hinohikari' and heat stress-tolerant cultivars 'Genkitsukushi', 'Nikomaru', 'Sagabiyori', 'Otentosodachi', and 'Kumsan no chikara'. Harvested seeds were obtained from Tanamachi et al. (2016), who used exactly the same cultivation methods as in this study. Seeds were germinated at 27°C in the dark. Significant differences are shown as  $P < 0.05^*$ ,  $P < 0.01^{**}$ , and  $P < 0.001^{***}$  according to Student's  $t$ -test ( $n = 3$ ). Error bars, SD.



**Supplemental Figure 3. Expression of DOG1-like genes in control and heat.** **a.** Relative gene expression of *DOG1-like* genes in developing seeds under control (25°C) and heat (30°C) conditions. **b.** Relative gene expression of *DOG1-like* genes in embryos during imbibition. Significant differences are shown as  $P < 0.05^*$ ,  $P < 0.01^{**}$ , and  $P < 0.001^{***}$  according to Student's *t*-test ( $n = 3$ ). Error bars, SD.



**Supplemental Figure 4.** DNA methylation levels of *OsABA8'OH1*, *OsABA8'OH3*, *OsAmy1C* and *OsAmy3B* promoters. Methylated DNA was precipitated and analyzed via MeDIP-qPCR. Significant differences are shown as  $P < 0.05^*$  and  $P < 0.01^{**}$  according to Student's *t*-test ( $n = 3$ ). Error bars, SD.