Suppression of OsMADS7 in rice endosperm stabilizes amylose content under high temperature stress

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Summary

High temperature significantly alters the amylose content of rice, resulting in mature grains with poor eating quality. However, only few genes and/or quantitative trait loci involved in this process have been isolated and the molecular mechanisms of this effect remain unclear. Here, we describe a floral organ identity gene, OsMADS7, involved in stabilizing rice amylose content at high temperature. OsMADS7 is greatly induced by high temperature at the early filling stage. Constitutive suppression of OsMADS7 stabilizes amylose content under high temperature stress but results in low spikelet fertility. However, rice plants with both stable amylose content at high temperature and normal spikelet fertility can be obtained by specifically suppressing OsMADS7 in endosperm. GBSSI is the major enzyme responsible for amylose biosynthesis. A low filling rate and high expression of GBSSI were detected in OsMADS7 RNAi plants at high temperature, which may be correlated with stabilized amylose content in these transgenic seeds under high temperature. Thus, specific suppression of OsMADS7 in endosperm could improve the stability of rice amylose content at high temperature, and such transgenic materials may be a valuable genetic resource for breeding rice with elite thermal resilience.

Introduction

Global mean surface temperatures continue to increase in this century (IPCC, 2007), resulting in many negative impacts on crop production (Lesk et al., 2016). Rice, one of the most important cereal crops feeding to more than half of the global population, is relatively sensitive to HT (high temperature). HT stress during the grain filling stage causes not only low yield but also poor quality in rice (Peng et al., 2004; Yamakawa et al., 2007). When the filling temperature is higher than 28 °C, a dramatic decrease in AC (Amylose Content) and increase in chalky appearance occur in some Japonica cultivars (Lyman et al., 2013; Sreenivasulu et al., 2015; Yamakawa et al., 2007). Abnormal starch granules were observed in the endosperm, suggesting that starch metabolism is greatly impaired under HT (Yamakawa et al., 2007; Zhang et al., 2016).

Accordingly, gene expression profiles reveal significant changes in starch metabolism pathways at HT. Most of the starch biosynthesis genes, such as GBSSI (granule-bound starch synthase I), SBE (starch branching enzyme) and cyPPDK (cytosolic pyruvate orthophosphate dikinase), are down-regulated, while some starch hydrolysing genes, such as α-amylases (Amy1A, Amy1C, Amy3A, Amy3D and Amy3E), are induced under HT in developing seeds (Hakata et al., 2012; Liao et al., 2015; Wang et al., 2015; Yamakawa et al., 2015). It has been suggested that the alteration of expression in these starch metabolism-related genes in rice might be the cause of poor grain quality under high filling temperatures (Cheng et al., 2005; Yamakawa et al., 2007). Overexpression of Amy1A or Amy3D results in more chalky grains even under normal growth conditions, while suppression of some α-amylase genes in the endosperm could improve rice quality by reducing grain chalkiness at HT (Hakata et al., 2012). Thus, down-regulation of α-amylase expression could be a potential strategy for ameliorating grain chalkiness caused by HT at the grain filling stage. However, suppressed expression of α-amylase genes had no effect on AC in rice seeds (Hakata et al., 2012).

AC, as an important physicochemical property for rice eating and cooking quality, is mainly determined by GBSSI (Liu et al., 2014; Sano, 1984; Wang et al., 1995). Previous data showed that the Wx gene encoding GBSSI was down-regulated at HT, which led to a considerable reduction in AC (Lin et al., 2005; Yamakawa et al., 2007; Zhang et al., 2014). In addition, SBE also plays important roles in amylose biosynthesis (Zhu et al., 2012). Overexpression of Wx and down-regulation of SBE both result in very high AC in rice seeds, while suppression of GBSSI expression leads to extremely low AC (Liu et al., 2005; Tian et al., 2009; Zhu et al., 2012). As modest AC (15%–25%) is a significant index for high-quality rice, simply overexpressing GBSSI or suppressing SBE may not be suitable for rice breeding. Therefore, fine-tuning of starch metabolic genes might be an alternative strategy for high-quality rice breeding in a globally warming climate.

Expression of the Wx gene can be regulated at multiple levels. Several transcription factors involved in Wx expression have been identified. OsBP-5 and OsEBP-89 were found to synergistically regulate the transcription of the Wx gene. Disturbing OsBP-5 expression by RNAi resulted in a mild reduction in AC in transgenic seeds (Zhu et al., 2003). OsZIP58 is another endosperm-specific transcription factor involved in the regulation of both the Wx and SBEI genes. A null mutant oszip58 showed decreased AC and altered seed morphology (Wang et al., 2013). Furthermore, expression of the Wx gene is finely controlled at the
post-transcriptional level. Alteration of Wx splicing efficiency by a SNP at the splicing site (Cai et al., 1998) or mutations in the splicesome (Isshiki et al., 2006; Zeng et al., 2007) could affect its mRNA abundance, which in turn results in reduced AC in their seeds. Our recent data showed that four major quantitative trait loci (QTLs) from Indica cultivar 9311 could reduce the deleterious effects of high temperature on the AC of the Japonica cultivar Nipponbare by increasing the splicing efficiency of Wx pre-mRNA (Zhang et al., 2014). However, no genes have yet been reported to maintain a stable AC at HT during the ripening stage.

The MADS-box genes belong to a large transcription factor family including 75 members in the rice genome (Arora et al., 2007). Most of them were found to be essential for floral organ identity (Li et al., 2011; Liu et al., 2013). Some play critical roles in rice endosperm development. For instance, OsMADS29 was presumed to be a key regulator of rice seed development (Nayar et al., 2014; Yang et al., 2012; Yin and Xue, 2012). OsMADS7 was found to be involved in endosperm cellularization and the regulation of seed size (Chen et al., 2016). Moreover, some MADS-box members also play important roles in stress responses. OsMADS26 negatively controls plant resistance to pathogens and drought stress (Khong et al., 2015). A set of type I MADS-box genes including OsMADS87 show responses to heat stress during the early stage of endosperm development (Chen et al., 2016).

OsMADS7 (LOC_Os08 g41950), an important transcription factor regulating floral identity, is highly expressed in rice reproductive organs, such as spikelet meristems, developing lodicules, stamens and pistils (Cui et al., 2010). Based on gene expression data (http://rice.plantbiology.msu.edu), OsMADS7 is also expressed abundantly at the early and middle milk stages (Kawahara et al., 2013). However, the biological function of OsMADS7 in seed development remains unknown. Here, we found that OsMADS7 can be highly induced by HT in rice endosperm. Plants with suppression of OsMADS7 exhibited less reduction in AC than WT under HT, suggesting a potential role of OsMADS7 in heat stress response in rice seeds. Specific down-regulation of OsMADS7 in endosperm which avoiding the reduction in fertility could be a potential strategy for improving the thermal resilience of rice for better quality and stable yield.

Results

Expression of OsMADS7 in rice endosperm is induced by HT

To isolate more regulators involved in seed quality control at HT, the transcription factor highly expressed in rice endosperm (Fu and Xue, 2010) was selected and subjected to expression analysis under long-term HT treatment at milk stage as described before (Xu et al., 2015). OsMADS7 is one of the HT responsive transcription factors screened out from above assay. According to a public database (RGAP, www.rice.plantbiology.msu.edu), published data (Kawahara et al., 2013) and our RNA-seq data (Xu et al., 2015), OsMADS7 is abundantly expressed in floral organs (inflorescence, anther and pistil), seed and endosperm. To further verify the expression pattern of OsMADS7, different vegetative tissues and reproductive tissues were analyzed by quantitative RT-PCR assay. As expected, OsMADS7 showed the highest expression in panicles and intermediate expression in endosperm (Figure 1a). To further examine whether OsMADS7 expression could be altered by HT stress at the milk stage, Nipponbare rice plants (Oryza sativa L. Japonica) were either subjected to HT treatment (day/night temperature of 35 °C/28 °C) or were maintained at RT (room temperature, day/night temperature of 28 °C/22 °C) at three DAP (day after pollination). Developing endosperm from different growth temperatures were collected at 6, 9, 12, 15, 18, 21, 24 and 30 DAP. As shown in Figure 1b, OsMADS7 was expressed mainly in early development stages in rice endosperm (6, 9, 12 and 15 DAP), and its expression level increased more than sixfold at HT.

Seed quality assay

To test the biological function of OsMADS7 in endosperm development, especially under the HT condition, independent constitutive RNAi lines of OsMADS7 were obtained from the Chinese Academy of Sciences, Beijing (Cui et al., 2010). Three lines with constitutive suppression of OsMADS7 were selected in this study (Figure 2a) and named M714, M721 and M734. After HT treatment, mature seeds from these lines and their WT counterpart (ZH11, O. sativa L. Japonica) were harvested for quality measurements (Figure 2b).

Figure 1 OsMADS7 is expressed intermittently in rice endosperm and induced by HT at the milk stage. Transcripts were measured using qRT-PCR and normalized to the level of the control sample (6 DAP endosperm) using UBQ10 as internal control. (a) Expression pattern of OsMADS7 in different tissues. Endosperm (g) indicates endosperm at the seed germination stage; endosperms (6 and 9) indicate developing endosperm at 6 and 9 DAP at the filling stage; panicle (b and a) indicate panicles before and after flowering, respectively. (b) Expression of OsMADS7 in developing endosperm is induced by HT. Data represent means ± SE, n – 3 biological replicates, 6–10 developing seeds in each replicate. DAP: day after pollination. H and R indicate seeds harvested from HT and RT growth conditions, respectively.
The AC from WT and OsMADS7 RNAi lines decreased significantly at HT (Figure 2c). However, the AC in RNAi seeds was slightly higher than in the WT seeds at HT, especially in M721 and M734 seeds (Figure 2c). As we mentioned in Zhang et al. (2014) and in the Materials and methods, the DD-value was used to measure AC stability at HT. A greater DD-value indicates higher stability. As shown in Figure 2d, DD-values of all three RNAi transgenic seeds were positive, suggesting that the AC of transgenic seeds is more stable than that of WT under HT. Thus, suppression of OsMADS7 expression could ameliorate the reduction in AC caused by HT.

Starch GT (gelatinization temperature) is another important physicochemical index of rice quality. To determine whether OsMADS7 has any effect on GT under HT, we conducted a DSC (differential scanning calorimetry) analysis between the M734 line (the line with highest DD-value) and WT (Figure 2c). Consistent with a previous report (Zhang et al., 2016), the Tp (peak transition temperature, Figure 2e) and ΔH (gelatinization enthalpies, Figure 2f) of rice starch were increased under HT, which suggests that the GT of rice seeds grown at HT is elevated. ΔH primarily reflects the loss of double helical order and its value is mainly determined by proportion of amylose double helices and AC value (Cooke and Gidley, 1992; Matveev et al., 2001). Increase in GT and ΔH, together with the reduction in AC, implies that the structure of amyllopectin might be changed and the proportion of amylose double helices should increase at HT. The significantly higher AC (P < 0.001, Figure 2c) and similar or slightly higher ΔH value (P = 0.26, not significant, Figure 2f) of M734 than that of WT suggested amylose double helices in M734 might be higher than that of WT.

Moreover, GT is mostly affected by amylose starch, amylose double helices and the B-type crystalline form of amyllopectin (Richardson et al., 2000; Shi et al., 1998). M734 got significantly lower Tp (P < 0.01, Figure 2e) but higher AC than WT at HT suggesting M734 is different from WT in terms of amyllopectin structure and the proportion of B-type crystalline form of amyllopectin in M734 might be lower than that of WT.
Grain filling dynamics

To better understand the change in rice quality caused by the alteration of OsMADS7 expression, a dynamic study of grain filling rate was carried out in the M734 and WT lines at different temperatures. As shown in Figure 3a, both genotypes had a higher grain filling rate at HT than at RT during the early filling stage (7–12 DAP), which might be the cause of impaired rice quality under HT. However, the difference in grain filling rate between HT and RT is smaller in M734 than that in WT, especially during the early filling stage (6–10 DAP) (Figure 3a); this might be the reason for relatively stable AC in M734 under HT.

As many starch synthase genes may be modulated by HT (Yamakawa et al., 2007), a dynamic expression analysis of starch biosynthesis genes was carried out during the filling stage. Most of the genes tested, such as GBSSI (Wx), SBEI, AGP2b, AGP2L and AGP3L (Figure S1), were suppressed by HT during the entire filling process. GBSSI (Figure 3d) is the only gene whose expression was slightly induced by HT. When considering genetic background, GBSSI (Wx), AGP2L and AGP3L showed different expression patterns between the M734 RNAi line and WT, while SBEI, SBEIIb, SSSI and AGP2b showed similar expression patterns between them. Although both AGP2L and AGP3L showed lower expression levels in M734 at RT than those of the WT, AGP3L showed higher level at HT than those of the WT, while AGP2L showed no big difference with WT (Figure S1). Moreover, the expression intensity of GBSSI (Wx) was higher in M734 than in WT at peak point (12 or 13 DAP) under both temperature conditions (Figure 3b).

Post-transcriptional regulation is one of the most important mechanisms for modulation of Wx expression (Cai et al., 1998). As we mentioned in Zhang et al. (2014), the splicing efficiency of the first intron of Wx is represented by the ratio of Wx10 (mature Wx transcript) to Wx2 (total Wx transcript). qRT-PCR results revealed that both the splicing efficiency of the first intron and the mature transcript products of Wx gene were higher in M734 than in WT under HT during almost the entire filling stage (Figure 3e, f), which might result in high AC and partially explain the stability of AC in OsMADS7 RNAi lines under HT. Therefore, our results suggest that suppression of OsMADS7 causing improved AC stability under HT might be mediated through regulation of Wx expression.

Suppression of OsMADS7 expression affects rice spikelet fertility

OsMADS7 is an important transcription factor involved in flower development. Constitutively knocking down of this gene resulted in low spikelet fertility in rice (Cui et al., 2010). To test the effect of high temperature on this process, plants were moved into chambers at the booting stage. Consistent with a previous report (Cui et al., 2010), all three OsMADS7 RNAi lines (M714, M721 and M734) displayed lower spikelet fertility (56%–60%) than that of WT (76%) at the control temperature. High temperature showed an additive effect. Under HT, the fertility of the OsMADS7 RNAi lines was further decreased to 13.8%–34.9%, while it was decreased only 42.7% in WT (Figure 4a). These data indicate that constitutive down-regulation of OsMADS7 expression also results in low fertility. Additionally, we found that HT...
affected seed width (Figure 4b) and length (Figure 4c) slightly and reduced the seed weight significantly \((P < 0.001, \text{Figure } 4d)\). However, no significant difference in seed size was found between RNAi lines and WT (Figure 4b–d). All results indicated that constitutive suppression of OsMADS7 led to reduced spikelet fertility under HT, which is not suitable for breeding plants with better quality.

**Endosperm-specific down-regulation of OsMADS7 increases the stability of rice AC at HT**

Low spikelet fertility in OsMADS7 RNAi lines may be due to the down-regulation of OsMADS7 in floral organs. To avoid this effect, we generated a set of endosperm-specific RNAi lines by using a rice endosperm-specific promoter from GluC (LOC_Os02g25860) (Qu et al., 2008).

Nineteen independent T\(_0\) RNAi lines (M7130–7148) were generated and the expression levels of OsMADS7 in the developing seeds at 9 DAP were examined. No obvious developmental deficiency was observed in these lines. Five transgenic lines (M7134, 7138, 7140, 7142 and 7145) with more than 17-fold down-regulation of OsMADS7 (less than 6% of WT, Figure 5a) in rice endosperm were selected, and their T\(_2\) plants were used for HT treatment. Mature seeds from these RNAi lines and a WT control (Nipponbare, O. sativa L. Japonica) were collected for AC measurement. The results showed that all the transgenic seeds had a similar AC to WT at RT. However, the AC from WT seeds was decreased approximately 35.6% at HT, while the reduction in AC in the RNAi seeds was much smaller (16.2%, 26.6%, 19.5%, 13.7% and 14.6%, Figure 5b). Additionally, one line (M7134) showed no significant difference in AC between HT and RT \((P = 0.06, \text{Figure } 5b)\). The DD-values of these RNAi lines ranged from 14% to 36% (Figure 5c). Four lines (M7134, 7140, 7142 and 7145) had DD-values above 30%, higher than any line with a constitutive RNAi construct (Figure 2b). These data suggested that endosperm-specific down-regulation of OsMADS7 was more tolerant to HT in terms of AC stability.

Spikelet fertility of endosperm-specific RNAi lines was further examined at HT. Compared to WT (76.9 ± 4.4%), lines M7134, 7138, 7140 and 7145 showed low fertility (from 30% to 60%), while the M7142 line (69.1 ± 6.7%) displayed only a slight decrease \((P = 0.31, \text{Figure } 5d)\). Interestingly, the M7142 line also had the highest DD-value (35.5 ± 1.1%) among the five transgenic lines (Figure 5c). Similar results were obtained from the T\(_3\) generation (data not shown). Therefore, genetic materials that produce better rice quality at HT without significant fertility problems can be obtained by knocking down OsMADS7 in an endosperm-specific manner.

Thus, specific suppression of OsMADS7 in rice endosperm is a potential strategy to increase the stability of rice AC at HT. The transgenic line M7142 might be a good genetic resource for thermal resilience rice breeding.

**Discussion**

HT during the filling stage may greatly impair rice seed quality, especially in Japonica varieties that are more sensitive to HT (Yamakawa et al., 2007; Zhang et al., 2016). The deleterious effects manifest mainly in two aspects: increase in chalky appearance and decrease in AC (Hakata et al., 2012; Larkin and Park, 1999). Previous studies showed that suppression of \(\alpha\)-amylose genes reduces the amount of chalky grains under HT stress (Hakata et al., 2012). Thus, it might be a potential strategy to improve rice quality under global warming conditions. However, no genes or strategies have been reported to stabilize AC at HT without deleterious side effects. This study identified a transcription factor, OsMADS7 involving in AC regulation at HT.

![Figure 4](image-url)
OsMADS7 belongs to the class E of floral organ identity genes and is highly expressed in floral organs (Cui et al., 2010). Constitutive silencing of OsMADS7 resulted in weak developmental deficiency including one carpel with three stigmas and low spikelet fertility. In this study, we confirmed that OsMADS7 is moderately expressed in rice endosperm and found that it can be highly induced by HT stress at the early grain filling stage. Furthermore, RNAi-mediated suppression of OsMADS7 produced a relatively stabilized rice AC under HT stress, suggesting that OsMADS7 may also play an important role in grain filling. In addition to impaired rice quality, HT during the reproductive stage can particularly affect rice yield. In this study, we found that the spikelet fertility of *Oryza sativa* L. cv. *Japonica* cultivars ZH11 and Nipponbare was significantly decreased under HT stress. ZH11 (42.7%, Figure 4a) seems to be more sensitive to HT than Nipponbare (76.9%, Figure 5d) in terms of spikelet fertility. Moreover, we also evaluated the effect of HT on rice seed size and weight. Seed weight was significantly reduced under HT, while the seed length and width did not change significantly (Figure 4b–d). The results further suggest that HT produces a negative effect on storage material accumulation during seed filling. Consistent with previous reports (Yamakawa et al., 2007; Zhang et al., 2016), lower AC, higher GT and more chalky grains were found in rice seeds grown at HT than at RT conditions in this study (Figure 2b–f). Dynamic analysis (Figure 3a) further revealed that the grain filling rate of rice seed was higher under HT than that under RT at early stages (6–12 DAP). The fresh weight of WT seeds peaked much earlier at HT (17 DAP) than at RT (≥21 DAP), while that of the RNAi seeds (M734) peaked at 21 DAP under both temperature conditions (Figure 3a). These results indicated that RNAi seeds had higher grain filling rate than WT at late milky stage under HT, which may also explain the stabilized AC in RNAi lines at HT.

As starch is the main content in rice seeds, expression patterns of many starch biosynthesis genes under different temperatures were examined in a dynamic manner. Except for GBSSI, most of the starch biosynthesis genes were down-regulated under HT. As in Hakata’s reports (Hakata et al., 2012), starch hydrolysing enzyme was greatly up-regulated at HT. We deduced that the high filling rate and the alteration of gene profiles involved in starch metabolism might be the major causes of poor rice quality under HT.

It is well documented that the reduction in rice AC might be attributed to the down-regulation of *GBSSI* (*Wx*) in developing rice seeds. Previous studies also showed that HT can modulate *Wx* expression at both transcriptional and post-transcriptional levels (Larkin and Park, 1999; Zhang et al., 2014). To determine the possible mechanism of OsMADS7 in AC regulation at HT, dynamic analyses of starch biosynthesis gene expression were performed in the *OsMADS7* RNAi line M734 and its WT counterpart ZH11. Both the total *Wx* transcript and the mature *Wx* transcript were higher in M734 than in ZH11 at HT (Figure 3b, e, f), suggesting that OsMADS7 may negatively regulate *Wx* expression at both transcriptional and post-transcriptional levels. The stable AC in OsMADS7 RNAi seeds at HT might be due to the high expression of the *Wx* gene. How OsMADS7 participates in *Wx* gene regulation needs to be clarified in the future.

Consistent with Cui’s observation (Cui et al., 2010), constitutive suppression of OsMADS7 resulted in weak phenotypes including low fertility (~60%, Figure 4a). When plants are grown at HT, the spikelet fertility of the OsMADS7 RNAi line is decreased (<35%, Figure 4a). Thus, this kind of transgenic material may not be suitable for rice breeding, although it may improve rice quality at HT. As OsMADS7 plays an important role in floral organ development, we speculated that the low spikelet fertility might be due to down-regulation of OsMADS7 in floral organs.

![Figure 5](image-url)
Therefore, using an endosperm-specific promoter to drive the suppression might be the best alternative. Indeed, OsMADS7 was down-regulated mainly in rice endosperm and very slightly in floral organs (Figure S2), using the new strategy. Although some transgenic lines also exhibited low spikelet fertility, others, such as M7142, were found to have the same spikelet fertility as the WT (Nipponbare) at HT (Figure Sd). OsMADS8, showing functional redundancy with OsMADS7 (Cui et al., 2010), was up-regulated in these transgenic lines (Figure S3), which may partially compensate the deficiency of spikelet fertility. It will be interesting to further check the insertion site of the RNAi construct or the possibility of the expression of other OsMADS7 homologs altered in these RNAi lines to elucidate the different fertility levels between M7142 and other RNAi lines. Moreover, the DD-values from these endosperm-specific RNAi lines (14%–36%, Figure 5) were higher than that of constitutive RNAi lines (4.4%–8.7%, Figure 2), suggesting that endosperm-specific suppression of OsMADS7 might be more tolerant of HT in terms of AC stability. Thus, OsMADS7 is a valuable candidate gene for maintaining a stable AC in rice at HT. Specific down-regulation of OsMADS7 in rice endosperm should be an effective strategy to breed HT-tolerant rice with high eating quality in the future.

**Experimental procedures**

**Plant materials and growth conditions**

Rice varieties Nipponbare and ZH11 (O. sativa L. japonica) were used in this study. Rice plants were grown in the experimental fields of Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang Province. At the initial heading stage, rice plants were transplanted into pots and then transferred to growth chambers for HT or control treatment. The HT condition in this study was 35 °C, 12-h light/28 °C 12-h dark; the control condition was set as 28 °C, 12-h light/22 °C 12-h dark.

**Phenotyping of rice seed**

Spikelet fertility, the ratio of filled grains versus total spikelets, was calculated from five panicles. Morphology of the rice seed (n ≥ 300), such as length and width, was measured using the SC-I test system (Wseen, China).

**Expression analysis of OsMADS7**

More than 100 dehulled rice seeds with no disease spots were selected and incubated in distilled water in a chamber (28 °C, 12-h light/22 °C 12-h dark). 5 days after soaking, endosperm (g) and shoots were dissected. Endosperm (g) from 6 to 8 germinated seeds and shoots from approximately 20 germinated seeds were selected in each replicate, respectively. Seedling, root and stem were harvested at three-leaf stage, and three plants were used in each replicate. Panicles before/after flowering were harvested from three rice plants. More than 10 developing rice seeds in each replicate were harvested at various DAPs, as the rice caryopse was marked on the initiation of pollination. Total RNAs were extracted from above tissues using TRIzol reagent (Invitrogen, USA) and reverse transcribed into cDNA with the GoScript™ Reverse Transcription System (Promega, USA) as previously described (Zhang et al., 2014). For RT-PCR analysis, the rice actin (She et al., 2010) gene was used as an internal control and gene-specific primers MADS7C2-F1&R1 were used to analyze the expression of OsMADS7. qRT-PCR assays were performed with iQ™ SYBR® Green Supermix (Bio-Rad, USA) on a CFX96™ Real-Time System (Bio-Rad) with UBQ10 (Jain et al., 2006) as the reference gene. Primer pairs MADS7C2-F5&R5 and MADS8-F3&R3 were used to specifically identify transcripts of OsMADS7 and OsMADS8, respectively, in the qRT-PCR assays (Table S1). All experiments were repeated three times. Data are means ± SE (n = 3).

**Analysis of rice quality**

 Mature seeds were dried in the 39 °C oven for at least 5 days. Rice seeds (n = 48) were then cut into two parts. The part with embryo was germinated in water, and the other part was used for rice quality analysis. Seven days after germination, genomic DNAs were extracted from germinating part and PCR with primer pair HYG-F&R. Positive and negative seeds were selected as transgenic and control (WT) samples, respectively.

Before rice quality analysis, the starch was purified from polished rice using the alkaline protease method (Zhu et al., 2010). Rice AC was measured using an iodine colorimetric method (Juliano, 1971) with slight modification (Zhang et al., 2014). The D-value of WT seeds was set to zero and the AC of RNAi lines was represented by the difference (D-value = ΔACRNAi – ΔACWT/ΔACWT) between the transgenic lines and WT. For each transgenic line, two D-values (DHT and DWT) were obtained. The difference in the D-values (DHT – DWT) between DHT and DWT, named as DD-value, was used to display the AC stability of the transgenic lines under HT. Starch gelatinization and retrogradation temperatures were measured by a differential scanning calorimeter (DSC 200F3, Netzsch Instruments NA LLC, Burlington, MA, USA) as previously described (Zhang et al., 2013).

**Generation of OsMADS7 endosperm-specific knockdown transgenic plants**

To construct an endosperm-specific RNAi vector, the promoter of the GluC (2331 bp) (Qu et al., 2008) was amplified by PCR with primer pair GluCp-1F&1R. The promoter was then inserted into the HindIII and PstI restriction sites of the vector pCAMBIA1301. Subsequently, the rice intron from vector PTCK303 (Wang et al. 2004) was fused into the above vector at restriction sites PstI and SpeI to yield the endosperm-specific RNAi vector pGluC-RNAi. The specific fragment of OsMADS7 used for the RNAi construct was amplified with primer pair MADS7C2-F6&R6. Then, the fragment of OsMADS7 was cloned into two sides of the rice intron with restriction enzymes SalI and PstI, SacI and SpeI, respectively, resulting in the final OsMADS7 endosperm-specific RNAi vector (GluC-MADS7-RNAi). The RNAi vector was introduced into Agrobacterium strain EHA105, and rice transformation was performed as previously described (Hiei et al., 1994).

**Dynamic analysis of developing rice seeds**

At the heading stage, more than 30 rice plants from transgenic line M734 or WT were transplanted from the field into pots. Caryopse were marked on the initiation of pollination. At 3 DAP, rice plants were transferred to chambers for HT or RT treatment. Thirty developing seeds were harvested at each time point (6–15, 17, 19, 21, 23, 25 and 27 DAP). The fresh weight was measured for dynamic filling rate analysis. The seeds were then immediately frozen in liquid nitrogen and stored at –80 °C for dynamic analysis of gene expression. Specific primers were used in the dynamic expression analysis for starch biosynthesis genes (Table S1).
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Conflict of interest

The authors declare no conflict of interest.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Expression of starch biosynthesis genes in developing seeds grown at different temperatures. Related expression levels of SBEIIb, AGP2L, AGP3L and AGP2b to UBQ10 in developing seeds under HT and RT conditions.

Figure S2 Relative expression of OsMADS7 in rice panicles of endosperm-specific suppression lines and WT (NIP). Transcripts were measured using qRT-PCR and normalized to the level of WT control using UBQ10 as internal control. Data represent means ± SE, n = 3 biological replicates.

Figure S3 Relative expression of OsMADS8 in rice panicles of endosperm-specific suppression lines of OsMADS7 and WT (NIP). Transcripts were measured using qRT-PCR and normalized to the level of WT control using UBQ10 as internal control. Data represent means ± SE, n = 3 biological replicates.

Table S1 Oligonucleotide primer sequences used in this study.