F1-ATPase relieves the damage caused by a high temperature stress during seed development

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High-temperature stress during seed development resulted in reduced grain quality with chalky endosperm, which was caused by insufficient storage starch synthesis because of shortage of ATP supply and inadequate energy status in developing seeds. In this case, since expression of genes involved in F1-ATPase was significantly decreased, it is suggested that reduced amount of F1-ATPase largely affected the grain quality. We created the transformants in which an F1-ATPase β gene was overexpressed in immature seeds. They confer the tolerance to the high-temperature stress and produced normal-shaped grains as well as those developed in the normal conditions. This indicated the possibility of acquisition of a tolerance to the high-temperature damage.

Grain production is sensitive to abiotic stresses such as high or low temperatures, submergence, and desiccation. Future global warming may lead to high temperature stress during the grain-filling stage of rice seed development, which will reduce the crop yield and quality. In rice fields, a high temperature environment during seed development leads to many changes in the characteristics of rice grains, such as a chalky texture, milky appearance, and reduced grain weight. These changes are caused by the formation of large airspaces in the endosperm because of inadequate growth of starch granules. If rice seeds develop in high temperature conditions, the expression of multiple genes, such as those responsible for starch biosynthesis in the endosperm, is repressed; this results in a decreased amylose content and an aberrant amylopectin structure. Thus, starch deposition may be impaired because of the downregulation of sucrose import/degradation and starch biosynthesis, and/or the upregulation of starch degradation, and inefficient ATP production because of inhibition of the cytochrome respiratory chain.

Rice grains developed under normal temperature condition

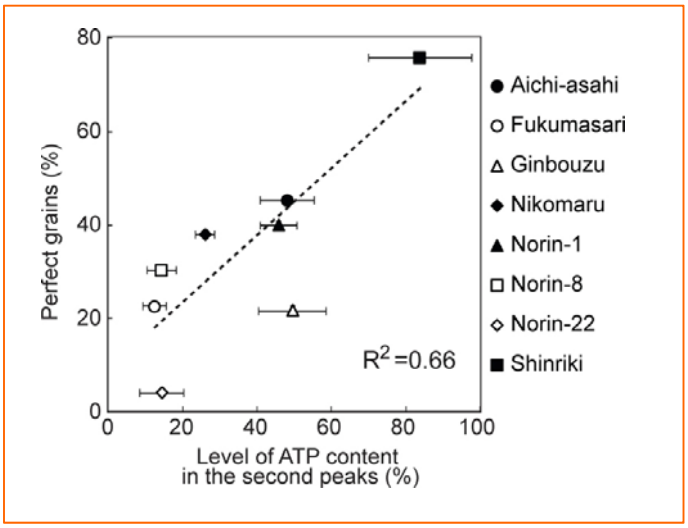
Rice grains developed under high temperature condition

Table 1. Proportion of defective grain produced in normal and high temperature conditions by eight rice cultivars.

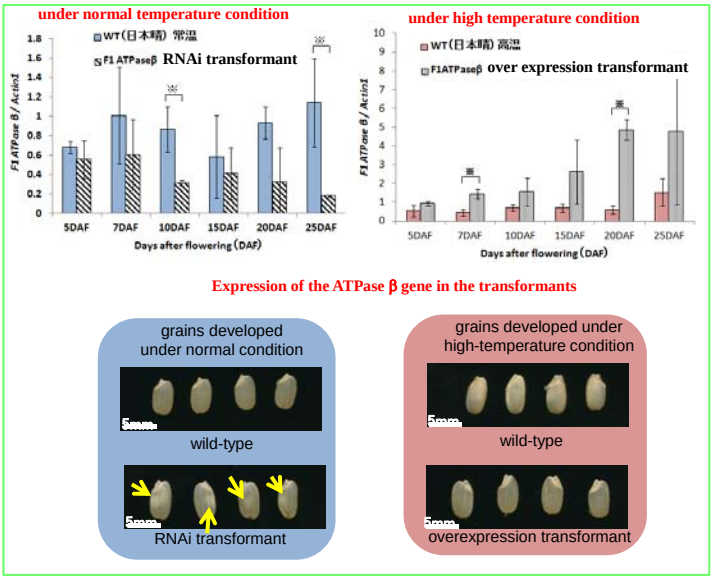
Grain feature	Shinriki		Aichi-asahi		Norin-1		Nikomaru	
	Normal	High	Normal	High	Normal	High	Normal	High
Perfect grain	96.0	76.0	96.0	45.5	96.2	40.0	91.3	38.1
Basal white	0	12.0	4.0	0	0	28.0	0	28.6
White back	0	12.0	0	40.9	0	32.0	4.3	14.3
White belly	4.0	4.0	0	31.8	3.8	16.0	0	4.8
White core	0	8.0	0	13.6	0	12.0	4.3	9.5
Milky white	0	0	0	0	0	0	0	14.3
Grain feature	Norin-8		Fukumasari		Ginbozu		Norin-22	
	Normal	High	Normal	High	Normal	High	Normal	High
Perfect grain	95.8	30.4	82.6	22.7	100	21.7	92.3	4.2
Basal white	0	17.4	13.0	36.4	0	0	7.7	25.0
White back	4.2	34.8	0	50.0	0	30.4	0	50.0
White belly	0	4.3	0	9.1	0	4.3	0	8.3
White core	0	26.1	4.3	13.6	0	26.1	0	41.7
Milky white	0	0	0	4.5	0	30.4	0	25.0

The proportion of grains with defective characteristics is indicated. For this analysis we used randomly selected 25 grains that were harvested from three individual pots of each line. Values are expressed as a percentage of the total grain. The sum of the values was not 100% because several grains exhibited two or more characteristics. "Normal" and "High" indicate the results for grains grown in normal and high temperature conditions, respectively.

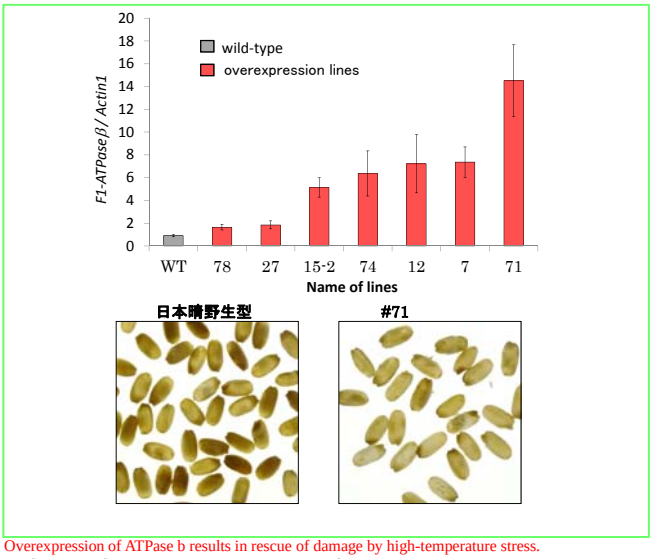
We evaluated the high temperature stress tolerance of eight representative rice cultivars. All eight cultivars produced normal-shaped, good quality grains when grown in normal conditions. By contrast, when grown under high temperature conditions, the rice grains of many of these cultivars exhibited major defective characteristics such as having basal white and white back grains, a chalky area on the dorsal and proximal sides, or white core endosperm.



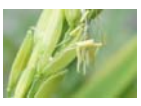
Correlation between ratio of perfect grains and ATP content in developing seeds.
We detected the correlation between the proportion of the perfect grain and the ratio of the level of the ATP contents at the second peaks in the high temperature conditions to those in the normal conditions (Figure 4). This suggests that reduction of ATP content corresponding to the second peak causes insufficient grain filling in the high temperature conditions.



Phenotype of the transformants containing RNAi and overexpression genes for ATPase β.
We created rice transformants in which the ATPase β gene was repressed by RNAi and overexpressed by the developing seed specific promoter, respectively. The RNAi transformants produced grains with reduced quality such as white core seeds even when they were developed under the normal temperature conditions. In contrast, the transformants in which the ATPase β gene was efficiently expressed in immature seeds showed significant tolerance to the damage by the high-temperature stress during seed development.



Overexpression of ATPase b results in rescue of damage by high-temperature stress.
We found significant correlation between strength of tolerance to the high-temperature stress and the expression levels of ATPase β gene in the transformants. This indicated that high-level expression of the ATPase β can rescue the damage by the high-temperature stresses during seed development. Our results suggest that potentials of ATP supply in developing seeds are very important to acquire the tolerance to the high-temperature damage. Strength of ATPase activity or high-level expression of the ATPase gene was available for the usable marker for breeding of the new rice lines.



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