

A novel mutation induced by Ar-ion-irradiation affect grain length and improve yield in rice

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Rice serves as an important staple food for more than half of the world's population. The size of a rice grain, which is one of the most important agronomic traits, is correlated with its yield. The grain size is a polygene-controlled quantitative trait, resulting in substantial variations in the size and shape of grains. Although several genes influencing the grain size have been cloned from rice cultivars, it is necessary to identify novel genes that determine the grain size to meet the increasing food demands of the growing world population.

To identify a novel gene that can determine the grain size, we isolated a rice mutant that exhibited a significant increase in grain weight from 159 independent M2 lines raised from Ar-ion-irradiated (5 Gy, 290 keV μm^{-1}) rice seeds. In the mutant, the grain length increased (5.46 ± 0.01 mm, Avr. \pm SE) in comparison with that of the wild-type (WT) Nipponbare (5.26 ± 0.02 mm, Fig. 1). Given that the grain width and thickness were not altered in the mutant, the increased grain weight was considered to be the result of the increase in grain length. We designated this mutant as *long grain1* (*lin1*).

We tested whether the yield increased in the *lin1* mutant under field conditions in 2014 and 2016 in a paddy field. Three plots of the *lin1* mutant and wild-type Nipponbare plants were formed in a paddy field. In each plot, 49 plants (7 \times 7 plants, 30 cm inter-plant spacing) were planted. The outermost plants were excluded from sampling to avoid the border effect. Therefore, 25 plants (5 \times 5 plants grown in the 1.44 m²) in the center of each plot were used to calculate the yield. The 1000-grain weight was 27.6 g and 25.9 g in 2014, and 27.1 g and 26.3 g in 2016 in the *lin1* mutant and Nipponbare, respectively (Table 1). The number of panicles, spikelets, and percentage fertility were not significantly different between the *lin1* mutant and WT in both years, suggesting that there was no tradeoff between grain length

Table 1. Agronomic traits and grain yield of WT and *lin1*.

Year	Line name	Panicle number	Spikelet number	Fertility (%)	1000-grain weight (g)	Yield (g/m ²)
2014	WT	23.8	124.9	93.7	25.9	803.5
	<i>lin1</i>	24.8	120.6	94.3	27.6	869.3
2016	WT	20.8	121.0	87.0	26.3	642.3
	<i>lin1</i>	21.3	126.5	92.0	27.1	747.8

and number of panicles, number of spikelets, and percentage fertility in this mutant. Consequently, the grain yield (g/m²) was 8.2% and 16.4% higher in the *lin1* mutant compared with that of the WT in 2014 and 2016, respectively. These results demonstrated that the mutated *lin1* allele could improve the rice grain yield.

We performed the whole-genome sequencing and genetic linkage analyses to identify the yield-related gene, *LIN1*. Thus, a 1 bp deletion in the coding sequence of *Os06g0675200* gene on chromosome 6 was ascertained to be linked with the grain length. To confirm that *Os06g0675200* corresponded with the *LIN1* gene, we introduced a 1 bp insertion in the gene of Nipponbare using the CRISPR/Cas9-mediated genome editing system. As with the *lin1* mutant, the transgenic plants displayed enhanced grain length compared with that of WT, thereby indicating that the inactivation of the gene by the 1 bp insertion and subsequent frameshift reproduced the observed phenotype of the *lin1* mutant and supported the conclusion that *Os06g0675200* is the *LIN1* gene.

To investigate whether the mutant allele of *LIN1* has already been utilized in rice breeding, we conducted the sequence polymorphism analysis using the TASUKE genome browser,¹⁾ which collectively provides polymorphisms among rice cultivars. We used information from 25 varieties (15 temperate *japonica* and ten *indica*), for which the sequencing coverage of the entire coding sequence of *LIN1* was available. All temperate *japonica*- and six *indica*-varieties harbored the wild-type *LIN1* allele. In contrast, four *indica* varieties shared an identical T to C substitution, which caused a non-synonymous substitution in the amino acid sequence of *LIN1*. These results revealed that the increase in grain yield achievable by the inactivation of *LIN1* might be limited in such variants that already harbor mutant *LIN1* alleles. Thus, the *lin1* mutation reported in this study may be useful to further increase the grain yield in both temperate *japonica* and *indica* rice varieties.



Fig. 1. Seeds and brown grains of Nipponbare (a and c) and *lin1* mutant (b and d). Bar = 5 mm.

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Reference

- 1) M. Kumagai *et al.*, *Bioinformatics* **29**, 1806 (2013).