

## Effect of Rolled Leaf Gene $Rl_{(t)}$ on Grain Quality in Hybrid Rice

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**Abstract:** Effects of rolled leaf gene  $Rl_{(t)}$  on grain quality characters of hybrid rice were analyzed by using three pairs of rolled leaf near-isogenic lines under two fertilizer treatments. Under normal fertilizer level (e.g. 450 kg urea per ha), head rice rates and milled rice recovery of rolled leaf hybrids were significantly higher than those of corresponding non-rolled crosses, while the chalky rice rate and chalkiness were all lower. Of the RVA profiles, the peak viscosity, the hot paste viscosity and the breakdown viscosity of the rolled were all higher than those of the corresponding non-rolled ones to various degrees. Increasing fertilizer application for promoting panicle development increased the brown, milled and head rice rates except for Shanyou 63, furthermore, significant difference of head rice rates existed between the rolled leaf Shanyou 559 and Shanyou 559; while the peak viscosity, the hot paste viscosity and the breakdown viscosity all decreased to different levels; changes of values of other characters had no apparent regularity. It suggested that  $Rl_{(t)}$  could improve rice quality under certain conditions.

**Key words:** hybrid rice; rice quality; rolled leaf; gene effect; starch viscosity; rapid visco analyzer

Rolled-leaf gene  $Rl_{(t)}$  was a semi-dominant gene. Leaves with homozygote  $Rl_{(t)}Rl_{(t)}$  were heavily incurved, while those with heterozygote  $Rl_{(t)}rl_{(t)}$  were semi-rolling<sup>[1]</sup>. Leaf rolling enhanced light availability, which probably due to erect leaves, smaller leaf angle, increased chlorophyll content and leaf area index, and reduced light extinction coefficient (LEC)<sup>[2]</sup>. Furthermore, the rolled leaf gene  $Rl_{(t)}$  had relation with increased effective tillers, declined number of grains per panicle, and the increased seed-setting rate and yield of individual plant<sup>[3]</sup>. This paper reported the effects of the rolled leaf gene  $Rl_{(t)}$  on grain quality characters by using three pairs of near-isogenic lines for rolled leaf gene.

## MATERIALS AND METHODS

### Plant materials

Rolled leaf Zhenshan 97B and Zhenshan 97B were crossed with Minghui 63, Yanhui 559 and 6078, respectively. Rolled leaf Zhenshan 97B carried the rolled leaf gene  $Rl_{(t)}$ , was a near-isogenic line of Zhenshan 97B. The hybrids were denoted as rolled

leaf Shanyou 63, Shanyou 63, rolled leaf Shanyou 559, Shanyou 559, rolled leaf Shanyou 6708 and Shanyou 6708, respectively.

### Experimental design

All materials were seeded on 5 May and then transplanted to a paddy field on 6 June with one seedling per hill. Each plot included 200 plants (10 rows×20 plants per row) spaced at 13.3 cm×25.0 cm. Two fertilizer treatments were arranged: 1) normal fertilization level, i.e. total urea was applied at a rate of 450 kg/ha, 80% as basal fertilizer and for promoting tillering, 20% for promoting panicle development; 2) increased fertilizer application, i.e. 540 kg/ha urea was supplied, 360 kg/ha as basal fertilizer and for promoting tillering, and 180 kg/ha for promoting panicle development.  $F_1$  from six crosses were grown in a randomized complete design with three replications.

### Measuring methods

#### *Grain milling and appearance quality characters*

Grain quality characters were measured after harvest. Brown rice rate and head rice rate were measured referred to the National Standards of P. R.

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China *GB1350-1999 Paddy*; milled rice rate referred to Standards of Ministry of Agriculture, P. R. China *NY22-86 High Quality Edible Paddy*; grain shape, the chalky rice rate and chalkiness referred to the National Standards of P. R. China, *GB/T17891-1999 High Quality Paddy*.

#### Viscosity of rice starch

Viscosity of rice starch was measured using a 3-D Rapid Visco Analyser (Newport Scientific Company, Australia) and the results were analyzed by the self-contained software of TCW (thermal cycle for Windows). According to the approved methods of AACC [4, 5], when moisture content was 14%, 3.00 g sample and 25.0 mL distilled water were needed. In this experiment moisture content was about 13.5%, so 2.95 g sample and 25.0 mL distilled water were needed. In the process of mixing, temperature in vessel changed as following: held at 50°C for 1 min; heated to 95°C at 12°C/min (3.75 min); held at 95°C for 2.5 min; cooled to 50°C at 12°C/min (3.75 min); held at 50°C for 1.4 min. The agitator ran at 960 r/min in 10 s at the beginning, and then kept in 160 r/min. RAU (RAV viscosity unit) was the value of viscosity of rice starch. Characteristics of RAV profile included peak viscosity, hot viscosity, cool viscosity, breakdown (peak viscosity minus hot viscosity), setback (cool viscosity minus peak viscosity) and consistence (cool viscosity minus hot viscosity).

## RESULTS

### Rice milling quality

Results of rice milling quality were showed in Table 1. Under normal fertilizer level, compared with non-rolled leaf crosses, rolled leaf crosses had similar brown rice rate, significantly higher milled rice rates and higher head rice rate, especially that of rolled leaf Shanyou 559. Increasing fertilizer for promoting panicle development, milled rice rate and head rice rate of rolled leaf crosses were both higher than those of corresponding non-rolled leaf ones, and significant difference of head rice rate existed between rolled leaf Shanyou 559 and Shanyou 559. It indicated that *RI<sub>(1)</sub>* had beneficial effect on rice quality under certain conditions. The brown rice, milled rice and head rice rates of the other crosses all increased at the increased fertilizer for promoting panicle development than at normal fertilizer level, except head rice rate of Shanyou 63 (Table 1). It indicated that increasing fertilizer level for promoting panicle development could improve rice-milling quality.

### Rice appearance quality

At normal fertilizer level, characters of grain shape had no significant differences between rolled leaf crosses and non-rolled leaf crosses (Table 2). Increasing fertilizer level, the characters had no

**Table 1. Milling quality of rice grain in different crosses.**

Cross	Treatment	Brown rice rate (%)	Milled rice rate (%)	Head rice rate (%)
Rolled leaf Shanyou 559	1	81.5*	67.1 <sup>*(*)</sup>	48.3**
	2	82.5	72.1	56.1 <sup>(*)</sup>
Shanyou 559	1	81.2*	64.6**	48.0
	2	82.5	71.4	50.0
Rolled leaf Shanyou 63	1	81.1*	69.2 <sup>(**)</sup>	48.4
	2	82.4	68.6	48.4
Shanyou 63	1	81.6	63.9	47.0*
	2	82.5	66.8	43.8
Rolled leaf Shanyou 6078	1	82.5**	69.0 <sup>***)</sup>	47.5 <sup>**(**)</sup>
	2	83.5	71.6	51.6
Shanyou 6078	1	82.4**	65.4**	45.7**
	2	83.4	70.3	50.6

\*, \*\*: The difference between two treatments of the same cross was significant at  $\alpha = 0.05$  and  $\alpha = 0.01$ , respectively.

(\*), (\*\*): The difference between two corresponding crosses of the same treatment was significant at  $\alpha = 0.05$  and  $\alpha = 0.01$ , respectively.

Treatment 1 means normal fertilizer level (450 kg urea per ha) and treatment 2 means increased fertilizer for promoting panicle development (540 kg urea per ha).

significant changes. Except chalky rice rate of rolled leaf Shanyou 63 was significantly lower than that of Shanyou 63, chalky rice rate and chalkiness of the other crosses had no significant differences. It indicated that leaf rolling had little effect on rice appearance quality. Under the increased fertilizer level, no differences in grain length and grain shape were observed between non-rolled ones and the corresponding rolled leaf crosses. However, compared with the normal fertilizer level, the chalky rice rates of Shanyou 63 and Shanyou 6078 both decreased significantly under the increased fertilizer application. While no differences were found in chalky rice rate

and chalkiness of the other crosses and in chalkiness of all the crosses between the two fertilizer levels. It showed that increasing fertilizer level has less effect on rice appearance quality, and the effects were various among different crosses.

### Rice starch viscosity

At normal fertilizer level, the peak viscosity, hot viscosity and breakdown of rolled leaf crosses were all higher than those of non-rolled leaf crosses (Table 3); The setback of rolled leaf Shanyou 559 and rolled leaf Shanyou 63 were smaller, while that of rolled leaf Shanyou 6078 was greater. At increased fertilizer

**Table 2. Appearance quality of milled rice grain in different crosses.**

Cross	Treatment	Grain length (mm)	Length-width ratio	Chalky rice rate (%)	Chalkiness (%)
Rolled leaf Shanyou 559	1	5.8	2.5	69.8	15.4
	2	5.8	2.5	71.5	18.1
Shanyou 559	1	5.8	2.5	74.8	17.4
	2	5.7	2.5	73.5	20.0
Rolled leaf Shanyou 63	1	6.2	2.7	66.8	20.1
	2	6.2	2.7	67.8 <sup>(**)</sup>	23.3
Shanyou 63	1	6.1	2.6	69.5 <sup>**</sup>	22.6
	2	6.0	2.6	54.3	22.9
Rolled leaf Shanyou 6078	1	6.2	2.7	76.8	24.4
	2	6.1	2.7	70.5	23.3
Shanyou 6078	1	6.2	2.7	86.5 <sup>*</sup>	30.9
	2	6.1	2.7	71.0	24.9

Treatment 1 means normal fertilizer level (450 kg urea per ha) and treatment 2 means increased fertilizer for promoting panicle development (540 kg urea per ha).

**Table 3. Characteristic values of RVA profile in different crosses.**

Cross	Treatment	Peak viscosity	Hot viscosity	Cool viscosity	Breakdown	Setback	Consistence
Rolled leaf Shanyou 559	1	254.8	168.8	247.2	86.0	-7.6	105.4
	2	218.7	140.2	245.3	78.5	26.6	105.1
Shanyou 559	1	226.4	148.1	255.0	78.3	28.6	106.9
	2	201.9	134.3	238.7	67.6	36.8	104.4
Rolled leaf Shanyou 63	1	247.6	167.5	271.3	80.1	23.6	103.7
	2	209.9	141.7	252.0	68.2	42.2	110.3
Shanyou 63	1	224.0	154.1	260.9	69.9	36.9	106.8
	2	213.2	145.2	273.8	68.0	60.6	128.6
Rolled leaf Shanyou 6078	1	234.0	154.7	265.8	79.3	31.7	111.0
	2	197.3	137.2	242.3	60.1	45.0	105.1
Shanyou 6078	1	230.5	153.3	254.2	77.2	23.7	100.8
	2	219.1	141.8	247.9	77.3	28.8	106.1

Treatment 1 means normal fertilizer level (450 kg urea per ha) and treatment 2 means increased fertilizer for promoting panicle development (540 kg urea per ha).

level, differences in characters of RAV profile were different in various crosses. Except for the decreased breakdown in Shanyou 6078, those of other crosses were similar at the two fertilizer levels. Therefore, increased fertilizer application for promoting panicle development, peak viscosity, hot paste viscosity and breakdown viscosity of the rolled leaf crosses all decreased, while the setback all increased. It indicated that increasing fertilizer for promoting panicle development made rice physical and chemical quality, rice-cooking quality become worse in this experiment.

## DISCUSSION

Previous study on the effects of  $Rl_{(t)}$  showed that rolled leaf crosses had more effective panicle numbers, less grain numbers per panicle and 1000-grain weight than the corresponding non-rolled leaf ones<sup>[3]</sup>; Furthermore, leaf rolling could improve leaf posture for absorbing light, delay leaf senescence, improve efficiency for solar energy utilization at middle and late growth stages, and grains of rolled leaf crosses were uniform and plump. These effects might improve rice milling quality and appearance quality. A similar result was got in this experiment that milled rice and head rice rates were higher, the chalky rice rate and chalkiness were lower than those of corresponding non-rolled leaf ones. Shu et al<sup>[6, 7]</sup> analyzed the relationship between characters of RAV profile and rice physical-chemical quality and rice cooking quality, and found that the breakdown and viscosity had distinctly positive correlation, the hardness of rice positively correlated to the setback with a significant coefficient  $r$  of 0.902, and negatively to the breakdown with a significant coefficient  $r$  of  $-0.772$ , the negative correlation between rice viscosity and the setback was significant at  $\alpha=0.01$ . In this experiment, a conclusion was made that rice physical-chemical quality and rice cooking quality of rolled leaf crosses were superior to those of corresponding non-rolled leaf ones due to big breakdown and little setback of the rolled leaf crosses.

Much research on the effect of N fertilizer on rice quality have been reported, but different studies had different results, furthermore, a reverse result of a few quality characters has been got. For example,

Zhou<sup>[8]</sup> and Jin et al<sup>[9]</sup> considered that at increased nitrogen fertilizer level, the chalky rice rate and chalkiness decreased significantly; while a reverse conclusion also was made that chalky decrement and nitrogen fertilizer amount had positive correlation<sup>[10]</sup>; Jin et al<sup>[11]</sup> considered that different varieties had different responses to nitrogen fertilizer. For instance, the chalky rate and chalkiness in Wuyujing 3 increased with the increasing nitrogen amount, but decreased in Shanyou 63. Nagato et al found that the chalky rate and chalkiness were higher than contrast when applying nitrogen at 5 d and 15 d after heading, that was to say the conditions which was favor to grain filling might increase chalky<sup>[12]</sup>; but some researchers considered that the chalky rate and chalkiness decreased with the increasing of nitrogen fertilizer amount at middle and late growth stages, delaying the applying time of the fertilizer for promoting panicle development could decrease the chalky rate and chalkiness<sup>[10]</sup>; Mu<sup>[13]</sup> also considered that with the increase in the proportion of fertilizer applied at middle and late growth stage, the grain yield increased, the chalky rate and chalkiness decreased, and rice quality improved. A similar result was got in rice viscosity. Those different results may be attributed to different materials and conditions. In this study, increasing fertilizer for promoting panicle development improved rice-milling quality, but made rice physical-chemical and cooking qualities become worse. Changes of chalky rate and chalkiness were different in different crosses. Compared with normal fertilizer level, most of the quality characters of rolled leaf crosses were better, or close to those of non-rolled leaf crosses under high fertilizer level. Therefore, it could be concluded that rolled leaf crosses were in favor of cultivation methods for high yielding and high quality.

## REFERENCES

- 1 Gu X Y, Gu M H. Genetic analysis of a rolled leaf character in rice. *Hereditas*, 1995, **17**(5): 20–23. (in Chinese with English abstract)
- 2 Chen Z X, Chen G, Hu J. Genetic expression and effects of rolled leaf gene  $Rl_{(t)}$  in hybrid rice (*Oryza sativa* L.).

- Acta Agron Sin*, 2002, **28**(6): 847–851. (in Chinese with English abstract)
- 3 Chen Z X, Chen G, Hu J. Effects of rolled leaf gene *Rl<sub>10</sub>* on economic traits of hybrid rice. *Acta Agron Sin*, 2004, **30**(5): 465–469. (in Chinese with English abstract)
- 4 American Association of Cereal Chemistry (AACC). Methods 61-02 for RVA. In: Approved Methods of the AACC. 9th ed. St. Paul, MN: the Association, 1995.
- 5 Gravois K A, Webb B D. Inheritance of long grain rice amylograph viscosity characteristics. *Euphytica*, 1997, **97**: 25–29.
- 6 Shu Q Y, Wu D X, Xia Y W. Relationship between RAV profile character and eating quality on *Oryza sativa* L. *Sci Agric Sin*, 1998, **31**(1): 1–4. (in Chinese with English abstract)
- 7 Wu D X, Shu Q Y, Xia Y W. Rapid identification of starch viscosity property of early indica rice varieties with different apparent amylase content by RVA profile. *Chinese J Rice Sci*, 2001, **15**(1): 57–59. (in Chinese with English abstract)
- 8 Zhou R Q. Effect of fertilizer on quality and yield. *Hunan Agric Coll J*, 1989, **15**(3): 1–5. (in Chinese)
- 9 Jin Z X, Qiu T Q, Sun Y L. Effect of nitrogen fertilizer on rice chalkiness ratio, cooking and eating quality of rice grain. *Plant Nutr & Fert Sci*, 2001, **7**(1): 31–35. (in Chinese with English abstract)
- 10 Tashiro T, Ebata M. Studies on white-belly rice kernel: IV. Effects of nitrogen topdressing at heading stage on the occurrence of white belly kernel. *Japan J Crop Sci*, 1978, **48**: 99–106.
- 11 Jin J, Xu D Y, Cai Y X. Effect of N-fertilizer on main quality characters of rice and RAV profile parameters. *Acta Agron Sin*, 2004, **30** (2): 154–158. (in Chinese with English abstract)
- 12 Nagato K, Ebata M, Ishikawa M. Protein contents of developing and mature rice grain. *Japan J Crop Sci*, 1972, **41**: 472–479.
- 13 Mu Y H, Sun H Y, Sun J Y. Effect of proportion of nitrogen on rice yield and quality. *Heilongjiang Agric Sci*, 2000, (3): 18–19. (in Chinese with English abstract)