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REVIEW

Breeding of a Blast Resistant Multiline Variety of Rice, Sasanishiki BL

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Abstract

Sasanishiki BL, a multiline variety of Sasanishiki for blast resistance was developed at Miyagi Prefectural Furukawa Agricultural Experiment Station in Japan. Sasanishiki BL is composed of 7 isogenic lines, Sasanishiki BL1 to BL7 with different resistance genes to blast disease. Sasanishiki BL was released to Miyagi Prefecture in 1994. The main characteristics of each component line are almost the same as those of Sasanishiki except for the resistance gene to blast disease. Sasanishiki BL has been cultivated in farmers' fields without rice blast damage since the released year.

Discipline: Plant breeding
Additional key words: multiline variety, rice blast resistance

Introduction

The once predominant rice variety in Tohoku region, Sasanishiki was released in 1963 and became one of the most popular varieties in Japan, because of its high yielding ability and excellent grain and eating quality. Since climatic fluctuation intensified in summer seasons after 1970, Sasanishiki was frequently damaged by blast disease. Although every effort to improve the blast resistance of Sasanishiki had been made, it was very difficult to attain the objective. Even if the objective could be attained, it seemed very difficult to replace Sasanishiki with an unknown new variety, because of the great popularity of Sasanishiki in the rice market. In such conditions, the rice breeders at Miyagi Prefectural Furukawa Agricultural Experiment Station tried to develop a blast resistant multiline variety of Sasanishiki so as to produce and supply a new rice variety with the famous brand name of Sasanishiki. In the present paper, the breeding procedures of Sasanishiki BL and its cultivation after being released in Miyagi Prefecture are reviewed based on the reports of Sasaki et al[4]. and Sasahara & Koizumi[3].

Breeding procedure

The breeding was started in 1977 by back crossing method. In segregating generations, the resistant plants were selected by spray innoculation of the blast fungus (*Pirycularia grisea*) avirulent to the target resistant gene. After the last back crossing, individual selections and pedigree selections were repeated until fixed lines were obtained. In 1986, 9 isogenic lines named Tohoku IL1 to Tohoku IL9 were developed. Each line's genotype of blast resistance was identified *+, Pii Pia, Pik Pia, Pik-m Pia, Piz Pia, Piz-t Pia, Pita Pia, Pita-2 Pia* and *Pib Pia*, respectively. We tried at first to develop isogenic lines without *Pia*, however, the plan was abandoned since it would take too long to attain[4].

After these procedures, the performance tests for a recommendable variety using these lines were conducted in Miyagi Prefecture. In 1994, Tohoku IL3, Tohoku IL4, Tohoku IL5 and Tohoku IL8 were identified to have an adequate similarity with Sasanishiki and were registered and named Sasanishiki BL1, BL2, BL3, and BL4, respectively as component lines of a multiline variety Sasanishiki BL by the Ministry of Agriculture, Forestry and Fisheries of Japan. Sasanishiki BL was released to Miyagi Prefecture. In order to increase the similarity of the other lines further back crossings of Sasanishiki were carried out and Tohoku IL7 in 1997, Tohoku IL6 in 1998, and Tohoku IL9 in 2002 were registered and named Sasanishiki BL5, BL6, and BL7, respectively. These were added as component lines of Sasanishiki BL in each reg-

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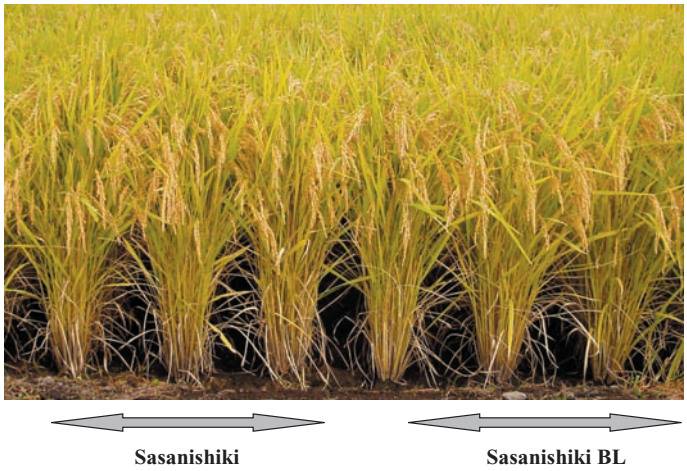

Fig. 1. Sasanishiki BL and Sasanishiki

istration year. The number of crossings of Sasanishiki BL1 to BL7, and resistance gene sources are shown in Table 1.

Main characteristics

Differences were not identified for almost all of the characteristics between each line and Sasanishiki (Fig. 1). The differences between each line and Sasanishiki for the main characteristics were not significant by t-test, except for heading time of BL4, panicle length of BL5, panicle number of BL3, panicle number of BL6, 1,000-grain weight of BL5, and the grain quality of BL7 (Table 2). But these differences were not assumed to be serious problems for practical use as a multiline variety.

The genotype of blast resistance of each line was identified to have *Pik*, *Pik-m*, *Piz*, *Piz-t*, *Pita*, *Pita-2*, and *Pib*, respectively, based on the seedling reactions of each

Table 1. The number of back crossings and resistance gene source in the component lines of Sasanishiki BL

Designation	Former designation	Resistance source	Resistance genes involved	No. of back crosses	First crossing year	Last crossing year	Registered year
Sasanishiki BL1	Tohoku IL3	Kyokukei780	*Pik*	6	1977	1983	1994
Sasanishiki BL2	Tohoku IL4	Tsuyuake	*Pik-m*	5	1978	1983	1994
Sasanishiki BL3	Tohoku IL5	Fukunishiki	*Piz*	6	1977	1983	1994
Sasanishiki BL4	Tohoku IL8	$76F_6$-88	*Piz-t*	5	1977	1979	1994
Sasanishiki BL5	Tohoku IL7	Kyokukei737	*Pita-2*	6	1977	1983	1997
Sasanishiki BL6	Tohoku IL6	Kyokukei808	*Pita*	11	1978	1989	1998
Sasanishiki BL7	Tohoku IL9	Kyokukei872	*Pib*	6	1977	1994	2002

Table 2. T-test of similarity of the component lines to Sasanishiki

Designation	Heading time	Maturing time	Lodging index	Culm length	Panicle length	Panicle no.	Total weight	Brown rice weight	1,000 grain weight	Grain quality
Sasanishiki BL1	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Sasanishiki BL2	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Sasanishiki BL3	n.s	n.s	n.s	n.s	n.s	*	n.s	n.s	n.s	n.s
Sasanishiki BL4	*	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Sasanishiki BL5	n.s	n.s	n.s	n.s	*	n.s	n.s	n.s	*	n.s
Sasanishiki BL6	n.s	n.s	n.s	n.s	n.s	*	n.s	n.s	n.s	n.s
Sasanishiki BL7	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	**

* : $\alpha = 0.05$. ** : $\alpha = 0.01$.

line to the spray infection of 15 fungi (Table 3). In addition to the target resistance gene, BL1, BL2 and BL6 were identified to have *Pia* derived from Sasanishiki. In another test, BL3, BL5 and BL7 were also identified to have *Pia*. However, BL4 was not evaluated because the blast fungi to distinguish *Piz-t* from *Piz-t, Pia* were not available. BL4 might have *Pia*, since the recurrent parent Sasanishiki has *Pia*.

The leaf blast severity of each line was very low and lesions were hardly found in natural infection tests. The panicle blast severity of each line was also very low in natural infection tests. The resistance level of each line was as susceptible as Sasanishiki in inoculation tests using the blast fungus virulent to each line in a greenhouse. The resistance level of each to panicle blast was also very susceptible like Sasanishiki. The cold tolerance level at the booting stage of each line was almost equal to Sasanishiki. The viviparity of each line was as susceptible as Sasanishiki. The shape of brown rice of each line was very similar to that of Sasanishiki. The amylose and protein contents of each line were similar to the levels of Sasanishiki. The eating quality of each line was as excellent as that of Sasanishiki.

The process of spreading Sasanishiki BL to farmers' fields

1. The cultivation guideline of Sasanishiki BL

The cultivation of Sasanishiki BL in farmers' fields started in Miyagi Prefecture in 1995. Farmers are advised to keep the following guidelines.

(1) The seeds must be completely disinfected.
(2) For leaf blast control, blasticide should not be used.
(3) For panicle blast control, blasticide should be sprayed only once at heading time because blast races avirulent to the leaf sometimes may attack panicles.
(4) The seeds must be completely renewed every year, since the proportion of components will be changed, in case blast races virulent to Sasanishiki BL are predominant in farmers' fields.

Table 3. The identification of blast resistance genes

Designation	Isolates (race*) of blast fungus															Resistance genes identified
	Ken 54–20 (003)	Naga 69–150 (007)	1104 –3 (013)	2601 –4 (017)	Ina 72–2 (031)	TH68 –126 (033)	Yu –01 (033b+)	Naga 68–182 (035)	Ken 60–19 (037)	F67 –57 (047)	TH80 –8 (073)	Ina 168 (101)	Ken 53–33 (137)	P2b (303)	138A (403)	
Sasanishiki BL1	R	R	S	S	R	S	S	R	S	R	S	R	S	R	R	*Pik*, *Pia*
Sasanishiki BL2	R	R	R	R	R	S	S	R	S	R	S	R	S	R	R	*Pik-m*, *Pia*
Sasanishiki BL3	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	*Piz*, *Pia***
Sasanishiki BL4	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	*Piz-t*, *Pia****
Sasanishiki BL5	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	*Pita-2*, *Pia***
Sasanishiki BL6	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	*Pita*, *Pia*
Sasanishiki BL7	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	*Pib*, *Pia***
Sasanishiki	S	S	S	S	R	S	S	R	S	S	S	R	S	S	S	*Pia*

R: resistant reaction. S: susceptible reaction.
*: identified race by Japanese differential varieties.
**: *Pia* was detected in another test.
***: *Pia* was not able to be identified. But BL4 possibly has *Pia* because recurrent parent Sasanishiki has *Pia*.

Table 4. Proportion of the component lines and cultivated area

Year	Proportion (%) of component lines				Cultivated area
	BL1	BL2	BL3	BL4	(ha)
1995	40	30	30	0	845
1996	30	30	40	0	4,513
1997	10	10	40	40	5,454
1998	10	10	40	40	3,234
1999	10	10	40	40	1,524
2000	10	10	40	40	845
2001	10	10	40	40	633
2002	10	10	40	40	524

(5) Sasanishiki BL should be cultivated by registered farmers in groups for effective blast control.

2. Seed production

Sasanishiki BL seeds are produced as follows. In breeders' stock and original seed farms, each component line is planted separately. The proportion of the component lines is decided based on the blast races observed in Sasanishiki BL fields, and then the seeds of each component line are mixed for seed production farms. The Sasanishiki BL seeds are produced by mixed cultivation in seed production farms, and then the seeds are supplied to farmers.

3. Proportion of the component lines and the cultivated area

Table 4 shows the changes in component lines of Sasanishiki BL and the cultivated area from 1995 to 2002. In 1995, BL1 (*Pik*), BL2 (*Pik-m*), and BL3 (*Piz*) were mixed in the proportion of 4:3:3. This proportion was changed to 3:3:4 in 1996, because blast races virulent to the the *Pik* and *Pik-m* lines had been observed in farmers' fields. In 1997 the proportion of these lines was reduced and BL4 (*Piz-t*) was added. Since 1997 BL1, BL2, BL3 and BL4 have been mixed in the proportion of 1:1:4:4. The cultivated area of Sasanishiki BL at first was about 800 ha in 1995, then increased to over 5,000 ha in 1997, however, less than 550 ha were cultivated in 2002, because Sasanishiki has become unpopular.

Blast severity of Sasanishiki BL compared to common varieties

Fig. 2 shows the leaf blast severity of Sasanishiki BL and common varieties in farmers' fields. Generally, the common varieties were treated with blasticides several times for blast control, whereas Sasanishiki BL was not sprayed with any blasticide. The leaf blast severity of Sasanishiki BL, however, was lower than that of common varieties in almost all of the fields. Fig. 3 shows the changes in the panicle blast severity of Sasanishiki BL and common varieties of farmers' fields in Miyagi Prefecture. Sasanishiki BL has been treated with blasticide only once for panicle blast control. The panicle blast

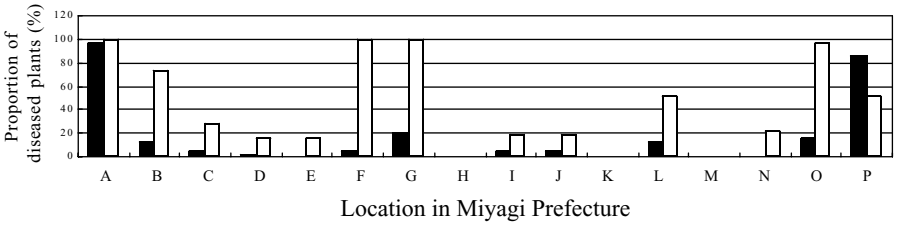

Fig. 2. Leaf blast severities of Sasanishiki BL and common rice varieties in farmers' fields (1995)
■ **:** Sasanishiki BL, □ **:** common rice varieties.
Sasanishiki BL has not been sprayed with any blasticides for leaf blast control while the common rice varieties have been treated with blasticides several times.

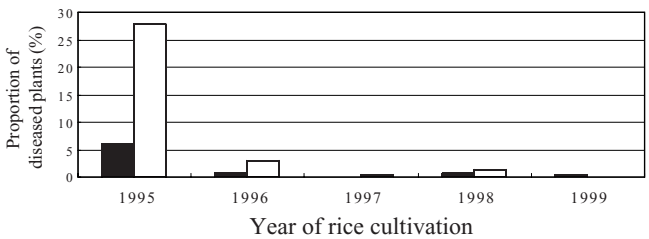

Fig. 3. Changes in panicle blast severity of Sasanishiki BL and common rice varieties in farmers' fields from 1995 to 1999
■ **:** Sasanishiki BL, □ **:** common rice varieties.
Proportion values were average of A to P in Fig. 2. BL has been sprayed only once with a blasticide for panicle blast control, while the common rice varieties have been treated with blasticide several times.

severity showed a similar tendency to leaf blast severity. Severe panicle blast epidemics have not broken out in Sasanishiki BL since 1996. Blast control effect of the multiline may seem small with such low epidemic levels since 1996. However, the multiline is sprayed only once with a blasticide while common varieties are sprayed several times. Consequently we think that Sasanishiki BL has had both a blast control effect and a reduced blasticide application effect since 1996.

Blast races isolated from Sasanishiki BL fields

The isolated blast races from the panicle blast lesions of Sasanishiki BL fields and their adjoining common varieties fields are shown in Table 5. The most predominant blast race isolated from common varieties was 007, which is avirulent to all component lines of Sasanishiki BL. Blast race 037, virulent to 2 components BL1 (*Pik*) and BL2 (*Pik-m*), was only isolated from one place. On the other hand, the most predominant race isolated from Sasanishiki BL was 037. This race is virulent to the 2 components and the race 077 or 073 virulent to the 3 components BL1 (*Pik*), BL2 (*Pik-m*) and BL3 (*Piz*) was also isolated at one place. Blast race 007, which is avirulent to all components of Sasanishiki BL, was also isolated from Sasanishiki BL and its isolation rate was relatively high. The main reasons for the high isolation rate of blast race 007 may be attributed to the facts that blast races avirulent to the leaf sometimes can attack the panicle of Sasanishiki BL, or other varieties accidentally get mixed in Sasanishiki BL. Table 6 shows the changes in blast races isolated from Sasanishiki BL. Races 037 and 033, which are virulent to 2 components BL1 (*Pik*) and BL2 (*Pik-m*), were predominant in the Sasanishiki BL-cultivated area in 1995 and 1996. However, since 1997, the number of places where race 037 was isolated

Table 5. Races of blast fungus from panicle blast lesions of common varieties and Sasanishiki BL in farmers' fields (1996)

Place	No. of isolates examined	Isolated races from common varieties		No. of isolates examined	Isolated races from Sasanishiki BL			
		007	037		007	037	073	077
A	6	6		12		12		
B	6	6		10			1	9
C	6	6		8	6	2		
D	6	6		10	10			
E	6	6		10		10		
F	6	6		10	10			
G	6	6		10	6	4		
H	6	6		10	4	6		
I	6	4	2	10	2	8		
J	6	6		10		10		
K	6	6		10	4	6		
Total	66	64	2	110	42	58	1	9

Table 6. Races of blast fungus from panicle blast lesions of Sasanishiki BL in farmers' fields

Year	No. of places examined	No. of places isolated races							
		003	007	013	033	037	047	077	407
1995	29	2	11	1	2	13			
1996	39	4	18			15	1	1	
1997	3		2			1			
1998	25	1	20			2	1		1
1999	4		4						
2000	2		2						

has declined. This may be caused by the decreased proportions of BL1 (*Pik*) and BL2 (*Pik-m*) in Sasanishiki BL. Blast races 047 and 077, virulent to the BL3 (*Piz*), appeared in the year after the proportion of the BL3 was increased. Blast race 407, virulent to BL4 (*Piz-t*), was isolated from Sasanishiki BL after the introduction of BL4. Currently, races virulent to components of Sasanishiki BL have been detected, but no race virulent to all components has been detected in farmers' fields of Miyagi Prefecture.

Discussion

Cultivation of Sasanishiki BL started in 1994. The blast severity of Sasanishiki BL has been at low levels in farmers' fields, and no change in the 4 components of Sasanishiki BL has been needed since 1997. At the time of the release to farmers' fields, the staff thought, it would be necessary to replace resistant lines in case the resistance would break down. However, we realized that drastically changing the components was practically difficult, very expensive and laborious when the cultivated area increased. Since the component varieties of Sasanishiki BL have increased from 4 to 7, the mixed cultivation of all components will be able to continue without replacement, because the resistance of 7 mixed components to blast disease will be more stable than 4. Nakajima et al.[2] reported that the multiline has a reduced density effect, a barrier effect and a possibility of induced resistance to rice blast, by using a multiline composed of 9 isogenic lines and Sasanishiki (*Pia*). In their investigation, the race (race 007 or race 037) infected the mixture of *Pia* line and *Pii* line. The disease severity in that mixture was less than that predicted. They claimed that the resistance of the multiline did not break down, even though a super race of the blast fungus was selected. Van der Plank[5] reported that a race with a wider spectrum of virulence became less aggressive to a host plant than a race with a narrower virulence in potato late blight (*Phytophtora infestans*). Koizumi & Kato[1] proposed that the factors of disease suppression occurrence were due to a reduced density effect and a barrier effect of the rice multiline.

As mentioned above, it may be possible to stably control the rice blast disease with the multiline mixed with all components without exchange. It would eliminate the work of tracing blast races in farmers' fields and the exchange of the components which requires a lot of expense and labor.

In breeding of multiline varieties, popular varieties in the rice market are generally used as the recurrent parent in Japan, because it is very difficult for an unknown new variety to become popular. By the time a multiline variety is developed, popularity will not always be guaranteed because the breeding procedure is a long term process. The developed multiline variety may be out of date by the time it is released. In the case of Sasanishiki BL, when it was released in Miyagi Prefecture, the recurrent parent Sasanishiki was on the way to becoming unpopular. Consequently the area of Sasanishiki BL has decreased with the decrease of Sasanishiki. This is the biggest problem of multiline varieties.

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