Spontaneous Occurrence of the Sekiguchi Lesion in Two American Rice Lines: Its Induction, Inheritance, and Utilization

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ABSTRACT

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Occurrence of the Sekiguchi lesion in rice is conditioned by a single recessive gene and can be induced either by infection with *Bipolaris oryzae* and *Pyricularia oryzae*, or by exposure to certain chemical agents. Rice plants with Sekiguchi lesions were found both as naturally occurring mutants and in X_2 populations from ^{137}Cs-irradiated seed. The lesions first appear on leaves and sheaths as gray water-soaked spots that turn orangish brown and become zonate as they enlarge, coalesce, and finally occupy the entire plant. Histopathological observations suggest that the Sekiguchi lesion represents a flaw in the biological mechanisms that normally regulate the hypersensitive response of rice to invasion by potential pathogens. Rice lines having the Sekiguchi lesions have been used successfully as spreaders to increase inoculum levels of *B. oryzae* in field studies of brown leaf spot of rice.

Additional key words: *Helminthosporium oryzae*.

Sekiguchi and Furuta, in 1965, described a unique lesion type in a mutant of the rice cultivar Asahi (9). The lesions are first visible as 1- to 2-mm-diameter water-soaked gray spots that enlarge rapidly into diurnally zonate orangish brown areas, gradually coalescing until finally the whole plant is affected (Fig. 1). These "Sekiguchi" lesions can be induced by infection by either *Bipolaris oryzae* (B. de Haan) Shoemaker (=*Helminthosporium oryzae* B. de Haan) or *Pyricularia oryzae* Cav., or with various atomized chemical agents, including sodium hypochlorite, pentachlorphenol, and certain organophosphate insecticides (9).

Kiyosawa, who named the mutation after Sekiguchi, determined that the Sekiguchi lesion in Asahi was conditioned by a single recessive gene, which he designated *sl* (6). He observed no linkages between the *sl* gene and the major blast resistance genes *Pi-z, Pi-k,*

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Pi-i, or *Pi-a*. Linkage was indicated between the *sl* and *Pi-ta* loci (cross-over value of 0.095).

In 1975, in the rice breeding nursery at Beaumont, TX, six plants of an F_8 line from the cross B689A (Taichung Native 1/CI9545//IR-8) exhibited large (1 × 3 cm) zonate orangish brown foliar lesions (8). The plants were potted and moved to a screenhouse for study and seed production. At that time we were unaware of the reports from Japan.

In 1978, two X_2 plants from ^{137}Cs-irradiated (25 krad) cultivar Labelle seed showed lesions similar to those found in 1975 and to those described by Kiyosawa. In addition, several plants with large purplish brown (PB) lesions were found among the Labelle X_2 plants (Fig. 2). The PB lesions appeared to be slower growing and more restricted in size than the Sekiguchi lesions. The X_2 plants were being screened for mutants resistant to race IC-17 of *P. oryzae* (4) when the Sekiguchi and PB mutants were observed.

The results of pathological, histological, and genetic studies of the Sekiguchi lesion occurring in United States rice breeding lines and the cultivar Labelle are reported in this paper. We also discuss the implications of these results in rice disease resistance research.

MATERIALS AND METHODS

Standard pathological, histological, and plant breeding procedures were employed to elucidate the relationships between pathogen and host and to study inheritance and linkages.

Pathology. Leaf sections with Sekiguchi lesions and normal brown spot lesions were incubated for 36–60 hr on moist filter paper in petri dishes at room temperature under alternate 12-hr periods of darkness and light supplemented by near-UV (black light) to promote sporulation (7). Subsequently, the conidia were picked off with a sliver of agar and transferred to 2% rice polish agar (RPA) slants (11). Isolations were made similarly from normal blast lesions incubated 16–20 hr at 27 C under continuous cool-white fluorescent illumination (4,240 lux).

Rice seedlings (four- to five-leaf stage) were inoculated by atomizing aqueous spore suspensions from 8- to 10-day-old cultures of *P. oryzae* or *B. oryzae* incubated on 2% RPA in 125-ml Erlenmeyer flasks at 27 C under continuous cool-white fluorescent light (4,240 lux). Two days prior to anticipated use, the *B. oryzae* cultures were exposed to alternate 12-hr periods of darkness and light supplemented with near-UV to induce prolific sporulation. Inoculated plants were incubated overnight (16–18 hr) at 25 C in dew chambers, then moved to a greenhouse. Symptoms were evident in 4–7 days.

Genetics. The B689A Sekiguchi mutant was crossed with the cultivars Tetep, Starbonnet, and Nortai to determine the inheritance of the Sekiguchi mutation and possible linkages. The Labelle Sekiguchi and PB mutants were crossed to determine if the two mutant genes were allelic or linked and to determine the effect of both genes in the same plant. The Labelle Sekiguchi mutant and the B689A1 Sekiguchi mutant were crossed to determine whether their respective mutant genes were allelic.

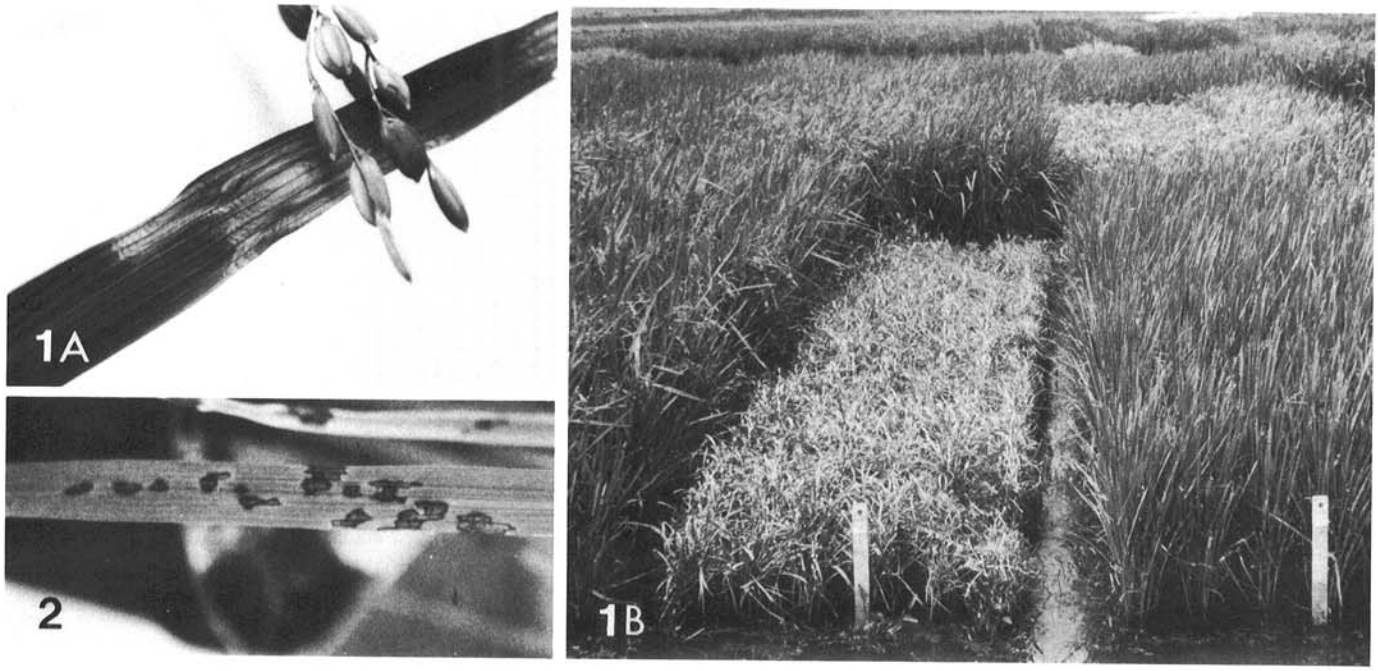
Histology. From the Labelle Sekiguchi × Labelle PB cross, normal F_2 plants and plants showing each lesion type were prepared for histological comparisons to determine whether some physical differences in leaf structure might account for the development of the mutant lesions, and the extent of colonization by the fungal pathogens.

Leaves with 5-day-old lesions were cut into 2- to 3-mm sections across the width of the leaf, killed in Farmers' fluid for 10 min, evacuated and fixed in 4% paraformaldehyde (5), and shipped to Beltsville for sectioning and study. The material was dehydrated in ethanol and embedded in Spurr's medium (10). The embedded material was sectioned at 3-μm thickness on a rotary microtome with glass "Ralph" knives (3), and stained with a mixture of basic fuchsin and toluidine blue in polyethylene glycol 400 (1).

RESULTS AND DISCUSSION

Pathology. *B. oryzae* was isolated from the Sekiguchi lesions on the B689A1 F_8 mutant plants. Inoculations of eight commercial cultivars with an isolate of *B. oryzae* from a Sekiguchi lesion resulted in typical brown leaf spot lesions (2). Conversely, progeny of the Sekiguchi mutants produced typical Sekiguchi lesions when inoculated with a field isolate of *B. oryzae* from Starbonnet rice, thus demonstrating the uniqueness of the Sekiguchi rice line and the type-character of the isolate from the Sekiguchi lesion.

Genetics. Of 20 asymptomatic sister plants of the six Sekiguchi mutants, from the same panicle row, which were progeny-tested by



Figs. 1 and **2.** The "Sekiguchi" and purplish brown lesions on mutant rice plants. **1A,** Sekiguchi lesion on leaf of a B689 A mutant rice plant 14 days after infection by *Bipolaris oryzae*. **1B,** A yield plot of B689A mutant rice collapsing from the effects of Sekiguchi lesions induced by natural infections of *B. oryzae*, compared flowering normal rice lines on either side. Within 20 days the mutant plot had practically disappeared below the water line. **2,** Purplish brown lesions on leaf of ^{137}Cs-induced mutant of cultivar Labelle rice 10 days after infection by *Pyricularia oryzae*.

inoculation with *B. oryzae*, four produced populations segregating for the *sl* mutation. Four sister lines, from sister plants in the F_7 generation, of the line having the six mutants showed no mutants. We hypothesized from these observations that the Sekiguchi lesion was conditioned by a single-gene recessive mutation that occurred in the F_6 generation and was carried heterozygotically in an F_7 plant from which a panicle was selected for a panicle row in F_8. The five F_8 panicle-rows, one of which had the six mutant plants, originated from the same panicle row in F_7, the same panicle in F_6 (where the mutation probably occurred), and the same seed in F_5.

The reactions of F_2 plants from crosses between the B689A-*sl* mutant and several other rice lines confirmed the hypothesized inheritance of the mutation (Tables 1 and 2). Results from the B689A-*sl*/Nortai cross also confirmed a phenomenon reported by Kiyosawa (6), namely that inoculation of the *sl* mutant with virulent races of *P. oryzae* resulted in the production of many susceptible-type blast lesions. Race IG-1 of *P. oryzae* is virulent against Nortai and avirulent against the mutant parent. In the B689A-*sl*/Nortai cross, resistance to race IG-1 was conditioned by a single dominant gene (*Pi*) that operated independently of the *sl* gene (Table 2). Presumably the effective *Pi*-gene was inherited from IR-8, since the other components of the mutant parent, Taichung Native 1 and CI9545, are susceptible to race IG-1.

In the B689A-*sl*/Nortai cross, race IG-1 induced only Sekiguchi lesions on resistant F_2 plants and a mixture of normal susceptible-type blast lesions and Sekiguchi lesions on susceptible F_2 plants, among F_2s that expressed the *sl* trait. Normal (*Sl*) F_2 plants produced either typical susceptible-type blast lesions mixed with pinpoint necrotic lesions (susceptible to race IG-1) or only the pinpoint lesions, which are indicative of a hypersensitive reaction and typical of the reaction of a normal rice line with major-gene resistance. This suggests that the Sekiguchi lesion is a manifestation of a hypersensitive reaction that continues unchecked by host mechanisms that normally prevent necroses from spreading beyond the localized area of intrusion. That this process can be induced by abiotic agents such as sodium hypochlorite and organophospate insecticides (9) supports the conclusion that the Sekiguchi lesion is solely a host response once the reaction has been triggered. The disruption of lysosomes and the consequent release of hydrolases and proteases has been postulated as the mechanism for protein degradation and necrosis associated with hypersensitivity (12). Perhaps the Sekiguchi lesion represents a flaw in the biochemical mechanisms that control cell-to-cell movement of hydrolytic enzymes. A chain reaction may be set in motion in which the hydrolases of one cell diffuse into adjacent cells and "decompartmentalize" their hydrolases, thus killing those cells, and so on.

Inoculations of F_2 populations from the Labelle-*sl*/Labelle-PB cross with an avirulent isolate of *P. oryzae* showed both mutations to be conditioned by single recessive genes operating independently (Table 3). It was difficult to distinguish clearly between plants homozygous for both the *sl* and purplish brown lesion (*pbl*) genes and those homozygous for the *sl* gene only. Therefore, the linkage analysis was conducted as if *sl* were epistatic to *pbl*, ie, 9 normal:4 *sl*:3 *pbl* phenotypes (Table 3).

Exactly 800 F_2 seeds were planted, of which 682 survived to be inoculated. The probability of seedlings homozygous for either mutation being extra vulnerable to microbial destruction during emergence would explain the higher-than-expected ratio of normal plants to those expressing either the *sl* or *pbl* genes. The observed ratio of *sl* to *pbl* plants agreed with the expected 4:3 ratio assuming *sl* was epistatic to *pbl* (Table 3).

All of six F_1 plants from the cross of the Labelle-*sl* mutant and the B689A-*sl* mutant developed the Sekiguchi lesions in the greenhouse without artificial inoculation. Since the *sl* gene in each parent was recessive and was expressed in the F_1 plants, we concluded that the "natural" B689A mutation and the ^{137}Cs-induced mutation in Labelle are allelic.

Histology. Sections of 5-day-old Sekiguchi and PB lesions (5–7 × 2–3 mm) revealed no evidence of fungal hyphae in affected leaf tissue. Cells involved in the Sekiguchi lesion were collapsed and necrotic. In contrast, cells involved in the PB lesions appeared turgid, contained dark-staining globules and darkened walls, but did not appear necrotic until later stages of lesion development (Fig. 3).

There were no visible anatomical differences, such as in structure of epidermis or cell wall thickness, between unaffected leaves from mutant plants and those from normal segregants. Anatomical features apparently played no obvious role in the mutants' unusual responses to infection.

The histological evidence for nonproliferation of the pathogen from the original infection sites of Sekiguchi lesions was supported by pathological observations. Normally blast lesions sporulate after 8–12 hr on wet filter paper in a petri dish. The young Sekiguchi lesions showed no evidence of *P. oryzae* or *B. oryzae* sporulation even after 48 hr, when saprobes began to flourish. However, older lesions frequently sporulated in their central areas,

TABLE 1. Segregation of the Sekiguchi lesion in the F_2 progeny of crosses between rice line B689A-*sl* and plants of cultivars Tetep or Starbonnet, inoculated with avirulent cultures of *Bipolaris oryzae* or *Pyricularia oryzae*, and grown at Beaumont, TX, 1977

		Number of plants		χ^2	P
	Pathogen	Normal	Sekiguchi	(3:1)	(3:1)
B689A-*sl* × Tetep	*B. oryzae*	248	92	0.77	>0.25
B689A-*sl* × Tetep	*P. oryzae*	360	114	0.23	>0.50
B689A-*sl* × Starbonnet	*P. oryzae*	151	50	<0.01	>0.90
B689A-*sl*		0	28		
Tetep		51	0		
Starbonnet		45	0		

TABLE 2. Inheritance and linkage analysis of the Sekiguchi lesion (*sl*) gene and the *Pi*-gene conditioning resistance to race IG-1 of *Pyricularia oryzae* in 98 F_2 progeny from the cross B689A-*sl*/Nortai[a], Beaumont, TX, 1977

	Number of plants		3 *Sl*:1 *sl*	
Phenotypes	Normal (*Sl*)	Sekiguchi (*sl*)	χ^2	P
Resistant (*Pi*)	52	21	0.12	>0.50
Susceptible (*pi*)	20	5[b]		
3 *Pi*:1 *pi*				
χ^2	0.01		9 *Pi*/*Sl*:3 *Pi*/*sl*:3 *pi*/*Sl*:1 *pi*/*sl*	
P	>0.90		$\chi^2 = 0.90$, $P > 0.75$	

[a]Nortai is susceptible to race IG-1 of *P. oryzae*; B689A-*sl* is resistant.
[b]Plants susceptible to race IG-1 of *P. oryzae* and carrying the *sl* gene produced both normal susceptible-type blast lesions and Sekiguchi lesions.

TABLE 3. Inheritance and linkage analysis of the *sl* gene and *pbl* genes in an F_2 population from the cross Labelle-*sl*/Labelle-*pbl*, mutants from ^{137}Cs-irradiated Labelle rice seed, inoculated with avirulent race IG-1 of *Pyricularia oryzae* in plants grown at Beaumont, TX, 1981

	Number of plants		3 *SL*:1 *sl*	
Phenotypes	*SL*	*sl*	χ^2	P
Pbl	412	155[a]	1.89	>0.10
pbl	115			
13[b] *Pbl*:3 *pbl*[a]				
χ^2	1.60		9 *Pbl*/*Sl*:3 *pbl*/*Sl*:4-/*sl*	
P	>0.10		$\chi^2 = 4.81$, $P > 0.05$	
			3 *pbl*:4 *sl*[b] $-\ \chi^2 = 0.01$, $P > 0.90$	

[a]Plants carrying both *sl* and *pbl* recessive genes homozygously were phenotypically indistinguishable from plants carrying only the *sl* gene; therefore, χ^2 tests were applied assuming 13:3 and 3:1 ratio for the *pbl* and *sl* phenotypes, respectively.
[b]χ^2 test applied to the phenotypic 3 *pbl*:4 *sl* ratio.

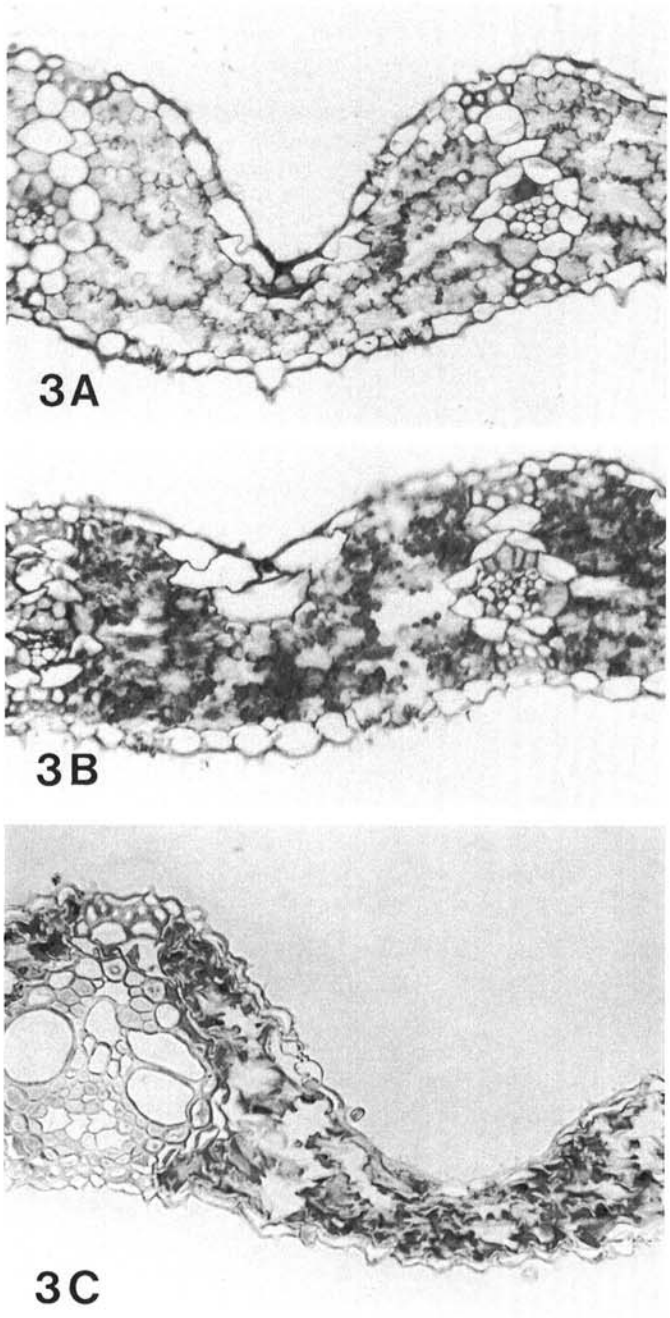


Fig. 3. Cross sections of leaves of cultivar Labelle mutant rice plants 5 days after infection by *Pyricularia oyrzae*. **A,** Unaffected area of the Sekiguchi (*sl*) mutant (×330). **B,** Purplish brown lesion from a purplish brown (*pbl*) mutant, showing dark-staining cellular inclusions, but only limited disorganization of tissue (×380). **C,** Sekiguchi lesion from the *sl* mutant showing collapse and deterioration of mesophyll and epidermal layer (×380).

usually producing spores of *B. oryzae* whether the primary inoculum came from *P. oryzae* or *B. oryzae* isolates, suggesting that the ubiquitous *B. oryzae* colonized the necrotic tissue as a saprobe. In fact, examination of rice leaves and florets under a dissecting microscope (×70) usually revealed abundant *B. oryzae* conidia that had settled on the surface.

The Sekiguchi mutant as a spreader. The *sl* mutant has been used successfully as a spreader to increase the inoculum level of *B. oryzae* in screening nurseries and in studies of the impact of *B. oryzae* on yield, floral abortion, and grain discoloration. Data showing the increased incidence of foliar brown spot, discolored grains, and discolored grains colonized by *B. oryzae* in six-row yield plots in which the windward row was planted to the Sekiguchi mutant are indicated in Table 4. The resulting heavily infected plants also provided, as a by-product, grain samples heavily infested and spotted by *B. oryzae* for fungicidal seed treatment and seedling disease studies.

TABLE 4. Mean effect of the Sekiguchi mutant as a spreader on the incidence of foliar brown spot, discolored grains, and discolored grains colonized by *Bipolaris oryzae*, in eight rice lines grown at Beaumont, TX, 1980

	Plots with spreader	Check plots
Lesions per 10 flag leaves	282	126
Discolored grains (%)[a]	3.8	2.2
Discolored grains colonized by *B. oryzae* (%)[b]	29.0	7.0

[a] 1,000 hulled rice grains per sample (32,000 grains per treatment).
[b] 25 discolored hulled rice grains per sample (800 grains per treatment).

In summary, the Sekiguchi lesion is conditioned by a single recessive gene, *sl*, and can be induced by a number of biotic and abiotic agents. It has been reported from both Japan and the United States in rice lines of diverse genetic backgrounds, both naturally occurring and ^{137}Cs-induced mutations. Histological and pathological evidence suggests that the Sekiguchi lesion is a manifestation of a flaw in the biological mechanisms that regulate the hypersensitive response of plants homozygous for the *sl* gene to pathogenic agents. The Sekiguchi rice lines are useful tools in the study of brown spot disease, caused by *B. oryzae*, and could prove valuable in the study of the hypersensitive response to attack by plant pathogens. Seed is available from the Beaumont location to interested researchers.

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