SHORT COMMUNICATION

FREQUENCY AND PATHOGENICITY DISTRIBUTION OF *RHIZOCTONIA* spp. CAUSING SHEATH BLIGHT ON RICE AND BANDED LEAF DISEASE ON MAIZE IN YUNNAN, CHINA

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SUMMARY

Sheath blight on rice and banded leaf disease on maize are epidemic in Yunnan, China, causing severe losses of both crops. Isolates from maize sheaths showing symptoms of sheath blight, were collected from five representative counties in Yunnan. These isolates were identified as *Rhizoctonia* spp. based on their morphological characteristics. Four anastomosis groups (AGs) of *R. solani* were identified: AG-1 IA (10 isolates, 28.3% of the total), AG-1 IC (8.3%), AG-4 (11.4%) and AG-5 (11.4%). The binucleate isolates of *Rhizoctonia* spp. belonged to AG-A. Thirteen multinucleate isolates failed to anastomose with the AG testers of *R. solani,* but they possessed morphological characteristics of *Rhizoctonia zeae* and anastomosed with the tester isolate of anastomosis group Z of the *Waitea* spp. (WAG-Z). Isolates of AG-1 IA were highly pathogenic at heading stage on maize, while isolates of AG-5, AG-1 IC, and WAG-Z were considerably less pathogenic. Isolates of AG-4 and AG-A were the least pathogenic. Among the sheath samples of rice with sheath blight symptoms, forty-nine isolates from 15 counties in Yunnan were collected. They were identified as four AGs of *R. solani*, AG-1 IA (31 isolates, 65.3% of the total), AG-1 IB (6.1%), AG-1 IC (6.1%) and AG-4 (2.1%). Binucleate isolates belonged to AG-Bb. Tested isolates of AG-1 IA, AG-Bb, and AG-4 were highly pathogenic at the heading stage on rice; isolates of AG-1 IC were moderately pathogenic, whereas isolates of AG-1 IB were non-pathogenic on rice.

Key words: Rice, sheet blight, *Rhizoctonia*, maize, banded leaf.

Rice sheath blight occurs throughout temperate and tropical rice production areas and is most prominent where the plant is grown intensively. Since it was first reported in Japan in 1910, the disease has become endemic in many countries in the Orient (Hashiba and Kobayashi, 1996). The disease has become an important constraint on rice production in Yunnan Province, China, especially under intensive cropping.

In recent years, sheath blight of maize has also become a major disease in Yunnan province. The causal organism is known. In Florida, Voorhees (1934) reported a sclerotial rot of corn (maize) caused by *Rhizoctonia zeae*. Gao (1987) obtained 77 *Rhizoctonia* isolates from sheath blight-diseased tissue of maize in Northern China and assigned them to six anastomosis groups (AGs). These included *R. solani* AG-1 IA (68.8% of the total samples), AG-1 IB, AG-3 and AG-5, and binucleate *Rhizoctonia* AG-A and AG-K (Zhang and Chen, 1986). Li *et al*. (1998) assigned *Rhizoctonia* spp. from maize in Sichuan province to four anastomosis groups: AG-1 IA (71.6% of the total), AG-1 IB (1.4%), AG-4 (6.4%) and AG-5 (2.8%). Two isolates of binucleate *Rhizoctonia* were identified as AG-K. Yan *et al*. (1984) confirmed by inoculation tests that certain isolates of *Rhizoctonia* caused sheath blight of maize, rice, and wheat under optimum temperature conditions for disease development. Rotation systems commonly used in Sichuan are rice-wheat and wheat-maize, which facilitate the increase of inoculum in the soil.

Yield losses of rice and maize have increased with large acreages of a single cultivar of rice or maize. For controlling sheath blight of rice and maize, it is vital to unravel the species of the pathogens causing these diseases. The objective of this study was to characterize isolates of *Rhizoctonia* spp. recovered from rice and maize in Yunnan province. To identify the *Rhizoctonia* spp. morphological observation, pathogenicity tests, and anastomosis tests were done.

Rice and maize sheath samples were collected in 2003 from rice and maize fields in Yunnan (Fig. 1). Specimens were rinsed gently in tap water for approximately 2 min, cut into small pieces (2-5 mm), washed three times in sterile distilled water (1 min each) and blotted dry on sterile paper towels. Sheath pieces were placed on 2.0% water agar (WA) and incubated at 25°C for one or two days. Emerging hyphal tips were transferred to plates of potato dextrose agar (PDA) and pure cultures were transferred to PDA slants for storage at 25°C until use.

Hyphal anastomosis groups were determined using

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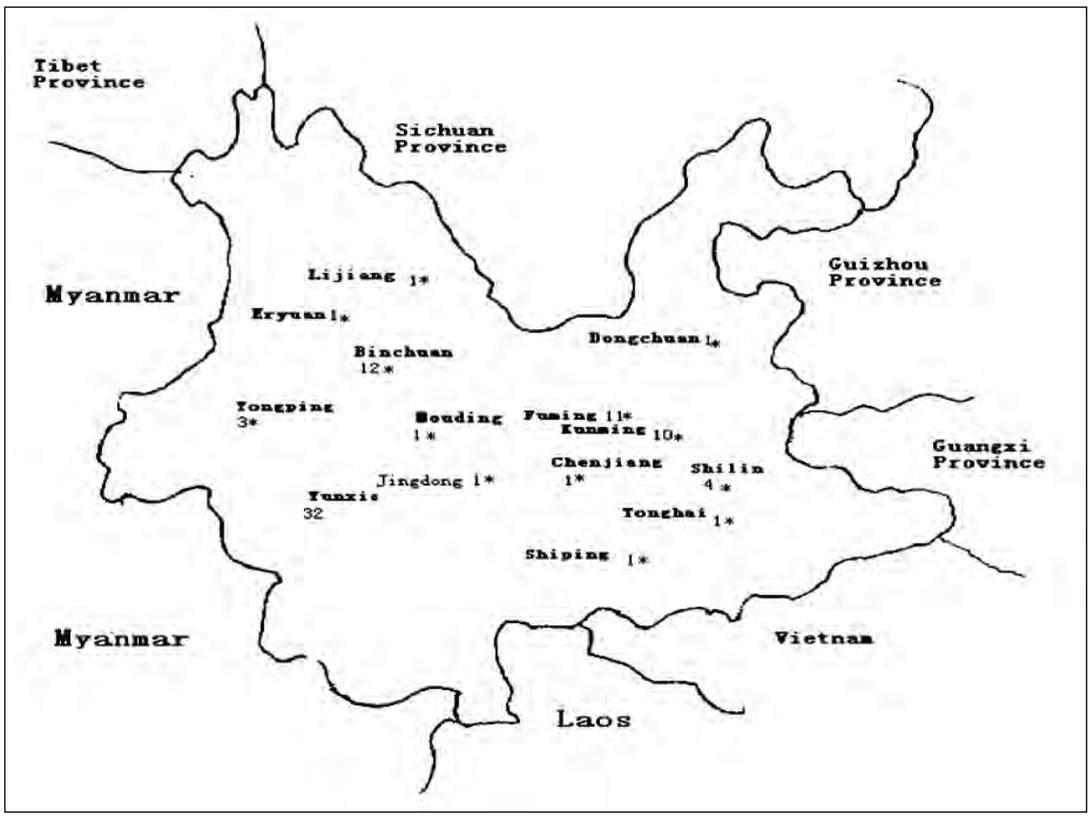

Fig. 1. Counties where isolates of *Rhozoctonia* spp. were sampled from rice and maize field in Yunnan Province, China, in 2003-2005. (*: The number of isolates from locations).

Table 1. List of tester isolates used.

Isolate	AG	Host	Origin	Isolate	AG	Host	Origin
C-325	AG-1 IA	Rice	Japan	C-484	AG-Ba	Rice	Japan
CS-Ka	AG-1 IA	Rice	Japan	C-455	AG-Bb	Rice	Japan
CB74-22	AG-1 IB	Sugar beet	Japan	C-511	AG-Bb	Rice	Japan
B-19	AG-1 IB	Sugar beet	Japan	OR-706	AG-C	*Gymnadenia conopsea*	Japan
BV-7	AG-1 IC	Sugar beet	Japan	STC-5	AG-C	Soil	Japan
SH-3	AG 2-1	Pea	Japan	W-12	AG-D	Wheat	Japan
PS-4	AG 2-1	Pea	Japan	C-46	AG-D	Mat rush	Japan
C-96	AG 2-2 IIIB	Mat rush	Japan	F-18	AG-E	Flax	Japan
C-104	AG 2-2 IIIB	Mat rush	Japan	RH-155	AG-E	Sugar beet	Japan
RI-64	AG 2-2 IV	Sugar beet	Japan	AH-6	AG-F	Peanut	Japan
C-6	AG 2-2 IV	Sugar beet	Japan	YT-2-1	AG-F	Taro	China
ST 11-6	AG-3	Potato	Japan	AH-9	AG-G	Peanut	Japan
ST-9	AG-3	Potato	Japan	RJ-2	AG-G	*Rumex* sp.	Japan
RH-165	AG- 4 HG II	Sugar beet	Japan	STC-9	AG-H	Soil	Japan
RH-131	AG-4 HG II	Sugar beet	Japan	AV-2	AG-I	*Artemisia*	Japan
78-23 R-3	AG- 4 HG I	Peanut	Japan	Cre-v-3a	AG-I	Unknown	Japan
GM-10	AG-5	Soybean	Japan	AC-1	AG-K	Onion	Japan
SH-4	AG-5	Soybean	Japan	STc-27	AG-K	Soil	Japan
NTA3-1	AG-6HG I	Soil	Japan	FKo-2--26	AG-L	Soil	Japan
OHT-1-1	AG-6HG I	Soil	Japan	STc-43	AG-N	Soil	Japan
KNB2-2	AG-6GV	Soil	Japan	FKo-6-2	AG-O	Soil	Japan
1535	AG-7	Soil	Japan	C-578	AG-P	Tea	Japan
HO-1556	AG-7	Soil	Japan	C-620	AG-Q	Bermuda grass	Japan
W-565	AG-8	Wheat	U.S.A	Bn-37	AG-R	*Cucumis*	U.S.A
SN1-2	AG-BI	Soil	Japan	J-03-7	AG-R	Ginger	China
C-157	AG-A	Rice	Japan	S-5	AG-S	*Pittosporum* sp.	U.S.A
Spi-3	AG-A	Unknown	Japan	C-505	WAG-Z	Rice	Japan

dual cultures of the Yunnan isolates and tester strains (AG-1 to AG-8 and AG A to AG-S) (Table 1) according to the procedure of Zhang and Dernoeden (1995). Sterilized cellophane rectangles measuring 1 × 8 cm were dipped in sterile water and then two strips were laid parallel on WA. The Yunnan isolates and the testers of each anastomosis group (AG) actively growing on PDA were paired on the rectangular strips placed on 2% WA in a Petri dish (9 cm) separated by a distance of 2-3 cm in all possible combinations. A mycelial disk (0.5 cm diameter) of a tester isolate was placed in the center of the strips, and one disk (0.5 cm diameter) of an unknown isolate was placed on each end of the two strips. The Petri dishes were incubated at 25°C in the dark until the advancing hyphae from the opposite disks overlapped. The overlapped portion was examined under the microscope at ×160 magnification for hyphal fusion. When paired isolates freely fused (fusion rate >30%), they were considered to belong to as the same AG. Tester strains used in this study are listed in Table 1.

The cultural characteristics of the Yunnan isolates were observed 10-14 days after inoculation on PDA. The number of nuclei per cell was determined in a 7-day-old culture. Mycelium was stained with safranin O and 3% KOH (Bandoni, 1979) and nuclei were examined microscopically at × 400 magnification.

The pathogenicity of Yunnan isolates was evaluated on maize seedlings. Isolates were cultured on PDA in a 9 cm Petri dishfor 3-4 days at 25°C and then covered with about 20 g autoclaved soil (120°C, 50 min), and maintained at 25°C for 3-4 days; this was used as the inoculum source. Maize seedlings of cv. Huidan 1, about 5 cm high, were planted in potting soil, one per vinyl pot (15 cm diameter, 20 cm height). Each seedling was inoculated in the root zone with about 7 g of infested soil. Control plants were inoculated with autoclaved soil. The experiments were conducted 3 times with 3 replicates in a greenhouse maintained at 30°C and 16°C with a photoperiod of 16 h light and 8 h dark, respectively. After 7 days, disease severity was recorded on a scale in which 0 = healthy, 1 = a few brown or black speck lesions on the hypocotyl, 2 = lesions covering between 10% to <49% of the hypocotyl, 3 = lesions covering ≥50% of the hypocotyl, and 4 = seedling dead.

Representative isolates were tested for pathogenicity on rice (cv. Youzhi Dao) and maize (cv. Huidan 1). Healthy seedlings grown in the greenhouse were transplanted to field trial plots with spacing 0.18 × 0.2 m, three to four seedlings were transplanted per hill and crops were raised according to standard cultural methods at the Yunnan Agricultural University. In order to avoid possible natural infection by soil pathogens, the upper nodal sheath was inoculated at the ear formation stage. A hyphal disk (4 mm diameter), cut from the margin of an actively growing colony of each isolate, was transferred to the inner side of the first leaf sheath that grew above the largest ear of the plant. Each plot was represented by three plants per isolate, and non-inoculated plants served as control. The surface of the inoculated area was covered with a piece of water-soaked cotton to retain moisture. Symptoms and lesion length on each plant were recorded after full development of the lesions. After 14 days, disease severity was recorded based on a scale, in which 0 = no symptom, 1 = brown sheath, 2 = large lesion on sheath with local spread, 3 = larger lesion on sheath with upward and downward spread. Koch's postulates were then fulfilled by re-isolation of the fungus from sheaths with lesions.

Data were analyzed by analysis of variance, and means compared using Duncan's multiple range test.

From the 400 rice sheath samples showing symptoms of sheath blight and banded leaf disease, collected from 16 representative counties (or cities) in Yunnan (Fig. 1), 49 isolates were obtained with morphological characteristics of the genus *Rhizoctonia* (Parmeter and Whitney, 1970). Among them, 37 isolates were multinucleate and 10 were binucleate. All isolates with multinucleate cells anastomosed with one of the AG-1 or AG-4 tester isolates of *R. solani*, and AG-1 was divided into three subgroups (AG-1 IA, -IB, -IC) according to their cultural characteristics: AG-1IA isolates were characterized by the production of large (1 to 3 mm) relatively spherical sclerotia, AG-1 IB produced small, irregular-shaped sclerotia; and AG-1 IC had small (0.2-0.8 mm) round sclerotia similar to the description of Sneh *et al.* (1998) (Fig. 2) The following *Rhizoctonia* spp. AGs were detected: AG-1 IA (32 isolates, 65.3% of the total), AG-1 IB (3, 6.1%), AG-1 IC (3, 6.1%) and AG-4 (1, 2.0%). The ten binucleate *R.* spp. isolates belonged to AG-Bb.

Three isolates belonging to *R. solani* AG-1 IA induced rice sheath blight symptoms on the upper nodal sheaths of inoculated plants at the heading stage. These symptoms were similar to the typical field symptoms of rice sheath blight, which initially appeared as oblong water-soaked lesions on sheaths, and later as an enlarged, greyish-white center surrounded by a dark

Table 2. Pathogenicity of Yunnan isolates of *Rhizoctonia* spp. at heading stage on rice (cv. Youzhi Dao).

AG	Isolate	Disease severity[(a)]
AG-1 IA	R-03-54	3.0a
AG-1 IA	R-03-55	3.0a
AG-1 IA	R-03-60	3.0a
AG-1 IB	R-03-1	0.0d
AG-1 IB	R-03-46	0.9c
AG-1 IC	R-99-3	1.0c
AG-1 IC	R-99-4	1.0c
AG-4	R-03-25	0.0d
AG-Bb	R-03-76	2.8a
AG-Bb	R-03-28	2.2b

[(a)]Means sharing the same letter are not significantly different according to Duncan's new multiple range test (P=0.05).

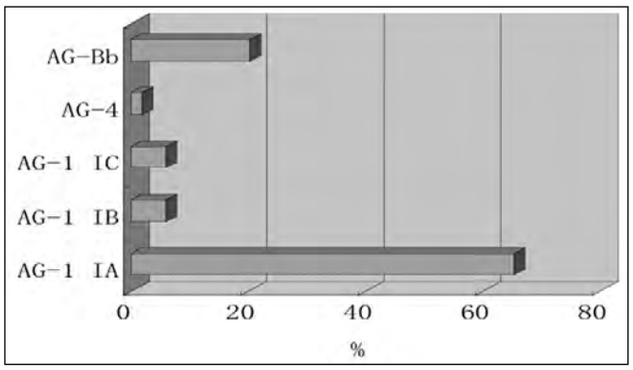

Fig. 2. Anastomosis groups and subgroups of *Rhizoctonia* spp. obtained from rice with symptoms of sheath blight.

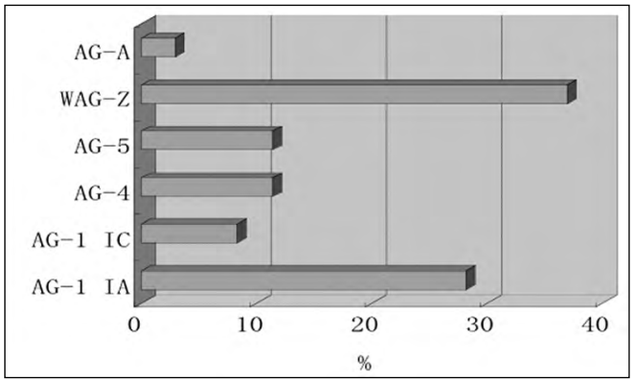

Fig. 3. Anastomosis groups and subgroups of *Rhizoctonia* spp. obtained from maize with symptoms of banded leaf disease.

brown or reddish brown margin reaching 2-5 cm and occasionally 7-8 cm long. Sometimes cottony or light brown mycelia appeared on or beyond the lesions and dark brown sclerotia were produced in the lesions. Sometimes spots also developed on the leaves, ears and stems. The two AG-Bb isolates caused symptoms similar to those of AG-1 IA. Lesions caused by isolates of AG-1 IB were smaller than those caused by AG-1 IA, and AG-4 isolates were not pathogenic on rice. The pathogens were re-isolated from sheath lesions. The upper nodal sheaths of the non-inoculated rice plants did not show any symptoms. AG-1 IA and AG-Bb isolates were highly pathogenic at the the heading stage of rice. AG-1 IC was less pathogenic than AG-1 IA (Table 2).

Thirty-five isolates of *Rhizoctonia*-like fungi were obtained from 100 maize samples with symptoms of banded leaf from five counties (or cities) in Yunnan. All isolates possessed dolipore septa and were multinucleate except for one binucleate isolate.

The anastomosis grouping of maize-derived isolates was as follows: AG-1 IA (10 isolates, 28.3% of the total), AG-1 IC (3, 8.3%), AG-4 (4, 11.4%) and AG-5 (4, 11.4%). The single binucleate isolate belonged to AG-A. Thirteen multinucleate isolates (37.1%) failed to anastomose with any of the AG testers of *R. solani*, but they possessed morphological characteristics of the anamorph *R. zeae* and anastomosed with the tester isolate of WAG-Z (Voorhees, 1934; Martin and Lucas, 1983). The *R. zeae* isolates had typical *Rhizoctonia* mycelial morphology, but mycelia of cultures grown on PDA were yellowish brown or orange and they formed small (0.3-1.0 mm) spherical sclerotia that were initially cream-colored, then turned red-orange, and eventually red-brown as the cultures aged. These sclerotia were submerged in the culture medium, unlike those of *R. solani* or binucleate *Rhizoctonia* which formed on the surface.

In inoculation tests, all isolates belonging to AG-1 IA, AG-1 IC, WAG-Z, AG-4, AG-5 and AG-A, were weakly pathogenic or avirulent on maize seedlings (Table 3). Two AG-1 IA isolates tested induced symptoms of maize banded leaf at the heading stage, similar to those occurring on maize in the field and similar to typical lesions of rice sheath blight. Lesions (5 to 10 cm in diameter) sometimes developed on the leaves, ears and stems. Lesions on the upper nodal sheath caused by isolates AG-4, AG-5, AG-1 IC and binucleate *Rhizoctonia* AG-A were smaller and darker than those of AG-1 IA. The three isolates of *R. zeae* usually produced discontinuous lesions with dark brown margins and brown central areas on the leaf sheaths; they also caused ear rot. Sclerotia developing on the husks and sheaths were reddish or black-brown, 0.1-0.5 mm in diameter, which is characteristic of sclerotial rot of corn (maize) caused by *R. zeae* (Voorhees, 1934). The pathogens were re-isolated from sheath lesions. The upper nodal sheaths of non-inoculated maize plants showed no symptoms.

Isolates of *R. solani* AG-1 IA were highly pathogenic on heading-stage maize, and those of AG-5, AG-1 IC and WAG-Z were considerably less pathogenic. *Rhizoctonia* isolates AG-4 and AG-A were the least pathogenic (Table 3).

Table 3. Pathogenicity of Yunnan isolates of *Rhizoctonia* spp. on maize (cv. Huidan 1) at the seedling and heading stage.

AG	Isolate	Disease severity[(a)]	
		Seedlings	Heading stage
AG-1 IA	M-03-48	1.2ab	3.0a
AG-1 IA	M-03-34	1.1ab	2.8a
AG-1 IC	M-03-14	0.7bc	2.8a
AG-1 IC	M-03-76	0.7bc	0.2d
AG-1 IC	M-03-77	0.7bc	0.2d
WAG-Z	M-03-56	0.7bc	1.1c
WAG-Z	M-03-59	0.7bc	1.9b
WAG-Z	M-03-28	0.4bc	0.9c
AG-4	M-03-18	1.2ab	0.2d
AG-5	M-03-3	0.6bc	2.1a
AG-5	M-03-2	0.5bc	2.1a
AG-A	M-03-23	0.4bc	1.1b
Not inoculated		0	-

[(a)]Means sharing the same letter are not significantly different according to Duncan's new multiple range test (P=0.05).

Table 4. Frequency and distribution of *Rhizoctonia* spp. anastomosis groups from rice and maize in Yunnan.

Locations	Anastomosis groups and number of isolates in each group								
	AG-1 IA	AG-1 IB	AG-4	AG-Bb	AG-1 IC	AG-5	AG-4	WAG-Z	AG-A
Fuming	7	1	1	1					1
Shilin	1	2						1	
Yunxian	25							6	
Binchuan	1			5				6	
Chenjiang	1								
Mouding	1								
Yongping	3								
Dongchuan					1				
Shiping					1				
Tonghai					1				
Eryuan				1					
Jingdong				1					
Lijiang				1					
Luliang				1					
Kunming					2	4	4		
Stone forest	2				1				

Rice yield losses as high as 50% occur in Yunnan province on susceptible cultivars when all leaf sheaths and leaf blades are infected. Isolates from diseased plants from Yunxian county, where sheath blight was consistently severe, belonged to AG-1 IA and isolates from Fuming county, in central Yunnan, belonged to AG-1 IA, AG-Bb and AG-4.

The banded leaf disease on maize is also very severe. The isolates from the diseased plants in Shilin county, where most plants were infected with banded leaf, belonged to AG-1 IA, AG-1 IC, and WAG-Z. Isolates from Yunxian county, in the southwest of Yunnan province, belonged to AG-1 IA, and WAG-Z. The difference in AG composition among the various locations was probably caused by differences in weather and soil type.

The predominant groups of *Rhizoctonia* spp. from rice were AG-1 IA and AG-Bb and those from maize, AG-1 IA and WAG-Z. The results are similar to those reported from neighboring Sichuan province (Li *et al*., 1998). This similar composition of *Rhizoctonia* pathogens is probably due to comparable climatic conditions in the two provinces.

The AG composition obtained in this study is similar to that found in previous studies (Gao, 1987), in which isolates of *R. solani* AG-1 IA were predominant.

Although in Japan AG-2-2 IIIB was the causal pathogen of brown sheath blight of paddy rice, in our study no isolates of this group were detected from diseased sheaths of rice in Yunnan.

The results of this study indicate that the *Rhizoctonia* isolates associated with rice sheath blight and maize banded leaf disease, form a diverse species complex. Rice isolates belonged to *R. solani* AG-1 IA, AG-1 IB, AG-1 IC, AG-4, and binucleate *Rhizoctonia* AG-Bb, and those from maize belonged to *R. solani* AG-1 IA, AG-1 IC, AG-4, AG-5, *R. zeae*, and binucleate *Rhizoctonia* AG-A. The frequency and distribution of AGs of *Rhizoctonia* spp. are shown in Table 4. These results will be important for controlling these diseases in China and the rest of Asia.

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