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The resistance to banded leaf and sheath blight in maize of 282 inbred lines

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Rhizoctonia solani **causal agent of banded leaf and sheath blight (BLSB), is widely distributed in the South China and Southeast Asian in maize causing severe yield losses. In this study, we identified the disease resistance of 282 maize inbred lines to BLSB. The results showed that no immune and highly resistant germplasm was found and four moderately resistant inbred lines were identified. These four moderately resistant inbreds had good performance in grain yield, combining ability and a suitable growth period; they could be good donor of disease resistance gene to improve the other maize lines. The environments, *R. solani* isolate and the evaluation method were main factors that affect the evaluate results. In order to evaluate the disease resistance of maize lines to BLSB, environments beneficial to the disease, *R. solani* isolate have stronger pathogenic force, artificial infection and proper disease nurseries were necessary. The analysis on the disease resistant inbred lines from different heterosis groups showed that lack of resistance germplasm in SS array was more serious and it was more effective to seek resistance germplasm from the NSS array, especially from the PB and tropical maize germplasm.**

Key words: Maize, inbred lines, resistance, banded leaf and sheath blight (BLSB).

INTRODUCTION

Banded leaf and sheath blight (BLSB) of maize has been a serious soil-borne disease caused by *Rhizoctonia solani* in maize production in the South China and Southeast Asian in the last decades. The high temperature, high humidity, long rainy and sunless weather during the maize growing season provide favorable conditions for the disease (Sharma et al., 1993; Asano et al., 1987; Huang et al., 2007). With the popularize and extensive planting of compact hybrids, the heavy use of nitrogen fertilizer, the increase in planting density and the application of plastic film mulching in maize production, the BLSB developed and spread rapidly, which caused a decrease of production of 10 to 20% or even higher (Huang et al., 2007; Tang and Tao, 1991; Yan et al., 2008). Some chemical control and preventive treatment to BLSB of maize has been advanced according to the occurrence pattern and prevalence condition of the disease, while these treatments have the shortage to use, high in cost or effect less in same condition (Yan et al., 2008; Chen et al., 2011; Ascual et al., 2000; Cheng et al., 2008; Huang et al., 2006). Therefore, breeding of cultivar with high resistance to BLSB were thought to be the most effective and economy method, but the lacks of resistance material hinder the breeding progress (Huang et al., 2007). The researchers have done a lot of screening test

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Table 1. The criterion of recording maize sheath blight.

Resistant type	Resistant level	Disease index	Symptom of disease
IM	0	0	No
HR	1	0.1 - 20	Disease spots below 4th sheath under ear
R	3	20.1 - 40	Disease spots below 3th sheath under ear
MR	5	40.1 - 60	Disease spots below 2th sheath under ear
S	7	60.1 - 80	Disease spots below 1st sheath under ear
HS	9	80.1 - 100	Disease spots over sheaths under ear

Im = Immune, HR = highly resistant, R = resistant, MR = moderately resistant, S = susceptible, HS = highly susceptible.

to identify the resistance of maize germplasm, same materials have better resistance in BLSB have been obtained, while only very few of them could be used in maize hybrid breeding for the reasons of bad combining ability, extreme long or short growth period and some other shortages (Sharma and Saxena, 2001; Zhao et al., 2006). In this paper, 282 maize inbred lines have been used as materials, which were used as the parents of commercial hybrids in China. We identified their resistance to BLSB by artificial infection of *R. solani* with the object to evaluate the disease resistance of the existing inbred lines and broaden the genetic base of the maize germplasm in breeding for disease resistance.

MATERIALS AND METHODS

The tested inbred lines

A total of 282 maize inbred lines which have been used as the parents of commercial hybrids were involved in this test (Table 2). These inbred lines were collected and provided by Maize Institute of Sichuan Agriculture University and Institute of Crop Science, Chinese Academy of Agricultural Science.

The setout of disease nursery

The four disease nurseries were located in Babu in Ya'an, Bifengxia in Ya'an, Fenjiang in Ya'an and Wenjiang in Chengdu; it was of high temperature, high humidity, long rainy and sunless weather during the maize growing season in these regions, which provide favorable conditions for the invasion and spread of BLSB of maize. Before sowing, 300 kg wheat and *R. solani* co-culture for 1 ha were broadcast sowed.

The preparation of inoculums

The *R. solani* AG1-IA isolate, which have strong pathogenic force, was collected and provided by the Plant Pathology Institute of Sichuan Agricultural University, and was cultured on standard potato dextrose agar (PDA) medium (potato, 200 g; dextrose, 20 g; agar, 10 g; H_2O, 1000 ml) and incubated at 26 to 28°C for 5 days (Zhao et al., 2006; Yang et al., 2003). For inoculations use in the field, the inoculums were prepared by transferring the pure bacterial strain to sterilized wheat grain and co-culture for 7 days.

The field experimental designs and artificial infection

The 282 lines (Table 2) were planted in a randomized complete block design with two replicates in each of the four disease nurseries. Each plots consisting of single row 3 m long with 7 holes and spaced 0.7 m apart. The plots were overplanted and thinned to 2 plants each hole with a density of 66600 plant/ha. At each location, the field management followed local practices for maize production. At the jointing stage, two wheat seeds inoculums were placed into the first and second sheath of each individual plant. After inoculating, the soils were kept moist for a week.

Data collection and analysis

Twenty (20) days after inoculation, the symptom of disease were collected on 10 plants per plot following the method introduced by Wang and Dai (2001) (Table 1). For a single individual, no disease spots visible were recorded as immune (0), the disease spots below the 4th, 3rd, 2nd and 1st sheath under ear were recorded as highly resistant (1), resistant (3), moderately resistant (5) and susceptible (7), respectively, and disease spots over sheaths under ear were recorded as highly susceptible (9). The disease resistance of each line was evaluated by the disease index (DI), and six disease severity classes were established. The DI was calculated according to the following formula:

DI = [∑(severity level × plant numbers of this level)/the highest severity level × the total numbers of the investigated plants] × 100

RESULTS AND ANALYSIS

The disease resistance of different inbred lines

The results of various analyses showed that there were significant difference among the lines, and the evaluation results in different locations varied. It indicated that the environments affect the resistance evaluation results strongly.

The resistance evaluations of the 282 inbred lines are listed in Table 2. It showed that no immune or highly resistant line was found. Only four inbred lines namely Qi318, 18-599, Shen3336 and PA23 were moderate resistant, accounting for 1.42% of the 282 lines. The lines of susceptible and highly susceptible were 47 for 16.67% and 231 for 81.91%, respectively. The results indicated

Table 2. The resistance to BLSB in maize of 282 inbred line and the heterosis information of the lines.

Lines	DR	HG	Lines	DR	HG	Lines	DR	HG	Lines	DR	HG	Lines	DR	HG
Qi318	MR	PB	7595-2	HS	BSSS	D46	HS	LRC	K14	HS	PA	Y8G	HS	PB
R18	MR	PB	803	HS	BSSS	Dan340	HS	LRC	K22	HS	PA	ZhongZi01	HS	PB
Shen3336	MR	PB	835	HS	BSSS	Dan360	HS	LRC	Liao184	HS	PA	196	HS	SPT
PA31	MR	T	8415	HS	BSSS	Dan598	HS	LRC	Liao2345	HS	PA	434	HS	SPT
T8	S	BSSS	8902	HS	BSSS	DH34	HS	LRC	Liao3053	HS	PA	444	HS	SPT
416	S	LAN	B104	HS	BSSS	J9206	HS	LRC	Liao371	HS	PA	4Z4	HS	SPT
C416	S	LAN	B73	HS	BSSS	Ji53	HS	LRC	Liao5114	HS	PA	502	HS	SPT
Dan1324	S	LAN	B84	HS	BSSS	Liao138	HS	LRC	Liao540	HS	PA	7379-2	HS	SPT
HZ85	S	LAN	Ji4112	HS	BSSS	LV9	HS	LRC	Liao6082	HS	PA	B467	HS	SPT
Ji992	S	LAN	Ji477	HS	BSSS	OH43	HS	LRC	Liao9586	HS	PA	CN962	HS	SPT
Mo17	S	LAN	Ji63	HS	BSSS	RedM	HS	LRC	Lu2548	HS	PA	DH212	HS	SPT
PH4CV	S	LAN	Ji81162	HS	BSSS	Sx707	HS	LRC	M14	HS	PA	H10	HS	SPT
Zi330	S	LRC	LX11	HS	BSSS	Tie9010	HS	LRC	M3005	HS	PA	H1124	HS	SPT
4379	S	PA	Qi205	HS	BSSS	W138	HS	LRC	N528-1	HS	PA	H152	HS	SPT
65232	S	PA	Si387	HS	BSSS	WF9	HS	LRC	PH6WC	HS	PA	H201	HS	SPT
BJ005	S	PA	U8112	HS	BSSS	Z106	HS	LRC	PI10	HS	PA	H21	HS	SPT
CAL70	S	PA	XZ153-2	HS	BSSS	Zong31	HS	LRC	PI41	HS	PA	H428-3	HS	SPT
CML206	S	PA	XZ218	HS	BSSS	ZZ4C1	HS	LRC	S7913	HS	PA	Hungye4	HS	SPT
CML292	S	PA	Ye107	HS	BSSS	1029	HS	PA	Shen118	HS	PA	HZ4	HS	SPT
CML51	S	PA	Z29	HS	BSSS	48-2	HS	PA	Shen5003	HS	PA	JH63	HS	SPT
Dan9046	S	PA	ZH68	HS	BSSS	4866	HS	PA	Si144	HS	PA	JH73	HS	SPT
ES40	S	PA	1263	HS	LAN	488	HS	PA	SiD105	HS	PA	JH76	HS	SPT
Ji046	S	PA	200B	HS	LAN	5022B	HS	PA	T5	HS	PA	Ji35	HS	SPT
Liao311	S	PA	374	HS	LAN	653	HS	PA	TS6278	HS	PA	Jing7	HS	SPT
Qi209	S	PA	485	HS	LAN	706F	HS	PA	W24	HS	PA	K12	HS	SPT
Z30	S	PA	5213	HS	LAN	7165-1	HS	PA	Ye478	HS	PA	Luyuan133	HS	SPT
141	S	PB	77	HS	LAN	7167-1	HS	PA	Ye52106	HS	PA	N28	HS	SPT
698-3	S	PB	C351	HS	LAN	7537-1	HS	PA	Ying64	HS	PA	P6Co	HS	SPT
89-1	S	PB	CA091	HS	LAN	7884	HS	PA	YML102	HS	PA	PI143	HS	SPT
Dan599	S	PB	D185	HS	LAN	7922	HS	PA	Z28	HS	PA	PI31	HS	SPT
Dan988	S	PB	D387	HS	LAN	8001	HS	PA	Z35	HS	PA	Shuang105	HS	SPT
JH59	S	PB	F19	HS	LAN	8129	HS	PA	Z451	HS	PA	Shuang741	HS	SPT
Ji1037	S	PB	H3	HS	LAN	81565	HS	PA	Z58	HS	PA	Si273	HS	SPT
L102	S	PB	He344	HS	LAN	832	HS	PA	ZH64	HS	PA	Si279	HS	SPT
P138	S	PB	J001	HS	LAN	888-9	HS	PA	8002	HS	PB	Si287	HS	SPT

Table 2. Contd.

P178	S	PB	J002	HS	LAN	B234	HS	PA	31778	HS	PB	SX605	HS	SPT
R15	S	PB	Ji412	HS	LAN	BHP44	HS	PA	C273	HS	PB	TangSPT	HS	SPT
y75	S	PB	Ji419	HS	LAN	BM130	HS	PA	C321	HS	PB	Te70	HS	SPT
ZM28	S	PB	Ji465	HS	LAN	C11-8	HS	PA	Dan3130	HS	PB	Tianya4	HS	SPT
H21	S	SPT	Ji495	HS	LAN	C28	HS	PA	DH25	HS	PB	Wenhuang	HS	SPT
Ch7-2	S	SPT	Ji842	HS	LAN	C8605-2	HS	PA	DH29	HS	PB	Wu314	HS	SPT
Guan17	S	SPT	LK11	HS	LAN	CA156	HS	PA	H9-21	HS	PB	Z22	HS	SPT
H66	S	SPT	MQ17	HS	LAN	Chang3	HS	PA	Han23	HS	PB	ZH204	HS	SPT
HuoTH	S	SPT	Si533	HS	LAN	Chong72	HS	PA	JH55	HS	PB	Zi495	HS	SPT
Ji846	S	SPT	SiF1	HS	LAN	CN165	HS	PA	JH96B	HS	PB	S37	HS	T
Ji853	S	SPT	Sx701	HS	LAN	D237	HS	PA	JiA-034	HS	PB	5311	HS	UN
LX9801	S	SPT	Yu12	HS	LAN	DH02	HS	PA	Lian87	HS	PB	5Gong	HS	UN
PI42	S	SPT	Zao49	HS	LAN	Dian11	HS	PA	Liao68	HS	PB	812	HS	UN
Si419	S	SPT	ZC546	HS	LAN	Dong156	HS	PA	LY92	HS	PB	CWFS8	HS	UN
F06	S	T	Zhong17	HS	LAN	Dong91	HS	PA	M0113	HS	PB	CWMS9	HS	UN
PA212	S	T	53-3	HS	LRC	Du321	HS	PA	Qi319	HS	PB	Nanwu	HS	UN
F17	HS	BSSS	8107	HS	LRC	E28	HS	PA	SH15	HS	PB	PI36	HS	UN
F22	HS	BSSS	C1073-7	HS	LRC	G649	HS	PA	Shen135	HS	PB	Q1261	HS	UN
3189	HS	BSSS	CA112	HS	LRC	H014	HS	PA	Shen136	HS	PB	Qing795	HS	UN
32	HS	BSSS	CA335	HS	LRC	HC	HS	PA	Shen137	HS	PB			
501	HS	BSSS	CA339	HS	LRC	Ji818	HS	PA	TZI8	HS	PB			
515	HS	BSSS	CA375	HS	LRC	K10	HS	PA	Y7	HS	PB			

DR = Disease resistance, HG = heterosis groups. PA, PB, BSSS, SPT, LRC and LAN presented the 6 temperate maize heterosis groups in China as described by Xie et al. (2007). T = Tropical, UN = the heterosis information unknown or unclear.

that most of the inbred lines did not have good resistance to BLSB of maize, and the germplasm of high resistance were extremely poor.

The comparison of the disease resistance of inbred lines from different heterosis groups

The heterosis groups of the lines were clustered according to the pedigree information and the previous study results of other researchers (Li and Wang, 2010; Wang et al., 2012; Feng et al., 2009; Gao et al., 2005; Shi, 2007; Liu et al., 2009). The 282 lines were clustered to seven heterosis groups which were commonly used in China. The results showed that the four lines of moderate resistant contain three lines from PB group and one line from the tropic population Suwan1. The 47 lines of susceptible contain 1, 13, 1, 13, 10, 7 and 2 lines from BSSS, PA, LRC, PB, SPT, LAN and tropic group, respectively. The 231 lines of highly susceptible contain 27, 73, 25, 42, 25, 29 and 1 lines from BSSS, PA, LRC, PB, SPT, LAN and tropic group, respectively (Table 3).

Further analysis showed that among the 28 BSSS lines, 1 and 27 of them were susceptible and highly susceptible to BLSB, respectively. Among the 86 PA lines, 13 and 73 of them were susceptible and highly susceptible to BLSB, respectively. Among the 27 LRC lines, 1 and 26 of them were susceptible and highly susceptible to BLSB, respectively. Among the 41 PB lines, 3, 13 and 25 of them were moderately resistant, susceptible and highly susceptible to BLSB, respectively. Among the 52 SPT lines, 10 and 42 of them were susceptible and highly

Table 3. The disease resistance of different heterosis group and different heterosis array.

Heterosis group	MR	S	HS	Total	Heterosis array
BSSS	0	1	27	28	SS
PA	0	13	73	86	SS
LRC	0	1	25	26	SS
PB	3	13	25	41	NSS
SPT	0	10	42	52	NSS
LAN	0	7	29	36	NSS
T	1	2	1	4	NSS
UN	0	0	9	9	
Total	4	47	231	282	

SS = The heterosis alignment presented by Borer Stiff Stalk Synthetic (BSSS) maize population, it contains the sub-groups of BSSS, PA and LRC in China.NSS = The heterosis alignment presented the maize germplasm did not belonged to the SS, it contains the sub-groups of LAN, PB and SPT in China.

susceptible to BLSB, respectively. Among the 36 LAN lines, 7 and 29 of them were susceptible and highly susceptible to BLSB, respectively. Among the 4T lines, 1, 2 and 1 of them were moderately resistant, susceptible and highly susceptible to BLSB, respectively (Table 3). These 282 lines were cluster to SS and NSS according to Xie et al. (2007), the results showed that 15 and 125 of the 140 SS lines were S and HS, respectively, and 4, 32 and 97 of the 133 NSS lines were MR, S and HS, respectively (Table 3).

DISSCUSSION

The factor effect the results of disease resistance evaluation

In our study, 282 maize inbred lines were identified for resistance to sheath blight by artificial infection of *R. solani* in four environments. The results showed that the evaluation results of same line in different locations varied. This could be explained by the reason that, the resistance to BSLB was quantity trait controlled by minor polygene, and it was easily affected by environments (Zhao et al., 2006).

In addition, the results of our study on some lines varied from that of the previous study, and the disease resistance were more weak in our study; the possible reason was that the environments in our study was more beneficial to the disease, the *R. solani* AG1-IA isolate have stronger pathogenic force, and the setout of disease nursery and artificial infection increase the chance of the happen of BLSB. From what has been mentioned above, in order to evaluate the disease resistance of maize lines to BLSB accurately, environments beneficial to the disease, *R. solani* isolate have stronger pathogenic force, artificial infection and proper disease nurseries were necessary.

The resistance inbred lines

Since the discovery of BLSB of maize in 1960s, many studies on this disease were carried out to improve the resistance of the hybrid in maize production; same progress has been made while the situation was not optimistic. The lack of resistance germplasm resource, extremely the resource could be applied in hybrid breeding, which was the biggest limitation in breeding for disease resistance (Cheng et al., 2009). The results of Huang et al. (2005) indicated that no immune or highly resistant germplasm to sheath blight caused by *R. solani* was found in the 161 maize germplasm evaluated. The results of the other study indicated that the immune or highly resistant germplasm were extremely poor (Zhao et al., 2006; Yang et al., 2003). The results indicated that the lack of commercial inbred resistant SLB to improve the disease resistance of the existing inbred lines and the hybrids. In our study, we identified the disease resistance of 282 inbred lines to BLSB, no immune or highly resistant germplasm was found and four moderately resistant inbred lines were identified. These moderately resistant inbred have been used as the parents for commercial hybrid, they have good performance in grain yield, combining ability and have a suitable growth period, could be good donor of disease resistance gene to improve the other maize lines.

The disease resistance of inbred lines from different heterosis groups

Although, there were some researches on the evaluation of disease resistance to BLSB in maize germplasm, while the analysis on the disease resistance of inbred lines from different heterosis groups were rarely for the reason that, the heterosis group information of the maize germplasm were difficult to obtain (Cheng et al., 2009). In our study, we consulted a large number of references and a lot of analyses were done to get the heterosis group information of the 282 maize lines (Li and Wang, 2010; Wang et al., 2012; Feng et al., 2009; Gao et al., 2005; Shi, 2007; Liu et al., 2009).

The results showed that all the lines belong to SS array

(including BSSS, PA and LRC group), which were commonly used as parents in commercial hybrids, and were susceptible or highly susceptible to the disease of BLSB. However, four lines (three PB and one T) from NSS array (including PB, LAN, SPT and TRP), which were commonly used as pollen parents in commercial hybrids, were moderately resistant to the disease of BLSB. The PB groups were the temperate maize germplasm introduced to tropical germplasm. The results showed that the lack of resistance germplasm in SS array was more serious and it was more effective to seek resistance germplasm from the NSS array, especially from the PB and tropic maize germplasm.

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