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Resistance Evaluation of Soybean Germplasm from Huanghuai Region to *Phytophthora* Root Rot

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Abstract

The aim of the study was to establish a set of differential strains and to identify soybean resistant genes to *Phytophthora* root rot and then to apply those strains for analysis of the resistant genes *Rps1a*, *Rps1c*, and *Rps1k* that soybean cultivars or lines may carry. Virulence formula of 125 *Phytophthora sojae* isolates were determined using the hypocotyls inoculation technique, the strains, which includ 6 isolates with different virulence formulas, were applied to identify the resistance of 55 soybean cultivars or lines and resistant genes were analyzed using the gene postulating procedure. Eighteen reaction types occurred in 55 cultivars or lines and results of gene postulation indicated that 2 cultivars or lines probably carried gene *Rps1c* and no cultivar may carry genes *Rps1a* or *Rps1k*. A few of soybean cultivars or lines from Huanghuai Region carry *Rps* genes *Rps1a*, *Rps1c* and *Rps1k* and tend to infect by *P. sojae*, so resistant cultivars or lines need to be bred and popularized actively.

Key words: *Phytophthora sojae*, differential strains, soybean, *Phytophthora* root rot, resistance gene

INTRODUCTION

Soybean root and stem rot, which was first described in America in 1951, caused by the oomycete pathogen *Phytophthora sojae*, is one of the most important diseases of soybean with an annual cost worldwide of 1-2 billion dollars. (Erwin and Ribeiro 1996; Wang *et al*. 2001a, b; Zhu *et al*. 2003; Tyler 2007). China is one of the major soybean producing countries in the world and soybean plays a main role in the national agricultural production. Unfortunately, this diease also occurres in Heilongjiang and Fujian provinces in China. Since *P. sojae* could be spread as oospores carried in soybean seed, as well as oospores in soil and diseased residues. It poses a serve threat to the soybean production in the two regions especially in Heilongjiang Province (Han *et al*. 1998). It is a soil-borne disease and very difficult to control except for resistant soybean cultivars or lines which are proved to be the most economical and effective (Zhu *et al*. 1999; Chen *et al*. 2008). Interaction between soybean and *P. sojae* follows the the so-called "gene-for-gene" model and more than 14 *Rps* genes at eight loci conferring to individual races of *P. sojae* have been identified (Anderson and Buzzell 1992; Buzzell and Anderson 1992; Burnham *et al*. 2003). *Rps* genes, especially *Rps1k*, have been successfully used in commercial cultivars (Tyler 2007)

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in America but not in China (Chen *et al*. 2008). Though abundant virulence diversity has been found in *P. sojae* in China (Zhu *et al*. 2003) and some common characteristics are found, the breeding and application of resistant cultivars or lines should be based on the common and different characteristics of *P. sojae* in different regions. At present, quantities of resistant soybean cultivars or lines evaluated are not effective for breeding resistant soybean varieties and controlling the disease as their resistant levels, *Rps* genes (Chen *et al*. 2008), and their target genes are not certain. Soybean cultivars or lines which carry *Rps* genes such as *Rps1a*, *Rps1c*, and *Rps1k*, could be widely used in the control of soybean root and stem rot based on our results that virulent frequency of 115 *P. sojae* isolates in China was not more than 10% on differential hosts which carry *Rps1a*, *Rps1c*, and *Rps1k* genes. The aim of this study was to screen effective germplasm for breeding and application of resistant soybean cultivars through establishing a set of differential strains which can identify the *Rps* genes and then analyze the 3 target genes and characteristics of soybean cultivars or lines from Huanghuai Region in China.

MATERIALS AND METHODS

Plant materials

Fifty-five soybean cultivars (lines) used in this study are listed in Table 1, while the differential cultivars or lines and *Rps* genes they carry are listed in Table 2.

Table 1 55 soybean cultivars or lines used in this study

Region	Number	Cultivar (line)
Beijing	6	Zhonghuang 22, Zhonghuang 25, Zhonghuang 15, Zhonghuang 3, Zhonghuang 7, Zhonghuang 14
Shanxi	10	Jinda 74, Fendou 55, Jinda 53, Jindou 11, Jindou 3, Jindou 14, Jindou 20, Jindou 21, Jindou 22, Jindou 26
Shandong	13	Wenfeng 5, Yuejin 10, Dabaima, Qinghuang 1, Wenfeng 7, Yuejin 5, Shanning 11, He 7308, Qihuang 2, Weimin 1, Qihuang 29, Zihuadou, Yanhuang 1
Henan	13	Yudou 5, Zhoudou 5, Zhoudou 11, Yudou 12, Huadou 20, Yudou 16, Yudou 26, Yudou 7, Zhengchangye 7, Zheng 126, Zhengzhou 135, Pingdou 1, Yudou 17
Hunan	2	Xiangchundou 16, Xiangchundou 23
Hubei	4	Edou 7, Aijiaozao, Zhongdou 3, Zhongdou 8
Zhejiang	1	Baiqianming
Fujian	2	Gutiandou, Changtinglüxie
Guangxi	1	Gui 119
Guizhou	2	Qiandou 2, Qiandou 6
Yunnan	1	Yuenfeng 5

Table 2 Virulence of differential lines on the differential soybean cultivars or lines for *P. sojae*

Differential host	*Rps* gene	Isolate[1)]					
		Pm2	Pm8	Pm17	Pm19	P1020	P1040
Harlon	*1a*	R	S	R	S	R	R
Harosoy13xy	*1b*	S	R	S	S	R	R
Williams79	*1c*	R	R	R	S	R	S
PI103091	*1d*	R	S	S	S	R	R
Williams82	*1k*	R	R	R	S	S	R
L76-1988	*2*	S	S	S	R	S	S
Chapman	*3a*	R	R	S	S	R	R
PRX146-36	*3b*	R	R	S	S	S	S
PRX145-48	*3c*	R	S	R	R	S	S
L85-2352	*4*	R	S	S	R	S	R
L85-3059	*5*	R	S	S	S	S	S
Harosoy62xx	*6*	R	S	S	R	S	S
Harosoy	*7*	S	S	S	R	R	R
William	*rps*	S	S	S	S	S	S
Virulence formula		1b, 2, 7	1a, 1d, 2, 3c, 4, 5, 6, 7	1b, 1d, 2, 3a, 3b, 4, 5, 6, 7	1a, 1b, 1c, 1d, 1k, 3a, 3b, 5	1k, 2, 3b, 3c, 4, 5, 6	1c, 2, 3b, 3c, 5, 6
Origin		America	Canada	Canada	America	Heilongjiang of China	Heilongjiang of China

[1)] R, resistant; S, susceptible.

P. sojae isolates

A set of identification strains used for analyzing *Rps* genes which carry in soybean cultivars or lines of a total of 6 *P. sojae* isolates (Table 2) were screened form 115 isolates and 10 races (data not shown).

Virulence test

The pathogenicity test was carried out by the hypocotyl injection technique as described by Wang (2006). Disease investigation was carried out by the method described by Chen *et al*. (2008). 0-30%, 31-69% and 70-100% seedlings out of a cultivar or line being killed in the test indicated a resistant, intermediate and susceptible response, respectively. Cultivars or lines showing intermediate response were then continued for the pathogenicity test for another 2 to 3 times.

RESULTS

Establishment of differential strains

Virulence of 115 *P. sojae* isolates in China was determined in 2008 and the proportion of isolates which could overcome the soybean cultivar carrying *Rps* genes, *Rps1a*, *Rps1c* or *Rps1k* were 6.1%, which showed that *P. sojae Avr* genes (*Avr1a*, *Avr1c*, and *Avr1k*) were widely distributed in *P. sojae* isolates in China. Therefore, we propose that soybean cultivars or lines carrying *Rps1a*, *Rps1c* and *Rps1k* genes will help to control the *Phytophthora* root rot of soybean on the basis of the so-called "gene-for-gene hypothesis". So 115 isolates and 10 races preserved in our lab were screened, a set of differential strains composed of six isolates were established finally and their virulence on differential host is listed in Table 2. Strains Pm2 (P6497), Pm8, Pm17, and Pm19 are standard physical races kept in our lab and P1020 and P1040 are single-zoospore isolates obtained from Heilongjiang Province.

Postulation of resistance gene

***Rps1a*, *Rps1c*, and *Rps1k* genes** Resistance of 55 soybean cultivars or lines was evaluated, then resistance genes were postulated based on 18 reaction types produced ultimately (Fig.). 23 soybean varieties or lines such as Zhengzhou 135, Pingdou 1 and so on showed a reaction type of SSSSSS (on isolate Pm2, Pm8, Pm17, Pm19, P1020 and P1040, respectively) as the same as soybean Williams which was a negative control. It indicated apparently that they did not carry any known *Rps* gene. The remaining was resistant to 1 to 5 isolates with 17 reaction types. Zhoudou 5 and Jinda 74 may carry *Rps* genes *Rps1c* or combinations of *Rps1c* and *Rps5* or *Rps1c* and *Rps3b* according to the genes postulated, so they may contain *Rps1c* gene. However, no cultivars or lines may carry *Rps1k* or *Rps1a* as they did not produce the reaction types of RRRSSR or RSRRRS, respectively. Yudou 5 and Wenfeng 5 showed a reaction type of RRRSRR, which indicated that they may hold obviously one of the 3 target genes as they may carry combinations of *Rps* genes *Rps1a* and *Rps3b* or *Rps1c* and *Rps3a* or *Rps1k* and *Rps3a*. The result also showed that only 4 to 5 cultivars or lines carry the 3 target genes with a proportion of 7.3 to 9.1%, which made us come to the conclusion that *Rps* genes *Rps1a*, *Rps1c* and *Rps1k* are not widely distributed in soybean germplasm from Huanghuaihai region. Similarly, 20.0% of 55 cultivars or lines were resistant to strain Pm17, which also support our conclusion above as strain Pm17 (race 17) carries *Avr* genes, *Avr1a*, *Avr1c*, and *Avr1k*.

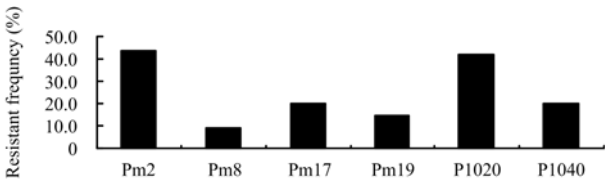

Fig. Resistance frequency of 55 soybean cultivars or lines on different *P. sojae* isolates.

Other resistant genes According to the results of genes postulated, three varieties, Yudou 16, Yudou 26 and Dabaima, may carry *Rps5* while Zhongdou 8, Wenfeng 7, and Yuejin 5 may contain *Rps1d* or a combination of *Rps1d* and *Rps5*. The results also indicated that Zhonghuang 22 may contain a combination of resistance genes *Rps1a* and *Rps7*, or *Rps1a* and *Rps3c*, or *Rps5* and *Rps7*, while Yuejin 10 and Fendou 55 may carry a combination of *Rps1d* and *Rps7*, or *Rps1d* and

Rps2, or *Rps5* and *Rps7*. Additionally, 19 soybean cultivars or lines such as Xiangchundou 16, Zhonghuang 15, etc., may carry unknown genes or combinations as they produced a total of 13 reaction types, which were different from any specific type that resulted by a known single gene or combinations of several genes.

Distributions of resistant genes

Resistance of 55 soybean cultivars or lines to the 6 isolates of the differential strains was assessed (Table 3). The result showed that resistant frequencies to the 6 isolates were 43.6, 9.1, 20.0, 14.5, 41.8 and 20.0%, respectively. The highest and lowest frequency of resistance was for the isolates P1020 and Pm8, respectively. The result also indicated that virulent frequency, which ranged from 16.7 to 100% on the same soybean cultivar or line, varied with the 6 isolates. Although frequencies of some soybean cultivars or lines were the same, their virulent spectrum was different, which indicated that they may carry different *Rps* genes. For example, virulent frequencies of Zhongdou 3, Zhoudou 11, Jindou 11, Qihuang 1, Yudou 7, Yuejin 5, Wenfeng 7, Gutiandou, Huadou 20, Yudou 12, Yanhuang 1, Zheng 126 and Zhengchangye 7 were 33.3%, however their resistant spectrum were not the same. At the same time, 32 soybean varieties were resistant to 1 to 5 isolates. The results above indicated that resistant soybean germplasm resources were rich in Huanghuai region in China.

Table 3 Result of resistance genes from 55 soybean cultivars or lines

Reaction type	Cultivar (line)	Resistant gene
RRRSRR	Yudou 5, Wenfeng 5	*1a+3b*, *1c+3a*, *1k+3a*
RRRSRS	Zhoudou 5, Jinda 74	*1c*, *1c+3b*, *1c+5*
RRRSSR		*1k*, *1k+3b*, *1k+5*
RSRRRR	Zhonghuang 22	*1a+7*, *1a+3c*, *2c+7*
RSRRRS	Jinda 53	Unknown
RSRSRR		*1a*, *1a+5*
RSRSRS	Zhoudou 11	Unknown
RSRSSS	Edou 7, Zhonghuang 25	Unknown
RSSRRR	Yuejin 10, Fendou 55	*1d+7*, *1d+2*, *5+7*
RSSRRS	Qihuang 1, Zhonghuang 15, Jindou 11	Unknown
RSSRSS	Jindou 3	Unknown
RSSSRR	Zhoudou 8, Wenfeng 7, Yuejin 5	*1d*, *1d+5*
RSSSRS	Yudou 12, Huadou 20, Zhonghuang 3	Unknown
RSSSSS	Yudou 16, Yudou 26, Dabaima	*5*
SRSSSS	Xiangchundou 16	Unknown
SSRSRS	Zhongdou 3, Zhonghuang 7	Unknown
SSSSRR	Yudou 7	Unknown
SSSSRS	Yanhuang 1, Gutiandou	Unknown
SSSSSR	Zhengchangye 7, Zheng 126	Unknown
SSSSSS	Zhengzhou 135, Pingdou 1, Shanning 11, Yunfeng 5, Changtinglüxie, Gui 119, Qiandou 2, Qiandou 6, Aijiaozao, Xiangchundou 23, Zhonghuang 14, Baiqianming, Yudou 17, He 7308, Qihuang 2, Weimin 1, Jindou 14, Jindou 20, Jindou 21, Jindou 22, Jindou 26, Qinghuang 29, Zihuadou	*rps*

The resistant results showed *Rps* genes carried in soybean varieties from Henan Province are the highest, followed by Beijing, Shandong, and Shanxi. The proportion of resistant varieties varied with different provinces, which indicated that there are regional differences in resistance level of soybean cultivars or lines from different provinces. For insistence, the proportion of reaction types resulted by soybean varieties from Beijing, Henan, Shandong, and Shanxi was 33.3, 44.4, 38.9, and 33.3%, respectively.

DISCUSSION

Definition of differential strains and value of *Rps* genes, *Rps1a*, *Rps1c*, and *Rps1k*

"Differential strains" is a concept presented for identification of *Rps* genes, *Rps1a*, *Rps1c*, and *Rps1k* that soybean cultivars may carry in this study, namely, a set of *P. sojae* isolates used to test the *Rps* genes car-

ried by the soybean cultivar, for example 12 and 7 isolates were used by Chen *et al*. (2008) and Sun *et al*. (2008) to test the *Rps* genes, respectively. The concept is different from a genealogy of single-zoospore lines or single-oospore progenies; the former is a combination of strains or isolate with different genetic backgrounds.

Growing resistant cultivars or lines is the most economical, safe and effective method for controlling Phytophthora root rot of soybean. Presently, resistant genes have been successfully applied to commercial soybean varieties and those with a single *Rps* gene including *Rps1k* have been applied extensively in disease control in the United States (Cui *et al*. 2004; Tyler 2007), unfortunately, such researches advance slowly in China. We propose in this study that soybean varieties containing *Rps1a*, *Rps1c* or *Rps1k* genes will be effective in reducing the damage of the disease based on our results of virulence test carried in 2008. Soybean cultivars or lines Zhoudou 5 and Jindou 74 may carry *Rps1c*, however, Wenfeng 5 and Yudou 5 possibly contain one of the 3 target genes, while Zhonghuagn 22 may carry *Rps1a* according to the final results. Similarly, reaction types caused by 19 cultivars or lines were different not only from types resulted by any known single *Rps* gene but also types led by combinations of two known genes, which indicated that they may carry a new *Rps* genes or a combination of genes.

Distributions of resistant soybean germplasm

Results obtained in this study indicated that there were quantities of resistant soybean cultivars or lines in Huanghuai region in China. 23 varieties were totally susceptible to all the 6 *P. sojae* isolates and the remainders were resistant to only 1 to 5 isolates, which indicated that resistant cultivars or lines need to be bred and popularized actively as soybean varieties in this region tend to be susceptible to different isolates. A number of studies indicated that there is an approximate similarity in genetic backgrounds and resistance levels for most resistant varieties from the same regions (Kyle *et al*. 1998; Chen *et al*. 2008; Sun *et al*. 2008) and our results support the conclusion too, such as Zhongdou and Yudou series which from Beijing and Henan provinces, respectively. Additionally, we found that there were regional differences in quantities of resistant varieties and resistance level which was similar with that of Kyle *et al*. (1998), and some scholars pointed that this differences were associated with narrow scope of parents used by breeding organizations and regional pressure brought by *Phytophthora* root rot of soybean (Zhu *et al*. 2003; Chen *et al*. 2004; Cui *et al*. 2004; Chen *et al*. 2008). The results of 115 isolates identified also indicated that virulence formula of *P. sojae* isolates in China was complex, as the same time there are features of a rich diversity of resources and resistance in soybean germplasm in Huanghuai Region (Wang *et al*. 2001a, b). Therefore, scientific distribution of resistant varieties in the same area should be directly carried out to prevent from the prevalence and damages of soybean root and stem rot.

Advantages and disadvantages of gene postulation procedure

Gene postulation procedure is a simple and rapid approach for analyzing resistant genes carried in soybean varieties. However, effectiveness of this method could be influenced by factors, for insistence, the combinations of *Avr* gene in differential strains, genetic stability, quantity of *Rps* genes in the host, inoculation methods, environmental conditions and genetic background of the hosts, etc. Similarly, genetic genealogy procedure must be applied for those soybean varieties who have unclear genealogies, complicated genetic backgrounds and multi-resistant genes (Kyle *et al*. 1998). Additionally, a *Rps* gene which make cultivars or lines resistant to all isolates used in one study can only be postulated, however it can not be distinguished by this procedure. In conclusion, resistance genes can be firstly postulated using this procedure then be confirmed by conventional genetic methods and molecular biology techniques for some resistant soybean germplasm.

It is sufficiently effective for screening new resistant germplasm and genes through gene postulation procedure in spite of its limitations (Dorrance and Schmitthenner 2000; Zhu *et al*. 2006; Chen *et al*. 2008). The application of molecular markers closely linked with the genes, such as sSSR can not only improve the accuracy of the results postulated, speed up the process of fine mapping for new genes, but also greatly shorten the schedule for the identification and application of new genes, a case in point is that *Rps8* gene which took researches only 3 years to mapping it after *Rps8* was discovered (Hegstad *et al*. 1998; Demirbas *et al*. 2001; Diers *et al*. 1992).

CONCLUSION

Applying scientifically available resistant varieties and breeding new germplasm actively are the most economical and effective methods for control of soybean root and stem rot as it is a soil-borne disease for which chemical control measures are always ineffective. Our study showed that resistant genes *Rps1a*, *Rps1c*, and *Rps1k*, which could be effective for controling this disease, are not widely distributed in soybean germplasm in Huanghuaihai region, consequently, it is important to breed resistant cultivars or lines actively. As our research indicated that there are dramatic differences in quantity and level of resistant germplasm in this region, it will be beneficial to the sustainability and validity for the control of soybean root and stem rot by the measures as follows, namely, to breed resistant varieties using broad-spectrum resistant varieties, especially cultivars or lines carrying *Rps* genes *Rps1a*, *Rps1c*, or *Rps1k*, as parents from different regions, to improve resistant level and broaden the genetic backgrounds then widely grow them.

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