Laboratory manual for studies on soybean rust resistance

Soybean [*Glycine max* (L.) Merrill] is an economically important legume crop, with more than 80 million tons exported to the world market mainly from North and South American countries. Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* Sydow & P. Sydow, is one of the biggest threats to stable soybean production in South America and in other tropical and sub-tropical regions.

Various studies related to the pathogenic variations of ASR and the development of ASR-resistant cultivars in each country have been done. Since ASR pathogens are widespread across borders, each country has had to cope with this disease based on information using a common resistance evaluation method. However, it was difficult to compare the pathogenicity of ASR pathogens and the degree of ASR resistance in soybean genotypes among countries because the evaluation method related to ASR resistance was not standardized. Therefore, a uniform procedure for conducting ASR studies is necessary.

First, we standardized the experimental protocols – i.e., 1) multiplication of ASR urediniospores, 2) single-lesion isolation, 3) inoculation of soybean with spore suspension, 4) evaluation of ASR pathogenicity, 5) evaluation of ASR resistance in soybean genotypes, and 6) evaluation of ASR tolerance of soybean genotypes (Figure 1, Table 1) -- to obtain experimental results that are reproducible. South American ASR pathogens and the differential varieties were utilized for this work (Table 2). Then, we optimized the experimental protocols related to SSR marker analysis for marker-assisted selection (MAS) so that domestic institutions in South America can carry out their soybean breeding programs for ASR resistance. Finally, we compiled these standardized experimental protocols into a single manual, titled “Laboratory manual for studies on soybean rust resistance,” which can be accessed from the JIRCAS website: http://www.jircas.affrc.go.jp/english/manual/soybean_rust/JIRCAS_manual_soybean_rust.pdf.

The data for the pathogenicity of ASR pathogens and for the degree of ASR resistance in soybean genotypes obtained by following this manual can be compared with previously obtained data (Akamatsu et al., 2013). Therefore, this manual is expected promote research related to pathogenic variations of ASR pathogens and marker-assisted soybean breeding for ASR-resistant cultivars.

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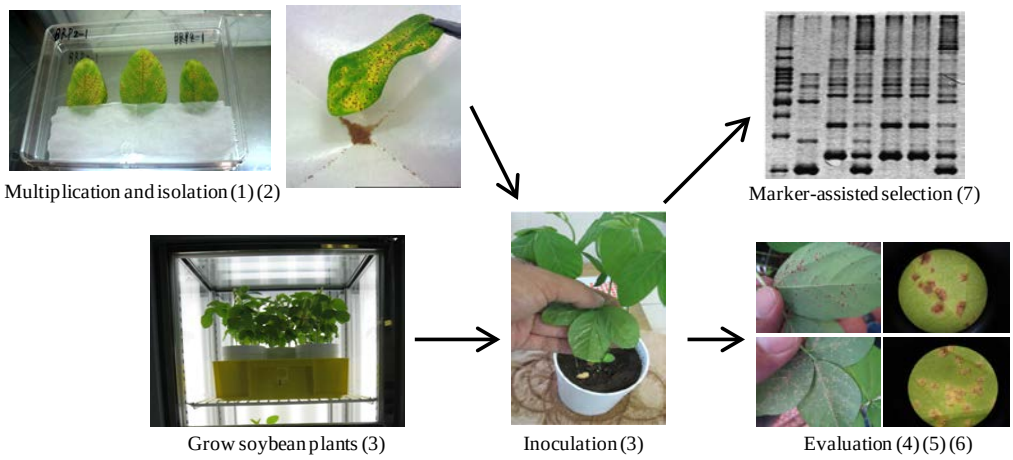

Fig. 1. Evaluation process for Asian soybean rust (ASR) resistance. The numbers in the figure correspond to that of Table 1.

Table 1. Contents of the manual for Asian soybean rust (ASR) resistance

No.	Item	Details
1	Multiplication of ASR urediniospores	Method to multiply ASR urediniospores for the following experiments
2	Single-lesion isolation	Method to isolate ASR pathogens from the ASR population that may contain various races
3	Inoculation of soybean with spore suspension	Methods to grow soybean plants, to prepare urediniospore suspension, and to inoculate urediniospore suspension to soybean plants
4	Evaluation of ASR pathogenicity	Method to evaluate virulence of ASR pathogens based on the lesion type
5	Evaluation of ASR resistance in soybean genotypes	Method to evaluate resistance of soybean genotypes based on the lesion type
6	Evaluation of ASR tolerance of soybean genotypes	Method to evaluate tolerance of soybean genotypes based on infection index and degree of leaf-yellowing
7	Marker-assisted selection of ASR resistance	Method of SSR marker analysis for the marker-assisted soybean breeding of ASR resistance

Table 2. Example of pathogenic data for Asian soybean rust (ASR) pathogens based on the resistance reactions of the differential varieties

Country	Location	Season	Differential varieties* No.	PI 200492 1	PI 368039 2	PI 230970 3	PI 417125 4	PI 462312 5	PI 459025 6	PI 200562 7	PI 416764 8	PI 587855 9	PI 587880A 10	PI 587886 11	PI 587905 12	PI 594767A 13	BRS 154 14	TK5 15	PI 548628 16
Argentina	Pergamino,	2007/200		S	S	S	S	nd	S	R	R	nd	R	S	R	R	nd	S	S
	Buenos Aires	2009/201		S	S	S	S	S	R	IM	S	I	R	S	S	IM	S	S	S
Brazil	Passo Fundo,	2007/200		S	S	R	IM	S	S	R	IM	IM	S	S	IM	R	S	S	S
	Rio Grande do	2008/200		S	S	IM	S	I	IM	R	S	I	I	S	I	R	S	S	S
		2009/201		S	S	S	IM	I	R	R	S	I	I	S	R	R	S	S	S
Paraguay	Capitán Miranda,	2007/200		S	S	S	S	S	S	R	S	I	R	S	I	R	S	S	S
	Itapúa	2008/200		S	R	S	S	S	R	R	R	I	I	S	I	R	S	R	S
		2009/201		IM	S	IM	IM	I	IM	R	IM	I	I	S	I	R	S	R	S
Japan	Tsukuba	2007		R	R	S	S	R	R	R	R	nd	I	S	R	R	nd	S	S
		2008		I	I	R	R	R	R	I	I	nd	R	R	R	nd	nd	S	R

I: Immune; R: Resistant; IM: Intermediate; S: Susceptible; nd: no data
* Latest set includes 17. PI517602B and 18. No6-12-1.