

# Detached Leaf Assay: A Rapid Screening Technique to Study Foliar Diseases of Soybean

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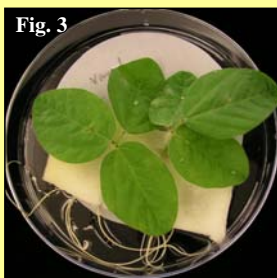
## INTRODUCTION



Host plant resistance may offer a realistic solution to identify resistance sources to soybean powdery mildew (Fig. 1) and rust (Fig. 2). Evaluating response of soybeans to these diseases usually involves inoculating whole plants with the pathogen. This method, however, is often undesirable in screening for multiple disease resistance. Therefore, we present the detached leaf assay an alternative to whole plant evaluations, which facilitates handling large numbers of genotypes, and also allows an individual plant/genotype to be challenged with several foliar pathogens. We present the results of our investigations on the development of a rapid screening technique to evaluate soybean for resistance to rust and powdery mildew.



## MATERIALS AND METHODS



### Inoculation, Incubation and Evaluation

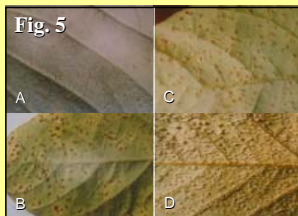
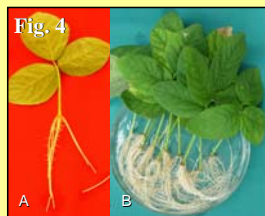
**Rust:** Ten soybean cultivars (Table 1) were tested in both detached leaf assay (DLA) and whole plant assay (WPA) for resistance against six isolates of *Phakopsora pachyrhizi* at Guangxi Academy of Agricultural Sciences, China.

**In DLA,** 12 trifoliolate leaves cultivar<sup>-1</sup> isolate<sup>-1</sup> were horizontally placed in plastic Petri dishes (@ 3 leaves/dish) containing a thin layered moist sponge. All 40 Petri dishes per isolate were spray inoculated using freshly prepared spore suspension ( $1 \times 10^4$  ml<sup>-1</sup>) and the plates were then incubated in dark for 24 h at 24±1C. Subsequently, the plates were incubated for a month in the laboratory with fluorescence light at 24±2C.

**In WPA,** at 40 cells per isolate were spray inoculated as detailed in DLA. The inoculated plants were incubated in dark for 24 h at 24±1C. Subsequently, the plants were uncovered and incubated for a month under fluorescence light at 24±1C. Three seedlings per genotype were maintained as un-inoculated control. The severity of soybean rust both in DLA and WPA was assessed following the severity scale developed by the Institute of Oil Crops Research, Chinese Academy of Agricultural Sciences, China (Table 1)

**Powdery Mildew:** Three soybean cultivars were tested in DLA (Figs. 3 and 6B) and WPA (Fig. 6A) for resistance against three concentrations ( $10^5$ ,  $10^4$ ,  $10^3$  conidia ml<sup>-1</sup>) of *Microsphaera diffusa* at ISU following a similar method as was used with rust. Number of patches/lesions plant<sup>-1</sup> or leaf<sup>-1</sup> were evaluated.

## RESULTS AND SUMMARY



**Root regeneration:** While testing for rust and powdery mildew resistance following DLA, roots were regenerated from the trifoliolate leaves (Figs. 4A, 4B and 6B).

**Symptoms:** Rust pustules were observed on adaxial surface of the leaves (Figs. 5A to 5D). Response of cultivars evaluated in DLA and WPA against six isolates is given in Table 1. Powdery patches/lesions were observed on the abaxial surface of inoculated leaves (Figs. 6A and 6B). P93M42 showed higher severity compared to other two cultivars (Fig. 7).

Table 1. Response of 10 soybean cultivars against six isolates of *Phakopsora pachyrhizi*

Cultivars	Rust reaction* of six isolates** in DLA					
	1	2	3	4	5	6
Ankur	S-L	S-L	S-S	S-L	S-L	S-L
T K5	S-L	S-S	S-S	S-L	S-S	S-L
T N4	S-L	S-L	S-S	S-L	S-L	S-L
P I 459025	R-N	R-N	R-N	R-N	R-N	S-L
P I 230971	R-N	R-N	S-L	R-BR	S-L	S-L
P I 200492	R-BR	R-BR	S-L	S-L	S-L	R-BR
Hou-Zi-Mao	S-L	S-L	S-S	S-L	S-L	S-L
Liu-Dou No.1	R-N	R-N	R-N	R-N	R-N	S-L
SRE-B-15B	S-L	S-L	S-L	S-L	S-L	R-N
Ye-Qing × Bu-Lei-Mei	S-S	S-S	S-S	S-S	S-S	S-S

\* Based on a scale developed by the Institute of Oil Crops Research, CAAS, China; Where, O= No symptoms; R-BR= Hypersensitive reaction with needle size black spot, no pustule; R-N= Necrosis symptoms with red or purple colored necrosis surrounded by reddish color tissue, small pustule normally appear 14-20 days after inoculation, later, a few or no visible spores; S-L= Restricted type with blackish and restricted lesions, pustule collapse, and large amount of spore exposed, leaves remain deeper green; S-S= Spread type with many pustules formed and collapsed after 1 week; spreading lesions with thick spores; leaf turned yellow quickly.

\*\* Isolate 1 & 2 were collected from genotype Yangsuo Xiao Mao duo and Yangsuo Huang-Dou grown at Guilin; 3 & 4 were collected from Liu-Dou No.1 & Xiao-Li-Liu-Ye-Hung grown at Liuzhou and isolate 5 & 6 were collected from Gui-Dou No.1 and Ping-Guo-Dou grown at soybean rust nursery, Nanning, China.

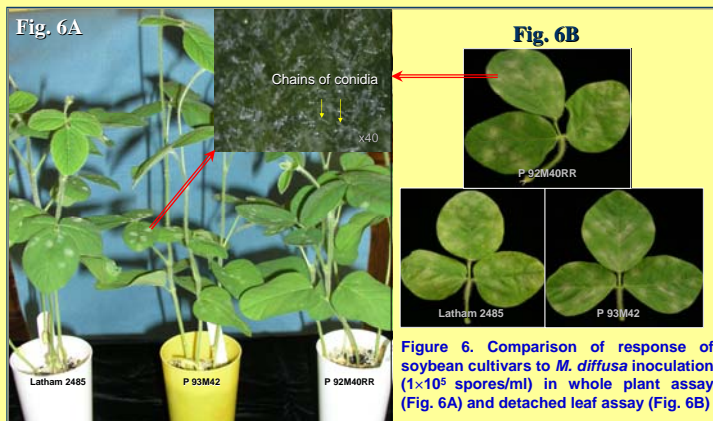


Figure 6. Comparison of response of soybean cultivars to *M. diffusa* inoculation ( $1 \times 10^5$  spores/ml) in whole plant assay (Fig. 6A) and detached leaf assay (Fig. 6B)

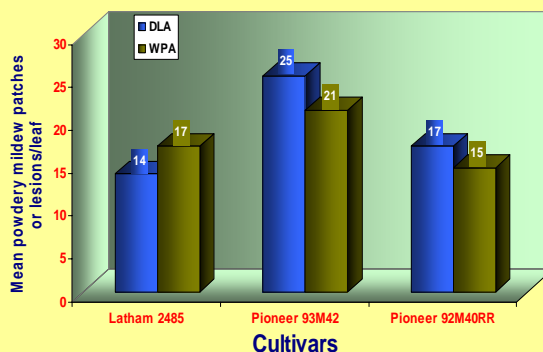


Figure 7. Response of soybean cultivars to powdery mildew in detached leaf assay (DLA) and whole plant assay (WPA) across three spore concentrations

## LITERATURE

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 Herath, et al., 2001. Evaluating faba beans for rust resistance using detached leaves. Euphytica. 117:47-57.

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