A detached leaf method to evaluate soybean for resistance to rust

Introduction

Soybean rust has the potential to cause severe yield losses worldwide. The causal agent, *Phakopsora pachyrhizi*, is an obligate parasite and a highly variable pathogen. Host plant resistance may offer a potential practical solution. Rapid screening methods to evaluate host resistance and pathogen variability are needed to hasten progress in developing rust-resistant cultivars. An alternative to whole plant evaluations for rust resistance is a detached leaf assay (1) which has a number of advantages, including accurate quantification of disease development and pathogen reproduction.

Objectives

1. Optimize the detached leaf assay for evaluating soybean lines for rust resistance.
2. Compare rust infection parameters in field, greenhouse and detached leaf assays.

Materials and Methods

1. a. Concentration of hormones to retard senescence of detached leaves: Gibberellic acid (5 and 15 µg/ml), kinetin (10, 20 and 50 µg/ml), benzimidazole (20, 40 and 60 µg/ml), and IBA (3 µg/ml) incorporated in 1% technical agar.
   b. Leaf age: Mid-canopy leaves from 1-2, 3-4 and 5-6 week-old plants.
   c. Spore concentrations for inoculation: 1×10^2, 1×10^3, 1×10^4, 1×10^5 and 1×10^6 spores ml^{-1}.
2. Fourteen genotypes with varying levels of resistance were evaluated using detached leaves, and in greenhouse and field to determine the correspondence of results between the three assays.

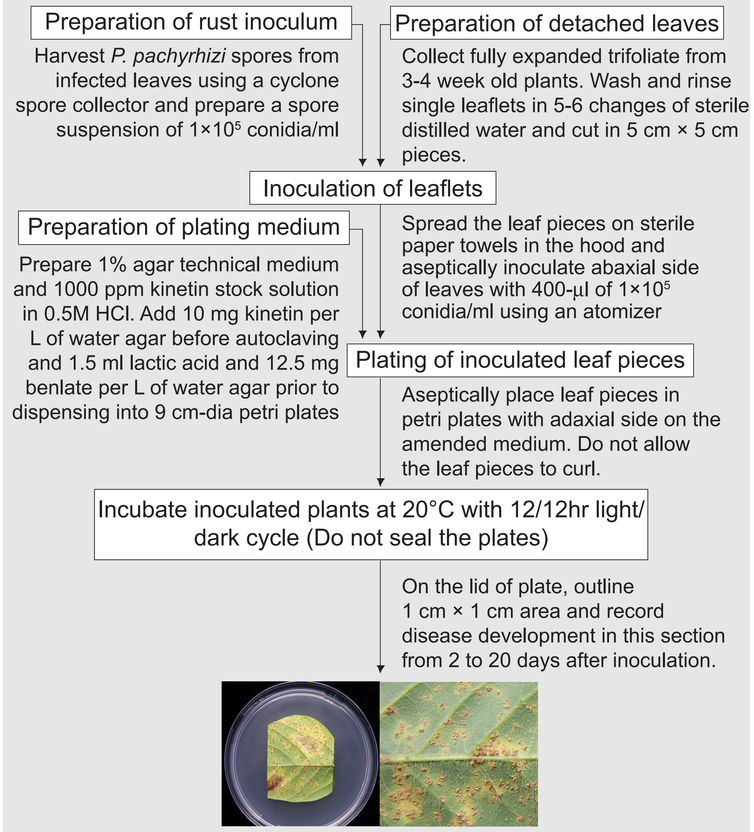

Figure 1. Flow diagram of detached leaf technique

3. Data collection: Leaf area infected and proportion of sporulating lesions were recorded at R6 stage in the field. Various infection parameters were observed in the greenhouse and detached leaf assays (Table 1). Correspondence among the various infection parameters from field, greenhouse and detached leaf was determined using correlation analysis.

Table 1. Infection parameters of selected soybean lines evaluated for resistance to soybean rust (*Phakopsora pachyrhizi*) 18 days after inoculation of detached leaves in Petri dishes and plants in the greenhouse

Soybean lines	Evaluation method	Number of days to			Number of lesions per cm^2	Pustules per lesion	Spores per pustule	Lesion diameter (µm)	Lesion type[a]
		Lesion appearance	Pustule formation	Pustule eruption					
PI594538A	Detached leaf	7	No pustule formation		9	0.0	0	171	HS
	Greenhouse	7	No pustule formation		3	0.0	0	280	HS
UG5	Detached leaf	7	14	16	8	1.0	60	352	RB
	Greenhouse	6	14	16	5	1.4	66	682	RB
TGx 1903-3F	Detached leaf	5	8	11	24	2.2	194	260	TAN
	Greenhouse	5	7	9	26	2.4	277	370	TAN
TGx 1485-1D	Detached leaf	5	7	10	28	2.9	318	344	TAN
	Greenhouse	5	7	9	31	3.5	406	387	TAN
LSD (α = 0.05)	Detached leaf	0.1	0.3	2.6	3.2	0.4	22.9	34.5	..
	Greenhouse	0.1	0.5	0.4	5.5	0.6	24.2	42.7	..

[a] HS = hypersensitive; RB = reddish brown; TAN = tan-colored lesions.

M. Twizeyimana[1], Bandyopadhyay R[1], Ojiambo P. S.[1], Paul C[2], and Hartman G. L.[2,3]
1. International Institute for Tropical Agriculture, PMB 5320, Ibadan, Nigeria,
2. National Soybean Research Center, 1101 W. Peabody Drive, Urbana, IL 61801, and
3. USDA-ARS.

Results

- Pieces of detached leaves from 3 to 4-week-old plants, 1% agar amended with 10 mg/l kinetin and inoculum at $1x10^5$ spores per ml were the optimal components for the detached leaf assay.
- The detached leaves remained green for ~30 days when plated on amended medium. Sufficient disease developed within 18 days to differentiate susceptible and resistant lines (Fig. 2).
- Infection parameters from greenhouse and detached leaves were similar (Table 1).
- Lesion types in all three assays were identical. Pearson correlation coefficients across genotypes were highly significant ($P < 0.01$) between detached leaf and:
  - Greenhouse for:
    - Days to lesion appearance ($r = 0.86$)
    - Days to pustule formation ($r = 0.98$)
    - Days to pustule eruption ($r = 0.89$)
    - Lesion number per cm^2 ($r = 0.86$)
    - Pustules per lesion ($r = 0.99$)
    - Spores per pustule ($r = 0.99$)
    - Lesion diameter ($r = 0.81$)
  - Field screening for sporulation ($r = 0.82$)

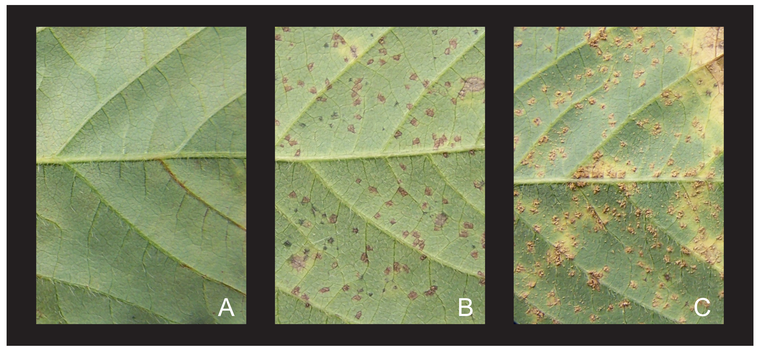

Figure 2. Rust development on detached leaves of soybean lines PI594538A (hypersensitive, A), UG5 (Red-Brown, B) and TGx 1485-1D (TAN, C) 18 days after inoculation

Conclusion

The detached leaf assay is a rapid and reliable method to evaluate germplasm, breeding lines and mapping population for rust resistance, and *P. pachyrhizi* isolates for pathogen variability in a short time with minimal cost and under uniform infestation. The method can be also used to monitor symptom development and infection parameters over time.

Reference

1. Burdon, J.J., and Marshall, D.R. 1981. Evaluation of Australian native species of *Glycine* for resistance to soybean rust. Plant Dis. 65:44-45.

Acknowledgement

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