POTENTIAL OF DETACHED SOYBEAN LEAVES FOR EVALUATION OF RUST RESISTANCE

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Overview

Soybean rust is one of the most important foliar diseases of soybean. It is caused by Phakopsora pachyrhizi, a fungal pathogen that produces copious amounts of spores that can spread via wind currents (1). Various management techniques have been employed to control rust; one of these is host resistance.

An alternative to whole plant evaluations for rust resistance are detached leaf assays which have a number of advantages. including accurate quantification of disease development and pathogen reproduction.

Objectives

- 1) Optimize the detached leaf assay for screening soybean lines for rust resistance.
- 2) Optimize concentration of inoculum and detached leaf age.
- 3) Compare rust reaction among Nigerian (IITA) lines.

Materials and Methods

- 1) Treatments consisted of water agar medium amended with benzimidazole (10 mg/l), kinetin (10 mg/l), gibberellic acid (5-15 ppm), indole butyric acid (IBA) (0.5 mg/ml) and 6-benzylaminopurine (BAP) (5 mg/ml) in various combinations (Table 1). Unifoliolate leaves from 14-day-old seedlings were removed, immersed in sterile water, placed on the agar media in petri dishes with 5 mm of the petiole pressed into the agar, and incubated at 27 °C under a 12-hour photo period. Fourteen days after inoculation, inoculated areas were scored on a 0-9 scale for green area deterioration (Table 1), where 0 is less and 9 is greater deterioration.
- 2) Detached leaves were used to evaluate five concentrations of inoculum and leaf age differences using two IITA lines.
- 3) Detached leaves in petri dishes were inoculated with 10ul of a mixture of Nigerian isolates. Days to pustule formation, days to spore release, number of pustules/cm², and number of spores/pustule were recorded (Table 2).

Results

Fig. 1. Detached leaf assays



Pieces of sovbean leaves in plastic plates remain green for 20 days (upper left). When inoculated, both reaction types can be observed (upper and lower right). With BAP, leaves stayed green longer than 30 days and root production was stimulated (lower left).

Fig. 2. Evaluation of different urediniospores concentrations using detached leaves (14 DAI).



Fig. 3. Leaf age effect on the number of pustules on TGx 1485-1D and TGx 1448-2E (14 DAI).





(susceptible)



RB reaction (resistant)

Green color

deterioration of detached leaves amended with plant hormones.

Treatment	(scale 0-9) 18 DAP	
Agar+BAP (2.5ppm)	1.0	
Agar+Kin 10ppm	1.3	
Agar+Kin 20ppm	1.8 1.8 2.3 2.8 4.5	
Agar+Gibb 5ppm & Kin 10 ppm		
Agar+Kin 50ppm		
Agar+Gibb 5ppm & Kin 50 ppm		
Agar+Gibb 5ppm		
Agar	4.8	
Agar+Gibb 5ppm & IBA 3/ml)	5.3	
Agar+IBA 3/ml)	5.5	
Agar+Kin 10ppm & Gibb 5ppm & IBA 3/ml)	6.0	
Agar+Kin 10ppm & IBA 3mg/ml)	6.3	
Agar+Sucrose 25% pH9	8.0	
Agar+Sucrose 10%	8.3	
Agar+Sucrose10% pH9	8.3	
Agar+Sucrose 50% pH9	8.5	
Agar+Benz 20ppm	8.7	

Table 2. Evaluation of IITA lines using detached leaves.

and the second se	Number of days to		Number of	
Line	Pustule formation	Spore release	Pustule per cm ²	Spores per pustule
UG-5	10	12	6	100
TGx 1903-9 3F	9	10	9	188
TGx 1895-7 50F	7	9	17	241
TGx 1897-7 17F	7	9	18	323
TGx 1895-7 49F	7	9	19	358
TGx 1871-8 12E	8	13	20	290
TGx 1740-7 2F	7	8	21	355
TGx1895-7 6F	7	9	31	400
TGx 1864-6 17F	6	8	32	369
Samsoy-2	5	7	55	419
LSD (P=0.05)	0.6	0.6	4	46

- 1) The combination of water agar and 2.5 ppm BAP retarded senescence (leaves remained green longer than 30 days) and stimulated root growth. Ten ppm kinetin also delayed senescence (Fig. 1 & Table 1).
- 2) Number of pustules increased linearly with concentration of spores. There were significant differences in number of pustules produced between the genotypes regardless of age (Fig. 2 & 3).
- 3) There were significant differences in days to pustule formation, days to spore release, number of pustules/cm² and number of spores/pustule among different genotypes (Table 2).

Conclusion

The detached leaf assay may be used as a rapid screening technique to evaluate germplasm, breeding materials, and mapping populations for resistance to rust in a short span of time with minimal cost, and under uniform infestation.

Reference

1. Sinclair, J.B. and Hartman, G.L., eds. 1996. Soybean Rust Workshop. College of Agricultural, Consumer, and Environmental Sciences. National Soybean Research Lab, Publication 1.





Table 1. Green leaf area after 18 days on water agar