An Inoculation Technique to Assess Soybean Plants for Response to *Xanthomonas campestris* pv. *glycines*

A. A. COOK, SRI HARDANINGSIH, and YUSMANI, Malang Research Institute for Food Crops, Malang, Indonesia 65101

ABSTRACT

Cook, A. A., Sri Hardaningsih, and Yusmani. 1990. An inoculation technique to assess soybean plants for response to *Xanthomonas campestris* pv. *glycines*. Plant Dis. 74:309-310.

Cotyledons on 1-wk-old susceptible soybean plants inoculated by infiltration with 1×10^4 cfu of the pustule bacterium abscised within 6 days, whereas cotyledons on resistant plants remained green and firmly attached. Bacterial populations in cotyledons on susceptible plants were 250 times greater than in cotyledons on resistant plants after 144 hr of incubation.

Bacterial pustule, caused by *Xanthomonas campestris* pv. *glycines* (Nakano) Dye, is one of the most prevalent diseases of soybean (*Glycine max* (L.) Merr.) in Java. Warm temperatures and frequent rains make field control other than by host resistance very difficult.

Resistance to the pustule bacterium has been available for many years (1,3), and cultivars with such resistance have been developed in the United States (4), where their use has significantly reduced the impact of this disease (5). To introduce resistance into the soybean breeding program in Java, it was necessary to ensure that available resistance was effective against the Indonesian pathogen and to identify individual plants with qualitative resistance. The usual technique employed to inoculate plants with the bacterial pustule organism has been to apply inoculum, sometimes with an abrasive added to the leaves. Variations of this basic technique do not really alter the configuration of lesions that subsequently develop on inoculated leaves unless distinctive pathotypes of the organism are involved. However, accurate evaluation and interpretation of symptoms that result from such inoculations may be difficult. Individual lesions on resistant and susceptible plants can vary in size and number as a result of: 1) age of the leaf at inoculation; 2) actual time of infection, i.e., when the applied inoculum actually reaches the site of infection; and 3) number of bacterial cells available at the infection site. Thus, assessment of individual plants based on symptoms that result from the usual technique of inoculation may be subject to error. We wanted a technique to qualitatively distinguish susceptible from resistant plants using only basic laboratory and greenhouse facilities.

Accepted for publication 19 October 1989.

© 1990 The American Phytopathological Society

MATERIALS AND METHODS

In early experimentation to develop a qualitative technique to evaluate test plants, we determined that cotyledons on 1-wk-old plants could easily be inoculated by hypodermic injection. Cotyledons on 1-wk-old plants infiltrated with approximately 1×10^8 cfu/ml consistently abscised within 3 days, irrespective of cultivar. Progressively less concentrated inocula caused corresponding delays in

abscission of infiltrated cotyledons. Inoculum that contained approximately 1×10^4 cfu/ml caused abscission within 6 days after inoculation of cotyledons on presumed susceptible cultivars (5), whereas cotyledons on resistant plants remained green and firmly attached.

The patterns of bacterial multiplication in vivo were followed in cotyledons of AGS 181 (susceptible) and AGS 129 (resistant) infiltrated with 1×10^4 cfu/ml. Concentrations of bacteria in vivo were determined by collecting 50 mm^2 of inoculated cotyledon tissue (three disks cut with a 4-mm-diameter cork borer) immediately after inoculation and every 48 hr thereafter from inoculated cotyledons of other plants of the same cultivars through 144 hr of incubation. Replicated tissue samples were triturated in 0.5 ml of sterile distilled water, and 9:1 dilutions were made therefrom. Appropriate dilutions were spread on PDA or NA plates, and numbers of colonies that developed after 3 days of incubation at room temperature (about 26 C) were recorded. These numbers were considered to reflect in vivo bacterial populations during incubation and were reduced for comparison of cultivars to colony-forming units per square millimeter of cotyledon tissue assayed.

RESULTS AND DISCUSSION

Bacterial populations in cotyledons of both resistant and susceptible plants were essentially the same for the first 48 hr after inoculation. After 96 and 144 hr, bacterial populations were approximately 20 and 250 times greater, respectively, in susceptible cotyledons than in resistant cotyledons. No difference was noted in pathogenic capability of eight bacterial isolates used in more than 25 inoculation experiments (2), and there was no evidence of systemic disease development from inoculated cotyledons. Both the preinoculation period required for full cotyledon expansion before inoculation and the post-inoculation incubation period for cotyledon abscission had to be extended by a day each in cooler (June–July) weather.

By use of the described technique, sizable numbers of plants can be assessed under restricted conditions of space and with limited laboratory and plant growth facilities. The technique has the distinct advantages of: 1) symptom development not being affected by variation in atmospheric humidity during incubation, 2) known concentration of inoculum at infection, and 3) accurate timing of the incubation period. With this technique, qualitative identification of resistant and susceptible plants 2 wk after seeding—when plants are still small enough for additional testing, transplanting to the field, or subsequent use as breeding stock—can become routine.

LITERATURE CITED

1. Chamberlain, D. W. 1962. Reaction of resistant and susceptible soybean to *Xanthomonas phaseoli* var. *sojensis*. Plant Dis. Rep. 46:407-409.
2. Cook, A. A., and Stall, R. E. 1969. Differentiation of pathotypes among isolates of *Xanthomonas vesicatoria*. Plant Dis. Rep. 53:617-619.
3. Lehman, S. G., and Woodside, J. W. 1929. Varietal resistance of soybeans to the bacterial pustule disease. J. Agric. Res. 39:795-805.
4. Shanmugasundaram, S. C., Yang, C. H., and Cheng, Y. H. 1988. Breeding for bacterial pustule resistance. Soybean Rust Newsl. 9:6-8.
5. Tisselli, O., Sinclair, J. B., and Hymowitz, T. 1980. Sources of resistance to selected fungal, bacterial, viral and nematode diseases of soybean. Pages 88-92 in: Univ. Ill. Int. Agric. Publ. INTSOY Ser. 18.