

# *Theobroma cacao*, a Host for *Agrobacterium tumefaciens*

L. H. PURDY and E. R. DICKSTEIN, Plant Pathology Department, University of Florida, Gainesville 32611

## ABSTRACT

Purdy, L. H., and Dickstein, E. R. 1989. *Theobroma cacao*, a host for *Agrobacterium tumefaciens*. Plant Disease 73:638-639.

Bacterial pathogens have not been reported in association with *Theobroma cacao*. However, three strains of *Agrobacterium tumefaciens* isolated from chrysanthemum induced galls in seedling cacao plants grown in a greenhouse. Since *T. cacao* is thus a proven host for the crown gall bacterium, the Ti plasmid of *A. tumefaciens* may possibly be used as a vector for the introduction of selected DNA into cacao.

Additional keywords: cocoa crown gall

There are no reported bacterial pathogens of *Theobroma cacao* L. under natural field conditions (4-6). Brunt and Wharton (1) refer to inoculations of *T. cacao* by W. J. Dowson and A. C. Hayward with a known strain of *Agrobacterium tumefaciens* (Smith & Townsend) Conn. in their research to identify the causal agent of a cushion gall disease of cacao in Ghana. Galls suggestive of crown gall developed on cotyledon halves and in stems of seedlings, but their results were not published. Brunt and Wharton (2) identified the etiologic agent of the cushion gall disease as *Calonectria rigidiuscula* (Berk. & Br.) Sacc. Although they refer to gall formation in cacao seedlings after inoculation with a culture of *A. tumefaciens*, they stated that only *C. rigidiuscula* produced galls on test cocoa beans.

## MATERIALS AND METHODS

We inoculated cacao plants with three strains of *A. tumefaciens* biovar 1 to determine if cacao can be infected by this pathogen. The three strains used were isolated from chrysanthemum by R. E. Stall (Plant Pathology Department, University of Florida, Gainesville). For one set of plants, sterilized straight pins contaminated with bacteria of 2-day-old cultures on malt extract-yeast extract agar of one of the three strains were inserted into and through unhardened green stems about 1 cm below the apical bud of 3-wk-old plants from open-pollinated seed of SCA 6, SCA 12, and Catango cacao. Ten plants of each seedling population were inoculated with each of the three crown gall strains, and 10 noninoculated plants of each seedling group were controls. Sterilized noncontaminated straight pins were inserted into

and through control plants in the same manner as for inoculated plants. In addition, two seedling tomato plants were inoculated with each bacterial strain in a manner similar to the method used for cacao as a check on the pathogenicity of the strain. All plants were grown and kept in a greenhouse at 23-30 C before and after insertion of the straight pins.

A second set of plants consisting of 40 of each seedling group was inoculated with bacteria from 2-day-old cultures of *A. tumefaciens*, strain Chry 9 (the most virulent in the first experiment). Bamboo skewers were used instead of straight pins; after 20 hr, the first set of bamboo skewers was removed and replaced with freshly contaminated and noncontaminated skewers for inoculated and noninoculated plants, respectively.

## RESULTS AND DISCUSSION

Nearly 7% of the first set of inoculated plants developed galls. A gall 4 cm in

diameter was observed after 5 mo on a Catango seedling inoculated with strain Chry 9 (Fig. 1); this strain was the most aggressive of the three strains and caused large galls in tomato plants within 4 wk. Smaller galls formed in four other cacao plants—three of SCA 6 and one of SCA 12 inoculated with other strains. The second inoculation technique was more efficient, because after 4 mo, nine of 40 SCA 6 plants, nine of 40 SCA 12 plants, and 16 of 40 Catango plants developed galls of similar size, about 1 cm. All inoculated tomato plants developed galls. None of the noninoculated cacao seedlings developed any symptoms of crown gall.

The pathogen was isolated from five galls from the second experiment, then was streaked on Kado and Haskett's DIM medium (3). Bacteria from the resulting growth were then spotted (1-cm-diameter spots) on a medium of 1% lactose, 0.01% yeast extract, and 2% agar. To test for the presence of 3-ketolactose, the plates were flooded with Benedict's reagent after 48 hr. A yellow halo developed around each of the colonies, a positive test for *A. tumefaciens* biovar 1.

Pieces of galls from cacao and tomato inoculated with strain Chry 9, as well as sections of stems from control plants, were inserted into the stem of healthy seedlings of tomato cv. Bonnie Best. Tomato plants were also inoculated with a culture of *A. tumefaciens* isolated from



Fig. 1. *Theobroma cacao* plant with 4-cm-diameter gall at the point of inoculation with strain Chry 9 of *Agrobacterium tumefaciens*.

the cacao plant with the 4-cm-diameter gall.

Plants inoculated with the cacao galls, the tomato galls, and the bacterial culture all developed symptoms of crown gall. Galls failed to develop in any of the control plants inoculated with healthy tissue pieces from the cacao and tomato control plants.

Responses of cacao plants from open-pollinated seed to *A. tumefaciens* did not demonstrate any differential with respect to host resistance to the pathogen.

We conclude that *T. cacao* is a host for *A. tumefaciens* under the conditions employed in the two experiments. Technology is not yet available to utilize the Ti plasmid of *A. tumefaciens* as a vector for the transfer of selected DNA to *T. cacao*, but the susceptibility of cacao to *A. tumefaciens* has been demonstrated.

#### LITERATURE CITED

1. Brunt, A. A., and Wharton, A. L. 1961. Galls of cocoa—a correction. *Commonw. Phytopathol. News* 7:44-45.
2. Brunt, A. A. F., and Wharton, A. L. 1962. Etiology of a gall disease of cocoa in Ghana caused by *Calonectria rigidiuscula* (Berk. & Br.) Sacc. *Ann. Appl. Biol.* 50:283-289.
3. Schaad, N. W., ed. 1980. Laboratory guide for identification of plant pathogenic bacteria. American Phytopathological Society, St. Paul, MN. 72 pp.
4. Thorold, C. A. 1975. Diseases of Cocoa. Clarendon Press, Oxford. 423 pp.
5. Wellman, F. L. 1977. Dictionary of Tropical American Crops and Their Diseases. Scarecrow Press, Inc., Metuchen, NJ. 495 pp.
6. Wood, G. A. R., and Lass, R. A. 1985. Cocoa. Longman, New York. 620 pp.