Isozyme Patterns in Gravity-Compensated Crown Gall Tissue

Penelope Hanchey, Bonnie Lou Baker and Ralph Baker

Associate Professor, Research Technician, and Professor, respectively, Department of Botany and Plant Pathology, Colorado State University, Fort Collins 80523.

Supported in part by NAS Contract 2-6615, NASA Ames Research Center, Moffett Field, California.
Accepted for publication 30 April 1975.

ABSTRACT

Isozyme patterns of gravity-compensated carrot crown galls were compared with patterns from galls grown in stationary or vertically rotated positions. Both horizontally rotated (gravity-compensated) and vertically rotated galls exhibited identical esterase zymograms differing from either horizontal or vertical stationary controls. Peroxidase patterns of each treatment were different. Differences in either enzyme were not associated with numbers of bacteria. Results demonstrate that the gravitational field influences isozyme activity in carrot crown gall.

Phytopathology 65:1136-1138

Conditions simulating weightlessness can be achieved by slowly rotating a plant about a horizontal axis on a clinostat (5). This provides continuous reorientation giving rise to a multidirectional gravitational field. The directional component of the field thus becomes nullified or "compensated" and the plant tissue is referred to as gravity-compensated.

Crown gall tumors were reportedly larger (fresh and dry weights) when inoculated carrot root disks were horizontally rotated on a clinostat (8). It is known that both metabolic rates and hormone responses are altered by gravity compensation (3, 4). Although not affected by gravity compensation (8), a polarity effect has also been observed. Larger tumors developed on inoculated apical-facing surfaces of excised disks than on inoculated basal-facing surfaces (6). In this paper we report comparisons of peroxidase and esterase isozyme patterns of compensated and noncompensated carrot crown galls.

MATERIALS AND METHODS.—Two disks (1-cm high) were excised from the center of several fresh carrot roots and surface sterilized in 10% acidified Clorox. Disks were suspended and sealed in water agar (1.5%) in the centers of plastic plates (8). The apical- or basal-facing surface of the disk was inoculated with a 0.1 ml suspension containing 10^{10} cells of *Agrobacterium tumefaciens* B-6 in physiological saline.

Dishes containing disks were placed on clinostats and rotated at a speed of 2 rpm about the vertical axis of the root (vertically rotated), or placed in a horizontal position and rotated about that horizontal axis (horizontally rotated), the latter resulting in gravity compensation. Horizontal and vertical stationary controls were also used. Noninoculated tissues from mature or juvenile roots, inoculated tissues on which galls failed to develop, and tissues located directly beneath galls were also studied.

All disks were incubated at 25 ± 2 C for 21 days after inoculation. Following incubation, gall samples were homogenized in physiological saline and bacterial numbers were determined by dilution platings.

For electrophoretic studies, tissues were ground in a chilled mortar with 0.1 M Tris [tris (hydroxymethyl)aminomethane]-HCl containing 16% sucrose, 0.1% cysteine-HCl, and 0.1% ascorbic acid, pH 8.0. Samples containing 50-100 mg protein were used for electrophoretic separation in an Ortec vertical slab electrophoresis apparatus. Peroxidase visualization was achieved by Ornsteins' method with benzidine-HCl and 0.01% H_2O_2 (1). Esterase activity was determined using $\alpha + \beta$ naphthyl acetate (7). After 15-30 minutes of incubation the gel slabs were photographed.

RESULTS.—Ten different isozyme bands of $\alpha + \beta$ esterase activity were detected in crown gall-infected carrot root disks. Eight bands were found in noninoculated disks and seven bands in both juvenile carrot roots and mature inoculated disks which failed to develop galls.

Four different banding patterns were found in the eight crown gall treatments. Galls removed from disks incubated in a vertical, stationary position, equivalent to the normal growing position of a carrot root, exhibited nine bands of activity (Fig. 1-D). No difference was found between galls formed on the apical or basal inoculated surfaces. Compared with noninoculated carrots (Fig. 1-E), staining was decreased in band 2 and increased in bands 3, 4, 6, and 10. A band not detected in noninoculated carrots was found at position 8. Galls from

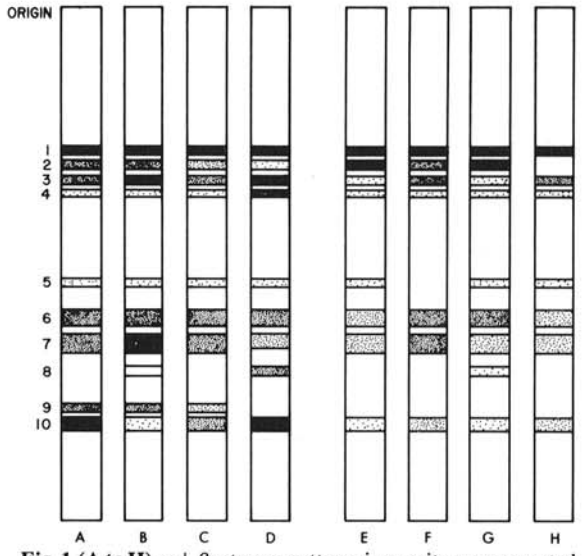

Fig. 1-(A to H). $\alpha + \beta$ esterase patterns in gravity-compensated carrot tissue disks inoculated with *Agrobacterium tumefaciens* (causal agent of crown gall). **A)** Horizontally (gravity-compensated) and vertically rotated, apical or basal face inoculated with *A. tumefaciens*. **B)** Horizontal stationary, basal face inoculated. **C)** Horizontal stationary, apical face inoculated. **D)** Vertical stationary, apical or basal face inoculated. **E)** Noninoculated, nontreated mature carrot. **F)** Gravity-compensated disk which failed to develop galls. **G)** Tissue beneath gall on gravity-compensated disk. **H)** Juvenile carrot root.

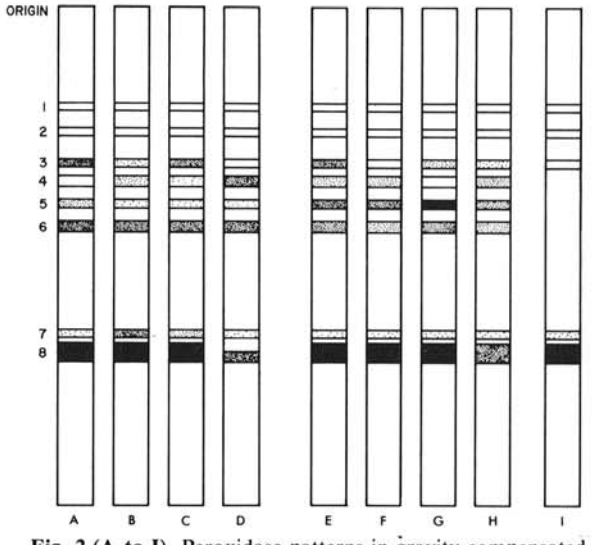

Fig. 2-(A to I). Peroxidase patterns in gravity-compensated carrot tissue disks inoculated with *Agrobacterium tumefaciens* (causal agent of crown gall). **A-D)** Apical face inoculated with *A. tumefaciens*. **E-H)** Basal face inoculated. **A, E)** Gravity-compensated. **B, F)** Vertically rotated. **C, G)** Horizontal stationary. **D, H)** Vertical stationary. **I)** Noninoculated, stationary mature carrot.

disks inoculated on the apical-facing surface and incubated in a horizontal stationary position (Fig. 1-C) showed decreased activity in band 2 and increased staining in bands 3, 6, 7, and 10. A previously undetected band was found at position 9. Inoculation of the basal-facing surface followed by incubation in the horizontal, stationary position led to a decreased activity of band 2 and increased activity of bands 3, 6, and 7 (Fig. 1-B). Bands at both the 8th and 9th position also were detected.

Galls from disks which were inoculated on either the apical or basal-facing surface and then either horizontally (gravity-compensated) or vertically rotated showed identical banding patterns of esterase activity (Fig. 1-A). Compared with mature, noninoculated, nonrotated tissue, these galls showed decreased activity in band 2 and increased activity in bands 3, 6, 7, and 10. Staining at position 9 was also detected. For all galls, decreased activity in band 2 and increased activity in bands 3 and 6 were found. In all treatments except the vertical stationary calluses, a previously undetected band at position 9 was found. These results show that crown gall infection of carrot roots leads to changes in isozyme activity which are dependent upon both the position in which the disks are incubated and the surface inoculated.

That these changes are not solely due to the presence of bacteria or proliferating gall tissue is shown by comparison with other noncallused carrot tissues. Compared with noninoculated mature carrot roots, juvenile roots showed no activity at position 2 and increased activity at positions 3 and 10 (Fig. 1-H). Bands 3, 6, 7, and 10 increased in activity in gravity compensated disks on which galls failed to develop (Fig. 1-F). The increased activity of these bands in callused tissue therefore is not solely due to cell proliferation. The only difference between tissue located beneath galls and noninoculated carrots was the increased activity of bands 6 and 8 in the former (Fig. 1-G). Thus, only changes in the activity of band 6 was consistently associated with the probable presence of the bacterium. Differences in activity of other bands was associated neither with inoculation nor with callusing.

Each of the treatments exhibited a different pattern of peroxidase activity among the eight different bands detected (Fig. 2). Five bands were distinguished in noninoculated, nonrotated disks (Fig. 2-I). Activity in 2 or 3 additional bands, depending upon the treatment, was detected in galls. Changes in activity of two of the bands found in noninoculated tissue occurred in some of the treatments. To determine whether the differences found in isozymes and gall size were related to differences in bacterial growth in the eight different treatments, bacterial counts were obtained from crushed galls. All galls, regardless of treatment, contained between 1×10^7 and 4×10^7 bacteria per gram fresh weight. These results demonstrate that both gravitational field and position of inoculation influence peroxidase isozymes in crown-gall infected carrots.

DISCUSSION.—There are but two reports on the effects of gravity compensation on disease development (2, 8). Disease development was accelerated in compensated beans infected with *Uromyces phaseoli,* although pustule number was not affected (2) and larger galls developed on compensated carrot roots infected with *A. tumefaciens* (8). Effects on disease development may result from metabolic changes during gravity compensation. Dedolph et al. (4) demonstrated increased phosphate accumulation in compensated oat seedlings, but the relative proportions of organic to inorganic phosphate remained unchanged. They further showed an enhanced coleoptile curvature response to unilaterally applied auxin, although compensation did not affect the amount of auxin produced or transported (3). Their results suggest that gravity compensation results in an enhancement rather than a change in metabolism.

In this study, both enhancements and decreases in activity of several isozymes were found. But no new isozymes, previously undetected in other treatments, resulted from compensation. Gravity-nullified galls were distinguished by a unique peroxidase isozyme pattern, as were treatments in any other position. Whether enhanced total peroxidase activity resulted from gravity-compensation was not determined. Esterase isozyme patterns were not affected by gravity nullification. Instead, rotation on either axis produced similar effects. These results demonstrate that metabolic changes occurring during gravity compensation were selective in nature, and that not all metabolic systems of the cell were affected.

LITERATURE CITED

1. CANAL INDUSTRIAL CORPORATION. 1963. Special subject. Enzyme analysis. Canal Industrial Corp., Bethesda, Md. 12 p.
2. CURTIS, C. R. 1967. Bean rust development in relation to gravity. Phytopathology 57:1025-1027.
3. DEDOLPH, R. R., S. M. NAQVI, and S. A. GORDON. 1966. Role of indole-3-acetic acid in modification of geotropic responses in clinostat rotated Avena seedlings. Plant Physiol. 41:897-902.
4. DEDOLPH, R. R., B. R. WILSON, W. CHORNEY, and J. J. BREEN. 1966. Simulated low-gravity environments and respiratory metabolism in Avena seedlings. Plant Physiol. 41:1520-1524.
5. GORDON, S. A., and J. SHEN-MILLER. 1971. Simulated weightlessness studies by compensation. Pages 415-426 *in* S. A. Gordon and M. J. Cohen, eds. Gravity and the organism. University of Chicago Press, Chicago. 473 p.
6. KLEIN, R. M., and I. L. TENENBAUM. 1955. A quantitative bioassay for crown-gall tumor formation. Am. J. Bot. 42:709.
7. THOMAS, D. L., and R. M. BROWN, JR. 1970. New taxonomic criteria in the classification of Chlorococcum species. III. Isozyme analysis. J. Phycol. 6:293-299.
8. WELLS, T. R., and R. BAKER. 1969. Gravity compensation and crown gall development. Nature 233:734-735.