Cytokinin Activity in Plasmodiophora brassicae-Infected Cabbage Tissue Cultures

M. N. Reddy and P. H. Williams

Postdoctoral Fellow and Associate Professor, respectively, Department of Plant Pathology, University of Wisconsin, Madison 53706.

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ABSTRACT

On modified Murashige and Skoog's medium containing 0.5 mg/liter of α-naphthalene acetic acid and 1 mg/liter of kinetin, the dry wt of *Plasmodiophora brassicae*-infected cabbage tissue cultures doubled every 3 days; whereas that of noninfected tissues doubled every 5 days. Noninfected tissues had an absolute requirement for exogenous auxin and kinetin; whereas infected tissues required only an exogenous auxin but not kinetin. Extracts from infected tissue cultures grown on kinetin-free media contained cytokinin activity based on tobacco callus growth, barley leaf senescence, and barley root inhibition bioassays. Three components having cytokinin activity in the tobacco pith bioassay could be isolated from infected callus grown on kinetin-free medium. Phytopathology 60:1463-1465.

Additional key words: clubroot, plant hormones.

During the course of our studies on the hormone requirements of *Plasmodiophora brassicae*-infected and noninfected cabbage tissue in culture (10), we noticed that infected tissues grew well in the absence of exogenous kinetin, whereas noninfected tissue failed to grow (5). In the clubroot disease of crucifers, Matsubara & Nakahira (8) reported that gall tissue contained higher amounts of cytokinin activity than did noninfected tissue. It was of interest to us, therefore, to compare the degree of kinetin independence of the parasitized tissue in culture with that of nonparasitized tissue. Isolation of cytokinin from infected tissue would implicate cytokinin autotrophy resulting from the parasitic relationship.

MATERIALS, METHODS, AND RESULTS.—Two callus clones originally isolated from noninfected (H-4) and *P. brassicae*-infected (CR-2) cabbage hypocotyls were used in this study and maintained on a modified Murashige and Skoog's (MS) medium with naphthalene acetic acid (NAA) (0.5 mg/liter) and kinetin (1 mg/liter) as previously described (10). The requirements of tissues for NAA or kinetin or both were determined by growing the tissues on the basal MS medium deficient of these compounds alone and in combination. The noninfected and infected tissues were grown for two and four successive passages, respectively, and dry wt were obtained at 10 days after the last passage. Weights represented the average of at least 12 tissue pieces/experiment. At the end of two successive transfers, noninfected tissues failed to grow in the basal medium deficient of either kinetin or NAA or of both (Fig. 1). Tissues turned brown, and no visible growth was observed in any cases investigated. On basal medium containing both NAA and kinetin, cream-colored, solid, rounded tissues continued to grow after many successive transfers (10).

As with the noninfected tissues, *P. brassicae*-infected callus explants failed to grow in the absence of NAA or NAA and kinetin (Fig. 1); however, they took four successive transfers, at 7- to 12-day intervals, before they became brown and stopped growing.

Unlike noninfected tissue, infected tissue grew rapidly when only kinetin was absent from the basal medium. After four successive passages on kinetin-deficient medium, the mean growth rate of infected callus was only slightly less than the rate of infected callus on complete medium (Fig. 1). Even after 20 successive transfers on kinetin-free medium, infected callus was growing as rapidly and looked identical to tissue growing on the complete medium.

Infected callus which had been grown for 20 successive transfers in kinetin-free medium was extracted for cytokinin activity using the method of Király et al. (7) modified to include passage of the ether-extracted acidified fraction over a Dowex-50 column as described by Miller (9). Washed and frozen callus in the amount of 200-300 g (fresh wt) were used in each extraction. For bioassay, the final butanol-soluble fractions were made up to 10 g of tissue/ml in hot water. For paper chromatography, the final fractions were concd to 50 g of tissue/ml, then 100 μliters were spotted on Whatman No. 1 filter paper and subjected to descending chromatography using *n*-butanol:acetic acid:water (12:3:5) solvents. After the solvent moved 45 cm from the origin, the chromatograms were dried and cut in 2.5-cm squares. The pieces were extracted with 5 ml 70% ethanol in a test tube, the extract was then dried, and 5 ml of MS medium without kinetin was added to the tube. The tube was autoclaved, then inoculated with a single piece of tobacco callus 15 to 20 mg fresh wt. After 30 days the callus pieces were weighed.

Various concn of the butanol-soluble fractions were bioassayed on kinetin-free medium using the pith callus from *Nicotiana tabacum* 'Wisconsin No. 38' (2) supplied by J. P. Helgeson. After 30 days, dry wt was recorded from triplicate samples of four pieces each.

The barley leaf senescence test for cytokinin activity was run on the butanol-soluble fractions following the method of Kende (6). *Hordeum vulgare* L. 'Trophy' was used in the assay. In order to preclude the possibility of "cytokininlike" contaminants in the

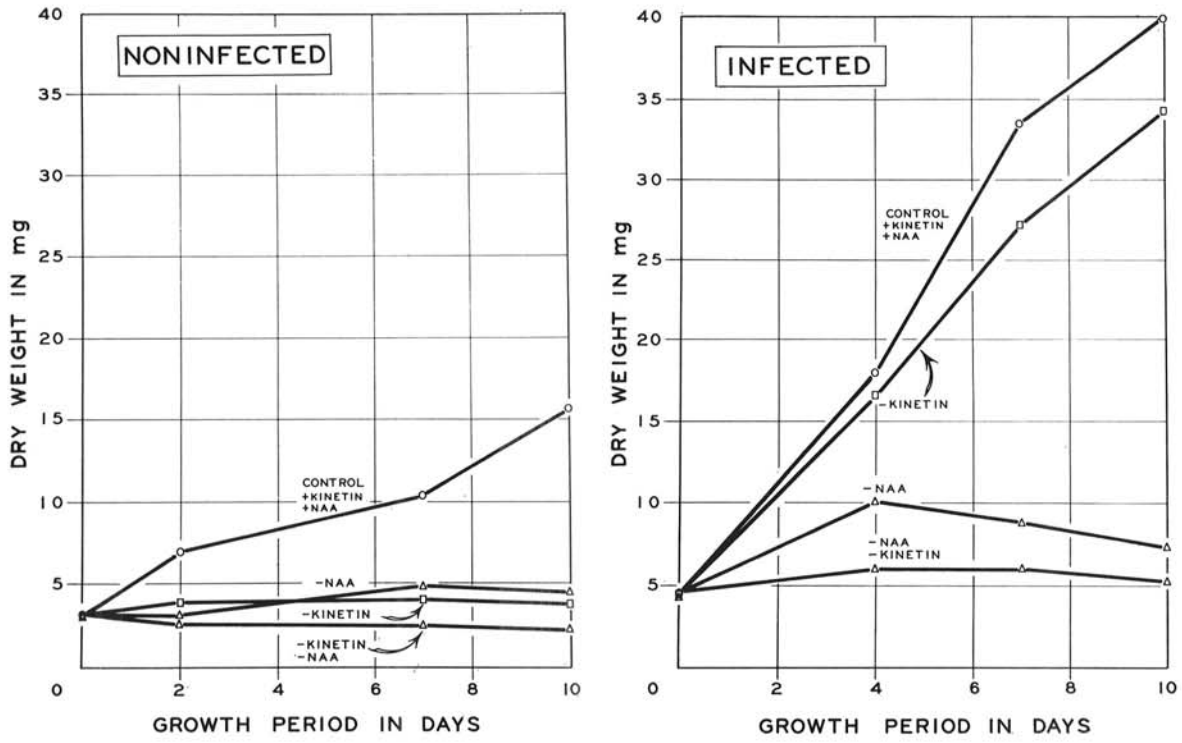

Fig. 1. Growth of noninfected and *Plasmodiophora brassicae*-infected cabbage callus on complete medium and on medium deficient in naphthalene acetic acid or kinetin or both.

butanol solvents used, 200 ml of butanol without added cytokinins were evaporated at 40 C, then made up in hot water to 20 ml. When this extract was bioassayed, it did not differ appreciably from the water control.

Barley root growth inhibition for cytokinin activity was measured on various concn of the butanol-soluble extracts of infected callus following the method of Király et al. (7). Data given are on barley 'Trophy' and represent averages of three experiments, four replicates each.

Ultraviolet absorption spectra on the butanol-soluble fractions were obtained using 0.1-ml samples (10 g tissue equivalents/ml) made up to 3 ml in either water, 1 N HCl or 1 N NaOH (Table 2).

In all three bioassays, the biological response was greatest to an extract concn of 10 g fresh wt/ml (Table 1). When concn were 25 g fresh wt/ml and higher, callus growth slowed and leaf senescence was enhanced above controls. At extract concn of 1 g fresh wt/ml, no cytokinin activity was detected above the level of the controls. Although the cytokinin activity for the clubroot fraction was about 10 times less than that for 1 μg of kinetin in the tobacco callus bioassay, our chromatographic data would suggest that there are at least three components which have cytokinin activity (Fig. 2). These observations are similar to those of Dekhuijzen & Staples (1), who noted several mobilization components in bean rust urediospores and

TABLE 1. Cytokinin activity in the butanol extracts from *Plasmodiophora brassicae*-infected cabbage callus cultures growing on kinetin-free medium as measured by three different bioassays

		Bioassay[b]					
		Barley root inhibition		Chlorophyll retention		Tobacco pith callus growth	
Treatments	Concn[a]	Length, mm	% Decrease over control	A at 665 nm	% Increase over control	Dry wt in mg of single callus	% Increase over control
Tissue culture extract	1	66.3	18	0.20	5	8.6	—11
	10	52.9	34	0.28	48	15.4	59
	25	34.6	56	0.15	—21	9.0	—7
Kinetin standard (1 mg)		55.1	31	0.27	42	115.5	1,089
Water control		79.6		0.19		9.7	

[a] In g equivalents, representing the extract obtained from g (fresh wt) of cabbage callus tissue.
[b] All the results are an average of at least two experiments with three replications each.

TABLE 2. Ultraviolet absorption max and min of the butanol extract fraction from *Plasmodiophora brassicae*-infected callus grown on kinetin-free medium

	Absorbance					
	In 1 N HCl		In water		In 1 N NaOH	
Fraction	Max mμ	Min mμ	Max mμ	Min mμ	Max mμ	Min mμ
Infected tissue	256	234	254	234	264	238
Adenine	260	230	258	222	268	236
Kinetin	274	234	266	234	270	238
Zeatin	277	237	268	231	276	242

rust-infected bean leaves. Although it is clear from our chromatographic data that the butanol-soluble fraction from the infected callus contains more than one compound possessing cytokinin activity, our ultraviolet absorption spectra suggest that these compounds are likely to contain substituted purines (Table 2).

DISCUSSION.—In the past few years, there have been numerous reports of high cytokinin activity associated with chlorophyll retention (green islands) and nutrient mobilization toward infection sites of a number of parasites (1). Although rigorous proof has not been established for the role of cytokinins in most diseases, the production and involvement of dimethylallyladenine (3) by *Corynebacterium fascians* in the fasciation disease of peas have been well-documented. Similarly, the work of Wood et al. (11) has implicated the role of both auxins and cytokinins in the induction of crown gall tumors. In crown gall, the alteration of tissue to full hormonal autonomy has been associated with increased cytokinin synthesis within the tissues. Though the clubroot gall is strictly a self-limiting type of gall, the presence of the parasite has in a sense provided a measure of autonomy to the parasitized tissue in that it is capable of providing itself adequate cytokinin for sustained growth in the absence of exogenous kinetin. The question as to whether the parasite or the parasitized host is responsible for the increased cytokinin, though unresolved, has been considered by Ingram (4), who observed results similar to our own with respect to the kinetin independence of *P. brassicae*-infected *Brassica rapa* callus. Ingram noted that so long as the parasite remained vegetative the infected callus was capable of growth in the absence of either kinetin or NAA or both. Although these clones were autotrophic for both NAA and kinetin, their growth on media deficient in these compounds was considerably slower than on a complete medium (4). It is clear that the parasite is providing for or stimulating the host to provide factors essential for cell proliferation and enlargement. Exactly how these factors are acting on the host and what their source may be remains to be examined.

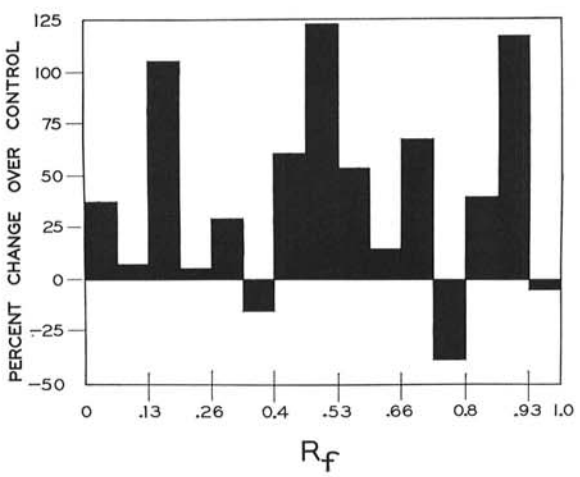

Fig. 2. The paper chromatographic separation of cytokinin activity from extracts of *Plasmodiophora brassicae*-infected cabbage callus growing for 20 transfers on cytokinin-free media. Activity was detected by the tobacco pith bioassay.

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