THE CYTOLOGICAL EFFECTS OF GROWTH INHIBITORS ON EXCISED ROOTS OF VICIA FABA AND PISUM SATIVUM

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INTRODUCTION

The effect of inhibitors on plant growth has been demonstrated by a number of investigators. One such inhibitor, trans-cinnamic acid, was isolated from Gualyule by Bonner and Galston (1) and has been shown to be inhibiting in very small concentrations to Guayule and to be unstable in non-sterile soil by Bonner (2). Trans-cinnamic acid acts as an antiauxin in inhibiting plant growth according to Van Overbeek (3). Most inhibitors have at least a dual effect on cells to produce the inhibition according to Hughes (4).

Extracts of hulls and other parts of walnut trees *(Juglans nigra)* have been shown to be inhibitory to some species of plants while being without effect on others. These are reviewed very adequately by Solomon (5).

Juglone, another inhibitor, was isolated from the roots and aerial parts of walnut by Davis (6). The effect of Juglone on cell physiology is unknown. It has not been demonstrated that Juglone is the inhibitory substance in walnut hulls.

The purpose of this investigation was to determine the effects of trans-cinnamic acid, a water soluble extract from *J. nigra* hulls and juglone on the nucleus and the size of the cells in excised roots of *Vicia faba* and *Pisum sativum.*

METHODS AND MATERIALS

Vicia faba Grown in White's Media

Seeds of *V. faba* were sterilized in a manner modified from White (7). Sterile conditions were maintained in all phases of the experiment. The seeds were wetted in an "Alconox" solution from 3 to 5 minutes and then placed in a 1% hypochlorite solution of "Clorox" for 30 minutes which served as a sterilizing agent. They were then rinsed with sterile distilled water and placed on wet filter paper in petri dishes. When the primary root reached a length of 2 cm to 3 cm, the tips from .5 cm to 1 cm long were excised and placed in the culture media.

White's media (7) was adjusted to pH 6.5 using .1N HCl and .1N NaOH. Fifteen ml of this solution was placed in each culture

tube (25mm X 150mm test tubes), cotton plugs were inserted in each tube and covered by paper held with a rubber band. After a 15 minute period of autoclaving, the excised roots were placed in the culture media and the cotton plug replaced by a 2 inch by 2 inch piece of aluminum foil folded over the end of the tube. The root tips were grown in diffuse light at room temperature. The root tips were measured initially and then at the end of one and two weeks. From 10 to 20 root tips were used in each series. At the end of the two week period five or more root tips, 3 mm to 4 mm long, were fixed and stained with proprio-carmine for cytological examination.

The water soluble extract of *J. nigra* hulls was prepared by drying the hulls at 95°C for 72 hours and homogenizing for 15 minutes in a Waring blender. The extract was kept under refrigeration.

Juglone (5-hydroxy-1, 4-naphthoquinone) was dissolved in alcohol. It was then added to 500 ml of water and heated at 80°C until below the original volume. A control of alcohol and water was run along with each juglone concentration.

Pisum sativum and 1/4% Hoagland's Solution Plus 2% Sucrose

The media was made according to Wilson (8) which consisted of 1/4% Hoaglands solution plus 2% sucrose. Alaska variety seeds of *P. sativum* were germinated in petri dishes on moist filter paper at room temperature. When the primary roots reached from 2.5 cm to 3 cm in length they were excised 1.5 cm from the tip and floated on two liters of the media in a 4-quart battery jar. Each series was started at 10:45 A.M. to eliminate any hourly fluctuation in the mitotic cycle. Root tips were removed from the culture media at 2, 4, 8, and 12 hour periods. Five or more root tips were fixed and stained with propric-carmine for cytological examination from each time period. A count was made to determine the mitotic rate and the percentage of mitotic cells in each stage of mitosis. The method of counting cells was modified from Bowen and Wilson (9). Five hundred meristimatic cells were counted an each slide to determine the mitotic rate. Forty cells on each slide were scored according to the stage of division. The mitotic stages used were prophase, metaphase, anaphase and telophase.

RESULTS AND DISCUSSION

Vicia faba Grown in White's Media

Large cells are produced in cultured tissue and have been described by Mitra and Steward (10) and other workers. These large cells were found in all series run with no apparent increase in number with any of the inhibitors used. Normal non-cultured root tips were also stained and cells measured. The largest cells in the

non-cultured root tips, of which there were very few, were 250μ by 28μ in size. In the cultured root tips the longest cells were about 340μ. The large cultured cells always had a much greater diameter, reaching 80μ.

Figure 1 shows the percentage of inhibition of *V. faba* root tips in White's media with increasing molar concentration of trans-cinnamic acid.

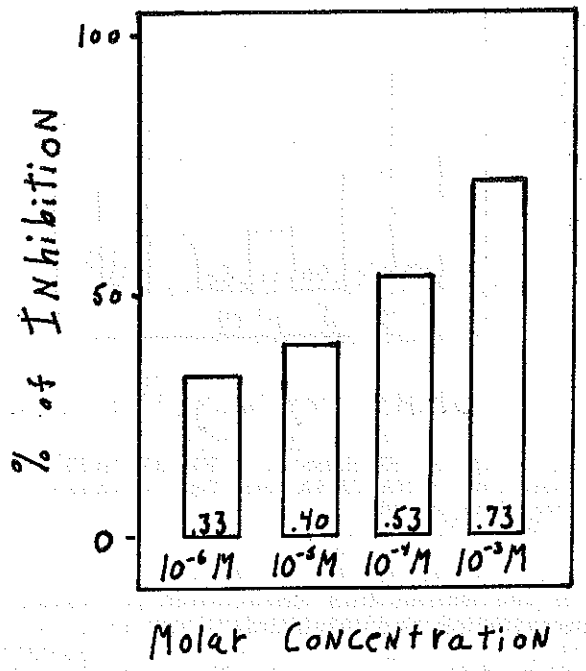

Figure 1. Percentage of Inhibition of Trans-cinnamic Acid on VICIA FABA Root Tips in White's Media Over a Two Week Period.

As shown the inhibition of growth increased with an increase in the concentration of trans-cinnamic acid. No apparent chromosomal abnormalities were produced. Using Students "t" test (11) the differences in the means of all the series were below the 5% level of significance when compared to the control. In comparing the differences in the means of the 10^{-6}M to 10^{-5}M and the 10^{-5}M to 10^{-4}M all figures are above the 5% level, but none exceeding the 18% level. When comparing the 10^{-6}M to 10^{-4}M, 10^{-5}M to 10^{-3}M and 10^{-4}M to 10^{-3}M all figures were below the 5% level.

Figure 2 shows that inhibition occurred at all levels of con-

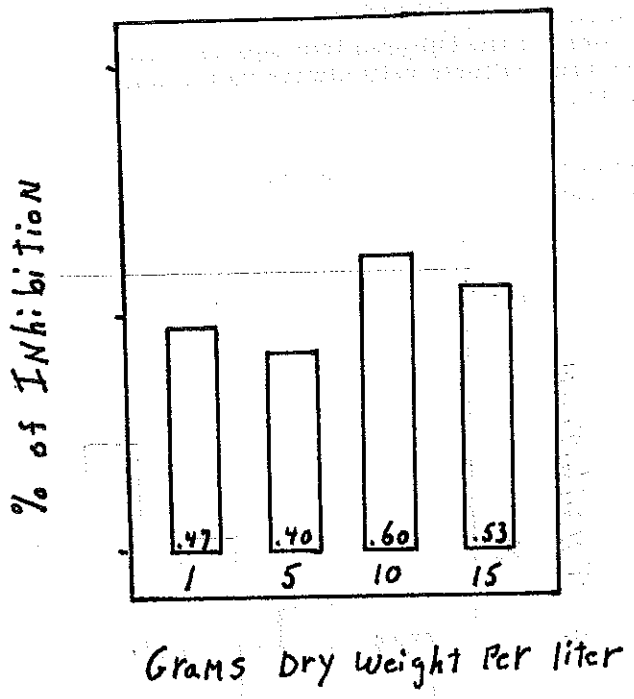

Figure 2. Percentage of Inhibition of J. NIGRA Hulls Water Soluble Extract on VICIA FABA Root Tips in White's Media Over a Two Week Period.

centration of the walnut hull extract but no correlation existed between concentration and inhibition.

Explanation of these results may lie in the fact that such an additive contains many other materials in addition to the inhibitor (s), including growth promoting substances. No chromosomal abnormalities were observed. With the extract at 10 g per liter and 15 g per liter many nuclei were stained brown. The material involved in the staining probably acts as a vital stain.

Students "t" test applied to the 1 g dry weight per liter and the control gave a figure below the 5% level.

Figure 3 shows the inhibition produced by juglone on excised roots of *V. faba*. As shown, the inhibition was greater with an increase in concentration. There were no apparent chromosomal abnormalities. With juglone at 10^{-5}M some nuclei were stained light brown. It apparently acts as a vital stain.

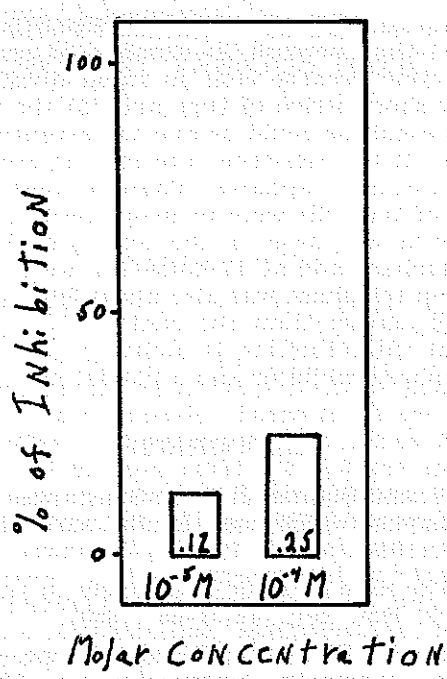

Figure 3. Percentage of Inhibition of Juglone on VICIA FABA Root Tips in White's Media Over a Two Week Period.

Pisum sativum in 1/4% Hoagland's Solution Plus 2% Sucrose

Trans-cinnamic acid at 10^{-4}M gave progressive inhibition from 30% at 2 hours to 76% at 12 hours. *J. nigra* hulls water soluble extract at 1 g per liter was without effect at 2 hours but gave progressive inhibition up to 76% at 12 hours. Juglone at 10^{-4}M was without effect at 2 hours and only produced 17% inhibition at 12 hours. The root tips in the juglone stained progressively toward a light yellow in the different time periods. This was observed macroscopically in the root tips and microscopically in the nuclei.

Students "t" test applied to the number of cells in mitosis in the controls and from the different time periods showed only 4 hours and 8 hours series of *J. nigra* hull extract and trans-cinnamic acid to be below the 5% level.

A count of cells in mitosis showed no differences in any of the inhibitors as opposed to the control with one exception. With walnut hulls water soluble extract at 1 g dry weight per liter at 8 hours 29% of the cells in mitosis were metaphase and at 12 hours 18% of the cells were in metaphase. In the control no metaphases were

observed in these time periods. The inhibition is probably due to two factors: (1) the increase in number of cells in metaphase show a slowed cycle because cells in rapid division are in metaphase for a very short period of time and (2) the increase in number of cells in metaphase could be due to preventing the cells from entering mitosis. If no cells enter the mitotic cycle this would result in an increase in metaphases. There is some support for this; at 8 hours 10% of the cells were in telophase and at 12 hours 12% of the cells were in telophase. In the control at 8 hours 1% of the cells were in telophase and at 12 hours 1% were in telophase. The number of cells in telophase was also about 30% less in the 1 g per liter walnut hull extract than the control. It appears from these observations that the inhibition is probably due to at least a dual effect, a pre-prophase inhibitor and a partial metaphase posion.

BIBLIOGRAPHY

1. Galston, A. W. and Bonner, J. Toxic Substances from the Culture Media of Guayule which may Inhibit Growth, Bot. Gaz., 106 (2): 185-198 (1944).
2. Bonner, J. The Role of Toxic Substances in the Interactions of Higher Plants, Bot. Rev., 16:51-65 (1950).
3. Van Overbeek, J., Blondeau, R., and Korne, U. Trans-cinnamic Acid as an Antiauxin, Amer. Jour. Bot., 38:589-595 (1951).
4. Hughes, A. "The Mitotic Cycle", Butterworths Scientific Publication, London (1952).
5. Solomon, G. Differential Growth-Inhibitors Produced by Plants, Bot. Rev., 27:422-443 (1961).
6. Davis R. F. The Toxic Principal of *Juglans nigra* as Identified with Synthetic Juglone and its Toxic Effects on Tomato and Alfalfa Plants, Amer. Jour. Bot., 15:620 (1928).
7. White, Philip R. "The Cultivation of Animal and Plant Cells", The Ronald Press Company, New York, New York (1954).
8. Wilson, G. B., Morrison, J. H. and Knoblack, N. Studies on the Control of Mitotic Activity in Excised Roots. 1. The Experimental System, Jour. Biophys. and Biochemical. Cytol., 5 (3): 411-420 (1959).
9. Bowen and Wilson, G. B. A Comparison of the Effects of Several Antimitotics, J. Hered., 45:3-9 (1954).
10. Mitra, J. and Steward, F. C. Growth Induction in Culture of *Haplopappus gracilis*. 11. The Behavior of the Nucleus, Amer. Jour. Bot., 48(5):358-368 (1961).
11. Snedecor, G. W. "Statistical Methods", Fourth Edition. The The Collegiate Press, Inc., Ames, Iowa (1946).