Plant Physiol. (1978) 61, 855-857

Short Communication

Effect of Triacontanol on Plant Cell Cultures *in Vitro*[1]

Received for publication July 5, 1977 and in revised form December 12, 1977

ROGER HANGARTER AND STANLEY K. RIES
Department of Horticulture, Pesticide Research Center, Michigan State University, East Lansing, Michigan 48824

PETER CARLSON
Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

Triacontanol [$CH_3(CH_2)_{28}CH_2OH$] increased growth *in vitro* of cell cultures of haploid tobacco (*Nicotiana tabacum*). The fresh weight of cell cultures of tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*), bean (*Phaseolus vulgaris*), and barley (*Hordeum vulgare* × *H. jubatum*) was also increased. The increase in growth of tobacco callus seems to have been due to an increase in cell number. Another long chain alcohol, octocosanol [$CH_3(CH_2)_{26}CH_2OH$], did not increase the growth of tobacco cell cultures.

Coarsely chopped alfalfa hay used as a band application increased growth and yield of cucumbers, lettuce, tomatoes, and wheat (13). Several other crops accumulated dry wt more rapidly following applications of small amounts of alfalfa under greenhouse and growth chamber conditions. Triacontanol, a straight chain, 30-carbon, saturated alcohol [$CH_3(CH_2)_{28}CH_2OH$], was isolated from alfalfa (15). Applications of 10 μg/l triacontanol increased the dry wt and leaf area of rice plants in nutrient cultures. Both triacontanol isolated from alfalfa and synthetic triacontanol (Analabs) were active at extremely low concentrations (23 nM). Several long chain alcohols exhibit growth-regulating activities on *Avena* coleoptiles (4). Other alcohols promote growth of excised wheat roots (8). The objective of this study was to determine if triacontanol affects the *in vitro* growth of plant cell cultures in the hope that cell cultures would provide a system for studying the mode of action.

MATERIALS AND METHODS

Growth of Cell Cultures. Cell cultures were grown in plastic disposable Petri dishes on the basic medium of mineral salts described by Linsmaier and Skoog (11), except for bean cultures which were grown on the basic medium of Gamborg *et al.* (7). Vitamins and hormones were varied for each tissue in order to maintain the tissues in an undifferentiated state. For haploid tobacco (*Nicotiana tabacum* cv. Wisconsin 38) these were thiamine (1 mg/l), IAA (3 mg/l), and kinetin (0.3 mg/l); for bean (*Phaseolus vulgaris* cv. Seafarer) thiamine (10 mg/l), pyridoxine (1 mg/l), nicotinic acid (1 mg/l), and 2,4-D (2 mg/l); for tomato (*Lycopersicon esculentum* cv. Marglobe), potato (*Solanum tuberosum* cv. Advera), and barley (*Hordeum vulgare* × *H. jubatum*) thiamine (1 mg/l), pyridoxine (0.5 mg/l), nicotinic acid (0.5 mg/l), 2,4-D (0.5 mg/l), IAA (5 mg/l), and kinetin (0.3 mg/l). Inositol (100 mg/l) was used in all cultures. Agar was used at a concentration of 1% and sucrose was used at 3%, except for the bean which was 2%.

Callus of tobacco, potato, and tomato was produced from pith. Bean callus was produced from hypocotyl sections and barley callus from immature ovarian tissue. Subcultures were maintained on the appropriate media in the dark at 25 to 27 C.

Experimental Procedures. Because of the low solubility of triacontanol and octocosanol in aqueous solutions the following procedure was devised for treatment application. Stock solutions (100 μg/l) of triacontanol and octocosanol were prepared in glass-distilled benzene. Lower concentrations were obtained by serial dilution. Aliquots (100 μl) were applied to sterile Whatman No. 1 filter paper discs (diameter 4.5 cm); controls received 100 μl of glass-distilled benzene. The solvent was allowed to evaporate for approximately 15 min, then the papers were placed on the agar medium. Callus tissue was broken into pieces of approximately the same size in each experiment and one piece was placed on the filter paper in each dish.

A randomized complete block design was used for all experiments with at least four replications. For experiments conducted in the light, the intensity at the growing surface was approximately 2 $\mu w/cm^2$ supplied from fluorescent bulbs. Tissue weights were measured after 10 to 15 days. Preliminary studies showed that fresh wt and dry wt responded similarly for both treated and nontreated tissue, therefore, only fresh wt data are reported in the figures. Studies on differentiation were carried out for 30 days. Total N was determined by the automated micro-Kjeldahl procedure of Ferrari (6), and converted to protein using the conversion factor of 6.25. Fresh callus was sectioned by hand and cell diameter was examined with wet mounts.

RESULTS AND DISCUSSION

Triacontanol promoted growth of tobacco callus at concentrations as low as 0.01 μg/dish (Fig. 1). Callus cultures of four other plant species also showed increased growth over the controls in response to triacontanol at 10 μg/dish (Table I).

Although the effect of triacontanol on whole plants does not require light (14), tobacco callus responded to triacontanol only in the light (Fig. 1). In addition, the stage of growth of the tissue at the time of treatment affected the response. The tissue usually responded more to triacontanol when the stock cultures were actively growing (log phase) than when they were in the slower growing "plateau phase." Response of plateau phase tissue was more variable making statistically significant differences difficult to obtain.

Tobacco callus treated with triacontanol for 30 days did not show any visible tissue differentiation. Neither root nor shoot development occurred in any of the experiments and the apparent

[1] Michigan Agricultural Experiment Station Journal article No. 8112.

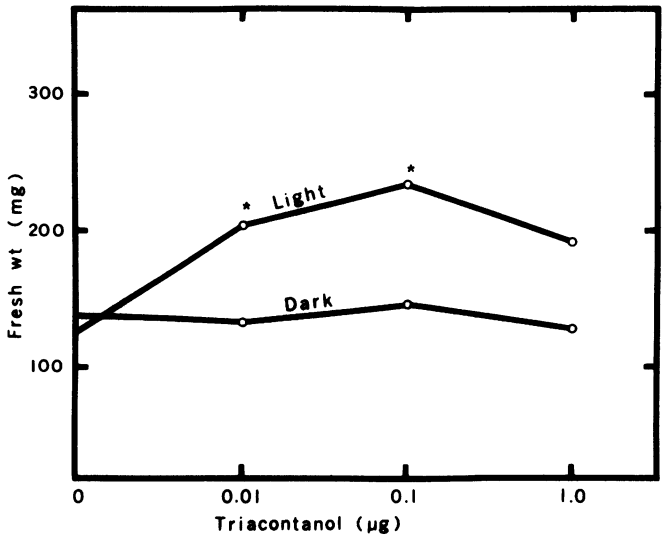

FIG. 1. Effect of triacontanol on the growth of tobacco callus in light and in dark. Treatments were applied to filter paper in 100-μl aliquots of benzene and placed on the agar medium. Approximately 20 mg of tissue was placed on each filter paper. Light intensity was approximately 2 μw/cm². Data are the mean fresh wt/dish for four replicates after 12 days of growth. *F value for comparison of control with treatments significantly different at 0.05 level.

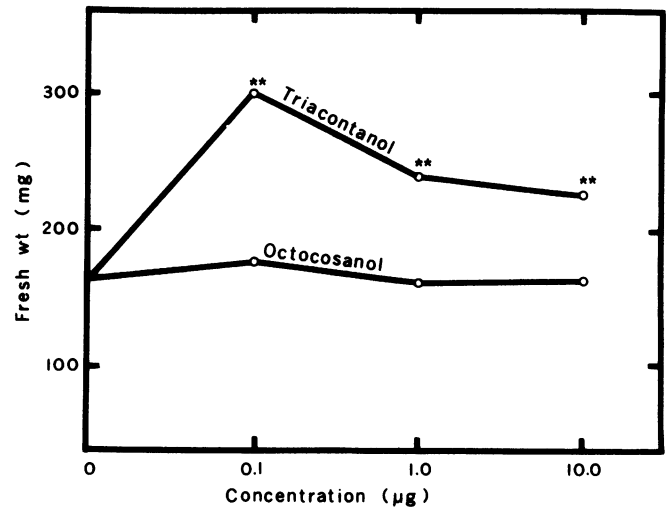

FIG. 2. Effect of triacontanol and octocosanol on the growth of tobacco callus. Treatments were applied to filter paper in 100-μl aliquots of benzene and placed on each filter paper. Approximately 20 mg of tissue was placed on each filter paper. Light intensity was approximately 2 μw/cm². Data are the mean fresh wt/dish for four replicates after 12 days of growth. **F value for comparison of control with treatments significantly different at 0.01 level.

Table I. Effect of triacontanol on growth in vitro of cell cultures of four plant species in light.

Treatments were applied to filter paper in 100 μl aliquots of benzene and placed on agar media. Approximately 5 mg of tissue was placed on each filter paper. Data are the mean fresh wt/dish for 4 to 6 replicates after 10 days growth.

Tissue	μg triacontanol/dish 0.0	10.0	Increase (% of control)
	Fresh wt (mg/tissue)		
Tomato	12.0	16.8	140
Potato	19.5	23.5	121
Barley	21.0	35.0	167
Bean	13.0	19.8	149

Table II. Effect of triacontanol on the growth of tobacco callus in light.

Treatments were applied to filter paper in 100 μl aliquots of benzene and placed on agar media. Approximately 100 mg of the tissue was placed on each filter paper. Data are the means of 4 replicates after 15 days' growth.

Triacontanol (μg/dish)	Fresh wt (mg/tissue)	Dry wt (mg/tissue)	Dry wt/Fresh wt
0.0	1190.0	54.0	0.045
0.1	1120.0*	73.0*	0.045

*Indicates F value for comparison of control with treatment significantly different at the 0.05 level.

friability of the tissue remained unaffected. Greening was uniform in treated and nontreated tissues grown in the light.

The dry wt to fresh wt ratios were similar in treated and nontreated tissues (Table II). Studies of free-hand sections by light microscopy showed no difference in cell size. These data suggest that increased growth caused by triacontanol is not simply caused by water uptake and cell enlargement but rather by an increase in cell number.

Octocosanol, a 28-carbon analog of triacontanol and also a major component of leaf wax (12), did not affect the growth of tobacco callus (Fig. 2) which suggests that a specific chain length may be required for activity. Similar results were found with whole plants (15).

It is generally believed that wax components of the leaf are excreted to the leaf surface as rapidly as they are synthesized, and reabsorption of such water-insoluble compounds is unlikely (10). Occasionally, compounds considered to be cuticular components are found in organelles such as chloroplasts (9). Moreover, Chibnall (1, 2) stated that a group of noncuticular leaf waxes exist which are not cuticle excretions, but constitute an integral part of the general fat phase of leaf cells. The long chain, primary components of the "inside wax" included C_{30} compounds, however, it is possible that there was contamination from the cuticle during extraction. Certain long chain alcohols were shown to exhibit growth-regulating activities in the *Avena* coleoptile test (4), and several long chain alkanes have been shown to have growth-promoting effects using the pea stem assay (16).

Leaf wax appears to serve several passive functions such as protecting the plant from desiccation, mechanical injury, pathogens, insects, and excessive UV radiation (5). The growth-promoting effect of triacontanol, a component of leaf waxes (3), on whole plants (14, 15) and on plant cell cultures *in vitro*, as reported here, suggests that some components of leaf wax may also serve an active role in growth-regulating activities. To what extent endogenous triacontanol is active in controlling the growth of plants still remains to be determined. Tissue culture, in the meantime, may be useful for studying the mode of action of triacontanol.

LITERATURE CITED

1. CHIBNALL AC 1934 The constitution of the primary alcohols, fatty acid and paraffins present in plant and insect waxes. Biochem J 28: 2189–2208
2. CHIBNALL AC 1934 The metabolism of plant and insect waxes. Biochem J 28: 2209–2219
3. CHIBNALL AC, EF WILLIAMS, AL LATNER, SH PIPER 1933 The isolation of *n*-triacontanol from Lucerne wax. Biochem J 27: 1885–1888
4. CROSBY DG, AJ VLITOS 1959 Growth substances from Maryland mammoth tobacco: long chain alcohols. Contrib Boyce Thompson Inst 20: 283–292
5. EGLINTON C, RJ HAMILTON 1967 Leaf epicuticular waxes. Science 156: 1322–1335
6. FERRARI A 1960 Nitrogen determined by a continuous digestion and analysis system. NY Acad Sci 87: 792–800
7. GAMBORG OL, RA MILLER, K OJIMA 1968 Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50: 151–158

8. GUDJÓNSDÓTTIR S, H BURSTRÖM 1962 Growth-promoting effect of alcohols on excised wheat roots. Physiol Plant 15: 498–504
9. GULZ PG 1968 Normale und verzweighte Alkane in Chloroplastenpräparaten und Blättern von *Antirrhinum majus*. Phytochemistry 7: 1009–1017
10. KOLATTUKUDY PE 1970 Biosynthesis of cuticular lipids. Annu Rev Plant Physiol 21: 163–192
11. LINSMAIER E, F SKOOG 1965 Organic growth factor requirements of tobacco tissue cultures. Physiol Plant 18: 100–127
12. POLLARD A, AC CHIBNALL, SH PIPER 1933 The isolation of *n*-octocosanol from wheat wax. Biochem J 27: 1889–1893
13. RIES SK, H BITTENBENDER, R HANGARTER, L KOLKER, G MORRIS, V WERT 1977 Improved growth and yield of corps from organic supplements. *In:* W Lockevetz, ed, Energy and Agriculture. Academic Press, New York, pp 377–384
14. RIES SK, V WERT 1977 Growth responses of rice seedlings to triacontanol in light and dark. Planta 135: 77–82
15. RIES SK, V WERT, CC SWEELEY, RA LEAVITT 1977 Triacontanol: a new naturally occurring plant growth regulator. Science 195: 1339–1341
16. STOWE BB, MA DOTTS 1971 Probing a membrane matrix regulating hormone action. I. The molecular length of effective lipids. Plant Physiol 48: 559–565