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EFFECT OF AUXINS ON THE FORMATION OF UBIQUINONE BY TOBACCO PLANT CELLS IN SUSPENSION CULTURE*

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Key Word Index—*Nicotiana tabacum*; Solanaceae; cell suspension culture; formation of ubiquinone-10; effect of auxins.

Abstract—Ubiquinone (UQ) formation in BY-2 tobacco cells was especially promoted by a high concentration of 2,4-D. 2,4,5-T, MCP and NAA also promoted UQ formation in these cells. The UQ content in the cells cultured at high concentrations of 2,4-D was higher than that of controls throughout the culture period. The addition of 2,4-D at an early period in cell growth was very effective in promoting UQ formation, but addition at the stationary phase was ineffective. Cell growth was improved by adding phosphate to the medium but UQ content was decreased. UQ content decreased slowly during subculturing, whereas cell growth recovered gradually.

INTRODUCTION

It has been recognized that the cultured cells of *Nicotiana tabacum* L. cv BY-2 contain a relatively high amount of UQ-10 [1, 2], comparable to that found in certain microorganisms [3, 4]. In our previous investigations, it was recognized that a high concentration of 2,4-D brought about a marked increase in UQ formation [5, 6]. The influence of auxins on the formation of secondary metabolites has been widely investigated in tissue culture and in general 2,4-D represses their formation [7–12]. On the other hand, there are very few reports about the accumulation of a primary metabolites in plant cells with varying auxin concentrations [13, 14]. The influence of auxin and its derivatives on UQ formation in tobacco cells was therefore examined and this paper deals with the results.

RESULTS AND DISCUSSION

Effect of 2,4-*D concentration on UQ formation*

The influence of 2,4-D concentration on UQ formation was examined using four kinds of tobacco culture cells (Fig. 1). The UQ content by BY-2 cells tends to increase at higher concentrations of 2,4-D. The maximum content of UQ was 580 μg g^{-1} with 5.0 mg $l.^{-1}$ of 2,4-D in the medium, which was about 60% higher than that of the subcultured control cells (360 μg g^{-1}). However, a higher concentration of 2,4-D caused a significant decrease in cell yield. In the cells of *N. tabacum* L. cv Xanthi, the increase of UQ content due to higher concentrations of 2,4-D, was less than that in BY-2 cells. The maximum content of UQ was 400 μg g^{-1} with 2.0 mg $l.^{-1}$ 2,4-D in the medium, which was about 20% higher than that of the subcultured control cells (340 μg g^{-1}). The UQ content of BY-2 auxin-independent cells was 213 μg g^{-1} in the basal medium without auxin, which was about 60% that of parent BY-2 cells (360 μg g^{-1}). The UQ content of BY-2 auxin-indepedent cells was not promoted by the addition of 2,4-D but slightly suppressed. On the other hand, cell growth was significantly limited by the addition of more than 0.2 mg $l.^{-1}$ 2,4-D. In the basal medium without auxin, the UQ content of crown gall cells was 167 μg g^{-1} and this was not increased by the addition of 2,4-D as in the case of BY-2 auxin-independent cells. Of the four kinds of tobacco cells investigated, BY-2 cells were especially affected by higher concentrations of 2,4-D in UQ content and growth.

There are few other reports on the effects of 2,4-D concentration on metabolite formation in plant cells. Brain reported in the production of L-Dopa in *Mucuna pruriens* suspension culture that the maximum growth rate and L-Dopa levels were observed at the highest 2,4-D level (25 mg $l.^{-1}$) [15]. Sugano *et al.* examined the effect of different 2,4-D concentration levels on carotenoid accumulation in carrot cells [11] and found that the carotenoid content in a strain GD-2 cells was dependent on 2,4-D concentration as in our BY-2 cells. Since the biosynthetic pathway of the polyisoprenoid side chain of UQ is similar to that of carotenoid [16], it is presumably this pathway which is promoted by 2,4-D.

* Part 10 in the series "Studies on the Culture Condition of Higher Plant Cells in Suspension Culture". Abbreviations used: UQ, ubiquinone; 2,4-D, 2,4,4-dichlorophenoxyacetic acid; 2,4-D·E, 2,4-D ethyl ester; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; MCP, 4-methyl-2-chlorophenoxyacetic acid; MCP·E, MCP ethyl ester; MCPP, 2[(4-chloro-*o*-toryl) oxy]propionic acid; MCPB·E, 2[(4-chloro-*o*-toryl)oxy] butylic acid ethyl ester; IAA, 3-indoleacetic acid; IPA, 3-indolepropionic acid; IBA, 3-indolebutyric acid; NAA, α-naphthaleneacetic acid; NOAA, β-naphthoxyacetic acid. UQ content throughout is expressed as μg per g dry wt of cultured cells.

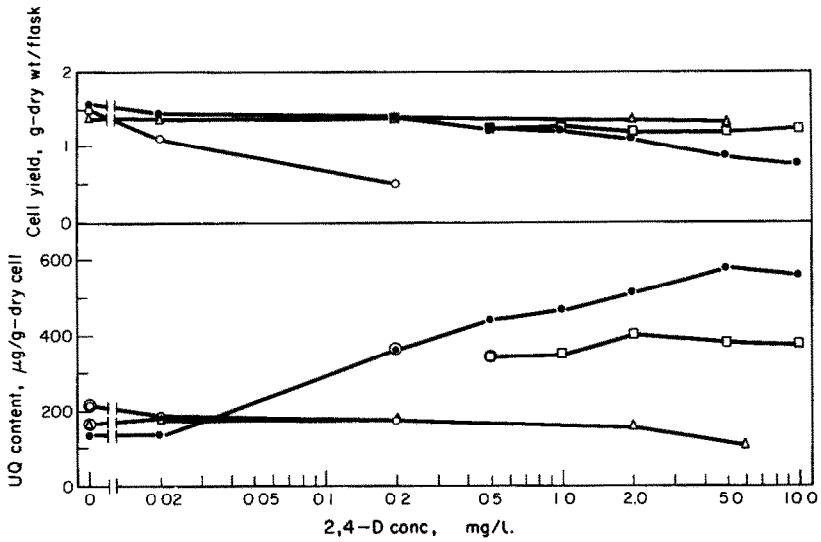

Fig. 1. Effect of 2,4-D concentration on UQ formation in cultured tobacco cells. Seed cells cultured in the control medium were inoculated in the fresh media with designated 2,4-D concentrations. Cultured cells were harvested at 8 days after inoculation. BY-2 auxin-independent cells did not grow in the medium with more than 0.2 mg $l.^{-1}$ of 2,4-D and crown gall cells also did not grow in the medium with 10 mg $l.^{-1}$ of 2,4-D. The culture condition and media except for the 2,4-D concentration, see the text. (○) indicates the control culture of the tobacco cells respectively. —●—, BY-2; —○—, BY-2 auxin-independent; –□—, Xanthi; —△—, Crown gall.

Effect of various auxins on UQ formation in BY-2 cells

While production of phenolics in cell culture is often inhibited by addition of auxins [9, 19, 17], this is not necessarily true for polyprenylquinone. Thus Lichtenthaler and Straub reported that IAA application (0.4 ppm) to a *Pimpinella* tissue culture pormoted the formation of plastoquinone-9, carotenoids and chlorophylls, but had no influence on the α-tocopherol synthesis [14]. The influence of some auxins and their derivatives on UQ formation in BY-2 cells was examined by us (Fig. 2). When BY-2 cells were cultured with another auxin instead of 2,4-D, the cell growth was markedly restrained. Therefore, the effects of other auxins on UQ formation were examined by the addition of these auxins to the control medium containing 0.2 mg $l.^{-1}$ of 2,4-D. 2,4-D·E, 2,4,5-T, MCP and MCP·E promoted UQ formation similarly to 2,4-D. In all three cases, the UQ content was the highest at the concentration of 3.2×10^{-5} M. The cell yield tended to decrease with increase of auxin concentration. MCPP and MCPB·E were ineffective on UQ formation. Furthermore, IAA and IPA were almost ineffective on UQ formation but IBA was slightly effective at higher concentration.

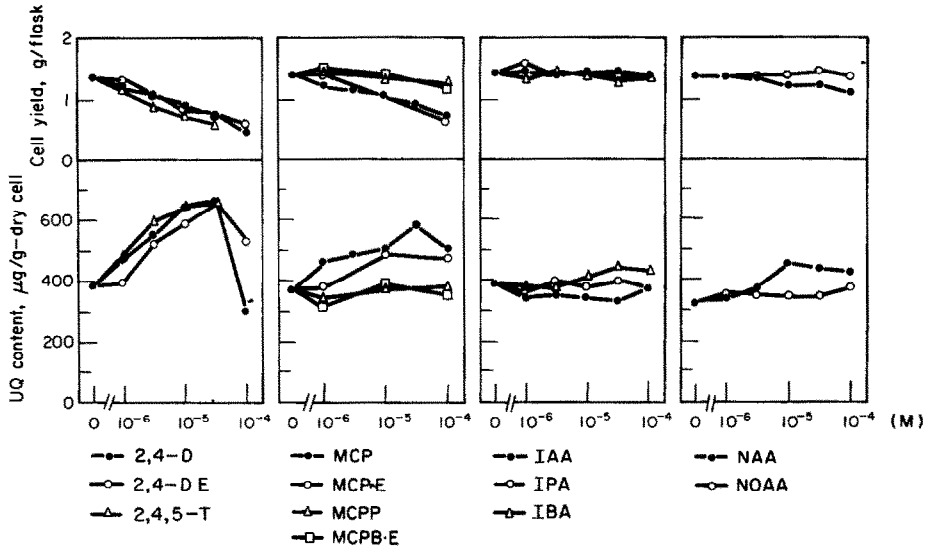

Fig. 2. Effect of auxins on UQ formation by BY-2 cells. Cultured cells were harvested at 8 days after inoculation. For the culture condition and medium except for added auxins, see the text. BY-2 cells did not grow in the medium with 10^{-4} M of 2,4,5-T.

Although NAA affected UQ formation, the effect seems to be inferior to that of 2,4-D. The cell yield tended also to decrease with increasing concentration of NAA. On the other hand, NOAA was not effective on UQ formation. The patterns of changes in UQ content with those auxins which were active were almost similar to those in the media with 2,4-D; on the other hand, the same auxins inhibited cell growth.

Effect of 2,4-D *concentration on the time-course of UQ formation*

The time-course of UQ content during the growth of BY-2 cells in a high concentration of 2,4-D (2.0 mg/l.) was compared (Fig. 3) with that in the standard concentration (0.2 mg/l.). UQ productivity of the cells cultured in the high concentration of 2,4-D was *ca* 30% higher on the 4th day, 60% higher on the 6th day, and 70% higher on the 8th and 10th days than that in the standard concentration. The UQ content in the cells cultured in the high concentration of 2,4-D was higher than that cultured in the standard concentration even at an early stage in growth, although the cell growth was significantly restrained. The cell growth of the control culture reached the stationary phase at the 6th day, while the growth of cells cultured in the high concentration of 2,4-D did not reach the stationary phase even at the 10th day.

Tabata [18] classified production-growth patterns in cultured cells into three major types and UQ formation in the control culture seems to fall into the second type: product formation is delayed until cell growth declines or ceases. In the high concentration of 2,4-D, UQ formation seems to be of the first type; product formation proceeds almost in parallel with cell growth.

Effect of delayed addition of higher concentration of 2,4-D *on UQ formation*

As the cell growth was considerably restrained at high concentrations of 2,4-D, the effect of delayed addition of 2,4-D on the growth and UQ formation was investigated. (Fig. 4). The UQ content in cultures supplemented on zero and the 2nd days, and on the 4th day were about 70% and 50% higher than those in the control culture, respectively. However, the UQ content in the culture supplemented on the 6th day was almost the same as that in the control culture. In our BY-2 cells, the addition of a high concentration of 2,4-D during lag phase was most effective on UQ formation. However, addition at the early stage brought about the inhibition of cell growth. On the other hand, addition of 2,4-D in the stationary phase was not effective on UQ formation.

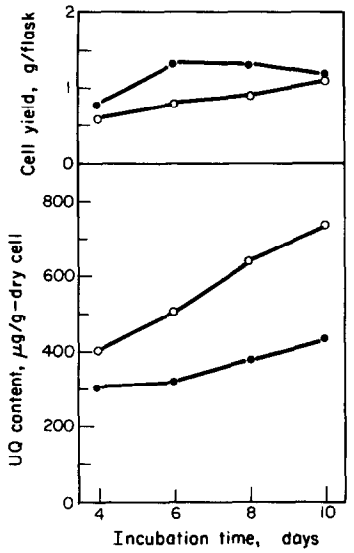

Fig. 3. Effect of 2,4-D concentration on the time-course of UQ formation. For the culture condition and medium except for 2,4-D concentration, see the text. —○—, 2.0 mg $l.^{-1}$, —●—, 0.2 mg $l.^{-1}$ (control).

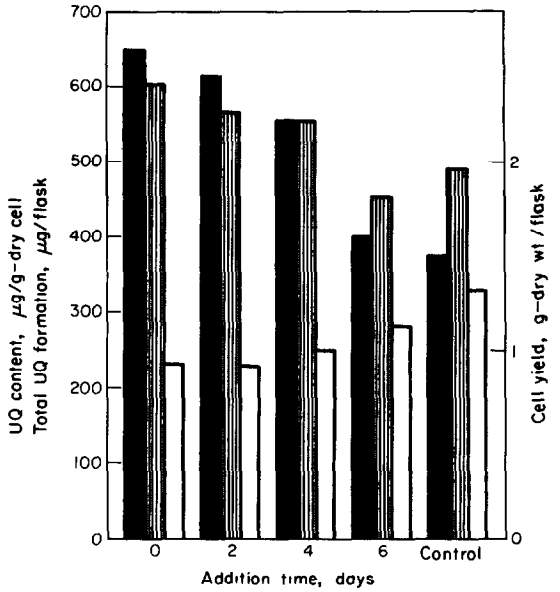

Fig. 4. Effect of delayed addition of 2,4-D on UQ formation. Additions (1.8 mg $l.^{-1}$ of 2,4-D) were made on the day 0, 2, 4 and 6. The unsupplemented medium contained 0.2 mg $l.^{-1}$ of 2,4-D. Cultured cells were harvested at 8 days after inoculation. For the culture condition and medium except for the 2,4-D addition, see the text. ■, UQ content, ▥, Total UQ; □, Cell yield.

Table 1. Combined effect of 2,4-D and phosphate concentration on UQ formation

Age (days)	2,4-D concn (mg $l.^{-1}$)	KH_2PO_4 concn (mg $l.^{-1}$)	Cell yield (g-dry wt/flack)	UQ content (μg g^{-1})
6	0.2 (control)	170	1.60	320
	2.0	170	0.96	513
	2.0	510	1.48	313
8	0.2 (control)	170	1.48	387
	2.0	170	1.07	587
	2.0	510	1.13	427

For the culture condition and medium except for 2,4-D and phosphate concentration, see the text.

Combined effect of 2,4-D *and phosphate concentration on UQ formation*

It has been reported that an increase of phosphate content in the medium markedly accelerates cell growth [19, 20]. In any attempt to promote the cell growth without any decrease in UQ content, BY-2 cells were cultured in a higher concentration of 2,4-D with phosphate 3 times as high as that of the basal medium, the results being shown in Table 1.

Addition of phosphate caused cell growth to recover to a considerable extent. However, UQ content was almost the same as that of the control culture on the 6th day. Even on the 8th day, it was only *ca* 10% higher than that of the control culture. The effect of 2,4-D on UQ formation was not significant in the cultures with a high concentration of phosphate.

Changes in UQ content during subculturing

Changes in UQ content and growth improvement were examined during successive subculturing at a high concentration of 2,4-D (5.0 mg $l.^{-1}$) (Fig. 5). In the first generation, the cell yield was 1.02 g-dry wt per flask and UQ content was 513 μg g^{-1}. In the 61st generation, the cell yield was slightly recovered to 1.19 g-dry wt. On the other hand, the UQ content was decreased to 320 μg g^{-1} which was almost the same as that of the parent BY-2 cells in the control medium. Thus a high concentration of 2,4-D had no effect on UQ formation after successive subculturing. The recovery of cell growth in the presence of a high concentration of 2,4-D may be due to the selection of variants with high growth rate in the given medium during subculturing [21, 22] and the UQ content of those cells might be lower. Generally, cultured cells are not uniform concerning cell growth [23] and biosynthetic activity [24–26].

EXPERIMENTAL

Cultured cells and culture medium. BY-2 cells were derived from the pith of *Nicotiana tabacum* L. cv BY-2. BY-2 auxin-independent cells were made by decreasing NAA gradually from parent BY-2 cells [22]. Xanthi cells were induced from seedling of *N. tabacum* L. cv Xanthi [27]. Crown gall cells, which are auxin-independent cells, were obtained from the tumor gall formed on a plant of *N. tabacum* L. cv Hicks-2 after infection by *Agrobacterium tumefaciens* [20, 28]. Linsmaier–Skoog inorganic medium [29] containing ($l.^{-1}$) 30 g sucrose and 1.0 mg thiamine–HCl was used as the basal medium. This was supplemented either with ($l.^{-1}$) 0.2 mg 2,4-D for BY-2 cells, or with 100 mg *myo*-inositol, 0.2 mg kinetin and 0.5 mg 2,4-D for Xanthi cells. For BY-2 auxin-independent cells and crown gall cells, basal medium was used without auxin. The initial pH of these control media were adjusted to 6.0 with 0.5 N NaOH. Stock suspension cultures were routinely subcultured in the control medium at intervals of 7 days.

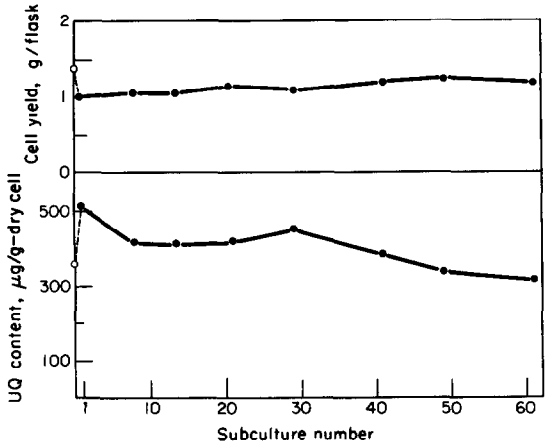

Fig. 5. Changes in the UQ content during series of successive subculturing. Cells were routinely subcultured on the basal medium supplemented with 5.0 mg l^{-1}. of 2,4-D with the interval of 7 days. The inoculum size was one-tenth. The cells for determination were harvested at 8 days after inoculation (O) indicates the UQ content and cell yield of parent BY-2 cells. For the culture conditions and medium except for 2,4-D concentration, see the text.

Culture conditions and harvesting. Incubation was carried out in 500 ml flasks containing 100 ml medium at 28° in the dark on a reciprocal shaker (100 strokes/min; 2.0 m in length). Seed cultures were incubated for 7 days, and 10 ml of the culture was inoculated into each flask. Cultured cells were separated from culture media by filtration through filter paper and weighed for fr. wt determination and then lyophilized. Extraction and determination of UQ were carried out as described in the previous paper [2].

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