A comparison of effects of several herbicides on photoautotrophic, photomixotrophic and heterotrophic cultured tobacco cells and seedlings

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Abstract

The effects of herbicides with different primary modes of action were examined on the growth of photoautotrophic, photomixotrophic, and heterotrophic cultures of tobacco cells. These responses were compared with those of tobacco seedlings to the same herbicides. Herbicides, which primarily inhibit or disturb photosynthetic processes, suppressed the growth of all types of cultured cells at similar concentrations (2,4-D, diuron, paraquat, bialaphos, DTP), but the photoautotrophic cells were still the most sensitive to all kinds of herbicides except sodium chlorate. Furthermore, photoautotrophic cells responded to most of the herbicides as did the seedlings, with the exception of glyphosate and diphenamid. The possibility of photoautotrophically cultured cells as a model system to study the effects of herbicides are discussed.

Abbreviation: bialaphos, (2-amino-4-methylphosphino-butyryl)alaninalanine sodium salt; diuron, 3-(3,4-dichloro-phenyl)-1,1-dimethyl-urea; 2,4-D, 2,4-dichlorophenoxy-acetic acid; DTP, 1,3-dimethyl-4-(2,4-dichlorobenzoyl)-5-hydoxy-pyrazolate; dinoseb, 2-sec-butyl-4,6-dinitrophenol

Introduction

Photoautotrophism is a unique characteristic of plant metabolism which has been investigated using intact plants, detached leaves, isolated mesophyll cells (Ashton et al., 1977), green algae or isolated chloroplasts as model systems. Each of these systems has its advantages as well as some problems; intact plants are excessively complicated for analyses while isolated cells may exhibit abnormal metabolic responses as a result of the isolation procedure (Paul et al., 1978). While unicellular green algae are useful simple systems for photosynthesis research, they may not be fully representative of the metabolism in higher plants. Alternatively cultured photoautotrophic higher plant cells offer a comparatively homogeneous, sterile, cuticle free system with little need for intercellular transport in which all cells are metabolically active. Thus, cultured green cells from higher plants could provide a useful new system for research on the cellular basis of photosynthesis in higher plants (Yamada, 1985).

Several photoautotrophic cultures of higher plants have been isolated (Hüsemann and Barz, 1977, Yamada and Sato, 1978, Horn et al., 1983, La Rosa et al., 1984). Studies on these photoautotrophic cultures show that the cells have well developed chloroplasts with a chemical composition and photosynthetic activity similar, but not identical to, mesophyll cells (Hüsemann et al., 1979, Yamada et al., 1982, Horn and Widholm, 1984). While the cultured cells photosynthetically fix carbon dioxide mainly through the Calvin cycle, they have an additional carboxylation pathway, namely, active PEP carboxylase (Sato et al. 1980, Hüsemann et al. 1979). These results indicate that cultured green cells, even photoautotrophic cells, have similarities to and differences from mesophyll cells. Because of these differences it remains to be determined if photoautotrophic cells may be used as a model system for mesophyll cells.

Herbicides have been developed to control weeds due to their selective action on the plant metabolism. The mode of action of several herbicides is known to be specific to certain metabolic processes. Based on this knowledge, we compared the response of both cultured cells and seedlings to a wide spectrum of herbicides. We used 12 herbicides which are thought to have different primary modes of action (see reviews, Ashton and Crafts, 1981, Dodge, 1983). Atrazine and diuron primarily affect the photosynthetic electron transport in chloroplasts. Prepanil inhibits photosynthesis, but an inhibitory effect on root growth was also reported. Paraquat diverts part of the electron flow in chloroplasts by the competition with ferredoxin for electrons from Photosystem I, producing superoxide by the reaction with oxygen. Nitrofen is thought to be activated in the light and inhibit electron transport and photophosphorylation, 2,4-D is thought to disturb the auxin action in plant cells although the exact mode of action still remains to be elucidated. Diphenamid is phytotoxic mainly, as a result of the inhibition of cell division. Glymphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase, one of the key enzyme in the biosynthesis of aromatic amino acids (Amrhein et al. 1980). Bialaphos, an antibiotic produced by Streptomyces hygroscopicus SF-1293 (Kondo et al., 1973), is used as a nonselective contact action-type herbicide in Japan. Its catabolic product (phosphinothricin) inhibits glutamine synthetase, a key enzyme of nitrogen assimilation (Leason et al., 1982). Dinoseb is an uncoupler which inhibits respiration as well as photosynthesis. Sodium chlorate is a strong oxidizer.

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The primary mode of action of DTP is postulated to be the inhibition of chlorophyll biosynthesis (Kawakubo et al., 1979).

While heterotrophically cultured cells have been used to investigate the effects of some herbicides or to select herbicide resistant cells or plants (Zilkah et al., 1979), Grassel et al., 1978, Davis and Shimabukuro, 1980, Nafziger et al., 1984, Chaleff and Ray, 1984), the photosynthesis-inhibiting herbicides are not particularly effective in inhibiting the growth of these cells with low photosynthetic rates. However, recently Cséplé and Medgyesy (1986) were successful in observing the effect of photosynthesis-inhibiting herbicides in photomixotrophic cultured tobacco cells when they were grown in medium with a low sucrose concentration. The report on photoautotrophic cells was limited to atrazine (Horn and Widholm, 1985). The present study compares the response of tobacco seedlings and photoautotrophically cultured tobacco cells to several herbicides with different modes of action, and discusses the possibility of using photoautotrophically cultured cells as a model system to investigate the effects of herbicides in higher plants.

Materials and Methods

Chemicals

The herbicides examined were atrazine, glyphosate, bialaphos and DTP (kind gifts from Ciba-Geigy, Monsanto, Meiji Seika Co., Ltd. and Sankyo Co., Ltd. respectively), propanil, nitrofen and diphenamid (kindly provided by Sumitomo Chemicals Co Ltd. as analytical reagents), diuron, paraquat, dinoseb and 2,4-D (purchased as analytical reagents, except that diuron was further purified by the recrystalization from benzene/ethylacetate solution before use). Their structures are shown in Fig. 1.

Cultured cells

Photoautotrophic, photomixotrophic and heterotrophic cell lines of Nicotiana tabacum var Samsun NN, were used for these experiments. The photoautotrophic cell line, which can grow in the light without organic carbon sources, also exhibits considerable photosynthetic activity at high sucrose concentration in liquid medium (Yamada and Sato, 1978).

The photomixotrophic and heterotrophic cultures of tobacco were maintained in liquid Linsmaier-Skoog basal medium containing double concentration of inositol and thiamine with 10 μM 1-naphthylacetic acid, 1 μM kinetin, and 3 % sucrose in the light (ca 8000lx; ca 120 μE/m2/sec) and the dark, respectively. Photoautotrophic cultures were maintained in the light (ca 8000lx; ca 120 μE/m2/sec) in same liquid medium as described above but without sucrose. Double tier flasks with 2M carbonate buffer in the lower part, as described by Hüsemann and Barz (1977), were employed to elevate the concentration of CO2 to 1–2 % during photoautotrophic culture. Photomixotrophic cells were subcultured every two weeks. Heterotrophic and photoautotrophic cells were subcultured every three weeks. All cells were cultured at 26±0°C with reciprocal or gyrotrary shaking (100rpm).

Seedlings

Tobacco (Nicotiana tabacum var Samsun) seeds were surface-sterilized by the sequential treatment with 70% ethanol for 30 seconds, 0.2% benzalkonium chloride for 15 minutes and 2% sodium hypochlorite for 15 minutes. After washing with sterile water, seeds were germinated on water-moistened sterile filter papers. Healthy seedlings were utilized for the experiments about two weeks after germination.

Test of herbicide toxicity

Herbicides in methanol or in filter-sterilized aqueous solution were added to culture medium at the final concentrations of 1:1 μM-1 mM. Fresh weight of 0.5g of cultured cells was inoculated in 12.5m1 of culture medium in 50 m1 flasks. Photoautotrophic cultures were incubated in a chamber in which the CO2 concentration was kept at about 1 % by flushing with CO2 enriched air (Fig. 2). In this photoautotrophic culture, silicone sponge cap (Silico-sen®, Sin-Etsu-Polymer Co., Ltd., Japan) was used to facilitate the gas exchange. Cultured cells were harvested after 2 weeks (photomixotrophic cells) or 3 weeks (photoautotrophic and heterotrophic cells) of incubation when the controls reached early stationary phase. The average increase in fresh weight of photoautotrophic, photomixotrophic and heterotrophic cells at harvest was 1.0 g, 5.5 g and 5.0 g, respectively. The effect of the herbicides on the cultured cells was evaluated by determining the increase in fresh weight of control and herbicide-treated cells and calculating relative growth where: Relative growth = (Increase in fresh weight of cells treated with herbicide/Increase in fresh weight of control cell) x 100 (%).

The effects of herbicides were examined with seedlings grown on moistened filter papers in petri dishes. The filter paper contained ten ml of half strength Linsmaier and Skoog basal medium with different concentrations of herbicides. The effect of the

Fig. 1
Structure of the herbicides used.

Fig. 2
System for photoautotrophic culture of tobacco cells consisting of a) CO2 gas, b) air compressor, c) flow control, d) reservoir for mixed gas, e) safety valve, f) distilled water to humidify and wash gas, g) air line filter to keep sterile condition, h) illumination, i) shaker, j) carbonate buffer.