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Comparative Potency of Different Plant Growth Retardants in Cell Cultures and Intact Plants

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Abstract. A comparison of the efficiency of a broad range of plant growth retardants on cell division growth of 13 cell suspension cultures is presented. The results show that (1) the new plant bioregulator tetcyclacis (NDA) is the compound with the highest activity in inhibiting cell division of all cultures tested, and (2) cell cultures react species-specifically to various compounds. Significant correlations between the results from suspension cultures and intact seedlings of the same plant species demonstrate the usefulness of cell cultures for identifying substances with a growth-regulating potency. Futhermore, the usefulness of cell cultures for establishing structure-activity relationships was shown with structural analogues of chlormequat and mepiquat chloride.

It is generally accepted that plant cell cultures offer advantages for biochemical studies of plant growth and for investigating the effects of biologically active substances, such as herbicides and plant growth regulators at the cellular level. Cultured cells represent a comparatively homogeneous axenic and cuticle-free system with almost all cells metabolically active and little need for intercellular transport. Growth can be easily monitored by cell counting, by determining packed cell volume, and by turbidimetric measurements.

Apart from the use of cell cultures for studies on metabolism and mode of action, it should also be possible to identify the biological activity of certain compounds on cell growth. With regard to herbicides, Gressel and co-workers have presented detailed information evaluating and comparing the phytotoxic effects on growth of seedlings, calli, and cell suspensions (for review see

Gressel 1980, 1984). It could be argued that cell cultures can reflect the whole plant but in a more complicated manner than expected before. Differences between the influence of herbicides on cell cultures and on whole plants could be traced back to a hampered penetration and translocation of the compounds in the intact plants. Furthermore, a sole interaction of a herbicide with the photosynthetic system resulted in an effect on green cells, but not on achlorophyllous ones. Herbicides, however, represent chemical substances which individually develop their phytotoxic activities via interfering with many different biochemical pathways and developmental processes of a plant. In contrast, most growth retardants are compounds with a more homogeneous action in modifying plant growth. They reduce elongation growth without exerting phytotoxic effects. The mode of action of many synthetic plant growth retardants is generally seen in reducing the gibberellin content (for review see Graebe and Ropers 1978) and/or in an inhibition of sterol biosynthesis (Douglas and Paleg 1981, Buchenauer and Roehner 1981, Grossmann et al. 1983), thereby slowing or inhibiting cell division and cell enlargement in the subapical meristems of the plants (Dicks 1980). Exponentially growing cell cultures correspond to plant meristems since cell division is the major cellular event (Sloan and Camper 1981). Likewise, cultured cells growing heterotrophically must be regarded as a sink for nutrients similar to cells in meristematic tissues. Thus, cell cultures may be used as a model system well suited to identify and to compare the activity of plant growth retardants. However, so far only a few experiments with growth retardants in cell cultures have been reported. Zilkah and Gressel (1978) have shown that daminozide and chlormequat chloride inhibit growth of suspension cultures without having phytotoxic effects. Recently, we described experiments for characterizing the phytostatic effects of growth retardants on growth in suspensions of soybean, cotton, and maize monitored by cell counting, packed cell volume, and turbidity (Grossmann et al. 1982).

The aim of this communication is to present comparative data about the influence of a broad range of plant growth retardants on cell division growth of 13 cell suspension cultures derived from various crop plants. In addition to the known growth regulators chlormequat chloride, AMO-1618, daminozide, and ancymidol, quaternary ammonium compounds (Jung 1967, 1970, Jung and Dressel 1977), the norbornenodiazetine derivative tetcyclacis (Jung et al. 1980), and three triazole compounds were used. The results obtained with maize, rice, soybean, and sunflower cultures were compared with the effects of the compounds on intact seedlings. Furthermore, the usefulness of cell cultures for establishing structure-activity relationships was investigated with structural analogues of chlormequat and mepiquat chloride.

Materials and Methods

Chemicals

The following plant growth retardants were used: N-trimethyl (β-chloroethyl)-ammoniumchloride (chlormequat chloride, chlorocholine chloride, CCC); N-

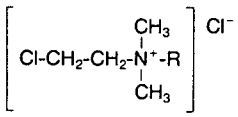
dimethyl-(β-chloroethyl)-hydrazoniumchloride (CMH); 1,1-dimethyl-morpholiniumchloride (DMC); 1,1-dimethyl-piperidiniumchloride (mepiquat chloride, DPC); (2-isopropyl-5-methyl-4-trimethyl-ammoniumchloride)-phenyl-1-piperidinium-carboxylate (AMO-1618); succinic acid 2,2-dimethyl hydrazide (daminozide); α-cyclopropyl-α(4-methoxyphenyl)5-pyrimidine methanol (ancymidol); 5 - (4-chlorophenyl) - 3,4,5,9,10-pentaaza-tetra-cyclo-5,4,1,$0^{2,6}$,$0^{8,11}$-dodeca-3,9-diene(tetcyclacis, NDA, BAS 106 W, LAB 102 883); 3-[1,2,4-triazolyl-(1)]-1-(4-chlorophenyl)-4,4-dimethyl-pentan-1-on (LAB 117 682); 3-[1,2,4,-triazolyl-(1)]-1-(4-chlorophenyl)-1-hydroximino-4,4-dimethyl-pentane (LAB 129 409); 3-[1,2,4-triazolyl-(1)]-1-(4-chlorophenyl)-4,4-dimethyl-2-methyl-pentan-1-on (LAB 130 827). The structural formulae of the growth retardants used are listed in Fig. 1.

Cell Suspension Cultures

Freely suspended callus cells from *Zea mays* L. (cv. Black Mexican Sweet), *Oryza sativa* L. (cv. Bahia), *Gossypium hirsutum* L. (cv. Stoneville), *Glycine max* (L.) Merr. (cv. SRF 400), *Brassica napus* L. (cv. Kasan), *Lycopersicon esculentum* Mill. (cv. Grosse Fleischtomate), *Arachis hypogaea* L. (cv. Tamnut), *Beta vulgaris* L. (cv. Polybeta), *Helianthus annuus* L. (cv. Spanners Allzweck) and *Acer pseudoplatanus* L. derived from shoots, *Glycine max* (L.) Merr. (cv. Mandarin), *Nicotiana tabacum* L. (cv. Maryland Mammoth), and *Daucus carota* L. derived from roots were cultivated in a Murashige-Skoog medium (1962) modified according to Seitz and Richter (1970) with amino acids (Koblitz and Hagen 1962), vitamines (Bergmann 1967), indole-3-acetic acid and kinetin (Murashige and Skoog 1962), and dichlorophenoxyacetic acid (Bergmann and Berger 1966). The cells, grown in culture for several years, were subcultivated at intervals of 7 d in the exponential growth phase. The culture conditions were as follows: 250-ml Erlenmeyer flasks containing 80 ml of cell suspension were shaken on a rotary shaker at 110 rpm in the dark at 25°C. Under these conditions the duration of the growth cycle of the suspension cultures is 10–14 days with an average rate of increase in cell number of 6.

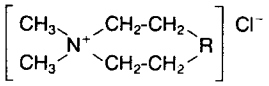
Solutions of the plant growth retardants were prepared in acetone and added to the flasks shortly before inoculation. The final acetone concentration in the medium (1%) had no adverse effects on the growth of each of the cultures.

As the measure of growth in the suspension cultures the cell number was determined during growth cycle as described previously (Grossmann et al. 1979). The differences in activity of the various compounds were calculated from the obtained growth curves and expressed in Z_{max} and $Z_{max/2}$ values. These values reflect the inhibition of the increase in cell number in percent with reference to the times during growth cycle of the maximum (Z_{max}; early stationary phase) and half maximum ($Z_{max/2}$; exponential phase) increase in cell number of the control. Thus, Z_{max} provides information about growth-inhibiting, and $Z_{max/2}$ about growth-retarding effects of a compound. Therefore, the mean of both values includes the respective inhibiting and retarding potency to characterize the efficiency of a compound in a suspension culture (Grossmann



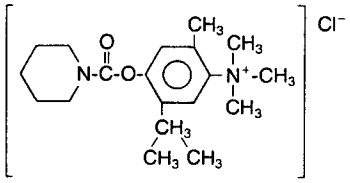
$R = CH_3$ Chlormequat chloride (CCC)

$R = NH_2$ Dimethylhydrazoniumchloride (CMH)

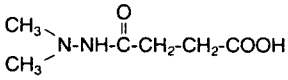


$R = CH_2$ Mepiquat chloride (DPC)

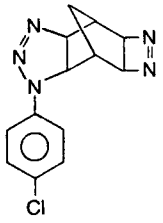
$R = O$ Dimethylmorpholiniumchloride (DMC)



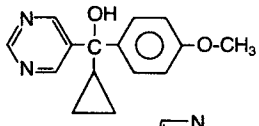
AMO-1618



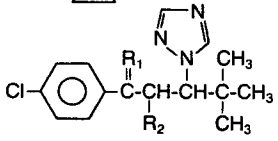
Daminozide



Tetcyclacis (NDA, BAS 106..W)



Ancymidol



Triazole-type compounds

$R_1 = O$; $R_2 = H$	LAB 117682
$R_1 = O$; $R_2 = CH_3$	LAB 130827
$R_1 = N\text{-}OH$; $R_2 = H$	LAB 129409

Fig. 1. Structural formulae of the active ingredients used.

et al. 1982). In Table 1 these mean values represent the values of growth inhibition in percent with reference to the control.

Seedling Treatments

For experiments with seedlings, seeds of *Glycine max* (L.) Merr. (cv. Gieso), *Zea mays* L. (cv. Inra Korn), and *Helianthus annuus* L. (cv. Spanners Allzweck) were pregerminated in vermiculite for 4–6 d, and the seedlings cultivated hydroponically on a nutrient solution according to Linsmaier and Skoog (1964) (14 h/day, $1.45 \cdot 10^{20}$ Q · $m^{-2} \cdot s^{-1}$, 400–750 nm; fluorescent lamps Radium HLRV, 1,000 W; at 25°C). After 3 days of adaptation the growth retardants were added to the medium. After 2 weeks the length of the sixth leaf sheath was measured in the case of maize, and after 3 weeks the shoot length above the cotyledons was determined in soybean and sunflower. These were compared to controls as a measure of growth reduction. For testing the growth retardants on *Oryza sativa*, seeds of the cultivar Girona were pregerminated on wet filter paper. Seedlings were cultivated in 100-ml cylinders with a 50% Linsmaier-Skoog nutrient solution containing the growth retardants (continuous light from Osram Universal White incandescent tubes; light intensity 5.5×10^{19} Q · $m^{-2} \cdot s^{-1}$, 400–750 nm). The length of the second leaf sheath was determined after 6 days and taken as a measure of growth reduction (Rademacher and Jung 1981). The values determined represent the mean of 6–8 test plants. Individual standard errors were less than 10%.

Cotton plants (*Gossypium hirsutum* L. cv. Delta Pine) were grown under standardized greenhouse conditions in a commercial peat substrate (200 mg N, 87 mg P, and 249 mg K in 1 l) with 6 plants per 500-ml pot. Seedlings 12 cm in height were sprayed by an automatic dispenser with solutions of several quaternary ammonium compounds, indicated in Fig. 2, in a concentration of 1.5 mg/pot. Each compound tested was dissolved in water with 0.015% Citowett. Control plants were treated with blank solutions. The shoot lengths of the plants were determined after 6 weeks. The relative cation lipophilicity of the compounds was calculated according to Rekker (1977) as the sum of the individual fragmental constants f neglecting a constant f-value for N^+.

Results and Discussion

A comparison of the biological effects evoked by the broad range of chemically different plant growth retardants (listed in Fig. 1) on cell division growth of the different cultures is presented in Table 1. Summarizing the data, the new plant growth retardant tetcyclacis proved to be the compound with the highest activity in inhibiting cell division of all cultures tested. The compound is also very active in intact plants of all higher plant species hitherto studied. Recently, it was shown that tetcyclacis could inhibit cell-free gibberellin biosynthesis of pumpkin endosperm (Graebe 1982) and that tetcyclacis is assumed to influence membrane permeability and protein synthesis in maize suspension cells by altering sterol biosynthesis (Grossmann et al. 1983). Other compounds with high efficiency in most of the cultures tested are ancymidol (except in peanut and

Table 1. Effects of 11 plant growth retardants on cell division growth of 13 suspension cultures

Compounds (10^{-4} M)	Cell suspension cultures (growth inhibition in % with reference to the control; mean values of Z_{max} and $Z_{max/2}$												
	Derived from shoots										Derived from roots		
	Maize	Rice	Cotton	Soybean	Rape	Tomato	Peanut	Sugarbeet	Sunflower	Sycamore	Soybean	Tobacco	Carrot
CCC	14	10	25	28	16	10	19	0	7	15	0	15	8
CMH	14	3	20	9	8	9	16	6	4	22	0	8	0
DMC	5	9	24	29	7	15	0	7	7	16	0	—	6
DPC	10	20	57	35	11	11	19	3	2	32	8	34	0
AMO-1618	7	13	28	89	51	28	25	6	18	8	26	22	0
Daminozide	14	13	13	40	15	25	7	9	27	7	0	31	4
LAB 117 682	90	84	73	100	96	74	77	74	70	96	81	40	36
LAB 129 409	64	50	76	63	95	61	41	58	58	88	65	36	24
LAB 130 827	78	59	86	78	73	53	53	59	47	97	60	21	26
Ancymidol	82	74	88	79	76	72	27	60	68	79	86	63	25
Tetcyclacis	100	99	100	100	100	100	100	100	96	97	93	100	100

Suspension cultures, grown under identical conditions, were treated with 10^{-4} M of each compound. Cell division growth was monitored by cell counting during the growth cycle. The efficiency of the different compounds was calculated from growth curves and expressed in mean values between Z_{max} and $Z_{max/2}$, which reflect the percent inhibition of the increase in cell number with reference to the times during growth cycle of maximum (Z_{max}; early stationary phase) and half maximum ($Z_{max/2}$; exponential phase) increase in cell number of the control. Data are mean values from at least two separate experiments.

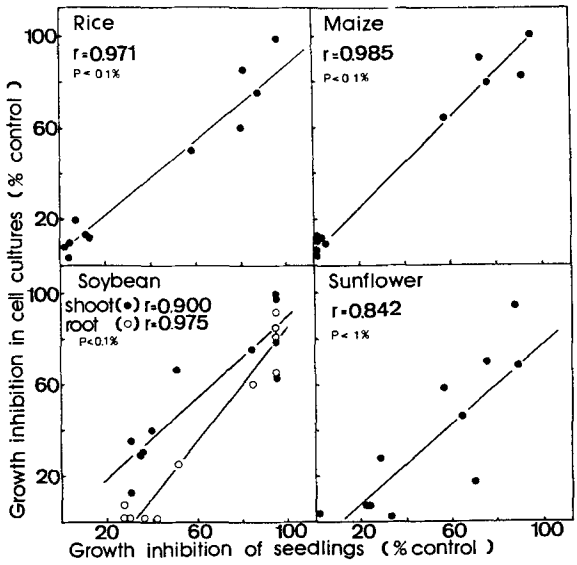


Fig. 2. Correlation of the effects of plant growth retardants on cell division of suspension cultures and growth of whole plants of four species. Suspension cultures and hydroponically cultivated seedlings were treated with 10^{-4} M of each compound. Inhibition of cell division in suspensions (data presented in Table 1) versus growth inhibition of seedlings and the corresponding correlation coefficients are shown. In the case of soybean, results from suspensions of cultivar SRF 400 derived from shoot (●) and of cultivar Mandarin derived from root (○) were correlated with data from seedlings (cv. Gieso).

carrot) and the triazoles in order of decreasing activity LAB 117 682, LAB 130 827, and LAB 129 409 except in tobacco and carrot. The triazole compounds caused growth-retarding effects with extended lag phase followed by an increase of cell division in some of the less sensitive, root-derived cultures. This might indicate metabolism of the substances to inactive forms. Cell cultures of peanut, tobacco, and carrot showed especially marked effects with high $Z_{max/2}$ values and low Z_{max} in response to triazole compounds. The quaternary ammonium derivative DPC with strong growth-retarding effects in cotton (Grossmann et al. 1982) and AMO-1618 in rape and soybean cultures derived from shoot must be regarded as compounds with species-specific activity. Comparatively low biological activity in cell cultures can be attested to CCC, CMH, DMC, and daminozide at a concentration of 10^{-4} M. Among the cell suspensions, cultures from the dicots soybean, cotton, rape, and sycamore, all originating from shoot tissue, respond most sensitively to growth retardants. In contrast, suspensions derived from root seem to be less sensitive than cells from shoot tissue. This finding is confirmed in soybean by comparing the data from suspensions of cultivar SRF 400 derived from shoot with data of cultivar Mandarin derived from root. However, an influence of genetic differences on the response to growth retardants cannot be ruled out.

To demonstrate the usefulness of cell suspension cultures for identifying growth retardants, the potencies of the compounds in suspensions of maize, rice, soybean, and sunflower were compared with findings of the corresponding intact plants (Fig. 2). Data from inhibition of cell division in the suspensions were plotted against the reduction in shoot or length of leaf sheath of seedlings. Highly significant correlations were obtained with rice, maize, and soybean and significant correlations with sunflower. In the case of soybean results from suspensions of cultivar SRF 400 derived from shoot as well as of cultivar Mandarin derived from root correlated highly with data from seedlings. (cv. Gieso).

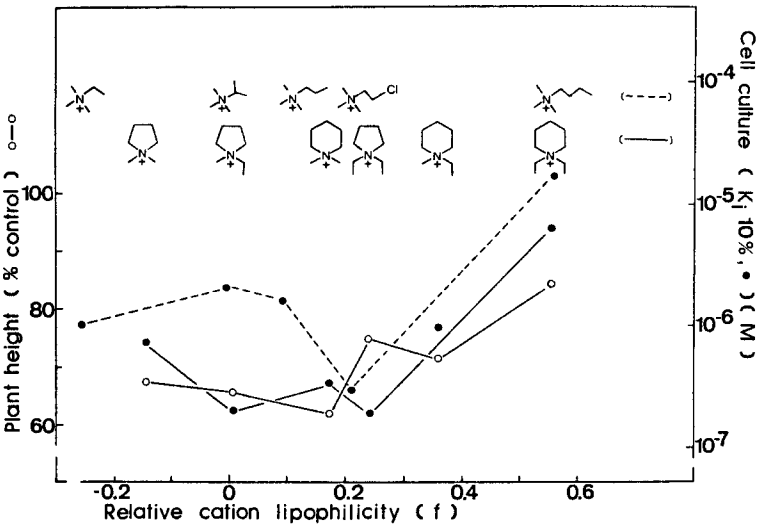


Fig. 3. Structure-activity relationships between cation lipophilicity of quaternary ammonium analogues of chlormequat and mepiquat chloride with activity as reduction of shoot length of seedlings and cell division growth in suspension cultures of cotton. Cotton plants were grown under greenhouse conditions and sprayed with 1.5 mg compound per pot. The plant height was measured 6 weeks after application. The effects on cell suspension growth were indicated by K_i 10%, which is the concentration resulting in a growth reduction of 10%. All substances were applied at 10^{-4}, 10^{-5}, 10^{-6}, and 5×10^{-7} M and the effects on cell division measured after 14 days.

The quaternary ammonium derivative DPC, which is used in agriculture to reduce plant height and vegetative growth in cotton, also had species-specific activity in cotton suspension cultures. Thus, it should be possible to establish structure-activity relationships using cell cultures. An experiment comparing the influence of several structural analogues of DPC on shoot length of seedlings and cell division growth in suspension cultures of cotton is summarized in Fig. 3. The growth-influencing potency of the derivatives apparently depends on their cation lipophilicity in intact plants as well as in suspensions of cotton. Compounds having high relative lipophilicity showed reduced growth-influencing potency. A similar result could be received for the effects of several structural analogues of CCC in cotton cell cultures (Fig. 3).

To sum up, suspension cultures appear well suited for identifying substances with a growth-retarding potency. This could be demonstrated both by the significant correlations in the spectrum of action of the substances tested in cell cultures and the seedlings of the same species, and in the experiments evaluating structure-activity relationships of growth retardants in cotton.

These data support the hypothesis that the mode of action of growth retardants at the cellular level is similar in cell cultures and in whole plants.

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