THE EFFECT OF CCC ON GROWTH AND ENDOGENOUS GROWTH SUBSTANCES IN WEDGWOOD IRIS.

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SUMMARY

The growth retardant (2-chloroethyl) trimethylammoniumchloride (CCC) was incorporated in a nutrient medium on which isolated buds and scales of Wedgwood iris were cultivated. CCC inhibited elongation of the older of the two leaf primordia that were left on the explant. In the process of leaf elongation a competitive interaction relationship with gibberellic acid could not be established, although both growth regulators act in opposite directions. CCC did not retard flower formation, which process is promoted by gibberellic acid. CCC promoted the production of gibberellin-like substances in excised scales.

1. INTRODUCTION

Bulbs of Wedgwood iris contain several growth substances (RODRIGUES PEREIRA 1964). Three of them, for the time being indicated as A, B, and C, seem to be important for the formation of the flower primordium. When chromatographed on paper with 80% isopropanol they have R_f values of 0.25, 0.45, and 0.75, respectively. On the strength of various bio-assays the two latter fractions are supposed to be gibberellin-like. The first fraction probably has no gibberellin-like nature.

The growth substances are found both in the scales and in the bud of the bulb, but in the bud higher concentrations are attained (RODRIGUES PEREIRA 1964). It was therefore conceivable that these substances are stored in the scales, either in their final form or in the form of a precursor, and that they are transported to the vegetation point under the influence of the temperature treatment required for flower formation. However, also in isolated scale fragments that are incubated on nutrient medium the amounts of these substances increase (RODRIGUES PEREIRA 1965).

NINNEMANN *et al.* (1964) found that synthesis of gibberellin by the fungus *Gibberella fujikuroi* is inhibited by the growth retardant (2-chloroethyl) trimethylammoniumchloride (CCC or Cycocel). HARADA & LANG (1965) compared CCC and seven analogs with respect to their effect on growth of wheat, squash, and cucumber, and on gibberellin synthesis in *Gibberella fujikuroi*. They found that the two activities parallelled each other. ZEEVAART (1965), who sprayed plants of *Ipomoea nil* with CCC shortly before and after anthesis, reported that gibberellin activity in the seeds of the treated plants was much

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lower than in those of the controls. More arguments for the inhibitory action of CCC on gibberellin synthesis are summarized in the review by CROSS (1968).

On the other hand there are also reports on growth promotion and increase of gibberellin content under the influence of CCC. HALEVY & WITTWER (1965) state that growth of snapdragon (*Antirrhinum majus*) is promoted by a foliar spray of CCC. VAN BRAGT (1969) treated young tomato plants with CCC by a single application via the roots. Initially there was a growth inhibition, but after 25 days the difference between treated and control plants had disappeared. Five days after application of CCC the total gibberellin content of the plants, as determined with the dwarf pea assay, was slightly larger than that of the controls. HALEVY & SHILO (1970) observed that application of CCC to gladiolus plants caused an increased growth and a greater number of flowers. The content of endogenous gibberellins in the aqueous fraction of their extract had also increased.

Considering these seemingly contradictory results, and seeing that flower formation in Wedgwood iris seems to be controlled by endogenous gibberellin-like substances, we decided to study the role of CCC in developmental processes in this plant. Using the technique of organ culture developed in earlier studies, we examined the effect of CCC both on leaf growth and flower formation in excised buds, and on the synthesis of endogenous growth substances in scale fragments.

2. MATERIAL AND METHODS

But for minor modifications the bulb material and the methods of culture and of determination of growth substances were as described earlier (RODRIGUES PEREIRA 1964). Contrary to previous years the bulbs were not stored at 25.5 °C but at 30–32 °C. This resulted in a much stronger inhibition of all developmental activities. Basal Medium (BM) was according to NITSCH & NITSCH (1967). Gibberellic acid (GA) and CCC were sterilized by millipore filtration.

3. RESULTS

3.1. The influence of CCC on growth and development of excised buds

CCC in three different concentrations was added to BM without and with 50 mg/l GA. Buds were excised from bulbs that had been stored at high temperature or from bulbs that, after high temperature storage, were activated by a pretreatment at 13 °C for a number of weeks. Incubation of the explants lasted seven weeks in the first and five weeks in the second case, i.e. the duration of pretreatment and incubation together was seven weeks in all cases. In every explant the two youngest recognizable leaf primordia were left in place.

In *table 1* the length of the longest leaf at the end of the experimental period is given both in millimeters and in percentage of the control. In each of the four series absolute leaf growth is increasingly retarded by higher concentrations of

Table 1. Influence of CCC and GA on leaf growth in excised buds. There were 15 buds per treatment; incubation temperature 13 °C.

Treatment	Leaf length			
	no pretreatment 7 weeks incubation		2 weeks pretreatment at 13 °C 5 weeks incubation	
	mm	% of control	mm	% of control
BM	110	100	51	100
BM + 100 mg/l CCC	91	83	48	94
BM + 300 mg/l CCC	86	78	48	94
BM + 1000 mg/l CCC	36	34	29	57
BM + 50 mg/l GA	154	100	80	100
BM + 50 mg/l GA + 100 mg/l CCC	150	97	65	81
BM + 50 mg/l GA + 300 mg/l CCC	120	78	61	76
BM + 50 mg/l GA + 1000 mg/l CCC	60	39	52	62

CCC. As soon, however, as we express these growth retardations in percentages of the respective controls, we see that actually the growth rates are the same in the presence and absence of gibberellic acid. We also calculated the percentage of growth promotion by gibberellic acid for each CCC concentration applied (*table 2*). In relative terms growth promotion was the same in the presence and absence of CCC. This seems to lead to the conclusion that there exists no genuine interaction between these two growth regulators. It should be noted that the leaves, the growth of which is measured in the two experimental series without and with bulb pretreatment, are not identical. Owing to the fact that new leaf primordia are initiated during the pretreatment itself, and that only two primordia are left on the apex, the leaves of the first series are older than those of the second. Moreover, they have been left to grow for two more weeks.

There was never any effect of CCC either on fresh weight of the explant or on flower induction or developmental stage of the flower primordium. Also gibberellic acid had no significant effect on fresh weight.

Table 2. Growth promotion by 50 mg/l gibberellic acid of the leaves of excised buds of Wedgwood iris cultivated on CCC containing media. Experimental data as in table 1.

Treatment	Growth promotion in %	
	bulbs not pretreated	2 weeks pretreatment at 13° C
no CCC	140	154
100 mg/l CCC	165	135
300 mg/l CCC	140	127
1000 mg/l CCC	167	179

Table 3. Influence of CCC on growth substance activities in scale fragments of Wedgwood iris. Scale fragments were incubated on basal medium for one week at 13 °C. Paper chromatograms of methanolic extracts of untreated and incubated scales were tested with the Avena mesocotyl test.

Fraction	Untreated scales	IAA equivalents in μg/l		
		Amount of CCC in medium (g/l)		
		0	0.1	1
A (R_f 0.25)	1	1	2	3
B (R_f 0.45)	–	–	5	3
C (R_f 0.75)	2	2	5	2

3.2. The influence of CCC on the content of growth substances in scale fragments

In the second part of our study we incubated scale fragments of non-pretreated bulbs during one week on BM in which various quantities of CCC were incorporated. Afterwards, both scales and medium were separately freeze-dried and the growth substance spectrum determined in 100 mg of dry matter. As controls were used, on the one hand, scales of non-treated bulbs and, on the other, agar medium without CCC. The experiment was carried out three times, each time with six bulbs per treatment. The increase in length of *Avena* mesocotyl segments, which is admittedly not a specific assay for gibberellins, under the influence of the growth substances present in the extracts was compared with that of known quantities of indoleacetic acid (IAA) and expressed as IAA-equivalents in μg/l (*tables 3* and *4*). There is no effect of CCC in the mesocotyl test. CCC promoted the production of the endogenous growth substances in the scales of the iris bulb. The fact that these substances were also found in the nutrient medium confirms our earlier findings (RODRIGUES PEREIRA 1964) that flower formation in excised buds is promoted on a medium on which scales have previously been incubated.

The amount of growth substances found in the medium seems to be much larger than in the scales themselves, but this difference is only apparent. In both cases 100 mg of dry matter was analyzed. The scales, however, contain about

Table 4. Influence of CCC on growth substance activities in agar medium at the end of a one week incubation period of scale fragments of Wedgwood iris. Paper chromatograms of methanolic extracts were tested with the Avena mesocotyl test.

Fraction	IAA equivalents in μg/l		
	Amount of CCC in medium (g/l)		
	0	0.1	1
A (R_f 0.25)	2	2	5
B (R_f 0.45)	1	3	7
C (R_f 0.75)	1	13	13

35% of dry matter, whereas in the medium only about 5% of dry matter is incorporated, *viz.* 4% of sucrose and 1% of agar. The results are thus more or less according to expectations.

4. DISCUSSION

In the experiments described here CCC acted as a growth retardant and GA as a promotor of leaf growth in isolated buds, but an interaction of the two growth regulators, let alone an antagonism or a competitive inhibition as proved by LOCKHART (1962) for bean plants, could not be established. Further, CCC had neither a positive nor a negative effect on flower formation, but it seemed to cause an increase in the amount of the two fractions B and C of scale extracts and of extracts of media on which these scales had been incubated. Diffusates of scales had previously been shown to promote flower formation in explants (RODRIGUES PEREIRA 1964). In addition it should be considered that gibberellic acid also promotes flower formation in Wedgwood iris, both in the whole bulb (HALEVY & SHOUB, 1964) and in isolated buds (RODRIGUES PEREIRA 1962). It could be objected that it was not stringently proved that it is the fractions B and C that possess the gibberellin-like activity found in the extract as a whole. But also if this growth substance activity would be more auxin-like, or if an auxin-like activity would be of additional influence, the observation remains that the mesocotyl effect of these fractions is enhanced by the presence of CCC in the medium.

This latter observation seems to confirm the results of VAN BRAGT (1969), HALEVY & WITTWER (1965) and HALEVY & SHILO (1970). Yet experimental data are always too few to permit a synthetic view of the mode of action of CCC, GA and endogenous growth substances in the process of flower formation in Wedgwood iris.

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