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A comparison between the effect of gibberellin and 2-chloroethyl trimethylammonium chloride (CCC) on some biochemical processes in bean plants

IV. Effect on the content of the inhibitor of absicisic acid properties

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Abstract:

In shoots and in roots of bean seedlings the inhibitor of ABA-like properties was stated. Its level in shoots was increased by CCC and decreased in roots by GA_3 treatment. Both substances had neither synergistic nor antagonistic or additive effects on the level of ABA-like inhibitor. The effect of GA_3 and CCC on the level of inhibitor was independent of the infuence of these substances on the growth processes. It has been concluded that the mode of action of GA and CCC in processes regulating the level of abscisic acid in plants is different for each of these substances.

INTRODUCTION

Exogenous gibberellins and growth retardants have a strong effect on the processes of plant growth and development. They have also some influence on the level of endogenous growth regulators (for review, Galston and Davies, 1969).

We have only very scarce informations concerning the effect of exogenous growth regulators on the content of abscisic acid (ABA). It is a natural plant growth regulator, gibberellin antagonist, widely distributed in the plant kingdom and playing an important role in the processes of growth and development of plants (for review, Addicott and Lyon, 1969).

The influence of gibberellic acid (GA_3) on the level of ABA was studied by Tietz and Dörffling (1969) who found that gibberellin increases the amount of ABA in basal parts of pea stems and decreases it in apical parts of plants.

There are also some data indicating the infuence of GA_3 on the inhibitor β-complex, which according to M i l b o r r o w (1967) includes also ABA. According to the data of B o o (1961), GA_3 decreases the level of inhibitor β-complex in the potato tubers. Similar results were also obtained by G w a n at al. (1964) in experiments with pea seedlings.

The purpose of this work has been to study the influence of gibberellin and growth retardant on the level of endogenous ABA in bean seedlings.

MATERIAL AND METHODS

Ten day old selected bean plants var. "Sachsa" were transferred to Knop's nutrient solution containing gibberellic acid (GA_3) at concentration of 30 ppm, 2-chloroethyl trimethylammonium chloride (CCC) at concentration of 250 ppm or mixture of these two growth regulators and were cultivated on long day conditions (16 hrs). The details concerning the method of the cultivation of plants are given in part I of this paper (M i c h n i e w i c z and S t a n i s ł a w s k i, 1965).

The measurements of growth of the first pair of leaves, shoots (without the first pair of leaves) and root system of plants were made two weeks after the transformation of plants to the nutrient solutions. This time, the measurements of the activity of abscisic acid-like substance in all mentioned plants organs were also made.

The abscisin-like inhibitor was determined in 40 g samples. Plant material was extracted using the method described by T o m a s z e w s k a (1968). Methanol extracts of plant material were acidified to pH 3 (with HCl) and shaken with ethyl ether. The ether fraction was evaporated and residue was diluted in water and then filtered through the alumina column (Al_2O_5) 5 cm high and of 3 cm diameter. The concentrated water extracts were chromatographed on Whatman 3 in water. On each chromatogram an equivalent of 0.5 g of fresh weight of plant material was located.

The bioassays which have been used for the inhibitor determination included: a) inhibition of growth of wheat (var. Ostka Chłopicka) coleoptile segments (R u d n i c k i, 1969), b) inhibition of lettuce seeds germination (I r v i n, 1969), c) acceleration of abscission of bean leaf petioles (M i c h n i e w i c z, 1970).

The physiological and chromatographic properties of the isolated inhibitor were compared with the properties of synthetic ABA. The chromatographic properties were tested using the paper (Whatman 3) as well as thin layer chromatography (Silica Gel H according to S t a h l, 1962) and six different solvents (tab. 2). Also the UV spectrum using Universal Spectrophotometer VSU-1 and the fluorescence with sulphuric acid on the plates coated with Silica gel (Milborrow, 1967, Antoszewski i Rudnicki, 1969) were compared.

The growth inhibiting activity of both inhibitors was also assayed using the microbiological test (Strzelczyk and Michniewicz, 1967, Michniewicz, 1970).

All experiments were repeated three times and the least significant differences were determined.

RESULTS AND DISCUSSION

The data concerning the influence of GA_3 and CCC on the growth of bean seedlings are presented in table 1.

Table 1

Effect of GA and CCC on the growth of bean plants

Organs	Kind of measurement		Treatment				LSD at P = 0.05
			Control	GA	CCC	GA+CCC	
The first pair of leaves	area in	cm^2	27.5	10.8	18.5	13.4	0.203
		%**	100	39.3	67.2	48.7	—
	fresh weight in	g	0.336	0.135	0.225	0.170	0.002
		%	100	40.1	66.9	50.5	—
Shoots*	length in	cm	357	619	311	770	102.1
		%	100	173.3	87.1	215.6	—
	fresh weight in	g	0.267	0.345	0.210	0.518	0.001
		%	100	129.2	78.6	194.0	—
Roots	volume in	cm^3	0.22	0.20	0.33	0.20	0.022
		%	100	90.9	150.0	90.9	—
	fresh weight in	g	0.300	0.250	0.350	0.264	0.007
		%	100	83.3	116.6	88.0	—

* without the first pair of leaves
** in relation to control = 100%

Similarly as in our previous experiments (Michniewicz et al. 1965, 1969) GA_3 stimulated and CCC inhibited the elongation of bean stems. However, unlike to the earlier results it was foud now that stem elongation induced by GA_3 was increased substantially by concurrent application of CCC. It is also in discordance with the general opinion predicated on the antagonism of these two growth regulators in plant growth processes (for review, Cathey, 1964).

The reaction of plants to the growth regulators depends of course on the internal as well as external factors. It is possible that although care was taken of keeping the same experimental conditions and the same variety of beans were used as those in previous experiments, they might have differred at some degree.

It must be underlined that similar synergistic effect of GA and CCC on plant growth processes are given also by other authors. Namely, Wittwer and Tolbert (1960) observed a synergistic growth rate in tomato ovaries, and Guttridge (1966) found synergism between these both substances in stem growth of strawberry.

Growth of roots was increased by CCC and slightly decreased by GA_3. The promotive effect of CCC was removed by gibberellin.

Both growth regulators, especially GA_3 inhibited the growth of the first pair of leaves.

Table 2

Chromatographic properties of the inhibitor isolated from bean plants and of synthetic ABA

Kind of chromatography	solvents v/v	R_f	
		inhibitor	ABA
Paper (descending)	water	0.8—1.0	0.90
paper (ascending)	isopropanol:ammonia:water 10:1:1	0.75—0.85	0.80
thin layer	benzene:ethylacetate:formic acid 70:30:5	0.3—0.4	0.30
thin layer	benzene:chloroform:formic acid 20:100;10	0.0—0.1	0.12
thin layer	benzene:acetone:acetic acid 70:30:1	0.4—0.5	0.40
thin layer	isopropanol:butanol:ammonia:water 6:2:1:2	0.6—0.7	0.65

In all tested plant organs the presence of growth inhibitor was stated. Its chromatographic behaviour on paper and thin layer plates (tab. 2), the fluorescence with sulphuric acid and the UV absorption curve were the same as the properties of synthetic ABA.

Similarly as synthetic ABA, inhibitor isolated from bean plants inhibited the growth of wheat coleoptile sections, the germination of lettuce seeds and accelerated the abscission of bean leaf petioles. Both compared substances did not inhibit the growth of *Agrobacterium radiobacter, Arthrobacter globiformis, Bacillus cereus, Bacillus subtilis, Corynebacterium michiganense, Escherichia coli, Erwinia carotovora, Flavobacterium aurantiacum, Proteus vulgaris, Pseudomonas fluorescens* and *Sarcina lutea* in the microbiological test.

So, it may be assumed with high probability that the inhibitor isolated from bean plants was identical with ABA. Abscisic acid in bean plants was also dedected by other authors (Milborrow, 1967, Barnes and Light, 1969).

As we see from the data given in table 3 and figure 1, gibberellin had

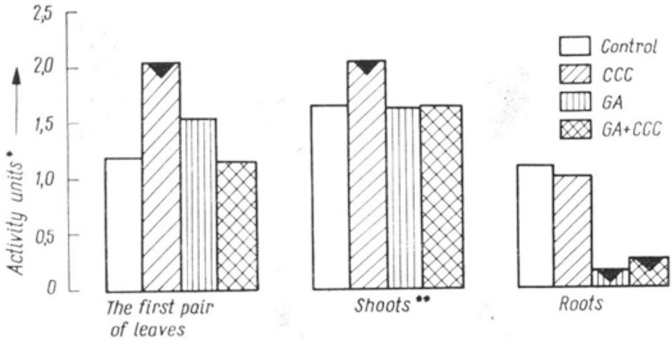

Fig. 1. The effect of GA and CCC on the ABA-like inhibitor isolated from bean content of plants

* — activity unit = 10 p.c. of growth inhibition of the wheat coleoptile section
** — without the first pair of leaves
□ — significant differences in relation to control at P = 0.05.

no influence on the level of the inhibitor in the overground parts of plants but significantly decreased the amount of the inhibitor in roots. On the other hand, CCC increased the inhibitor level in leaves and shoots but had no significant influences on the level of ABA in roots.

Table 3

The effect of GA and CCC on the content of ABA — like inhibitor isolated from bean plants (the content of inhibitor present at R_f 0.8—1.0 is expressed as the growth inhibition of the wheat coleoptile sections in p.c.)

Organs	Treatment				LSC at P = 0.05
	control	CCC	GA	GA + CCC	
The first pair of leaves	13.5	20.6	13.8	12.7	3.12
Shoots*	17.8	20.4	17.0	17.2	2.02
Roots	11.1	9.1	2.4	4.2	3.20

* without the first pair of leaves.
Underlined type indicates significant differences.

The comparison of the results with the data given in table 1 shows a lack of correlation between the rate of growth processes and the level of ABA-like inhibitor in plants.

The inhibition of growth of shoots and the first pair of leaves caused by CCC treatment was coincided with the increase of abscissin-like inhibitor level. On the other hand GA_3 which also inhibited the growth of the first pair of leaves did not influence the amount of this inhibitor.

Also the lack of correlation between the stimulation of growth and the level of ABA has been stated. The amount of abscisin-like inhibitor in shoots stimulated by GA_3 was the same as in control and as in plants more elongated, treated jointly with both growth regulators. Also the roots of plants treated by CCC growing more intensively, contained similar amounts of ABA-like inhibitor as the roots of control plants.

The results of these experiments showed that gibberellin and growth retardant used together had neither antagonistic nor additive or synergistic effects on the level of ABA-like inhibitor in bean plants. Similar results were also obtained in earlier experiments devoted to the effect of GA_3 and CCC on the level of vitamin E (Michniewicz and Kamieńska, 1969).

The lack of synergistic either additive or antagonistic effects of these two growth regulators on the level of ABA-like inhibitor lead to the supposition that the mode of action of GA_3 and CCC in the processes regulating the amount of ABA in plants is different for each of these substances.

CONCLUSIONS

In bean seedlings the inhibitor of abscisic acid properties was stated. This inhibitor occurred in shoots and in roots of plants.

Gibberellic acid did not significantly influence the level of ABA-like inhibitor in overground parts of plants but strongly decreased the level of this inhibitor in roots.

Chlorocholine chloride increased the level of ABA-like inhibitor in overground parts of plants and had no significant infuence on the amount of this substance in roots.

It was stated that gibberellin and growth retardant used jointly had neither synergistic nor antagonistic or additive effects on the level of ABA-like inhibitor.

The effect of gibberellin and growth retardant on the amount of ABA--like inhibitor was independent of the influence of these growth regulators on the growth processes.

It has been concluded that the mode of action of GA_3 and CCC in the processes regulating the level of abscisic acid in plants is different for each of these growth regulators.

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Porównanie wpływu gibereliny i chlorku 2-chloroetylotrójmetyloamoniowego (CCC) na niektóre procesy biochemiczne u fasoli

IV. Wpływ na zawartość inhibitora o właściwościach kwasu abscysynowego (ABA)

Streszczenie

Siewki fasoli hodowano na pożywce Knopa zawierającej kwas giberelowy (30 ppm), chlorek chlorocholiny (250 ppm) lub obie te substancje łącznie oraz na pożywce bez dodatku tych związków.

Giberelina stymulowała a chlorek chlorocholiny hamował wzrost łodygi. Najsilniejsze wydłużenie łodyg obserwowano przy łącznym stosowaniu obu regulatorów wzrostu roślin. Wzrost korzeni był stymulowany przez CCC i słabo hamowany przez GA_3. Stymulacja wzrostu korzeni wywołana CCC była znoszona działaniem gibereliny. Oba regulatory wzrostu roślin, szczególnie zaś GA_3 hamowały wzrost pierwszego liścia.

Zarówno w pędach jak i korzeniach fasoli wykazano obecność inhibitora którego właściwości chromatograficzne, spectrum w UV, zdolność do fluorescencji oraz właściwości fizjologiczne były identyczne jak właściwości syntetycznego kwasu abscysynowego.

Kwas giberelowy nie wpływał w sposób istotny na poziom inhibitora w nadziemnych częściach rośliny natomiast silnie obniżał ilość inhitora w korzeniach.

Chlorek chlorocholiny zwiększał poziom inhibitora w nadziemnych częściach i nie wpływał w sposób istotny na jego zawartość w korzeniach.

Wykazano brak synergicznego, antagonistycznego i sumującego się oddziaływania gibereliny i retardanta wzrostu na poziom inhibitorów w siewkach fasoli.

Wpływ GA_3 i CCC na poziom inhibitora był niezależny od wpływu tych związków na procesy wzrostowe.

Wnioskuje się, że drogi które prowadzą do zmian w poziomie kwasu abscysynowego w roślinach pod wpływem GA_3 i CCC są różne dla obu tych regulatorów wzrostu roślin.

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