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

I. Effect on catalase and peroxidase activity

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Catalase and peroxidase play a considerable role in the growth processes of plants because they affect the level of auxin-like substances. After Pilet and Galston (1955), peroxidase oxidises indoleacetic acid in the presence of H_2O_2 and therefore stops the physiological activity of auxin. Catalase, causing a destruction of hydrogen peroxide, protects auxin against the oxidizing effect of peroxidase.

Gibberellin has a great effect on the level of endogenous auxin. Both these substances show an interaction in the growth processes of plants (comp. Brian, 1959). It has been also found that under the influence of gibberellin there occurs in plants an increase of auxin-like substances (Michniewicz 1962).

The effect of gibberellin on the activity of catalase and peroxidase is, as yet, not explained. Data, regarding this topic, quoted in literature express a wide difference of opinion.

An increase of the catalase activity, as an effect of gibberellin treatment, has been found by: Munekata and Kato (1957, cit. Berquist at all. 1959) in germinating barley, Michniewicz and Stanisławski (1962) in germinating wheat, Gukova and Faustov (1963) in maize, oats and barley seeds, and Istakov (1963) in maize.

Opposite results were gained by Hayashi at all (1956) with rice, Halevy (1962, 1963) with cucumber seedlings, and Raspevin (1964) with tomatoes.

A similar aspect has the effect of gibberellin on the activity of peroxidase. An increase of the activity of this enzyme, as a result of gibberellin treatment, has been shown in experimens of: Yabuta at all. (1943, comp. Stowe and Yamaki 1957) with tea, Hayashi at all. (1956) with rice, Istakov (1963) with maize, and Raspevin (1964) with tomatoes. The last author cited also Grebinski (1961) who pointed towards an increase of the activity of peroxidase as an effect of gibberellin treatment.

Several authors have shown a diminished peroxidase activity under the influence of gibberellin. Such results were gained by McCune and Galston (1959) with dwarf pea and maize, Monselise and Halevy (1962) with citrus seedlings, Halevy (1962, 1963) with cucumber seedlings, and Gukova and Faustov (1964) with maize, oats and pea seeds.

A great influence on the growth processes of plants exhibit also some retardants, such as CCC, that retards the elongation of plants and also shows many other, properties of an antigibberellic nature (comp. Michniewicz 1963, and Leh 1964). These substances do cause a decrease of the auxin level (Kuraishi and Muir 1963) and inhibit the biosynthesis of gibberellin in higher plants (Lang 1964; Michniewicz 1964). Hence arises the problem in which way do retardants affect the activity of catalase and peroxidase.

Studies on this problem were made by Monselise and Halevy (1962), and Halevy (1962, 1963) with citrus and cucumber seedlings. Results of their experiments led to the conclusion that those substances, contrary to gibberellin, induce an increase in the activity of catalase and peroxidase. There also exists a reversed correlation between the effect of retardants on the growth of plants and the activity of these enzymes.

Data gathered by these authors with regard to the effect of gibberellin and also regarding the correlation between the activity of these enzymes and the growth intensity proved to be contrary to our results (1962) and also the results of other authors quoted above. It is also important to note that Monselise and Halevy (1962), in their experiments with citrus seedlings did not observe any antagonistic action between gibberellin and the retardant Amo — 1618 on the growth processes, which is a typical phenomenon for the activity of both these growth substances (comp. Michniewicz 1963; Leh 1964). We considered it therefore as being appropriate to carry out similar studies to check the effect of gibberellin and one of the most typical retardants, viz. CCC on the activity of catalase and peroxidase in beans — a plant reacting prompt to gibberellin and being very sensitive to the influence of CCC.

METHOD

Beans var. 'Saxa' were used for experiments. Kernels of uniformed size sprouted in garden soil, in darkness at a temperature of 21°C for 10 days. Morphologically selected plants were then transferred to Knop's nutrient solutions containing gibberellic acid (Merk's production) in a concentration of 300 ppm, and CCC (produced by the American Cyanamid Company) concentrated to 250 ppm, or without any addition of

these substances. The corresponding concentrations of the given compounds were chosen after some preliminary experiments.

The plants grew in a thermoluminostat in long day conditions (16 hours) at a light intensity of about 5000 lux and an humidity of 70% in glass containers carrying 250 ml nutrient. Five plants were placed in each container, and each experimental variant counted 40 plants.

Morphological observations and measurements regarding the activity of enzymes were made after 5 and 9 days after the transfer of plants to the nutrient solutions. The activity of enzymes was detected only in leaves, and was repeated consecutively 3 times. For one sample 10 g of fresh plant material was used. Three independent measurements were made for each of them. Euler's and Josephson's method modified by Jolles (comp. Sumner and Somers 1947) was used for the indication of catalase activity. The activity of peroxidase was defined by the method described by Reifer and Grabianowska (1952) modified by Bokučov (comp. Belozerski and Proskuriakov 1954). Its principle is to transform pyrogallol into purpurogallin. The standard curve of purpurogallin was checked by a spectrophotometer (Uvispek Photoelectric Spectophotometer H 700.308) independently on measurements taken by a photocolorimeter KF-2. Spectrophotometric measurements confirmed the identical character of the curve shown by the photocolorimeter KF-2.

A detailed description of the method to determinate catalase was given in a paper by Michniewicz and Stanisławski (1964).

All experimental results were subject to statistical analysis whereby the least significant difference was determined.

RESULTS AND DISCUSSION

Data given in table No 1 show clearly that gibberellin stimulated the growth of shoot and leaf, but inhibited the growth of root in length. Contrary to this was the effect of CCC. The morphological effects of these experiments were therefore characteristic for the used substances.

An opposite effect both substances have shown also in relation to the catalase and peroxidase activity (Table 2 and 3). The activity of both enzymes was strongly stimulated through gibberellin application and clearly retarded through CCC. The results of these experiments are therefore not in accordance with the data supplied by Monselise and Halevy (1962), and Halevy (1962, 1963) who, in experiments with citrus and cucumber seedlings gained a decrease of the activity of these enzymes as an effect of GA treatment and an increase of their activity under the influence of retardants.

Table 1

Effect of GA and CCC on the growth of bean plants
(GA in concentration 30 ppm, CCC in concentration 250 ppm)

Days after treatment	Kind of measurement (average)		Kind of growth regulators			L.S.D. at P=0.05
			C*	GA	CCC	
5	Lenght of stem in	cm	12.53	27.51	9.22	1.07
		%**	100	219.55	74.58	
	Lenght of root in	cm	8.59	6.88	9.89	1.34
		%	100	80.09	115.13	
	Leaf area in	cm^2	5.97	8.43	4.71	1.16
		%	100	141.21	78.89	
9	Lenght of stem in	cm	16.57	37.81	12.48	1.78
		%	100	228.18	75.32	
	Lenght of root in	cm	10.37	8.92	11.60	0.75
		%	100	86.01	111.86	
	Leaf area in	cm^2	11.83	13.67	8.61	1.63
		%	100	115.55	72.78	

*Control.

**Control = 100%.

All differences in relation to control are significant.

The divergent results may be doubtlessly explained by the different plant material chosen for experiments. It must be underlined that even different varieties of the same species may react to CCC in different ways. We may cite as example the results of Tso and Jeffrey (1961, comp. Leh 1964) who showed that this substance caused in some varieties of tobacco a decrease, in others an increase of the nicotine content in leaves.

Table 2

Effect of GA and CCC on catalase activity
(GA in concentration 30 ppm, CCC in concentration 250 ppm)

Days after treatment	Catalase activity	Kind of growth regulators			L.S.D. at P=0.05
		C*	GA	CCC	
5	in activity units	31.58	59.98	24.71	14.16
	in %**	100	189.93	78.25	
9	in activity units	38.49	64.93	29.56	6.19
	in %	100	168.69	76.80	

*Control.

**Control = 100%.

The fact must be stressed that during our experiments, CCC showed a stimulative effect on the growth of roots. This coincides with the results of some experiments with other plants (Linser and Kühn 1963; Supniewska 1963). Halevy (1962), however, who experimented with retardant Amo — 1618 found that this substance caused a retarding effect on the growth of roots of cucumber seedlings.

Also Monselise and Halevy (1962) did not achieve any antagonistic reaction typical for gibberellin and retardants in growth processes. In this way, the effect of retardants applied by these authors differed with the results of our experiments with beans.

The divergence between Monselise's and Halevy's (1962) and Halevy's (1962, 1963) and our results might have been also caused by some differences in the methods which were used for the determination of enzymes. It should be pointed out that Galston and McCune (1961), who studied the effect of gibberellin on the peroxidase in beans and maize, have found that the results depended on the fact whether, in order to determine this enzyme, they used pyrogallol or gauiacol as substrate.

A great influence on the activity of both enzymes in plants have also the light conditions. This was showed by Chailakhian and Bojarski (1955) with regard to peroxidase, and Michniewicz and Stanisławski (1962) regarding catalase. From the experiments of Jermyn and Thomas (1954), and Keilin and Hartree (1955) we know that peroxidase is not a homogeneous enzyme but it consists of several components showing peroxidase activity. The appearance of the particular peroxidase depends on seasonal changes (Jermyn and Thomas 1954). Periodical changes in the activity of peroxidase substances was also found by Ettori (1949). Thus, it may be concluded that differences in the outer environmental conditions might also cause differing results of experiments here discussed.

Table 3

Effect of GA and CCC on peroxidase activity
(GA in concentration 30 ppm, CCC in concentration 250 ppm)

Days after treatment	Peroxidase activity	Kind of growth regulators			L.S.D. at P=0.05
		C*	GA	CCC	
5	in mg purpurogallin	364.91	427.39	343.96	16.54
	in %**	100	117.12	94.26	
9	in mg purpurogallin	422.38	475.17	392.75	22.63
	in %	100	112.50	92.98	

*Control.

**Control = 100%.

Summarising, it may be said that the present state of knowledge does not permit any general conclusion about the possible mode of action of gibberellins and retardants on the activity of catalase and peroxidase, because the effectiveness of these substances depends on many factors, both, of environmental and internal nature. It is possible, that different kind of plants react differently depending on their genetic and physiological properties as well as their outer environmental conditions.

In the light of known literature and our results, a strict correlation between the influence of growth substances on growth, and the activity of peroxidase suggested by the fact, that dwarfed forms characterizes a higher activity of this enzyme than normal and high forms (Kamerbeek 1956; McCune and Galston 1959; Galston and McCune 1961; and cited by the last — Overbeek 1935; Hoan and Gorter 1936; and Ross 1941) can not be considered as sure. It is possible, that the effect of gibberellin and of retardants on the activity of peroxidase is independent on their action regarding the growth processes in plants.

The only certain conclusion seems to be that the influence of gibberellin on the activity of catalase and peroxidase is opposite to the influence of retardants on the activity of these enzymes.

SUMMARY

The effect of gibberellin and CCC on the activity of catalase and peroxidase in the leaves of beans, var. 'Saxa' was studied.

Ten days after sowing, the bean seedlings were transferred to Knop's nutrient solutions containing gibberellin in a concentration of 30 ppm or CCC in a concentration of 250 ppm, and also to control solutions.

After 5 and 9 days, counting from the day of transfer to nutrients, some morphological observations and measurements of enzyme were made.

Both substances caused typical changes in plant growth. Gibberellin stimulated the growth of stem and leaves but retarded the growth of root length. CCC application gave an opposite effect.

It has been found that, under the influence of gibberellin, the activity of catalase and peroxidase increased, whereas CCC inhibited the activity of both enzymes.

The results of the present investigations were compared with the data of other authors. The considerable differences existing between experimental results of various authors regarding the effect of gibberellin and retardants on the activity of catalase and peroxidase, were discussed.

After summarising the discussion it has been established that the present state of knowledge does not permit to draw the conclusion whereby gibberellin and retardants may be considered as factors affecting the activity of catalase and peroxidase in any strictly defined directions, causing an inhibiting or stimulating activity of these enzymes. Accordingly, the opinion of a strict correlation between

the influence of growth substances on the growth processes and the activity of catalase and peroxidase cannot be accepted. It seems however to be certain that the effect of gibberellin on the activity of catalase and peroxidase is opposed to the influence of retardants on the activity of these enzymes.

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

I. Wpływ na aktywność katalazy i peroksydazy.

Streszczenie

Zbadano wpływ gibereliny i CCC na aktywność katalazy i peroksydazy w liściach odmiany 'Saxa'.

Fasolę w 10 dni po wysiewie przeniesiono na pożywki Knopa zawierające giberelinę w stężeniu 30 ppm, lub CCC w stężeniu 250 ppm i na pożywki kontrolne.

Po 5 i 9 dniach po przeniesieniu roślin na pożywki dokonano obserwacji morfologicznych i pomiarów aktywności enzymów.

Oba zastosowane tu preparaty wywołały typowy dla nich efekt wzrostowy. Giberelina stymulowała wzrost łodygi i liści, a hamowała wzrost korzenia na długość. Działanie CCC było przeciwne.

Stwierdzono, że pod wpływem gibereliny zwiększa się aktywność katalazy i peroksydazy, natomiast CCC wpływał hamująco na aktywność obu enzymów.

Dane uzyskane w pracy niniejszej porównano z wynikami innych autorów. Stwierdzono wielką rozbieżność w wynikach różnych autorów odnośnie wpływu gibereliny i retardantów na aktywność katalazy i peroksydazy. Próbowano wyjaśnić przyczyny tych rozbieżności.

W wyniku dyskusji stwierdzono, że dotychczasowy stan wiedzy nie pozwala wnioskować, że giberelina i retardanty wpływają na aktywność katalazy i peroksydazy w ściśle określonym kierunku, wywołując hamowanie czy stymulację aktywności tych enzymów. Nie można wobec tego przyjąć istnienia ścisłej korelacji pomiędzy wpływem substancji wzrostowych na procesy wzrostowe i na aktywność katalazy i peroksydazy. Pewnym natomiast jest, że wpływ gibereliny na aktywność katalazy i peroksydazy jest przeciwny, aniżeli wpływ retardantów na aktywność tych enzymów.

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