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

III. Effect on vitamin E content

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Vitamin E plays an essential role in the processes of growth and development of plants especially in those leading to flower formation (cf. Sironval 1957; Baszyński 1964; Drożdżewska-Teske 1966). It was stated that the role of tocopherols in flower induction is in some cases similar to the role of gibberellin (Michniewicz and Kamieńska 1964, 1965), and on the other hand, the role of CCC in these processes is opposite to the role of gibberellin (Zeevaart and Lang 1963; Zeevaart 1964). There are also some data showing that flowering of plants is strictly connected with the increase of the level of vitamin E in plants (Green 1958; Sironval and El Tannir-Lomba 1960; Baszyński 1964). Then it was interesting to study the influence of gibberellin and CCC on the level of vitamin E content in bean plants which are very sensitive to both of these growth regulators (Michniewicz and Stanisławski 1965; Michniewicz and Lamparska 1965).

This problem is also interesting if we take into consideration the fact of the relationship between tocopherols and chlorophyll content in leaves (Booth 1963, 1964) and the opposite effect of gibberellin and CCC on the level of chlorophyll (Halevy and Wittwer 1965; Czarnolewski 1967).

METHODS

Similarly as in the previously described experiments (Michniewicz and Stanisławski 1965; Michniewicz and Lamparska 1965), 10 days old, selected bean plants var. ‚Saxa' were transferred to Knop's nutrient solution containing gibberellic acid (GA_3) and 2-chloroethyl trimethylammonium chloride (CCC) at concentrations of 30 and 250 ppm respectively, and cultivated on long day (16 hrs). In such conditions these substances caused typical growth modifications. More details concerning the methods of plant cultivation are given in part I of this paper (Michniewicz and Stanisławski 1965).

The measurements of the levels of tocopherols in leaves and roots were made after 5, 9, 15, and 25 days after the transfer of plants to the nutrient solutions. So, at the time of analyses, the plants were respectively 15, 19, 25, and 35 days old.

The determination of tocopherols was carried out in 10 g samples according to the method described by the Analytical Methods Committee (1959). The details of extraction, purification, and estimation of vitamin E are given in paper of Michniewicz *et al.* 1967. The content of tocopherols was expressed as μg/g of dry weight.

The chlorophylls a + b were estimated in 100 mg samples on a Spekol-type spectrocolorimeter (Zeiss, Jena) by the method described by Bruinsma (1963).

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

RESULTS AND DISCUSSIONS

It was found that the bean leaves contained α-and γ-tocopherols, and the traces of δ-tocopherol, whereas the roots showed the presence of α--tocopherol, and the traces of γ-and δ-tocopherols.

As we see from the data given in table 1 and in figure 1 γ-tocopherol appeared in leaves of 25 days old beans, immediately before flower bud formation. In the leaves of plants beeing in this phase of development as well as in older plants, γ-tocopherol represented about 80% of total tocopherols. Our data stating greater amounts of γ-tocopherol when compared with the amount of α-tocopherol are in discordance with the data known from literature pointing out that the green leaves contained mainly α-tocopherol (Green 1958; Baszyński 1959; Dilley and Crane 1963). However, it must be stressed that the compound determined by us as γ-tocopherol was not only localized on chromatograms at the position of this tocopherol but also gave typical colour reactions with three different reagents (Emmerie-Engel, diazotised o-dianisidine, and Brown reagents). May be one of the reasons of these differences are due to the specific laboratory conditions of plant cultivation in our experiments. It might be also possible, that some amount of interferring substances reducing $FeCl_3$ had been present on the chromatograms at the position of γ-tocopherol and they could not have been removed in our experimental conditions.

The level of vitamin E in bean leaves increased with the age of plants. This statement is in accordance with the data of other authors who

Table 1

Effect of GA and CCC on vitamin E content in bean plants

Plant organs	Treatment: Tocopherol	Value in:	Control 15	Control 19	Control 25	Control 35	GA 15	GA 19	GA 25	GA 35	CCC 15	CCC 19	CCC 25	CCC 35	GA+CCC 15	GA+CCC 19	GA+CCC 25	GA+CCC 35	LSD at P = 0.01	LSD at P = 0.05
			Age of the plant in days																	
Leaves	α	μg/g dry wt	1.65	3.19	15.52	26.01	22.60	36.14	42.87	57.13	6.53	6.83	16.62	45.13	11.29	6.84	29.18	45.31	5.66	4.28
		% of total	100.00	100.00	18.55	19.58	100.00	100.00	24.53	30.90	100.00	100.00	10.92	22.18	100.00	100.00	15.58	21.03	—	—
	γ	μg/g dry wt	0	0	68.17	106.85	0	0	131.92	127.75	0	0	135.61	158.31	0	0	158.17	170.17	5.66	4.28
		% of total	0	0	81.45	80.42	0	0	75.47	69.10	0	0	89.08	77.82	0	0	84.42	78.97	—	—
	total	μg/g dry wt	1.65	3.19	83.69	132.86	22.60	36.14	174.79	184.88	6.53	6.83	152.23	203.44	11.29	6.84	187.35	215.48	10.28	7.70
Roots	α	μg/g dry wt	2.00	4.76	6.23	1.47	24.20	33.52	14.99	3.45	8.06	13.49	6.90	1.38	13.73	16.69	8.33	3.14	7.65	5.73

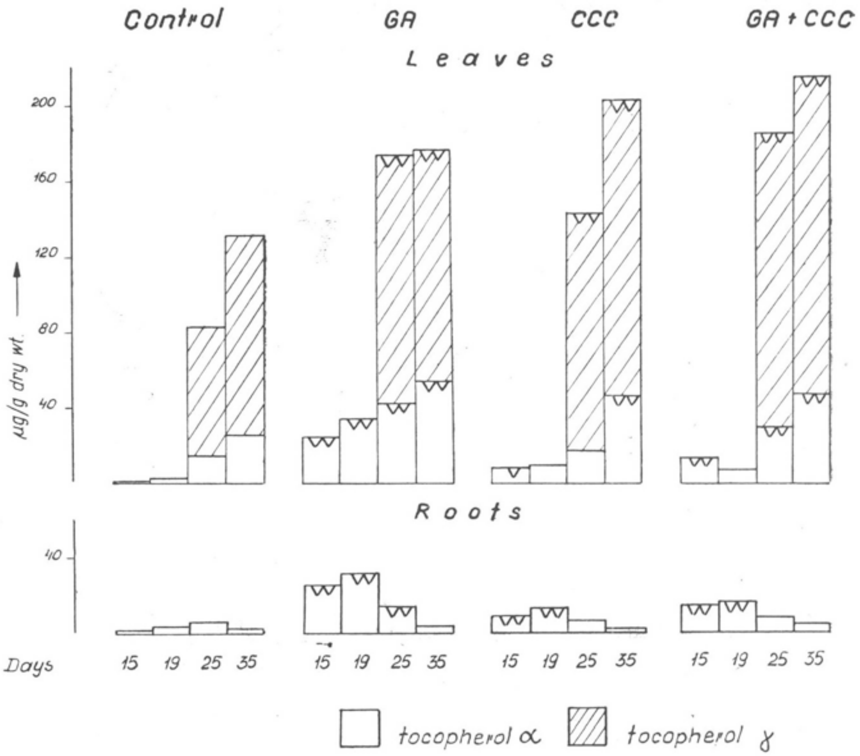

Fig. 1. The level of vitamin E in bean plants treated with GA and CCC
Significant differences in relation to control: △ at P = 0.05, △△ at P = 0.01

stated the increasing amount of vitamin E in plants till the period of flower formation (G r e e n 1958; Z w o l i ń s k a-Ś n i a t a ł o w a 1959; S i r o n v a l and E l T a n n i r-L o m b a 1960).

The amount of this vitamin was very small in the roots and increased reaching its maximum in 25 days old control plants or in 19 days old treated bean plants, then the level of vitamin E decreased.

The results concerning the control variant of experiments show that the amount of tocopherols in 15 and 19 days old plants was very small in leaves as well as in roots. In older plants the level of vitamin E in leaves exceeded many times the level of this vitamin in roots. The higher level of tocopherols in overground parts of peas than in their roots was reported also by N o r c i a *et al.* (1964). Both growth regulators i.e. GA and CCC caused a significant raise of tocopherols in leaves and roots of plants. The level of vitamin E in treated plants was, in some variants of experiments, even above 13 times higher in relation to control plants. However this effect was more distinct in leaves than in roots.

The results of our experiments do not fully confirm the data obtained by Norcia *et al.* (1964). They stated that gibberellin only sligthly increased the level of vitamin E in overground parts of pea, and not even in each of the three tested pea varieties, but its effect on vitamin E level in roots was a little stronger.

Gibberellin increased the tocopherol level more effectively than CCC. This could be remarked especially in organs containing only α-tocopherol i.e. in leaves of 15 and 19 days old plants and in roots.

The effect of GA and CCC on the level of vitamin E differred markedly from the effect of these growth regulators on the vitamin C level. In the previous experiments (Michniewicz and Lamparska 1965) GA decreased and CCC increased the amount of vitamin C in bean plants. In addition GA lowered the promotive effect of CCC on the ascrobic acid level.

The results of this part of experiments in which CCC was used together with gibberellin showed neither antagonistic nor additive effects of these two growth regulators on the level of vitamin E.

It could be expected that the reason for the lack of additive effect of both growth regulators on the level of vitamin E was due to the optimal concentration of these substances. However, the results of special experiment with four times increased CCC concentration gave a negative answer (tab. 2). The level of vitamin E in bean leaves treated with CCC

Table 2

Effect of CCC used in different concentrations on vitamin E level in leaves of bean plants

Plant age in days	CCC treatment (in ppm)		
	0	250	1000
15	0.91	4.46	6.27
19	3.81	7.96	12.46

All differences are statistically significant at $P = 0.01$

in concentration of 1000 ppm was significantly higher than in plants treated with this growth retardant in concentration of 250 ppm. These results lead also to the supposition, that the mode of action of GA and CCC promoting the increase of vitamin E in plants is different for each of these substances.

As was mentioned above, there exist some data showing the opposite effect of CCC and GA on the chlorophyll level. This was fully confirmed in our experiments. We stated that GA increased and CCC decreased the level of chlorophyll in leaves (tab. 3) whereas both these growth regulators increased the amount of tocopherols. Consequently, these results are in disagreement with the suggestion of Booth (1963) on the tocopherol co-occurrence with chloroplasts. They are also not in line with the results obtained by Dilley and Crane (1963) who found that α-tocopherol is sited inside the chloroplasts.

Table 3

Chlorophyll content in leaves of bean plants treated with GA and CCC (in mg/100 mg of dry matter)

Plant age in days	Treatment			
	Control	GA	CCC	GA+CCC
15	1.198	1.150	1.160	1.116
19	1.407	1.382	1.586	1.209

LSD at P = 0.01 = 0.097
LSD at P = 0.05 = 0.073

The results of our experiments are also in discordance with the data of Booth and Hobson-Frohock (1961) who stated that α-tocopherol content in leaves is inversely related to growth rate. It is known that the growth of bean plants is inhibited by CCC and stimulated by GA treatments (Michniewicz and Stanisławski 1965) but the action of these two growth regulators on the level of vitamin E is similar.

It has been already mentioned that tocopherols play a very essential role in the processes leading to flower initiation. Vitamin E, similarly to GA has induced the flower initiation in cold-requiring winter rye (Bruinsma and Patil 1963), and in *Cichorium intybus* (Michniewicz and Kamieńska 1964). Similarly to gibberellin it may also „substitute" the photoinduction in some long day plants like *Arabidopsis Thaliana* (Michniewicz and Kamieńska 1965), and *Calendula officinalis* (Baszyński 1967). In our previous papers (Michniewicz and Kamieńska 1964, 1965) we have suggested the possibility that the role of vitamin E in flower initiation consists in controlling the level of endogenous gibberellins in plants. This hypothesis was not confirmed in our further experiments with *Cichorium* and *Arabidopsis* (Michniewicz and Kamieńska 1967).

The results of the here presented experiments do not however support the possibility that gibberellin controls the level of tocopherols in the process of flower induction, because we have obtained a similar increase of vitamin E content under the influence of CCC treatment, though it is known that CCC inhibits the gibberellin-induced flower formation (Zeevaart and Lang 1963; Zeevaart 1964).

However, the results of our experiments support the previously suggested idea (Michniewicz and Kamieńska 1967) that the tocopherol- or gibberellin-induced flower formations are realized on different metabolic ways.

Evidently the role of gibberellin and tocopherol in the processes leading to flower formation would be better explained in experiments carried out with plants requiring photo- or thermoinduction.

CONCLUSIONS

1. The level of vitamin E in bean leaves was greater than in their roots and increased with the age of plants.

2. Gibberellin and chlorocholine chloride caused a significant raise of tocopherols in leaves and roots. This effect was more distinct in leaves than in roots.

3. The lack of additive effect of GA and CCC on the level of tocopherols has been stated.

4. It has been concluded that the mode of action of GA and CCC promoting the increase of vitamin E level in plants is different for each of these substances.

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

III. Wpływ na zawartość witaminy E

Streszczenie

Porównywano wpływ GA_3 (w stężeniu 30 ppm) i CCC (w stężeniu 250 ppm) na zawartość witaminy E w liściach i korzeniach fasoli rosnącej na pożywce Knopa. Analizy wykonywano metodą podaną przez Analytical Methods Committee (1959) na roślinach będących w wieku 15, 19, 25 i 35 dni.

Doświadczenia powtórzono czterokrotnie, a wyniki poddano analizie statystycznej.

W wyniku doświadczeń stwierdzono:

Poziom witaminy E w liściach był większy niż w korzeniach i wzrastał wraz z wiekiem rośliny.

Traktowanie roślin gibereliną i chlorkiem chlorocholiny wywoływało znaczne zwiększenie poziomu witaminy E zarówno w liściach jak i w korzeniach. Wpływ ten był wyraźniejszy w przypadku liści niż korzeni.

W przypadku łącznego stosowania GA i CCC nie stwierdzono sumującego się wpływu obu tych regulatorów wzrostu na poziom witaminy E.

Autorzy wnioskują, że drogi które prowadzą do zwiększenia ilości witaminy E pod wpływem GA i CCC są różne dla obu tych substancji.

W pracy dyskutowane jest zagadnienie zależności pomiędzy wpływem GA i CCC na zawartość witaminy E i chlorofilu oraz na wzrost liści. Omówiono również rolę gibereliny i witaminy E w procesach prowadzących do zakwitania.

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