ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XXXIV — nr 3
1965

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

II. Influence on the content of vitamin C

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Experiments with beans and tomatoes have shown that CCC induces plants to resist frost (Michniewicz et all., 1965). It is also well known, that an important feature of frost resisting plants is to contain great amount of vitamin C (Lvov and Altuchova 1958) and that hardening of plants causes an increase of the amount of this vitamin (Zaraulov 1957). Mountain plants which are adapted to low temperatures are also characterized by a high level of vitamin C (Ovčarov 1958). Further on, it has been found that the amount of ascorbic acid increases under the influence of low temperatures (Povolockaja 1937; Berezovskaja 1949; Viktorov 1950; Franke 1957; Chinoy and Nanda 1959) and in the course of vernalization (Michniewicz 1961 and authors quoted there).

It was also shown that phosfon, a retardant of antigibberellic properties, led to an increased vitamin C content in beans (Michniewicz 1963 a).

It might be therefore assumed that CCC, a substance inducing a similar effect on the growth of plants as phosfon does, and making beans frost resisting, should also raise the amount of vitamin C in this plant.

The aim of this work was to examine this problem and to compare the effect of CCC and gibberellin treatment on the level of vitamin C in plants. It also aims to explain the effect of gibberellin and CCC on the level of this vitamin in relation to the light conditions during plants cultivation. For, in experiments regarding the effect of gibberellin and phosfon (Michniewicz 1963a) on the amount of vitamin C in beans, it was found that gibberellin diminished the amount of this vitamin, which differs with data supplied by Yabuta and Yamaki (1941, after Stowe and Yamaki 1957), Guseinova and Mamedov (1960), Michniewicz (1960) and Gukova and Faustov (1963). In order to explain these differences it was supposed that the results of experiments with beans were due to the fact that the plants were cultivated in a luminostat of a relatively low light intensity (about 5000 lux). For, Lockhard (1958) has indicated an influence of the light conditions on the response of bean plants to gibberellic acid.

METHOD

Beans var. 'Sax' grown in Knop's nutrient solutions containing gibberellin at a concentration of 30 ppm, CCC at a concentration of 250 ppm, or withouth any of these substances. The method of cultivation was identical to that one described in part I of this paper (Michniewicz and Stanisławski 1965). The plants were kept on long day (16 hours) in a thermoluminostat at a light intensity of about 5000 lux. After two weeks, at the time when the plants developed alredy their first pair of leaves, ascorbic acid and dehydroascorbic acid was estimated, separately in leaves and stems.

5 g fresh material were taken for each analysis. Ascorbic acid was estimated by Tillman's method modified by Niemyski and Gubański (1958). Dehydroascorbic acid was identified as ascorbic acid previously reduced by H_2S using the method of Pijanowski (1954).

Each result represents the average of three measurements.

Also the effect of a simultaneous treatment of plants with CCC and gibberellin in concentrations of 5, 10 and 30 ppm was examined by means of a special experiment, to define the content of ascorbic acid in leaves.

The effect of light conditions on the content of vitamin C in bean leaves was also examined. During this experiment one part of the plants was kept in thermoluminostat on 8 hours day, the other half was kept on a 16 hours day. At the latter light variant, the plants were grown for about 10 hours in natural day light conditions between 8.00—18.00 o'clock from about the middle of April till the middle of May, and 6 hours in a thermoluminostat.

All experiments were repeated three times. The results were statistically analysed, and the least significant difference was determined.

RESULTS

It might be seen from data given in the first part of this paper (Michniewicz and Stanisławski 1965), that gibberellin and CCC caused typical growth modifications. Gibberellin stimulated considerably the growth of stem and inhibited the length of root. Treatment with CCC gave just an opposite effect. Plants treated with this retardant characterized an intense green colour of leaves.

Data presented in Table 1 show, that CCC caused a significant raise of ascorbic acid in leaves, whereas treatment with gibberellin resulted in a decreased amount of this acid. The effect of both growth regulators on the ascorbic acid content in the stem was similar, but still more distinct.

Table 1

Effect of GA and CCC on vitamin C content in leaves and stems of kindey bean

Organs of plant	Names of investigated substances	Value in	Kind of growth regulators			L.S.D. at P =	
			C[1]	GA	CCC	0.05	0.01
Leaves	AA[2]	mg%	55.89	49.55	109.51	12.52	18.98
		%[4]	100	88.66	195.76	—	—
	DAA[3]	mg%	180.51	59.94	229.18	25.56	38.72
		%	100	33.21	126.96	—	—
Stems	AA	mg%	1.83	0.93	4.22	0.92	1.39
		%	100	50.82	230.60	—	—
	DAA	mg%	23.46	8.57	30.23	5.87	8.90
		%	100	36.53	128.86	—	—

[1] Control
[2] Ascorbic acid
[3] Dehydroascorbic acid
[4] Control = 100%

Similar resutls were obtained when the effect of these growth regulators on the content of dehydroascorbic acid was examined. Under the influence of gibberellin, however a far greater decrease of this substance in plants took place whereas the stimulating effect of CCC was far weaker.

Results of experiments for which CCC together with gibberellin (Table 2) was used showed a clearly antagonistic reaction to both these growth regulators on the level of vitamin C. Gibberellin diminished proportionally to its concentrations in the nutrient solution, the effect of CCC treatment.

Results of experiments concerning the influence of growth regulators on the level of vitamin C in plants, growing in various light conditions, are shown in Table 3. We may see here, that plants, growing on long day, are characterized by a higher level of this vitamin. Gibberellin led so a decrease of vitamin C in the leaves of beans, whereas CCC caused a significant increase of this substance on long day as well as on short day at a low intensity of light. The increase of ascorbic and dehydroascorbic acid in the leaves among plants from both light variants was very similar as compared with their controls. It was however found that under the influence of gibberellin the decrease of dehydroascorbic acid was, in relation to control, smaller in short day conditions.

Table 2

Effect of simultaneous treatment of kidney bean plants with CCC and GA on ascorbic acid content in leaves

Concentration of CCC in ppm	Value in	Concentration of GA in ppm			
		0	5	10	30
0	mg%	97.1	74.5	69.3	54.6
	%*	100	76.7	71.4	56.2
250	mg%	129.8	81.6	75.1	65.2
	%	133.7	84.0	77.3	67.1

L.S.D. at P = 0.05 = 4.99
P = 0.01 = 6.87
* Control = 100%

Table 3

Effect of GA and CCC on vitamin C content in leaves of kidney bean in different light conditions

Names of investigated substances	Light period in hrs	Value in	Kind of growth regulators			L.S.D. at P = 0.01
			C[1]	GA	CCC	
AA[2]	16	mg%	76.5	35.1	122.0	20.6
		%[4]	100	45.9	159.5	—
	8	mg%	46.3	24.9	65.4	17.5
		%	100	53.8	141.3	—
DAA[3]	16	mg%	309.7	153.7	390.0	44.9
		%	100	49.6	125.9	—
	8	mg%	149.0	121.6	187.3	26.9
		%	100	81.6	125.7	—

[1] Control.
[2] Ascorbic acid.
[3] Dehydroascorbic acid.
[4] Control = 100%.

DISCUSSION

The results of the presented experiments have fully confirmed all data obtained by Michniewicz (1963a) viz., that gibberellin, inducing a very strong elongation of the stem, acts as an agent which lowers the level of vitamin C in plants. This decrease was here however more distinct and statistically significant not only in the leaves but also in the stems.

All experiments made at different times and in different environmental conditions confirmed the presented results. Although in the various experiments the effect did not appear at the same degree, it was nevertheless everywhere very distinct.

The above described results, therefore, do not confirm the data of Yabuta and Yamaki (1941, after Stowe and Yamaki 1957) obtained in experiments with etiolated soya beans, Guseinova and Mamedov (1960) with maize, Michniewicz (1960) with germinating wheat kernels and Gukova and Faustov (1963) with gibberellin applied as pre-sowing treatment to kernels of maize, oats and peas. Only partly do our results confirm also data supplied by Key (1962) who connected the growth stimulating effect on plants with the raise of ascorbic acid and with the decrease of the level of dehydroascorbic acid.

This divergence of results may not only be explained by differences of the plant material used, but also by conditions in which the experiments took place. This seems to be indicated by the findings of Lona and P. Giovanola (1951), who could observe a great variability in the level of ascorbic acid depending on the outer environmental conditions. It should be underlined that even growth changes obtained by us and Guseinova and Mamedov (1963) were not identical. These authors observed but in experiments with maize a growth promoting effect a gibberellin on the growth of roots, whereas during our experiments with beans gibberellin treatment caused a distinctive inhibitory effect on the roots elongatien (comp. Table 1, part I, Michniewicz and Stanisławski 1965).

Data obtained in the present investigations confirmed distinctly the fact that CCC, as phosfon does, induced a distinct increase of vitamin C in beans. All experiments confirmed this fact without any doubt.

The effect of gibberellin and of retardants is therefore contradictory, not only with regard to growth processes, but also with regard to their effect on the level of vitamin C in plants. The influence of these growth regulators in plants is clearly antagonistic (Table 2) like the effect of these substances on the growth of plants (comp. Michniewicz 1963 b).

Our results are identical with the data presented by Tonzig and Marre (1961). They quote many facts from various publications according to which the increase of vitamin C is connected with the inhibiting of growth. This phenomenon was caused by an inhibition of cell division and also cell elongation.

The results of our experiments seem to be also confirmed by the facts, cited in the literature quoted above, that the increase of vitamin C under the influence of CCC was connected with a simultaneous increase of frost resistance. It should be underlined that the experiments which have shown an increase of this vitamin and frost resistance faculties, as due to the effect of CCC, were made with the same variety of beans, cultivated in the same way and in the same environmental conditions.

Towards the trustworthiness of our results points also the comparison between the effect of the given light conditions and the applied substances on the level of vitamin C, chlorophyll and growth. As can be seen from data included in table 3 and also from appropriate literature, light promotes the biosynthesis of vitamin C (for review, Wysokińska, 1962). It is also known that the biosynthesis of chlorophyll depends on the light factor. It has been shown, that the amount of chlorophyll decreases under the influence of gibberellin (Stowe and Yamaki 1957), whereas CCC causes an increase of this dye (Damaty et all., 1964). The stimulation of plants growth, as an effect of gibberellin treatment, is also similar to the effect caused through lack of sufficient illumination, whereas growth inhibition, as a consequence of CCC activity, is similar to the action of light of a high intensity.

Our experiments have also shown, that the effect of growth regulators on the level of vitamin C was independent on the existing light conditions. This is in accord with data supplied by Wittwer and Tolbert (1960) who obtained an identical growth inhibiting result with tomatoes under the influence of CCC and by applying light of different intensity. The results are also in accord with data obtained by Downs and Cathey (1961) who, in experiments with beans, applying retardant Amo-1618 having some proprieties similar to CCC, did not find any differences in the reaction of plants in relation to the different light conditions.

The results of this work are pointing clearly towards the fact that growth stimulation was connected with a decrease of vitamin C whereas plants with inhibited growing abilities have shown an increase of this vitamin. As already mentioned, not all the data published in this matter are in accordance with this observation. It seems therefore correct to agree with the conclusion of Michniewicz (1960) that changes in the level of vitamin C in plants caused by action of growth regulators do not depend on these regulators affecting the growth processes of plants.

SUMMARY

Examined was the effect of gibberellin at a concentration of 30 ppm and CCC at a concentration of 250 ppm on the content of ascorbic and dehydroascorbic acid in leaves and stems of beans, in the stage of two leaves, growing in Knop's nutrient solution in a thermoluminostat having a light intensity of about 5000 lux.

The effect of a simultaneous application of CCC and gibberellin, at various concentrations, on the level of ascorbic acid in leaves, and also the influence of light conditions on the effectiveness of the growth regulators has been studied.

All experiments were three times repeated and the results were statistically analysed.

Gibberellin, stimulating the growth of stem and leaves, caused a decrease of ascorbic and dehydroascorbic acid in these organs.

CCC caused an opposite effect. It inhibited the growth of stem and leaves and caused a significant raise of the level of both the acids.

The addition of gibberellin, to the nutrient solution containing CCC, lowered the promotive effect of this retardant on the biosynthesis of ascorbic acid.

A raise in the amount of vitamin C under the influence of CCC, and a decrease of this substance, as an effect of gibberellin treatment, was obtained on long-day as well as on short day of low light intensity.

Comparing our results with data of known literature it may be concluded, that changes in the content of vitamin C, due to the effect of gibberellin and CCC, did not depend on the influence of these substances on the growth of plants.

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(Entered: 18.XI.1964.)

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

II. Wpływ na zawartość witaminy C

Streszczenie

Zbadano wpływ gibereliny w stężeniu 30 ppm i CCC w stężeniu 250 ppm na zawartość kwasu askorbinowego i dehydroaskorbinowego w liściach i w łodygach fasoli będącej w fazie dwóch liści, rosnącej na pożywkach Knopa w termoluminostacie o natężeniu światła około 5000 lux.

Zbadano też wpływ jednoczesnego stosowania CCC i gibereliny o różnym stężeniu, na poziom kwasu askorbinowego w liściach oraz wpływ warunków świetlnych na efektywność działania stosowanych tu regulatorów wzrostu.

Wszystkie doświadczenia powtórzono trzykrotnie, a wyniki poddano analizie statystycznej.

W wyniku doświadczeń stwierdzono:

Pod wpływem gibereliny stymulującej wzrost łodygi i liści nastąpiło obniżenie zawartości kwasu askorbinowego i dehydroaskorbinowego w tych organach.

CCC wywoływało efekt przeciwny. Pod wpływem tego preparatu następowało hamowanie wzrostu łodygi i liści i znaczne zwiększenie poziomu obu kwasów.

Giberelina, wprowadzona do pożywki zawierającej CCC, obniżała stymulujący efekt działania tego retardanta na biosyntezę kwasu askorbinowego.

Zwiększenie ilości witaminy C pod wpływem CCC oraz zmniejszenie ilości tej substancji jako efekt działania gibereliny występowało zarówno na dniu długim, jak i na dniu krótkim o słabej intensywności światła.

Porównanie wyników z danymi z literatury upoważniają do wniosku, że zmiany, jakie nastąpiły w zawartości witaminy C pod wpływem gibereliny i CCC, nie były uwarunkowane wpływem tych związków na wzrost rośliny.

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