The effect of 2- chloroethyl trimethylammonium chloride (CCC) on the frost resistance of tomato and bean plants

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Among retardants, i.e. substances inhibiting the growth of plants without causing any toxic damages is CCC of special interest in comparison with other compounds of this type. It is active in a lot of more species than any other retardant, like Amo 1618, or phosfon (Cathey and Stuart 1961). It is further of great interest because, like other retardants, it makes plants resistant against the unfavourable environmental conditions. This was shown by some experiments in which the effect of CCC on the plant growth by a reduced humidity (Lindstrom and Tolbert 1960; Halevy and Kessler 1963; Damaty at al. 1964) and physiological drought (Michniewicz and Chromiński 1955) was examined.

CCC increases also the resistance of plants against exceeded salt contents (Marth and Frank 1961; Miyamoto 1962a; Damaty at al. 1964), unfavourable pH (Miyamoto 1962b) and also, as found by Teubner and O'Keefe (1960, compare Marth and Frank 1961) against the harmful influence of high temperatures.

It seems, therefore, probable that through CCC application may be also induced a positive effect in the plant resistance against low temperatures. A positive answer to this question gives the fact, that retardants increase remarkably the amount of vitamin C (Michniewicz 1965). It is well known that a great amount of vitamin C are characteristic for frost resisting plants. (Lvov and Altuchova 1951; Zarauslov 1957).

The present study was undertaken to test this possibility in experiments with tomato and bean plants. Principal results with tomatoes have been published in a preliminary note (Michniewicz and Kentzer 1965).

METHODS

Tomatoes: variety 'Sukces Mor' and beans: variety 'Saxa', were used. The effect of CCC produced by the American Cyanamid Company on the process of seeds germination in a reduced temperature, and the frost resistance of seedlings at different stages of their growth was studied.

In experiments with germination, a solution of CCC in concentrations of 10, 100, 500 and 1000 ppm was used. Germination took place on Petri dishes (100 plants per dish), in normal and reduced temperatures. Temperatures were 25°C and 12°C for tomatoes and 19°C, 12°C and 9°C for beans. CCC, in appropriate concentrations, or water, was applied by soaking the seeds for 24 hours in darkness, at 8—10°C. The seeds were rinsed and dried before placed in dishes.

The experiments were repeated six times. Those experiments which aimed towards testing the effect of CCC on frost resistance took place in the time from November 1963 till March 1964.

CCC was applied by soaking the seeds in a water solution of this compound as described above. Then they were sown into boxes filled with soil and placed in the greenhouse at a temperature of 19—20°C. After 10—14 days, one part of the plants was transplanted into pots with soil, another to Knop's nutrient solutions containing CCC in a concentration of 100 and 200 ppm. Both groups of plants were then left in the same greenhouse conditions. The concentrations of CCC were chosen according to preliminary experiments during which also lower and higher concentrations were used.

CCC in concentrations of 100 and 200 ppm caused typical morphological changes, characteristic for the effect of retardants, and made plants resistant to low temperatures. The experiments were carried out in field conditions. The plants were exposed to low temperatures between -2°C and -10°C up to 12 hours when temperatures were higher and up to 20 minutes at their lowest. All plants exposed to low temperatures were afterwards transferred to a room with a temperature of +4 to +6°C, kept there for several hours and then brought back to the same greenhouse in which they grew before. Results were evaluated after 24 hours counted from the end of chilling. There were 10 plants in each experimental variant, and each experiment was repeated four times.

Some investigations were also conducted in order to find out any effect of above described experiments upon the contents of sugars in plant leaves.

The sugar contents in 10 g samples of bean leaves were examined after the method of Samogyi (1945). Defined were total sugar contents and simple sugars. The analysis were repeated six times.

The results of all experiments were statistically analysed and the least significance difference was determined.

RESULTS

1. The effect of CCC on the germination of seeds dependent on temperature

The results of experiments concerning the influence of CCC on the process of germination are shown in Table 1. They clearly indicate that CCC, even when applied in high concentrations, did not have any influence on the process of germination if it took place in optimal temperatures. The positive effect of CCC on germination was observed only in lower temperatures. In a minimum temperature of 12°C, CCC increased the germination energy but did not induce any changes in the germination capacity, because the general amount of germinated seeds, after 9 days for beans and 13 days for tomatoes, had been identical in all variants.

Table 1

Effect of CCC on the germination of seeds in dependence to temperature
(Number of germinated seeds expressed per cent)

Kind of plant	Temperature in °C	Days of germination	Concentration of CCC - ppm					L.S.D. at P=0.05
			0	10	100	500	1000	
Tomatoes	25	5	75.1	75.1	75.5	77.3	-	5.59
	12	6	9.6	10.5	10.2	<u>14.0</u>	-	3.46
		11	49.6	53.8	49.6	<u>57.0</u>	-	6.38
		13	60.6	60.6	58.1	63.6	-	7.54
		15	65.0	63.8	63.5	<u>68.6</u>	-	5.63
Beans	19	2	88.5	87.5	89.5	88.5	89.1	5.26
	12	5	2.1	<u>17.0</u>	7.3	9.3	<u>15.1</u>	7.86
		7	25.8	<u>55.0</u>	34.6	<u>38.5</u>	<u>38.8</u>	11.81
		9	60.5	64.5	50.6	56.3	66.6	8.44
		12	70.6	69.6	57.8	62.3	69.5	8.84
	9	8	5.5	6.1	6.8	6.6	3.5	2.83
		10	8.3	<u>12.0</u>	10.1	9.1	6.6	3.01
		12	10.1	<u>16.8</u>	<u>15.0</u>	12.3	9.8	3.51

Significant differences in relation to control are underlined.

In a temperature below minimum, tomato as well as bean seeds began to germinate not until a week after, and at this to a very small percentage. In these conditions the seeds were affected by some micro-organisms which forced us to resign from a prolongation of the experiments with tomatoes. Experiments with beans in a temperature of 9°C had to be stopped too after 12 days, although seeds germinated, after that time, only up to a small percentage. Nevertheless, also these experiments showed that CCC has the ability to accelerate the process of germination.

It should be pointed out that CCC, at its highest concentration of 500—1000 ppm, prevented growing of mould on tomato as well as on bean seeds.

2. Frost resistance effect of CCC

In the experiment with frost resistance, plants treated previously with CCC were examined, after they developed the first and second pair of leaves. In the investigations with tomatoes, plants at the cotyledons stage were also used.

Table 2

The effect of cold treatment on tomato and bean seedlings from seeds treated by CCC before sowing
(The number of frozen plants in %)

Kind of plant*	Temperature and period of cold treatment in hrs	Concentration of CCC - ppm		
		0	100	200
Tomatoes	-2°C 12	60	80	80
Beans	-4°C 3	50	70	50

*First pair of leaves. All differences were not significant.

As a result of these experiments it has been found that plants from seeds which were given a presowing hormonization did not show exactly features characteristic for plants treated with retardants. The resistance of such plants was identical with the resistance of control plants not treated with CCC. As an example may serve the results of one experiment showed in Table 2.

Table 3

The influence of CCC on stem growth of tomato and bean seedlings
(The growth of plants in cm)

Kind of plant	Growth stage	Concentration of CCC - ppm		
		0	100	200
Tomatoes	Cotyledons	44.4	36.8	31.4
	First pair of leaves	46.4	37.2	33.0
	Two pairs of leaves	82.0	43.0	36.0
Beans	First pair of leaves	11.3	7.9	5.8
	Two pairs of leaves	26.1	14.8	11.1

Significant changes in plant growth were achieved in experiments with plants grown in Knop's nutrient solutions with an addition of CCC.

Such plants were characterized by a remarkable shortening of their internodes, the stems were thicker, the leaves greener (Table 3).

Table 4
Frost resistance of tomato seedlings at different stages of their growth as influenced by CCC treatment

Experiment No.	Growth stage	Date, temperature and period of cold treatment	Growth in nutrient solution (days)	Number of frozen plants in %			L.S.D. at P=	
				Concentration of CCC in ppm			0.05	0.01
				0	100	200		
1	Cotyledons	6.I.64 -2°C 12 hrs	4	80.0	20.0	22.0	15.36	22.10
2		27.I.64 -4°C to -8°C 1 hr	6	93.0	55.0	27.5	17.20	24.80
3	First pair of leaves	13.XII.63 -4°C 1 hr	16	97.5	97.5	12.5	10.97	15.76
4		20.XII.63 -4°C 6 hrs	20	97.5	27.5	5.0	8.41	12.10
5	Two pairs of leaves	6.I.64 -2°C 12 hrs	32	92.5	27.5	0	16.49	23.72
6		11.I.64 -10°C 20 min	37	92.5	52.5	25.0	22.89	32.92
7		11.I.64 -10°C 20 min	37	97.5	60.0	30.0	26.21	37.70

Results of experiments regarding the effect of CCC on frost resistance are summarized: for tomatoes in Table 4, for beans in Table 5. They indicate that plants treated with CCC were more resistant against injurious effects of low temperatures than control plants. This phenomenon was especially remarkable in the variants treated with more concentrated CCC. A stronger resistance was observed also in plants being in a later stage of growth for which the CCC treatment was prolonged. The differences between the number of control plants killed by frost and those treated with CCC (in concentration of 200 ppm) were statistically significant in all experiments, with tomatoes as well as

Table 5

Frost resistance of bean seedlings at different stages of their growth as influenced by CCC treatment

Experiment No.	Growth stage	Date, temperature and period of cold treatment	Growth in nutrient solution (days)	Number of frozen plants in %			L.S.D. at P=	
				Concentration of CCC in ppm				
				0	100	200	0.05	0.01
1	First pair of leaves	4.XII.63 -4°C to -2°C 3 hrs	5	100.0	92.5	65.0	15.54	22.33
2		22.XII.63 -4°C 3 hrs	5	100.0	72.5	42.5	22.30	32.04
3		22.I.64 -4°C to -2°C 3 hrs	6	95.0	90.0	70.0	17.69	25.42
4	Two pairs of leaves	20.XII.63 -4°C 3 hrs	18	100.0	70.0	17.5	23.09	33.18
5		4.I.64 -5°C to -2°C 3 hrs	18	60.0	32.5	12.5	20.99	30.16
6		6.I.64 -4°C to -2°C 3 hrs	20	62.5	27.5	7.5	23.99	34.88
7		6.II.64 -4°C to -6°C 1 hrs	19	100.0	72.5	25.0	24.86	35.72

Table 6

The influence of CCC on the sugar contents in leaves of bean seedlings (values in per cent)

Sugars contents	Concentration of CCC - ppm			L.S.D. at P=	
	0	100	200	0.05	0.01
Total	0.322	0.313	0.263	0.195	0.141
Simple sugars	0.233	0.137	0.095	0.120	0.087

beans. As shown by data summarized in Table 6, the leaves of beans treated with CCC in a concentration of 200 ppm had a smaller amount of sugars.

Besides above described results it has been found that CCC showed a positive influence on the plants resistance against infections caused by some microorganisms. This phenomenon appeared clearly in one of the experiments with tomatoes affected by fungus. All control tomatoes perished, whereas those tomatoes which grew in nutrients with an addition of CCC were untouched, except of about 5% of these plants. The species of the fungus was not determined because, in order to stop the spreading of the disease, the whole affected experimental series was removed at once.

DISCUSSION

The results described in this paper indicate that CCC caused morphological changes similar to those which were achieved by Wittwer and Tolbert in experiments with tomatoes (1960a) and beans (1960b). The results are also identical with data supplied by other authors, quoted above, showing that retardants make plants resistant against unfavourable environmental conditions. This has been clearly ascertained in our experiments with germination of seeds in reduced temperatures as well as in experiments in which the effect of CCC on frost resistance of plants was examined.

The results described in this paper impose the question, which role actually does play CCC in the resistance of plants against low temperatures.

The resistance of plants against frost is often correlated with an increase of sugars (Maksimov 1950), and vitamin C (Lvov and Altuchova 1951; Zarauslov 1957).

As was seen in experiments with beans, CCC which increased the frost resistance caused not great but significant reduction of the amount of sugars in leaves. These data confirm the view of Heber (1958) that an increased frost resistance is not always related with an increase of the sugar level in plants.

It seems be possible that the increased frost resistance of plants, as induced by CCC treatment, might be connected with the increasing of vitamin C in plants.

There is some evidence that plants resisting low temperatures are characterized by a high level of vitamin C.

On the other hand it was found that phosfon, a compound which showed certain properties closely related to those of CCC, increased to a great extent the amount of vitamin C in beans (Michniewicz 1965). Not yet published data of Michniewicz supply also evidence

regarding a considerable increase of vitamins C in beans treated with CCC. It has to be pointed out that experiments were made with the same variety of beans, in the same conditions, and with similar concentrations of CCC as those used in the studies about frost resistance in plants. This indicates that the effect of CCC, making plants resist against low temperatures, might be correlated with an increase of vitamin C.

CCC may also influence certain other processes associated with the frost resistance of plants. It may be connected with the influence of CCC on the level of auxin, and this seems to be confirmed by Kuraishi and Muir (1963) who found a sixfold decrease of the auxin level in pea shoots as caused by CCC treatment. On the other hand, it was shown that the increased frost resistance of some plants might be just associated with a decrease of the auxin level (Sulakadze 1959 and Tumanov cited by him, 1945).

Experiments described in this paper showed also that CCC makes plants resistant against fungal infections similarly to the results of experiments, whereby Amo — 1618 was used, by Marth and Mitchell (1960).

SUMMARY

The effect of CCC on the germination of tomato seeds, variety 'Sukces Mor', and of bean seeds, variety 'Saxa' in reduced temperatures, was examined. The frost resistance of tomato and bean seedlings at the cotyledons stage and the first and second leaves pair, treated with CCC was also studied.

CCC did not influence germination in optimal temperatures. In reduced temperatures, CCC increased the germination energy without causing any change in the germination capacity of seeds. Significant differences, in relation to control, were obtained in the germination of tomatoes by using CCC in a concentration of 500 ppm, and in beans within concentrations of 10 to 1000 ppm.

Plants grown on Knop's nutrient solutions with an addition of CCC up to 100 and 200 ppm were found to be to a great extent frost resistant. The resistance was particularly strong in variants treated with CCC of 200 ppm. More resistant proved plants being in a later stage of growth on which CCC had a longer effect. Frost resistance induced by CCC treatment was associated with an increase of vitamin C.

The leaves of beans treated with CCC had a smaller amount of sugars in relation to control plants. Plants treated with CCC were much more resistant to some fungal infections.

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Wpływ chlorku 2-chloroetylotrójmetyloamoniowego (CCC) na odporność na mróz pomidorów i fasoli

Streszczenie

Zbadano wpływ CCC na proces kiełkowania nasion pomidorów odmiany 'Sukces Mor' i fasoli odmiany 'Saxa', stosując różne temperatury. Badano także mrozoodporność siewek pomidorów i fasoli będących w fazie liścieni, pierwszej i drugiej pary liści poddanych uprzednio działaniu CCC.

Stwierdzono, że preparat ten nie wpływał na kiełkowanie nasion, jeżeli proces ten przebiegał w temperaturze optymalnej. W temperaturach obniżonych CCC powodował zwiększenie energii kiełkowania, nie wywołując zmian w sile kiełkowania nasion. Istotne różnice w stosunku do kontroli uzyskano u pomidorów kiełkujących w roztworach CCC o stężeniu 500 ppm, a u fasoli w granicach stężeń od 10 do 1000 ppm.

Rośliny, które rosły na pożywkach Knopa z dodatkiem CCC 100 i 200 ppm, okazały się w znacznym stopniu odporne na mróz. Odporność ta była szczególnie duża w wariantach, w których stosowano CCC w stężeniu 200 ppm. Bardziej odporne okazały się również rośliny będące w późniejszej fazie wzrostu, na które preparat działał dłużej.

Odporność roślin na mróz wywołana działaniem CCC, łączyła się ze znacznym wzrostem zawartości witaminy C.

Analiza soku komórkowego na zawartość cukrów, którą przeprowadzono u fasoli, wykazała nieznaczne obniżenie poziomu tych związków u roślin potraktowanych CCC.

Rośliny poddane działaniu chlorku chlorocholiny, były znacznie bardziej odporne na choroby infekcyjne wywołane grzybami.

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