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INCREASE OF COLD TOLERANCE IN COTTON PLANT (GOSSYPIUM HIRSUTUM L.) BY MEPIQUAT CHLORIDE

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16. Abstract

Three mepiquat chloride (MC) concentrations - 40, 70, and 100 g a.i./ha - were used to spray cotton (Gossypium hirsutum L., cultival 'McNair 220') plants to determine whether or not MC would increase their cold tolerance. Seven to ten days after the spray, the plants were exposed to three different cold treatments. No important difference in cold damage was noticed between the control and the MC-treated plants when they were exposed repeatedly to 4.5°C. No plants died when exposed to 0.5°C for 12 h; however, 90% of the 1st and 2nd leaves of the control plants were damaged. This was three times more damage than those leaves of plants treated with 70 and 100 g a.i./ha MC concentrations; 60% of the control and 10-20% of the MC-treated plants died when the plants were subjected to a cold hardening process with 15.5°C day (12 h) and 1.7°C night (12 h) for 10 days, and then, held at -2.2°C for 24 hours. The electrolyte leakage and reflectance measurement data showed that the cell membranes of the MC-treated plants sustained much less damage than those of the control. Freezing injury was easily assessed by reflectance measurements at the 1.65-μm wavelength

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ABSTRACT

Three mepiquat chloride (MC) concentrations--40, 70, and 100 g a.i./ha--were used to spray cotton (*Gossypium hirsutum* L., cultivar 'McNair 220') plants to determine whether or not MC would increase their cold tolerance. Seven to ten days after the spray, the plants were exposed to three different cold treatments. No important difference in cold damage was noticed between the control and the MC-treated plants when they were exposed repeatedly to 4.5°C. No plants died when exposed to 0.5°C for 12 h; however, 90% of the 1st and 2nd leaves of the control plants were damaged. This was three times more damage than those leaves of plants treated with 70 and 100 g a.i./ha MC concentrations; 60% of the control and 10-20% of the MC-treated plants died when the plants were subjected to a cold hardening process with 15.5°C day (12 h) and 1.7°C night (12 h) for 10 days, and then, held at -2.2°C for 24 hours. The electrolyte leakage and reflectance measurement data showed that the cell membranes of the MC-treated plants sustained much less damage than those of the control. Freezing injury was easily assessed by reflectance measurements at the 1.65-μm wavelength.

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INTRODUCTION

Application of growth retardant chemicals have been shown to increase the tolerance of some plants against effec of physiological stress (6, 12, 16). In laboratory experiments, Roberts (12) demonstrated that wheat plants treated with chloromequat increased frost or cold tolerance; Freeman and Carne (2) showed that cold injury in strawberries was reduced by using succinic acid 2,2-dimethylhydrazide (alar). Recently, prevention of chilling injury in cotton plants by abscisic acid (ABA) was shown (11); however, the lowest temperature used was 4°C.

Cold hardiness is often believed to be accompanied by a great reduction in the gibberellin activity (14), and most growth retardants are known to interfere with gibberellin biosynthesis or utilization (3). Field research has proven that mepiquat chloride (1,1-dimethylpiperidinium chloride) is very effective in reducing vegetative growth of cotton (4). Since mepiquat chloride (MC) is a growth retardant and has an antigibberellin activity (15), its relation to cold resistance of plants needs investigating.

We conducted this study to determine whether or not MC would protect cotton plants from cold injury, and if so, to collect data to evaluate the optimal concentration of MC to be used for further laboratory and field investigations.

MATERIALS AND METHODS

Cotton plant seeds (cultivar 'McNair 220') were planted in peat pellets (Jiffy-7 a/s Jiffy products LTD. Norway).[6] After germination, emerged seedlings were transplanted into plastic pots containing a 1:200 mixture of perlite and Hidalgo sandy clay loam (Typic Calciustoll). A 10-20-5 fertilizer was added to the mixture at the equivalent of 67.2 kg/ha of N. All pots were surface irrigated every 2 days with equal amounts of rain water.

From August 20 to October 30, 1980, the plants were kept in an open greenhouse until they were subjected to a cold treatment, and then they were returned to natural conditions. Day temperatures ranged from 28°C to 35°C and night temperatures ranged from 17°C to 24°C.

The plants were separated into three experimental groups, each with a different time of MC application to plants and cold hardening exposure. Each group consisted of control (nontreated) plants, which were sprayed with distilled water, and plants hand sprayed with three concentrations of MC: 40, 70, and 100 g a.i./ha. The total volume was equivalent to 252 liter/ha. Experimental conditions and times of application (ages) are summarized below:

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[6] Mention of a company name or trademark is for the readers' benefit and does not constitute endorsement of a particular product by the USDA over others that may be commercially available.

Experimental Group No.	Age (days) of plants at MC spray	No. of plants	No. of days of cold treatment	Day/night temp., °C	Daily photo period
1	5	120	6	15.5/4.5	12 h
2	4	60	1	15.5/0.5	12 h
3	19	40	10	15.5/1.7	12 h
			1	-2.2	(24 h dark period)

Two hours after the chilling treatment, the plants were photographed with conventional Kodacolor[6] II color negative film to record injury. Number of cotyledons and leaves damaged were counted, and leaves of the same age were randomly selected for light reflectance and electrolyte leakage measurements.

A Beckman Model DK-2A[6] spectrophotometer, equipped with a reflectance attachment, was used to measure total diffuse reflectance on upper (adaxial) surfaces of single leaves over the 0.5 to 2.5-μm waveband. Reflectance at the 1.65-μm wavelength was used for cold injury assessment (10).

Five discs (each 15 mm in diameter) from the leaves used for reflectance measurements were punched out with a cork borer, weighed, and floated on 20 ml of distilled water. The amount of electrolyte that leaked out after 6 h at room temperature was measured with a Model 31 YSI Conductive Bridge (Scientific Products[6]).

The analysis of variance was applied to reflectance and electrolyte leakage data. Duncan's multiple range test was used to compare mean differences statistically.

All plants were kept outdoors for three weeks after the chilling treatment to observe the survival and regrowth of injured plant parts.

RESULTS

About 50 to 60% of the cotyledons were damaged when cotton plants were exposed repeatedly to 4.5°C (Table 1). None of the plants died, and cold injury to the leaves was negligible as shown by gross examination and electrolyte leakage data (Fig. 1), but reflectance measurements did indicate that there was a significant difference ($P<0.05$) in light reflectance between the control leaves and the MC-treated leaves at the 1.65-μm wavelength (Fig. 2). The effect of MC on true leaves of the cotton plants was not apparent with this level of cold treatment.

The results from groups 2 and 3 clearly demonstrated that MC modified the cotton plants in a way (yet unknown), which enabled the plants to be less susceptible to cold injury. Though no plants in group 2 died, almost 90% of the 1st and 2nd leaves of the control plants were destroyed. This was three

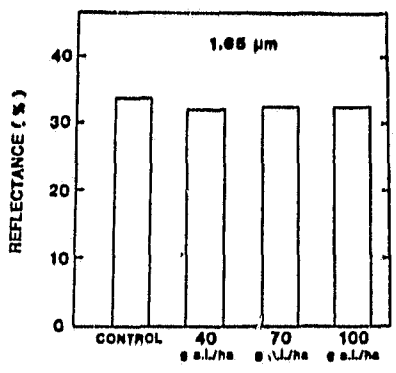

Fig. 2. Percent reflectance of cotton leaves from control and MC-treated plants. The plants were exposed to 4.5°C night (12 h) for 6 days.

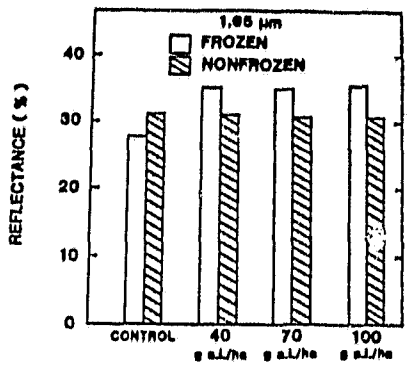

Fig. 4. Percent reflectance of cotton leaves from control and MC-treated plants with or without exposure to 15.5°C day (12 h) and 1.7°C night (12 h) for 10 days and then to -2.2°C for 24 hours in the dark.

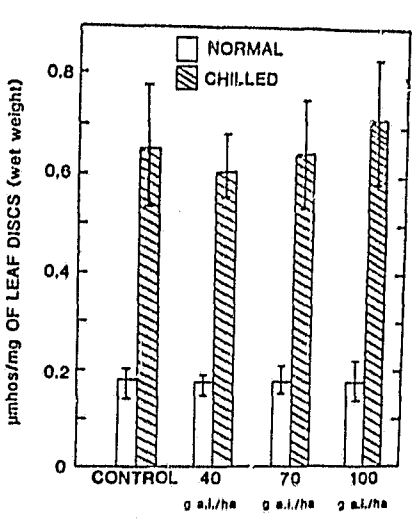

Fig. 3. Electrolyte leakage of leaf discs from control and MC-treated cotton plants. The chilled plants were exposed to 0.5°C night temperature for 12 hours. The mean and range of conductance measurements from 3 replications are given.

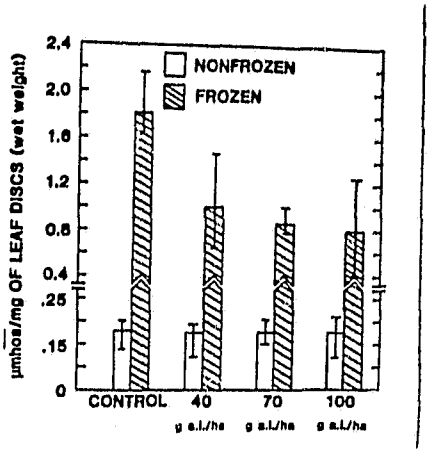

Fig. 5. Electrolyte leakage of leaf discs from control and MC-treated cotton plants with or without exposure to 15.5°C day (12 h) for 10 days and then to -2.2°C for 24 hours in the dark. The mean and range of conductance measurements from 3 replicates are given.

times more damage than plants treated with MC at concentrations of 70 and 100 g a.i./ha. The reason is unknown, but the younger leaves (3rd & 4th) were damaged less than the more mature leaves (1st & 2nd) (Table 2). The injury inflicted by cold stress above freezing was mainly leaf wilting, burn (desiccation), and abscission. The leaves from chilled plants released approximately 4 times ($P<0.5$) more electrolytes than those from normal (nonchilled) plants, indicating that they had some cold induced injury (Fig. 3). No significant difference in electrolyte leakages between the control and MC-treated leaves was detected and the final outcome of the leaves (abscission and death), could not be predicted definitely by the electrolyte leakage data.

After chilling at 1.7°C for 10 nights, little damage (other than slight wilting) was observed visually in group 3 plants. Group 3 plants were two weeks older than group 1 and 2 plants. Cold exposure for 10 days might have induced some cold hardening in group 3 plants, but when the same plants were exposed to -2.2°C for 24 hours, 60% of the control plants were killed and only 10 to 20% of the MC-treated plants appeared to be dead (Table 3). The frozen dead plants showed typical freezing injury--a darkened, limp, and water-soaked appearance after thawing. The damage ratio of control to MC-treated plants was 3 to 1. The reflectance and the electrolyte leakage of leaf discs from the dead and surviving plants are shown in Figs. 4 & 5. The largest difference in reflectance between frozen MC treated and untreated plants occurred over the 1.65-μm (near infrared) wavelength. The leaf discs from the dead control plants leaked 10 times more electrolytes than those of nonchilled plants and two times more electrolytes than those of the surviving MC-treated plants. These results were all statistically significant ($P<0.05$). Freezing injury could be assessed easily by either the reflectance or the electrolyte leakage measurements; however, the differences in the degree of freezing damage among the three different concentrations of MC-treated plants were difficult to distinguish with either method of measurement. Some plants assumed dead in group 3 regrew during the three-week period after freezing treatment (Table 3).

DISCUSSION

A review of several papers indicated that there is no agreement as to the natural resistance of cotton plants to cold temperature, especially the threshold of killing temperature. Christiansen and Ashworth (1) reported that approximately 40% of control cotton seedlings were killed by chilling 2 days at 8°C. Yet in another study, only 3% of cotton seedlings died after chilling for 4 days at 8°C (13). Guinn (5) demonstrated that chilling at 5°C for 24 hours caused cotton seedlings to wilt and increased their cotyledonary membrane's permeability but no seedling death was indicated. Rikin et al. (11) showed that most of the 12-day-old cotton seedlings covered with polyethylene bags and exposed to chilling at 4°C in the dark for six days died. In our study, no cotton plants (12 to 26 days old when exposed to cold) died even after chilling at 1.7°C for 10 nights or 0.5°C for 12 hours. Different experimental conditions --day/night temperature, photoperiod, stage of development of plants, different strains (cultivars) and physiological conditions of plants before chilling exposure--probably all contribute to the survival and death of plants and may explain the discrepancies described above. For evaluating plant resistance

to chilling injury, criteria other than death of plants can be used. In our study, the number of cotyledons and leaves that were damaged and became desiccated were recorded, and the results showed that MC-treated plants had their resistance to cold injury increased about threefold. The same ratio was established for the resistance to freezing injury.

According to our results, 70 to 100 g a.i./ha concentrations of MC could give the maximal protection against cold injury, but it is very important to study the combined influence of MC spray and low temperature on the long term effect on cotton growth and development.

The cold protection level that we found is comparable to other investigations that used ABA (11) and antitranspirants (1). The increased resistance to cold stress induced by ABA and antitranspirants is attributed to a reduced water loss. The increased ABA levels are believed to induce stomatal closure, and ABA applications can result in complete and reversible stomatal closure (8). Whether the same mechanism is involved in MC-induced protection should be investigated.

It is generally agreed that one of the most universal measures of plant temperature injury has been electrolyte leakage (7) and that, after severe chilling, the tissues leak internal electrolyte and die (9). Our results indicated that a totally damaged tissue (frozen and thawed) did leak a large amount of electrolytes. However, the method is not specific and sensitive enough to predict a partially damaged tissue with no visually observable injury that can either revive or die.

It was demonstrated through our reflectance data that detecting freeze damage in a large scale field operation may be feasible using a technique such as remote sensing photography.

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