Water Relations in Young Growing Wheat Leaves after Application of (2-Chloroethyl)trimethyl-ammoniumchloride (CCC) to the Roots of Wheat Seedlings

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The influence on the water relations of the third developing leaf of (2-chloroethyl)trimethylammoniumchloride, a synthetic growth regulator, applied to the roots of young wheat plants (*Triticum aestivum* L.) has been investigated. The tissue water potential and the pressure potential were found to be reduced by several bars in comparison to the untreated controls, whereas the osmotic potential remained unchanged. The content of soluble reducing sugars was considerably increased in the cell sap of CCC-treated leaves. With this accumulation, however, the turgor was not maintained. Additionally, CCC-treated leaves showed considerably lower transpiration rates and higher diffusive resistance than the controls. Thus, the application of CCC to the roots causes alterations in the water relations of developing wheat leaves, which resemble those induced by water deficiency.

Introduction

Preliminary investigations have shown that after the application of (2-chloroethyl)trimethylammoniumchloride (CCC) to the roots cellular content of soluble proteins and soluble reducing sugars is intensified and *in vitro* activity of isolated ribulose-1,5-bisphosphate carboxylase is increased in the leaves of young wheat plants (*Triticum aestivum* L.). Root growth was stopped immediately and the growth of the leaves, CO_2 fixation capacity and leaf conductance were reduced [1]. The inhibition of both cell division and cell enlargement was found to be responsible for the 40% reduction in leaf area of CCC-treated plants in comparison with the untreated control plants.

Water deficiency can cause similar changes in higher plants. Sugars and free amino acids, for example, are often accumulated during water stress. Stomata may close and transpiration and CO_2 fixation will be reduced in response to water deficits (for review see [2–4]). Therefore, a study was made of the water relations of growing wheat leaves after CCC-treatment.

Abbreviation: CCC, (2-chloroethyl)trimethylammoniumchloride.

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Materials and Methods

Plants of *Triticum aestivum* L. (var. Kolibri) were cultivated in hydroculture boxes as described earlier [1]. 11 days after the seeds were sown, CCC was added to the nutrient solution so that the final concentration was 10^{-2} mol · l^{-1}. At this time the third leaf was just emerging. During its development the following measurements were made. Samples were taken 1 hour after the light period had begun and transpiration was measured during the afternoon. The leaf water potential (Ψ) was measured using the pressure bomb technique developed by Scholander and colleagues [5]. The osmotic potential (Ψ_s) was calculated from the cryoscopically measured osmolarity (Osmomat 030, Fa. Gonotec, Berlin) of cell sap obtained from rapidly frozen (in liquid N_2) and thawed wheat leaves [6]. For details about the determination of soluble reducing sugars with the dinitrosalicylate reagent see Wild and Zerbe [7]. The determination of transpiration and diffusive resistance of the adaxial surface of attached wheat leaves with a steady state porometer (LI-1600, LICOR, Lincoln, Nebraska, USA) followed after the calibration of the cuvette under the conditions of the climatic chamber. In each experiment 10 to 20 leaves were investigated. The presented data are mean values taken from three experiments.

Results and Discussion

CCC applicated to the roots of young wheat plants significantly influenced the water potential (Ψ) of the developing third leaf (Fig. 1). The Ψ remained relatively unchanged in the control leaves during leaf expansion, whereas a slight but continuous decrease of Ψ in the treated leaves could be observed.

Osmotic potential (Ψ_s) and turgor pressure (Ψ_p) are two other parameters closely related to tissue water status [2]. Changes in Ψ_s could be brought about by changes in the solute content of the symplasm, or in amount of water in the symplasm [6]. With the relative water content of the CCC-treated wheat leaves being depressed by about 5 to 10% as opposed to the control leaves (data not shown) and the concentration of soluble reducing sugars in the

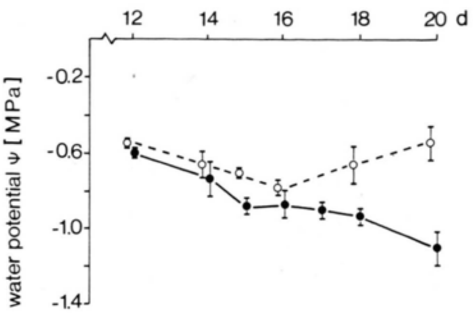

Fig. 1. The water potential (Ψ) of the third growing leaf of untreated (○---○) and CCC-treated (●——●) wheat plants. 10^{-2} mol · l^{-1} CCC were added to the nutrient solution at the 11th day of development. The bars represent the standard deviation.

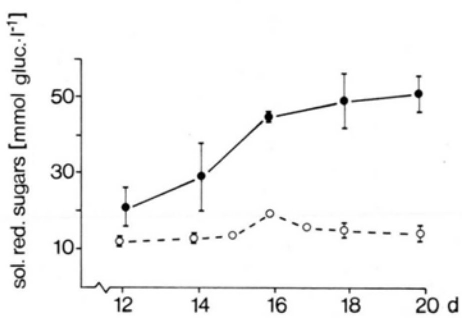

Fig. 2. The increase of the content of soluble reducing sugars in the cell sap extracted from the third wheat leaves after CCC application at the 11th day (●——●); (○- - -○): untreated controls. The concentration of sugars is given as equivalents to a glucose standard.

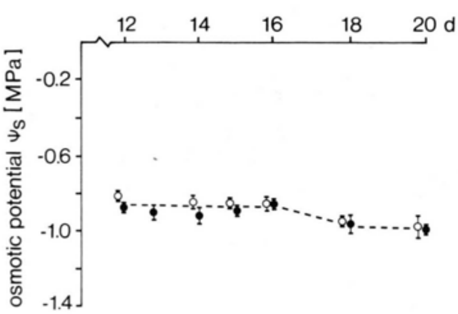

Fig. 3. The effect of CCC on the osmotic potential (Ψ_s) of the third wheat leaf during its development. The open symbols represent the control values, the closed symbols represent the data obtained from leaves after CCC application at the 11th day. Ψ_s was estimated on extracts of sap obtained from rapidly frozen leaves by the cryoscopic technique.

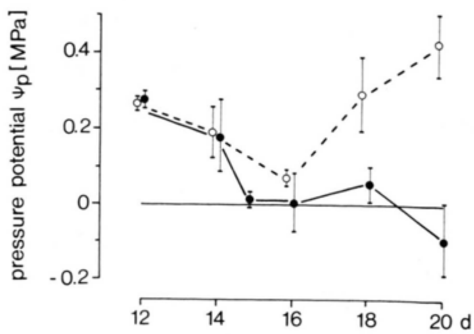

Fig. 4. The pressure potential (Ψ_p) of the third wheat leaves after CCC application to the plant roots (closed symbols). The open symbols represent the control values. Ψ_p was calculated from the cryoscopically measured data of Ψ_s: $\Psi_p = (-)\ \Psi - (-)\ \Psi_s$.

cell sap being markedly raised (Fig. 2), one might expect a lowering of the osmotic potential of the cell sap after CCC had been applicated. But as shown in Fig. 3, the growth retardant did not affect the Ψ_s of the third wheat leaf.

As a consequence of the unchanged osmotic potential and the decrease of leaf water potential, the turgor of the third leaf was lowered from the third day after the addition of CCC (Fig. 4). Calculated from the cryoscopically measured data, Ψ_p even assumed negative values in the CCC-treated tissue. The actual existence of negative turgor pressures in wheat plants, however, has to be doubted. These probably arise from dilution errors in the measurement of the osmotic potential on cell sap from frozen and thawed tissue [6, 8].

The transpiration determined at the adaxial surface of attached wheat leaves under the conditions of the climatic chamber, was less in the CCC-treated leaves as compared to the control leaves (Table I).

Table I. Transpiration and stomatal resistance in CCC-treated wheat plants and untreated controls. The measurements of transpiration and stomatal resistance were performed with the adaxial surface of the third attached leaves at the 20th day of development in the climatic chamber. The data presented are mean values (mv) from 50 different plants, s = standard deviation.

	Untreated control		10^{-2} mol · l^{-1} CCC	
	mv	s	mv	s
Transpiration [g · m^{-2} · h^{-1}]	249	± 6	35	± 11
Stomatal resistance [s · cm^{-1}]	0.9	± 0.03	9.1	± 3.3

Our results suggest that the application of CCC to the roots causes alterations in the water status of developing wheat leaves which resemble those induced by water deficiency. These might be a consequence of the sudden stop of root growth observed in our experiments after addition of CCC to the nutrient solution [1].

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