Stomatal Responses to Changes in Atmospheric Humidity and Water Supply: Experiments with Leaf Sections of Zea mays in CO₂-Free Air

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Summary. Leaf sections were exposed to CO₂-free air, thus excluding interference by the CO₂-sensitive system in the guard cells. Stomates did not close in response to change from moist to dry air, whether it passed over the leaf or was forced through the intercellular spaces. In contrast, the stomatal apertures became narrower if the water potential in the liquid supplying the leaf was lowered. Of solutions with the same osmolality, those with the higher viscosity produced the larger responses.

Transient stomatal movements in the opposite direction to the final response were observed upon any sudden change in the water status of the leaf sections, whether caused by varying the moisture content of the air passing around or through the leaf sections, or by varying the water supply. Increased load on the water supply caused temporary opening movements, while improvements in water supply led to closing movements of varying duration. When dry air was forced through the leaf sections, non-sinusoidal oscillations with large amplitudes were sometimes observed.

It is concluded that the guard cells are tightly coupled to the water-supply system of the leaf and only indirectly to the conditions in the atmosphere by a negative feedback of transpiration on the water potential in the water-conducting system.

Introduction

Stomata respond to changes in intercellular CO₂ concentration in the manner of a negative feedback system, namely, decreases in the CO₂ content are answered by proportional increases in stomatal conductivity for CO₂ (RASCHKE, 1965b).

Stomatal aperture reflects also the water status of the plant. STÄLFEI] suggested as early as 1929 that stomatal width is the result of an “automatic balancing” between two reaction systems, a “photoactive” one and a “hydroactive” one. This is equivalent to the assumption of another feedback loop, in addition to the CO₂-feedback system. SÉYBOLD (as quoted by MAÈRECKER, 1965) thought that direct water loss from guard cells (“peristomatäre Transpiration”) might provide a feedback
channel for the guard cells, enabling them to respond quickly to changes in atmospheric humidity, and thereby to control transpiration. MAERCKER (1965) claims to have provided experimental support for this hypothesis by demonstrating that guard cells surrounding open pores have lower cuticular diffusion resistances than guard cells surrounding closed pores. STÅLFEKT (1967), experimenting with leaf sections floating on water, interpreted wider openings in the epidermis in contact with water as favoring SEYBOLD's suggestion. However, the difference in pore sizes could equally well be a consequence of a concentration gradient of CO₂ across the leaf sample. This was demonstrated by repeating STÅLFEKT's experiments in CO₂-free air which produced lesser openings of stomates in contact with water (RASCHEKE, unpublished data).

In this paper we report experiments designed to provide evidence for or against the presence of a stomatal negative feedback system in maize leaves which stabilizes the relation between water loss and water supply, and if one is present to determine whether it involves peristomatal transpiration.

In order to exclude any interference from the CO₂-feedback system, the experiments were done in CO₂-free air. Under this condition the CO₂-control circuit calls for maximum aperture at all times. The width of the stomatal pores is then a function of the energy supply to the guard cells; it will also depend on the state of the water control system in the plant, if one is present.

**Materials and Methods**

*Zea mays* L cv. Asilo (= Pioneer 395) was cultivated in solution and under controlled conditions as described previously (RASCHEKE, 1966). The 4th and 5th leaves (counted in the sequence of their emergence) were used when the plants were 20 to 25 days old. Leaf sections about 3 cm long were clamped into plexiglas chambers giving an area of 2.5 cm² exposed to the air (RASCHEKE, 1965a). The leaves were supplied with water through their cut edges. Thermocouples (0.1 mm in diameter) were run along the lower surfaces of the leaf cuttings. Leaf temperatures were kept at or near 30°C (with the exception of one experiment, Fig. 2). The air conducted into the chambers was dried by condensation at 0°C, and freed from CO₂ and further dried by passing it through an NaOH tower. Air with a defined moisture content was obtained by saturating the CO₂-free air with water and subsequent passage through a temperature-controlled condenser. Changes in water-vapor concentration were measured with a differential infrared gas analyzer (“Uras”, Hartmann & Braun, Frankfurt a. M., Germany), calibrated with air saturated with water vapor at several constant temperatures, and by using mixing pumps (Wösthoff, Bochum, Germany). Stomatal aperture was recorded with the porometer system previously described (RASCHEKE, 1965a), and the osmolality of the solutions supplying the leaf sections was determined with a freezing-point osmometer (Knauer, Berlin, Germany). Light was produced by high-pressure xenon arcs and passed through 3 mm KG1 Schott filters. The irradiance in the plexiglas chambers was 20 mW cm⁻² (if not stated otherwise). All experiments were