HYDROGEN ION CONCENTRATION OF THE
ROOT MEDIA IN RELATION TO
TRANSPIRATION RATES

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ABSTRACT

The transpiration rate of tobacco (Nicotiana tabacum L.) with roots immersed in a
solution at pH 3.0 was lower than that of plants with roots in a solution at pH 4.0 or 6.5
(control). Since wilting occurred in the pH 3.0 treated plants, it was apparent that water
absorption was inadequate to compensate for transpiration losses and to maintain turgor.
This disruption of the internal water balance was attributed to the physiological distortion
of the root tissue. Plants subjected to a pH 4.0 treatment maintained turgor and
transpired at rates comparable to the controls. It is suggested that pH 4.0 is within the
physiological range for water absorption by the tobacco plant.

INTRODUCTION

Hydrogen ion effects are directly related to the survival and adaptation of crop
plants to their nutritional environment (1, 4). Adverse effects of low pH on the growth of
some crop plants have been recognized (1, 2); however, whether the failure of plants to

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thrive in an acid medium is due to physiological disorders or unbalanced absorption of essential nutrients has not been verified. There are reports on the effect of $H^+$ on various physiological processes in plants (6, 10, 11, 13, 14), but not relative to transpiration and water metabolism.

The loss of water vapor from plants is defined as transpiration (8, 9). Since the internal water balance of the plant is the net result of the amount of water absorbed and transpired (8), the factors influencing water absorption may affect transpiration (8, 9). The rate of transpiration is extremely variable (9) because of the influence of internal and external factors. In spite of extensive research conducted on the effects of the environmental factors on transpiration, no studies on the effect of $H^+$ concentration on transpiration have been reported. A short statement, however, with no experimental evidence (12), indicated that the ability of the roots to absorb water may be reduced due to the acidity of the medium.

The objective of this research work was to study the effect of $H^+$ concentration on the transpiration of tobacco plant with further efforts to evaluate the physiological pH range for transpiration, specifically in the acid region.

**MATERIALS AND METHODS**

Seeds of tobacco (*Nicotiana tabacum* L., cv. Havana 501) were germinated and grown in a 3:1 soil vermiculite mixture. After 4 weeks, the plants were transferred to a complete nutrient solution and grown for another 4 weeks. Gallon size, opaque, plastic containers with 3 l of solution were used. Prior to the start of transpiration measurements, the plants were removed from the nutrient solutions, the roots were rinsed with distilled water and placed in pH adjusted solutions. To determine the $H^+$ effect, three pH values of 3.0, 4.0 and 6.5 were tested. The solutions with pH 3.0 and pH 4.0 were prepared by addition of appropriate quantities of HCl to distilled water. Pure distilled water with pH 6.5 was used for control.

One ml of tritiated water with specific activity of 0.5 mc/ml was added to each treatment solution and mixed thoroughly. Tritiated water was used to follow the rate of water absorption and movement as well as the time of replacement of internal water of plants by that of the external solution. Proper sealing was made around the stem to prevent the entrance of tritiated water into the chamber except via the plant.

For each experiment, 2 uniform plants, one for the control (pH 6.5 treatment) and the other for the low pH treatments, were selected and transferred into the transpiration measurement chambers. Comparability of the experimental plants was assured by measuring their transpiration rates for a period of 12 to 18 hours before subjecting the plant roots to the pH treatments. The culture solutions were aerated.

The transpiration measurement apparatus consisted of 3 parts: a plant chamber, a freezing unit to capture the water vapor evolved in transpiration, and a flow meter to measure the rate of flow and the total volume of air passing through the system. Cylindrical bell jars, 22 cm in diameter and 37 cm in height with an approximate volume of 14 L, were used as chambers. A thermo-couple was installed inside each chamber with its tip located a short distance above the uppermost leaves of the plant. Temperatures were recorded every 6 hours by means of a Minneapolis-Honeywell Model 126 W2P potentiometer.

Fig. 1. Schematic representation of the transpiration measurement apparatus.

The schematic representation of the transpiration measurement apparatus used in these experiments is shown in Fig. 1. Reproducibility of the results obtained from repeated experiments indicated that the apparatus was suitably designed and operated satisfactorily.

Pyrex cold-traps were used for collecting the transpired water by freezing. The freezing units were placed in thermos jugs containing a dry ice and acetone mixture (−78 °C). At 6-hour intervals the freezing units were detached and replaced by others to melt the ice and measure the volumes of water transpired. Air flow at a rate of 4 l/min was used to remove the water vapor evolved by transpiration from the chamber to the freezing unit. Pipeline air was used for passing through the chambers and for aeration of culture solution. The average moisture of the air supply was 0.4 mg/l. This amounted to approximately 0.6 ml for each 6-hour interval which was subtracted from the total volume of water collected during this period.

Carbon dioxide concentration of the air entering and leaving the plant chambers was determined to the nearest ±5 ppm at each sampling time by means of a Liston-Bec-ker, Model 15-A, gas analyzer.

A 1 ml sample of the transpired water was mixed with 10 ml of a dioxane-based scintillator mixture for radioactive counting. All radioactivity determinations were at 40% efficiency in a Beckman LS 200 scintillation spectro-meter.

RESULTS AND DISCUSSION

Values for transpiration rate (ml/plant/6 hours), CO₂ concentration (ppm) of the air entering and leaving the plant chambers, and specific activity (dpm X 10⁹/ml) and total activity (dpm X 10⁶) of the transpired water from the treated and control plants are reported in Figs. 2 and 3 for pH 3.0 vs. pH 6.5 and pH 4.0 vs. pH 6.5, respectively. On the horizontal axes for time intervals, the broken and solid lines indicate light and dark periods, respectively.

As shown in Fig. 2, the transpiration rate of tobacco with roots immersed in a solution at pH 3.0 was markedly below that of a comparable transpiration rate of the control plant (pH 6.5). Expressed as the percent of the control, transpiration rate of the pH 3.0 treated plant ranged from 38.5% to 94% and the total quantity of water transpired during the 48-hour period of the experiment was 74%. Generally, the magnitude of the decrease in the transpiration rate was greater during the light than the dark period (Fig. 2).

The loss of turgidity in leaves of pH 3.0 treated plants occurred within approximately 1 hour. Although no experimental data was gathered, it was observed that under sunny skies and high temperatures, wilting appeared faster and was more severe than under cloudy and cool conditions. Frequently the wilted plants recovered at night and regained their turgidity. The plant subjected to a pH 4.0 treatment transpired at rates comparable to the control plants and maintained turgor.

![Graph showing CO₂ concentration and water transpired over time intervals.](image)

Fig. 2. Transpiration rate of *Nicotiana tabacum* L. and CO₂ concentrations in the air supply and inside the plant chambers in relation to the pH of root media.

Carbon dioxide concentration of the air inside the chamber of the pH 3.0 treated plant was higher than the control plant during the light hours and lower during the dark hours (Fig. 2). Carbon dioxide concentrations inside the chambers of the pH 4.0 and 6.5 treated plants were almost the same and showed similar diurnal patterns (Fig. 3).

Fig. 3. Specific and total activity of tritium in the water transpired by *Nicotiana tabacum* L. in relation to the pH of root media.

Specific activity of the tritium in the transpired water increased consistently in both acid-treated and the control plants and reached a maximum at approximately 36 hours after the start of the treatments and leveled off thereafter. Maximum specific activity of the transpired water at 36 hours was about 90 - 95% of the specific activity of the external root solution. Specific activity of the transpired water for the pH 3.0 treated plant was consistently higher than that for the control plant. However, values for the pH 4.0 and control plants were similar.

Total activity of tritium in the transpired water was a function of the transpiration rate irrespective of treatments. The total radioactivity of the transpired water from the control plant was greater than the pH 3.0 treated plant except in the early stages of the experiment, i.e., until approximately 12 hours after the start of the experiment. Total activity of the transpired water in the pH 4.0 and 6.5 treated plants was similar.

A disruption in the internal water balance of the pH 3.0 treated plants was evident since wilting occurred in the leaves indicating that water was not made available to the leaves in sufficient quantities to replace the transpiration losses. In some experiments the pH 3.0 treated plants were transferred to the pH 6.5 solution upon the termination of transpiration measurements to find out whether they would recover and return to normal. The plants generally failed to recover. Two possibilities are suggested as the causative factor:

1) the water absorption by the roots was not sufficient to compensate for the transpiration losses, due to the physiological and morphological distortions of the roots, possibly changes in the permeability of the cell membranes, or

2) the translocation of water through stems was not fast enough to replace the water lost by the leaves to maintain turgor.

Changes in the permeability of root membranes at low pH accompanied by an outward diffusion of cell material and a decrease in dry weight of the roots have been reported (5, 13). Dry weight determination of the roots at the end of transpiration experiment consistently showed lower values for pH 3.0 treated plants as compared to those of controls. This perhaps indicated outward diffusion and loss of cell material from the roots of pH 3.0 treated plants, since their culture solutions became turbid. The second possibility was not fully verified. However, anatomical studies of the water conducting tissues in the pH 3.0 treated plants showed no indication of damage to the xylem vessels of the stem. Evidently, the main site of disruption in water movement was in the plant root system.

Regardless of the exact nature of the H⁺ effect on transpiration, the ability of the roots to absorb water was greatly reduced and water was not made available to the leaves sufficiently enough to compensate for transpiration losses and to maintain turgor. Stomatal closure as a consequence of loss of turgidity may have partially been involved in

the decreased transpiration rates, since there is a close association between the turgidity of the guard cells and stomatal movement. With decrease in the pH of the cell sap in the acid treated plants (1) and also the possible role of the pH of the guard cells in controlling stomatal movement (7, 15), it is speculated that the reduction in the transpiration rate of the pH 3.0 treated plants could in part be due to the low pH effect on stomatal closure.

Specific activity of the transpired water of the pH 3.0 treated plant was consistently higher than the control even though the total activity was usually greater in the control. Whether this could be attributed to differences in the size of their internal water pools which was anticipated to be smaller in the pH 3.0 treated plants remains to be further resolved. The specific activity of the transpired water reached equilibrium with the specific activity of the external solution after approximately 36 hours. This indicated that the major replacement of the internal water of the tobacco plant occurred within 36 hours.

Differences in the CO₂ concentration inside the chambers of the pH 3.0 and control plants suggest that the rates of photosynthesis and perhaps respiration may also have been reduced as the result of the low pH.

The plant subjected to a pH 4.0 solution transpired at rates similar to that of the control; thus pH 4.0 was considered within the physiological pH range for the tobacco plant. In various crop plants and for certain physiological processes, pH 4.0 is considered by several investigators to be within the physiological range for plant growth and pH 3.5 by others (14).

LITERATURE CITED


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