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THE EFFECT OF HYDROGEN ION CONCENTRATION ON THE ANATOMY OF TOBACCO[1]

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ABSTRACT

The study of the anatomy of tobacco (*Nicotiana tabacum* L.) in relation to H^+ concentration in the external root medium revealed that pH 2.0 or below was lethal to the plant. Serious tissue damage occurred in both roots and stems. At pH 3.0 tissue injury occurred only in the roots. At pH 4.0 indications of damage were not detected either in the roots or stems. Thus, pH 4.0 was considered within the physiological range for the tobacco plant as far as the direct effect of H^+ to the plant is concerned.

INTRODUCTION

The influence of pH of the root medium on plant growth has been attributed to both direct and indirect effects of H^+ (6). There is evidence that pH 2.0 and below was lethal to the plant; at pH 3.0 some plants persisted with little or no growth (6) and some completely failed to grow (1, 2). At pH 4.0 satisfactory growth or even maximum yeild of several crop species was obtained (1, 2, 12).

Retardation of root growth at high concentrations of H^+ has been reported (3). The growth depression of roots at low pH values was attributed by Audus (3) to auxin dissociation.

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A decrease in the absorption of cations with increased concentrations of H^+ within certain pH range has been recognized (12). Two types of effects of H^+ on the ion absorption mechanism have been suggested by Rains *et al.* (13) : 1) a competition between H^+ and other cations for the available exchange sites, and 2) a direct injury to the absorption mechanism itself resulting in irreversible alterations. Denaturation of proteins, nucleic acids, phospholipids, and other polymers associated with the structure and function of the cell membranes were suggested as some of the possible damages induced by high concentrations of H^+ (13).

Although there is lack of information on the anatomy of plant tissues in relation to the acidity of root medium, recently an investigation on the fine structure of meristematic cells of corn root tips treated with acid was made (11). According to these investigators, cells of 5 - day old root tips exposed to solutions at pH 4.4 and below for several hours displayed serious damage. Vacuole - like structures with well defined membranes were observed. The damage to the root cells was attributed to the loss of K and possibly Ca (11). They found that the presence of Ca in the external solutions greatly reduced the adverse effect of H^+. A mild acid treatment to roots to withdraw Ca caused serious injuries and even death. Withdrawal of Ca also caused serious damage to the cell membranes resulting in the impairment of the ion absorption and retention mechanism (7, 15).

Normal anatomy of the roots, stems, and stem apices of various plant species are described by several investigators (4, 8). The anatomy of tobacco plant was investigated to study the nature of the tissue damage occurring in certain plant organs due to the acidity of the root medium.

MATERIALS AND METHODS

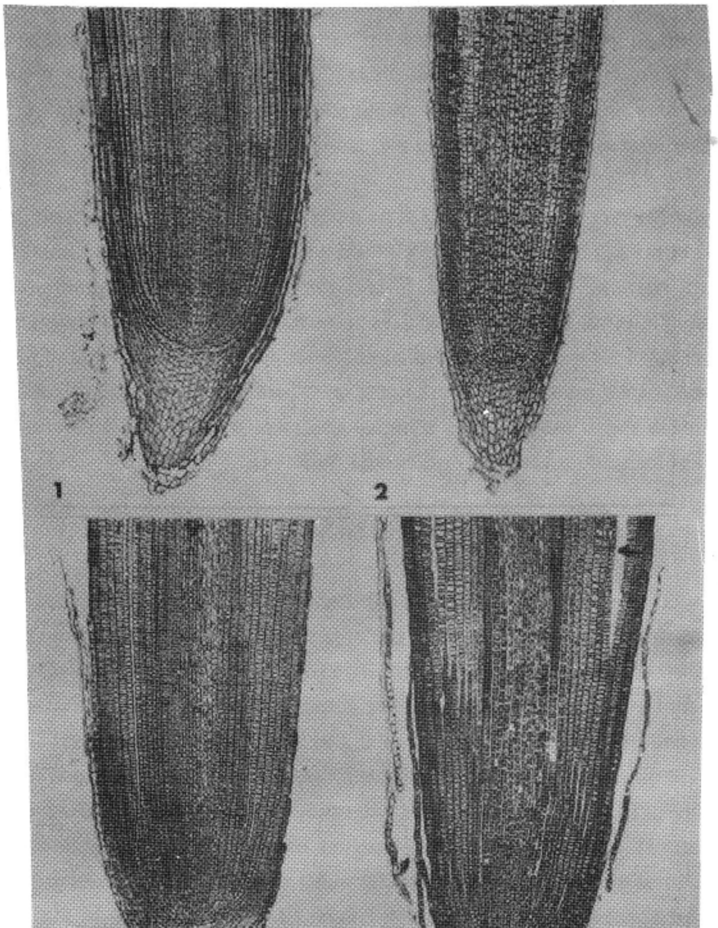
The roots of eight-week old tobacco plants (*Nicotiana tabacum* L., cv. Havana 501) grown in complete nutrient solution were rinsed with distilled water and the plants transferred to non-nutrient solutions at pH 1.0, 2.0, 3.0, 4.0, and 6.5 (control) for either 6 or 24 hours. Redistilled HC1 was used for pH adjustments, and pH values were measured to the nearest ± 0.1 unit.

The concentrations (ppm) of 13 essential elements in the complete nutrient solution in decreasing order were K 234, N 210, Ca 200, S 64, Mg 48, P 31, Cl 1.77, B 0.27, Mn 0.27, Fe 0.22, Zn 0.13, Cu 0.03, and Mo 0.01. The nutrient solution was changed twice a week and the pH was maintained within the range of 5.5 to 6.0 when necessary, using 1 N KOH or redistilled HCl. Each treatment was replicated four times. Root tips and stems (sixth internode from the stem tip) of the plants were sampled 6 hours after the exposure of the roots to the acid solutions. This period was sufficient to detect the root tip and stem injuries induced by high concentrations of H^+. Sampling of stem apices, however, was made 24 hours after exposure to low pH.

To study the recovery of the pH 3.0 treated plants, their roots previously immersed in a solution at pH 3.0 for 24 hours, were rinsed with distilled water and transferred back to a complete nutrient solution. After 5 days the plants appeared normal and samples were taken at this time. Plant tissue samples were fixed in formalin-aceto-alcohol, dehydrated with n-butyl alcohol, and infiltrated and embedded in paraplast. Longitudinal and transverse sections were cut 10 μ in thickness on a rotary microtome. The serial sections were fixed to the slides with Haupt's adhesive and stained with safranin 0 and fast green FCF. The microtechniques used are outlined by Sass (14).

RESULTS

The structure and tissue organization in the root tips of control plants were normal (Fig. 1). No differences were detected in the organization of the root tissue in plants subjected to pH 4.0 as compared to the control. The roots of pH 3.0 treated plants were considerably smaller in diameter and cells of the rootcap were fewer and elongated (Fig. 2). The cortical cells contained less cytoplasm, and the nuclei were abnormally shaped. Serious root damage in the pH 2.0 treated plants was evident. The rootcap was much less conspicuous (Fig. 3) with the outer layer extending acropetally and epidermis separated from the cortical layer and gradually sloughed off. Cortical cells began to separate possibly due to loosening of the intercellular substances. Cells in the stelar region were vacuolated and contained little cytoplasm. Nuclei appeared abnormal in shape, and the nucleoli were inconspicuous. The size and number of rootcap cells in pH 1.0 treated plants were considerably reduced. The rootcap layer adjacent to the protoderm separated and sloughed off (Fig. 4). Cells of the cortical and stelar regions contained small amounts of coagulated cytoplasm. Individual cells and files of cells were separated. Separations between the layers of cells resulted in large spaces in the cortical and stelar regions (arrow). Nuclei were small, distorted, and abnormally shaped. Generally, the impact of the destructive effects of H^+ to the root tissues within the pH range of 1.0 to 3.0 increased with increases in the concentration of H^+.



Figs. 1 to 4 : Longitudinal sections of the root tips of *Nicotiana tabacum* L. treated with solutions at different pH values for 6 hours. Fig. 1. pH 6.5 (control). Fig. 2. pH 3.0. Fig. 3. pH 2.0. Fig. 4. pH 1.0. Magnification : X50.

Although tissue damage occurred at various locations in the stems of pH 1.0 and 2.0 treated plants, the injury was most severe in the vascular system. The stem tissues of plants subjected to pH 6.5 were normal (Fig. 5). Tissue injury was not apparent in the stems of plants with roots immersed in a solution at pH 3.0. Cambial activity and xylem differentiation were normal (Fig. 6). Stems of plants treated with pH 2.0 and 1.0 solutions showed serious damage to all tissues. Cortical cells were misshapen and collapsing and the cambium and external phloem were completely collapsed. Xylem vessels with wall thickenings were still apparent (Figs. 7, 8). Some differentiating vessels had expanded at pH 2.0 (Fig. 7). The internal phloem displayed the least extent of damage.

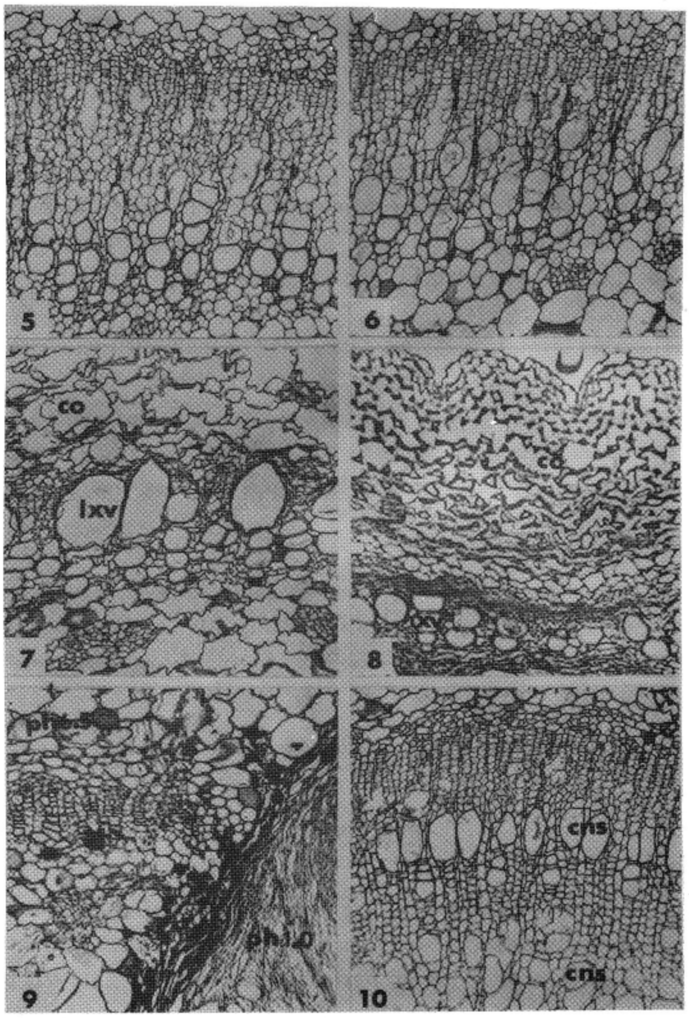
In a split-root experiment, approximately 1/10 of the plant root system was transferred to a solution at pH 1.0 for 24 hours, while the remaining roots were immersed in distilled water. Examinations showed presence of both normal and injured tissues in the same stem. The tissue damage was similar to that found in the stems of plants with entire roots rubjected to pH 1.0 solution, but the injury was apparent only in part of the stem (Fig. 9).

In the recovery study, however, stems of plants revealed normal xylem vessels which differentiated prior to the pH 3.0 treatment (Fig. 10). The experiment further substantiated that stems of plants with roots subjected to pH 3.0 solution showed no adverse effects.

The anatomy of the stem apices at all H^+ concentrations tested was similar (Figs. 11 - 14).

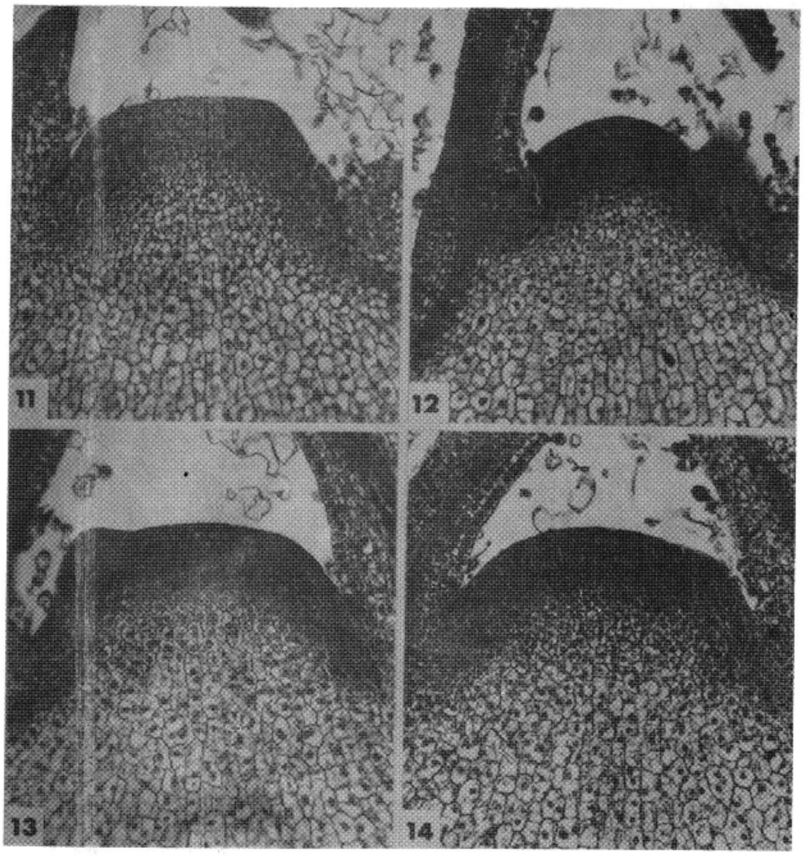
DISCUSSION

Serious tissue damage occurred in roots and stems at pH 1.0 and 2.0 within 6 hours. At pH 3.0 noticeable damage occurred in the roots but not in the stems. Plants treated at pH 1.0 and 2.0 wilted. However, pH 3.0 treated plants wilted during the day but recovered at night which indicated that xylem elements were still functional and the translocation of water to the leaves continued so that they became turgid at night. Daytime wilting of the pH 3.0 treated plants was undoubtedly the result of reduced water absorption by the roots. During the light period, when transpiration loss was greatest, the roots did not absorb water in quantities sufficient enough to compensate for the losses through the leaves. Reduced diameter of the root tips at pH 3.0, their vacuolated cells and small amounts of cytoplasm indicated changes in the permeability of membranes and outward



Figs. 5 to 10 : Transverse sections of the sixth internode from the stem tip of *Nicotiana tabacum* L. with roots immersed in solutions at different pH values for 6 hours. Fig. 5. pH 6.5 (control). Fig. 6. pH 3.0. Fig. 7. pH 2.0. Fig. 8. pH 1.0. Fig. 9. Transverse section of the sixth internode from the stem tip of the tobacco plant with split-roots treated with a solution at pH 1.0 for 24 hours. Fig. 10. Transverse section of the sixth internode from the stem tip of the tobacco plant recovered from wilting upon the transfer from a pH 3.0 solution (24 hours) to a complete nutrient solution (10 days). Magnification : X50.

diffusion of cell material since the solution of the root medium became turbid. A reduction in the dry weights of the roots of pH 3.0 treated plants determined after 48 hours was noted in previous experiments (5).



Figs. 11 to 14 : Longitudinal sections of stem apices of *Nicotiana tabacum* L. with roots immersed in solutions at different pH values for 24 hours. Fig. 11. pH 6.5 (control). Fig. 12. pH 3.0. Fig. 13. pH 2.0. Fig. 14. pH 1.0. Magnification : X100.

The separation of root cells and cell tiers at pH 1.0 and 2.0 could be the result of loosening or disappearance of the Ca-containing intercellular substances. The presence of Ca ions in the treatment solution could have reduced the impact of root damage to a certain extent, since sufficient evidence exists for the beneficial role of Ca in perserving cell structure (9, 11).

The tolerance of different plant species to the same acidity of the root medium may vary greatly. It was reported (2) that unlike tomato and lettuce, bermuda grass showed no initial loss of Ca at pH 3.0 within several hours. The losses of K, Ca, and cell constituents from the roots to the ambient solutions as induced by H^+ were reported by several investigators (7, 9, 10). Therefore, any concentration of H^+ which results in a loss of K or other metallic cations could result in tissue injury.

LITERATURE CITED

1. Arnon, D.I. and C.M. Johnson. 1942. Influence of hydrogen ion concentration on the growth of higher plants under controlled conditions. Plant Physiol. 17:525-539.

2. Arnon, D.I., W.E. Fratzke and C.M. Johnson. 1942. Hydrogen ion concentration in relation to absorption of inorganic nutrients by higher plants. Plant Physiol. 17 : 515 - 524.

3. Audus, L.H. 1948. Studies on the pH relationships of root growth and its inhibition by 2, 4 - dichlorophenoxyacetic acid and coumarin. New Phytol. 48 : 97.

4. Esau, K. 1965. Plant Anatomy. John Wiley and Sons, Inc., New York.

5. Farhoomand, M.B. 1969. The effect of hydrogen ion on transpiration and anatomy of tobacco (*Nicotiana tabacum* L.) Ph. D. Thesis Univ. Wisconsin, Madison.

6. Guest, P.L. and H.K. Chapman. 1944. Some effects of pH on growth of citrus in sand and solution cultures. Soil Sci. 58 : 455 - 465.

7. Hansen, J.B. 1960. Impairment of respiration, ion accumulation and ion retention in root tissue treated with ribonuclease and ethylene-diamine-tetraacetic acid. Plant Physiol. 35 : 372 - 379.

8. Hayward, H.E. 1938. The Structure of Economic Plants. The MacMillan Co., New York.

9. Jacobson, L., D.P. Moore and R.J. Hannapel. 1960. Role of calcium in absorption of monovalent cations. Plant Physiol. 35 : 352 - 358.

10. Jacobson, L., R. Overstreet, H.M. King and R, Handley. 1950. A study of potassium absorption by barley roots. Plant Physiol. 25 : 639 - 644.

11. Marschner, H., R. Handley and R. Overstreet. 1966. Potassium loss and changes in the fine structure of corn root tips induced by H-ion. Plant Physiol. 41:1725-1735.

12. Nielsen, T.R. and R. Overstreet. 1955. A study of the role of hydrogen ion in the mechanism of potassium absorption by excised barley roots. Plant Physiol. 30 : 303 - 309.

13. Rains, D.W., W.E. Schmid and E. Epstein. 1963. Absorption of cations by roots, effects of hydrogen ions and essential role of calcium. Plant Physiol. 39 : 274 - 278.

14. Sass, J.E. 1958. Botanical Microtechnique. Iowa State Univ. Press, Ames, Iowa.

15. Sutcliffe, J.E. 1962. Mineral Salts Absorption in Plants. Pergamon Press, New York.