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Short Communication

Proton Flux and Elongation in Primary Roots of Barley (*Hordeum vulgare* L.)[1]

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ROGER A. O'NEILL AND TOM K. SCOTT
Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

ABSTRACT

The elongation zone of the primary root of barley (*Hordeum vulgare* L.) has been reported to be markedly basic in pH, in apparent contradiction of the acid-growth theory. We determined simultaneously the location of the elongation zone and the basic zone in these roots and found them indeed to be the same. However, sections of barley root elongation zones were found to respond to acidic, basic, and neutral solutions as predicted by the acid-growth theory.

Acidic solutions have long been known to enhance the rate of cell elongation in shoot tissues (1, 9). In 1971, it was suggested by Hager *et al.* (6) that auxin may exert its effect on shoot tissue cell elongation by causing the cell wall to become acidified. Edwards and Scott (2, 3), using corn, showed for the first time that root tissues respond to acidic solutions as do shoot tissues, and in fact that the response is of a considerably greater magnitude than observed in shoot tissues. The situation regarding auxin effects on root elongation is less clear (4) as enhancement of elongation rate can be demonstrated only under a more restricted set of conditions. That cell wall acidification may play a role in root cell elongation is suggested by the studies mentioned above (2–4), as well as by more recent work (5, 8).

In light of such evidence for the occurrence of acid-growth in root tissue, a report by Weisenseel *et al.* (12) that, in barley roots, the region thought to be the elongation zone was distinctly basic was somewhat surprising. Using barley primary roots, we have determined simultaneously the location of the basic region reported by Weisenseel *et al.* (12), as well as the precise location of the zone of maximal cell elongation. Additionally, we have determined the effect of acidic, neutral, and basic solutions on the rate of elongation in this root tissue.

MATERIALS AND METHODS

Plant Tissue. Seeds of barley (*Hordeum vulgare* L. cv 'Keowee') were surface sterilized with 0.525% NaOCl for 10 min, rinsed three times in distilled deionized H_2O, and soaked in distilled deionized H_2O for 6 h. They were then placed embryo side down on two sheets of Whatman No. 1 filter paper dampened with 'artificial pond water' consisting of 1.0 mM NaCl, 0.1 mM KCl, and 0.1 mM $CaCl_2$ at pH 5.8 (12), in covered Petri dishes and germinated in the dark for 72 h at 25°C in humid chambers. Primary roots from 1.5 to 2 cm in length were selected for growth studies, and 3.5-mm sections 0.5 mm from the tip were cut using a razor blade cutter, and were used as described below.

pH Indicator Dye Studies. The following operations were carried out in dim green light. To determine the locations of acidic and basic regions along the length of barley primary roots, seedlings were removed from the Petri dishes and placed directly on 3-mm thick slabs of agar containing pH indicator dyes, modified from Weisenseel *et al.* (12) as follows: the bromocresol purple concentration was increased from 0.71 to 1.00 mM, and the pH adjusted to 5.2 prior to the addition of agar, and heating of the mixture. Under these conditions, zones of different pH were more readily discerned. In addition to using bromocresol purple, we used phenol red at 1.00 mM concentration, adjusted to pH 6.8 prior to the addition of agar and heating of the mixture. The seedlings thus manipulated were placed in humid chambers in the dark at 25°C for a period of from 1 to 4 h after which results were scored. Such determinations were made more than 25 times with little variation.

Elongation Zone Localization. To establish the boundaries of the elongation zone and region of maximal elongation rate, a series of india ink dots were made at 0.5-mm intervals along the length of roots prior to their placement on pH indicator agar slabs as described above. Photographs were made at the same time as pH scoring which provided a permanent record of the relative amounts of elongation which occurred in different regions along the axis of the root.

Acid-Growth Response. To determine if the young primary roots of barley behave in a manner predicted by the acid-growth theory, the elongation zones of roots were exposed to a variety of acidic, neutral, and basic solutions, and their resulting growth rate was measured. To do this, 10 tissue sections including the zone of maximal cell elongation, made as described above, were stacked tip end downward within a capillary tube (1.0 mm i.d.) constricted at the bottom enough to retain a small cylindrical stainless steel bead which served to retain the tissue. A second bead was placed on top of the stack to insure its integrity during the experiment (Fig. 1). Aerated test solutions were passed through the capillary via a polyethylene tube attached to the unrestricted (top) end. Flow was established by gravity, and its rate was controlled using a thumb-screw clamp restricting the conducting tube. At varying intervals during an experiment, elongation was read from a ruler attached to the outside of the capillary. The solutions tested were 2 mM citrate-phosphate buffer (pH 5.0), HCl in distilled deionized H_2O at pH 5.0, 2 mM Hepes buffer at pH 7.0 and 8.2, and nonbuffered distilled deionized H_2O. These experiments were each repeated three times and the averages for each test solution (unless otherwise indicated) are significantly different than those of other solutions as deter-

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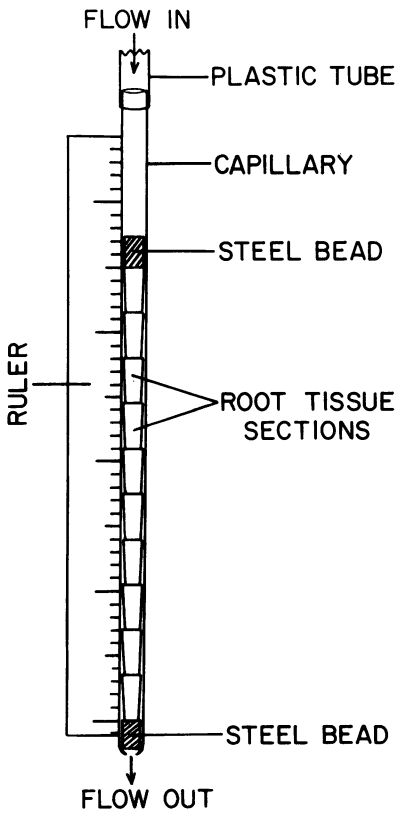

FIG. 1. Diagrammatic representation of the assemblage used in measuring root section growth (see text for details).

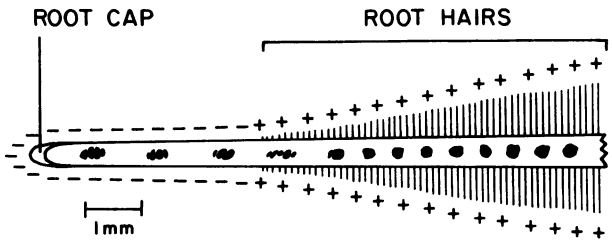

FIG. 2. Diagrammatic representation of the typical result when a seedling's root is placed in contact with an agar gel containing a pH indicator. (+), Areas of low pH; (−), regions of high pH, as indicated by the color change of the agar gel. Dots initially placed 0.5 mm apart indicate regions where elongation has occurred during an experiment by their increased separation. The diagram is a typical result of a 3-h experiment using either dye.

mined by Student's *t* test of the difference between two means (7).

RESULTS AND DISCUSSION

The results of our pH indicator dye-agar studies using both bromocresol purple and phenol red were in complete agreement with those obtained by Weisenseel *et al.* (12) using bromocresol purple; they showed that there is a markedly basic region extending from the root cap to the region where root hairs first appear. The results of our work (Fig. 2) emphasize that the demarcation between the basic zone (as indicated by −) and the acidic zone (as indicated by +) was a very sharp one. Further, in the case of phenol red, the acid color of the indicator appears below pH 6.8, and a few tenths of a pH unit above pH 6.8 the basic color of the dye intensifies markedly. In the case of bromocresol purple, the distinctly acid region is below pH 5.2 while the more basic

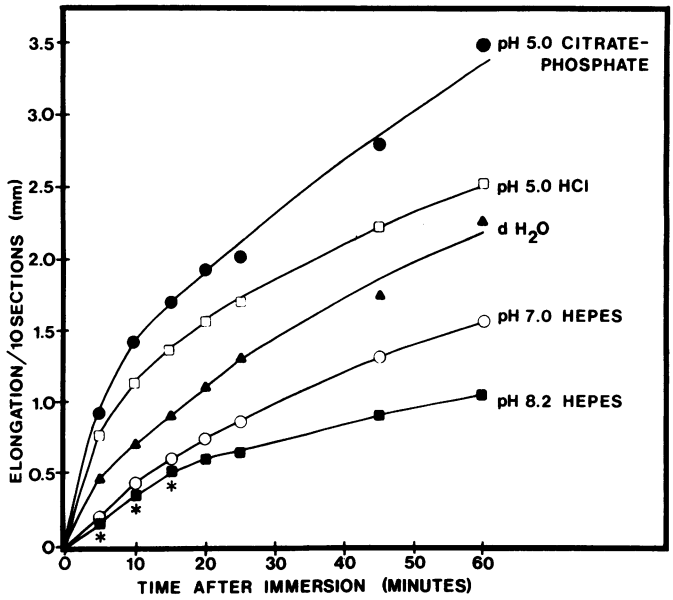

FIG. 3. Total elongation of ten 3.5-mm root sections in the presence of five solutions (noted at right). All averages were found to be significantly different than others taken at the same time with the exception of those shown by asterisks.

color of the indicator intensifies markedly a few tenths of a pH unit above pH 5.2. Both of these observations indicate that at least the external surface of the growing region of the barley root is basic, and that the external surface of the region immediately basal to it is distinctly acidic. Using india ink dots along the growing root, we have found that the basic region does in fact include the region of maximal cell elongation (Fig. 2).

Growth studies using the capillary tube apparatus indicate that excised sections of the region of maximal cell elongation, which is also within the basic region of the root, do in fact respond to acidic, neutral, and basic solutions as predicted by the acid-growth theory (Fig. 3). Exposure of these sections to buffered and nonbuffered solutions at pH 5.0 produced the greatest rates of elongation of the solutions tested. Further, neutral Hepes buffer produced an elongation rate less than that obtained using distilled deionized H_2O. Finally, the use of a basic solution of Hepes buffer resulted in the lowest rate of elongation of the solutions tested.

In the mid-1970's, Edwards and Scott showed (2–4) that acid-growth, first described in shoot tissues, could be demonstrated to occur in corn roots. This work on roots, as well as more recent work on geotropic curvature (8) and auxin effects on root elongation (5) suggests that acid growth, found to occur generally in all shoot tissues tested, can be generalized to include roots as well. The report of Weisenseel *et al.* (12) that the barley primary root elongation zone exhibits an uncharacteristic, basic pH is surprising in that it seems inconsistent with the acid-growth hypothesis. Also, the observation is unlike that made of corn by Mulkey and Evans (8), and additionally by us of corn and mung bean, which indicate that in these species the elongation zone is characteristically acidic.

Mulkey and Evans (8), in a note appearing within their paper, suggest that the case of the work of Weisenseel et al. (12) can be explained by their finding that the zone of maximal elongation in barley roots is in fact in the acidic region further from the tip than the basic region described by Weisenseel *et al.* Our elongation zone localization work using ink dots placed along the root does not concur with this finding, since it shows clearly that the zone of maximal cell elongation is directly within that region of the agar which indicates the most basic region of the barley root.

Our finding that the barley root elongation zone does respond to acidic, neutral, and basic solutions as predicted by the acid-growth theory suggests that this system may not represent an exception to the acid-growth theory in roots. One possible explanation is that barley roots, in the absence of such stimuli as gravity or pressure may be elongating at a submaximal rate. This system may thus remain poised until such stimulation occurs at which time modulation of growth is in some way altered. Alternatively, it may be that in barley, sufficient root cap slime is left behind on the surface of the elongation zone as the root grows, to cause this entire tissue region to appear to be basic when viewed from the outside as in the agar-dye method. In simple tests where root cap material was removed from the root and placed in contact with a pH indicator agar slab, we have found this material to be clearly basic. It would be desirable to determine the pH of the elongation zone independently of the root cap by physically removing the root cap slime of barley roots. Unfortunately, we have found that even the mild abrasion required to remove the cap, occurring anywhere along the root, caused an exceptionally great amount of apparent proton efflux. Were it possible to remove the root cap, it might not be desirable to do this since it is not clear how it or its chemical products affect growth *in vivo*. It should also be noted that there is the possibility that the tissue(s) controlling the rate of root growth may lie beneath the surface of the root, and that their pH may be controlled independently of the pH of surface tissues.

That there is good reason to have concern about pH and surface charge of growing roots is made further evident by the work of Tanada (10, 11) who showed that barley roots, when compared to mung bean roots, respond oppositely with respect to apparent surface charge to a variety of light and/or hormonal treatments. Additional experimentation is clearly necessary in order to determine if our observations and those of others concerning the unexpected pH of the elongation zone of barley roots are truly an anomaly or merely a result of inadequate separation of one developmental phenomenon from another. If, in fact, the immediate environment of the growing cells is basic, the mechanism(s) governing root growth will have to be reevaluated and further elucidated.

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