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Automated Recording of Lettuce Root Elongation as Affected by Auxin and Acid pH in A New Rhizometer with Minimum Mechanical Contact to Roots

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The elongation of many lettuce roots was separately recorded at the same time by a new rhizometer with minimum mechanical contact to root tips. The apparatus was operated by a microcomputer system interfaced with field-effect transistor circuits. Elongation responses of intact seedling roots (ISR), decotylized seedling roots (DSR) and excised apical roots (EXR) to different concentrations of H^+ and IAA were compared. A pH 4-induced acid growth was observed only in DER and EXR, whereas a pH 3-induced one was observed in all roots. Duration of pH 3-induced elongation was shorter in ISR than in DSR and EXR.

Growth curves suggested that ISR is more susceptible to acid-injury than EXR. The maximum acid growth was obtained at pH 2.5 in EXR which is comparable to thick roots of maize [Edwards and Scott (1974) *Planta* 119: 27]. Indole-3-acetic acid showed growth inhibition at concentrations higher than 10^{-8} M which is comparable to other reports on thick roots. It is concluded that fine lettuce roots have the same magnitude of sensitivity to external concentration of H^+ and IAA as thick roots. It is also suggested that root excision or decotylization makes roots less susceptible to acid-injury, resulting in greater acid-growth of EXR. Further characteristics and application of the new rhizometer are discussed.

Key words: Acid growth — Acid injury — Auxanometer — IAA (indole-3-acetic acid) — *Lactuca sativa* — Root elongation.

A microscopic device and an automated technique of growth measurement have been used in the time-course analyses of hormone action on plant growth (Ray and Ruesink 1962, Masuda and Wada 1967, Tanimoto et al. 1967, Evans and Ray 1969, Gordon and Dobra 1972, Edwards and Scott 1974, Macdowall and Sirois 1976, see also Evans 1974, Penny and Penny 1978, Zeroni and Hall 1980). A position-sensing transducer has been widely used for recording the growth of coleoptiles and stems (Dela Fuente and Leopold 1970, Murayama and Ueda 1973, Yamagata et al. 1974, also see Evans 1974, Penny and Penny 1978, Zeroni and Hall 1980) and of roots (Evans 1976). These methods require mechanical contact to plant materials which is unfavorable to the physiological study of root growth, since the mechanical stress itself is known to induce physiological responses in the root (Kuiper 1971, Veen and Boone 1981, Chastain and Hanson 1982, Feldman 1984, Biddington 1986).

The induction of rapid elongation by the treatment of tissue at low pH has been extensively

Abbreviations: DSR, decotylized seedling roots; EXR, excised apical roots; FET, field effect transistor; ISR, intact seedling roots.

studied in segments of coleptiles and stems to explain the mechanism of auxin-induced elongation (Rayle and Cleland 1970, 1972, Hager et al. 1971, Marrè et al. 1973, Rayle 1973, Barkley and Leopold 1973, Yamagata et al. 1974, Yamamoto et al. 1974a, b Vanderhoef et al. 1977, Vanderhoef and Dute 1981, Prat and Goldberg 1984, see also Evans 1974, 1984, Rayle and Cleland 1977, Cleland and Rayle 1978, Penny and Penny 1978, Zeroni and Hall 1980). It is important to determine the optimum pH value of acid-growth for each plant tissue in order to understand the biochemical or physicochemical process of cell wall extension induced by low pH.

The pH optimum of acid growth has been reported to be ca. pH 3.0 for unpeeled intact segments of coleoptiles and of stems, as compared to ca. pH 5.0 for peeled or abraded segments (Rayle and Cleland 1972, Cleland and Rayle 1975, 1978, Mentze et al. 1977). Since coleoptiles and stems have epidermis with thick outer cell walls covered by cuticles, hundreds-fold difference in optimal H^+ concentration for peeled and unpeeled segments could be ascribed to the efficiency of H^+ penetration into the tissue (Prat and Goldberg 1984).

While a root with no cuticle might be expected to have a higher pH optimum than unpeeled stem segments, it has been reported to be about pH 3.0 in maize root segments (Edwards and Scott 1974, 1976). Since a permeation barrier to exogenous H^+ or IAA has been reported to exist between the external medium and the hormone or H^+ responsive sites of the root (Cleland and Rayle 1975, Dreyer et al. 1981, Blakely et al. 1986), a fine root may be more sensitive to external IAA and H^+ concentrations than a thick root.

Kinetic studies on acid growth of root were conducted on excised maize root segments using a traveling microscope (Edwards and Scott 1974, 1976, 1977) and on intact pea roots using a transducer-based root auxanometer (Evans 1976). Edwards and Scott (1974) could trace the elongation magnified by stacking ten root segments using a travelling microscope.

A transducer-based auxanometer, however, could not be adapted for very fine roots such as those of lettuce because of the mechanical resistance of the apparatus.

We have developed a new "rhizometer", so named by Drs. T. K. Scott and Y. Masuda, which has no mechanical contact with the root tip. We used this apparatus to record the elongation of fine lettuce roots which are ca. 0.15 mm diameter at the elongation zone which is one tenth of that of pea roots (Tanimoto 1985). This paper presents the structure and function of the apparatus and the growth kinetics of intact and excised lettuce roots to determine the sensitivity of fine roots to exogenously applied IAA and/or acid solution. The principle of the new rhizometer has been presented before (Tanimoto and Watanabe 1982, Tanimoto et al. 1985).

Materials and Methods

Plant material

Seeds of lettuce, *Lactuca sativa* cv. Grand rapids, were surface sterilized in 0.1% sodium hypochlorite solution for five min, and rinsed with distilled water. Thirty to forty seeds with embryos downwards were lined on the upper longer margin of double filter papers (4.5×8 cm) wet with distilled water. A couple of these filter papers were vertically attached inside an air-tight polyethylene cup (100 ml) supplemented with 10 ml distilled water and incubated at 23°C under fluorescent light (300 lux). Two-day old seedlings with 15 ± 1 mm long roots were used for the experiments.

Growth solution

Roots were repeatedly dipped for five min at 10-min intervals in the following growth solution: KNO_3 (2 mM), $Ca(NO_3)_2 \cdot 4H_2O$ (2 mM), $MgSO_4 \cdot 7H_2O$ (2 mM), KH_2PO_4 (2 mM), $EDTANaFe \cdot 3H_2O$ (20 μM), H_3BO_3 (50 μM), KCl (40 μM), $MnSO_4 \cdot H_2O$ (10 μM), $ZnSO_4 \cdot 7H_2O$ (1 μM), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.2 μM), $CuSO_4 \cdot 5H_2O$ (0.5 μM) adjusted to different pHs with

0.5 M KOH or HCl unless otherwise indicated. The pH of solutions changed by less than 0.2-pH units during experiments.

Structure and function of the rhizometer

Principle of growth measurement—When water is poured at a constant rate into a box by a pump, the water level rises at a constant rate in the box (Fig. 1). If a root and two level detectors, black and white arrows, are fixed in the box, the apparatus is capable of measuring the root elongation as downward movement of the root tip by recording the two time data, one elapsed for raising water level from the lower detector to the upper one (td) and the other elapsed from the lower detector to root tip (tx) at two separate occasions (To and Ti). Dx and D in Fig. 1 stand for vertical distances from the lower level-detector to the root tip and from the lower to the upper level detector, respectively. The increase in root length (ΔL) during (Ti−To) can be calculated by the equation of Fig. 1.

Root holder—A commercially available hair clip was modified as shown in Fig. 2. The clip was enameled to prevent staining except for the platinum spikes soldered at the bottom edge. The upper part of the clip was screwed into a slit in a stainless steel plate which allowed for vertical sliding and inclining of the root clip. The basal part of the root was placed between small pieces of filter paper (2 × 7 mm) wet with the growth solution, which was pinched by the platinum spikes of the root holder.

Electronic device—In order to measure the time when root tips and level detectors contacted the water surface, a microcomputer monitored the logical signals from units of field-effect

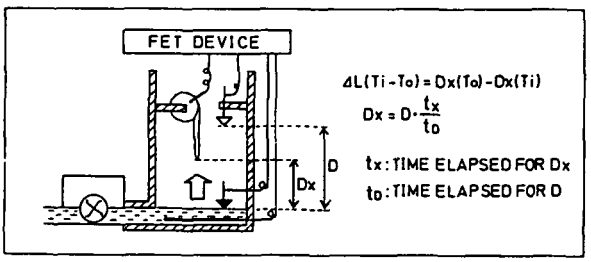

Fig. 1

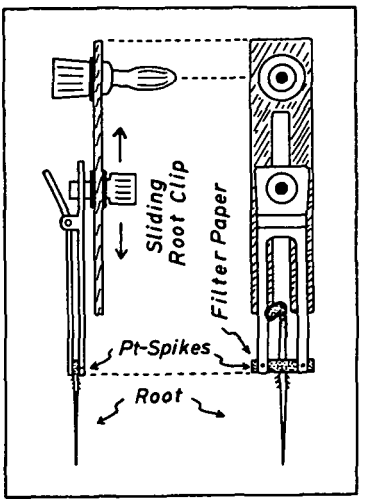

Fig. 2

Fig. 1 Schematic presentation of a new method of measuring root elongation. At time To, a mineral solution is poured into a box at a constant speed. A field-effect transistor (FET) device transmits signals to a microcomputer when the water surface contacts the lower level detector (black arrow), the root tip and the upper level detector (white arrow). A microcomputer records the time periods (tx and td) elapsed for distances Dx and D. After an appropriate period of dipping a root in the solution, the solution is drained and the root is kept in humid air for an appropriate period. At time Ti, the mineral solution is again poured into a box and tx and td are measured again. Root elongation (ΔL) during (Ti−To) is calculated by the equation shown in the figure.

Fig. 2 Root holder with a lettuce seedling pinched by wet filter papers at the bottom. Stainless steel plate with a slit allows sliding of root clip to adjust the position of a root. Root holder was fixed in the measuring box (Fig. 3) by inserting a jack attached at the top.

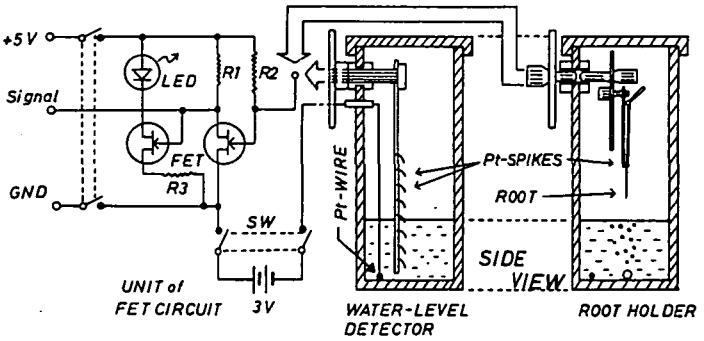

Fig. 3 Side view of the measuring box and unit of FET circuits. Unit of FET circuits is connected to each root and to each *Pt*-spike of the water level detector and transmits the signal to a microcomputer when any one of them contacts water. Resistance R1 = 20 kohm, R2 = 10 Mohm and R3 = 1 kohm. LED, light emitting diode to indicate which root or *Pt*-spike is in contact with growth solution. SW, Electromagnetic switch to break the circuits when it is out of use.

transistor (FET) circuits (Fig. 3) while raising the water level. We used simple and inexpensive circuits of FET (2SK30A) which gave electronic signals to a microcomputer by detecting tiny electric current of less than 0.8 μA through roots, when a root tip came into contact with the water surface.

A microcomputer also controlled the back-and-forth operation of tubing pumps (Masterflex Pump, Cole Parmer Ins. Co. Chicago, U.S.A.) and the electromagnetic valves (Dura Pinch Valve, Takasago Electric Inc. Nagoya, Japan) which were inserted, if necessary, between a tubing pump and reservoir tanks to change the growth solution to be poured into the measuring box (Fig. 4). All times (To, Ti, td and tx) described above were also recorded by a microcomputer. Times td and tx were measured by ten milliseconds.

Measuring box—Sixteen root holders were fixed by inserting their jacks inside the measuring box (14 × 40 × 4 cm) made of Sumipex acrylic plates (Sumitomo Chemical Industry), as shown in Fig. 3 and 4. The box was divided into several chambers by baffling plates with an open slit at the bottom in order to prevent vibration on the water surface. The surface of the growth

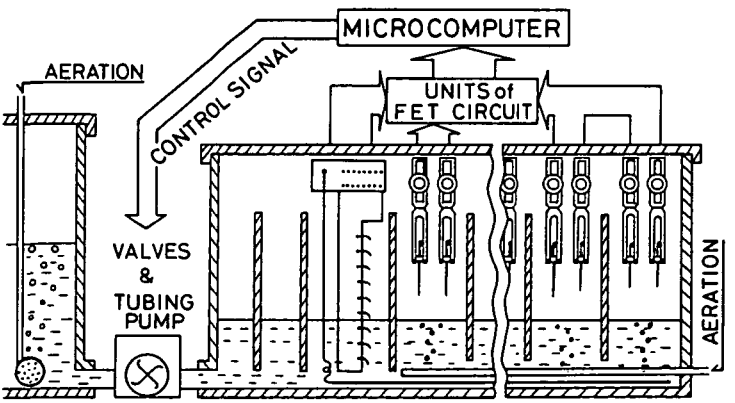

Fig. 4 Whole diagram of a rhizometer. A microcomputer receives electric signals from FET circuits and operates a tubing pump, electromagnetic valves to exchange the growth solution and an air pump to supply humid air to measuring box (900 ml max.) and to reservoir tank (1 liter). Two systems were controlled in parallel by a microcomputer to compare two lots of roots.

solution was detected by platinum spikes fixed at 5 mm intervals on the water-level detector which was vertically held in place by an IC-socket. We usually used two boxes in parallel to compare two lots of roots (maximum number of 16 per box). Growth measurement was carried out under fluorescent light (200 lux) at 20°C. All experiments were duplicated and elongation data were means of 12 roots with S. E.

Results

Accuracy of rhizometer

Time data, tx and td of Fig. 1, were measured twenty times in two measuring boxes to confirm the reproducibility of pumping the growth solution, where Dx was fixed at four different distances, 5, 10, 15 and 20 mm, and D was set at 20 mm. The mean tx of twenty measurements is shown in Table 1 with its standard deviation and with the percent of standard deviation to the mean (SD/M%). The relative value of tx/td was calculated for each time of measurement, and the means of twenty values are shown with their standard deviation and with the percent of deviation to the mean. Although tubing pumps gave highly reproducible values of net dx, SD/M of relative values were significantly smaller than those of net elapsed time, thus we used relative values of tx/td to calculate the elongation by the equation of Fig. 1.

Correlation between time tx (sec) and distance Dx (mm) is also indicated in Table 1. Mean tx of relative values (tx/td) was strictly proportional to Dx in the two measuring boxes.

Effect of electric current on the elongation

An electric current through a resistance (R2), a root and growth solution (Fig. 3) was assumed to be less than 0.8 μA, since 8 V was applied between them and R2 was 10 Mohms in addition to some megohms resistance of a root itself. Sixteen roots were divided into two lots of eight roots. An electric current was given to one lot but not to the other. Total electric power given to a root was less than 17 mW·sec in 108 measurements during 18 h, since the electric current was allowed to flow by turning on the switches of Fig. 2 only if the level of growth solution was rising in the measuring box. After 108 repetitive incubations in the growth solution and in humid air, the length of two lots of eight roots was measured by a ruler. The mean elongation during 18 h was 9.38 ± 0.42 (S. E.) mm with electricity supplied, whereas it was 9.25 ± 0.84

Table 1 Repetitive measurement of tx and td

Dx (mm)	Net elapsed time (sec)				Relative value (tx/td)		
	5	10	15	20 (D)	5	10	15
Box-1				(td)			
Mean tx	19.55	38.00	57.79	76.86	0.254	0.494	0.752
STD.DEV.	0.134	0.232	0.348	0.439	0.00054	0.00060	0.00070
SD/M (%)	0.684	0.611	0.602	0.571	0.213	0.122	0.0931
Box-2				(td)			
Mean tx	18.22	36.98	55.74	74.24	0.245	0.498	0.751
STD.DEV.	0.143	0.218	0.302	0.351	0.00089	0.00091	0.00070
SD/M (%)	0.785	0.589	0.542	0.473	0.363	0.183	0.0928

Time (tx) elapsed for four fixed distances (Dx) was repetitively determined 20 times in two measuring boxes in parallel. Relative values (tx/td) were calculated every time of measurement. Standard deviation (STD.DEV.) of the mean and percent of STD.DEV. to the mean (M) were indicated.

(S. E.) mm without electricity. Electric current applied during the 108 measurements gave no significant effect on the elongation of intact roots.

Elongation response to the pH change

Responses of intact seedling roots (ISR)—The growth rate of ISR was quite constant and the maximum growth rate fallen within 0.45–0.55 mm/h was obtained at pH 5 and 6 over a period of 20 h (Fig. 5). Increasing the pH from 6 to 7 rapidly suppressed the elongation, while growth inhibition became gradually pronounced when the pH decreased from 6 to 4.5 and to 4.0. Since buffering action of the growth solution (2 mM KH_2PO_4) might not be strong enough to cause acid growth, the concentration of KH_2PO_4 was increased to 4 mM or McIlvain's buffer was added to the growth solution (Fig. 6). Fig. 6 shows that growth-suppression by pH 4 or by pH 7 and growth recovery by pH 6 can be obtained repeatedly by alternating between pH 6 and 4 or 7 in the growth solution. Fig. 6 also showed that growth suppression by pH 4 occurred after 30–40 min lag whereas pH 7 rapidly suppressed the elongation. Although Evans (1976) found rapid and prolonged growth stimulation by pH 4 solution in intact pea roots, no growth promotion was observed by the pH drop from 6 to 4 in ISR of lettuce. Thus the effect of pH drop from pH 7 to pH 3 and to pH 2.5 was further examined.

Rapid and short period of acid growth was detectable when the pH was decreased from 7

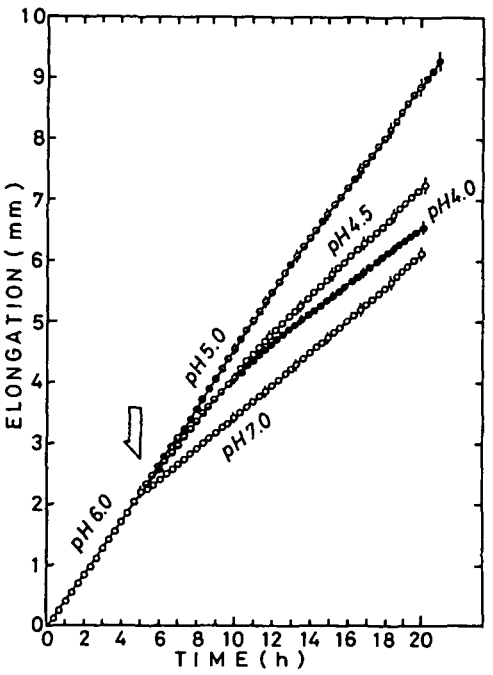

Fig. 5

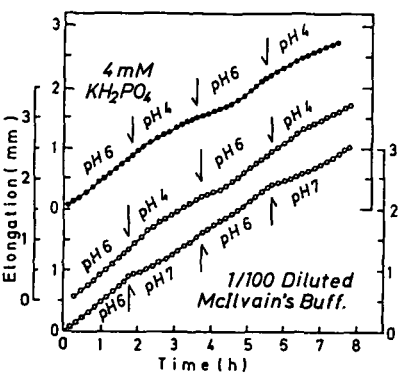

Fig. 6

Fig. 5 Effect of pH of the growth solution on the elongation of intact seedling roots. The elongation of intact roots was first measured every 20 min at pH 6 and then (arrow) at different pH. Mean of 12 roots with S.E. (vertical bars) indicated every five dots.

Fig. 6 Regulation of intact root elongation by alternating changes of pH. Buffer strength of pH 7 and 4 was increased by doubling the concentration of KH_2PO_4 to 4 mM in the growth solution (uppermost curve) or by adding 10 ml mixture of citrate (0.1 M)-phosphate (0.2 M) buffer to 1 liter standard growth solution (lower curves). Mean elongation of 12 roots at 10 min intervals. S.E. was omitted since it was close to or less than the size of circles.

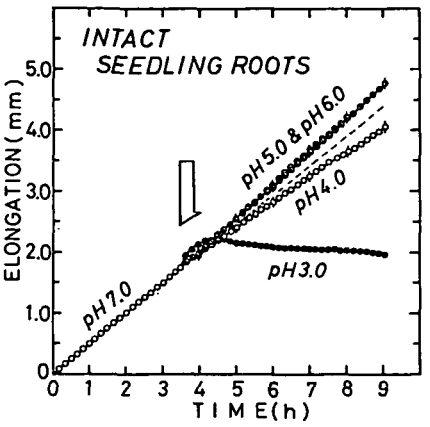

Fig. 7

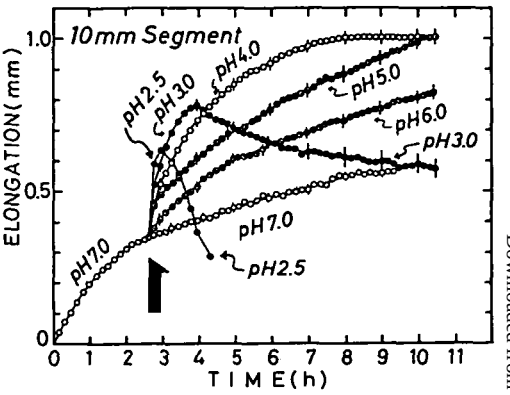

Fig. 8

Fig. 7 Response of intact seedling roots to the acidic pH. The pH of the growth solution was changed from pH 7 to lower pH at the arrow. Means of 12 roots with S.E. (vertical bars) were plotted at 10 min intervals. Dotted line indicates the elongation of control roots kept at pH 7.

Fig. 8 Acid-growth response of excised apical roots. Apical 10-mm long segments were excised from 15-mm long roots and set in the measuring box. It took about 40 min to prepare 32 root segments for two boxes. The pH of the growth solution was changed at arrow after preincubation at pH 7. Means of 12 roots were plotted at 10 min intervals with S.E. (vertical bars).

to 3 (Fig. 7). Although the pH 5 and 6 significantly enhanced and the pH 4 suppressed the elongation, these effects appeared slowly but steadily.

Responses of excised apical roots(EXR)—The growth curves of EXR are illustrated in Fig. 8. When an apical 10-mm segment was excised from a 15-mm long root, the elongation rate of EXR was considerably reduced compared with ISR but the steady growth, ca. 0.04 mm/h, was obtained after about 2-h preincubation in the pH 7 growth solution. Lowering the pH from 7 rapidly enhanced growth. The greatest initial response was obtained at pH 2.5, as shown in Fig. 8, and pH 2.3 also induced acid growth, not shown in Fig. 8, which was smaller than that obtained at pH 2.5 (see Fig. 11). The reason why EXR exhibited profound response to the weak acidic solution in contrast to the intact roots may be explained by the penetration of H^+ through the cut surface of EXR.

In the next experiment the roots were kept intact but cotyledons were removed to slow down the spontaneous growth of the roots.

Responses of decotylized seedling roots(DSR)—Root growth was significantly suppressed to ca. 0.14 mm/h at pH 6 by decotylization after 30 min lag period. Clear acid-growth response was observed at pH 4 in DSR (Fig. 9). Response of DSR to different pH was similar to those of EXR (Fig. 10). Thus the penetration of H^+ through cut surface of EXR may not give the reason why EXR shows clear response to weak acid.

Initial acid-growth responses of three types of roots were compared in Fig. 11 which indicates net elongation after pH shift as a function of H^+ concentration. Initial 10-min response was maximal at pH 2.5 in EXR, 2.5–3.0 in DSR and 3.0 in ISR. The 80-min response of EXR indicated that growth response was greater as the H^+ concentration increased up to pH 3, whereas 80-min elongation was smaller at pH 3 than at pH 4 in DSR and ISR. EXR exhibited the longest duration of pH-3-induced acid growth and ISR did the shortest one (Fig. 7, 8, 10). These results may indicate that ISR was the most susceptible to acid-injury and that EXR was the least, i.e., the greater the spontaneous growth, the stronger the growth suppression by acid.

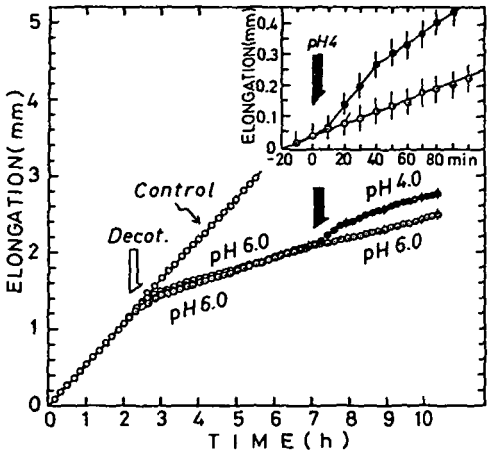

Fig. 9

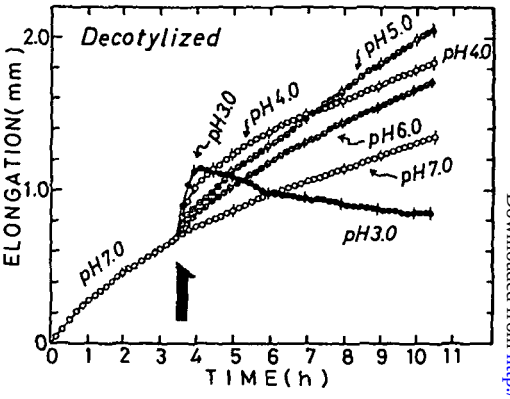

Fig. 10

Fig. 9 Effect of cotyledon excision and low pH on root elongation. Cotyledons were excised using de Wecker's scissors at open arrow, and the pH of the growth solution was then changed from 6 to 4 at closed arrow. Means of 12 roots were plotted every 10 min with S.E. (vertical bars). Inserted is the expanded figure of pH-shift region.

Fig. 10 Acid-growth response of decotylized seedling roots. Decotylized seedling roots were preincubated at pH 7 and then treated with different pH at the arrow. Means of 12 roots were plotted every 10 min with S.E. (vertical bars).

Responses of ISR to IAA and pH 4 growth solution

Effect of IAA at wide range of concentration for 10^{-6} M to 10^{-15} on the growth of ISR was illustrated in Fig. 12. Strong and rapid inhibition was observed at concentrations higher than 10^{-8} M. This concentration was comparable to the previously reported one in maize root (Batra

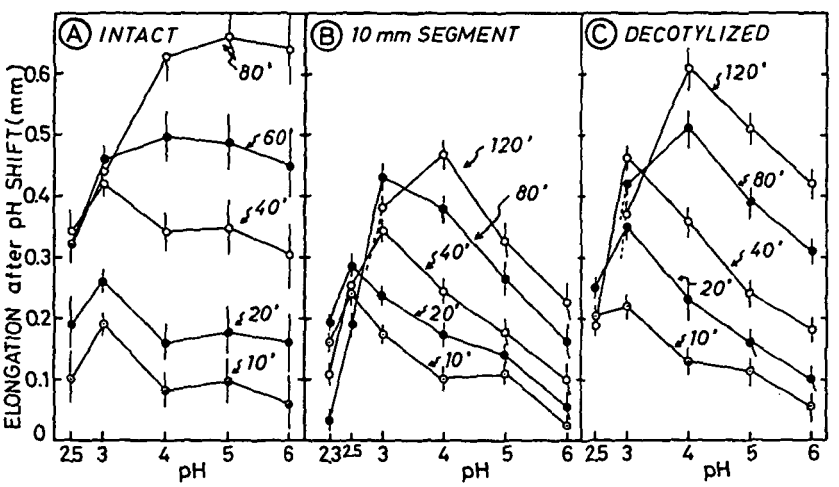

Fig. 11 Initial growth response of three types of roots to the pH change from 7 to lower pH. The net elongation after the pH shift from 7 to various lower pH was obtained from the data of Fig. 7, 8 and 10 and plotted against H^+ concentration. The growth periods in min were indicated by numbers in the figure. Means of 12 roots with S.E. (vertical bars).

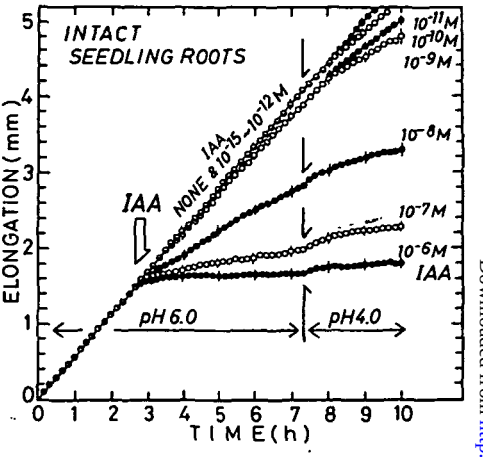

Fig. 12 Effect of various concentration of IAA on the elongation of intact seedling roots. Intact seedling roots were incubated in the pH-6 growth solution and in various concentration of IAA at open arrow. The pH of these growth solutions were then shifted to pH 4 at the second fine arrow. Means of 12 roots were plotted every 10 min with S.E. (vertical bars).

et al. 1975, Mulkey et al. 1982a). Growth recovery was also observed at 10^{-7} M and 10^{-8} M about 80 min after IAA application. This phenomenon is also consistent with that reported in maize roots (Gougler and Evans 1981). The pH 4-induced acid growth was detectable in IAA-stunted roots treated with 10^{-8}–10^{-6} M IAA. It should be noted that acid-growth response was observed even if the growth was completely suppressed by 10^{-6} M IAA. Low concentrations of IAA which was prepared immediately before use showed no growth promotion.

Discussion

Only one automated method has been reported to record plant growth without mechanical attachment to plant materials in the dark (Gordon and Dobra 1972). Since they used a capacitance method which requires careful control of temperature and humidity, their method was not applicable to the physiological analyses of root growth which required dipping roots in growth solution. On the other hand, a position-sensing transducer has most widely been used for growth analyses of stems and roots but it requires mechanical contact with plant materials and this rules out application of the method to tiny or mechanically weak plant materials.

We have succeeded in recording the elongation of very fine roots by weak electric current which has little effect on the elongation. The growth solution was required to contain some electrolytes which permit the electric current to turn on the FET. If the present growth solution was diluted two-fold the electric current through lettuce roots longer than 15 mm was not enough for the FET. But two-fold-diluted growth solution could be used for thick roots such as pea and maize. The present apparatus was able to measure two lots of 16 roots at the same time whereas previously reported apparatus measured only one specimen. Also, using this method, we could compare, at the same time, two averages of growth in different physiological and environmental conditions such as intact or excised and in different growth solution. Thus we estimated the average elongation rate at steady state as 0.45–0.55 mm/h for ISR, ca. 0.15 mm/h for DSR and 0.07–0.1 mm/h for EXR in pH 6-growth solution at 23°C.

The present growth kinetics indicated that the sensitivity of lettuce roots to exogenous H^+ and IAA is as high as thicker roots of maize (Edwards and Scott 1974, Mulkey et al. 1982a, b). However, when the responses to H^+ concentration were compared between intact seedling roots and excised roots, the latter showed greater response and the former seemed more susceptible to acid-injury. Since pH lower than 4 cause an injury on roots (Marshner et al. 1966, Zsoldos and Erdei 1986, Erdei et al. 1986), the magnitude and the duration of acid-growth may be

determined by the balance of growth-promoting and growth-inhibiting injury effects of acid. The present result that maximum initial response (10 min after acid application in Fig. 11) was obtained at pH 2.5 in fine excised roots suggests that maximum cell-wall extension is brought about by the cell-wall acidification lower than pH 3, as suggested in thicker roots of maize (Edwards and Scott 1974).

Intact stem segments have been reported to show little growth response to low pH, whereas abraded segments, stripped epidermis, frozen-thawed segments and peeled segments exhibited extension in response to low pH (Rayle 1973, Yamagata et al. 1974, Yamamoto et al. 1974a, b, Cleland and Rayle 1978, Pearce and Penny 1983, 1986, Prat and Goldberg 1984). Dreyer et al. (1981) directly indicated that epidermal cuticles are barriers against exogenously applied H^+. Since the epidermis is known to play an important role in the elongation growth of stem segments (Tanimoto and Masuda 1971, Masuda and Yamamoto 1972, Tanimoto 1978, Pearce and Penny 1983, 1986), the acidification of epidermal cell walls is thought to cause acid growth of stems. In roots, however, the importance of epidermal tissue in acid growth is not well established. Since a root has no cuticle on its outer surface, the importance of the outer surface of the root in acid growth was also stressed by the fact that fine lettuce roots with one tenth diameter of maize root exhibited rapid growth responding to the same range of H^+ concentration, pH 2.5–3.0, as maize roots. Correlation studies between surface pH and growth rate of the elongation zone of maize roots (Pilet et al. 1983, Versel and Mayor 1985) indicated that the acidification of outer surface of the root is strongly correlated with the spontaneous growth of the root.

The components of buffer solution is known to affect the root elongation (Edwards and Scott 1976, Gabella and Pilet 1978). We compared pH effect without changing buffer components using large amounts of growth solution containing 2 mM KH_2PO_4, 700–1,000 ml against 16 roots, pH adjusted by KOH and HCl.

According to the study on the relation of endogenous and exogenous IAA to spontaneous and induced growth of roots (Scott 1972, Batra et al. 1975, Pilet et al. 1979, Pilet and Saugy 1985), endogenous auxin content is as high as growth-inhibitory concentration in maize roots. Thus exogenously applied IAA is usually inhibitory on the root elongation. In the present data (Fig. 12) exogenous IAA did not promote but inhibited the elongation, suggesting that endogenous auxin content is as high as supraoptimal concentrations in intact lettuce seedling roots. Supraoptimal concentration of IAA has been reported to cause H^+ influx (Evans et al. 1980) and inhibit growth through ethylene production (Mulkey et al. 1982a, b). Both mechanisms may be working in IAA-induced growth suppression of lettuce roots because the pH 4-induced acid growth was detectable in IAA-stunted roots but the magnitude of acid growth was smaller than excised roots with no IAA applied (Fig. 8, 12). The induction of acid growth in IAA-stunted lettuce roots is explainable by the findings of Evans et al. (1980) and of O'Neill and Scott (1983) that supraoptimal IAA caused H^+ influx and spontaneously H^+-influxing barley roots exhibited acid growth. Root elongation was strongly suppressed by decotylization after 30 min lag period (Fig. 9). Some cotyledon factors have been reported to play an important role in the regulation of hypocotyl growth by gibberellin (Katsumi et al. 1965, Kamisaka 1973, Kamisaka et al. 1974). These factors conceivably play an important role in root elongation, since the lettuce root has been found to require gibberellic acid for normal elongation (E. T. in preparation). Acid-enhanced elongation was also apparent in decotylized seedling roots with maximum initial response at pH 3.0, whereas maximum response of excised roots was at pH 2.5. These results, together with the comparison of the duration of acid-growth among intact seedling roots, decotylized seedling roots and excised roots (Fig. 7, 8, 10), suggested that sensitivity of decotylized seedling roots to acid-injury is considerably decreased and hence the roots showed greater initial response to acid than intact seedling roots. It was also suggested that acid-labile components of cell-wall extensibility are as much at work as those caused by pH 4

in intact seedling roots and that these dormant components are awakened by weak acid in decotylized seedling roots and excised roots.

The present apparatus can be used with either excised or intact roots of other plants such as maize, pea and azuki bean simply by exchanging the root holder (Tanimoto et al. 1985).

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