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Short Communication

A New Sensitive Root Auxanometer

PRELIMINARY STUDIES OF THE INTERACTION OF AUXIN AND ACID pH IN THE REGULATION OF INTACT ROOT ELONGATION[1]

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ABSTRACT

A new sensitive root auxanometer is described. The auxanometer represents an adaption of the position-sensor transducer method to measurement of intact root elongation and has the advantages of simplicity and high sensitivity. Experiments with the auxanometer show that auxin begins to inhibit intact pea root elongation within 10 minutes and continues to inhibit elongation for at least 1 hour following a 1-hour treatment with the hormone. Exposure of pea roots to pH 4 results in a 2- to 3-fold increase in elongation rate beginning about 1 minute after acid treatment. Acid-induced elongation continues at a steady rate for at least 160 minutes and can be reinitiated repeatedly by shifting between pH 4 and 6.5. Auxin inhibits acid-induced elongation whether given before or after acidification, and a transient exposure to auxin renders intact roots relatively insensitive to acid for at least 1 hour after withdrawal of the hormone.

There have been numerous reports dealing with the interaction of acid and auxin in the regulation of stem tissue elongation (4, 7, 12). It is clear that acidic solutions strongly promote the elongation of stem segments and it seems possible that hydrogen ions may be the mediator in at least a portion of auxin-induced elongation of some tissues.

Much less attention has been given to the role, if any, of hydrogen ions in the modification of root elongation by auxins. It is well established that auxins are inhibitors of root elongation (10, 15), but there is evidence that stimulation of root elongation does occur in some roots at very low concentrations of auxin (1, 3). Edwards and Scott (6) have demonstrated that the acid growth response is even stronger in corn root segments than in coleoptile segments, but they noted significant differences in the nature of the acid growth response in root segments as compared to stem segments. This paper describes the use of a new type of root auxanometer in a preliminary study of the influence of auxin and H^+ ions on the elongation of intact primary roots of pea.

MATERIALS AND METHODS

Plant Material. Pea seeds (*Pisum sativum* L. var. Alaska) were soaked for 16 hr in tap water at room temperature. The seeds were then inserted into holes in a sheet of Plexiglas (20 × 28 cm) standing on 5-cm legs in a plastic tray. The holes were slightly smaller in diameter than the swollen seed and had a notch to one side through which the emerging radicle could grow. The trays were filled with Meyer's solution (11) to the bottom surface of the Plexiglas platform and the solution was aerated during the subsequent growth of the roots. Roots were allowed to grow at room temperature in the laboratory under normal room lighting for 3 to 6 days at which time straight roots from 3 to 4 cm long were selected for experimentation. Although light is reported to inhibit root elongation (16, 17), there was no obvious effect of darkening the room on root elongation in this system during these short term experiments so these initial experiments were carried out in the laboratory under room lighting.

Measurement of Elongation. Root elongation was measured by adapting the position-sensor transducer method (5) of recording stem elongation so that it could be used with intact roots. The pea seedling was mounted (Fig. 1) in a Plexiglas platform (D) fixed to the side of a specially constructed growth chamber 450 ml in volume so that the root protruded through a 3-mm hole in the bottom of the holder. The tip of the root was positioned in a glass capillary (E) 1.5 mm high and 2 mm in diameter fixed to the end of a copper wire (F) which passed through the rotating arm of a Metripak model 33-04 angular position-sensor transducer (G) and extended beyond to a counterweight (H) of 102 mg. The glass capillary was fixed to the end of the copper wire with fingernail polish which was also used to coat the submerged portion of the copper wire. The position-sensor transducer was provided with a constant 25 v power input from a Hewlett-Packard model 6215A DC power supply while output from the transducer was recorded using a Sargent-Welch model SRLG recorder. The chamber was filled with half strength Meyer's solution to the bottom of the seedling platform (D). The solution was continuously oxygenated through a 21 gauge stainless steel hypodermic needle (B) which was separated from the seedling platform by a Plexiglas baffle (C) with two 13-mm holes to allow circulation but minimize turbulence from oxygenation. Solutions were drained from the chamber through drainage tubing (A) connected to a solenoid valve. Solutions to be tested were added directly to the chamber by pipette. Small volumes of concentrated solutions were used so that the desired concentration was established after mixing with the half strength Meyer's solution in the chamber. Uniform mixing was obtained within 90 sec as measured by dye dispersal.

The recorder sensitivity was adjusted so that full scale displacement on the recorder was accomplished by an increase in root length of about 2 mm. The recorder pen was then returned to the zero position by lowering the position-sensor transducer 2 mm by means of a microscope chassis rack and pinion gear upon which the transducer was mounted. Since the distance between the transducer shaft and root tip was large (120 mm) and total

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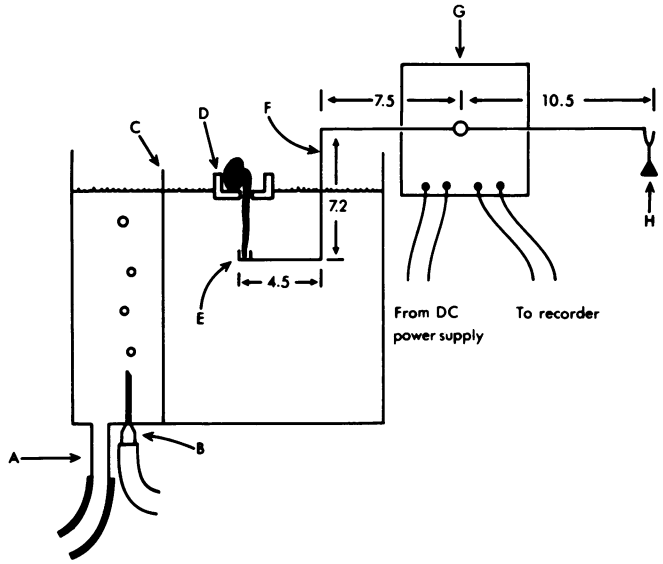

FIG. 1. Position-sensor transducer used as a root auxanometer. A: solenoid operated drainage port; B: stainless steel syringe needle (21 gauge) for oxygenation; C: baffle; D: seedling platform; E: glass capillary to guide root tip; F: transducer arm; G: transducer; H: counterweight. Numbers shown represent length in cm. Total volume of chamber to bottom of seedling platform = 450 ml.

displacement before resetting was small (2 mm), the displacement angle varied only between 0° and 1° so that tangent error (14) was negligible and recorder output could be directly related to elongation without introducing a correction factor. That recorder output did vary linearly with elongation over this angle of displacement was verified by inserting a micrometer screw in place of the root. The micrometer was also used to calibrate the recorder at the end of each experiment.

RESULTS AND DISCUSSION

Inhibition of Root Elongation by Auxin. The growth rate of intact roots was usually quite constant over a period of many hours and fell within the range of 0.4 to 0.9 mm/hr from seedling to seedling. In some cases a rhythmic increase and decrease in growth rate was observed with a period of about 20 min. This represented an exception to the usual steady rate of growth, and seedlings with roots showing steady growth rates were selected for experimentation.

Figure 2 illustrates the time course of the effect of 0.01 mM IAA on intact pea root elongation. In this experiment there was a detectable decrease in the rate of elongation beginning about 11 min after introduction of auxin. Since mixing after addition requires approximately 90 sec in this system, the latent period in inhibition of pea root elongation by this concentration of IAA can be estimated to be about 9.5 to 10 min. A similar rapid inhibition of intact corn root elongation by auxin has been reported by others. List (10) observed a latent period of 5 to 10 min in the inhibition of corn root elongation by auxin while Hejnowicz and Erickson (9) found a latent period of about 15 min in the inhibition of root elongation by auxin in either corn or pea.

Stimulation of Root Elongation by Acid. Figure 3 presents evidence that intact pea roots show an acid growth response as has been described for isolated segments of corn root (6). Citric acid solution was added to the root medium (initially pH 6.5) to lower the pH to 4 (upper curve). The increase in growth rate began about 2 min after addition of acid which, allowing for mixing, suggests a latent period in the acid growth response of intact pea roots of about 1 min, *i.e.* comparable to that reported for coleoptile segments (7) and for corn root segments (6). The magnitude of the increase in growth rate induced by acid was about 2-fold in this experiment and fell within the range of 2- to 3-fold in most experiments. This is smaller than that reported under similar conditions in isolated coleoptile segments (5- to 8-fold see refs. 8 and 13) or corn root segments (about 5-fold see ref. 6). In the experiment of Figure 3, acid-induced elongation was recorded for 160 min with no sign of a decline. Thus acid-induced elongation in intact pea roots is much more long lived than in coleoptile or corn root segments in which growth rate begins to decline 15 to 30 min after initiation (6, 13). Figure 3 also shows that the acid growth response in pea roots can be obtained repeatedly by alternating between pH 6.5 and 4 in the root medium (lower curve). Such repeatability of the acid growth response is reported not to occur in coleoptile segments between pH 3 and 7 (13) but has been reported in corn root segments (6), in wheat nodal segments (2), and in oat internodal segments (Kaufman, personal communication). In corn root segments the magnitude of the acid growth response decreases with repeated induction, while in these experiments with intact pea roots, the magnitude of repeated acid growth responses over at least a 2-hr period is as great or greater than the initial growth response.

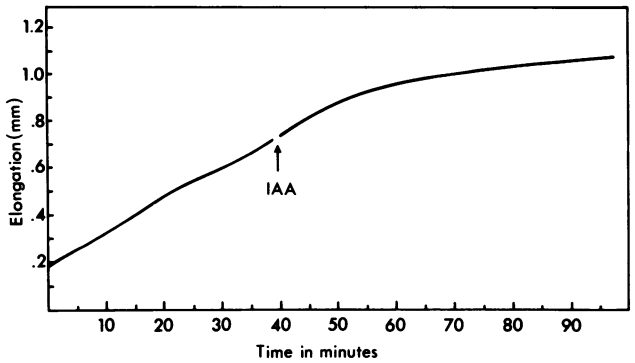

FIG. 2. Inhibition of intact root elongation by auxin. Root growing in half strength Meyer's solution. Auxin added to a final concentration of 0.01 mM at the arrow.

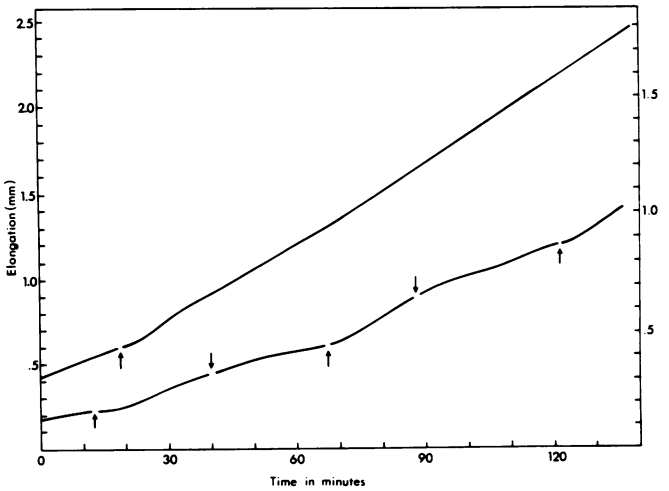

FIG. 3. Enhancement of intact root elongation by acid pH. Upper curve: citric acid added at the arrow to decrease pH from 6.5 to 4. Root continued to grow at accelerated rate for 160 min and growth was not recorded beyond that time. Lower curve: reinitiation of the acid growth response in intact roots. Medium was acidified to pH 4 (citric acid) at each upward pointing arrow and readjusted to pH 6.5 (NaOH) at each downward pointing arrow. On elongation axis, left-hand scale applies to lower curve, right-hand scale applies to upper curve.

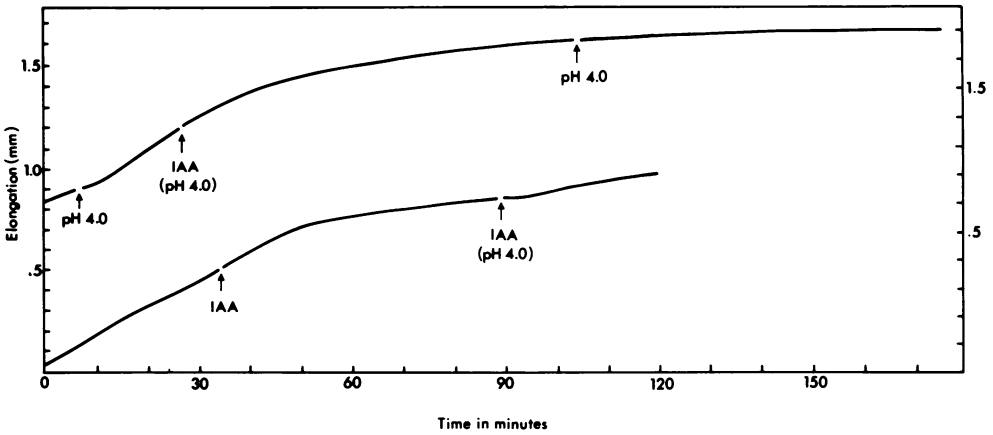

FIG. 4. Inhibition of the acid growth response by auxin. Upper curve: medium adjusted from pH 6.5 to pH 4 (citric acid) at first arrow. Medium changed to 0.01 mM IAA at pH 4 at second arrow and to pH 4 in the absence of IAA at third arrow. Lower curve: 0.01 mM IAA added at first arrow. IAA-containing medium adjusted to pH 4 at second arrow. On elongation axis, left-hand scale applies to lower curve, righthand scale applies to upper curve.

Inhibition of Acid-induced Growth by Auxin. Since auxin is observed to inhibit root growth strongly while acid is observed to promote growth strongly, the question arises as to which effect would dominate if auxin were offered at pH 4. Figure 4 shows that IAA is capable of strongly inhibiting acid-promoted elongation whether the hormone is added before or after acidification. The addition of auxin to the growth medium at pH 6.5 leads to maximal inhibition of root elongation in about 40 min (lower curve). If the auxin-containing medium is then acidified to pH 4, the resulting acid growth response is small (about 25% of normal). If IAA is applied to a root already growing at pH 4 (Fig. 4, upper curve) inhibition begins about 7 to 8 min later and is maximal about 50 min later. Subsequent removal of IAA and replacement with root medium at pH 4 is totally ineffective in restoring acid-induced growth. It appears that auxin inhibition of acid-induced elongation is irreversible, at least within 70 min following removal of the hormone. Hejnowicz and Erickson (9) have noted that the inhibition of corn root elongation by brief exposure to auxin is also expressed during the 60-min period following withdrawal of the hormone.

Further experiments on the nature of acid-induced elongation in intact roots are being carried out. Of particular interest is the question of the role H^+ ions might play in the normal regulation of root elongation.

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