Plant Physiol. (1978) 62, 161-162

Short Communication

Rapid Production of Auxin-induced Ethylene[1]

Received for publication September 13, 1977 and in revised form February 17, 1978

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ABSTRACT

The time course of auxin-induced ethylene production was determined in mesocotyl segments of etiolated sorghum (*Sorghum bicolor* L. Moench) seedlings. The latent period between addition of auxin and a detectable rise in ethylene release was 15 to 20 minutes in four different genotypes. This may indicate that the initial effect of auxin on ethylene production is too rapid to involve synthesis of an ethylene-producing enzyme. The technique devised for these experiments involves placing tissue segments end to end in a glass tube, and it allows simultaneous determination of growth and ethylene production.

The ability to determine accurately the early effects of auxin on growth has been very useful in refining the kinetics of auxin-induced growth and furthering the investigation of the mode of action involved (3, 4). Ethylene physiology has been hampered somewhat because the early time course of induced ethylene production has been impossible to determine. Further, the mechanism of induction of ethylene synthesis could be better understood if the kinetics of the process were more accurately known. The majority of studies on induced ethylene synthesis indicate a lag of more than 1 but less than 2 hr (2, 7, 14).

We have developed a method for accurate determination of ethylene production rates over short time periods, 5 to 15 min, and have used the method to establish that the apparent latent period in auxin-induced ethylene production in etiolated sorghum mesocotyl segments is less than 20 min.

MATERIALS AND METHODS

Four maturity genotypes (60M, SM60, 90M and SM90) of sorghum (*Sorghum bicolor* L. Moench) were used. These milo-type sorghums differ in the night length necessary for floral induction (13). The upper 1 cm of mesocotyls from 3-day-old etiolated seedlings both grew and produced ethylene in response to auxin, and this tissue segment was used in these experiments.

The technique involved placing tissue segments on a filter paper support and placing this into a glass tube which was then sealed with rubber serum vial caps. Gas samples could then be removed with a syringe.

Pieces of filter paper (1 × 24 cm) were folded median longitudinally and 15 tissue segments were placed end to end in the fold. The papers were then inserted into 7.5-ml tubes (cut from Pyrex glass tubing, o.d. 0.7 cm, final dimensions were 24.5 × 0.7 cm) placed in the horizontal position. Various filter papers and serum vial caps were tested for blank ethylene release; large sheets of Whatman No. 1 filter paper and new serum vial caps had satisfactorily low blank ethylene release values.

The tubes were then flushed with building compressed air that had passed through a scrubbing column containing Vermiculite coated with a saturated solution of $KMnO_4$ to remove contaminating traces of ethylene (verified effective by gas chromatography) and stoppered with serum vial caps (7 × 13 mm). A syringe needle (only) was inserted into one cap and 1 ml of the test solution, either 0.25 mM IAA in a pH 5 citrate-phosphate buffer or buffer alone for controls, was introduced via a syringe inserted at the other end of the tube. The solution rapidly flowed the length of the tube wetting the filter paper support in 5 sec. Both needles were then removed and the time clock started; ethylene production was measured every 15 min. All of the tubes were placed on a rack in a water bath of 27 C. Ethylene release in a blank tube containing filter paper and test solution without the plant segments was determined in each experiment and data were corrected for the small blank values. After 15 min, ethylene measurements were initiated by inserting a gas-tight syringe, mixing the air by pumping, inserting a syringe needle at the other end, and withdrawing a 1-ml air sample. Ethylene levels were determined by a gas chromatograph equipped with an activated alumina column and flame ionization detector (9). Both serum vial caps were then removed, the container flushed with air from the scrubbing column, caps replaced, and ethylene was then allowed to accumulate for another 15 min. The time for flushing and resealing was 15 to 30 sec.

RESULTS AND DISCUSSION

The four sorghum genotypes, 60M, SM60, 90M, SM90, responded to IAA identically with respect to: (*a*) the concentration dependence of the growth response and of the release of ethylene; (*b*) the 1-hr and 1- to 12-hr time course of ethylene release. Because of this uniformity of response the ethylene release data from all four genotypes were averaged allowing each data point in Figure 1 to represent 36 observations. In every experiment the observed ethylene synthesis rate was higher in treated than control segments 15 min after IAA was added (Fig. 1). The difference was consistent, but the standard errors overlapped with the control slightly. By 30 min the difference in ethylene production rates between IAA-treated and control segments were significantly different. From 30 to 60 min the ethylene production rate of IAA-treated segments rose linearly on a logarithmic scale and the back extrapolation of this line indicates a lag of about 20 min (Fig. 1, inset). Observation of the column of segments with a microscope indicated that growth had begun within 15 min, but growth was only quantitated at hourly intervals.

During the latent period in this system auxin must enter the tissue and have its initial action, then ethylene must be synthesized and exit the tissue to be measured. The small increase in the

[1] A preliminary report of the work has been published Plant Physiol. 1976 57: S-97.

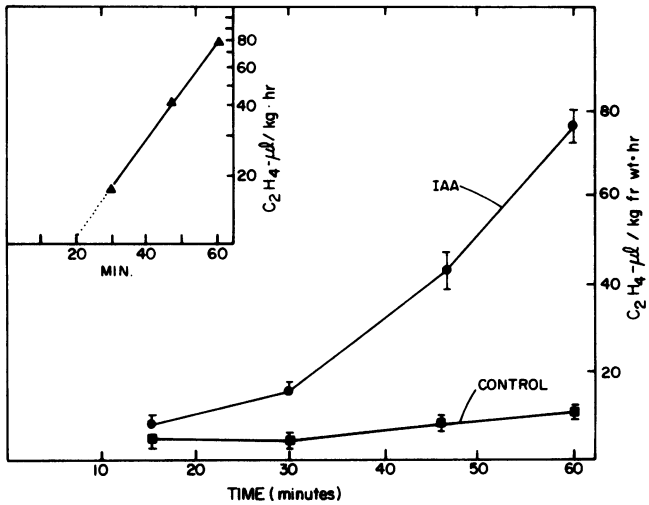

FIG. 1. Release of ethylene from etiolated sorghum mesocotyl segments treated with 0.25 mM IAA at time zero. Vertical lines through data points indicate standard errors. Data are averages of three experiments each with four milo genotypes with three replications/experiment (36 observations/data point). Inset: IAA data plotted on logarithmic scale.

ethylene production rate already apparent at 15 min suggests that auxin molecules began to modify ethylene production rapidly after entering the tissue and this action continued with time. The lag between initial action and ethylene synthesis would have to be considerably less than 15 min because increased ethylene synthesis was apparent at 15 min and some time was necessary for the preceding events. A more conservative statement is that the latent period was 20 min, but this estimate would also include some time for entry of the auxin and exit of the ethylene. In either case the latent period is considerably less than an hr or 2 and of the same order of magnitude as the "rapid" effects of auxin on growth (3, 4).

While this work was in progress others have reported relatively short latent periods for induced ethylene production. Using the approach of a large amount of tissue in a small flask, a 15-min latent period was found for auxin-induced ethylene production (5). A 28-min latent period was noted for ethylene production induced by wounding (6).

It has been argued that a 10- to 15-min latent period for auxin to promote growth indicates that the initial action is not induction of synthesis of proteins which have an enzymic role in cell wall elongation (3). Penny (11) and Pope and Black (12) have both obtained induction of growth with auxin in tissue in which protein synthesis is almost completely blocked. Continued growth did not occur. Therefore, with auxin-induced ethylene synthesis the initial action may not involve induction of synthesis of enzymes which convert a precursor to ethylene, but sustained ethylene production probably does require such synthesis. The requirement for protein synthesis for sustained growth is well documented (8, 10, 11).

The major evidence that the mechanism of induced ethylene synthesis involves a synthesis of the enzyme-producing ethylene is the effective blocking of auxin-induced and stress-induced ethylene production by cycloheximide (1, 7, 12). If the initial effect of auxin is too rapid to involve enzyme synthesis, then cycloheximide must have an effect which modifies auxin action and which is in addition to inhibition of protein synthesis.

LITERATURE CITED

1. ABELES AL, FB ABELES 1972 Biochemical pathway of stress-induced ethylene. Plant Physiol. 50: 496–498
2. BURG SP, EA BURG 1966 The interaction between auxin and ethylene and its role in plant growth. Proc Nat Acad Sci USA 55: 262–269
3. EVANS ML 1974 Rapid responses to plant hormones. Annu Rev Plant Physiol 25: 195–223
4. EVANS ML, PM RAY 1969. Timing of the auxin response in coleoptiles and its implications regarding auxin action. J. Gen. Physiol. 53: 1–20
5. IMASEKI H, K KONDO, S ODAWARA, A WATANABE 1976 Action of plant hormones in ethylene production. 9th Intern Conf Plant Growth Subst-Collected Abstracts, Lausanne Switzerland, pp 157–159
6. JACKSON MB, DJ CAMPBELL 1976 Production of ethylene by excised segments of plant tissue prior to the effect of wounding. Planta 129: 273–274
7. KANG BG, W NEWCOMB, SP BURG 1971 Mechanism of auxin-induced ethylene formation. Plant Physiol 47: 504–509
8. KEY JL 1974 Ribonucleic acid and protein synthesis as essential processes for cell elongation. Plant Physiol 39: 365–370
9. MORGAN PW, JI DURHAM 1972 Abscission: potentiating action of auxin transport inhibitors. Plant Physiol 50: 313–318
10. PATTERSON BD, AJ TREWAVAS 1967 Changes in the pattern of protein synthesis induced by 3-indolylacetic acid. Plant Physiol 42: 1081–1086
11. PENNY P 1971 Growth-limiting proteins in relation to auxin-induced elongation of lupine hypocotyls. Plant Physiol 48: 720–723
12. POPE D, M BLACK 1972 The effect of indole-3-acetic acid on coleoptile extension growth in the absence of protein synthesis. Planta 102: 26–36
13. QUINBY JR 1967 The maturity genes of sorghum. *In* AG Norman, ed, Advances in Agronomy, Vol 19, Academic Press, New York, pp 267–305
14. SAKAI S, H IMASEKI 1971 Auxin-induced ethylene production in mungbean hypocotyl segments. Plant Cell Physiol 12: 349–359