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Ethylene, Seed Germination, and Epinasty

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Abstract. **Ethylene activity in lettuce seed (*Lactuca satina*) germination and tomato (*Lycopersicon esculentum*) petiole epinasty has been characterized by using heat to inhibit ethylene synthesis. This procedure enabled a separation of the production of ethylene from the effect of ethylene. Ethylene was required in tomato petioles to produce the epinastic response and auxin was found to be active in producing epinasty through a stimulation of ethylene synthesis with the resulting ethylene being responsible for the epinasty. In the same manner, it was shown that gibberellic acid stimulated ethylene synthesis in lettuce seeds. The ethylene produced then in turn stimulated the seeds to germinate. It was hypothesized that ethylene was the intermediate which caused epinasty or seed germination. Auxin and gibberellin primarily induced their response by stimulating ethylene production.**

The production of ethylene and its role in plant growth regulation is becoming increasingly important. Ethylene activity has been demonstrated in tomato epinasty (7), fruit ripening (11), leaf abscission (13), disease resistance (14), root formation (7), flower induction (5), and auxin transport (12). Providing some insight into the activity of ethylene in these systems is the close relationship between auxin and ethylene in plants (3, 4, 7, 9). Probably the most significant observation is that ethylene is produced by plants themselves and that this ethylene synthesis can be auxin stimulated (1).

One of the problems encountered in studying ethylene activity is separation of ethylene production from its physiological response. By utilizing a selective heat inactivation of ethylene synthesis, first shown by Hansen (10) and Burg and Thimann (6), an attempt was made to separate ethylene synthesis from its effect. By separating these 2 processes the role of ethylene was studied in the germination of lettuce seeds and the epinasty of tomato petioles.

Materials and Methods

The tomato plants (*Lycopersicon esculentum* var. Marglobe) were 8 inch seedlings. These plants were grown in the greenhouse and were transplanted to individual 3 inch pots. The plants were allowed to adjust to experimental temperature and light conditions for 2 days before the start of the experiment.

Petiole explants were obtained by excising the petiole and attached stem 5 mm from the abscission zone. The explants consisted of 10 mm of stem and 5 mm of petiole. They were washed several times in distilled water and then introduced into the surface of agar previously poured into Warburg flasks.

The treatments with naphthaleneacetic acid (NAA) and indoleacetic acid (IAA) of whole plants was accomplished using lanolin paste applied to the cut surfaces after the apical meristem or leaf blade had been removed. In the explant experiments the auxin was added to the agar. Ethylene treatment of both whole plants and explants was achieved in a closed glass container receiving air with 100 ppm v/v of ethylene. The experiments were conducted at 25° under normal room lighting.

The tomato plants were heat treated in an oven at 40° along with several open petri dishes full of water. The water was added to prevent desiccation of the plants at the higher temperatures. Control plants were placed in the dark at 25° for the same period of time. A 4 hr heat treatment was used in all experiments involving tomato plants.

The lettuce seeds (*Lactuca satina* var., Grand Rapids) were held in a desiccator containing anhydrous calcium sulfate for 2 weeks prior to their use. The germination studies were conducted by placing a known number of seeds on filter paper in the bottom of 125 ml Erlenmeyer flasks. To these flasks were added a total of 2.5 ml of distilled water or a solution of gibberellic acid in distilled water. The flasks were then evacuated with an aspirator. The vacuum was released with air or air containing 100 ppm v/v of ethylene. The seeds were allowed to germinate in the dark for 2 days at 25°.

The heat treatment was accomplished in the same manner described by Havber (8). (The seeds were heated for 8 and 16 hr at 97° in a dry oven).

Ethylene production was measured on a flame ionization gas chromatograph. A dual flame Micro-Tek GC 2000R equipped with a 5 foot, one-eighth inch 30/60 mesh activated alumina column was used

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to detect ethylene at concentrations as low as 0.1 ppm. Nitrogen was used for a carrier gas. The oven temperature was kept constant at 30°. An Aerograph, 2 position, 6-way linear gas sampling valve with a 1 or 5 ml sample loop was used to inject the samples. Ethylene was determined by comparison of its residence time and peak areas with those of known standards and by absorption with mercuric perchlorate (15). Ethylene produced by the petiole explants was measured by accumulation in sealed Warburg flasks. Ethylene was accumulated from the lettuce seeds in sealed, 125 ml Erlenmeyer flasks.

Results

It was found that IAA in lanolin paste would cause epinasty of tomato petioles when applied to the cut surface of the stem. Petiole explants from similar plants were examined for their capacity to produce ethylene. As Fig. 1 shows, a small but consistent endogenous ethylene production was found in the tissues. The application of auxins (IAA and NAA) increased the rate of ethylene synthesis several fold.

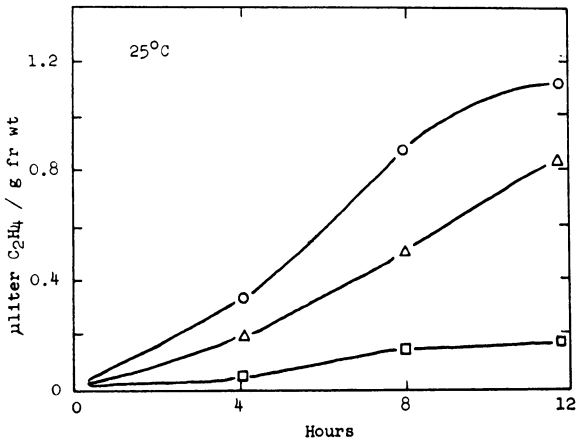

FIG. 1. Ethylene accumulation as a result of auxin addition to tomato petioles explants. □, control; ○, 0.1 % NAA; △, 0.1 % IAA.

The involvement of auxin stimulated ethylene synthesis in the epinasty process was then tested. A group of tomato plants were heat treated for 4 hr at 40° and sealed in desiccators. The apical meristem of all of the plants was then excised. To half of the plants lanolin paste containing 0.1 % w/v IAA was applied. Ethylene (100 ppm v/v) was then added to half of each group. Unheated controls were treated in the same manner. The results are presented in table I. The unheated tomato plants, that were treated with either ethylene or auxin, demonstrated epinasty. With the heat-treated plants, however, only those treated with exogenously applied ethylene showed epinasty. It was found that the auxin-stimulated epinasty which appears to be ethylene mediated was heat labile. The experiment was repeated 6 times with the results being the same in all except 2. In the 2 exceptions, an epinastic response was not obtained with the addition of auxin, therefore, the effect of heat on this response could not be determined.

Table I. *The Influence of IAA, Ethylene and Heat on Epinasty in Tomato Plants*

Heat treated (40° for 4 hr) and control (25° for 4 hr) tomato plants were examined for epinasty 24 hr after having been treated with 0.1 % IAA or 100 ppm ethylene at 25°.

Auxin or ethylene treatment	Heat treated	Control
Lanolin paste applied to the excised surface (control)	No epinasty	No epinasty
Lanolin paste containing 0.1 % IAA applied to the excised surface	No epinasty	Epinasty
Lanolin paste applied to the excised surface and placed in 100 ppm ethylene	Epinasty	Epinasty

Haber (8) indicated that gibberellic acid treatment of lettuce seeds caused a reversal of the effects of many of the known seed germination inhibitors. Gibberellic acid, however, was unable to overcome the temporary inhibitions resulting from heat treatments of the seeds. Ethylene participation in this process was tested. Lettuce seeds were subjected to gibberellic acid, ethylene, and heat, then allowed to germinate for 2 days in the dark. At the end of that period the percent germination and the ethylene produced was determined. As indicated in table II, all the flasks that contained germinating seeds also contained between 0.1 and 1.0 ppm v/v of ethylene. No ethylene was detected in flasks containing seeds which did not germinate. Further tests showed that the addition of 100 ppm v/v of ethylene to the flask resulted in a reversal of the inhibitory effects of heat. After repeating the experiment 3 times with the same results it appeared that gibberellic acid was active in reversing the effects of seed germination inhibitors through the synthesis of ethylene. In the case of the heat treatment, however, gibberellic acid was inactive in reversing the inhibition because the heat had stopped ethylene synthesis. Exogenously applied ethylene was, however, effective in reversing the heat inhibition.

Discussion

The number of reports of ethylene production in the plant kingdom (5, 11, 13, 14) and its association with other plant hormones (7, 13) indicates that ethylene may be a major controlling factor of plant activities. An intimate relationship may exist be-

Table II. *The Effect of Heat, Gibberellic Acid, and Ethylene on Seed Germination*

The percent germination and ethylene production was determined for lettuce seeds having been incubated in 3×10^{-4} M GA and/or ethylene (100 ppm) for 48 hr at 25°. The seeds had been pre-treated at 97° for 0, 8, and 16 hr.

Seed treatment	Results measured	Temperature treatment: Hr at 97° 0	8	16
H_2O	% Germination	82 ± 5	48 ± 4	1 ± 2
	Ethylene detected	1.3 ppm	0.1 ppm	0.0 ppm
3×10^{-4} M Gibberellic acid	% Germination	79 ± 5	51 ± 4	2 ± 2
	Ethylene detected	1.4 ppm	0.2 ppm	0.0 ppm
100 ppm Ethylene	% Germination	85 ± 5	87 ± 5	81 ± 5
100 ppm Ethylene and 3×10^{-4} M gibberellic acid	% Germination	86 ± 5	80 ± 5	78 ± 5

tween auxins and ethylene (3, 4, 9) since auxins can be shown to stimulate ethylene synthesis in many plant tissues (1).

By using the heat lability of ethylene synthesis first described by Hansen (10) and later by Burg and Thimann (6) it was noted that auxin-stimulated ethylene synthesis was heat labile in tomato petioles. The tissues could not produce large amounts of ethylene at 25° for several days following the heat treatment. The heat inactivation of ethylene synthesis was temporary and did not affect the action of ethylene. Thus a temporary separation of the production and action of ethylene was achieved and heat treatment could therefore be used as a tool in studying the role of ethylene as a plant growth regulator.

The work with epinastic responses in tomato plants demonstrated that ethylene and not auxin was closer to the active factor in inducing epinasty. Auxin was found to be active in producing epinasty only when ethylene synthesis could be detected. It was shown that epinasty did not occur in heat treated plants receiving auxin but did occur in heat treated plants receiving ethylene. The heat treatment inhibited the synthesis of ethylene which was normally stimulated by auxin. In the absence of this ethylene synthesis or exogenously applied ethylene, epinasty did not take place.

Other workers (2) have demonstrated similar relationships between ethylene and auxin. Ethylene, produced in response to auxin, caused the inhibition of flowering in *Xanthium*, a response which was previously attributed to auxin. Thus it is possible that ethylene may be an intermediate between auxin and the physiological responses attributed to auxin in other plants.

The work with the lettuce seeds further suggested the importance of stimulated ethylene production to a physiological response previously attributed to other hormones. In this case, gibberellic acid was credited with overcoming inhibitory effects of various seed germination inhibitors. Through the use of selective inhibition of ethylene synthesis it was demonstrated that ethylene was an active compound. Heat treatments were used to inactivate the ethylene synthesis without effecting ethylene action. The heat treatment eliminated gibberellic acid activity which presumably depended on ethylene synthesis. It did not, however, inhibit the activity of exogenously applied ethylene. The inhibition of ethylene synthesis and subsequently germination by heat treatment was only temporary. Five days following the heat treatment an average of 80% germination was noted in the samples that had been inhibited by heat.

The ethylene interrelationship in plants is far from a simple one. Its importance is not to be underestimated. In its complexity, lies the key to understanding a basic control mechanism of the plant kingdom. Since ethylene synthesis and activity are such general and yet specific processes involved in many plant responses, the understanding of ethylene biogenesis and role in metabolism is vital for the understanding of the physiological processes it controls.

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