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*Short Communication*

# Effect of Abscisic Acid and Its Interactions with Other Plant Hormones on Ethylene Production in Two Plant Systems[1, 2, 3]

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The influence of auxins (2, 3, 14), gibberellins (2), and cytokinins (8) on ethylene production in plants has already been established. It has been suggested that ethylene formation in seedlings is interrelated with the balance of auxins and cytokinins in the tissue (8). It is reasonable to assume that abscisic acid, which interacts with cytokinins and auxins in some other respects (11, 13), may also influence this balance. Recently, abscisic acid has been mentioned (1, 6, 7, 10) in connection with ethylene production. Herein is presented a study of the effects of abscisic acid on ethylene production in two different plant systems and its interactions with IAA, kinetin, and gibberellic acid.

## MATERIALS AND METHODS

Alaska pea seeds (*Pisum sativum* L. var. Alaska) were germinated on wet vermiculite for 72 hr at 25 C in the dark. Seedlings were transferred, in the dark, into 50-ml flasks containing 2 ml of the test solution and were incubated for 24 hr under the same conditions. Shamouti oranges (*Citrus sinensis* L.) were picked at the color turning stage and dipped as whole fruits in the test solutions; in other experiments, only peel plugs were used.

Growth regulators at various concentrations and DL-methionine at a final concentration of 5 mM, were applied in 1 mM phosphate buffer, pH 6.8; the same buffer was used for controls. RS-Abscisic acid was a gift from Hoffman La Roche and Co. Ltd. Two milliliters of solution and the plant material were placed in a 50-ml flask which was then sealed with a one-hole rubber stopper, fitted with a clamped capillary tube. Four whole seedlings or four Shamouti peel plugs were used per flask. In all experiments the sealed flasks were held for 24 hr at 25 C in the dark. The flasks were not shaken. At the end of the holding period the atmosphere in the flasks was sampled with a syringe for ethylene determination. Three whole fruits were enclosed in 10-liter jars for 24-hr periods for ethylene determination every other day for 2 weeks. Ethylene was determined by gas chromatography as described elsewhere (8). Rates of ethylene production are presented on a fresh weight basis. At least six replications were run for each experiment. Statistical analysis was carried out using analysis of variance and the Duncan multiple range test (9).

## RESULTS AND DISCUSSION

ABA in concentrations of 0.1 μM to 1 mM inhibited ethylene production in 3-day-old pea seedlings but stimulated the production in orange peel plugs (Fig. 1). Six-day-old apical pea-stem sections gave a response similar to that of the 3-day-old whole pea seedlings. IAA and kinetin-induced ethylene production in pea seedlings (3, 8) showed similar concentration curves to those of the ABA-induced inhibition. The greatest effect of ABA on the rate of ethylene production was observed between the 5th and 9th hr of incubation; after 16 hr, the production rate approached zero and it did not change for 48 hr (Fig. 2). The results were not significantly changed when vials of 20% potassium hydroxide were included in the 50-ml flasks.

When methionine, at a concentration of 5 mM, was added to both systems, it stimulated ethylene production (Table I). In the pea seedlings IAA and kinetin enhanced the methionine conversion to ethylene, as already reported (4, 8); GA had no influence in this respect and ABA inhibited the conversion of methionine to ethylene. None of the plant growth substances used in this study stimulated significantly the conversion of methionine into ethylene in the orange peel system. IAA, GA, and ABA significantly inhibited this process, while kinetin did not influence it at all. The fact that methionine is converted into ethylene in both systems and that the effective range of concentrations of ABA is similar in both sytems (Fig. 1) supports the suggestion that the mechanism of production is similar but the control mechanism is probably different.

As shown in Table I, ABA inhibited ethylene production in pea seedlings and stimulated the production of ethylene in the orange peel plugs. However, in both systems, when ABA and GA were added together (Table I), ethylene production was reduced relative to production in the growth hormone alone. Likewise in both systems, when kinetin and ABA were added together, ethylene production was reduced. IAA and ABA reduced ethylene production in the peas but it did not alter much the stimulating effect of ABA on ethylene production in the orange peel plugs. It should be mentioned that GA in combination with IAA had a stimulating effect on ethylene production in the orange system but not in the seedlings. It is difficult to conclude whether the differences between the two systems are due only to a different endogenous hormonal balance of the systems, which is very complex in itself, or to possibly completely different mechanisms of ethylene production in the various systems. Orange peel plugs are similar to cotton and bean explant (7) in their response to ABA (0.5 mM). As in other respects and in other systems (5, 12) there is a distinct antagonism between ABA and GA with respect to ethylene production in both pea seedlings and orange peel plugs systems. As in

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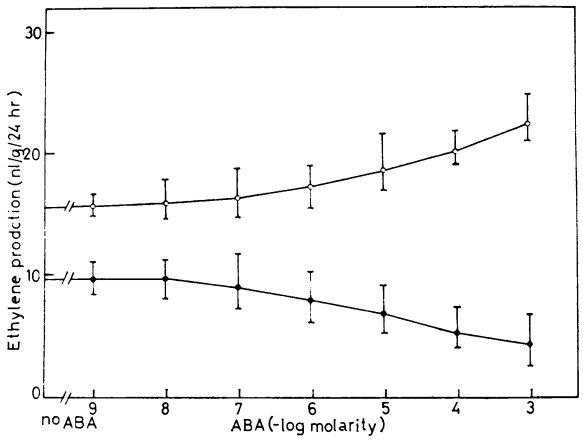


FIG. 1. Effect of ABA concentration on ethylene production by pea seedlings and orange peel plugs. Four 3-day-old pea seedlings (●), or four orange peel plugs (○), were kept for 24 hr in sealed 50-ml flasks in the dark at 25 C. SE (six replications) is indicated by vertical bars.

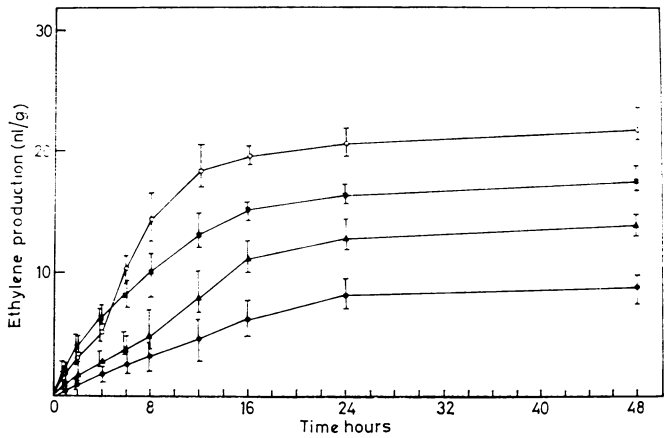


FIG. 2. Ethylene production by pea seedlings and orange peel plugs as affected by 0.1 mM ABA. Four 3-day-old pea seedlings were kept for different time periods with ABA (●) or without it (▲), in 50-ml flasks in the dark at 25 C. Four orange peel plugs were kept under similar conditions with ABA (○), or without it (■). SE (six replications) is indicated by vertical bars.

Table I. *Effects of ABA, IAA, Kinetin, GA, and* DL-*Methionine on Ethylene Evolution by Alaska Pea Seedlings and Shamouti Orange Peel Plugs*

Seedlings and peel plugs were treated with growth substances as described in the text. The final concentration of each of the participating growth substances was 0.1 mM at pH 6.8. The final concentration of methionine was 5 mM. The experiments were replicated six times. Statistical analysis was done separately for pea seedlings and orange peel plugs. Figures with different superscript letters are significantly different at the 5% level. The control treatments of peas and orange plugs produced 9.13 and 16.11 nl of ethylene/g·24 hr, respectively.

| Incubation Solution | Ethylene Production | |
|---|---|---|
| | Pea seedlings | Orange peel plugs |
| | *% of control* | |
| Control | $100^{f}$ | $100^{c}$ |
| ABA | $60^{g}$ | $156^{b}$ |
| IAA | $281^{c}$ | $65^{d}$ |
| Kinetin | $183^{e}$ | $120^{bc}$ |
| GA | $123^{f}$ | $85^{c}$ |
| Methionine | $164^{e}$ | $206^{a}$ |
| IAA + ABA | $187^{e}$ | $142^{b}$ |
| IAA + kinetin | $494^{b}$ | $69^{dc}$ |
| IAA + GA | $318^{c}$ | $142^{b}$ |
| IAA + methionine | $463^{b}$ | $83^{c}$ |
| ABA + kinetin | $145^{e}$ | $93^{c}$ |
| ABA + GA | $89^{f}$ | $70^{dc}$ |
| ABA + methionine | $106^{f}$ | $167^{b}$ |
| Kinetin + GA | $170^{e}$ | $71^{dc}$ |
| Kinetin + methionine | $240^{d}$ | $248^{a}$ |
| GA + methionine | $141^{e}$ | $87^{c}$ |
| Methionine + IAA + ABA | $236^{d}$ | $156^{b}$ |
| Methionine + IAA + kinetin | $559^{a}$ | $107^{c}$ |
| Methionine + IAA + GA | $406^{b}$ | $143^{b}$ |
| Methionine + ABA + kinetin | $165^{e}$ | $140^{b}$ |
| Methionine + ABA + GA | $89^{f}$ | $180^{ab}$ |
| Methionine + kinetin + GA | $206^{de}$ | $131^{bc}$ |

other systems (12, 13) in which the effect of kinetin interferes with the effect of ABA, in our work, too, kinetin interfered with the effect of ABA on ethylene production. Whole orange fruits do not, normally, produce ethylene and ABA treatment did not induce its production. It seems that in combination with other plant hormones, ABA is playing a role in the regulation rather than in the induction of the ethylene-producing systems.

## LITERATURE CITED


1. ABELES, F. B. 1967. Mechanism of action of abscission accelerators. Physiol. Plant. 20: 442–454.
2. ABELES, F. B. AND B. RUBINSTEIN. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol. 39: 963–969.
3. BURG, S. P. AND E. A. BURG. 1966. The interaction between auxin and ethylene and its role in plant growth. Proc. Nat. Acad. Sci. U.S.A. 55: 262–269.
4. BURG, S. P. AND C. O. CLAGETT. 1967. Conversion of methionine to ethylene in vegetative tissue and fruits. Biochem. Biophys. Res. Commun. 27: 125–130.
5. CHRISPEELS, M. J. AND J. E. VARNER. 1966. Inhibition of gibberellic acid induced formation of α-amylase by abscisin II. Nature 212: 1066–1067.
6. COOPER, W. C., G. K. RASMUSSEN, B. T. ROGERS, P. C. REECE, AND W. H. HENRY. 1968. Control of abscission in agricultural crops and its physiological basis. Plant Physiol. 43: 1560–1576.
7. CRAKER, L. E. AND F. B. ABELES. 1969. Abscission: role of abscisic acid. Plant Physiol. 44: 1144–1149.
8. FUCHS, Y. AND M. LIEBERMAN. 1969. Effects of kinetin, IAA and gibberellin on ethylene production and their interactions in growth of seedlings. Plant Physiol. 43: 2029–2036.
9. LE CLERG, E. L. 1957. Mean separation by the functional analysis of variance and multiple comparisons. United States Department of Agriculture, Agricultural Research Service Publication. Beltsville, Md. pp. 20–23.
10. LIEBERMAN, M. AND A. T. KUNISHI. 1971. Abscisic acid and ethylene production. Plant Physiol. 47: S22.
11. PILET, P. E. 1970. The effect of auxin and abscisic acid on the catabolism of RNA. J. Exp. Bot. 21: 446–451.
12. REYNOLDS, T. 1970. A new method for separation of gibberellins and growth inhibitors in plant extracts using Sephadex columns. J. Exp. Bot. 21: 702–711.
13. SANKHLA, N. AND D. SANKHLA. 1968. Reversal of (±)-abscisin II-induced inhibition of lettuce seed germination and seedling growth by kinetin. Physiol. Plant. 21: 190–195.
14. ZIMMERMAN, P. W. AND F. WILCOXON, 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. Plant Res. 7: 209.