Plant Physiol. (1984) 75, 1165–1166
0032-0889/84/75/1165/02/$01.00/0

Short Communication

Introduction of Xylem Differentiation in *Lactuca* by Ethylene

Received for publication April 12, 1984 and in revised form June 8, 1984

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ABSTRACT

Evidence was obtained to support the hypothesis that ethylene is involved in xylem differentiation in primary pith explants of *Lactuca sativa* L. cv Romaine cultured *in vitro*. Xylem elements differentiated when explants were supplied indole-3-acetic acid (IAA) in combination with either the ethylene biosynthetic precursor 1-aminocyclopropane-1-carboxylic acid (ACC), the ethylene-releasing agent 2-chloroethylphosphonic acid (CEPA), or kinetin. In contrast, no xylem elements differentiated in the presence of IAA, kinetin, ACC, or CEPA alone, or when kinetin was supplied together with ACC or CEPA. These results show that ethylene will substitute qualitatively for cytokinin during auxin-induced xylogenesis, and suggest that both ethylene and auxin are required for xylem differentiation in *Lactuca*.

Xylem differentiation can be induced in cultured plant tissues by supplying an auxin and cytokinin to the growth medium (10). Considerable indirect evidence (6, 7, 11, 13) supports the hypothesis of Roberts (10) that ethylene also plays a role in xylogenesis *in vitro*. This evidence includes the observation that xylogenesis in lettuce pith explants was increased when the xylogenic medium was supplemented with the ethylene-releasing agent CEPA[2] (7) or the ethylene precursors L-methionine (7, 11), *S*-adenosylmethionine and ACC (7). Moreover, the ethylene inhibitors aminoethoxyvinylglycine, CO^{2+}, and Ag^{+} inhibited xylem formation, and these effects were partially reversed by ethylene precursors (7). These results indicate that auxin and cytokinin may induce xylem differentiation via ethylene formation. The experiments reported here were performed to determine whether ACC or CEPA could substitute for auxin or cytokinin in the induction of xylem differentiation in *Lactuca* pith explants.

MATERIALS AND METHODS

Sterile pith parenchyma explants were prepared from the core of lettuce (*Lactuca sativa* L. cv Romaine) heads (7) and cultured individually in 50-ml screw-cap culture tubes containing 10 ml of growth medium solidified with 1% (w/v) Bacto-Agar. Basal growth medium was prepared according to Murashige and Skoog (8) except that 2% (w/v) glucose was substituted for sucrose. IAA, kinetin, ACC, and CEPA were sterilized by membrane filtration and added as indicated to the autoclaved basal medium. Ethylene was measured by GC (4) after removing a 2-ml gaseous sample from the headspace of culture tubes which had been sealed for 6 h with Teflon-silicone laminated septa (Pierce Chemical Co., Rockford, IL). Ethylene peaks were quantitated by comparison with a 10.8 μl/l ethylene standard (Ideal Gas Products, Edison, NJ).

The number of xylem elements was determined by microscopic examination of squash preparations of safranin-O-stained explants (2, 11) or explants macerated in a chromic acid maceration fluid (11). Xylem elements in macerated explants were quantitated according to Dodds and Roberts (3). Xylem elements were identified as cells having scalariform-reticulate secondary walls (2).

Chemicals. Bacto-agar was from Difco Laboratories, Detroit, MI; glucose and CEPA were from Sigma Chemical Co.; IAA and kinetin were from ICN Nutritional Biochemicals; and ACC was from Calbiochem-Behring.

RESULTS AND DISCUSSION

Xylem elements formed in lettuce pith explants cultured in the presence of IAA plus kinetin, but not in explants cultured in the presence of either compound alone or in basal medium (Table 1). Tissues cultured on IAA plus kinetin also produced more ethylene than tissues cultured on IAA, kinetin, or basal medium (Table I).

To test whether ethylene may be involved in xylogenesis, tissues were cultured with ACC or CEPA singly or in combination with IAA or kinetin. Xylem elements developed in tissues cultured on ACC or CEPA in combination with IAA, but not in combination with kinetin or in the absence of IAA (Table I).

Although xylem elements induced by IAA plus kinetin or IAA plus ethylene were morphologically indistinguishable, several differences in xylogenesis were found with these treatments. First, the number of xylem elements formed was greater with IAA plus kinetin than with IAA plus ACC or CEPA (Table I), and their number could not be increased by varying the ACC or CEPA levels (not shown). Second, hand-sectioned material showed that xylem differentiation induced by IAA plus kinetin occurred primarily near the tissue surface in contact with the culture medium, while xylem elements were found near the tissue surface distal to the culture medium in tissues treated with IAA plus ACC or CEPA (not shown). Finally, whereas IAA plus kinetin treatment induced xylem differentiation in explants during the first week of culture, xylem elements did not appear until the third week of culture on IAA plus ACC (Table II).

Differences in the time course of xylogenesis and the location of xylem elements within the explants correlated directly with

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[2] Abbreviations: CEPA, 2-chlorethylphosphonic acid; ACC, 1-aminocyclopropane-1-carboxylic acid.

Table I. *Summary of the Effects of Various Hormone Regimes on Ethylene Production and Xylogenesis in Lactuca Pith Explants*

Treatment[a]	Ethylene Production at Following Days in Culture				Fresh Wt[b]	Xylem Elements[b]
	1	7	14	21		
	nl/h·explant				*mg/explant*	*no./g fresh wt*
No Hormones	0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	70 ± 2	0
IAA	0.3 ± 0.03[c]	1.0 ± 0.0	0	0	109 ± 3	0
Kinetin	0	0.1 ± 0.09	0.5 ± 0.03	0.6 ± 0.03	154 ± 9	0
ACC	0.8 ± 0.1	2.4 ± 0.06	1.5 ± 0.03	0.5 ± 0.0	73 ± 3	0
CEPA	3.6 ± 0.03	2.0 ± 0.03	2.2 ± 0.03	1.1 ± 0.09	79 ± 2	0
IAA + kinetin	0.4 ± 0.06	11.7 ± 0.3	11.8 ± 0.3	18.0 ± 0.5	364 ± 30	250,000 ± 30,000
IAA + ACC	0.6 ± 0.06	6.0 ± 0.8	9.4 ± 0.6	5.5 ± 0.7	100 ± 6	1,700 ± 600
IAA + CEPA	4.0 ± 0.1	1.8 ± 0.09	1.9 ± 0.06	0.9 ± 0.03	108 ± 6	3,900 ± 600
Kinetin + ACC	3.6 ± 0.06	0.8 ± 0.03	0.7 ± 0.0	1.0 ± 0.06	88 ± 3	0
Kinetin + CEPA	2.2 ± 0.06	2.5 ± 0.09	1.8 ± 0.06	0.8 ± 0.03	90 ± 6	0

[a] Explants were cultured on basal medium supplemented as specified with IAA (60 μM), kinetin (0.5 μM), ACC (10 μM), and CEPA (1 μM).

[b] Determined after 21 d. Initial fresh weight was approximately 52 mg.

[c] Values reported are from a representative experiment; each treatment was repeated three times. Values expressed ± SE (n = 10).

Table II. *Time Course of IAA-ACC-Induced Xylogenesis in Lactuca Pith Explants*

Treatment[a]	Xylem Elements at Following Days in Culture		
	7	14	21
	no./g fresh wt		
IAA	0	0	0
IAA + 0.1 μM ACC	0	0	0
IAA + μM ACC	0	50 ± 20	9,000 ± 1,600
IAA + 10 μM ACC	0	300 ± 75	28,000 ± 1,000
IAA + kinetin	55,000 ± 2,100[b]	188,000 ± 31,300	344,000 ± 62,100

[a] Explants were cultured on basal medium supplemented as specified with IAA (60 μM), kinetin (0.5 μM), and ACC.

[b] Values expressed ± SE (n = 8).

cell proliferation. Cell proliferation in lettuce explants cultured on an IAA plus kinetin medium initiated rapidly and occurred at the explant surface contacting the culture medium (2), whereas cell proliferation on IAA plus ACC or CEPA was slow to develop, was less extensive than with IAA plus kinetin, and occurred at the upper surface of the explant. Hence, xylem formation in lettuce pith explants was related to the appearance of dedifferentiated cells which presumably show a high capacity for xylem differentiation. Although it is not known whether ACC or CEPA may affect cytokinin metabolism in this system, experiments with ethylene inhibitors have shown that xylogenesis in the presence of IAA plus kinetin can be reduced without affecting the growth rate of the explants (7). Thus it appears that the effect of ethylene on the induction of xylem differentiation cannot be explained solely on the basis of cytokinin metabolism or cell proliferation.

From evidence presented here it seems reasonable that ethylene plays a role in the induction of xylem differentiation in lettuce pith explants. Increased ethylene production has been previously reported to occur coincident with reaction wood formation in *Pinus* and *Eucalyptus* (5, 9), and exogenous ethylene or CEPA has been shown to induce reaction wood formation (9). Moreover, ACC was recently identified in developing compressionwood of *Pinus contorta* Dougl. (12). Thus, the finding that ethylene can induce xylem differentiation in lettuce explants may be relevant to wood and xylem formation in general.

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