Gradients in ethylene metabolism along the growing mung bean hypocotyl

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RÉSUMÉ

KEVERS, C., R. GOLDBERG & T. GASPAR (1989). Métabolisme de l'éthylène le long de l'hypocotyle de Soya vert. *Saussurea* 19: 129-134. En anglais, résumés français et anglais.

Le métabolisme de l'éthylène (émission d'éthylène à l'extérieur et rétention à l'intérieur des tissus, capacité de conversion de l'ACC en éthylène, contenus en ACC et en m-ACC) a été mesuré dans le crochet et les segments adjacents de l'hypocotyle de plantules (issues de germination) de 3 jours de *Vigna radiata.* Un gradient croissant de l'émission et de la rétention d'éthylène, parallèle à un accroissement des teneurs en ACC et en m-ACC, va de la zone d'élongation la plus active vers la moins active. La capacité qu'ont les segments entiers d'hypocotyle de convertir l'ACC en éthylène correspond bien à une activité ACC-oxydase *in vitro* des fluides intercellulaires des mêmes fragments. Ces résultats sont en accord avec le rôle supposé de l'éthylène dans la limitation des possibilités de croissance des parois cellulaires en voie de maturation.

ABSTRACT

KEVERS, C., R. GOLDBERG & T. GASPAR (1989). Gradients in ethylene metabolism along the growing mung bean hypocotyl. *Saussurea* 19: 129-134. In English, French and English abstracts.

Ethylene metabolism (ethylene emission and retention, capacity to convert ACC to ethylene, ACC and m-ACC levels) was measured along the hook and adjacent hypocotyl segments of 3-d-old etiolated *Vigna radiata* seedlings. There was an increasing gradient of emission and retention of ethylene in parallel with an increase of ACC and m-ACC levels from the fast- to the slow- growing zone. The capacity of whole hypocotyl segments to convert ACC to ethylene correlated with similar activity in intercellular fluids extracted from the same segments. These results well fit with the notion that ethylene may be responsible in part for the decreasing potential of cell walls to expand as tissues mature.

Introduction

Decreasing growth rate along the mung bean hypocotyl had been shown to be related to the loss of plasticity of the cell walls (GOLDBERG & al., 1986c and 1986b). Modifications of cell wall structure and plasticity were attributed to increasing activity of two fast migrating anionic isoperoxidases along this axis (GOLDBERG & al., 1986a and 1989). Peroxidase-catalyzed integration of diphenyl phenolic crosslink wall polymer subunits

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into the cell wall indeed might limit cell wall plasticity (LAMPORT, 1986). Such crosslinkages might bind lignin (HIGUCHI, 1981), polysaccharides (FRY, 1986) or proteins (EPSTEIN & LAMPORT, 1984; COOPER & VARNER, 1984) to pectins or hemicelluloses (COOPER & VARNER, 1984). In different growth and morphogenesis processes involving wall rigidification (most often through lignification) in response to biological (VAN LOON, 1986), chemical (KEVERS & al., 1987) or physical stresses (BOYER & al., 1983; BIRO & JAFFE, 1984), the mediating role of ethylene has been suggested (ROBERTS & MILLER, 1983; GASPAR, 1986). In the model of plant cell development presented by TREWAVAS (1981), ethylene indeed is associated with the terminal phase. The aim of the present work therefore was to evaluate ethylene metabolism along the growth gradient of the mung bean hypocotyl.

Materials and methods

Plant material

Seeds of *Vigna radiata* (L.) Wilezek were soaked in tap water for 2 h, placed on moist vermiculite and covered with a wet cloth. After 3 days at 26°C in the dark, seedlings with hypocotyls 45 mm (± 5 mm) long were selected. Three successive segments were excised in the upper part of the hypocotyl: (I) the cross, 5 mm long, just below the cotyledons, (II) and (III) are the respectively following cm as previously described (GOLDBERG & al., 1986a).

Ethylene metabolism

Five excised hypocotyl segments were placed on a disc of Whatman n° 1 filter paper (20 mm diameter) and imbibed with 1 ml distilled H_2O or 2 mM ACC (1 aminocyclopropane-1-carboxylic acid) in 14 ml glass vials sealed with a serum cap and kept at room temperature. Ethylene release was determined after 24 h incubation in 1 ml air samples by gas chromatography, according to the technique used by KEVERS & GASPAR (1985). Endogenously retained ethylene was measured using mercuric perchlorate as a specific trap (± 250 mg fresh material in 1 ml mercuric perchlorate) and LiCl to destroy the stable ethylene mercury complex (ABELES, 1973).

ACC oxidase of the intercellular fluid was measured by the ethylene evolved (after 24 h) from a mixture of 400 µl fluid, 50 µl 20 mM $MnCl_2$ and 100µl 10^{-3}M ACC. Intercellular fluid was recovered after centrifugation (1500 *g*, 10 min) of whole plant segments previously "infiltrated" (RATHMELL & SEQUEIRA, 1974) either in H_2O, or in MOPS (N-morpholino-3-propanesulfonic acid) buffer (0.25 M, pH 6.1.) or in MOPS buffer with NaCl 1M, under vacuum (2 × 1 min).

For the ACC measurement, one g of fresh material was ground with 5 ml ethanol (80%) and centrifuged at 5000 *g* for 10 min. The supernatant was evaporated to dryness at 35°C. The flask was rinsed with chloroform and the residue resuspended by agitation in 1 ml distilled water after chloroform evaporation under N_2. ACC was estimated from the ethylene evolved after 2.5 min from 1 ml solution (in a sealed 35 ml — glass bottle) containing 800 µl extract, sodium hypochlorite 0.44%, $HgCl_2$ 10^{-3}M and 3.3% of a saturated NaOH solution, according to the method of LIZADA & YANG (1979).

Determination of conjugated ACC (malonyl-ACC, m-ACC) in the 1 ml ACC extract was carried out after its hydrolysis in 6 M HCl at 100°C for 1 h (HOFFMAN & al., 1983). The difference in ACC content after and before HCl-hydrolysis was taken as the amount of ACC conjugated in the extract.

Each set of experiments was repeated at least three times on different series of plants.

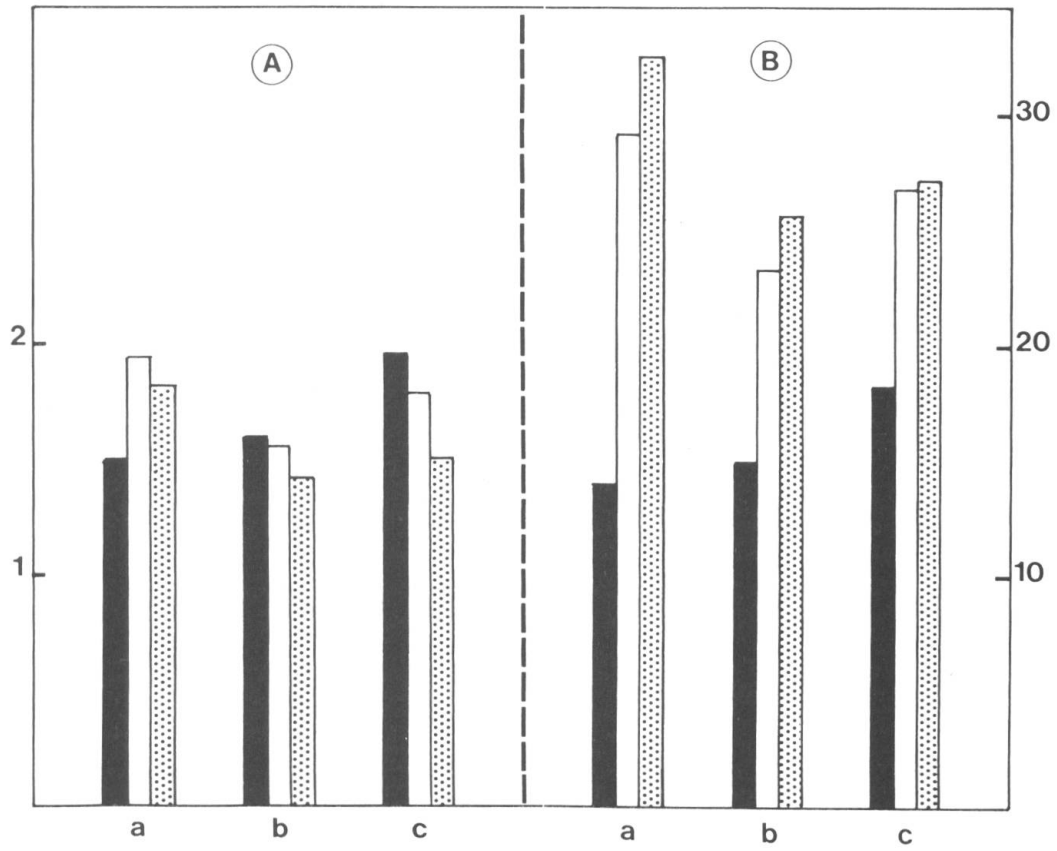

Fig. 1. — ACC-oxidase activities recovered after infiltration of the three successive segments with H_2O **(a)**, MOPS **(b)** and MOPS + NaCl **(c)**. Activities as 10^{-6} ml C_2H_4/24 h and per g fresh **(A)** and dry **(B)** matter. ■, □, ⊡: successive hypocotyl segments I, II, III.

Results

In early experiments, the three successive segments of the mung bean hypocotyl were analyzed for retained ethylene, ethylene emission rates, their content of ACC and m-ACC, and their capacity to convert ACC to ethylene. The results were expressed both on a fresh and a dry weight bases (Table 1). There was no clear gradient in ACC and m-ACC levels, ethylene retention nor in ethylene emission along the three hypocotyl segments when fresh weight was taken as reference. There was a clear gradient however, with increasing amounts of ethylene, ACC and m-ACC from segment I to III, on a dry weight basis. The whole fragments converted ACC to ethylene with a decreasing capacity from segment I to III on a fresh weight basis. On a dry weight basis this capacity was the greatest in the middle segment II. Although *in vitro* measured ACC-oxidase activity may not have physiological significance (VENIS, 1984), we have nevertheless estimated ACC-oxidase activity in the periplasmic and intercellular fluid recovered using different ionic strengths (Fig. 1), because the circulation pathway of ACC and location of conversion into ethylene could be the periplasmic and intercellular space (GASPAR & al., 1985). All values of periplasmic fluid ACC-oxidase activity were much lower than those corresponding to the capacity of whole segments to convert ACC to C_2H_4. Increasing ionic strength of the infiltration buffer did not significantly affect the recovery of ACC-oxidase activity. On a dry weight basis, there was a clear increasing gradient of intercellular fluid ACC-oxidase activity from segment I to segment III.

	Hypocotyl segments		
	I	*II*	*III*
Ethylene content			
10^{-6} ml/g FW	0.53 ± 0.06	0.46 ± 0.08	0.75 ± 0.07
10^{-6} ml/g DW	4.95 ± 0.56	6.87 ± 1.19	13.39 ± 1.25
Ethylene emitted			
10^{-6} ml/g FW/24 h	72.5 ± 8.5	54.8 ± 2.7	60.7 ± 10.7
10^{-6} ml/g DW/ 24 h	677 ± 79	818 ± 40	1084 ± 191
ACC level			
10^{-9} ml/g FW	0.646 ± 0.024	0.630 ± 0.025	0.690 ± 0.061
10^{-9} ml/g DW	6.04 ± 0.22	9.40 ± 0.37	12.32 ± 1.09
m-ACC level			
10^{-6} ml/g FW	1.042 ± 0.033	1.092 ± 0.113	2.067 ± 0.045
10^{-6} ml/g FW	9.75 ± 0.31	16.30 ± 1.69	36.91 ± 0.80
ACC to C_2H_4 conversion			
10^{-6} ml C_2H_4/FW/24 h	3794 ± 420	3289 ± 542	2066 ± 294
10^{-6} ml C_2H_4/g FW/24 h	35458 ± 3925	49090 ± 8090	36893 ± 5250

Table 1. — Ethylene content and emission, capacity of ACC conversion to ethylene, ACC and m-ACC levels in the three hypocotyl segments expressed per unit fresh (FW) or dry (DW) weight.

Discussion

We show that in more slowly growing parts of the hypocotyl compared to fast growing parts, emission and retention of ethylene increased in parallel with ACC and m-ACC levels. This was expected since ethylene can be considered as a regulatory agent of cell wall edification (ref. in GASPAR & al., 1985; GASPAR, 1986). Thus, cessation of growth might be considered a part of the senescence process, where ethylene is known to be involved (ABELES, 1973; LESHEM & al., 1986). The way ethylene controls the enzymes (phenylalanine-ammonia-lyase, cinnamyl-alcohol-dehydrogenase, syringaldazine-peroxidases) involved in cell wall edification therefore merits further examination. The capacity to convert ACC to ethylene in whole hypocotyl segments (Table 1) did not correlate with ethylene production but did with the disputed (VENIS, 1984; GUY & KENDE, 1984) *in vitro* ACC-oxidase activity. That ethylene metabolism can run in parallel with changes in cell wall bound peroxidases (GOLDBERG & al., 1986a) and the ACC-oxidase activity of the intercellular fluid correlated well with ascorbate peroxidase activity of this fraction but not with its guaïacol-peroxidase activity (GOLDBERG & al., in press). These relationships also are to be investigated further in the growing mung bean hypocotyl since some isoperoxidases might be involved in ACC to C_2H_4 conversion (BOYER & DE JAEGHER, 1986). The present work indicates that the already established biochemical gradients along growing organs must be re-appraised, taking into account the periplasmic and intercellular space, as already hypothesized (GASPAR & al., 1985). A recent study of HOREMANS & al. (1986) on the longitudinal gradients

of indole-3-acetic acid and abscisic acid in the hypocotyl of etiolated bean seedlings also came to the conclusion that further data on the inter- and intra- cellular distribution of the plant growth substances and their receptors is needed.

REFERENCES

ABELES, F. B. (1973). *Ethylene in Plant Biology:* 21-22. Academic Press, New York & London.

BIRO, R. L. & M. J. JAFFE (1984). Thigmomorphogenesis: ethylene evolution and its role in the changes observed in mechanically perturbed bean plants. *Physiol. Pl.* 62: 289-296.

BOYER, N. & G. DE JAEGHER (1986). Direct or indirect role of peroxidases in ethylene biosynthesis? *In:* GREPPIN, H., C. PENEL & T. GASPAR (Eds.), *Molecular and physiological aspects of plant peroxidases:* 47-60. University of Geneva, Switzerland.

BOYER, N., M. O. DESBIEZ, M. HOFINGER & T. GASPAR (1983). Effect of lithium in thigmomorphogenesis in Bryonia dioica. Ethylene production and sensitivity. *Pl. Physiol.* 72: 522-525.

COOPER, J. B. & J. E. VARNER (1984). Cross-linking of soluble extensin in isolated cell walls. *Pl. Physiol.* 76: 414-417.

EPSTEIN, L. & D. T. A. LAMPORT (1984). An intramolecular linkage involving isodityrosine in extensin. *Phytochem.* 23: 1241-1246.

FRY, S. C. (1986). Polymer-bound phenols as natural substrates of peroxidases. *In:* GREPPIN, H., C. PENEL & T. GASPAR (Eds.), *Molecular and physiological aspects of plant peroxidases:* 169-182. University of Geneva, Switzerland.

GASPAR, T. (1986). Integrated relationships of biochemical and physiological peroxidase activities. *In:* GREPPIN, H., C. PENEL & T. GASPAR (Eds.), *Molecular and physiological aspects of plant peroxidases:* 455-468. University of Geneva, Switzerland.

GASPAR, T., C. PENEL, F. J. CASTILLO & H. GREPPIN (1985). A two-step control of basic and acidic peroxidases an its significance for growth and development. *Physiol. Pl.* 64: 418-423.

GOLDBERG, R., A. IMBERTY & J. CHU-BA (1986a). Development of isoperoxidases along the growth gradient in the mung bean hypocotyl. *Phytochem.* 25: 1271-1274.

GOLDBERG, R., A. IMBERTY, M. LIBERMAN & R. PRAT (1986b). Relationships between peroxidatic activities and cell wall plasticity. *In:* GREPPIN, H., C. PENEL & T. GASPAR (Eds.), *Molecular and physiological aspects of plant peroxidases:* 209-220. University of Geneva, Switzerland.

GOLDBERG, R., M. LIBERMAN, A. M. CATESSON & C. MATHIEU (1986c). Localisation et propriétés des peroxydases épidermiques de l'hypocotyle de Vigna radiata. Relations avec la diminution de la plasticité pariétale. *Bull. Groupe Polyphénols, Compt. Rend. Journées Intern. d'Etude, Montpellier* 13: 195-200.

GOLDBERG, R., C. KEVERS & T. GASPAR (1989). Guaïacol- and ascorbate-peroxidase compartmentation and gradient along the growing mung bean hypocotyl. *Biochem. Physiol. Pflanzen.* In press.

GUY, M. & H. KENDE (1984). Conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene by isolated vacuoles of Pisum sativum L. *Planta* 160: 281-287.

HIGUCHI, T. (1981). *In:* TANNER, W. & F. A. LOEWUS (Eds.), *Plant Carbohydrates II. Extracellular carbohydrates:* 194-224. Springer, Berlin.

HOFFMAN, N. E., Y. LIU & S. F. YANG (1983). Changes in 1-(malonylamino)-cyclopropane 1-carboxylic acid content in wilted wheat leaves in relation to their ethylene production rates and 1-aminocyclopropane-1-carboxylic acid content. *Planta* 157: 518-523.

HOREMANS, S., H. A. VAN ONCKELEN & J. A. DE GREEF (1986). Longitudinal gradients of indole-3-acetic acid and abscisic acid in the hypocotyl of etiolated bean seedlings. *J. Exp. Bot.* 37: 1525-1532.

KEVERS, C. & T. GASPAR (1985). Vitrification of carnation in vitro: changes in ethylene production, ACC level and capacity to convert ACC to ethylene. *Plant Cell Tissue Organ Culture* 4: 215-223.

KEVERS, C., R. PRAT & T. GASPAR (1987). Vitrification of carnation in vitro: changes in cell wall mechanical properties, cellulose and lignin content. *Plant Growth Regulation* 5: 59-66.

LAMPORT, D. T. A. (1986). Roles for peroxidases in cell wall genesis. *In:* GREPPIN, H., C. PENEL & T. GASPAR (Eds.), *Molecular and physiological aspects of plant peroxidases:* 199-208. University of Geneva, Switzerland.

LESHEM, Y. Y., A. H. HALEVY & C. FRENKEL (1986). *Processes and control of plant senescence.* Elsevier Science Publ., Amsterdam.

LIZADA, C. C. & S. F. YANG (1979). A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid . *Anal. Biochem.* 100: 140-145.

RATHMELL, W. G. & L. SEQUEIRA (1974). Soluble peroxidase in fluid from the intercellular spaces of tobacco leaves. *Pl. Physiol.* 53: 317-318.

ROBERTS, L. W. & A. R. MILLER (1983). Is ethylene involved in xylem differentiation? *Vistas Pl. Sci.* 6: 1-24.

TREWAVAS, A. J. (1981). How do plant growth substances work? *Pl. Cell Environ.* 4: 203-228.

VAN LOON, L. C. (1986). The significance of changes in peroxidase in diseased plants. *In:* GREPPIN, H., C. PENEL & T. GASPAR (Eds.), *Molecular and physiological aspects of plant peroxidases:* 405-418. University of Geneva, Switzerland.

VENIS, M. A. (1984). Cell-free ethylene-forming systems lack stereochemical fidelity. *Planta* 162: 85-88.

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