BIOLOGIA PLANTARUM 35 (2): 229-236, 1993

Coleoptile removal-induced ethylene production in winter rye seedlings

G. IEVINSH, V. ILJIN and O. KREICBERGS

Laboratory of Plant Physiology, Institute of Biology, Latvian Academy of Sciences, 3 Miera Str., LV-2169 Salaspils, Latvia

Abstract

Coleoptile removal-induced ethylene production was investigated in light-grown winter rye seedlings. Removal of the coleoptile induced 1-aminocyclopropane-1-carboxylic acid (ACC) synthesis and ethylene production by primary leaves and caused an inhibition of elongation growth of the leaves. The activity of ethylene-forming enzyme (EFE) was associated with the increase in ethylene evolution. Both rise in ethylene and ACC production, as well as EFE activity were inhibited by cycloheximide. Wounding the tissue 40 min after the initial treatment resulted in the second increase in ethylene evolution. Derooting of the seedlings without coleoptile removal did not induce ethylene production. It is suggested that the coleoptile represents a barrier for wound-induced ethylene production from actively growing leaf tissue.

Introduction

Actively growing tissues usually produce ethylene at a high rate (Fuchs and Lieberman 1968, Saltveit and Dilley 1978a, Satler and Kende 1985). In grass seedlings, these tissues are confined to the nodes and are localized inside the coleoptile or the surrounding leaf (Kemp 1980). This suggests that wound-induced ethylene production in the leaf tissue of grass seedlings may be affected by the surrounding coleoptile.

Ethylene production can be regulated by the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase or ethylene-forming enzyme (EFE) (Yang and Hoffman 1984, Kende 1989). An increase in ACC synthase is responsible for the increase in ethylene production observed during ripening or stress (Yang and Hoffman 1984). Increase in EFE has also been noted as a result of stress and ethylene treatment (McKeon *et al.* 1982, Hyodo *et al.* 1985, Riov and Hausman 1988).

Received 27 March 1992, *accepted* 10 November 1992.

Abbreviations: ACC - 1-aminocyclopropane-1-carboxylic acid; EFE - ethylene-forming enzyme; MACC - 1-(malonylamino)cyclopropane-1-carboxylic acid.

The aim of the present investigation was to characterize the effect of coleoptile removal on ethylene production and EFE activity in light-grown winter rye seedlings and on elongation growth of their primary leaves.

Materials and methods

Winter rye (*Secale cereale* L. cv. Priekulu) seeds were surface sterilized in 0.1 M $KMnO_4$, imbibed in distilled water for 6 h and then sown in moist vermiculite. Plants were grown for 72 h in dark at 24 °C and then in white luminiscent light (irradiance 25 W m^{-2} for 16 h) with day/night temperature of 20/17 °C. Tap water was used for watering. The seedlings were used for experiments one day after transfer to light, when they reached length of about 50 mm and the primary leaf had emerged from the coleoptile and grown an additional 5-10 mm.

Plants were either left intact, wounded, or cut into appropriate parts. In some experiments the leaf was liberated from the coleoptile by slitting along its length without separating it from the seedling and the leaves were pulled free. The length of primary leaves was measured within 8 d after the treatment. The effect of wounding was also measured in detached primary leaves. Five leaves were cut into 2 to 14 segments. Ethylene production was measured by placing 5 seedlings (intact, wounded, or excised sections, approximately 300-400 mg) in 15 cm^3 serum bottles. The bottles containing 0.1 cm^3 water were sealed with rubber stoppers and incubated at 28 °C in darkness. Ethylene accumulation was measured after 1 h.

The time course of ethylene production and EFE activity was measured using 20 - 25 leaves (about 500 mg) without coleoptiles. The leaves were infiltrated with either water, 0.1 mM cycloheximide, 1 mM ACC or 1 mM ACC plus 0.1 mM cycloheximide. The infiltrated leaves were incubated in sealed bottles covered with aluminium foil at room temperature. Ethylene was measured every 10 min over the next 4 h. The bottles were flushed with air after each measurement.

Ethylene was measured with a gas chromatograph fitted with an Al_2O_3 column and a flame ionization detector (Ievinsh *et al.* 1990).

ACC extraction and measurements was performed as described previously (Ievinsh *et al.* 1990) using the method of Lizada and Yang (1979). The total amount of free and bound ACC was analysed after acidic hydrolysis (Schierle and Schwark 1988).

The content of 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC) was calculated by subtracting the ACC content before hydrolysis from that after hydrolysis.

Each experiment was repeated 2-3 times with similar results. All measurements were carried out in 3-5 replicates.

Results

Cutting winter rye seedlings in parts induced an increase in ethylene production (Table 1). Following the cutting, 80 % of the ethylene produced was by the primary

leaf. As shown in Fig. 1A, a lag period of about 30 min preceeded the increase in ethylene production by the leaves. Following the lag period ethylene production increased for 40 min, the maximum rate being 14-fold higher than the basal level. Infiltration of leaves with 0.1 mM cycloheximide immediately after cutting completely prevented the increase in ethylene production.

Tab. 1. Ethylene production by intact and segmented winter rye seedlings. Data shown are the means ± SE of 5 samples. Each assay was run in duplicate.

Plant part	C_2H_4 production [nmol h^{-1} g^{-1} (f.m.)]	[nmol h^{-1} seedling^{-1}]
Intact seedling	0.15 ± 0.02	0.010 ± 0.001
Primary leaf	1.09 ± 0.26	0.025 ± 0.005
Coleoptile	0.07 ± 0.01	0.002 ± 0.0
Root	0.11 ± 0.01	0.004 ± 0.001

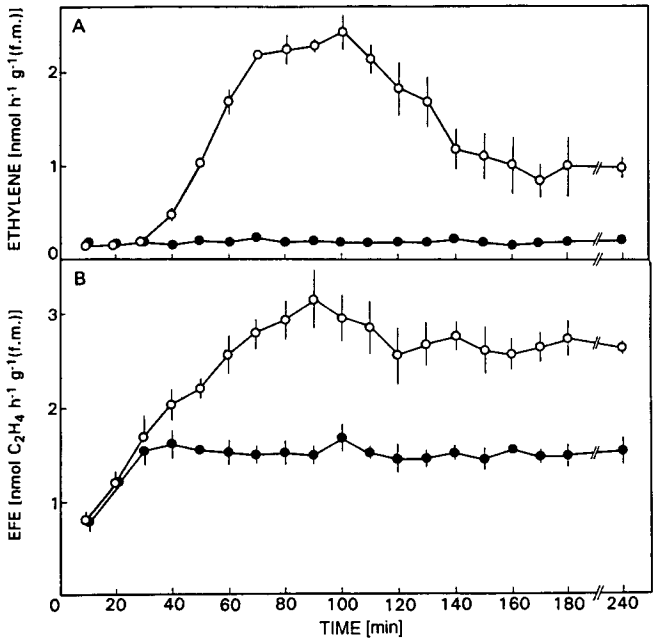

Fig. 1. Time-course of ethylene production (A) and EFE activity (B) in detached primary leaves of winter rye seedlings (controls - *open circles*, infiltrated with 0.1 mM cyclohexamide - *closed circles*). Values represent the mean of 3 samples ± SE, where SE bars are larger than symbols.

The increase in ethylene production was accompanied by an increase in EFE activity (Fig. 1B). No lag period was observed for the increase in EFE. The rise in EFE

occurred for 90 min after the detachement and remained high for the rest of the experiment. Cycloheximide prevented about one half of the increase in EFE activity.

Tab. 2. Effect of coleoptile on ACC and MACC content in winter rye primary leaves incubated in moist chamber. Means ± SE of 3 samples for each measurement.

Leaves	Time after detaching [min]	ACC [nmol g^{-1} (f.m.)]	MACC [nmol g^{-1} (f.m.)]
Covered	0	3.60 ± 0.0	5.20 ± 0.30
	90	4.74 ± 0.15	6.71 ± 0.38
Liberated	90	4.46 ± 0.18	5.06 ± 0.30
Liberated + cycloheximide	90	3.48 ± 0.20	5.13 ± 0.32

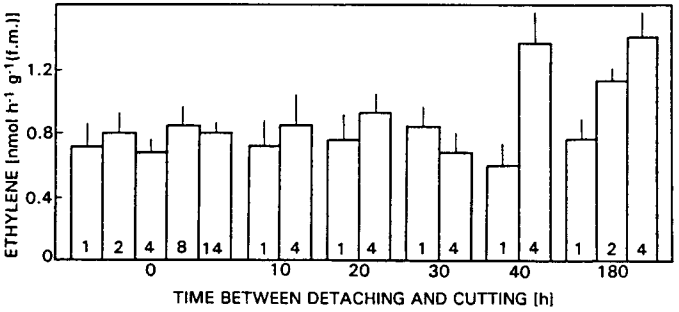

Fig. 2. Effect of the degree of tissue injury on ethylene production in primary leaves of winter rye seedlings. Numbers on columns indicate the number of segments. Leaves were detached, liberated from coleoptile and incubated in moist chamber for 0-180 min, then segmenting was performed. Values are means of 3 samples ± SE.

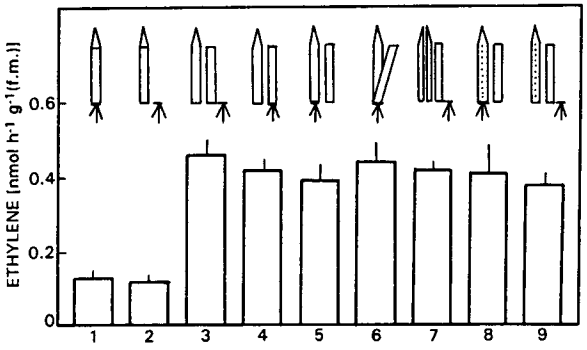

Fig. 3. Ethylene production in winter rye seedlings as a response to different types of wounding. The sketches show the type of wounding performed immediately before incubation. Values are means of 4 samples ± SE.

The detachement of leaves induced ACC synthesis irrespective of the presence or absence of coleoptile (Table 2). The rise in ACC content was completely inhibited by cycloheximide. Nevertheless the content of MACC was higher in detached leaves incubated with coleoptile in comparison to non-incubated or liberated leaves.

The effect of the extent of cutting the tissue on ethylene production is shown in Fig. 2. The results showed that the rate of ethylene production was not a function of the degree of tissue injury, if cutting the leaves was performed within 30 min after the detachement. However, 40 min after detachement, cutting the tissue promoted ethylene production.

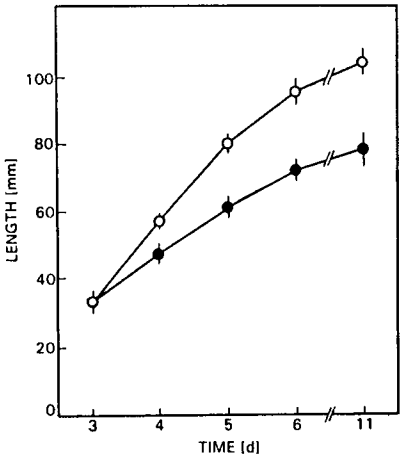

Fig. 4. Effect of coleoptile removal on growth of primary leaves of intact winter rye seedlings (controls - *open circles*, coleoptile removed on the 3rd day - *closed circles*). Values are means from 30 seedlings ± SE.

The data in Fig. 3 illustrate the observation that the increase in ethylene production was due to the removal of the coleoptile, rather than solely to wounding, as there were no differences in ethylene production between intact and derooted seedlings. However, removal of the coleoptile induced ethylene production irrespective of the presence or absence of the roots. Therefore, we investigated the effect of coleoptile removal on growth of primary leaves of intact seedlings. Removal of coleoptiles from the seedlings where the primary leaf had not yet emerged from the coleoptile, caused an inhibition of their elongation growth (Fig. 4).

Discussion

The results from the present study demonstrate that in winter rye seedlings two types of induced ethylene production can be defined. (*1*) The primary response was caused by separation of the primary leaves from the coleoptile and appeared after a lag phase of about 30 min. Wounding had no effect on this initial rise in ethylene production. (*2*) Wound-induced ethylene production was evident if cutting the tissues was done

40 min after the detachement, *i.e.* at the time when the actual increase in ethylene production due to coleoptile removal started. This type of response seemed to be dependent on the degree of wounding. It is thus possible that contradictory data according to a correlation between the degree of tissue injury and the rate of ethylene production (Imaseki *et al.* 1968, Imaseki *et al.* 1971, Saltveit and Dilley 1978b) are connected not only with the specificity of the object used but also with the differences in experimental conditions (*e.g.* period between primary and secondary wounding, duration of incubation period prior to ethylene determination).

In accordance with earlier studies (Abeles and Abeles 1972, Saltveit and Dilley 1979, Konze and Kwiatkowski 1981) we found that cycloheximide suppressed rapidly induced ethylene production in the leaves of rye seedlings (Fig. 1A). *De novo* synthesis of ACC synthase protein has been shown to occur as a result of tissue wounding (Boller and Kende 1980). Recently it has been reported that in some situations *e.g.* during ethylene treatment or pathogen stress the induced activity of EFE as well is blocked by cycloheximide (Schierle *et al.* 1989, Boller 1990). The data presented here suggest that both ACC synthesis and EFE in rye seedling leaves increased following removal of the coleoptile and that the effect may be due to protein synthesis. The increase in EFE activity during the first 30 min (with and without cycloheximide) seems to be due to a gradual increase in ACC supply after infiltration the tissues as a result of the relatively slow ACC diffusion.

The question still remains, what is the physiological significance of coleoptile removal-induced ethylene production in cereal seedlings? First, time-course of ethylene emanation after the leaf liberation from the coleoptile (Fig. 1A) represents the typical case of wound ethylene formation demonstrated previously (Saltveit and Dilley 1978a, Konze and Kwiatkowski 1981). Second, solely the derooting of seedlings without removal of the coleoptile did not induce an increase in ethylene production (Fig. 3). Third, the removal of the coleoptile from non-emerged leaves of intact rye seedlings caused the same effect of growth inhibition (Fig. 4) as demonstrated for ethephon- and ACC-treated seedlings (Ievinsh and Romanovskaya 1991). Consequently, the coleoptile of growing cereal seedlings seems to represent a barrier for wound-induced ethylene production from actively growing leaf tissues. In this respect, grass seedlings are different from dicot seedlings where juvenile tissues which are able to produce high level of stress-induced ethylene are unprotected by covering tissues. The initial reason for suppressed ethylene production from wounded coleoptile-covered leaves may be the restriction of ethylene formation from ACC leading to accumulation of MACC.

Ethylene is shown to be involved in an inhibition of elongation growth of mechanically stressed seedlings (Goeschl *et al.* 1966). It is possible to suggest that coleoptile-protected ethylene production in stressed grass seedlings supported the unchanged growth pattern of the seedlings.

It has been suggested earlier that the emerging leaf of grass plants does experience a sudden change in environment as it emerges from the protection of older tissues (Begg and Wright 1962). It is possible that leaf emergence from coleoptile represents a certain kind of stress for the leaf tissues. Indeed, our results show that leaf

emergence from the coleoptile of intact cereal seedlings is associated with a sharp transient increase in ethylene evolution (Ievinsh and Kreicbergs 1992).

References

Abeles, A.L., Abeles, F.B.: Biochemical pathway of stress-induced ethylene. - Plant Physiol. **50**: 496-498, 1972.

Begg, J.E., Wright, M.J.: Growth and development of leaves from intercallary meristems in *Phalaris arundinacea* L. - Nature **194**: 1097-1098, 1962.

Boller, T.: Regulation of the biosynthetic pathway of ethylene in response to pathogen stress. - Physiol. Plant. **79**(Suppl.): A150, 1990.

Boller, T., Kende, H.: Regulation of wound ethylene synthesis in plants. - Nature **286**: 259-260, 1980.

Fuchs, Y., Lieberman, M.: Effects of kinetin, IAA, and gibberellin on ethylene production, and their interactions in growth of seedlings. - Plant Physiol. **43**: 2029-2036, 1968.

Goeschl, J.D., Rappaport, L., Pratt, H.K.: Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. - Plant Physiol. **41**: 877-884, 1966.

Hyodo, H., Tanaka, K., Yoshiaka, J.: Induction of 1-aminocyclopropane-1-carboxylic acid synthase in wounded mesocarp tissue of winter squash fruit and the effects of ethylene. - Plant Cell Physiol. **26**: 161-167, 1985.

Ievinsh, G., Iljin, V., Kreicbergs, O., Romanovskaya, O.: Effect of ethephon on the activity of the ethylene-forming enzyme and the biosynthesis of ethylene in winter rye seedlings. - Biochem. Physiol. Pflanzen **186**: 221-228, 1990.

Ievinsh, G., Kreicbergs, O.: Endogenous rhythmicity of ethylene production in growing intact cereal seedlings. - Plant Physiol.**100**: 1389-1391, 1992.

Ievinsh, G., Romanovskaya, O.: Accelerated lignification as a possible mechanism of growth inhibition in winter rye seedlings caused by ethephon and 1-aminocyclopropane-1-carboxylic acid. - Plant Physiol. Biochem. **29**: 327-331, 1991.

Imaseki, H., Uritani, I., Stahmann, M.A.: Production of ethylene by injured sweet potato root tissue. - Plant Cell Physiol. **9**: 757-768, 1968.

Imaseki, H., Pjon, C.-J., Furuya, M.: Phytochrome action in *Oryza sativa* L. IV. Red and far red reversible effect on the production of ethylene in excised coleoptiles. - Plant Physiol. **48**: 241-244, 1971.

Kemp, D.R.: The location and size of the extension zone of emerging wheat leaves. - New Phytol. **84**: 729-737, 1980.

Kende, H.: Enzymes of ethylene biosynthesis. - Plant Physiol. **91**: 1-4, 1989.

Konze, J.R., Kwiatkowski, G.M.K.: Rapidly induced ethylene formation after wounding is controlled by the regulation of 1-aminocyclopropane-1-carboxylic acid synthesis. - Planta **151**: 327-330, 1981.

Lizada, M.C.C., Yang, S.F.: A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. - Anal. Biochem. **100**: 140-145, 1979.

McKeon, T.A., Hoffman, N.E., Yang, S.F.: The effect of plant-hormone pretreatments on ethylene production and synthesis of 1-aminocyclopropane-1-carboxylic acid in water-stressed wheat leaves. - Planta **155**: 437-443, 1982.

Riov, J., Hausman, R.: Regulation of water-stress induced ethylene in citrus leaves. - Israel J. Bot. **37**: 83-91, 1988.

Saltveit, M.E., Dilley, D.R.: Rapidly induced wound ethylene from excised segments of etiolated *Pisum sativum* L. cv. Alaska. I. Characterization of the response. - Plant Physiol. **61**: 447-450, 1978a.

Saltveit, M.E., Dilley, D.R.: Rapidly induced wound ethylene from excised segments of etiolated *Pisum sativum* L. cv. Alaska. III. Induction and transmission of the response. - Plant Physiol. **62**: 710-712, 1978b.

Saltveit, M.E., Dilley, D.R.: Studies of rapidly induced wound ethylene synthesis by excised sections of etiolated *Pisum sativum* L. cv. Alaska. IV. Requirement of a water-soluble, heat-stable factor. - Plant Physiol. **64**: 417-420, 1979.

Satler, S.O., Kende, H.: Ethylene and the growth of rice seedlings. - Plant Physiol. **79**: 194-198, 1985.

Schierle, J., Rohwer, F., Bopp, M.: Distribution of ethylene synthesis along the etiolated pea shoot and its regulation by ethylene. - J. Plant Physiol. **134**: 331-337, 1989.

Schierle, J., Schwark, A.: Asymmetric synthesis and concentrations of ethylene in the hypocotyl hook of *Phaseolus vulgaris*. - J. Plant Physiol. **133**: 325-331, 1988.

Yang, S.F., Hoffman, N.E.: Ethylene biosynthesis and its regulation in higher plants. - Annu. Rev. Plant Physiol. **35**: 155-189, 1984.

Communicated by I. MACHÁČKOVÁ