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The Regulation of Maize Mesocotyl Growth by Ethylene and Carbon Dioxide

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Abstract : Ethylene stimulated the elongation of maize mesocotyls of whole seedings under red light. Maximum elongation was obtained with $1\mu l\,l^{-1}$ ethylene. The length of mesocotyls was still much longer than in ethylene-free air when ethylene concentrations were increased to 10,100 or $1{,}000\mu l\,l^{-1}$. Ethylene also induced lateral expansion of mesocotyls at ethylene concentrations of $0.1\mu l\,l^{-1}$ or above and the diameter increased at higher ethylene concentratins. Carbon dioxide, in the range of 1-4%, also stimulated mesocotyl extension and expansion. Maximum growth of mesocotyls was obtained in a mixture of ethylene and carbon dioxide. Removal of either endogenously evolved ethylene or carbon dioxide or of both gases reduced elongation. In contrast to the effects in red light, ethylene inhibited the growth of mesocotyls in darkness while carbon dioxide inhibited this effect of ethylene. Thus, ethylene and carbon dioxide acted cooperatively under red light and antagonistically in darkness.
Key words : Carbon dioxide, Darkness, Ethylene, Maize, Mesocotyl, Red light.

エチレンと二酸化炭素によるトウモロコシの中茎の生長の制御：西沢武明・菅　洋（東北大学遺伝生態研究センター）
要　旨：エチレンは赤色光下で，トウモロコシ芽生えの中茎の伸長を促進した．最大伸長は，エチレンの1 $\mu l\,l^{-1}$ で得られた．中茎の長さは，エチレン濃度が，10,100又は1,000 $\mu l\,l^{-1}$ まで増大してもまだ，エチレンを除去した気中のそれよりは長かった．エチレンは，0.1 $\mu l\,l^{-1}$ より高い濃度で中茎の，横方向への伸展を引き起こした．二酸化炭素は，テストした1-4%の範囲内で同様に中茎の，縦方向への伸長と，横方向への伸展を引き起こした．中茎の最大生長は，エチレンと二酸化炭素の共存下で得られた．内生エチレン，あるいは二酸化炭素，又はその両者の除去は，伸長の低下をもたらした．赤色光下の影響と対照的に，エチレンは暗黒下で中茎の生長を阻害し，一方，二酸化炭素は，エチレンのこの効果を抑制した．このように，エチレンと二酸化炭素は，赤色光下では協同的に働き，暗黒下では拮抗的に働いた．
キーワード：暗黒下，エチレン，赤色光，中茎，トウモロコシ，二酸化炭素．

Germinating seedlings in soil are exposed to ethylene and carbon dioxide produced by the seedlings themselves and by soil microorganisms[2]. This production of ethylene and carbon dioxide is especially important for seeds located deep beneath the soil surface and for those in compacted or poorly aerated soils where gas exchange is limited[7]. Germination in soil may give mechanical stress on germinating seedings, and this increases ethylene production from the plants[8]. Ethylene as a factor regulating the growth of seedlings subjected to physical stress was first discussed by Goeschl et al.[4].

The mesocotyl, the internode between the scutellar node and the coleoptile in the embryo and seedling of a grass[3], has an adaptive significance in the germination process of grass since it functions to elevate the apical meristem up to near the soil surface.

It is important to study the role of ethylene and carbon dioxide in the elongation of mesocotyls. Herein, we examine this in maize seedlings. While ethylene is known to inhibit root growth in maize[6,18,19], no information is available on how ethylene controls the growth of mesocotyls, coleoptiles and the first leaf although the leaf extension and the growth of the whole maize plants were reported to be inhibited by ethylene[6]. We present findings which indicate that ethylene stimulates the growth of maize mesocotyls and acts cooperatively with carbon dioxide under red light. On the other hands, in darkness ethylene was found to retard the growth of mesocotyls and carbon dioxide exerted the opposite effect in darkness.

Materials and Methods

1. Plant material

Seeds of maize, *Zea mays* L, var Evata were obtained from Watanabe Seed Co., Kogota, Japan. The seeds were originally imported from the U.S.A. This type of maize is convenient for experiments because the seeds are small and can more readily be placed in capsules for gas treatments, compared with large

seeded types. Seeds were washed in running water to remove fungicide and placed on wet filter paper in large Petri dishes (12 cm in diameter) and incubated for 24 hrs at 25°C in the dark. Germinating seeds with 1-mm-long coleoptiles were selected for uniformity and planted in the centre wells of glass capsules made specially for ethylene treatment[11], filled with wet vermiculite. Eight seeds were placed 5 mm-deep in the centre well of each capsule. Duplicate capsules were used for each treatment. Measurements were made on 8 to 12 plants selected for their uniformity from 16 plants in duplicate capsules.

2. Ethylene and carbon dioxide treatments

Ethylene and/or carbon dioxide were introduced through a vaccine cap fitted to the lower mouth of the capsule with a gas-tight syringe. The capsule was separated into two parts connected to each other by a ground-glass seal coated with high vacuum silicone grease to make a gas-tight seal. In addition, both parts were secured by rubber bands attached to the outside of the capsule. The lower part had a centre well and an opening. When it was necessary to prevent the accumulation of endogenously evolved ethylene and/or carbon dioxide, a small tube (1.9x3.5mm) containing 2m*l* of mercuric perchlorate (0.25M in 2.0M perchloric acid) and/or KOH (20%) was attached to the outside of the centre well.

After transfer, these capsules were maintained for 7 days in darkness, or in red light, at 27°C. All procedures including sowing of seed were conducted under a green light when red light needd to be excluded.

3. Red light irradiation

Red light irradiation was provided by Mitsubishi coloured fluorescent lamps (RFL-20R-R) with a peak at 645nm (607-688nm); the level of irradiation was 5.43μmolm^{-2} s^{-1} at the glass surface. Sixty-five percent of the incident light was transmitted through the glass, with no spectral dependence, providing a radiant energy of 3.53μmolm^{-2} s^{-1} at the plant level. Under such irradiance plants were grown for 7 d until they were measured.

4. Statistical comparisons

All data were treated with Student-Newman-Keuls multiple range test. Different letters in the figures indicated significant difference ($P<0.05$).

Results

1. Effects of ethylene

The effects of different concentrations of ethylene on mesocotyl elongation are shown in Fig. 1. Ethylene stimulated both elongation and lateral growth of maize mesocotyls under red light. The volume of mesocotyls obtained by a formula, πx (1/2 diameter)^{2}x length, also increased with increased ethylene concentrations. The maximum volume was obtained at 100$\mu l l^{-1}$ ethylene and slightly decreased from this the maximum value in 1,000$\mu l l^{-1}$ due to an insignificant reduction of diameter. Mesocotyls were longest in 1$\mu l l^{-1}$ ethylene and were shorter in concentrations higher than 10$\mu l l^{-1}$, although they were still much longer than those in air without ethylene or in capsules in which only endogenously evolved ethylene was present. It should be noted that endogenously evolved carbon dioxide was present in all treatments shown in Fig. 1.

In contrast, the growth of coleoptiles was not greatly affected by ethylene although ethylene concentrations higher than 10$\mu l l^{-1}$ were slightly inhibitory. Elongation of the first leaf was more inhibited by high concentrations of ethylene, especially those above 10$\mu l l^{-1}$.

2. Effects of carbon dioxide

In some samples, endogenously evolved ethylene and/or carbon dioxide were removed by KOH or mercuric perchlorate. As shown in Fig. 2, carbon dioxide was found to stimulate the growth of mesocotyls under red light. Within the concentration 1-4% range tested, 2% carbon dioxide was most effective at stimulating growth (Fig. 2) although the differences were small. Lateral expansion was also promoted by carbon dioxide, and the diameter of mesocotyls was increased with increasing concentrations of carbon dioxide. Thus, the volume of mesocotyls increased considerably as carbon dioxide concentrations were increased from 0% to 4% compared with plants where these gases were allowed to accumulate. Removal of ethylene and/or carbon dioxide resulted in less mesocotyl growth (Fig. 2). However, the diameters of mesocotyls are not markedly altered by removal of ethylene and/or carbon dioxide. The growth of coleoptiles was not greatly altered by treatment with carbon dioxide, although the gas showed a

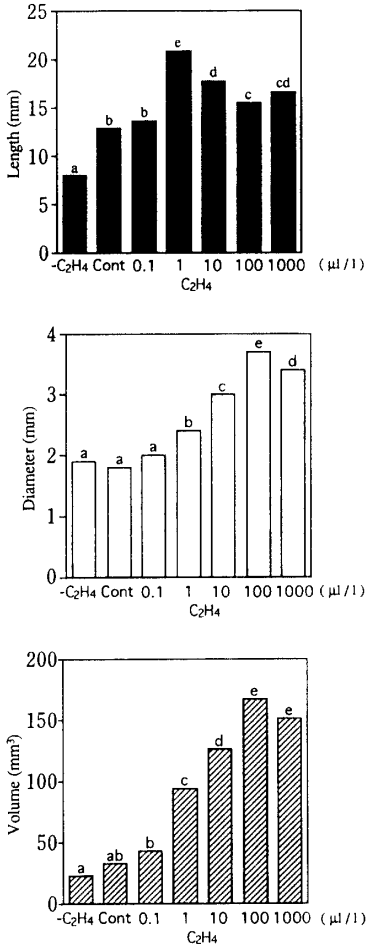

Fig. 1. Effects of different concentrations of ethylene on the growth of maize mesocotyls under **red light** for 7 days. $-C_2H_4$ indicates that endogenously evolved ethylene was removed by mercuric perchlorate. Control (Cont) contains endogenously evolved ethylene. In all treatments, endogenously evolved carbon dioxide was present. Different letters indicate significant difference ($P<0.05$).

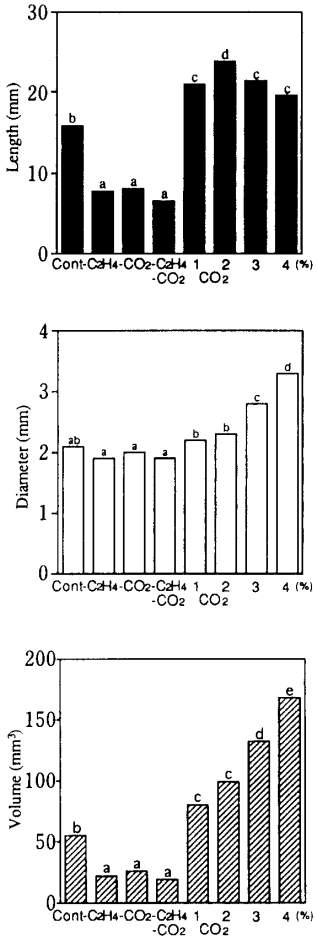

Fig. 2. Effects of different concentrations of carbon dioxide on teh growth of mesocotyls under **red light** for 7 days. Control (Cont) contains endogenously evolved ethylene and carbon dioxide. $-C_2H_4$ and $-CO_2$ indicate that endogenously evolved ethylene and carbon dioxide, respectively, were removed. In all treatments with different concentrations of carbon dioxide, endogenously evolved ethylene was present. Different letters indicate significant difference ($P<0.05$).

tendency to inhibit the growth as the concentration was increased. The growth of the first leaf showed a similar trend. Again it should be noted that both ethylene and carbon dioxide stimulated not only longitudinal growth but also lateral expansion in maize mesocotyls under red light (Fig. 3). High concentrations of ethylene and carbon dioxide interfered with the expansion of the first leaf (Fig. 3). In this experiment, the effect of different concentrations of carbon dioxide on the growth of mesocotyls was determined in the presence of endogenously evolved ethylene allowed to accumulate in the growth capsules.

3. Interaction between ethylene and carbon dioxide

Since endogenous carbon dioxide (first experiment) and ethylene (second experiment) were also present, it is necessary to determine the effects of added ethylene and/or carbon dioxide independently. Thus, a third experiment was undertaken. Ethylene and/or carbon dioxide were added at the concentrations shown to have the maximum effect in the previous experiments. Fig. 4 indicates that extension and volume growth of maize mesocotyls was maximal in the presence

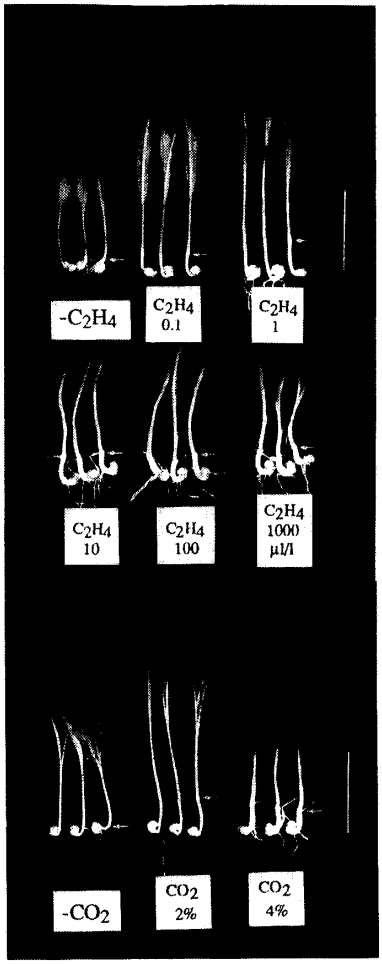

Fig. 3. Photographs showing the concurrent stimulation of longitudinal elongation and lateral expansion of maize mesocotyls under **red light** for 7 days. $-C_2H_4$ and $-CO_2$ indicate endogenously evolved ethylene or carbon dioxide, respectively, was removed. White arrows indicate the position of coleoptilar nodes. White scale bars indicate 5 cm.

of both ethylene and carbon dioxide, under red light. The length of mesocotyls was minimal in the absence of both gases ($-C_2H_4$ $-CO_2$). Addition of both gases at the optimal concentrations (1 $\mu l\,l^{-1}$ ethylene and 2% carbon dioxide) stimulated the growth in length of mesocotyls under red light (Fig. 5).

For an increase in diameter, the presence of either ethylene or carbon dioxide is important, but both treatment did not increase diameter of the mesocotyls beyond that of carbon dioxide or ethylene given alone. The numbers of secondary roots induced from the mesocotyl increased with the addition of ethylene, and there was some evidence that carbon dioxide inhibited root formation (Fig. 5). Growth of coleoptiles was affected to some extent by the various combinations of carbon dioxide and ethylene (Fig. 6). However, the growth of the first leaf was increased in ethylene-free air only when carbon dioxide was present (Fig. 6).

4. Effects of ethylene and carbon dioxide under darkness

Finally, we determined the effects of ethylene and carbon dioxide on the growth of maize mesocotyls in darkness. As shown in Fig. 7, ethylene inhibited the growth of mesocotyls in the dark, while carbon dioxide exerted the opposite effect. The length of mesocotyls was decreased when endogenously evolved or exogenous ethylene was present. The length of mesocotyls was shortest when ethylene was added to the air from which endogenously evolved carbon dioxide had been removed ($+C_2H_4-CO_2$). Removal of endogenously-evolved carbon dioxide ($-CO_2$) from control air that contained endogenously evolved ethylene also resulted in short mesocotyls indicating that small amounts of ethylene might be sufficient to inhibit the growth if carbon dioxide is absent. Thus, the inhibitory effect of ethylene was largely overcome by the presence of carbon dioxide. The effect of carbon dioxide itself was not clear since the addition of exogenous carbon dioxide in the absence of ethylene ($-C_2H_4+CO_2$) did not result in excess elongation as compared with the results obtained in the air where only endogenously evolved carbon dioxide was present ($-C_2H_4$). The diameter of mesocotyls was changed in some extent in various combinations of two gas factors, however these changes were not significantly different (Fig. 7).

Discussion

Ethylene has been known to stimulate the longitudinal growth in many aquatic and semi-aquatic plants, although it usually inhibits that of terrestrial plants[1]. Elongation of mesocotyls has adaptive significance in the germination process of seeds and the present findings that ethylene and carbon dioxide stimulates mesocotyl elongation even in land plants such as maize are very interesting since physical barriers have been shown to increase the shoot ethylene production[8].

When seeds of dicotyledonous plants germinate, plumular hooks push up soil to help the

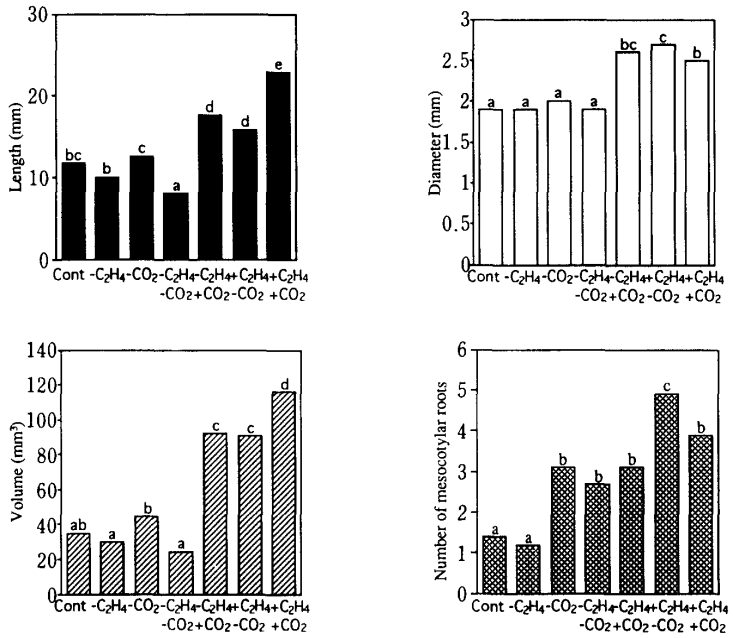

Fig. 4. Effects of endogenously and added ethylene and carbon dioxide on the elongation of maize mesocotyls under **red light** for 7 days. Cont: endogenously evolved C_2H_4 and CO_2 present. $-C_2H_4$: endogenously evolved C_2H_4 removed and endogenously evolved CO_2 present. $-CO_2$: endogenouslye volved CO_2 removed and endogenously evolved C_2H_4 present. $-C_2H_4-CO_2$: both endogenously evolved C_2H_4 and CO_2 removed. $-C_2H_4+CO_2$: endogenously evolved ethylene removed and CO_2 (2%) added. $+C_2H_4-CO_2$: endogenously evolved carbon dioxide removed and C_2H_4 ($1\mu l\,l^{-1}$) added. $+C_2H_4+CO_2$: both ethylene ($1\mu l\,l^{-1}$) and carbon dioxide (2%) were added. Different letters indicate significant difference ($P<0.05$).

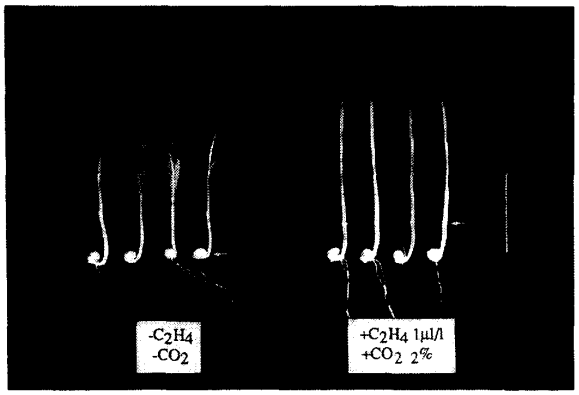

Fig. 5. Photograph showing the difference of seedling growth under **red light** between the control from which both endogenously evolved ethylene and carbon dioxide were removed and the experimental group in which both gases were exogenously added at the optimum concentrations. White scale bar indicates 5 cm.

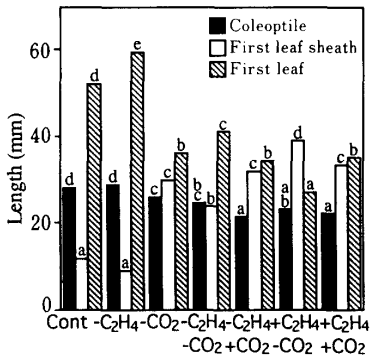

Fig. 6. Effects of endogenously and added ethylene and carbon dioxide on the elongation of coleoptiles and the first leaf under **red light** for 7 days. In each group of data within different organs, different letters indicate significant difference ($P<0.05$).

seedlings to emerge from the soil surface. Soil resistance stimulates ethylene biosynthesis by the seedlings. Ethylene inhibits the longitudinal elongation of the stem and promotes lateral expansion. Thus physical stress may control the radial growth of seedlings via ethylene biosynthesis[4].

In monocotyledonous plants, on the other

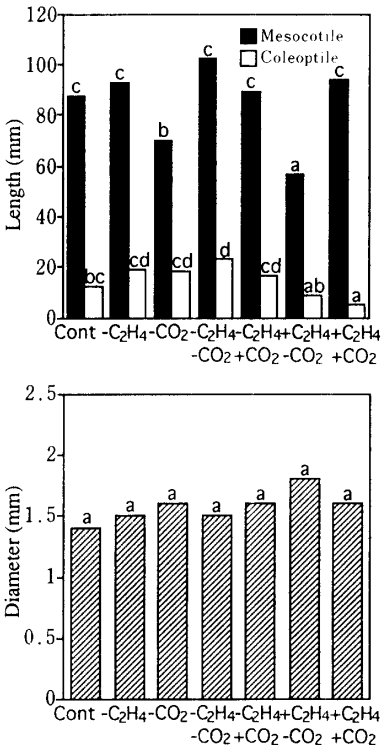

Fig. 7. Effects of endogenously and added ethylene and carbon dioxide on the elongation of maize mesocotyls and coleoptiles under **darkness** for 7 days. Different letters indicate significant difference ($P<0.05$).

hand, no hook, is formed, while the apices are held within the colepitle until they emerge. Ethylene evolution was found to increase in rice seedlings subjected to physical stress and this resulted in an increase in mesocotyl length [12].

The present findings that ethylene stimulates mesocotyl elongation even in land plants such as maize while inhibiting the growth of other plant organs of the same seedlings, i.e., coleoptiles and the first leaf, are interesting. This is easy to understand if we consider the adaptive role of mesocotyl in the process of germination. As mentioned above, the mesocotyl is an organ which elevates the apical meristem to a point near the soil surface, especially when the seed is located deep in the soil.

However, it generally appears that physiologically and ecologically significant amounts of light rarely penetrate more than 4-5 mm through the soil[14]. Under darkness, ethylene was found to inhibit the growth of maize mesocotyls, while carbon dioxide acted antagonistically to the effect of ethylene; thus more detailed study is needed to elucidate the ecological role of ethylene more precisely in relation to the growth of mesocotyls in soil.

Mesocotyls of maize can increase their length more than 100 mm under total darkness in air without any physical impedance. Ethylene inhibition of maize mesocotyl elongation in the longitudinal direction under darkness is not so great, as we showed there in the case of 1 $\mu l l^{-1}$ although much higher concentrations of ethylene may induce the greater inhibition; ethylene rather induced lateral expantion. This is rather favorable for overcoming the effect of the impedance given by compact soil structures as Goeschl et al.[4] suggested in dicotyledonous pea seedlings.

In land plants such as bluegrass, *Poa pratensis* L[10,15-17], ethephon, an ethylene releasing agent, can increase tiller internode length by increasing cell length. Since the growth of tiller shoots is always under the control of apical dominance of the main shoot, it is not easy to discuss those findings directly in relation to ethylene action. In oat seedlings, however, ethylene was reported to stimulate the growth of mesocotyls in darkness[9,11].

The maize plant usually has long mesocotyls[5] and mesocotyls are often seen above the soil surface. Indeed roots from the cotyledonary node or from higher nodes (brace roots) support maize plants. Thus, the ability of maize mesocotyls to elongate even under light in response to ethylene may have some ecological significance, although precise conclusions await further study. Especially it is important to define the boundaries of light energy between the inhibition and the promotion of mesocotyl elongation by ethylene. Remarkably, sorghum seedlings, which also have long mesocotyls, respond to ethylene in light and darkness in a similar way to ethylene[13].

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