

## Opposite Effects of Ethylene on the Growth of Sorghum Mesocotyl under Red Light and in Darkness

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**Abstract :** Ethylene inhibited the growth of sorghum coleoptile and mesocotyl in darkness. It also inhibited the development of mesocotylar roots in darkness. However, ethylene rather stimulated the growth of mesocotyl under red light in a certain range of concentrations, 0.1-10 ppm, although it inhibited the growth of coleoptile and first leaf under the same condition. The optimum concentration of ethylene for mesocotyl growth under the light was different depending on the type of sorghum variety. Ethylene also induced the radial expansion of mesocotyl, especially at concentrations higher than 100 ppm under red light. The volume of mesocotyl increased in all ethylene concentrations tested. Development of mesocotylar roots was rather stimulated by ethylene under red light. Carbon dioxide acted antagonistically with ethylene in the growth of mesocotyl and coleoptile in darkness. Thus, sorghum mesocotyl is a unique organ in which ethylene stimulates not only longitudinal growth but also radial expansion.

**Key words :** Carbon dioxide, Coleoptile, Ethylene, Mesocotyl, Mesocotylar root, Red light, Sorghum.

ソルガムの中茎の生長に対するエチレンの赤色光中と暗中ででの反対の作用 : 菅 洋・西沢武明 (東北大学遺伝生態研究センター)

**要 旨 :** エチレンはソルガムの子葉鞘と中茎の生長を暗中で阻害した。さらにエチレンは暗中で中茎根の発育を阻害した。しかし、エチレンはある一定の範囲の濃度 (0.01-10 ppm) で中茎の生長を光中で促進した。子葉鞘と第1葉の生長は同じ条件下で阻害した。中茎の生長に対するエチレンの最適濃度は、ソルガム品種のタイプにより異なった。エチレンはまた光中で特に 100 ppm 以上の濃度で中茎の横方向への拡大を誘起した。したがって、中茎の容積は供試したエチレンのどの濃度下においても、エチレンを除去したものに比べて増加した。中茎根の発育は光中においては、エチレンによりむしろ促進された。二酸化炭素は暗中所において、中茎と子葉鞘の生長にエチレンの作用に拮抗的に働いた。ソルガムの中茎はエチレンにより縦方向への生長と横方向への拡大が同じ器官において同時に起こるユニークな器官である。

**キーワード :** エチレン, 子葉鞘, 赤色光, ソルガム, 中茎, 中茎根, 二酸化炭素。

Mesocotyl of monocotyledonous plants has an important function to raise the meristem to near the soil surface, especially when the seed is located deep below the surface. Soil structure give physical stress to seedlings in the process of germination and this increases the production of ethylene from the seedling. In fact, the first demonstration on the role of endogenous ethylene as a growth regulator in intact plants was the finding of Goeschl et al.<sup>1)</sup> that a sharp increase in ethylene production and in radial expansion occurs when the elongation of the etiolated pea epicotyl is mechanically impeded as it would be while emergence from the soil.

On the other hand, it is reported that ethylene stimulated the growth of rice and oat mesocotyls<sup>8)</sup> and red light inhibited ethylene production from plants while far red light partially recovered the inhibition<sup>2)</sup>. Usually, red light inhibits the growth of mesocotyls<sup>5)</sup>. It

is important to study ethylene-light interaction in the growth of mesocotyls to uncover the role of ethylene in the germinating process. Here, we examined this by way of sorghum seedlings.

### Materials and Methods

Seeds of sorghum, *Sorghum vulgare* Pers., were soaked for one day in darkness at 27° C. Soaked seeds were selected for uniformity and 10 seeds were planted on a wetted vermiculite prepared in the center well (5 cm in diameter and 4 cm in depth) of a glass-made capsule (500 ml in volume), designed especially for ethylene treatment<sup>8)</sup>. Ethylene was introduced through a vaccine cap fitted to the lower mouth of the capsule with a gas-tight syringe.

In some cases, a small tube (18×35 mm) containing either 5 ml of 20% KOH to remove evolved CO<sub>2</sub>, or 5 ml of mercuric perchlorate to remove evolved ethylene, was placed in the

vermiculite of the center well.

Seedlings in the capsule were incubated either in darkness or under red light at 27°C. Light irradiation was provided by 3 Mitsubishi colored fluorescent lamps (RFL-20 R-F) with a peak at 645 nm (607-668 nm) and the irradiation constituted  $11 \text{ W cm}^{-2}\text{s}^{-1}$  at the glass surface. Sixty-five percent of the incidental light was transmitted through the glass, with no spectral dependence, providing a radiant energy of  $7.15 \text{ W cm}^{-2}\text{s}^{-1}$  at the plant level. Under such irradiance plants were grown for 7 days. Measurement of coleoptile, mesocotyl and first leaf sheath was made 7 days after the start of irradiation. Two types of varieties, grain sorghum type and grass sorghum type were used.

### Results

Effects of ethylene on the growth of mesocotyl and coleoptile of the grain sorghum variety CSH-2 in darkness are shown in Fig. 1. Ethylene inhibited the growth of intact sorghum mesocotyl and coleoptile in darkness, although inhibition was not so great. If ethylene is, indeed, a growth regulator for controlling sorghum mesocotyl and coleoptile growth, then the possibility is that growth of the mesocotyl and coleoptile may be affected when sorghum seedlings are grown in an ethylene-free atmosphere. A variety of conditions, with or without evolved or added ethylene, were tested therefore. Results with cv. Swarna, a grain sorghum variety, are shown in Table 1. Carbon dioxide acted antagonistically with ethylene in darkness. The growth inhibition induced by 20 ppm ethylene and by endogenously evolved ethylene in darkness

was increased when endogenously evolved carbon dioxide was removed. (Table 1). The development of mesocotylar roots was inhibited by ethylene (Fig. 2). The inhibition was observed even in low concentrations of ethylene. The growth was inhibited at 0.1 ppm although the number of roots was hardly affected in this concentration. Not only the development but also the growth of mesocotylar roots was inhibited at 1 ppm and 10 ppm. At 100 ppm, very small numbers of short mesocotylar roots were observed and almost no mesocotylar roots were found at 1,000 ppm ethylene (Fig. 2).

Irradiation of red light greatly reduced the growth of mesocotyl but red light did not completely suppress the growth of mesocotyl. Interestingly enough, ethylene, in the range of

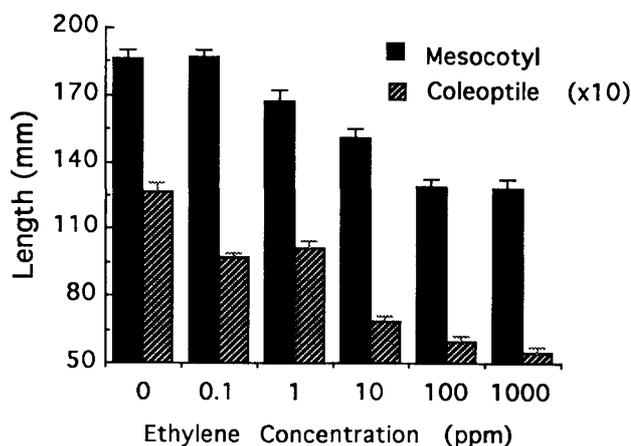


Fig. 1. Growth of sorghum (cv. CSH-2) mesocotyl and coleoptile as affected by different concentrations of ethylene in darkness. 0 ethylene indicates endogenously evolved ethylene removed with mercuric perchlorate. Vertical bars indicate standard error.

Table 1. Effects of ethylene and carbon dioxide on the growth of sorghum (cv. Swarna) and seedlings in darkness.

	Length of mesocotyl (mm)	Length of coleoptile (mm)
Air control (endogenous $\text{CO}_2$ and $\text{C}_2\text{H}_4$ )	$133.2 \pm 12.7$	$12.5 \pm 1.1$
No $\text{C}_2\text{H}_4$ (endogenous $\text{CO}_2$ )	$129.1 \pm 12.9$	$15.7 \pm 1.6$
No $\text{CO}_2$ (endogenous $\text{C}_2\text{H}_4$ )	$109.6 \pm 9.0$	$13.4 \pm 1.4$
No $\text{CO}_2$ , No $\text{C}_2\text{H}_4$	$121.1 \pm 10.8$	$13.4 \pm 1.5$
No $\text{CO}_2$ , 20 ppm	$89.2 \pm 9.2$	$7.2 \pm 0.8$
$\text{C}_2\text{H}_4$ 20 ppm	$118.7 \pm 9.1$	$6.9 \pm 0.4$

The values in the table show confidence limit at 95% confidence coefficient from the average of 10 plants.

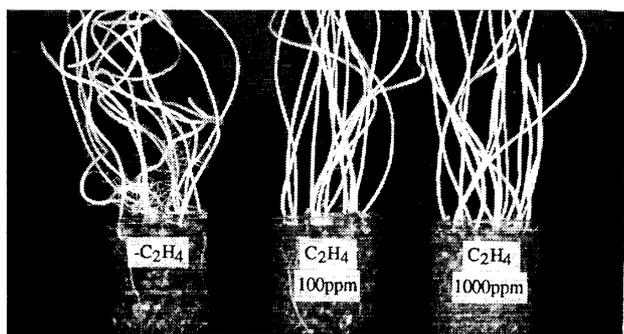


Fig. 2. Development of mesocotylar roots of sorghum seedlings (cv. CSH-2) as affected by ethylene in darkness. From right to left; No ethylene (endogenously evolved ethylene removed), 100 ppm ethylene added and 1,000 ppm ethylene added.

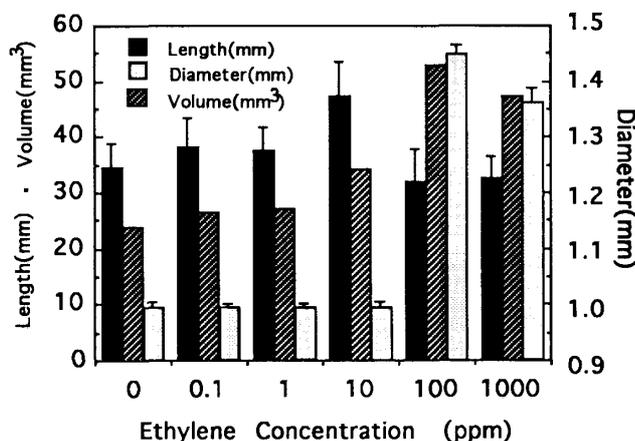


Fig. 3. Growth of mesocotyl as affected by different concentrations of ethylene under red light (cv. CSH-2). 0 ethylene indicates endogenously evolved ethylene was removed using mercuric perchlorate. Vertical bars indicate standard error.

0.1-10 ppm, promoted the growth of mesocotyl under red light (Figs. 3, 4 and 5) when comparison was made between seedlings grown in ethylene-removed air and those grown in ethylene-added air. Stimulation of mesocotyl growth was much greater in the grass variety, New Sorgo, with a slender stem than in the grain variety, CSH-2 with thicker stem. Optimum concentration for inducing maximum growth was lower in the former variety than in the latter variety. The growth of mesocotyl was maximum at 10 ppm ethylene in the grain type CSH-2 whereas that of grass variety New Sorgo was at a maximum at 1 ppm ethylene. Ethylene also stimulated the expansion of

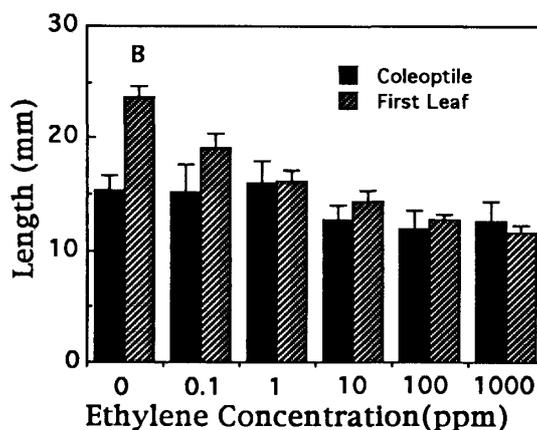
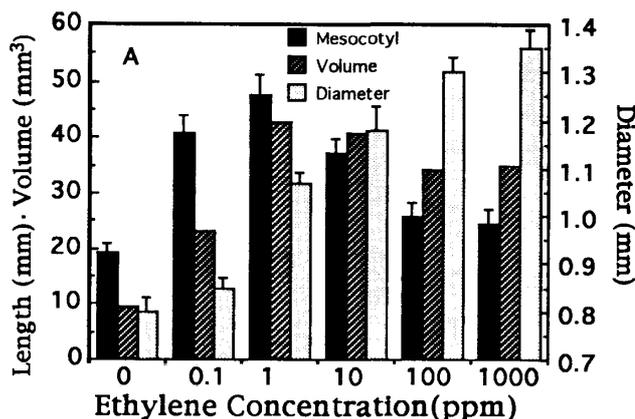


Fig. 4. Growth of sorghum seedlings (cv. New Sorgo) as affected by different concentrations of ethylene under red light. Vertical bars indicate standard error.

mesocotyls to lateral direction in all ranges of concentrations tested. The radial expansion was greater with the increase of ethylene concentration (Figs. 3 and 4). With the presence of 100 ppm ethylene, diameter of mesocotyl was increased to 155% of untreated control plant in the grain variety CSH-2. The volume of mesocotyl was calculated by multiplying length and area; area was calculated by multiplying square of the radius ( $r^2$ ) and the circular constant ( $\pi$ ). The volume of mesocotyl was increased in all ethylene concentrations tested. Furthermore, ethylene stimulated the development of mesocotylar roots under red light. Ethylene slightly inhibited the growth of coleoptile and first leaf sheath under red light (Table 2, Fig. 4).

### Discussion

Ethylene inhibited the growth of sorghum mesocotyl and coleoptile in darkness. However, under red light, ethylene stimulated the

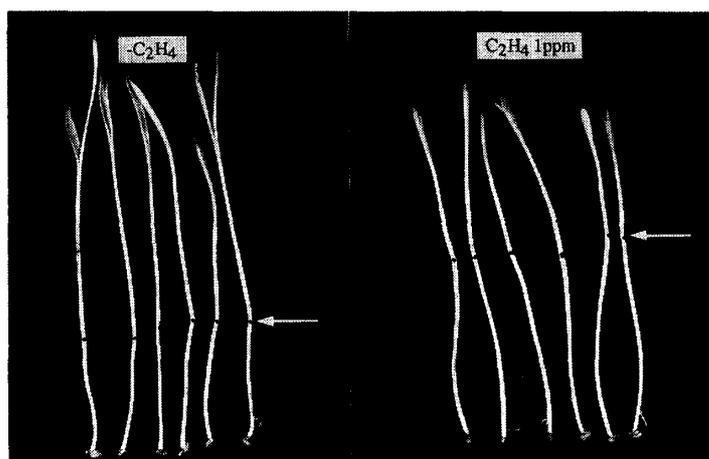


Fig. 5. Growth of sorghum seedlings (cv. New Sorgo) as affected by ethylene (1 ppm) under red light.  $-C_2H_4$  indicates endogenously evolved ethylene was removed using mercuric perchlorate. Arrows and black dots indicate the division between mesocotyl and coleoptile. The lower part is the mesocotyl and the upper part is the coleoptile. First leaf is already expanded from the coleoptile.

Table 2. Effects of ethylene on the growth of coleoptile and first leaf in sorghum (cv. CHS-2) seedlings under red light.

Ethylene (ppm)	Length of coleoptile (mm)	Length of first leaf sheath (mm)	Length of first leaf blade (mm)	Width of first leaf blade (mm)
0	$12.7 \pm 1.0$	$40.8 \pm 3.1$	$19.0 \pm 1.6$	$5.3 \pm 0.2$
0.1	$12.8 \pm 1.6$	$37.4 \pm 2.9$	$16.4 \pm 1.6$	$5.1 \pm 0.3$
1	$12.4 \pm 1.2$	$37.9 \pm 4.9$	$17.7 \pm 1.1$	$5.0 \pm 0.2$
10	$11.7 \pm 0.6$	$36.6 \pm 4.2$	$12.9 \pm 1.4$	$5.4 \pm 0.4$
100	$10.7 \pm 0.5$	$33.9 \pm 3.2$	$10.0 \pm 0.6$	$5.6 \pm 0.3$
1000	$10.2 \pm 0.7$	$16.9 \pm 5.3$	$8.4 \pm 0.9$	$4.6 \pm 0.3$

0 ethylene indicates endogenously evolved ethylene was removed using mercuric perchlorate. The values in the table show confidence limit at 95% confidence coefficient from the average of 10 plants.

growth of mesocotyl in a certain range of concentration, 0.1-10 ppm, although ethylene showed slight inhibition at much higher concentrations. Ethylene concentrations that induced maximum elongation under light deferred, depending on the sorghum variety. Mesocotyl growth in grain variety gave a maximum growth, with 10 ppm ethylene, however that in the grass variety gave a maximum growth and much lower concentrations of ethylene.

Moreover, if we define plant growth as volume increase, not as increase in length of longitudinal direction, ethylene stimulated the growth of mesocotyl in all concentrations tested, from 0.1 to 1,000 ppm, not only in the grain variety, but also in the grass variety of sorghum, although ethylene inhibited the growth of coleoptile and first leaf. Thus, the mesocotyl of sorghum is a very unique organ since ethylene stimulates not only longitudinal growth but also lateral expansion at the same time.

The elongation of etiolated *Avena* mesocotyls was reported to be inhibited in red light and this response is almost saturated at 30  $mj/cm^2$ . The inhibition of mesocotyl growth in red light can be reversed partially by subsequent exposure to far-red light<sup>5)</sup>. The growth inhibition of sorghum mesocotyl under red light might be saturated under the present experimental condition, although no kinetic analysis undertaken. Moreover, ethylene partially reversed the inhibition of mesocotyl growth induced by red light. Interestingly enough, effects of ethylene on the development of mesocotylar roots were also opposite under light and in darkness.

On the other hand, the growth of coleoptile and first leaf sheath was not stimulated by ethylene under red light. Carbon dioxide acted antagonistically with ethylene in the growth of mesocotyl and coleoptile in darkness. In rice seedlings, ethylene stimulated coleoptile growth both in darkness<sup>4)</sup> and in red light<sup>9)</sup> and

carbon dioxide acted synergistically with ethylene in the growth of mesocotyl and coleoptile in darkness<sup>4,8</sup>). Thus, the response of sorghum mesocotyl and coleoptile to carbon dioxide was different from rice seedlings, although ethylene also acted as a stimulator to mesocotyl growth under red light. However light inhibition of rice mesocotyl growth was not recovered even with ethylene.

The present findings showed the existence of another type of plant group for ethylene response. Suge<sup>8</sup>) found that ethylene stimulated the growth of oat mesocotyl, however the precise ethylene-light relationship is not clear in the previous experiment since ethylene treatment was done in darkness. Here the procedure for setting of soaked seeds into the glass-made capsule for ethylene treatment was done in room conditions under artificial light and light energy was not determined. Growth of *Avena*<sup>6</sup>) and wheat<sup>7</sup>) coleoptile was inhibited by ethylene.

Goeschl et al.<sup>1</sup>) have suggested that the mechanical loading of soil may cause the epicotyls of germinating pea seeds to produce additional quantities of ethylene and in turn regulate the force of emerging shoot can exert on the soil. Ethylene production was increased by non wounding physical stress and ethylene acts as an endogenous growth regulator, decreasing elongation and increasing diameter in response to increasing increments of stress.

In sorghum seedlings, the role of mesocotyl in the process of germination seems a little more complex because the growth was rather stimulated in a certain range of ethylene concentrations under red light, although it was inhibited in darkness. The role of ethylene in the germination process of monocotyledonous plants must be considered differently to that of dicotyledonous plants. Dicotyledonous plants form plumular hooks, and this hook formation is important to establish seedlings in natural environments. On the other hand, monocotyledonous plants do not form plumular hooks. The apical meristem, however, is protected within coleoptile and this is important to establish the seedlings in natural environments. Mesocotyl is also important to raise the position of meristems to the soil surface, especially when seeds are located deep below the soil surface. The role of ethylene in

monocotyledonous plants must be considered in this connection.

When plants grow within soil, it is necessary to obtain the force coming from physical stress of the soil. Thus, increased production of ethylene inhibited the growth in longitudinal direction and induced radial expansion. However, interestingly enough, the growth of sorghum mesocotyl in longitudinal direction was promoted under red light by a certain range of concentrations of ethylene. This indicates ethylene plays an important role in the process of seedling establishment, although more detailed information is needed to explain this response more precisely.

Hoshikawa<sup>3</sup>) widely surveyed the underground organs of seedlings in Gramineae plants. Sorghum seedling was classified as Type M that mesocotylar roots are the longest and are more than 8-10 in number. The development and growth of these mesocotylar roots was inhibited by ethylene in darkness and stimulated under light. Thus the effect of ethylene on the growth and development of mesocotylar roots was also found to be opposite in darkness and under light.

### References

1. Goeschl, J.D., L. Rappaport and H.K. Pratt 1966. Ethylene as a factor regulating the growth of pea epicotyl subjected to physical stress. *Plant Physiol.* 41 : 877—884.
2. Goeschl, J.D., H.K. Pratt and B.A. Bonner 1967. An effect of light on the plumular portion of etiolated pea seedlings. *Plant Physiol.* 42 : 1077—1080.
3. Hoshikawa, K. 1969. Underground organs of the seedlings and the systematics of Gramineae. *Bot. Gaz.* 130 : 192—203.
4. Ku, H.S., Rappaport H.L. Suge, and H.K. Pratt 1970. Stimulation of rice coleoptile growth by ethylene. *Planta* 90 : 333—339.
5. Loercher, L. 1966. Phytochrome changes correlated to mesocotyl inhibition in etiolated *Avena* seedlings. *Plant Physiol.* 41 : 932—936.
6. Marinos, N.G. 1960. Some responses of *Avena* coleoptiles to ethylene. *J. Exp. Bot.* 11 : 227—235.
7. Roberts, R.W.A. 1951. Some effects of ethylene on germinating wheat. *Can. J. Bot.* 29 : 10—25.
8. Suge, H. 1971. Stimulation of oat and rice mesocotyl growth by ethylene. *Plant Cell Physiol.* 12 : 831—837.
9. Suge, H. 1984. Synergistic action of ethylene with gibberellins in the growth of rice seedlings. *Proc. Crop Sci. Soc. Japan* 43 : 83—87.
10. Suge, H., N. Katsura and K. Inada 1971. Ethylene-light relationship in the growth of rice coleoptile. *Planta* 101 : 365—368.