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Ethylene Control of Anthocyanin Synthesis in Sorghum

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ABSTRACT

Light-induced anthocyanin synthesis in *Sorghum vulgare* L. seedlings was both promoted and inhibited by ethylene treatment. The rate of anthocyanin formation in sorghum tissue was dependent upon the time of ethylene treatment in relation to light exposure and the stage of the anthocyanin synthesis process. Those plants receiving ethylene treatment during the early lag phase of anthocyanin synthesis had higher anthocyanin content at 24 hours than control plants receiving no ethylene treatment. Plants receiving ethylene treatment after the lag phase had lower anthocyanin content at 24 hours than control plants receiving no ethylene treatment.

Light-induced anthocyanin formation in plants is influenced by the addition of ethylene to the gaseous atmosphere around the plant tissue. Early observations by Kropfitsch (8) on *Vicia faba* indicated that ethylene inhibited anthocyanin synthesis in the nectaries but had no effect on anthocyanin development in the stem. Other reports have indicated that ethylene inhibits anthocyanin formation in bean seedlings (9) and that ethylene promotes anthocyanin synthesis in cranberry fruit (2). It was the purpose of this paper to determine the role of ethylene in light-induced anthocyanin synthesis in sorghum tissue.

MATERIALS AND METHODS

Plant Material. *Sorghum vulgare* cv. DeKalb C45 was used in these studies. Seeds were surface-sterilized with 0.5% sodium hypochlorite solution for 10 min, rinsed with distilled water for 2 min, and planted on 10 ml of sterile agar contained in 125-ml Erlenmyer flasks. The flasks were wrapped with aluminum foil to exclude light, stoppered with cotton plugs to prevent airborne contamination, and placed in an incubator at 27 ± 1 C for germination and growth in the dark.

Experimental Procedure. Seedlings were used in experiments after 3 or 4 days of growth in the dark. Those flasks containing seedlings for ethylene treatments were sealed with rubber vaccine caps, and ethylene was injected into the gaseous atmosphere around the seedling tissue with a hypodermic needle and syringe (2). Control flasks had cotton plugs. For light treatment the aluminum foil was removed and the flasks were placed under NuLite No. 40WT12 fluorescent tubes at 10^4 ergs cm^{-2} sec^{-1} for the experimental treatment time. Temperature was maintained at 27 ± 2 C.

For anthocyanin analysis 10 sections of 10 mm each were cut from seedling tissue just proximal to the first node at the end of the experimental treatment time. Anthocyanin was extracted from the sections overnight in the refrigerator with 4 ml of methanol-HCl (99:1, v/v) (11). Absorbance was read in a spectrophotometer at the anthocyanin-sensitive wavelength of 525 nm (11).

The influence of light on ethylene production in the sorghum tissue was determined on 10-mm sections of tissue like those used for anthocyanin analysis. The sections were cut from dark-grown tissue under a safelight filter made of green Rohm and Haas acrylic that produced no detectable effect on anthocyanin synthesis in seedlings even after prolonged exposure. After cutting, sections were left on moist filter paper for 6 hr for dissipation of wound ethylene. The sections of tissue were then sealed in 10-ml Erlenmyer flasks containing 2 ml of sterile 1.5% agar solution with rubber vaccine caps. Light treatment was the same as for the other experiments. Ethylene concentration in the flask was measured by injecting a 1-ml sample of gas from the flask into a gas chromatograph equipped with a flame ionization attachment. Oven temperature was 150 C, and nitrogen flowed at 60 ml/min through a 6-mm outer diameter, 150-cm activated alumina column. Identity of the gas was established by cochromatography with standards.

Calculation of Rate of Synthesis. The rate of anthocyanin synthesis at different times for plants receiving no ethylene treatment was taken from data in Figure 1 and is the slope of the curve at 2-hr increments. The rate of anthocyanin synthesis at different times in the presence of ethylene was taken from data in Figures 1 and 3. It was assumed that anthocyanin followed the normal ethylene-free synthesis pattern until ethylene was added and that the slope of the line connecting this point with the measured amount of anthocyanin present at the end of 24 hr was representative of the rate of synthesis under ethylene at the time ethylene was added. The rate of anthocyanin synthesis at different times for plants receiving ethylene initially but with subsequent removal was taken from data in Figure 4. It was assumed that anthocyanin synthesis under ethylene followed a straight line connecting the initial amount of anthocyanin with the measured amount of anthocyanin after 24-hr treatment with ethylene. Synthesis would follow this line until ethylene was removed, and the line connecting this point with the measured amount of anthocyanin in the tissue at 24 hr would be representative of the rate of synthesis at the time ethylene was removed.

Unless otherwise stated, all experiments were repeated a minimum of three times with three replicates for each treatment.

RESULTS

Normal light-stimulated synthesis of anthocyanin in sorghum seedlings showed an initial lag phase of 8 hr after the dark-grown seedlings were placed in the light (Fig. 1). Rapid formation of anthocyanin began at the end of the lag phase and continued linearly throughout the time in which these experiments were conducted.

The measured ethylene inhibition of anthocyanin synthesis in sorghum after 24 hr at different ethylene concentrations is shown in Figure 2. Higher concentrations of ethylene in the

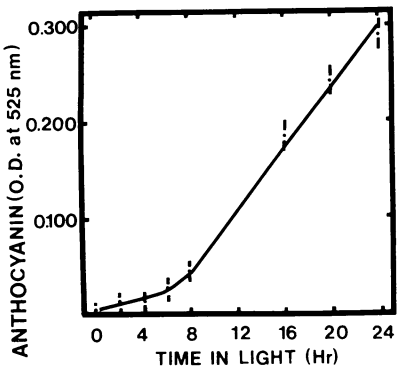

FIG. 1. Time course of anthocyanin synthesis in sorghum seedlings. Dark grown seedlings were placed in light at 0 hr and harvested at indicated times. Means ± SE.

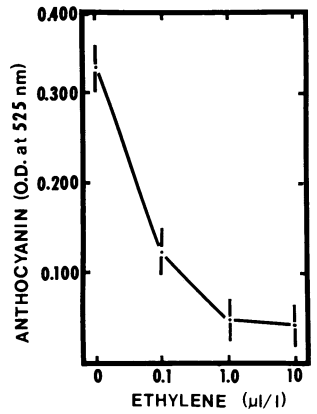

FIG. 2. Inhibition of anthocyanin formation by ethylene. Dark grown seedlings were exposed to ethylene and light for 24 hr. Initial is amount of anthocyanin in dark grown seedlings. Means ± SE.

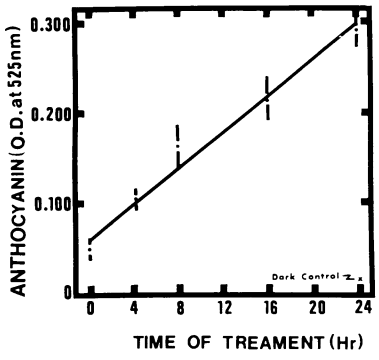

FIG. 3. Effect of ethylene on anthocyanin synthesis in sorghum. Dark grown seedlings were placed in light at 0 hr, treated with ethylene at indicated times, and harvested at 24 hr. Means ± SE.

gaseous atmosphere around the seedlings increased the inhibition of anthocyanin formation with a maximal response at about 1 µl/liter of ethylene.

Data in Figure 3 demonstrated that application of ethylene to the gaseous atmosphere around sorghum seedlings at various times after planting them in the light decreased the total anthocyanin content measured at 24 hr as compared to plants receiving no ethylene. However, the ethylene inhibition of anthocyanin synthesis does not follow the light-stimulated pattern shown in Figure 1. Ethylene applied during the lag phase did not keep the relative levels of anthocyanin low as in the lag phase of control plant anthocyanin synthesis. This suggested that ethylene did not inhibit processes in the lag phase of anthocyanin synthesis but was only effective during the rapid synthesis stage.

Data in Figure 4 shows the effects of application and removal of ethylene at different times during the light-stimulated synthesis of anthocyanin. Treatment with ethylene during the lag phase increased anthocyanin content of tissue measured at 24 hr as compared to control tissue. Treatment with ethylene after 8 hr decreased the amount of anthocyanin in the tissue at 24 hr as compared to control tissue.

The effects of different ethylene treatments on rates of anthocyanin synthesis in sorghum are shown in Figure 5. Treatment of sorghum seedlings with ethylene at different times and maintaining the ethylene treatment until plants are harvested at 24 hr indicated that the ethylene initially stimulated the rate of synthesis in the lag phase but inhibited the rate of synthesis compared to control plants as the seedlings enter the rapid synthesis stage at about 8 hr after placing

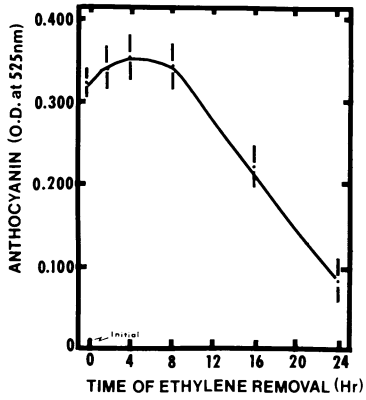

FIG. 4. Effect of ethylene on different stages of anthocyanin synthesis in sorghum. Dark grown seedlings were placed in light and treated with ethylene at 0 hr. Ethylene was removed from bottle at times indicated by a 3-min flush of air. Plants were harvested at 24 hr. Initial is amount of anthocyanin in dark grown seedlings. Means ± SE.

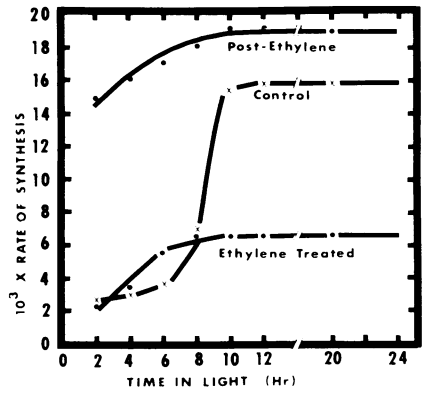

FIG. 5. The effect of ethylene on rate of anthocyanin synthesis. The control rates are from nonethylene-treated plants. The ethylene-treated rates are from ethylene-treated plants and represent the rate of synthesis under ethylene. The postethylene-treated rates are from ethylene-treated plants and represent the rate of synthesis after the ethylene is removed.

them in the light. Treatment with ethylene as sorghum seedlings are placed in the light with subsequent ethylene removal showed that ethylene accelerated the rate of anthocyanin synthesis as compared to control plants receiving no ethylene treatment.

Ethylene production by sections of light-treated sorghum seedlings is presented in Table I. Light increased ethylene production as compared with those sections kept in the dark.

Table I. *Ethylene Production by Light-treated Sorghum Seedlings*

Sections like those used for anthocyanin analysis were cut from dark-grown seedlings under green safelight, allowed 6 hr for wound ethylene dissipation, and sealed in 10-ml Erlenmyer flasks. In trials 1 and 2, ethylene content of the flasks was measured after 24 and 20 hr, respectively. After ethylene measurement in trial 2, the bottles were evacuated to remove ethylene, and the light and dark treatments were reversed with ethylene measurement 6 hr later on the same sections for trial 3. Means of four replicates ± SE.

Treatment	Ethylene Production		
	Trial 1	Trial 2	Trial 3
	nl/g·hr		
Light	5.8 ± 0.7	4.9 ± 0.4	5.7 ± 0.5
Dark	2.4 ± 0.5	3.9 ± 0.7	3.2 ± 0.3

DISCUSSION

Our observed time course of light-stimulated anthocyanin formation in sorghum tissue receiving no ethylene treatment was like that previously reported by Vince (12) showing two stages or phases of synthesis (Fig. 1). There was an initial lag phase followed by a second rapid synthesis phase.

As reported for other plants (8, 9), data presented in this paper demonstrated that ethylene could act as an inhibitor of light-stimulated anthocyanin synthesis in sorghum. The active ethylene concentrations were similar to those reported for other plant processes regulated by ethylene (10).

Although measurements presented in Figure 2 indicated that ethylene inhibited anthocyanin synthesis, data in Figures 3 and 4 demonstrated that this inhibition occurred only during the second or rapid synthesis stage of anthocyanin formation. Treatment with ethylene during the initial lag phase from 0 to 8 hr did not maintain low quantities of anthocyanin in the tissue (Fig. 3) comparable to the levels synthesized by that time from light treatment alone (Fig. 1). Anthocyanin synthesis continued in the presence of ethylene until the end of the lag phase of about 8 hr. These observations suggested that ethylene did not inhibit those processes occurring during the lag phase of anthocyanin synthesis, but that ethylene was inhibitory to anthocyanin formation only during the second rapid synthesis phase.

Treatment of sorghum seedlings with ethylene as they were initially placed in the light, with removal of ethylene before the lag phase was completed, increased the quantities of anthocyanin produced by 24 hr (Fig. 4). These results suggest that the ethylene increases the rate of anthocyanin formation in sorghum tissue if applied during the lag phase of anthocyanin synthesis. Ethylene production by sorghum tissue should have no influence in these experiments since ethylene treatment was at 10 μl/liter and any ethylene requirement should be satisfied by this level (Fig. 2).

Confirmation of an increased rate of anthocyanin synthesis was obtained by examining the rates of anthocyanin synthesis in light-stimulated tissue having different ethylene treatments. The rate of anthocyanin synthesis in control tissue increases as the seedlings are exposed to light and reaches a steady state by about 8 to 10 hr (Fig. 5). Application of ethylene at various times after initiation of the light treatment indicated that with ethylene present the resulting rate of synthesis is stimulated during the lag phase, but that the rate of synthesis becomes steady at a much lower level than plants receiving no ethylene treatment (Fig. 5). Treating the plants with ethylene as they are placed in light and subsequently removing the ethylene induce a very rapid rate of anthocyanin synthesis as measured after the ethylene is removed. The rate of synthesis becomes constant at a higher level than for tissue receiving no ethylene treatment.

These differences in rates explain the observations in Figures 3 and 4 as ethylene treatment changes the rate of anthocyanin synthesis by sorghum tissue. This rate is higher or lower than that of control tissue depending upon whether ethylene is present during phase I or phase II of the anthocyanin synthesis process. If ethylene is present in phase I, it promotes anthocyanin production and if ethylene is present in phase II, it inhibits anthocyanin production.

Previous work by Stafford (11) using excised sorghum tissue sections and metabolic inhibitors has established that light-stimulated cyanidin formation is dependent upon production of mRNA during the initial lag phase. Data in Figures 3 and 4 showed that ethylene accelerated the processes in the lag phase of anthocyanin production and are consistent with the idea that the ethylene stimulated mRNA generation involved with anthocyanin biosynthesis. This would agree with other reports of ethylene-induced increases in RNA production in soybean hypocotyls (5, 6) and abscission zone explants (4).

The mode of action of ethylene inhibition of anthocyanin synthesis in seedling tissue differs from that reported for auxin-like compounds in that the latter extend the lag phase (1, 12). It is similar to these compounds, however, in that IAA reportedly reduced the rate of anthocyanin synthesis in IAA-treated tissue. Further investigations are necessary to determine whether the differences and similarities of auxin and ethylene inhibition of anthocyanin synthesis are related to effects on light-stimulated nucleic acid and enzyme synthesis or enzyme activity.

Measurement of ethylene production from sections of sorghum seedlings showed increased ethylene production when tissue was treated with light. This observation agrees with data from another anthocyanin-producing tissue (2), and differences between these observations and those reported by other workers (3, 7) may be due to differences in the light treatment spectrum or differences in plant tissue. A complete explanation of increased anthocyanin production as related to tissue ethylene production cannot be determined with data reported in this paper. However, it appears that initial ethylene synthesis could promote rapid synthesis of anthocyanin in the tissue and that ethylene synthesis after the lag phase is completed could limit the rate of anthocyanin production by sorghum tissue.

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