Effect of ethylene treatment on ethylene production, EFE activity and ACC levels in peel and pulp of banana fruit

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Abstract

Exogenous ethylene treatment (100 ppm) induces preclimacteric bananas (Musa acuminata Collar. cv. Dwarf Cavendish) to ripen with increased respiration and endogenous ethylene production; 12 h treatment was slightly more effective than 6 h. In transverse sections of bananas the contribution of the peel to respiratory and ethylene increases after induction by exogenous ethylene was negligible and the pulp provided all the output. Increases in respiratory CO₂ and ethylene by banana slices were generally in line with the inductive period of ethylene treatment. Banana slices treated with ethylene show an increase in EFE activity in both peel and pulp, with that in the peel being shown earlier. The effect is related to the duration of treatment. Ethylene-treated slices contained higher levels of conjugated ACC, but not free ACC, than control slices, but there was no clear link with the length of exposure to exogenous ethylene. The increase in ACC conjugation activity could explain the initial lower ethylene production shown by slices treated with exogenous ethylene.

Key words: Bananas; Banana peel; Banana pulp; Ethylene induction; ACC; EFE activity

1. Introduction

In plant tissues, ethylene is produced from methionine via S-adenosyl methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) (Yang, 1981). ACC synthase and ethylene forming enzyme (EFE) are the key enzymes in the control of ethylene biosynthesis (McGlasson, 1985). In climacteric fruits, regulation of ethylene biosynthesis seems to depend as much on ACC availability as on the tissue capacity to convert ACC to ethylene (Yang, 1987). The ACC level can be regulated

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by the rate of its synthesis and its conversion to ethylene and by its conjugation to malonyl-ACC (MACC) (Yang and Hoffman, 1984). On the other hand, ethylene is capable of regulating the activity of EFE (Hoffman and Yang, 1982), ACC synthase (Bifler, 1984) and malonyl-ACC transferase (Liu et al., 1985b).

Treatment of climacteric fruits with ethylene induces ripening (Burg and Burg, 1962). Concentrations as low as 0.1 ppm ethylene can be effective in preclimacteric bananas (Peacock, 1972), although commercially treatments of about 1000 ppm are used (Inaba and Nakamura, 1988). The effect of any treatment depends on the sensitivity of the fruit, which increases with age, on ethylene concentration and on the period of exposure to ethylene (Inaba and Nakamura, 1986). The effect of ethylene seems to be related to its capacity to combine to receptors present in the tissue (Whitehead and Bossé, 1991).

In this paper, we show the effect of different periods of exogenous ethylene treatment on ethylene biosynthesis in whole banana fruit, transverse sections and pulp and peel tissue.

2. Material and methods

Plant material

Bananas (Musa acuminata Collar. cv. Dwarf Cavendish), slightly immature by commercial standards, were obtained from Tenerife. Fruits from the same hand (15–20) were used in each experiment to avoid differences in physiological development.

The bananas were washed with an aqueous fungicide solution containing 0.1% (w/v) Benlate and 0.2% (w/v) Ditane, and allowed to dry. Single fruits were placed in respiration jars and ventilated with humidified air (flow about 1.2 1 h⁻¹) at 24°C. In each experiment at least three whole fruits were kept as controls, measuring respiration and ethylene production.

Transverse 6 mm-thick whole slices were cut from whole fruits and handled as described by Palmer and McGlasson (1969). Sections of pulp or peel tissue were prepared by removing the peel from 6 mm-thick slices (Vendrell and McGlasson, 1971).

Ethylene treatment

Individual whole fruits or composite samples of slices (about 30 g FW) or of peel or pulp tissue were used. Fruit sections were treated 24 h after cutting. This is the time they take to recover from the mechanical stress of cutting. Samples were placed in closed 2-l jars, with a septum, through which ethylene (from a mixture of 5% in nitrogen) was injected to create a concentration of 100 ppm, which was verified by gas chromatography. Inside the jars a 20% solution KOH was placed in a small container to absorb the CO₂ produced, as described by Liu et al. (1985a). After treatment (6, 12, or 24 h), samples were ventilated to eliminate the ethylene and placed in ventilated jars at 24°C. Endogenous ethylene production and respiration were measured (see below). Some samples were used immediately after treatment to analyze EFE activity or ACC and MACC content.
Analysis of ethylene production

Single fruit or composite samples of slices were placed in ventilated jars and 2-ml samples were taken from the effluent air from each respiration jar and injected into a Perkin-Elmer 3920B gas chromatograph with a gas-tight syringe. Three fruit samples were used per treatment. The gas chromatograph was fitted with a flame ionization detector. Operating conditions were as follows: the column (2 m × 4 mm i.d.) was packed with 80–100 mesh activated alumina, oven temperature 110°C, nitrogen carrier gas 40 ml min⁻¹.

EFE activity

EFE activity was measured in vivo as described by Mansour et al. (1986). Peel and pulp discs, one of each, from three transverse whole slices, about 1 mm thick were incubated in the presence or absence of 5 mM ACC. Ethylene production was measured by gas chromatography after three hours of incubation at 24°C. Results are given in nmol C₂H₄ gFW⁻¹ h⁻¹.

ACC and MACC assay

ACC and MACC were measured in peel and pulp. In each measurement, tissue samples were taken from three transverse whole slices and placed immediately on dry ice. Samples of 1–2 g were extracted with 80% ethanol under reflux for 15 min. Ethanol was removed under vacuum at 40°C. Free ACC was measured directly on the aqueous extract following the method of Lizada and Yang (1979). Total ACC (free and conjugated) was measured from the aqueous extract hydrolysed with HCl (2N) at 100°C for 3 h, following the method of Hoffman et al. (1982). Results are given in nmol gFW⁻¹.

Analysis of respiration

Respiration was measured as CO₂ production. The effluent air from the ventilated jars was connected to a Cossma infrared gas analyzer, model Diamant 6000. Results from three fruit samples are given in μg CO₂ gFW⁻¹ h⁻¹.

Statistical analysis

ANOVA and two-way ANOVA were used to compare treatments. Differences between treatments are considered at a P < 0.05 level of significance.

3. Results

Effect of ethylene treatment on respiration and ethylene production in whole fruits

Fig. 1 shows the effect of treatment with 100 ppm ethylene for 6 or 12 h on respiration and ethylene production by whole bananas. Treatment with ethylene induces an increase in respiration similar to the respiratory climacteric. The rise in CO₂ production is immediate and related to the time of treatment (Fig. 1A). In the 12-h treatment the climacteric peak is reached in 3 days. The 6-h treatment has a similar effect but the climacteric peak is attained 1 day later. In both cases the
treated fruits show a slightly higher maximum of respiration (150 μg CO₂ gFW⁻¹ h⁻¹) than untreated fruits (130 μg CO₂ gFW⁻¹ h⁻¹), probably due to a higher synchronization in the induced respiration in the fruit tissue.

The effect of ethylene treatment on endogenous ethylene production shows similar results to those for respiration (Fig. 1B). The 12-h treatment induces an immediate increase in ethylene production, which reaches a maximum in 2 or 3 days (1.5 nl gFW⁻¹ h⁻¹), although this level is lower than that attained by control fruits (3–3.5 nl gFW⁻¹ h⁻¹). However, the ethylene production in treated fruits then increases steadily to reach the level of the controls, but showing clear symptoms of senescence (browning and softening). The 6-h treatment shows similar results than the 12-h, but with about 1 or 2 days delay.
**Effect of ethylene treatment on respiration and ethylene production by transverse whole slices**

Fig. 2 shows the effect of treatment with 100 ppm ethylene for 6, 12 or 24 h on whole slices, on respiration and on ethylene production. Treatment was applied 24 h after cutting, when the slices had already recovered from the mechanical stress. A treatment of 6 h induces an increase in respiration (100–110 µg CO₂ gFW⁻¹ h⁻¹) (Fig. 2A) that then decreases to lower levels, but higher than the controls. These samples show an advance of the climacteric rise of two days compared to controls. A treatment of 12 h induces a higher increase in respiration than the 6-h treatment, to the level of 130–140 µg CO₂ gFW⁻¹ h⁻¹ which, after a slight decrease, again returns to the levels of the climacteric peak. A treatment of 24 h has an even stronger effect on respiration.

![Graph](image_url)

*Fig. 2. Effect of the length of exposure to exogenous ethylene (100 ppm) on respiration rate (A) and ethylene production (B) of whole transverse banana slices. Treatment was applied 24 h after cutting, for 6 (○), 12 (△) or 24 (□) h and are compared to controls untreated (●). Three composite samples were used per treatment. In all cases, there are significant differences with the controls (P < 0.05, P < 0.001 and P < 0.001, respectively).*
The endogenous ethylene production of whole slices treated for 6 h (Fig. 2B) is similar to controls except that the increase to a peak characteristic of the climacteric is advanced 2 days, as the respiration increases. In whole slices treated for 12 or 24 h, ethylene production is not parallel to the increase on respiration. Immediately after treatment the production levels are low, and they increase steadily after about 5 days, but in a different pattern from that of normal ripening in controls.

Effect of ethylene treatment on respiration and ethylene production in peel and pulp sections

The effect of treatment with 100 ppm ethylene for 12 h on peel and pulp sections, obtained from the same fruits and treated simultaneously, is shown in Fig. 3. The pulp shows a quick rise in respiration that reaches the level of the normal climacteric peak in banana, and higher (Fig. 3A). However, in the peel there is an

Fig. 3. Effect of ethylene treatment (100 ppm for 12 h) on respiration rate (A) and ethylene production (B) in peel (△) or pulp (○) sections, obtained from banana fruit, compared to controls untreated (▲) peel and (●) pulp. Three composite samples were used per treatment.
initial increase in respiration, but afterwards it decreases to the low levels prior to treatment, and similar to that of control untreated peel.

The pattern of ethylene production is somehow similar (Fig. 3B). The peel does not show any effect. The pulp shows an increase in endogenous ethylene production but the pattern is different from that of controls. There is an initial increase (3.5–4 nl gFW⁻¹ h⁻¹) but not to the level of the climacteric ethylene peak of pulp controls (8 nl gFW⁻¹ h⁻¹); production then decreases, and then increases again.

Effect on EFE activity of peel and pulp of transverse whole slices

EFE activity, in peel and pulp of whole banana slices treated with ethylene (100 ppm) 24 h after cutting, was measured just after the different periods of treatment (3, 6, 12 or 24 h) (Fig. 4). There is an increase in EFE activity, in both peel and pulp, in treated slices compared to controls. This increase, in the peel (Fig. 4A), is evident after a 6-h treatment. In the pulp (Fig. 4B), where EFE activity is lower, the differences are not clearly discernible before a 12-h treatment. In both, peel and pulp, the differences compared to controls become larger in longer treatments.

Fig. 4. EFE activity in peel (A) and pulp (B) of whole banana slices treated with ethylene (100 ppm) 24 h after cutting, for different periods (■) compared with controls untreated (○). EFE activity was measured just after each period of treatment. Three composite samples were used per treatment. All results show a significant difference compared to controls (P < 0.05).
Effect on free and conjugated ACC in peel and pulp of transverse whole slices

Ethylene treatment (100 ppm) increases or prevents a decline in total ACC (free + conjugated) both in peel and pulp (Fig. 5). As in the measurement of EFE activity, the longer the treatment the differences compared to controls are slightly higher, but the link of the level of total ACC with the length of exposure to exogenous ethylene is not clear. However, free ACC in the pulp of the slices (Fig. 5B) does not differ significantly from that of the controls. In peel (Fig. 5A), free ACC in treated slices is slightly higher after a 12-h treatment.

4. Discussion

The use of 100 ppm ethylene for 24 or 48 h to induce ripening of bananas at a temperature between 15 and 25°C is a common commercial practice (Scriven et al., 1989). Several authors have used concentrations ranging from 1 to 1000 ppm (Vendrell and McGlasson, 1971; Inaba and Nakamura, 1988; Ke and Tsai, 1988).
From the results obtained after treatment of whole bananas with 100 ppm for 6 or 12 h, it can be deduced that endogenous ethylene production does not follow the same pattern as in natural ripening. The characteristic ethylene climacteric peak does not appear, and the level of ethylene production is lower, although increasing. Natural ripening and ethylene production seems to begin in the pulp, and ripening of the peel depends on the pulp (Vendrell and McGlasson, 1971; Garcia and Lajolo, 1988; Domínguez and Vendrell, 1993). There are differences in flavour and aroma in bananas ripened with exogenous ethylene compared to natural ripening (Scriven et al., 1989). Besides, inhibition of endogenous ethylene production caused by exogenous ethylene treatment (Vendrell and McGlasson, 1971), could be the cause of the different behaviour of treated fruits. The results obtained are similar to those described by Ke and Tsai (1988) and by Whitehead and Bossè (1991).

Transversal banana slices show a ripening behaviour similar to that of whole fruits. They exhibit the characteristic climacteric peak of respiration, autocatalytic ethylene production, chlorophyll breakdown in the peel and starch hydrolysis in the pulp (Palmer and McGlasson, 1969; Vendrell and McGlasson, 1971). The behaviour of whole slices treated with ethylene shows the need of a minimum treatment period to induce the climacteric respiration rise, although not initially the autocatalytic ethylene production probably due to the inhibition of endogenous ethylene production caused by ethylene treatment. With 100 ppm, the minimum treatment period is between 6 and 12 h. Shorter periods do not induce the climacteric, but make the tissue more sensitive to ethylene (Halevy and Mayak, 1981) and the onset of ripening is advanced. According to Yang (1987), the effect of exogenous ethylene could be on receptors of system I, also inducing the increase of respiration and disinhibiting the receptors of system II or their synthesis. In any case, sufficient effect to produce ripening is only obtained over time.

The data of Fig. 3 indicate that the peel is incapable of autocatalytic ethylene production. It does not respond to ethylene treatment unless this is continuously present and even then, changes are at the level of pigment development (Ke and Tsai, 1988). This is probably because the peel does not have the capacity of system II for autocatalytic ethylene production, but only that of system I, which produces lower amounts of ethylene related to senescence. However, the pulp responds irreversibly to ethylene treatment. Differences with controls in the pattern of ethylene production in ethylene-treated pulp slices could be due to the initial inhibition caused by ethylene treatment (Vendrell and McGlasson, 1971).

The effect of ethylene on EFE is to enhance enzyme activity. Enzyme activity is higher in the peel by about a factor of 5. Similar results have been obtained in tomatoes, melons (Liu et al., 1985a) and in apples (Bufler, 1986).

As total ACC is higher in slices treated with ethylene, while free ACC does not change (in the pulp) it follows that the MACC level increases. Then exogenous ethylene may increase ACC synthase activity (Bufler, 1984), but at the same time as, and faster than, the malonyl-ACC transferase activity (Liu et al., 1985b). ACC produced is converted almost immediately to MACC, increasing MACC level. If ACC does not accumulate, it has no access to EFE, whose activity is increased by exogenous ethylene, and is not metabolized to ethylene. This could explain the
initial inhibition of endogenous ethylene production observed in bananas treated with ethylene (Vendrell and McGlasson, 1971), and it is probably related to the low ethylene production rates observed later in banana slices treated with ethylene (Figs. 2B and 3B).

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References


