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PUTRESCINE ACCUMULATION IN WOUNDED GREEN BANANA FRUIT

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Key Word Index—*Musa* AAA group; Musaceae; banana; wound; polyamine; putrescine; ethylene.

Abstract—The concentrations of putrescine (Put) and ethylene, and the effects of biosynthesis inhibitors on the production of polyamines and ethylene were examined in wounded pulp tissue of green banana fruit. The Put concentration in the sliced banana fruit, and the arginine decarboxylase (ADC, EC 4.1.1.19) activity increased with the increase of ethylene evolution. Difluoromethylarginine (DFMA) suppressed Put accumulation by 72% while difluoromethylornithine (DFMO) had no effect on the Put content. Methylglyoxal bis(guanylhydrazone) (MGBG) decreased ethylene production and suppressed Put accumulation. Aminooxyacetic acid (AOA) and norbornadiene (NBD) also depressed Put accumulation. These findings suggest that ethylene produced in the wounded pulp tissue affects the induction of ADC and the accumulation of Put.

INTRODUCTION

The polyamines putrescine (Put), Spd and Spm are of widespread occurrence in plants, and the changes in their levels and biosynthesis are associated with growth, development, senescence and response to stress [1–3]. In particular, Put accumulation has been reported in various plants under stress conditions, such as low pH [4], low K^+ [5], osmotic stress [6, 7] and oxygen deficiency [8].

Exogenously applied polyamines have been shown to inhibit ethylene biosynthesis [9, 10], while ethylene application has been shown to inhibit polyamine biosynthesis [11, 12]. The polyamine content in various fruits generally decreased during normal fruit ripening, followed by ethylene evolution [13–15]. On the basis of these observations, it has been suggested that an antagonistic relationship between polyamines and ethylene exists in plants. However, we found that the increase in the Put content occurs simultaneously with an increase in climacteric ethylene evolution during the ripening process of stored green banana fruit (unpublished results). Ethylene is known to be generated in wounded plant tissue, and we expected that the Put accumulation would be correlated with the ethylene evolution in wounded banana fruit tissue.

The purpose of this study was to clarify the relationship between the polyamines and ethylene, and to examine the effects of inhibitors of polyamine and ethylene biosyntheses on Put accumulation in wounded banana fruit tissues.

RESULTS AND DISCUSSION

Ethylene evolution

The pattern of ethylene production by banana pieces after cutting exhibited a sharp increase in wound-induced ethylene, which commenced from *ca* 4 hr after cutting, reached a peak 6 hr later, and then gradually decreased (Fig. 1).

Putrescine and spermidine content

The Put content in the tissue increased markedly after a lag period of *ca* 8 hr, and was 231 nmol g^{-1} fresh weight after 24 hr of incubation, while the Spd content remained almost at the initial level throughout the entire incubation period (Fig. 2). The marked increase of Put content began at 4–5 hr after the initiation of ethylene evolution. Put accumulation may have been induced by an action of ethylene produced in response to wounding.

These increases in Put content and ethylene production are not found in most plants [13–15], although a few exceptions have been reported. During senescence of cut carnation flowers, the Put and ethylene contents increased concurrently, and the explanation is that the rate of ethylene biosynthesis is moderated by Put and would have exceeded the observed values if the rise in Put had not occurred [16]. During elongation of

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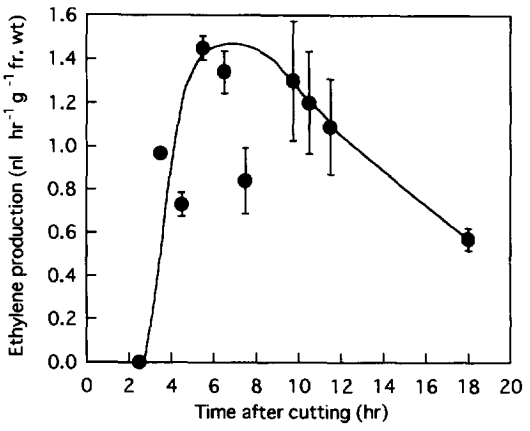

Fig. 1. Ethylene production of pieces of green banana fruit after cutting treatment. Data points are means ± s.e. ($n = 4$).

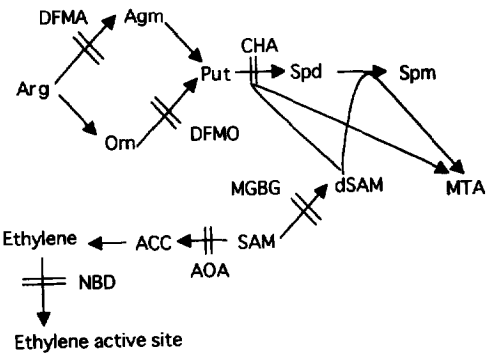

Fig. 3. Pathway of polyamine and ethylene biosyntheses showing points of inhibition by inhibitors used in the experiment. For abbreviations see the text.

rice coleoptiles, both ethylene and Put increases were also observed and this polyamine accumulation was found to be induced by ethylene [17].

Effects of polyamine and ethylene biosyntheses inhibitors

To examine the mechanism of Put accumulation, we examined the effects of several inhibitors on polyamine contents and ethylene evolution after 20 hr of incubation (Table 1). Figure 3 shows the pathway of polyamine and ethylene biosyntheses, and inhibition points of inhibitors used in this experiment.

DFMO and DFMA are irreversible inhibitors of ornithine decarboxylase (ODC) and arginine decarboxylase (ADC), respectively. Treatment with DFMO had little effect on the Put level, while DFMA depressed the Put level by 72% compared with the control. In general, Put is biosynthesized through two alternative pathways, directly by ODC, or by ADC through agmatine [2].

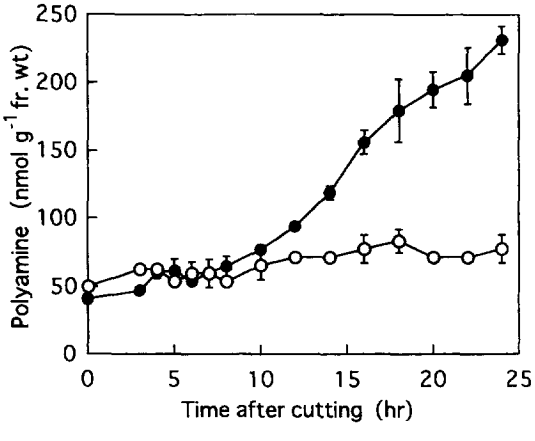

Fig. 2. Put (●) and Spd (○) contents in green banana fruit pieces after cutting treatment. Data points are means ± s.e. ($n = 4$).

Since DFMA but not DFMO suppressed the Put increment, Put was suggested to be biosynthesized mainly through the ADC mediated pathway in banana pieces.

The ethylene production of the pulp tissue treated with DFMO and DFMA was 14.5 and 15.8 nl g^{-1} fr. wt after 20 hr of incubation, respectively, being almost the same as that of the control. These findings suggest that the endogenous Put accumulation did not affect the ethylene biosynthesis in the banana pulp. This is different from the finding reported by Roberts *et al.* [16] who found that the application of DFMA to a cut carnation flower reduced the amount of Put but increased the total amount of ethylene production.

MGBG acts as an irreversible inhibitor of *S*-adenosylmethionine decarboxylase, which converts *S*-adenosylmethionine (SAM) to decarboxylated *S*-adenosylmethionine (dSAM). It inhibited the increase of the Put content in the banana fruit by 60% compared with the control, while this treatment suppressed the Spd content by 27% (Table 1). In addition, ethylene production was reduced by 41%.

The treatment with cyclohexylamine (CHA), which is a competitive inhibitor of spermidine synthase, increased the Put content by 24% compared with the control, and ethylene evolution was reduced by 17%. These findings concerning the treatment with MGBG and CHA deny the hypothesis that depression of spermidine synthesis increases SAM that is used for conversion to 1-aminocyclopropane-1-carboxylic acid (ACC) and thereby promotes ethylene production. Ethylene and polyamines may not compete for SAM for their synthesis in the banana fruit tissue. In the CHA treated banana, the Spd level could not be determined, since the CHA peak widely overlapped the Spd peak on the HPLC chromatogram.

The treatment with AOA, which is an ACC synthase inhibitor, completely prevented the increase in the levels of Put and ethylene in the banana pulp tissue (Table 1). Ethylene appeared to have affected the Put level. However, since AOA acts like pyridoxal phosphate [18, 19], AOA may block not only ACC synthase

Table 1. Effects of DFMO, DFMA, MGBG, CHA, AOA and NBD on polyamine accumulation and ethylene evolution in sliced banana tissues incubated for 20 hr

Treatment	Put (nmol g^{-1} fr. wt)	Spd (nmol g^{-1} fr. wt)	C_2H_4 (nl g^{-1} fr. wt)
7.5 mM DFMO	218±11	53±4	14.5±2.5
7.5 mM DFMA	64±8	55±5	15.8±1.3
10 mM MGBG	87±10	45±2	8.8±1.4
10 mM CHA	280±35	—	12.5±0.4
5 mM AOA	60±2	51±6	<1
6 ml l^{-1} NBD	71±6	57±1	28.5±4.3
Control	226±15	62±4	15.0±0.8
0 hr	60±4	53±4	<1

DFMO, Difluoromethylornithine; DFMA, difluoromethylarginine; MGBG, methylglyoxal bis-(guanylhydrazone); CHA, cyclohexylamine; AOA, aminooxyacetic acid; NBD, norbornadiene.

but also another pyridoxal phosphate dependent enzyme including ODC and ADC [16].

The ADC activity in pea seedlings has been reported to be depressed by endogenous ethylene [11, 12]. Thus, ethylene was considered to act as a suppressor of ADC activity. However, our findings with the treatment with DFMA, CHA, MGBG and AOA suggested that ethylene promoted rather than suppressed Put accumulation in the wounded banana pulp tissue.

NBD has an anti-ethylene effect due to competition with ethylene for binding sites [20]. Exposure of the pulp tissue to 6 ml l^{-1} NBD vapor depressed the increase in Put level by 38%, and increased the level of ethylene by 90% for 20 hr compared with the control (Table 1). This is also consistent with our hypothesis that ethylene promotes putrescine biosynthesis. The relationship between ethylene evolution and Put level with NBD treatment was apparently similar to that between ethylene and phenylalanine ammonia lyase (PAL) activity in the fruit tissue of *Cucurbita maxima*, in which endogenous ethylene induction is considered to be associated with the induction of PAL [21].

Arginine decarboxylase activity

ADC activity was assayed to ascertain that the increase of Put content was caused by the activation of ADC (Fig. 4). The initial ADC activity was 0.18 pkat g^{-1} fresh weight and then increased for 16 hr to a maximum of 0.86 pkat g^{-1} fresh weight and declined thereafter. ADC was obviously induced by the slicing treatment.

Therefore, we confirmed that the Put accumulation occurred with ethylene production in the wounded banana fruit tissue. The generated ethylene or ethylene mediated biochemical processes seemed to be responsible for the activation of ADC and Put accumulation in the wounded banana fruit tissue.

EXPERIMENTAL

Plant material. Mature, green, preclimacteric bananas (*Musa* AAA group, Cavendish subgroup cv. Giant Cavendish) from Philippines were supplied by an importer in Fukuyama, Hiroshima, Japan. For prepn of wounded tissue, banana pulp was sliced to 1.5 mm in thickness, then each disk was cut into 8 pieces. Prepd tissues (4 or 5 pieces, *ca* 1 g fr. wt) were incubated in a 30 ml gas-tight conical flask containing 1 ml H_2O or each test soln at 25°.

Ethylene analysis. The head space gas was assayed for C_2H_4 using GC (with a 2 m × 3 mm i.d. column of Porapak Q, 70° column temp with N_2 at a flow rate of 35 ml min^{-1}) equipped with an FID (injector and detector at 100°).

Extraction and HPLC determination of free polyamines. Each pulp tissue treated was homogenized in 5% ice-cold $HClO_4$ (0.1 g fr. wt ml^{-1}). Free polyamines in the homogenate were assayed by HPLC as described previously [22].

Arginine decarboxylase activity was estimated by measuring the release of $^{14}CO_2$ from L-[U-^{14}C]arginine by a modification of the method in ref. [7]. For extraction, 2 g fr. wt of tissue was homogenized in 4 ml of extraction medium consisting of 0.1 M K-Pi buffer (pH 8), 1 mM DTT, and 0.1 mM EDTA. The homogenate was clarified by centrifugation at 17 000 *g* for 20 min. The supernatant was used for the assay.

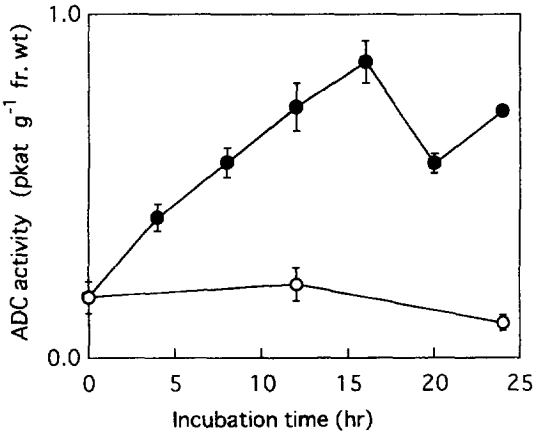

Fig. 4. ADC activity in green banana fruit after cutting treatment (●) and that of control (○). Data points are means ± s.e. ($n = 3$).

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