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Effect of Anoxia Treatment on the Placental Alcohol Fermentation of Oriental Melon (*Cucumis melo*) at Different Developmental Stage.

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Abstract : Effect of anoxia treatment on alcohol fermentation in the placenta of oriental melons (*Cucumis melo*) at different developmental stages was studied. Results showed that fruits at the rapid growth stage (stage III) contained the lowest amount of acetaldehyde and ethanol as compared with fruits at other developmental stages. During anoxia treatment, a steady increase in ethanol content was observed in the placenta of oriental melons, regardless of their developmental stages, while the increment of acetaldehyde content was relatively small. Alcohol dehydrogenase in growing and maturing stage fruits showed increased activity with the maximum value at one day after the onset of anoxia treatment and then decreased gradually. An increase in the activity of pyruvate decarboxylase was also observed during anoxia.(Received October 16, 1997; accepted December 1, 1997)

Introduction

Alcohol fermentation is a metabolic pathway that degrades pyruvate to produce ethanol, regenerating NAD^+ from NADH, under anaerobic conditions by the consecutive actions of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH). When plants, animals and aerobic microbes are placed under hypoxia or anoxia conditions, the normal respiratory pathway of the TCA cycle and mitochondrial electron transfer is blocked.[1)] As a result, ATP production through oxidative phosphorylation in mitocondria is severly impaired and build-up of the glycolysis products, pyruvate and NADH, occurs, which in turn results in inhibiting their glycolytic activity and thereby decreasing ATP production *via* glycolysis. Under such adverse cirumstances, cells should take tactics to dissipate the metabolite build-up so as to keep their glycolytic activity functioning at normal rates by enhancing such fermentation processes as lactic acid fermentation and alcohol fermentaion.[2,3)]

Since extended lactic acid fermentation causes acidosis of plant cells which leads to cell death, plants have been reported to quickly upregulate alcohol fermentation shortly after the induction of the lactic acid fermentation.[4)] Thus, the ability of a particular plant to induce alcohol fermentation is closely related to its ability to cope with hypoxia and anoxia stress.[5,6)] Alcohol fermentation has also drawn much attention from researchers in the field of postharvest storage of agricultural fruits.[7-9)] By storing fruits under reduced oxygen and elevated carbon dioxide conditions, called as CA storage, several advantages such as pest control and delay of fruit senescence are expected. However, alcohol fermentation has been a major drawback in CA storage of fruits due to the production of various volatiles and off-flavor.

In this study, it was tested whether or not the accumulations of acetaldehyde and ethanol are related to changes in the activities of pyruvate decarboxylase and alcohol dehydrogenase in the placenta of oriental melon fruits during anoxia.

Materials and Methods

Materials

Oriental melons (*Cucumis melo* L.) were grown in a

Key words : Oriental melon (*Cucumis melo*), alcohol fermentation, anoxia, volatiles, pyruvate decarboxylase, alcohol dehydrogenase
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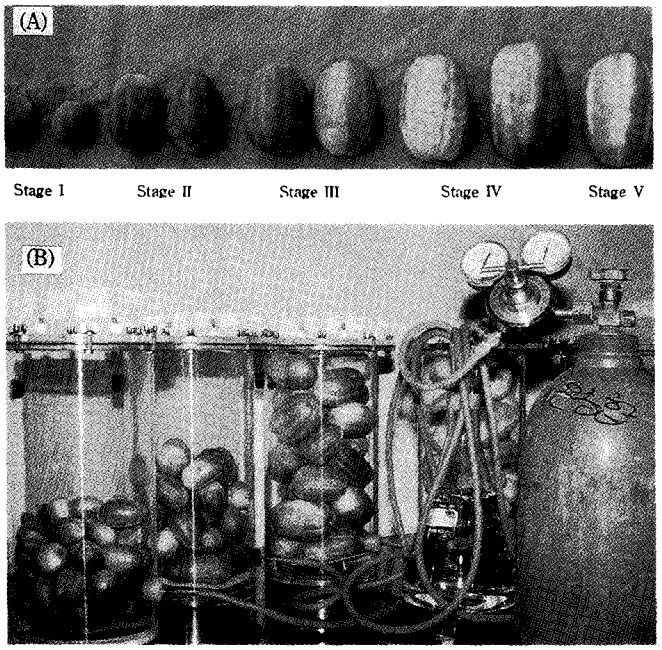

Fig. 1. Photographic illustrations of fruits of oriental melon at 5 different developmental stages (A) and anoxia treatment (B).

greenhouse at the Sungju Fruiting Vegetable Experiment Station, Gyeong-Sang Buk Do and fruits of five different developmental stages were collected as shown in Fig. 1a.

Anoxia treatments

Fruits were incubated in transparent plastic chambers 25℃ under the stream of N_2 gas for six days (Fig. 1b). During the anoxia treatment, two to three fruits were retrieved with one day interval. Flesh and placenta of fruits were then separated, frozen under liquid nitrogen, crushed to powder and stored at -80℃ until used. As a control experiment, fruits were also stored in air and harvested in the same way as anoxia treated fruits.

Analysis of volatiles[7]

About five grams of crushed placental tissues of oriental melon fruits were weighed and put in 10 m*l* glass bottles. After the mouth of the bottle was sealed with a serum cap and an aluminium seal, samples were incubated at 60℃ for one hour and 1 m*l* of the gas phase was taken by the use of a disposable syringe. Volatiles in the gas phase were then injected to a gas chromatograph (Hewlett Packard Series II Model 5890), resolved on a capillary column (J & W Scientific, DB-Wax 25 μm, 30 m×0.25 mm) and detected by a flame ionization detector. Volatiles were separated on a GC column at 40℃ with 1 m*l*/min of carrier gas (N_2) flow.

Enzyme preparation

Placental tissue was ground finely with liquid nitrogen by the use of a mortar and pestle[8]. Tissue was then homogenized in four volumes of 100 mM Mes buffer (pH 6.5) supplemented with 2 mM DTT and 1% polyvinylpyrrolidone (MW 40,000). Homogenate was centrifuged at 27,000×g for 10 min at 4℃ and the supernatant was retrieved. Protein contents in the supernatant was determined by the Bradford method[10], with bovine serum albumin as the standard, and stored in frozen at -80℃ until the enzyme activity was assayed.

Enzyme assay

Activities of PDC and ADH were assayed according to methods reported by Ke *et al.*[8] with minor modifications. For the assay of PDC, 0.45 m*l* of 100 mM Mes buffer (pH 6.5), 0.1 m*l* of 5 mM thiamine pyrophosphate (cocarboxylase), 0.1 ml of 50 mM $MgCl_2$, 0.05 m*l* of 2 mM NADH, 0.1 m*l* of 13.5 U/m*l* ADH and 0.1 m*l* of 50 mM pyruvate were mixed to make 0.9 m*l*. After the assay solution was warmed up to 25℃, the enzyme reaction was initiated by the addition of 0.1 m*l* of enzyme extract and monitored by recording the absorbance change at 340 nm overtime using a spectrophotometer. The assay of ADH activity was performed in the direction of ethanol production.[8] One m*l* of the enzyme reaction mixture consisted of 0.8 m*l* of 100 mM Mes (pH 6.5), 0.05 m*l* of 2 mM NADH, 0.05 m*l* of 50%(v/v) acetaldehyde, and 0.1 m*l* of enzyme extract. The enzyme reaction was initiated and monitored as was PDC assay.

Results and Discussion

Occurrence of "abnormally fermented fruits" is one of the major problems in the agricultural practice of oriental melon.[11-16] Since various factors affect the formation of the fermented fruits and numbers of symptoms are observed as indicators of the physiological disorder, it is difficult to fully understand the physiological and biochemical processes involved in the production of the fermented fruits. For the present study, we paid special attention to observations in which acetaldehyde accumulation and off-flavor occurrence take place in the fermented fruits of oriental melon; such biochemical changes have also been observed when fruits are treated under hypoxia to anoxia conditions.[7,8] This provided the rationale for an assumption that occurrence of abnormally fermented oriental melon fruits might be associated with hypoxia to anoxia effects.

When alcohol fermentation of anoxia-treated and -un-

Table 1. Alcohol dehydrogenase and pyruvate decarboxylase activities distributed in different parts of oriental melon fruits (unit; -△ 340 nm/min · g F.W)

Parts	Alcohol dehydrogenase	Pyruvate decarboxylase
Seeds	1.66	0.46
Placenta	39.2	14.44
Pericarp	3.58	1.54

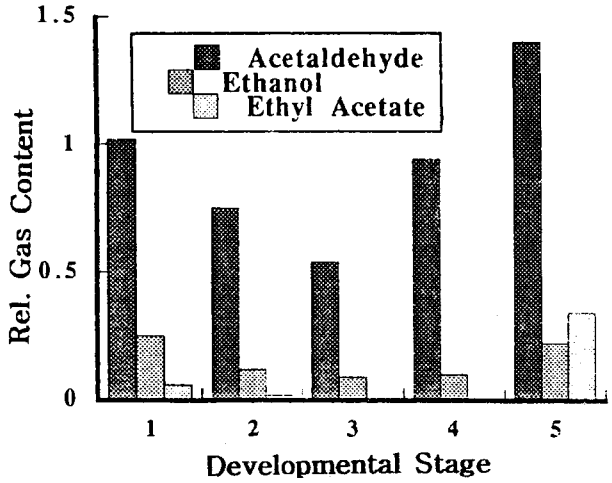

Fig. 2. Relative contents of acetaldehyde, ethanol and ethyl acetate in the placenta of oriental melons at 5 different development stages.

treated fruits were analyzed, it was found that the placenta showed higher ADH and PDC activities than the pericarp did (Table 1). These results are in agreement with previous observation that symptoms of "fermented fruit" occur especially at the placental portion of the fruit.[11-16] Prior to the anoxia treatment, the contents of volatile compounds in the placenta were compared among fruits of different developmental stages. As it turned out, fruits in stage III which corresponds to a period of late fruit enlargement showed the minimum level of acetaldehyde and ethanol (Fig. 2).

Changes in both acetaldehyde and ethanol contents were noted with the progress of the anoxia treatment (Fig. 3). Ethanol in the placenta of the stage III fruits increased rapidly with the increasing duration of the anoxia treatment while only a slight increase was seen in the acetaldehyde content. Similar observation was made with fruits of other developmental stages (data not shown).

The accumulations of ethanol and acetaldehyde were compared with changes in ADH and PDC activities during the anoxia treatment (Fig. 4 and Fig. 5). Fig. 4a showed that ethanol in the placenta increased throughout the period of anoxia treatment, regardless of the developmental stage of the fruits. However, ADH activities in developing fruits (stage I and III) rapidly in-

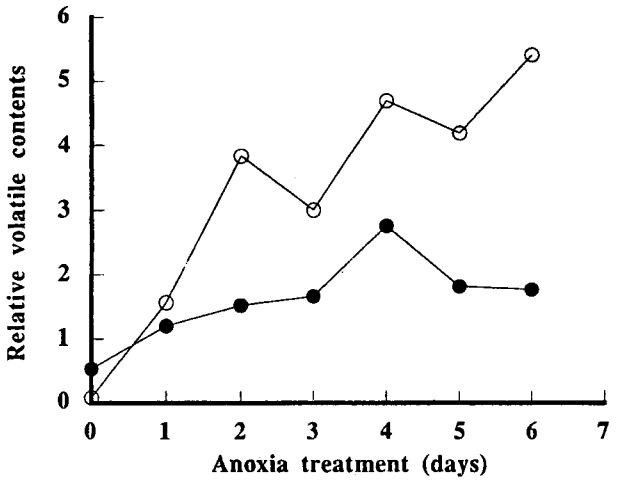

Fig. 3. Changes in acetaldehyde and ethanol contents in the placenta of oriental melon at developmental stage III during anoxia treatment. Symbols: closed circle,acetaldehyde; open circle,

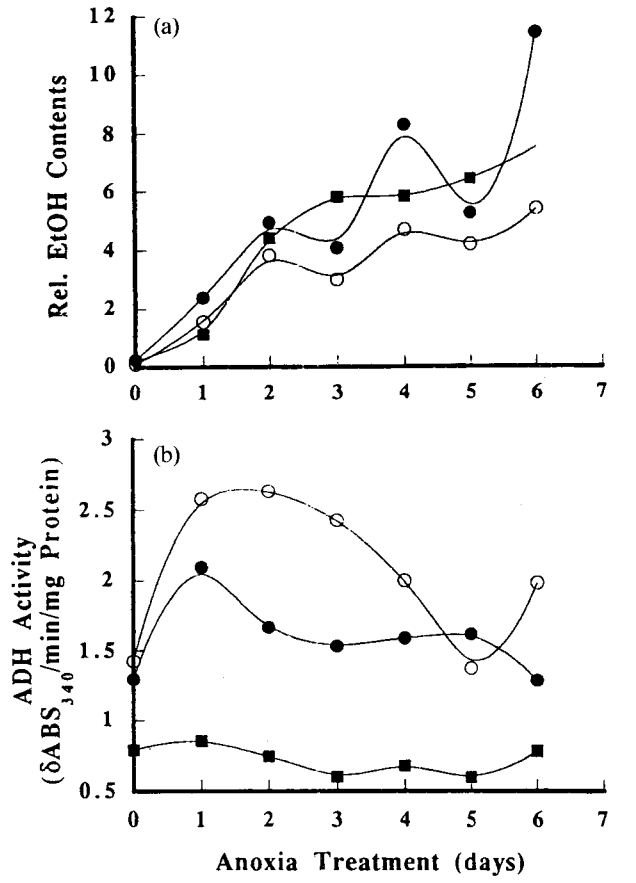

Fig. 4. Changes in ethanol content (a) and alcohol dehydrogenase activity (b) in the placenta of oriental melon at developmental stages I, III, and V during anoxia treatment. Symbols: closed circle, Stage I; open circle, Stage III; closed square, Stage V.

creased to reach the maximum level at one day after the anoxia treatment and gradually decreased afterward (Fig. 4b). Moreover, ADH activity in mature fruits did not increase during the anoxia treatment, implicating that the increase in ethanol content in the anoxia-treat-

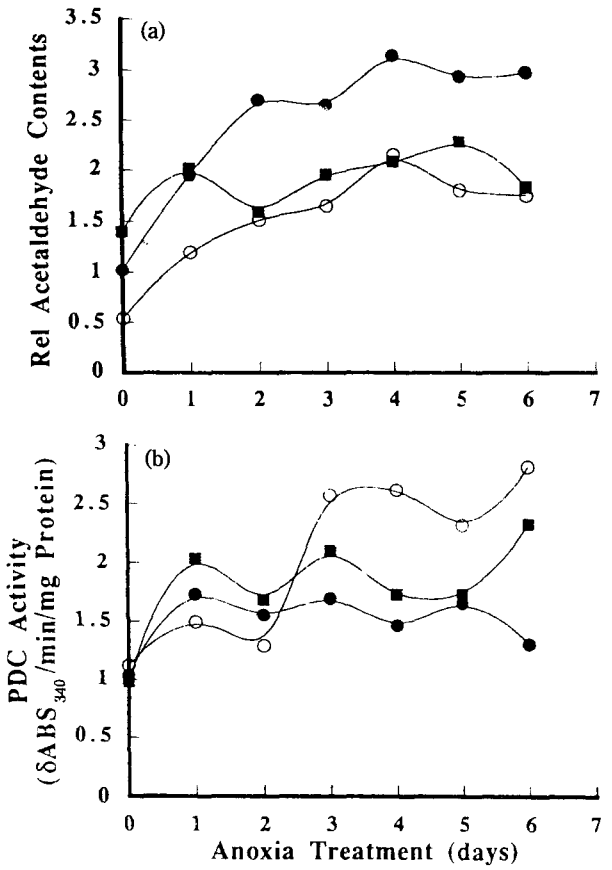

Fig. 5. Changes in acetaldehyde content (a) and pyruvate decarboxylase activity (b) in the placenta of oriental melon at developmental stages I, III and V during anoxia treatment. Symbols: closed circle, Stage I; open circle, Stage III; closed square, Stage V.

ed mature fruits was probably not due to the induction of ADH by anoxia treatment. On the contrary, the change in acetaldehyde content in the anoxia-treated fruits appeared well correlated to the upregulation of PDC activity in the placenta. As shown in Fig. 5a, acetaldehyde content in the placenta of stage III fruits increased to the maximum at about three days after the onset of the anoxia treatment and was then leveled off. Correlated to such change in acetaldehyde content, the maximum PDC activity was seen in stage III fruits when anoxia-treated for three days (Fig. 5b). However, the increase in acetaldehyde level as well as in PDC activity in mature fruits was not so apparent. In oriental melon fruits at the earliest developmental stage (Stage I), acetaldehyde accumulation occurred rapidly during the first two days of the anoxia treatment while the maximum activation of PDC was achieved by only one day of the treatment. It was also noteworthy that acetaldehyde content in Stage I fruits treated with anoxia for three to six days was about two times higher than that in anoxia-treated fruits of stage III, while the PDC activity of the stage I fruits was about a half of the PDC activity of the stage III fruits (Fig. 5a and b): this implies that the PDC activity of fruits at a particular stage does not necessarily represent the level of acetaldehyde contents.

Results obtained in the present study demonstrated that the upregulation of alcohol fermentation in anoxia-treated fruits of oriental melon is not straightforward. Acetaldehyde content in the placenta of fruits at stage I increased to a higher level during anoxia as compared with that of stage III fruits, while PDC activity in the younger fruits remained at lower level than that in the older fruits. Further, any upregulation of alcohol dehydrogenase was not detected in anoxia-treated mature fruits, even though the accumulation of ethanol was observed. Such results may imply that the increased alcohol fermentation and the accumulation of ethanol in anoxia-treated oriental melon fruits do not result directly from the induction of enzymes involved, but from the changes in carbon partitioning among metabolic pathways involved in producing and consuming pyruvate via glycolysis, TCA cycle, pentose phosphate pathway and mitochondrial electron pathway, etc. Therefore, in order to fully understand the upregulation of alcohol fermentation in the anoxia-treated oriental melon fruits, carbon flow not only to alcohol fermentation pathway but also to other carbohydrate metabolisms should be examined simultaneously.

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발달단계가 다른 참외 태좌부의 알콜발효에 미치는 무산소처리효과

김동헌*, 서동환[1], 최홍집[1], 최성국[1], 정 진[2], 황영수(441-707 경기도 수원시 권선구 서둔동 농업과학기술원 생화학과, [1]719-860 경상북도 성주군 대가면 경상북도 농촌진흥원 성주과채류시험장, [3]441-744 경기도 수원시 권선구 서둔동 서울대학교 농업생명과학대 농화학과

초 록 : 발달단계가 다른 참외 태좌부의 알콜발효에 미치는 무산소처리효과를 검정하였다. 참외의 발달단계별 acetaldehyde 및 ethanol 수준을 검정한 결과, 비대 후기 (stage 3)의 과실에서 가장 낮았다. 무산소 처리기간동안 태좌부 중의 ethanol은 꾸준히 증가 하였으며, acetaldehyde 함량의 증가는 상대적으로 미미하였다. 그러나 alcohol dehydrogenase 활성은 무산소 처리 1일 까지 급격히 증가한 다음 서서히 감소하였고, pyruvate decarboxylase 활성은 저온 처리기간 중 증가하였다. 완숙기과실의 경우 두 효소의 활성은 증가하지 않았으나, 발효산물의 축적은 관찰되어, 완숙기 과실의 알콜발효는 효소활성의 증가에 기인하는 것이 아님을 알 수 있었다.

찾는말 : 참외, 무산소처리, 휘발성물질, pyruvate decarboxylase, alcohol dehydrogenase
*연락저자