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Effect of Far infrared Rays emitted from the calcined Ceramics on Ethylene Production in Mungbean Hypocotyls

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Ethylene production was measured in mungbean hypocotyls in the presence of far infrared rays emitted from ceramic powder using a gas chromatography. Both IAA- and 1-aminocyclopropane-1-carboxylic acid (ACC)-induced ethylene production was inhibited by 20-30% of the control by indirect application of the ceramic powder. These data suggested that far infrared rays emitted from ceramic powder might act on the conversion step of ACC to ethylene. Furthermore, the activity of ACC oxidase, acting on the conversion of ACC to ethylene, was also inhibited by 20% of the control by indirect treatment of the ceramic powder. These results suggested the possibility that inhibition of ethylene production by far infrared rays emitted from ceramic powder could be used for increasing the period of storage and freshness of crops, fruits, and vegetables.

Keywords: ceramics, ethylene, far infrared ray, hypocotyl, mungbean

INTRODUCTION

Since the properties of far infrared rays emitted from ceramic powder are known, they have been applied to many industrial fields such as food industry and medicine. For example, far infrared rays from a hot ceramic surface are used to increase the storage period of vegetables by dryness or sterilization without deterioration of quality in the food industry (Ahn *et al.*, 1993; Sasamori, 1993; Shibukawa, 1993). It is also applied in the medical field, for example, hot treatment, surgical operation with laser thermography, and physical therapy (Maeda, 1987; Lee, 1992). All these applications are mainly based on the use of far infrared rays emitted from hot a ceramic surface.

However, our laboratory has preliminary data about the effects of far infrared rays emitted from ceramic powder in room temperature on plant senescence. That is, the soil mixture with ceramic powder seemed increase the storage period of garlic (data not shown). Furthermore, Lee *et al.* (1995) reported that the soil mixture with ceramic powder increased the growth of rice seedlings. These results suggested that far infrared rays emitted from ceramic powder or the ceramic powder itself might control the production of ethylene to regulate the growth of plants. Based on these data, we examined the effect of far infrared rays emitted from ceramic powder in room temperature on the ethylene production in mungbean hypocotyl segments to examine the possibility of ceramic powder application in the increase of storage period of crops, fruits, and vegetables.

Ethylene, a gaseous plant hormone, plays an important role in many physiological phenomena such as senescence and ripening in plant organs (Abeles *et al.*, 1992). The stimulation of ethylene production is associated with stressed or ripening plant tissues. Therefore, loss of or damage to plants in storage is associated with the accumulation of ethylene (Theologis, 1992).

It has been known that IAA stimulates ethylene production in plant tissues (Kende, 1993). IAA application inhibited root growth in maize, and the inhibited root growth by ethylene was reversed by the application of aminoethoxyvinylglycine (AVG), an inhibitor of ethylene production (Kim and Mulkey, 1997). These data suggested the possibility that the regulation of ethylene production might be related to the control of physiological phenomena such as growth and ripening.

Ripening of fruits occurs through many enzymes such as malic enzyme and carboxylase. These ripening enzymes are concerned with softening, respiration, and ethylene production (Abeles *et al.*, 1992). Therefore, the ethylene production is a very important factor

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for increasing the fruit storage period. Based on these facts, this study was focused on the effect of far infrared rays on the ethylene production in mungbean hypocotyl segments.

MATERIALS AND METHODS

Analysis of Ceramic Powder

Powder from the natural sericite mineral was used as a raw material. Sericite powder calcined at 1,200°C for 1 h was used in this study. Both the calcined and the uncalcined ceramic powders were analyzed by EDS (Energy Dispersion Spectrometer, Kevex Instrument, USA) for components and by XRD (X-ray Diffractometer, Rigaku, Japan) for mineral structures. Far-infrared emission characteristics of calcined and uncalcined ceramic powders were measured with a FT-IR Spectrometer (Bio-Rad, USA) at 50°C. Far-infrared emissivities of ceramic powders were measured as a ratio of black body emission intensity at every wave number from 2,200 cm^{-1} to 550 cm^{-1}.

Plant Material

Mungbean (*Vigna radiata*) seeds were soaked overnight in tap water and germinated in a 0.5% agar plate at 27°C for 2-3 days in darkness. The germination plate was placed in a box containing a 0.5 mM $KMnO_4$ solution to keep the humidity and to absorb the ethylene from germinating seedlings. The hypocotyl segments (1 cm) were obtained from the mungbean seedlings.

Measurement of Ethylene Production

The appropriate number of hypocotyl segments (1 cm) was incubated in a 2 mL potassium phosphate buffer (0.05 M, pH 6.8) in a 25 mL vial sealed with a silicon stopper at 27°C in darkness. Chloramphenicol (50 μg/mL) was added to inhibit microbial activity for long term incubation. The ceramic powder was placed outside of the vial containing the hypocotyl segments to prevent direct contact each other. The flask containing the vials and ceramic powder was wrapped with aluminum foil to prevent far infrared ray emission from the ceramic powder to the control flask (Fig. 1). A 1 mL gas sample was withdrawn and analyzed with a gas chromatography (DS 6200, Donam Instrument Inc., Korea) equipped with aluminum columns and a flame-ionization detector at 180°C.

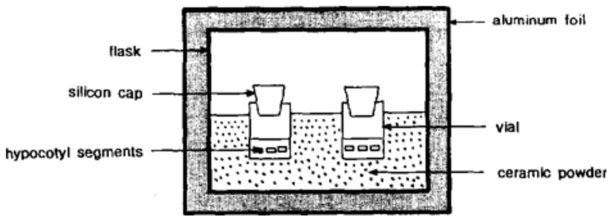

Fig. 1. Diagram of the indirect treatment of far infrared ceramic powder. Notice that the hypocotyl segments and the ceramic powder are not direct contact. The flask containing vials and far infrared ceramic powder was wrapped with aluminum foil to prevent emission from the ceramic powder to prevent affecting the other control flask.

The Assay of ACC oxidase Activity

The activity of ACC oxidase was measured *in vivo* as a modified method of Wang and Woodson (1989). Twenty-five hypocotyl segments were placed in a buffer for 12 h in the presence or absence of ceramic powder. After incubation, both treatments of hypocotyls were washed with distilled water, and then were infiltrated with 1 mM ACC for 30 min. ACC oxidase activity was deduced by measuring ethylene production from the infiltrated hypocotyl segments for 1 h.

RESULTS

Analysis and the Emission Properties of the Ceramic Powder

Table 1 shows the quantitative analysis of the ceramic powders used in this experiment. The components of uncalcined and calcined ceramic powders were oxides of Al, Si, and K, which are components of the sericite mineral. The XRD analysis of uncalcined and calcined ceramic powder showed the peaks of α-SiO_2 and unknown phases (Fig. 2). The peaks of α-SiO_2, the major crystal phases of the calcined ceramic powder, are taller than those of the uncalcined ceramic powder. Actually, the calcined

Table 1. The composition of far infrared ceramic powder by EDS analysis

Element	Weight (%)
O	89.60
Al	2.67
Si	6.17
K	1.56
Total	100.00

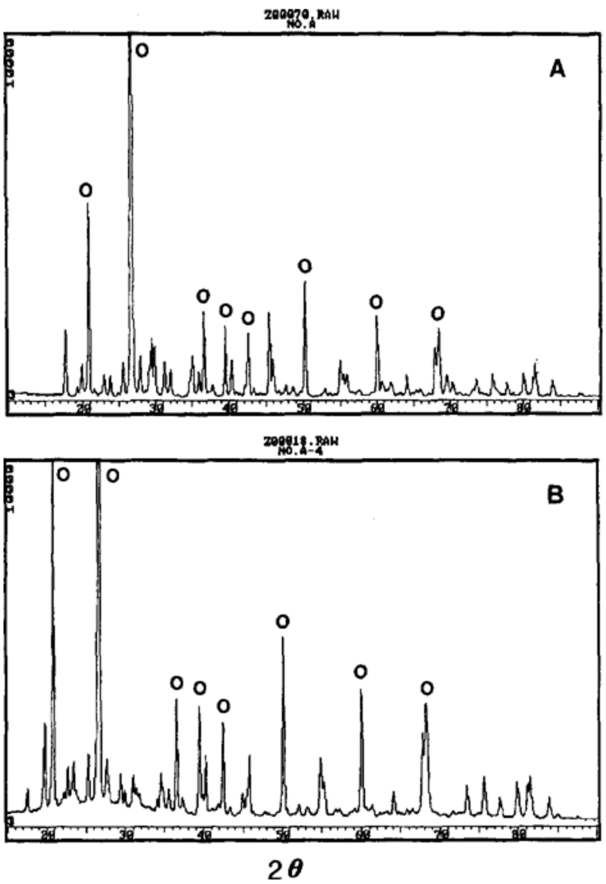

Fig. 2. XRD analysis of the uncalcined ceramic powder (A) and the calcined ceramic powder (B) (O: α-SiO_2). The ceramic powder was calcined at 1,200°C for 1 h. Notice that the peaks of α-SiO_2 in calcined ceramic powder significantly increased compared to the uncalcined one.

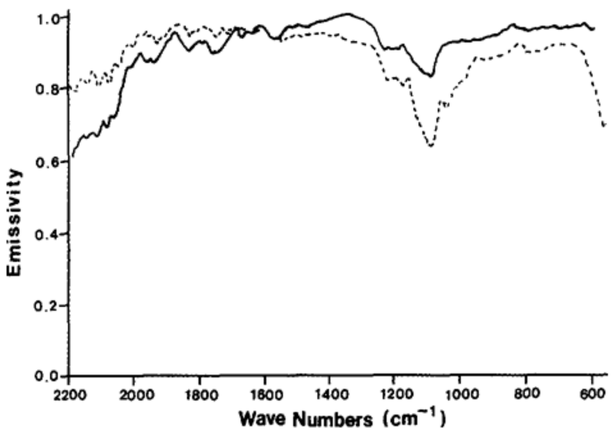

Fig. 3. Far infrared emissivity of the uncalcined (···) and calcined (—) ceramic powders, ranging from 2,200 cm^{-1} to 550 cm^{-1}. The ceramic powder was calcined at 1,200°C for 1 h. The average emissivities of uncalcined and calcined ceramic powder were 0.87 and 0.94, respectively.

ceramic powder was more effective than the uncalcined powder in the regulation of ethylene production in preliminary experiments. Therefore, we used calcined ceramic powder as the source of far infrared rays in this experiment, and this calcined powder was composed with α-SiO_2 as a major crystal phase.

The far-infrared ray emissivities of the uncalcined and calcined ceramic powders are shown in Fig. 3. The emissivity of the calcined ceramic powder is greater than that of uncalcined ceramic powder. Average emissivities of uncalcined and calcined ceramic powders were 0.87 and 0.94, respectively. Furthermore, calcined ceramic powder showed high average emissivities in the effective long wave number ranges, 1,600 cm^{-1} to 550 cm^{-1}.

Effect of Far Infrared Ray from Ceramic Powder on Ethylene Production

We measured ACC-induced ethylene production in the presence of ceramic powder. Hypocotyls were placed in a 0.1 mM ACC solution and ethylene production was measured at 2 h intervals. Ethylene production of the conthols was increased by the ACC treatment, but the indirect treatment of ceramic powder started to inhibit ACC-induced ethylene production after 6 h incubation (Fig. 4). The inhibition

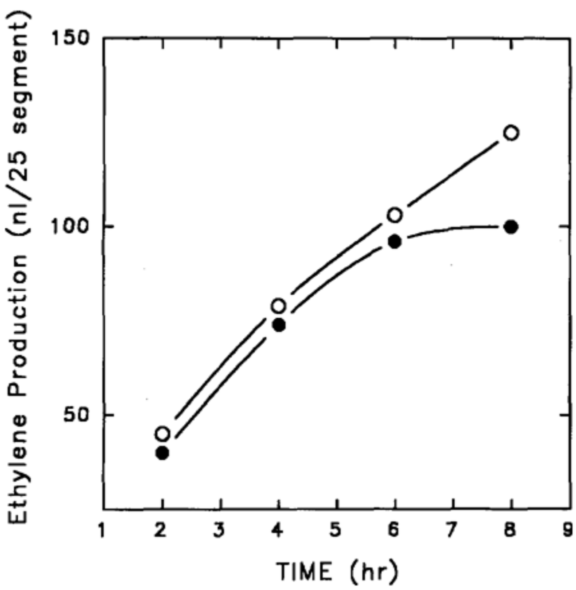

Fig. 4. Kinetics of 0.1 mM ACC-dependent ethylene production from the hypocotyl segments. The hypocotyl segments were incubated with 0.1 mM ACC. Closed circles indicate the indirect treatment of calcined far infrared ceramic powder. Open circles represent the control.

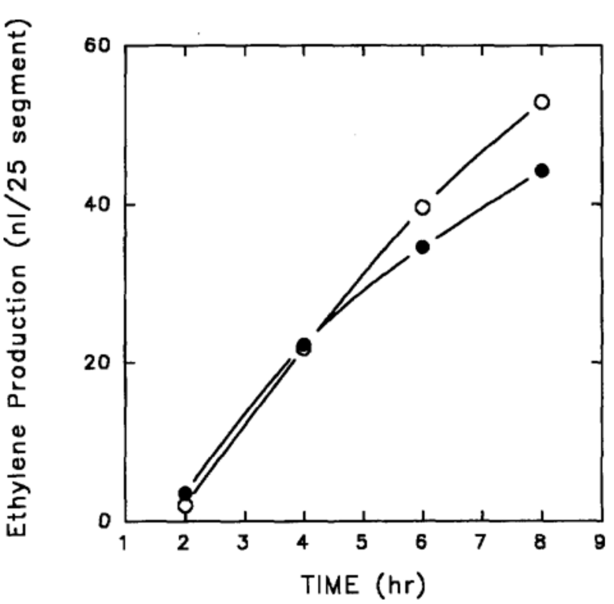

Fig. 5. Kinetics of 0.01 mM IAA-dependent ethylene production from the hypocotyl segments. The hypocotyl segments were incubated with 0.01 mM IAA. Closed circles indicate the indirect treatment of calcined far infrared ceramic powder. Open circles represent the control.

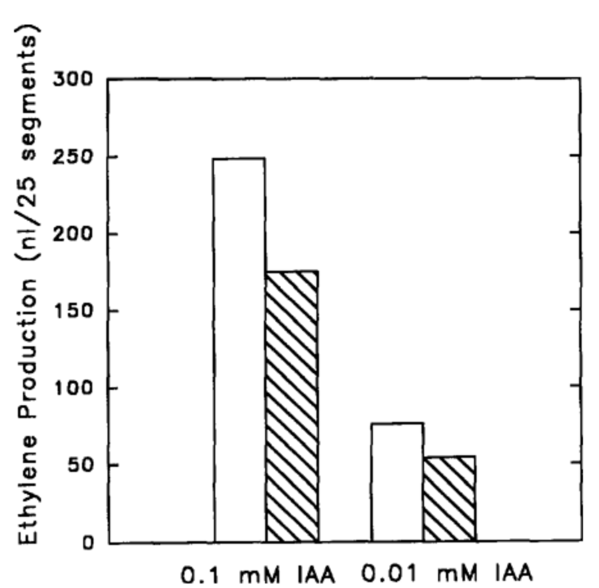

Fig. 6. IAA-induced ethylene production from the hypocotyl segments after 12 hr. Hatched columns indicate the treatment of calcined infrared ceramic powder. White columns indicate the control.

was by about 20% compared to the control after 8 h incubation.

IAA-induced ethylene production was not inhibited by the treatment of the ceramic powder within 4 h.

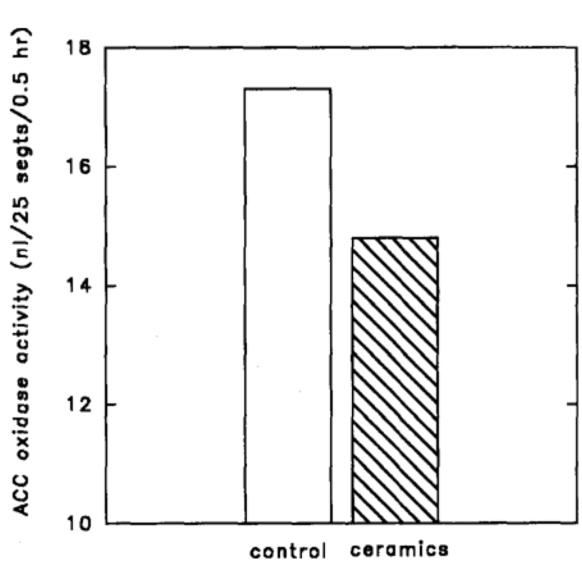

Fig. 7. Effect of far infrared ray emitted from ceramic powder on *in vivo* ACC oxidase activity in hypocotyl segments. Hypocotyls segments were incubated indirectly with or without calcined far infrared ceramic powder for 12 h, and then washed with distilled water. Ethylene production was measured after 0.5 h incubation with 1 mM ACC. The amount of ethylene production was used for in vivo ACC oxidase activity as described in Materials and Methods. Hatched column indicates the treatment of calcined infrared ceramic powder. White column indicates control.

However, the treatment showed approximtely 14% inhibition after 6 h (Fig. 5). This inhibition maintained until 12 h (Fig. 6). These results suggested that the far infrared rays emitted from the ceramic powder, not the ceramic powder itself, inhibited the IAA- or ACC-induced ethylene production. In the presence of the ceramic powder, ACC-induced ethylene production showed more inhibition than IAA-induced ethylene production within 4 h. This difference of kinetics might be a result of the regulation of ACC oxidase activity by producing ethylene. Therefore, we measured *in vivo* ACC oxidase activity in the treatment of ceramic powder as described in Materials and Methods.

Effect of Far Infrared Rays from Ceramic Powder on the Activity of ACC Oxidase

The treatment of ceramic powder inhibited the ACC oxidase activity by 15% (Fig. 7). This result supported the fact that far infrared rays emitted from ceramic powder inhibit ethylene production in the conversion step of ACC to ethylene in mungbean hypocotyl segments.

DISCUSSION

Far infrared rays are electromagnetic waves, ranging from 4 to 1,000 μm. It has been known that far infrared rays keep freshness in and increase the storage period of vegetables and crops. However, the action mechanism of far infrared rays in these physiological effects is not well understood. One possible explanation is as follows: Because the absorption spectrum of these rays belong to the same spectrum ranges as those of the macromolecules absorbed, far infrared rays are easily absorbed in the cells of an organism and affect freshness and storage, especially in vegetables, through resonance with the water in the cells (Sasamori, 1993; Shibukawa, 1993). Some results suggest that mixture of ceramic powder with soil enhances growth of crops such as corn and rice (Lee *et al.*, 1995). Therefore, far infrared rays seem to regulate senescence and growth in plants. Among the plant hormones, ethylene is involved in the maturation of fruit, tissue senescence, and inhibiting stem elongation. Based on these results, we examined the effect of far infrared rays emitted from ceramic powder on the ethylene production in mungbean hypocotyl segments.

In this study, we indirectly treated samples with ceramic powder as illustrated in Fig. 1 to examine the effect of far infrared rays. Therefore, the ceramic powder could not have absorbed the ethylene produced directly or acted on hypocotyl segments as a chemical. Both IAA- and ACC-induced ethylene production was inhibited by the indirect treatment of ceramic powder. These results suggested that only far infrared rays emitted from the ceramic powder inhibited the ethylene production, and that the inhibited step in the ethylene biosynthesis pathway might have heen the conversion step of ACC to ethylene. From these results, we measured the *in vivo* ACC oxidase activity, and far infrared rays inhibited the *in vivo* ACC oxidase activity. However, still there is a possibility that far infrared rays inhibited the other enzymes in the ethylene biosynthesis pathway, such as ACC synthase. Further studies are needed to elucidate the exact action of far infrared rays from ceramic powder such as ACC synthase activity and the content of ACC and malonyl-ACC in mungbean hypocotyl segments. Recently, there has been some data that ACC synthase and ACC oxidase are regulated at the molecular level (Kim *et al.*, 1992; Kim and Yang, 1994). Kim and Yang (1994) isolated 0.9 kb ACC-oxidase cDNA in mungbean hypocotyls. Using this cDNA, Lee *et al.* (1997) showed regulation of the ACC-oxidase transcript by ethylene. They suggested that a latent period was required to increase the amount of ACC-oxidase transcript by the treatment of spermin, which inhibits ethylene production. In this experiment, ACC-induced ethylene production was inhibited by treatment with ceramic powder within 4 h. However, IAA-induced ethylene production was not inhibited at this period. This result suggests that ACC oxidase activity might be regulated by ethylene. Woodson *et al.* (1992) suggested that ACC oxidase activity increased in an autostimulatory manner in the carnation flower. In addition, Lee *et al.* (1997) showed that ACC-oxidase was increased by the promoted ethylene production between 60 min and 120 min in mungbean hypocotyl protoplasts. Therefore, further experiments are needed to focus on the regulation of ACC oxidase activity by far infrared ray at the molecular level.

There are various types of ceramic powder, and the wave length of the emitted rays can be changed by some calcination temperatures. If effective methods of calcination of ceramic powder are developed in order to inhibit ethylene production, it will be possible to increase the storage and freshness period of crops, flowers, or vegetables. Actually, there has been a report that the ceramic powder (Zeolite) has been used in food wrapping materials to increase freshness, and the ceramic powder (Zeolite) absorbed the ethylene produced because of direct contact with the food (Park, 1995). In our system, however, ceramic powder did not absorb the ethylene produced because of indirect treatment of ceramic powder in our system. Therefore, these results suggested a possibility that ceramic powder can be use as a wrapping material without direct contact to the food, vegetables, or crops. Further study will elucidate the action mechanism of ceramic powder of several physiological roles in storage and freshness for long periods.

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