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**EFFECTS OF CARBON DIOXIDE ON THE ETHYLENE-FORMING
ENZYME IN JAPANESE PEAR AND APPLE FRUITS**

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of the requirements for the degree of
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ABSTRACT

The aim of this thesis was to investigate the effects of carbon dioxide on ethylene-forming enzyme (EFE) and its regulation in both Hosui and Granny Smith fruit discs.

1. From measurement of respiration rate and ethylene production after harvest and response of them to propylene treatment, it has been shown that Japanese pear ^{ed} Hosui grown in New Zealand is a nonclimacteric fruit. Granny Smith apple showed different respiration and ethylene production patterns in different seasons. Because fruit produced typical climacteric respiration and ethylene peaks, Granny Smith apple is a climacteric fruit.

2. The rapid rise of ethylene production and respiration measured at 21°C showed no time lag in Granny Smith fruit after storage for 20 days at low temperature ($1^{\circ}\pm 1^{\circ}\text{C}$) compared to those immediately measured after harvest at 21°C. Ethylene production and respiration rate also increased faster and to a greater extent in fruit exposure ^{ed} to low temperature than in fruit measured immediately after harvest. This ripening behavior is similar to that of European pears and Golden Delicious apple. In contrast to Granny Smith apple, ethylene production and respiration patterns of Hosui were not changed by low temperature storage.

3. A reliable method for testing ethylene forming enzyme (EFE) activity in fruit discs of Hosui and Granny Smith was developed.

4. At harvest time EFE activity was present in Hosui fruit, but not in Granny Smith apple. EFE development showed a similar pattern in both fruits, increasing steadily in fruit stored at low temperature.

5. Carbon dioxide stimulated EFE synthesis in fruit discs of preclimacteric Granny Smith, but not in those of Hosui. Carbon dioxide stimulated EFE activity in Hosui fruit discs during the short term storage at $1^{\circ}\pm 1^{\circ}\text{C}$, after ~~that~~ ^{which} CO_2 lost its stimulatory effect. In contrast to Hosui, CO_2 stimulated EFE activity in Granny Smith fruit discs through the measuring period tested.

out

6. Carbon dioxide was not able to reverse Co^{++} (an EFE activity inhibitor) inhibitory effect on EFE activity in ^{23/11/01} both types of fruit discs. This result showed that CO_2 could directly stimulate EFE activity.

7. The stimulatory effect of CO_2 on EFE activity was dependent on exogenous ACC. EFE activity in both types of fruit discs was lower in 0.4 M mannitol solution than in 0.8 M solution, but EFE lost the capability to respond to CO_2 in 0.8 M mannitol solution. This suggests that the EFE located in the plasma membrane is the main form to respond to CO_2 in both types of fruit discs.

8. Results from kinetic studies indicated that EFE in discs of both Hosui and Granny Smith fruits was not allosteric. The apparent K_m values of EFE for ACC were 0.166 mM for Hosui, and 0.193 mM for Granny Smith apple. Carbon dioxide increased the maximum reaction rate of conversion of ACC to ethylene without changing apparent K_m values of EFE for ACC in discs of both Hosui and Granny Smith fruits. It suggests that the mechanism of the direct stimulatory effect of CO_2 on EFE activity was due to the formation of a CO_2 -EFE-ACC complex and/or EFE-ACC- CO_2 complex which increased the maximum rate of the reaction which resulted in the conversion of ACC to ethylene.

9. Silver ions inhibited ethylene production in Granny Smith fruit discs, but not in Hosui fruit discs which did not produce detectable ethylene. Low concentrations (<0.25 mM) Ag^+ stimulated, but high concentrations (>0.5 mM) inhibited, EFE activity in both types of fruit discs. Carbon dioxide did not reverse the inhibitory effect of Ag^+ . Because only System I ethylene receptor is thought to occur in Hosui, a nonclimacteric fruit, and the inhibitory effect of Ag^+ on EFE activity in discs of both types of fruit were similar, results suggest that EFE activity was regulated by the System I ethylene receptor.

10. Norbornadiene (NDE) is a competitive ethylene action inhibitor, which inhibits ethylene synthesis by binding to the ethylene binding site of ethylene receptors. At 0.5% (v/v), NDE inhibited EFE activity in both types of fruit discs, and this inhibition was partially reversed by CO_2 in discs from unripe Hosui and preclimacteric Granny Smith fruit. These results suggest that CO_2 might indirectly stimulate EFE activity by binding competitively to the System I ethylene receptor at the ethylene binding site. When Granny Smith was at

the climacteric stage CO_2 did not reverse the NDE inhibitory effect on EFE activity.

11. According to the response of EFE to CO_2 and the mechanisms of the response, cells in Hosui and Granny Smith fruits were distinguished into four different types. During ripening, cells in fruits changed their type, thereafter the responsiveness of EFE to CO_2 changed. A model is presented to explain the mechanism of CO_2 on EFE synthesis and its activity through interactions with EFE directly and the ethylene receptors indirectly.

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