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## 学 位 論 文 要 旨

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### **Title: Studies on the ‘Tree Factor’ and Its Role in the Development and Ripening of Persimmon (*Diospyros kaki* Thunb.) Fruit**

題目: カキ果実の発育および成熟に伴う ‘樹体要因’ とその役割に関する研究

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Reports of ‘tree factors’ identified that some climacteric fruit on a tree receive inhibitors of ripening or ethylene synthesis from leaves on the parent plant. This thesis summarizes the studies on the ‘tree factor’ or ‘tree ethylene inhibitory factor’ in persimmon fruit, including its different aspects at their various stages of development according to the lowering of ethylene production, and the characterization of the factors at young stage.

#### *1. Physiological profile of persimmon fruit as the advance of fruit development and ripening*

Young fruit harvested in June to July, produced an extremely high rate of ethylene. At the later immature and mature stages, ethylene production of harvested fruit decreased a lot to a basal level, without any significant peak at all.

Calyx abscission took place exclusively at young stage; the calyx abscission rate decreased markedly according to the advance of development; there was no calyx abscission at all in immature or mature fruit from early September, instead, coloration and softening in detached fruit progressed, although it took a relatively long duration.

In overripe stage, there was little ethylene production in fruit with relatively high firmness, while in fruit with jelly-like softened texture, internal ethylene concentration reached to a relatively high level.

#### *2. Variation of effects of ‘tree ethylene inhibitory factors’ in persimmon fruit as the advance of fruit development and ripening according to the lowering in ethylene production*

The present study focused on the role of ‘tree factor’ in persimmon at the different developmental stages. In order to clarify the leaf factor, defoliation has been applied as a main trial through the whole study. Fruit drop, softening, and ethylene production of persimmon fruit with or without attachment of leaves to the bearing shoot were monitored in young, immature and mature fruit.

In young ‘Hiratanenashi’ persimmon, fruit dropped and the endogenous ethylene production increased continuously from 1 day to 4 days after defoliation or leaf-shading, reaching  $9.85 \text{ nL g}^{-1}\text{h}^{-1}$ , but remained low in the control. In intact, early immature ‘Fuyu’ fruit, neither fruit with nor without leaves dropped during a long period, which caused a difficulty in detecting the ‘tree factors’. So at the following mid immature stage, wounding treatment (cut 1 / 3 part from

fruit apex) was applied to induce ethylene production artificially, in order to magnify the effect of 'tree factors'. The results showed that wounded fruit without leaves began to drop 3-fold of that with leaves, also induced internal ethylene in fruit peaked at 60.5 ppm on day 4 after treatments, which was almost 3.5-fold of that with leaves. Afterwards, in wounded, late immature 'Hiratanenashi' fruit, same treatment barely induced fruit drop but only fruit softening, both fruit softening and ethylene production showed a certain increasing tendency in wounded fruit without leaves. Finally, in wounded, mature 'Matsumotowasefuyu' fruit, even the tendency became ambiguous, and the effect of 'tree factor' was unable to be confirmed.

Gene expression of five ethylene biosynthetic enzymes suggested that a large accumulation of *DK-ACS* and *DK-ACO* transcripts activated by wounding stress at cut surface was markedly inhibited in the presence of leaf attachment. In addition, effect of 'tree ethylene inhibitory factor' was more obvious in fruit core rather than in pulp section.

### 3. Characterization of 'tree ethylene inhibitory factors' in persimmon fruit at young stage

Infiltration of a crude leaf extract (extracted in ethanol and dissolved in phosphate buffer), and three leaf - extract fractions of different polarities (hexane, ethyl acetate, and aqueous fractions), and four plant hormones (ABA, GA<sub>3</sub>, IAA, and zeatin) into fruit flesh was conducted, while the results turned not to inhibit endogenous ethylene production in detached young 'Hiratanenashi' persimmons, thus, the leaf extracts contained none of the expected 'tree ethylene - inhibitory factors'.

We determined the effects of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU; a specific photosynthesis inhibitor) and defoliation treatments on photosynthetic rate, transpiration rate, sap flow, fruit drop, and ethylene evolution. DCMU treatment, which prevented photosynthate transport but allowed water flow, promoted fruit drop by 67% 10 days after treatment. The defoliation treatment, which prevented photosynthate and water transport, significantly hastened fruit drop. On average, fruit drop occurred 2 days earlier in the defoliation treatment than in the DCMU treatment.. Compared with that of the control, treated fruit attached to the tree showed an increasing trend in ethylene production, although at a low level ( $\leq 0.8 \text{ nL g}^{-1}\text{h}^{-1}$  in the flesh;  $\leq 20 \text{ nL g}^{-1}\text{h}^{-1}$  in the calyx). Fruit abscission is thought to be induced by a following ethylene burst, because fruit showing an abscission trend showed high rates of ethylene production (almost  $200 \text{ nL g}^{-1}\text{h}^{-1}$ ). Thus, blocking of photosynthate transport and water flow induced initial and autocatalytic increases in production of ethylene, which plays a crucial role in triggering fruit drop. Overall, our results suggest that transport of photosynthates, or both photosynthates and water, to fruit inhibits fruit drop by preventing induction of ethylene synthesis in young persimmon fruit

Further molecular biological research is required to elucidate the mechanisms that underlie the induction and regulation of ethylene biosynthesis, especially on the candidates for 'tree factor'. All these are hopeful to be utilized in improvement of postharvest fruit quality and storability.