INTRODUCTION

In strawberry fruit, it was suggested that IAA synthesized in the fruit migrated to the receptacle and promoted the fruit growth, because the application of auxin to the fruit after removal of the achenes can induce the growth of the fruit of normal size (Nitsch, 1955). However, in many fruits, the application of a combination of plant hormones induces normal fruit growth, and it has been established that fruit growth requires interaction between various plant hormones (Vivian-Smith and Koltunow, 1999). These plant hormones required for fruit growth are mainly synthesized in achenes (Naylor, 1984).

Non-climacteric fruits such as strawberries have been studied for the use of various plant hormones for uniform ripening and fruit quality improvement (Symons et al., 2012). In general, auxins, cytokinins (CK), gibberellins (GA), abscisic acid (ABA) plays a role in inhibiting maturation, while abscisic acid (ABA) is thought to play a role in promoting maturation (Leopold and Kriedemann, 1964). In the application study of in vitro culture of strawberry fruits, auxin and cytokinin inhibited maturation and GA promoted maturation in growing fruits (Kano and Asahira, 1978). In ripening fruits, ABA promoted ripening and CK suppressed ripening (Kano and Asahira, 1981). In strawberries, the presence of outer achenes suppresses receptacle maturation (Given et al., 1988).

In strawberries, bioassay for CK and ABA activity in fruits was reported (Kano and Asahira, 1979). Since then, instrumental analysis which is much more reliable than bioassays has been reported. IAA and ABA were determined by GC-MS in achenes and receptacles of developing strawberry (Archbold and Dennis, 1984). Endogenous levels of IAA, ABA, GA, and castasterone were quantified from flowers to the stage of maturity of developing strawberry by GC-MS or LC-MS / MS (Symons et al., 2012).

In this study, major plant hormones, IAA, abscisic acid (ABA), trans zeatin (Z), isopentenyl adenine (iP), gibberellin, (GA1), and gibberellin 4 (GA4) were analyzed in strawberry achenes and receptacles. The purpose of the study is to gain information about the physiological roles of these endogenous plant hormones.

MATERIALS AND METHODS

Plant material

The material is strawberry (Fragaria ×ananassa Duchesne ‘Echigohime’), cultivated in an elevated cultivation unit (75 cm high, 30 cm wide, 3 m long) installed in a glasshouse of a large scale farmhouse. Medium, peat moss; liquid manure, fertilizer for strawberry diluted 1000 times (N, 13.5; P, 10; K, 20, Otsuka Chemical Co., Ltd.)

Three growth stages were set based on size and appearance characteristics of the fruit (Fig. 1): Green, green and small (2.3 g / fruit); Breaker, red at the tip only (10.1 g / fruit); Turning, almost whole red, a pale green color remains on the side of the calyx (15.5g / fruit). The numbers of fruits collected are as follows: green stage, 307; breaker stage, 95; turning stage, 74. After harvest, they were transported in a cool box containing ice (about 6°C), frozen at –80°C, and freeze-dried (Freeze Drier, VD-800F, Taitec Corp., Saitama). After drying, Achenes were...
picked from the receptacles with tweezers, and stored at −80°C until extraction. Collected achenes and receptacles were homogenized and filtrated according to the procedure of Kojima et al. (2020).

**Analysis for IAA and ABA**

Extraction, separation and purification: Hormone analysis was performed according to the procedure of Kojima et al. (2020). Briefly, achenes and receptacles were homogenized and the three tissues were filtered into a stock solution of about 80% ethanol. We added 13C6-IAA and d6-ABA as internal standards, concentrated aqueous solution, adjusted pH to 2.8 and filtered. Partition extraction was performed with diethyl ether, which was concentrated and filtered.

First HPLC: The extracts were fractionated with a high-performance liquid chromatography (HPLC) system equipped with an ultraviolet detector (Kojima et al., 2002). The HPLC column (Inertsil ODS-3) was isocratically eluted with a solution of 40% ethanol. Eluates corresponding to the retention times of IAA and ABA were collected separately and dried under reduced pressure.

Second HPLC: After fractionation by “First HPLC” the extracts were further fractionated using the same method for “Second HPLC for IAA and ABA”. The fractions of GA1, GA4, Z, and iP were injected separately, and eluates were collected and dried.

**Analysis by LC-MS**

We used a LC-MS to identify the hormones according to Kojima et al. (2020): column, Cadenza CD-C18; flow rate, 0.1 mL min⁻¹; and eluent, 70% ethanol + 30% ultrapure water + 0.1% acetic acid. Selected ion monitoring (SIM) method was selected. Plant hormone concentrations were calculated from the ratio of the peak areas of natural and labeled ions as an internal standard.

**RESULTS AND DISCUSSION**

**Changes in pulp color and fresh weight**

The fruit weight of the receptacle increased as the stage progressed (Fig. 2). The achene weight increased rapidly up to the breaker and gradually increased until the turning stage. As reported by Perkins-Veazie (1995), color
maturation began in the breaker stage and continued during the subsequent maturation process.

Changes in IAA concentration
In the achenes, the IAA concentration was high in the green stage (1230 pmol / gFW), but decreased sharply in the breaker stage and increased slightly in the turning stage (Fig. 3). The receptacle had similar low concentration. Achenes maintained higher concentrations than receptacles throughout the entire period.

External application of auxin to a strawberry fruit inhibits anthocyanin synthesis, while removing the achenes (the source of auxin) during red coloration induces anthocyanin synthesis (Manning, 1993). It is thought that anthocyanin synthesis is induced by a decrease in auxin concentration. Our report supports the results of Manning (1993), since the concentration of IAA in the fruit in the turning stage decreases and coloring proceeds. However, since the receptacle had the same low concentration of IAA, the progress of coloring could not be explained by the changes of IAA alone.

When the IAA data of Archbold and Dennis (1984) were converted to a fresh weight basis, the fruits at the highest concentration were about 600 ng / gFW, which was comparable to our data. However, in the receptacle, it was about 520 ng / gFW, which was about 100 times higher than our data. Future research is expected to elucidate the cause of this order difference.

Changes in Z and iP concentration
In the achenes, Z concentration was low in the green stage, but increased sharply in the breaker stage and decreased slightly in the turning stage (Fig. 4a). The receptacle had similar low concentration. Achenes had higher concentrations than receptacles throughout the entire period.

Fig. 3 Endogenous IAA concentration profile in achenes and receptacles of strawberry at three stages of growth. At each stage, achenes and receptacles of the fruits collected were mixed. After grinding and filtration, internal standards were added. The solution was extracted with diethyl ether, fractionated, purified by HPLC, and quantified by LC-MS.

Changes in Z type cytokinin has an action of promoting the growth of above-ground parts such as leaves and flower stems, while iP type cytokinin does not have such an action (Kiba et al., 2013). Even in the strawberry fruit, Z and iP have different fluctuations, and Z and iP may have different functions.

Changes in GA1 and GA4 concentrations
The GA1 concentration was almost the same in the achene and receptacle, and increased as the stage progressed (Fig. 4b). The GA4 concentrations were varied in different studies.
Achene and receptacle, and was higher in the green stage, but decreased rapidly in the breaker stage, and was almost the same level in the turning stage (Fig. 5a). The GA4 concentration in the achenes decreased slightly in the breaker stage, and rose to the same level in the turning stage. (Fig. 5b). The receptacle decreased as the stage progressed. The GA1 and GA4 were generally at the same level in strawberry fruit (Fig. 5), indicating that the pathways for synthesizing these bioactive forms are working equally. Moreover, it is considered that the two bioactive forms work cooperatively.

The application of GA during fruit growth has been reported to delay ripening in many fruits (McDonald et al., 1997; Zilkah et al., 1997). The GA1 concentration decreased rapidly in the achene and receptacle in the breaker stage (Fig. 5a), and GA4 concentration decreased in the receptacle (Fig. 5b). These results suggest that a decrease in GA contributes to the promotion of fruit ripening. According to the report of Symons et al. (2012), the ABA concentration increased as the stage progressed as in our report, but the ABA concentration was 200−1800 ng / gFW, which is about 100 times higher. It is expected that the cause of the difference in the order of concentration will be clarified in the future research.

Higher levels of ABA in the fruit promote ripening (Coombe, 1976). Because the Z level of cytokinin in the fruits decreased in the later stages of fruit development (Fig. 4a), the suppression of ABA synthesis by Z may be weakened (Leopold and Kriedemann, 1964). Therefore, ABA increase and Z decrease may promote ripening jointly.

It was suggested that IAA diffused from the achene into the receptacle (flesh) and promoted the enlargement of the receptacle (Nitsch, 1955). At the Breaker stage, the IAA concentration in achenes decreased, but in receptacle was almost constant (Fig. 3). Furthermore, it has been thought that the interaction between various phytohormones is required for fruit growth (Vivian-Smith and Koltunow, 1999). It is considered that Z with increased concentration in the Breaker stage and ABA with increasing concentration also contribute to the promotion of
growth (Kojima et al., 1995).

In conclusion, these results suggest the following: 1) promotion of cell division in fruits by high Z concentration in achenes; 2) involvement of increased iP concentration in ripening process; 3) promotion of fruit maturation by reduction of GA; 4) Acceleration of red coloring by increasing ABA concentration in the receptacles.

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