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Comparison of Endogenous IAA and Cytokinins in Shoots of Columnar and Normal Type Apple Trees

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Summary

Seasonal changes of endogenous IAA and cytokinin concentrations in shoots of two columnar type cultivars, 'Maypole' and 'Tuscan', and a normal type apple tree, 'McIntosh', were compared by gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM). The IAA concentrations in both types were higher in June, when shoot growth was vigorous, than in July. However, distinct differences in the IAA concentrations between columnar and normal types were not found. Cytokinin concentrations, particularly zeatin riboside (ZR), in shoots were higher in columnar type trees than in the normal type during the whole growing season in June. These results suggest that the concentration of cytokinins, particularly ZR, in shoots may be related to the growth characteristics of columnar type trees.

Key Words: apple, columnar type, cytokinin, GC-MS-SIM, IAA.

Introduction

Many spur type mutants were discovered as bud sports from 'McIntosh' (Fisher, 1969). The 'McIntosh Wijcik', found in Canada in the latter half of the 1960's, is the most distinctive mutant (Fisher, 1970). This cultivar is called a columnar type tree because it has few lateral shoots and many spurs on the trunk with short internodes. 'McIntosh Wijcik' is unique because it transmits its growth characteristics to its progeny (Lapins, 1969, 1976), whereas those of spur type mutants except 'McIntosh Wijcik' are not. 'McIntosh Wijcik' and its progenies have been utilized for breeding materials at many research stations, and several cultivars have already been registered (Khanizadeh et al., 2002; Tobutt, 1986).

Differences in levels of endogenous growth regulators between columnar and normal type trees have been reported. Looney and Lane (1984) found that the concentration of free abscisic acid (ABA) in spur buds was lower, whereas the free indoleacetic acid (IAA) was higher in 'McIntosh Wijcik' than in 'McIntosh'. Looney et al. (1988) also showed that the levels of polar gibberellins (GAs) of shoots were lower in 'McIntosh Wijcik' than in other strains of 'McIntosh' mutants; less-polar GAs existed at the same level in all cultivars. Likewise, they showed that cytokinin-like substances were higher in 'McIntosh Wijcik' than in 'McIntosh'. Although several researchers compared the endogenous growth regulators in both type of trees, only a few have examined the relationship between the growth characteristics of columnar type and the dynamics of endogenous growth regulators.

Cook et al. (2001) showed that auxin undoubtedly plays a central role in the correlative phenomena, relating the determination of acrotony and tree architecture in apple trees; cytokinins are also related to the habits of lateral shoot growth. Furthermore there are clear differences in the distribution of zeatin riboside (ZR) in shoots between cultivars with different habits of lateral shoot growth. Seasonal changes and the distribution of auxin and cytokinins in columnar type trees might also determine their growth habits.

Therefore, in this study we compared IAA and cytokinin concentrations in shoots during the growth period in columnar and normal type apple trees, to seek the possible relationship between these phytohormones and the growth characteristics of columnar type trees.

Materials and Methods

Plant materials

Three- or four-year-old columnar type, 'Maypole' and 'Tuscan' apple trees on seedling rootstocks and 38- or 39-year-old normal 'McIntosh' growing at the Department of Apple Research, National Institute of Fruit Tree Science, were used for this study. In 11 July of 2000 and in 1 June of 2001, columnar type tree shoots developing from just below the previous year's pruning

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cut were harvested, and 'McIntosh' shoots developing on one- or two-year-old branch were harvested. These harvested shoots were separated into two subsamples for analysis: 0.5 cm shoot tips with expanding leaves removed and two cm of the subapical part including two lateral buds just below the tip. These samples were stored at −30 ℃ until analyzed.

Determination of shoot length

The lengths of ten shoots on one-year-old branch of 'Maypole', 'Tuscan' and 'McIntosh' were measured periodically from 21 May until 19 November of 2001 to determine their growth characteristic.

Extraction and purification procedure for IAA

A 500 mg (FW) sample macerated in cold 80% (v/v) ethanol containing 50 mg soluble polyvinylpyrrolidone (K-30, Tokyo Kasei Kogyo Co. , Ltd., Tokyo) with 5 nmol of [$^{13}C_6$]-IAA as an internal standard was incubated overnight at 4 ℃ . The homogenate was centrifuged (10,000 × g, 10 min, 4 ℃) and filtered. The filtrate was evaporated in vacuo at 40 ℃ and the residue dissolved in distilled water. The solution was adjusted to pH 2.5 with phosphoric acid and the acidic phase partitioned three times with petroleum ether. The organic phase was discarded, and the water phase was partitioned three times with diethyl ether. The diethyl ether phases were combined and evaporated to dryness in vacuo. The residue was dissolved in 20% (v/v) methanol and filtered through a millipore filter (0.45 μm). The filtrate was injected into a high performance liquid chromatograph (HPLC), equipped with an Inertsil ODS-2 column (10 mm i. d. × 150 mm; GL Science Inc., Tokyo), and eluted with linear gradient from 30% (v/v) to 80% (v/v) methanol in 0.5% (v/v) acetic acid for 50 min. The flow rate was 3 ml・min^{-1}. The eluates with retention times from 16 min to 20 min were collected as the IAA fraction. Each sample was prepared in duplicate.

Extraction and purification procedure for cytokinins

Cytokinins were analyzed for zeatin (Z), ZR, isopentenyladenine (iAde) and isopentenyladenosine (iAdo). A 500 mg (FW) sample was macerated in cold 80% (v/v) methanol, containing 100 ng each of [$^2H_5$]-Z, [$^2H_5$]-ZR, [$^2H_6$]-iAde, and [$^2H_6$]-iAdo as an internal standard, then extracted overnight at 4 ℃. The extract was centrifuged (10,000 × g, 10 min, 4 ℃) and then filtered. The filtrate was evaporated in vacuo at 40 ℃ and the residue dissolved in 0.03 N acetic acid. This solution was centrifuged (14,000 × g, 30 min, 4 ℃), and the supernatant passed through a column of polyvinylpoly pyrrolidone after which the column was washed with three volumes of 0.03 N acetic acid. The eluate was evaporated to dryness in vacuo; the residue was dissolved in phosphate buffer (pH 8.5) and adjusted to pH 8.5 with 1 N NaOH. The solution was partitioned four times with half volume of water-saturated *n*-butanol and the combined *n*-butanol phase was evaporated to dryness in vacuo. The residue was dissolved in 0.03 N acetic acid, passed through a Sep-Pak C18 cartridge, then washed with distilled water before the cytokinins were eluted with 70% (v/v) methanol. The eluate was evaporated to dryness in vacuo, and the residue was dissolved in 20% (v/v) methanol. The solution was filtered through a millipore filter (0.45 μm). The filtrate was injected into the HPLC under the same condition as for IAA analysis; the column was eluted with a linear gradient from 5% (v/v) to 80% (v/v) methanol in 0.5% (v/v) acetic acid for 85 min. The flow rate was 3 ml・min^{-1}. Eluates with retention times of 17, 27, 36 and 56 min were collected as fractions of Z, ZR, iAde and iAdo, respectively. The analysis of each sample was replicated.

Gas chromatograph-mass spectrometry-selected ion monitoring (GC-MS-SIM) analysis

The collected IAA fraction was methylated with diazomethane, and the collected cytokinin fractions were each permethylated with methyl iodide. The IAA and cytokinin derivatives were quantified with a QP5000 GC-MS-SIM (Shimadzu Inc., Kyoto) using a fused silica capillary column (CBP1, 0.22 mm i.d. × 25 m, Shimadzu Inc., Kyoto). Selected ion monitoring was conducted at m/z 130/136 for IAA/[$^{13}C_6$]IAA, 230/235, 216/221, 231/237 and 391/397 for Z/[$^2H_5$]Z, ZR/[$^2H_5$]ZR, iAde/[$^2H_6$]iAde, and iAdo/[$^2H_6$]iAdo, respectively.

Statistics

Data for IAA concentration was analyzed by two-way (cultivars × month) analysis of variance (ANOVA); data for total cytokinin concentration in June was analyzed by a one-way ANOVA. The data from July could not be statistically analyzed because some data were lacking. Furthermore, IAA concentrations between cultivars on the same date were compared using the least significant difference test at the 5% level.

Results

The shoots of two columnar type apple trees in this study grew until October, whereas those of 'McIntosh' ceased growing in July (Fig. 1).

The IAA concentration in the shoot tips and subapical parts of shoot was significantly different among cultivars and months (Fig. 2). In all cultivars, IAA concentration was higher in June than in July in both shoot tips and subapical parts. In early June, the IAA levels in shoot tip was highest in 'Maypole', but not significantly different between 'Tuscan' and 'McIntosh'. However, shoot tips of 'Maypole' and 'Tuscan' in early July had significantly higher IAA concentration than those of 'McIntosh' (Fig. 2A). In early June, all cultivars showed a similar concentration of IAA in subapical parts of the shoot. However, in early July, the levels in 'Maypole' and 'Tuscan' were significantly higher than that of

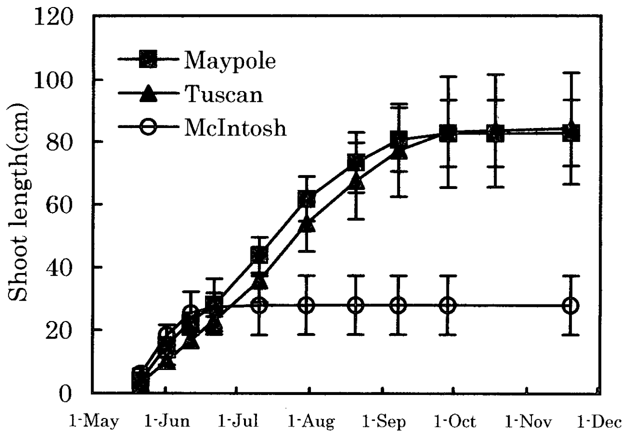

Fig. 1. Growth curves of current shoots of 'Maypole', 'Tuscan' and 'McIntosh' apple trees. Vertical bars indicate SD (n=10).

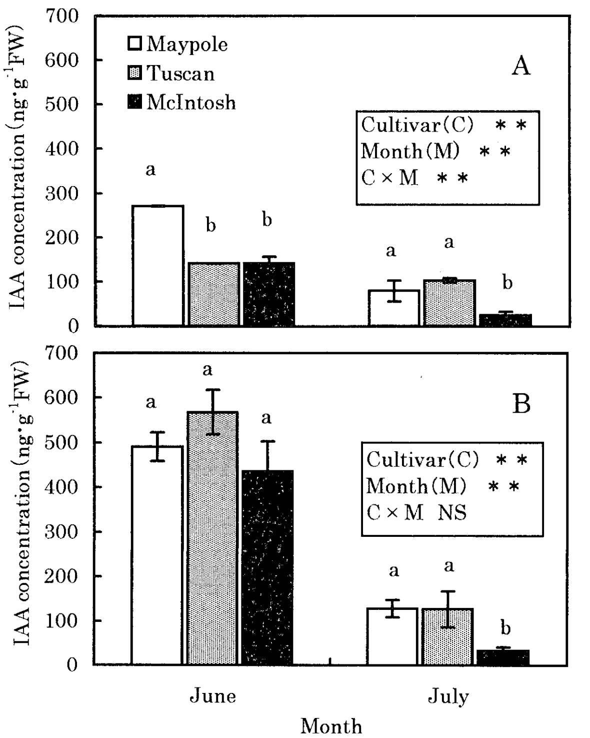

Fig. 2. IAA concentration in the shoot tips with expanding leaves removed (A) and the subapical parts of the shoot including two lateral buds (B) in 'Maypole', 'Tuscan' and 'McIntosh' in early June and early July. Vertical bars indicate SD (n=2). Different letters indicate significant difference (P<0.05) by LSD test on each date. NS,** Nonsignificant or significant at P<0.01, by two-way analysis of variance.

'McIntosh' in subapical parts of shoot (Fig. 2B).

Total cytokinin concentrations in shoot tips in early June were significantly higher in 'Maypole' and 'Tuscan' than in 'McIntosh' (Fig. 3A), whereas that in subapical parts of shoots in early June were highest in 'Tuscan' among the three cultivars (Fig. 3B). However, the mass spectrum of deuterated iAde and putative iAde in shoot tips of 'Tuscan' in July could not be measured (data not shown). Although the respective cytokinin

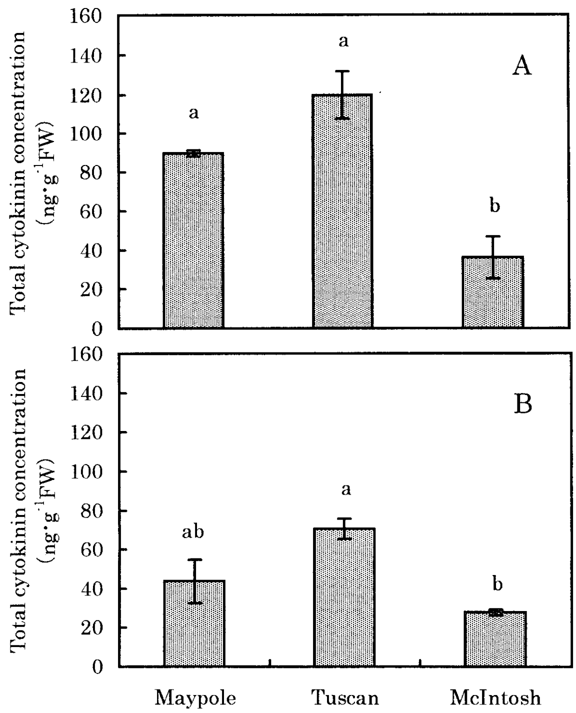

Fig. 3. Total cytokinin concentrations in the shoot tips with expanding leaves removed (A) and the subapical parts of the shoot including two lateral buds of shoots (B) in 'Maypole', 'Tuscan' and 'McIntosh' in early June. Vertical bars indicate SD (n=2). Different letters indicate significant difference (P<0.05) by LSD test.

concentrations and distribution in the shoots of 'Maypole' and 'Tuscan' had similar patterns in early June and early July, those of 'McIntosh' differed, depending on the sampling date (Fig. 4A, B). The main cytokinin was ZR in all cultivars. ZR concentration tends to be higher in columnar type trees than in the normal type, especially in June. The differences between ZR and other cytokinins were greater in 'Maypole' and 'Tuscan', compared to 'McIntosh'. Concentrations of ZR and iAdo in shoot tips of 'McIntosh' were higher in early July, compared to early June (Fig. 4A). In the subapical part of a shoot, the concentration of iAdo of 'McIntosh' was higher in early July, compared to early June (Fig. 4B).

Discussion

The relationship between the growth characteristics of columnar type trees and endogenous growth regulators, such as auxin (Lee and Looney, 1977; Looney and Lane, 1984), cytokinins (Looney et al., 1988; Sarwar et al., 1998) and gibberellins (Lee and Looney, 1977; Looney et al., 1988) have been reported, but their results were inconsistent. Our study on the seasonal changes of auxin and cytokinins suggests that they are involved with the growth characteristics of columnar types.

Shoot length, determined in 2001 reveal that shoots of normal type trees grew from budbreak in May and terminated in July, while the growth of columnar type

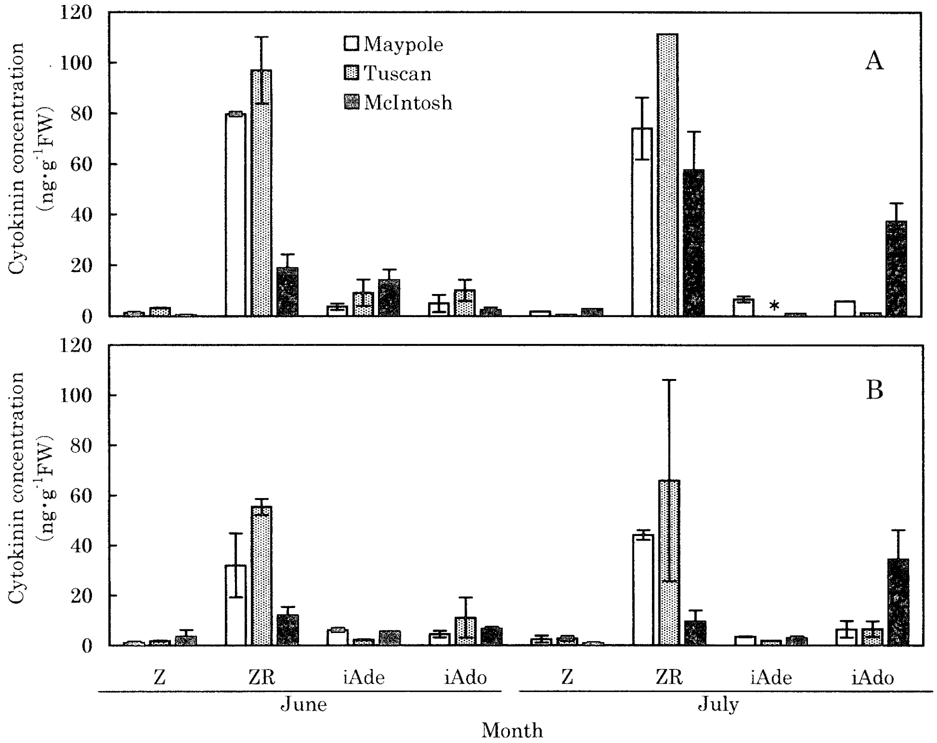

Fig. 4. Cytokinin (CK) concentrations in the shoot tips with expanding leaves removed (A) and the subapical parts of the shoot including two lateral buds of shoots (B) in 'Maypole', 'Tuscan' and 'McIntosh' in early June and early July. Vertical bars indicate SD (n=2). *data lacked.

cultivars continued until October as reported previously by Watanabe et al. (2002). From the observation that the growth of columnar type trees continues until autumn, we assume that shoot growth of both types observed in 2000 was similar to that in 2001. Therefore, although the IAA and cytokinins levels were measured in July of 2000 and in June of 2001, we compared these data and discussed the relationship.

IAA concentrations in both parts of columnar and normal type trees were equally high in June when shoot growth was vigorous; they decreased in July; that of the 'McIntosh' was lower than that in columnar type cultivars. Kamboj et al. (1997) reported that the polar transport of IAA was faster in rapidly extending shoots than in slower growing ones. Therefore, the differences in IAA concentration between the tree types in July may be attributable to the differences in the extension rates between the columnar and the normal types. IAA has been found to play a minor role in the regulation of growth habits of the columnar type (Lee and Looney, 1977); however, one report recognized a relationship between IAA concentration and shoot growth (Looney and Lane, 1984). The more rapid growth rate of columnar types in July may be attributed to the higher IAA concentration, but distinct differences in the IAA concentrations between columnar and normal types during the whole growing season did not exist.

Looney et al. (1988) also found cytokinin concentrations to be higher in 'McIntosh Wijcik' than in 'McIntosh'. ZR has previously been shown to be the predominant cytokinin associated with budburst in apple (Cutting et al., 1991; Tromp and Ovaa, 1990). The higher ZR concentrations in the columnar type compared to 'McIntosh' suggest that ZR concentrations in shoots may affect the growth characteristics of columnar type trees which have many spurs.

Application of BA to apple trees promotes lateral shoot development (Hrotko et al., 1997; Wertheim and Estabrooks, 1994); moreover, cytokinins play a central role in the budburst of apple trees (Cutting et al., 1991; Tromp and Ovaa, 1990). One might thus assume that columnar type trees in the present study produced more lateral shoots compared to 'McIntosh' because of higher cytokinin concentration. However, in many cases, most lateral buds in the columnar type undergo budbreak and subsequently grow into short spurs but not into long shoots. It also may be that the nutrient competition among the lateral buds and the lack of growth promoters, such as gibberellin in the lateral buds (Looney et al., 1988), promote spur formation of the columnar type trees. In this study, endogenous cytokinins levels in 3 to 4-year old columnar type trees were compared with those in nearly 38-year old normal type trees which may have biased the results. In the future, we should compare the levels of endogenous cytokinins in both growth types of the same age.

Cook et al. (2001) indicated that auxins appear to prevent the movement of cytokinins into lateral buds under the influence of apical dominance. Our results imply the possibility that there might be differences in the synthesis and metabolism of cytokinins in columnar type trees and normal type trees. Our data agree with the findings of Jones (1982) who suggested that 'Wijcik' has the capacity to metabolize excess cytokinins to levels required for growth.

In conclusion, the differences in growth characteristics of the columnar type trees and normal ones could not be ascribed to IAA levels in their shoot tips. However, the high concentration of total cytokinins, particularly ZR, of shoots in columnar type trees may be related to its growth characteristics. Current distal shoots, exhibiting relatively intense growth, were analyzed in this experiment, although the growth characteristics of columnar types were exhibited by lateral and spur buds on branches more than one year old. In the future, it is necessary to investigate the dynamics of endogenous growth regulators in trees of similar ages to compare the relationship between growth characteristics of columnar types and normal ones.

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カラムナータイプおよび普通タイプリンゴ樹間における新梢中の内生IAAおよびサイトカイニンの比較

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摘　要

カラムナータイプリンゴ樹2品種，‘メイポール’および‘タスカン’，および普通タイプ樹‘旭’の新梢中における内生インドール酢酸およびサイトカイニン含量の変化を調査した．両タイプのIAA含量は，7月よりも新梢伸長の盛んな6月において高くなる傾向がみられた．しかしながら，生育期間を通じたカラムナータイプ樹と普通タイプ樹間におけるIAA含量の明瞭な差異は認められなかった．サイトカイニン含量，特にZR含量は，6月のカラムナータイプ樹の新梢において普通タイプ樹よりも高かった．以上の結果から，カラムナー生長特性には，新梢中におけるサイトカイニンの量が関与していることが推察された．