Relationship between Indole-3-acetic Acid and Flowering in Two Apple Cultivars, Fuji and Ohrin

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Summary
The effect of indole-3-acetic acid (IAA) on flowering in apple was investigated by using ‘Fuji’ (biennial bearing cultivar) and ‘Ohrin’ (regular-bearing cultivar) cultivars (Malus pumila Mill.). IAA concentration in the seed of ‘Fuji’ was higher than that of ‘Ohrin’ 28 days after full bloom (DAFB). The IAA levels in ‘Ohrin’ peaked later than they did in ‘Fuji’. After 55 DAFB, IAA levels in the bourse of fruiting trees were higher than those of trees which had undergone non-fruiting deflorated treatment. The IAA levels in the seed and bourse of ‘Fuji’ peaked at 77 DAFB which coincide with the time of flower bud initiation. These results suggest that IAA may be related to flower bud formation in apple.

Key Words: indole-3-acetic acid, flowering, apple.

Introduction
In the relationship between plant hormone and flower bud differentiation in fruit trees. Marino and Greene (1981) found that bearing spurs had more GA activity than had vegetative spurs in ‘Early McIntosh’ apple, a biennial bearing cultivar. This suggests that the presence of fruit and GA influences flower bud formation in apple. Although the relationship between IAA and flower bud differentiation was established in the satsuma mandarin (Yahata et al., 1995), the same on apple (Buban and Faust, 1982) was not clear.

In this report, seeds and bourse of ‘Fuji’ (biennial bearing cultivar) and ‘Ohrin’ (regular-bearing cultivar) apples were analyzed for indole-3-acetic acid (IAA) and the relationship between IAA and flowering was investigated.

Materials and Methods
Twelve 25-27-yr old ‘Fuji’ and ‘Ohrin’ apple trees (Malus pumila Mill.) grafted onto a domestic rootstock (Malus prunifolia Borkh.) in a commercial orchard near Shobara city were randomly selected in 1994-1996 for this study.

Fruiting and non-fruiting treatments for each cultivar were made with three pairs of trees (n=6) per treatment. In the fruiting plot, the leaf/fruit ratio was about 9.5; in the non-fruiting plot, all flowers were removed annually at anthesis. The percentage of flowering spurs in each treatment in both cultivars was recorded each spring by dividing the number of blossom clusters by the total number of spurs \times 100. Seeds were collected from each cultivar between 28-108 days after full bloom (DAFB), at intervals of 7-16 days in 1994; bourses of trees from fruiting and non-fruiting treatments were collected between 35-108 DAFB, at intervals of 10-16 days. In the non-fruiting treatment, the non-flowering bourses were used.

IAA concentration was measured by the indolo-α-pyrene fluorescence method (Kamisaka, 1983; Hayata et al., 1996) in which a 2-g sample (dry mass) was extracted in 80% methanol containing [3H]-IAA. The solution was filtered and the filtrate reduced in a vacuum to the aqueous phase. After the aqueous phase was adjusted to pH 8.5 with 0.5 mol K2HPO4, it was then partitioned with petroleum ether. The ether fraction was discarded and the aqueous phase was adjusted to pH 3.5 with 0.1 mol phosphoric acid; it was then partitioned with dichloromethane. The mixture was shaken with 2% NaHCO3 and the organic layer discarded. After the aqueous phase was altered to pH 3.0 with 0.1 mol phosphoric acid, it was partitioned with dichloromethane again. This final dichloromethane fraction was reduced to dryness in a vacuum. The residue was redissolved with an ice-cooled 400 μL mixture of acetic anhydride and trifluoroacetic acid. The reaction mixture was analyzed by HPLC (Jasco Gulliver; Japan Spectroscopic Co., Tokyo) column = Inertsil ODS-2 (GL Sciences Inc., Tokyo, Japan; 4.6 mm I.D. \times 25 cm); column temperature = 35 °C; mobile phase = 60/39.5/0.5 (v/v/v; methanol/H2O/acetic acid); detection = fluorescence detector, excitation = 440 nm, fluorescence = 490 nm); and IAA concentration in the sample was calculated by correcting for the quenching effect (Kamisaka, 1983).

Recovery of IAA was determined by measuring the radioactivity of an aliquot of the final fraction with a liquid scintillation system (LSC-360; Aloka Co.,

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Results and Discussion

The data on the rate of flowering in trees with the fruited treatment and non-fruited treatment in 'Fuji' and 'Ohrin' over 3 years (Table 1) reveal that although flowering was nearly equal in both cultivars and treatments in 1994, the percentage was 16% less in the fruited treatment of 'Fuji' as compared to that of 'Ohrin' in 1995. This result indicates that 'Fuji' had a poorer return bloom and/or a greater biennial bearing tendency than 'Ohrin' had.

The IAA analyses in seeds of 'Fuji' and 'Ohrin' (Fig. 1) reveal that 'Fuji', the IAA level increased dramatically from 44 DAFB to 77 DAFB, reaching maximum and then decreasing to 108 DAFB. Contrarily, in 'Ohrin', its level increased gradually from 35 DAFB and reached a maximum at 92 DAFB, then decreased dramatically until 108 DAFB. IAA levels in 'Fuji' seeds were higher than in those of 'Ohrin', except for at 44 DAFB. Ramirez (1995) reported that GA content in seeds of 'Golden Delicious' and 'Rome Beauty' apples began to increase from about 42 DAFB, reached a peak at about 70 DAFB, and then declined. In our study, the changes of IAA levels in the seed approximately coincided with those of GA activity (Ramirez, 1995). Flower bud initiation in 'Fuji' and 'Ohrin' occurs from the beginning to the middle of July (Yokota, 1983). In our study, this period is 60–80 DAFB and corresponds to the peak of IAA levels in seeds of 'Fuji'. IAA levels in seeds of 'Ohrin' were lower than in those of 'Fuji' and reached a peak later in flower bud differentiation period. Therefore, it can be assumed that IAA levels in seeds may contribute to the difference in flowering between these two apple cultivars.

In the fruited treatment, the IAA levels in the bourse of 'Fuji' changed similarly to those in the seed. Although the level of IAA in the bourse of 'Fuji' fluctuated, there was an increase between 44 DAFB and 77 DAFB when it reached a maximum; it then decreased until 108 DAFB (Fig. 2). In contrast, the IAA levels in the bourse of 'Ohrin' that underwent the fruited treatment did not always coincide with those in the seeds. However, similar tendencies existed, that is, the levels increased gradually from 35 DAFB until 108 DAFB except at 92 DAFB (Fig. 3). The maximum IAA levels in the bourse of 'Fuji' in the fruited treatment was higher than in that of 'Ohrin', whereas, in the non-

Table 1. Effect of fruiting on the flowering in 'Fuji' and 'Ohrin' apple trees.

<table>
<thead>
<tr>
<th>Cultivar Treatment</th>
<th>Flowering</th>
<th>1994 (%)</th>
<th>1995 (%)</th>
<th>1996 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ohrin</td>
<td>Fruiting</td>
<td>71.8 ± 1.7</td>
<td>55.3 ± 1.1</td>
<td>61.0 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Non-fruited</td>
<td>73.2 ± 2.3</td>
<td>69.5 ± 1.9</td>
<td>81.6 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>NS</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Fuji</td>
<td>Fruiting</td>
<td>70.4 ± 0.7</td>
<td>38.7 ± 1.3</td>
<td>51.6 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Non-fruited</td>
<td>68.9 ± 1.2</td>
<td>64.6 ± 1.5</td>
<td>78.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>NS</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

Data are means ± SE of three trees.
NS * ** Nonsignificant or significant at P=0.05 or 0.01, respectively.

Fig. 1. Seasonal changes in IAA concentration in the seed of 'Fuji' and 'Ohrin' apples between 28 and 108 days after full bloom (DAFB). Data are means of three replications.

Fig. 2. Changes in IAA concentration in the bourse of 'Fuji' apple trees between 35 and 108 DAFB. Data are means of three replications.
fruiting treatment, although IAA levels were higher than those of the fruiting treatment until 44 DAFB in 'Fuji' and 55 DAFB in 'Ohrin', those levels subsequently became lower than those in the fruiting treatment. Thus, IAA in the seed which increases with seed development may translocate to the bourse; IAA may then regulate flower bud formation in the same manner as GA (Buban and Faust, 1982). Ito (1998) reported that in ‘Kosui’ Japanese pear, IAA concentration in the bud of the current shoot decreased when the shoot was bent and flower bud formation was promoted. This result indicated that the degree of shoot growth may be related to the regulation of flower bud formation by IAA. However, the effect of IAA in the seeds on shoot growth needs to be examined. Browning et al. (1992) showed that although (2RS, 3RS)-paclobutrazol (an inhibitor of GA biosynthesis) application decreased shoot growth and stimulated flower initiation of pear ‘Doyenne du Comice’, it did not reduce IAA production in the shoot apex. Hence, the interaction of IAA and GA on flower bud formation and shoot growth should be investigated.

**Literature Cited**


