Effects of Some Plant Growth Regulators on the Development of Strawberry Fruits in vitro Culture

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Summary

Fruits of strawberry, Fragaria x ananassa (Duch), cv. ‘Hokkoh-wase’, one day prior to anthesis, were cultured in vitro to determine the roles of seeds in fruit development in relation to plant growth regulators.

1. Fruits from which carpels were removed enlarged normally as intact fruits with carpels on the medium with α-naphthaleneacetamide (NAAnm). The ripening of intact fruits with carpels on the NAAnm medium was suppressed in comparison to that of fruits without carpels.

2. When fruits with or without carpels were cultured on the medium with gibberellic acid (GA3), in either fruit, only the basal portion, which was originally devoid of carpels, swelled, but the upper portion, where there were carpels originally, showed no growth. With addition of GA3 to the NAAnm medium, fruits with carpels enlarged into normal shape and their growth and ripening were promoted in comparison to those of fruits on the NAAnm medium.

3. When fruits with or without carpels were cultured on the medium with N6-benzyladenine (BA) and NAAnm, the growth and ripening were suppressed in either fruit as the concentration of BA increased.

4. When fruits with carpels were cultured on the medium with maleic hydrazide (MH) and NAAnm, the browning of the carpels became severe as the concentration of MH increased, and the growth and ripening were promoted.

Based on these results, it is suggested that auxin is more essential for the growth of strawberry fruits than gibberellin, and the carpel is the production site of cytokinin, which suppresses the growth and ripening of strawberry fruits.

Introduction

There is much evidence that the growth of many fruits is regulated by the plant growth regulators produced in their seeds. Nitsch (10) first demonstrated that an exogenously applied auxin sustained the growth of strawberry fruits, from which achenes had been removed. He (12) also showed that the auxin produced in fertilized carpels is a promotive factor for the growth of strawberry fruits (receptacles). Thompson (16), however, postulated that fertilization shifts a substance(s) in unfertilized mature carpels which inhibits the growth of strawberry fruits, and that this shifting triggers fruit growth.

Nitsch (11) succeeded in culturing the ova-
with detergent, then sterilized for 10 minutes with sodium hypochlorite solution (containing 2% active chlorine), to which 0.05% Tween-20 had been added, and rinsed three times with distilled water. The pedicel, calyx and corolla (and carpels if necessary) were removed and fruits with or without carpels were placed on 20 ml of the medium in 25×200 mm test tubes.

The basal medium contained Murashige and Skoog’s macro elements (9) and the following minor elements and organic substances (in mg/l; Ringe and Nitsch, (14)) : MnSO₄·2H₂O (35), H₂BO₃ (10), ZnSO₄·4H₂O (10), KI (1), Na₂MoO₄·2H₂O (0.25), CuSO₄·5H₂O (0.035), CoCl₂·6H₂O (0.035), myo-inositol (100), nicotinic acid (5), glycin (2), pyridoxine·HCl (0.5), thiamine·HCl (0.5), folic acid (0.1), biotin (0.05) and sucrose (50,000). The pH of the medium was adjusted to 5.5 with NaOH before adding 0.8% agar and autoclaving the whole at 1.1 kg/cm² for 10 minutes.

Ns-benzyladenine (BA), α-naphthaleneacetic acid (NAA), α-naphthaleneacetamide (NAAm), (3-naphthoxyacetic acid (NOA) and maleic hydrazide (MH) were added as growth regulators before autoclaving. Only gibberellic acid (GA₃) was added to the autoclaved medium through a sterilized Millipore filter.

Each culture consisted of 10 fruits. The cultures were placed under 3000 lux of 16-hour illumination by cool white fluorescent lamps at 25-30°C. Fruit weight and the number of days to ripening (number of days from explanting to red coloration of the whole surface of the fruits) were recorded.

Results

Effects of auxin.

To select a growth regulator suitable for inducing the normal growth of strawberry fruits in vitro, the effects of several synthetic auxins were investigated. When 1 mg/l of NAA or NOA was added to the medium, normally shaped fruits developed, but a large amount of callus was formed at the basal parts (Fig. 1-a). When 1 mg/l of NAAm was added, no callus was formed but fruits did not grow well (Fig. 1-b). By increasing the NAAm concentration to 10 mg/l in the medium, fruits grew as well as with 1 mg/l of NAA or NOA and no callus was formed (Fig. 1-c). Therefore, NAAm was added to the medium as auxin in the following experiments, unless otherwise stated. After a 17-day culture period, the optimum concentration of NAAm for the growth of fruits without carpels was 50 mg/l with a fruit weight...
of 420 mg. The optimum concentration for the growth of fruits with carpels was 10 mg/l and the fruit weight was 255 mg for the same culture period (Fig. 2).

In fruit ripening, fruits without carpels had turned red after the 17-day period on 50 mg/l of NAAm medium but on 0, 10 and 100 mg/l of NAAm they remained pale green, white and pink respectively. In contrast, all fruits with carpels remained pale green irrespective of the NAAm concentration (Table 1).

**Effects of gibberellin**

When fruits with carpels were cultured on the medium with 10 mg/l of GA₃ for 14 days, only the basal portion originally lacking carpels swelled, but the upper portion with carpels attached showed no growth (Fig. 3-a). Also when fruits without carpels were cultured on 10 mg/l of GA₃ medium, the basal portion originally lacking carpels grew, but the upper portion where carpels were removed did not grow (Fig. 3-b). The response of fruits without carpels to GA₃ differed from that of fruits without carpels to NAAm (Fig. 3-c). When both 10 mg/l of GA₃ and 10 mg/l of NAAm were added to the medium, fruits grew normally even with carpels attached (Fig. 3-d). The weight of the fruits cultured for 17 days on the medium with 10 mg/l each of GA₃ and NAAm was twice that of the fruits cultured on the medium with 10 mg/l of NAAm alone (Fig. 4). Thus, a supplement of GA₃ in the medium containing NAAm promoted the growth of normally shaped fruits.

In the ripening of fruit, GA₃ was promotive. Fruits with carpels cultured on the medium with 1 mg/l of GA₃ + 10 mg/l of NAAm or with 10 mg/l of GA₃ + 10 mg/l of NAAm had already turned red after a 17-day culture period.

### Table 1. Effect of auxin on the ripening of strawberry fruits after a 17-day culture period.

<table>
<thead>
<tr>
<th>Conc. of NAAm (mg/l)</th>
<th>Fruit color with carpels</th>
<th>Fruit color without carpels</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Light green</td>
<td>Light green</td>
</tr>
<tr>
<td>10</td>
<td>Light green White</td>
<td>White</td>
</tr>
<tr>
<td>50</td>
<td>Light green Red</td>
<td>Red</td>
</tr>
<tr>
<td>100</td>
<td>Light green Pink</td>
<td>Pink</td>
</tr>
</tbody>
</table>

![Fig.3. Comparison of the effects of gibberellin and auxin on the development of strawberry fruits after a 14-day culture period.](image)

**Fig.4. Effect of gibberellin on the growth of strawberry fruits with carpels after a 17-day culture period. All media contained a supplement of 10 mg/l of NAAm.**

<table>
<thead>
<tr>
<th>Conc. of GA₃ (mg/l)</th>
<th>Fruit color</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Light green</td>
</tr>
<tr>
<td>0.1</td>
<td>Light green</td>
</tr>
<tr>
<td>1</td>
<td>Red</td>
</tr>
<tr>
<td>10</td>
<td>Red</td>
</tr>
</tbody>
</table>

### Table 2. Effect of gibberellin on the ripening of strawberry fruits with carpels after a 17-day culture period. All media contained a supplement of 10 mg/l of NAAm.
Effects of cytokinin.

When fruits were cultured on the medium with BA alone, they did not grow. Therefore, 50 mg/l of NAAm was incorporated in all the media used to investigate the effects of cytokinin. The growth of fruits cultured on the medium with NAAm and BA (0.1, 1.0 and 10 mg/l) for 19 days was suppressed, compared to that on the medium with NAAm alone. This suppression by BA was especially pronounced when fruits without carpels were cultured (Fig. 5).

Fruits without carpels ripened earlier than those with carpels in each treatment with the growth regulators (Table 3). The incorporation of BA to the medium retarded fruit ripening irrespective of the presence or absence of carpels. Accordingly, the number of days to ripening for the fruit with carpels on 50

Table 3. Effect of cytokinin on the ripening of strawberry fruits cultured in vitro. All media contained a supplement of 50 mg/l of NAAm.

<table>
<thead>
<tr>
<th>Conc. of BA (mg/l)</th>
<th>Number of days to ripening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With carpels</td>
</tr>
<tr>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>0.1</td>
<td>37</td>
</tr>
<tr>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>10</td>
<td>&gt;45</td>
</tr>
</tbody>
</table>

mg/l of NAAm medium was the same as for the fruit without carpels on the 0.1 mg/l of BA + 50 mg/l of NAAm medium. In addition, the number of days to ripening increased as the concentration of BA increased (Table 3).

Effects of maleic hydrazide.

In the following experiments, all the media had supplements of 10 mg/l of NAAm. The weight of the fruits with carpels cultured on the medium with 0, 1, 10 and 50 mg/l of MH was 290 mg, 385 mg, 370 mg and 490 mg re-

Fig.5. Effect of cytokinin on the growth of strawberry fruits after a 19-day culture period. All media contained a supplement of 50 mg/l of NAAm. Solid bar: with carpels. Empty bar: without carpels.

Fig.6. Effect of MH on the growth of strawberry fruits with carpels after a 24-day culture period. All media contained a supplement of 10 mg/l of NAAm.
spectively. Thus, MH had a tendency to increase fruit weight as its concentration increased (Fig. 6).

In contrast, the number of days to ripening decreased as the concentration of MH in the medium increased (Table 4). The carpels of fruits cultured on the medium without MH remained green during a 24-day culture period but those on the medium with MH, particularly with 50 mg/l, turned dark brown.

**Discussion**

*Effects of auxin.*

Nitsch (10,12) reported that strawberry fruits, whose fertilized carpels (achenes) had been removed, grew normally, when the achenes were replaced by a lanolin paste containing NOA, and that the achenes really contained a large amount of free auxin. Thus, he was the first to demonstrate the important role of fertilized carpels from a hormonal viewpoint in the development of strawberry fruits. In the present study, when the receptacles of strawberries prior to anthesis were cultured on a medium with a synthetic auxin (NAAm), they developed into normally fruits, regardless of the presence or absence of unfertilized carpels. Thus, it is clear that auxin has the ability to develop strawberry fruits. The optimum concentration of NAAm for the growth of fruits with carpels was lower than that for the growth of fruits without carpels (Fig. 2). Thompson (18) postulated that the growth of auxin-induced parthenocarpic strawberry fruits was stimulated by growth substances produced in the nucellus and the integument which were developed by auxin treatment. For tomatoes, Asahira et al. (1) reported that the integument and pseudophembyro started to develop in auxin–treated ovaries, after which the ovaries developed into parthenocarpic fruits and that an equal amount of diffusible auxin was detected in both pollinated and auxin–induced parthenocarpic fruits. This suggested that an endogenous auxin(s) was produced in the developing carpels (achenes), also when strawberry fruits with carpels were cultured on NAAm medium. Therefore, it is reasonable to believe that the concentration of the exogenous auxin for the maximum growth of fruit with carpels is lower than that for the maximum growth of fruit without carpels, whose growth is sustained exclusively by exogenous auxin.

Supposing that fruit growth and ripening are regulated by auxin alone, then, at the optimum concentration of NAAm for fruit growth, there should be no difference in the growth and ripening between fruits with and without carpels. However, the growth and ripening of fruits with carpels were suppressed in comparison to those of fruits without carpels (Fig. 2 and Tables 1, 3). This suggests that an inhibitory substance(s) for fruit growth and ripening is produced, besides auxin, in the developing carpels.

*Effects of gibberellin.*

Creasy and Sommer (5) reported that strawberry receptacles without unfertilized carpels developed into normally shaped fruits on a medium with GA₃, but those with unfertilized carpels did not develop. They suggested that fruit growth is triggered when an anti-gibberellin-like substance in unfertilized carpels is removed by fertilization. Bajaj and Collins (3) showed that when strawberry fruits with unfertilized carpels were cultured on a medium with GA₃, the basal portion of the fruit grew well but the upper portion, with carpels attached, did not grow. These results may indicate that an anti–gibberellin–like substance is produced in unfertilized carpels. In the present experiment, however, the upper portion of the fruit did not grow on the medium with GA₃, the basal portion of the fruit grew well but the upper portion, with carpels attached, did not grow. These results may indicate that an anti–gibberellin–like substance is produced in unfertilized carpels. In the present experiment, however, the upper portion of the fruit did not grow on the medium with GA₃ even when fruits without carpels were cultured (Fig. 3). This does not indicate that strawberry fruits do not grow normally on a medium with gibberellin because anti-gibberellin–like substance produced in unfertilized carpels inhibits the growth of the upper portion of the receptacles where the carpels are attached. But it suggests more properly that gibberellin by itself is incapable of inducing
the growth of the portion stated above.

When fruits were cultured on the medium with both auxin and gibberellin, the upper portion of the fruits grew normally and the growth and ripening of whole fruits were promoted in comparison to fruits cultured on the medium with auxin alone (Fig. 3, 4 and Table 2). Thompson (18) reported that when a solution containing auxin and gibberellin was applied to strawberry fruits, fruit weight and ripening were promoted as the concentration of gibberellin increased. Bajaj and Collins (3) showed that gibberellin was one of the factors which promoted the ripening of strawberry fruits cultured \textit{in vitro}. Thus, gibberellin can be considered to promote the growth and ripening of strawberry fruits when it coexists with auxin.

\textbf{Effects of cytokinin and maleic hydrazide.}

In our experiments, an addition of BA to the medium suppressed the growth and ripening of strawberry fruits (Fig. 5 and Table 3). Creasy and Sommer (5) reported that the growth of strawberry fruits cultured on a medium with kinetin was suppressed. Thompson (18) also showed that a spray of kinetin combined with indolebutylic acid (IBA) given to the fruit of intact strawberry plants suppressed fruit growth and ripening in comparison to a spray of IBA alone. Thus, cytokinins appear to suppress the growth and ripening of strawberry fruits.

The growth and ripening of strawberry fruits without carpels on the NAAm medium were promoted in comparison to those of fruits with carpels (Fig. 2 and Table 3). In fruits with carpels, ripening was promoted when MH was added to the medium (Fig. 6 and Table 4). Judging from the color of the carpels of fruits cultured on the medium with MH, development of the carpels must have been severely inhibited. Thompson (17) also reported that in strawberry fruits treated with MH prior to anthesis, the tissues of the ovule showed little development.

Asahira \textit{et al.} (2) reported that cytokinin activity was also detected in auxin-treated parthenocarpic tomato fruits, and suggested that the source of cytokinin was the integument or pseudoembryo developed by auxin treatment. It is possible that cytokinin was also produced in the unfertilized carpels of strawberry fruits when they were cultured on the NAAm medium. It follows that the removal of the carpels or the inhibition of the development of the carpels by MH treatment promoted fruit growth and ripening because cytokinin was no longer produced.

Tukey (19) reported that the growth and ripening of pollinated peach fruits were promoted when the seed was destroyed mechanically by a drill. Crane and Nelson (4) reported that the growth and ripening of apricot fruits were promoted when auxin was sprayed after inhibiting seed development by an MH application. These observations suggest that the removal and mechanical destruction of seeds or the chemical inhibition of seed development prevent the production of inhibitory substance(s) for fruit growth and ripening. Therefore, it is suggested that one of the inhibitors to the growth and ripening of strawberry fruits is cytokinin. There are many publications (6, 8 and 13) showing that high cytokinin activity can be detected in immature seeds of various kinds of fruits. Kano and Asahira (7) reported similar results in strawberry fruits.

Thus, cytokinin, whose activity changes with seed development, can be considered to be one of the most important factors in regulating the development of strawberry fruits.

\textbf{Literature Cited}


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in vitro 培養におけるイチゴ果実の発育におよぼす数種の植物生長調節物質の影響

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摘要

イチゴ果実の発育過程における心皮（種子）の役割を植物生長調節物質の面から明確にするため、イチゴ品種“宝交早生”の開花1日前の果実の in vitro 培養を試みた。
1. ナフタレンアセトアミド (NAAm) を含む培地で心皮を除去した果実を培養した場合、その果実は心皮を除去しない果実に同様に正常な形に肥大した。また、心皮を除去せずに培養した場合、その果実の成熟は心皮を除去した果実にくらべ抑制された。
2. ジベンゾン酸 (GA₃) のみを含む培地で心皮を除去した果実あるいは除去しない果実を培養した場合、いずれも本来心皮の着生していた部分はまったく肥大せず、心皮の着生していない果実の基部のみが異常に肥大した。しかし、GA₃ と NAAm をともに含む培地で心皮を除去しない果実を培養した場合、果実は正常な形に肥大し、肥大および成熟は NAAm のみの培地で培養した果実にくらべ促進された。
3. ベンジルアデニン (BA) と NAAm を含む培地で心皮を除去した果実あるいは除去しない果実を培養した場合、いずれの場合も BA の濃度が高くなるにともない果実の肥大および成熟は抑制された。
4. メライン酸アデリド (MH) と NAAm を含む培地で心皮を除去しない果実を培養した場合、MH の濃度が高くなるにともない心皮の褐変が著しくなり、果実
の肥大および成熟は促進された。
以上の結果より、イチゴ果実の肥大にはジベレリンよりオーキシンのほうが重要であること、および心皮でイチゴ果実の肥大および成熟に抑制的に作用するサイトカイニンが生成されることが示唆される。