Promotion of Berry Ripening by 2, 3, 5- Triiodobenzoic Acid in ‘Kyoho’ Grapes

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Summary
We studied the effects of 2,3,5-triiodobenzoic acid (TIBA) on berry ripening of ‘Kyoho’ grapes (Vitis vinifera L. × V. labrusca L.). Compared with the untreated control, application of 200 mg·liter⁻¹ TIBA 30 days after full bloom (DAF) reduced berry weight and delayed ripening, increased the titratable acidity in the berry, and decreased the anthocyanin content in the peel. TIBA applied 45 DAF, at the beginning of veraison, resulted in higher soluble solids concentration (SSC) in the juice and a higher anthocyanin content in the peel, compared with the control. In a dosage experiment, grape clusters, sprayed with TIBA at 200 mg·liter⁻¹, had higher SSC and anthocyanin levels than those sprayed with 20 or 400 mg·liter⁻¹. The auxin naphthaleneacetic acid (NAA) clearly delayed berry ripening. Abscisic acid (ABA) enhanced peel coloration, but the SSC was equal to that of the control at harvest. When the effects of the anti-auxins: N- (1-naphthyl) phthalamic acid (NPA) and maleic hydrazide (MH) were compared with those of TIBA on gibberellin-induced seedless berries, NPA – and MH – treated berries had lower SSC and higher acidity but no effect on peel coloration. TIBA –treated berries had a significantly higher anthocyanin content and SSC than did those of the control. Thus, TIBA application at the beginning of veraison promotes the ripening of seeded and seedless ‘Kyoho’ grapes.

Key Words: abscisic acid, grapes ripening, maleic hydrazide, N- (1-naphthyl) phthalamic acid, TIBA.

Introduction
The growth curve of grape berries follows a double sigmoid pattern, consisting of two active growth stages (Coombe, 1992). Growth Stage II is a less active or no-growth lag phase between Stages I and III. The ripening of grape berries is characterized by rapid softening, sugar accumulation, reduction of acidity, and an increase in peel coloration after Stage III begins. The boundary point between Stages II and III marks the onset of ripening in grape berries; it is called ‘veraison’ by viticulturists.

Studies of changes in endogenous plant hormones in grape berries have found that indole-3-acetic acid (IAA) levels rapidly decrease and abscisic acid (ABA) levels significantly increase after veraison (Inaba et al., 1976; Niimi et al., 1977; Cawthon and Morris, 1982). Exogenous ethylene, gibberellin, and cytokinin had almost no effect on ripening ‘Delaware’ grapes (Inaba et al., 1974); exogenous ABA promotes anthocyanin accumulation in grape peel (Kataoka et al., 1982; Matsui et al., 1992). Inaba et al. (1976) pointed out that endogenous ABA must be accumulated to a certain level simultaneously with the inactivation of endogenous auxin before grape berries enter the ripening stage.

Exogenous auxins, including IAA and some synthetic auxins, inhibit softening and the accumulation of sugar and anthocyanin (Hale, 1968; Iwasaki et al., 1969; Inaba et al., 1974; Matsui et al., 1992; Weaver, 1962), by affecting the expression of genes that are involved in the ripening process (Davies et al., 1997). Therefore, it has been proposed that endogenous auxins in the berry represent a suppressing factor of ripening and must be inactivated before ripening can occur. This assumption could be tested by if the application of anti-auxins which enhance ripening. In pears, anti-auxins have been found to accelerate ripening (Frenkel and Haard, 1973), whereas in grapes, Coombe and Hale (1973) reported that 2,3,5-triiodobenzoic acid (TIBA) applied at 2 weeks before veraison caused a slight delay on berry ripening. However, TIBA applied at bloom or berry -set stages did not affect berry ripening in ‘Perlette’ grapes (Khajuria and Bakhshi, 1984). Because the question remains unsettled as to whether anti-auxin application at veraison can hasten ripening in grapes, the effects of the anti-auxins; TIBA, maleic hydrazide (MH), and N- (1-naphthyl) phthalamic acid (NPA) on the berry ripening were examined. In addition, the effects of abscisic acid (ABA) and naphthaleneacetic acid (NAA), a synthetic auxin, were compared with those of anti-
auxins.

**Materials and Methods**

**Plant materials**

‘Kyoho’ grapes (*Vitis vinifera* L. × *V. labrusca* L.), a tetraploid cultivar with a black purple-skin growing at the Persimmon and Grape Research Center in either an unheated glasshouse or in the open vineyard were used. Clusters, which flowered on the same date, were selected to obtain berries at the same developmental stage.

**Experiment 1. Effects of TIBA, ABA and NAA on the ripening of grape berries**

In 1995, 15 randomized clusters on three, 4-year-old vines grown in an unheated glasshouse were treated as follows: 1) TIBA (Na salt, Sigma Chemical Co.) 200 mg · liter⁻¹; 2) c - t ABA (Sigma Chemical Co.) 500 mg · liter⁻¹; 3) NAA (Sigma Chemical Co.) 200 mg · liter⁻¹; 4) untreated kept as the control. The chemicals were sprayed on the clusters at 45 DAF (beginning of veraison). All solutions contained 0.1% (v/v) Tween 80.

**Experiment 2. Effect of application time of TIBA on ripening of grape berries**

In 1996, TIBA, at 200 mg · liter⁻¹, containing 0.1% (v/v) Tween 80, was applied to clusters on three, 4-year-old vines grown in an open field at 30 DAF (end of Stage I), 45 DAF (beginning of veraison) and 60 DAF (middle of Stage III). Treatment was replicated on 5 clusters on each vine at each application time.

**Experiment 3. Effect of TIBA concentration at the beginning of veraison on berry ripening**

In 1996, three 4-year-old vines grown in an open field were sprayed with 20, 200, and 400 mg · liter⁻¹ TIBA containing 0.1% (v/v) Tween 80 at 45 DAF. The treatment was replicated for 5 clusters on each vine at each application time.

**Experiment 4. Effects of TIBA, NPA and MH on the ripening of seedless grape berries**

In 1998, flower clusters on three, 7-year-old vines grown in an unheated glasshouse were dipped in gibberellin (GA₃) 25 mg · liter⁻¹ at full bloom to induce seedless; then retreated with GA₃ 25 mg · liter⁻¹ at 18 DAF to promote berry size. At 45 DAF, the beginning of veraison, 15 GA₃-treated clusters were administered the following: 1) nothing; untreated control; 2) TIBA 200 mg · liter⁻¹; 3) NPA (Tokyo–Kasei Co.) 200 mg · liter⁻¹ and 4) MH (Nihon Nouyaku Co.) 1000 mg · liter⁻¹. All solutions contained 0.1 % (v/v) Tween 80.

**Pre- and Post-harvest handling of clusters**

In all the experiments, the clusters were thinned to 1 per shoot, and the number of berries per cluster was adjusted to 25–30 within 30 DAF. At each sampling date, ten berries were harvested from each cluster and peeled. The soluble solids concentration (SSC) in the juice was measured by a digital refractometer (PR-100, Atago, Japan). The titratable acidity was determined with 0.1 N NaOH and expressed as g of tartaric acid equivalent per 100 ml juice. For the anthocyanin analysis, 10 disks (8-mm-diameter) of berry skin were extracted in 50 ml methanol, containing 1% HCl at 4 °C for 12 hr, the absorbance of the extract was measured at 537 nm with a spectrophotometer (UV-260, Nihon-Bunkou, Japan). These measurements were replicated for each of the 15 clusters.

**Results**

**Experiment 1. Effects of TIBA, ABA and NAA on the ripening of grape berries**

Treatment with NAA at 200 mg · liter⁻¹ at 45 DAF retarded berry growth and a significantly lowering the SSC, while increasing acidity and lowering anthocyanin content than the control (Table 1). Treatment with ABA at 500mg · liter⁻¹ promoted anthocyanin accumulation in the peel but lowered SSC while having no effect on berry weight and titratable acidity. Although TIBA at 200mg · liter⁻¹ did not affect berry weight, it produced berries with the highest SSC and anthocyanin levels among the treatments.

**Experiment 2. Effect of application time of TIBA on ripening of grape berries**

Treatment of clusters at 30 DAF with 200 mg · liter⁻¹ TIBA resulted in small berries with a high acidity and low anthocyanin content, whereas application at 45 DAF promoted berry ripening by significantly increasing SSC and anthocyanin content over the control. TIBA, applied

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**Table 1. Effects of TIBA, ABA and NAA treatment 45 DAF on berry ripening of ‘Kyoho’ grapes (1995).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cluster weight (g)</th>
<th>Berry weight (g)</th>
<th>SSC (%)</th>
<th>Titratable acidity (%)</th>
<th>Anthocyanin content (OD₅₇₇)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIBA 200 mg · liter⁻¹</td>
<td>333.3b</td>
<td>12.8b</td>
<td>21.0d</td>
<td>0.46a</td>
<td>1.766c</td>
</tr>
<tr>
<td>ABA 500 mg · liter⁻¹</td>
<td>354.2b</td>
<td>13.4b</td>
<td>19.2b</td>
<td>0.44a</td>
<td>1.676c</td>
</tr>
<tr>
<td>NAA 200 mg · liter⁻¹</td>
<td>265.3a</td>
<td>10.4a</td>
<td>15.5a</td>
<td>0.76b</td>
<td>0.122a</td>
</tr>
<tr>
<td>Control</td>
<td>382.1b</td>
<td>12.7b</td>
<td>19.9c</td>
<td>0.47a</td>
<td>1.205b</td>
</tr>
</tbody>
</table>

*Separations were performed by Duncan’s multiple range test, and means (n=15) with dissimilar letters are significantly different at P<0.05.
Table 2. Effect of application time of 200 mg·liter⁻¹ TIBA on berry ripening in ‘Kyoho’ grapes (1996).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cluster weight (g)</th>
<th>Berry weight (g)</th>
<th>SSC (%)</th>
<th>Titratable acidity (%)</th>
<th>Anthocyanin content (OD₅₂₇)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 DAF</td>
<td>259.4a</td>
<td>8.9a</td>
<td>19.8a</td>
<td>0.55c</td>
<td>1.535a</td>
</tr>
<tr>
<td>45 DAF</td>
<td>299.9b</td>
<td>10.1b</td>
<td>21.1b</td>
<td>0.48a</td>
<td>2.580c</td>
</tr>
<tr>
<td>60 DAF</td>
<td>276.0b</td>
<td>10.4b</td>
<td>20.1ab</td>
<td>0.49a</td>
<td>1.946b</td>
</tr>
<tr>
<td>Control</td>
<td>276.8b</td>
<td>10.4b</td>
<td>19.5a</td>
<td>0.51b</td>
<td>2.013b</td>
</tr>
</tbody>
</table>

Separations were performed Duncan’s multiple range test, and means (n=15) with dissimilar letters are significantly different at P<0.05.

Table 3. Effect of concentration of TIBA applied 45 DAF on berry ripening in ‘Kyoho’ grapes (1996).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cluster weight (g)</th>
<th>Berry weight (g)</th>
<th>SSC (%)</th>
<th>Titratable acidity (%)</th>
<th>Anthocyanin content (OD₅₂₇)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg·liter⁻¹</td>
<td>266.4az</td>
<td>11.6a</td>
<td>18.8a</td>
<td>0.53a</td>
<td>2.405a</td>
</tr>
<tr>
<td>200 mg·liter⁻¹</td>
<td>253.3a</td>
<td>11.0a</td>
<td>19.6c</td>
<td>0.54a</td>
<td>2.776b</td>
</tr>
<tr>
<td>400 mg·liter⁻¹</td>
<td>260.1a</td>
<td>11.3a</td>
<td>19.4bc</td>
<td>0.54a</td>
<td>2.587ab</td>
</tr>
<tr>
<td>Control</td>
<td>239.9a</td>
<td>11.4a</td>
<td>19.1a</td>
<td>0.54a</td>
<td>2.317a</td>
</tr>
</tbody>
</table>

Separations were performed Duncan’s multiple range test, and means (n=15) with dissimilar letters are significantly different at P<0.05.

Table 4. Effect of TIBA, NPA and MH application 45 DAF on berry ripening in gibberellin-treated seedless ‘Kyoho’ grapes (1998).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cluster weight (g)</th>
<th>Berry weight (g)</th>
<th>SSC (%)</th>
<th>Titratable acidity (%)</th>
<th>Anthocyanin content (OD₅₂₇)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIBA 200 mg·liter⁻¹</td>
<td>259.2a</td>
<td>11.5a</td>
<td>18.6c</td>
<td>0.56a</td>
<td>1.661b</td>
</tr>
<tr>
<td>NPA 200 mg·liter⁻¹</td>
<td>266.2a</td>
<td>11.2a</td>
<td>16.8a</td>
<td>0.70b</td>
<td>1.244a</td>
</tr>
<tr>
<td>MH 1000 mg·liter⁻¹</td>
<td>250.6a</td>
<td>11.0a</td>
<td>18.5c</td>
<td>0.60a</td>
<td>1.481a</td>
</tr>
<tr>
<td>Control</td>
<td>273.7a</td>
<td>11.3a</td>
<td>17.9b</td>
<td>0.60a</td>
<td>1.303a</td>
</tr>
</tbody>
</table>

Separations were performed Duncan’s multiple range test, and means (n=15) with dissimilar letters are significantly different at P<0.05.

60 DAF, had similar effects on berry ripening at 45 DAF but the former treatment resulted in lower anthocyanin content than did the latter (Table 2).

Experiment 3. Effect of TIBA concentration at the beginning of veraison on berry ripening

Among the three concentrations of TIBA applied at 45 DAF, 20 mg·liter⁻¹ had no effect on berry weight and quality. Although the berry weights and titratable acidities of 200 and 400 mg·liter⁻¹ treatments were not significantly different, but the SSC and anthocyanin levels were significantly higher than those of the control (Table 3).

Experiment 4. Effects of TIBA, NPA and MH on berry ripening of seedless grape berries

In the gibberellin-treated seedless berries, application of TIBA at 200 mg·liter⁻¹ significantly increased the SSC and the anthocyanin content (Table 4). Although the application of MH at 1000 mg·liter⁻¹ increased the SSC of the juice, it had no effect on the anthocyanin content in the peel. Berries, treated with NPA at 200 mg·liter⁻¹, had a higher titratable acidity and lower SSC than did the control.

Discussion

The application of exogenous auxins just prior to veraison greatly delays ripening in grapes (Davies et al., 1997; Hale, 1968; Inaba et al., 1974; Matsui et al., 1992). In this study, we observed similar effects of NAA applied at 200 mg·liter⁻¹ to clusters at the beginning of veraison (Table 1). Endogenous auxin levels are high during Stage I, and then rapidly drop to a low level after veraison (Inaba et al., 1976; Niimi et al., 1977; Cawthon and Morris, 1982). Davies et al. (1997) showed that benzothiazole-2-oxacyclic acid (BTOA), a synthetic auxin-like compound, delayed berry ripening and affected the expression of genes related to invertase and anthocyanin synthesis. Therefore, veraison is a critical stage for berry ripening, and endogenous auxin levels play an important role in regulating the maturity of grape berries at this time.

The application of TIBA at the beginning of veraison increased the SSC content in the juice and the anthocyanin content in the peel (Table 1), thus, advancing berry ripening from 7 to 10 days. However, TIBA
applied at the end of Stage I produced smaller berries with lower SSC, higher acidity and lower anthocyanin content than when it is applied at veraison (Table 2). This indicates that the growth stage of the berries is an important determinant on the effect of TIBA on ripening. The production of smaller berries with TIBA application at 30 DAF indicates that an inhibition of cell enlargement in the end of Stage I may be caused by the reduction of endogenous IAA levels or interference with IAA activity.

TIBA, an auxin transport inhibitor, inhibits the auxin efflux carrier (Thomson et al., 1973). In this study, the TIBA treatment at the beginning of veraison enhanced berry ripening in seeded berries (Tables 1, 2, and 3). TIBA may inhibit IAA efflux from seeds into the flesh. The promotive effect of TIBA was also found in the GA3-induced seedless berries (Table 4) which suggests that the absence of seeds is unrelated to the effects of TIBA on berry ripening. In eggplant, the IAA levels of ovaries grown on TIBA-rich media decreased (Ikeda et al., 1999). Similarly, it is possible that TIBA affected the production of endogenous IAA in the berry flesh. Moreover, the diffusion of IAA, produced in shoot apex or other organs into a cluster, may have been inhibited by TIBA application to clusters. Thus, the promotion of berry ripening by TIBA may have been induced by decreasing endogenous IAA levels in the flesh.

In ‘Doradillo’ grapes, the application of 30mg·liter⁻¹ TIBA to clusters 2 weeks before veraison caused a slight delay in berry ripening (Coome and Hale, 1973). Khajuria and Bakhshi (1984) reported that the application of TIBA at 50 mg·liter⁻¹ at bloom and the berry set stage gave no significant differences over the controls. These results concur with ours on TIBA concentration and application timing (Tables 2 and 3). One reason for the lack of effect on berry ripening in both reports may be the use of lower concentrations used. Moreover, the timing of TIBA application was too early to stimulate berry ripening (Table 2).

ABA is likely to have a dominant role in the ripening of grapes as its application hastens ripening of GA3-treated seedless ‘Delaware’ grapes (Matsui et al., 1992). Endogenous ABA levels also rapidly increase after veraison in the flesh (Coome, 1973; Inaba et al., 1976) and peel (Kondo and Kawai, 1998). BTOA treatment delayed the increase in endogenous ABA level by 2 weeks (Davies et al., 1997). ABA application promotes peel coloration but it had little effect on sugar accumulation in ‘Kyoho’ (Kataoka et al., 1982) and seeded ‘Delaware’ grapes (Niimi et al., 1977). Likewise, in our experiments with ‘Kyoho’, ABA application increased the anthocyanin content in the peel, but not the sugar content of the berries (Matsui et al., 1980) which demonstrates that exogenous ABA stimulated pigment formation but not sugar accumulation in ‘Kyoho’ grapes. In contrast, TIBA application enhanced both peel coloration and SSC accumulation (Tables 1 and 2) by indirectly stimulating anthocyanin synthesis and altering endogenous ABA levels.

When applied at veraison, MH had no effect on berry ripening except to promote SSC accumulation (Table 4). Therefore, different effects of MH on berry ripening is attributable to its action as an auxin antagonist, but not by decreasing endogenous IAA levels as reported by Yamada (1966).

NPA, an auxin transport inhibitor similar to TIBA, inhibits the auxin efflux carrier (Thomson et al., 1973). Its application had an inhibitory effect on berry ripening by lowering SSC and increasing acidity (Table 4). NPA and TIBA seem to have different binding sites (Thomson et al., 1973; Depta and Rubery, 1984; Michalka et al., 1992). Moreover, TIBA is transported basipetally, while NPA is not (Thomson et al., 1973). These differences in the modes of action are possibly responsible for their effects on berry ripening. Additionally, NPA has a weak auxin activity (Kett and Baker, 1966), which may have been inhibitory for berry ripening. TIBA has not been registered as an agricultural chemical for commercial use.

In conclusion, it seems that a decline in endogenous IAA caused by TIBA applied at the beginning of veraison could promote berry ripening. Further investigations are needed to pinpoint where TIBA suppresses the endogenous IAA and ABA activities in grape berries.

Literature Cited


TIBA による‘巨峰’ 果実の成熟促進効果

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摘 要

抗オーキシン剤である 2, 3, 5-トリクロール安息香酸 (TIBA) がブドウ‘巨峰’ の果実品質に及ぼす影響を検討した。時期別の試験において滴下後 30 日目の TIBA 200 mg・liter^(-1) の果房処理では、果粒数が小さく果皮のアントシアニン含量が対照区より低かった。一方、ペレゾーン初期（滴下後 45 日目）の処理では、果粒肥大に差は認められなかったが、果汁糖度と果皮のアントシアニン含量が対照区より明らかに増加した。TIBA の処理濃度の影響を比較した結果、20 mg・liter^(-1) では対照区と果実品質に差はなかったが、200 mg・liter^(-1) 以上で成熟促進効果が認められた。ペレゾーン初期におけるナフタレン酢酸 (NAA) の果房処理は成熟を明らかに遅延した。アブジシン酸 (ABA) 処理では、果皮のアントシアニン含量は増加したが、果汁糖度の増加は認められなかった。

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