Regulation of Ripening in Grapes by Hormone Treatments

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Summary : Auxin (IAA, NAA, 2, 4-D, 2, 4, 5-T), GA, BA, CEPA and ABA were applied to the cluster of Delaware grapes at different stages during their development, and the effects on the ripening were followed by measuring changes in the fructose content of berries. GA and BA slightly delayed the ripening of grapes when applied immediately before veraison, but no effects were observed when applied at stages I and III. Auxin greatly delayed the ripening of grapes, and the greatest response to auxin was obtained by the application immediately before veraison. The effect became successively greater in the order of IAA, NAA, 2,4-D and 2,4,5-T. It seems, therefore, that auxin is prominent as a ripening retardant in grape berries. CEPA not only had almost no effect on the ripening even when applied at stage III, but also delayed the ripening at stages I and II. ABA clearly hastened the ripening of grapes when treatеd within two weeks before veraison, and its effect was partly reversed by 2,4-D. The data lead to the conclusion that an auxin-ABA relationship may be involved in the regulation of the ripening of grape berries.

It is well known that auxin, GA and cytokinin can delay the onset of senescence of intact or detached plant organs under certain conditions and these hormones have been used to delay the senescence of vegetables, flowers and fruits (27). On the contrary, it has been found that ABA content increases during a senescent process and plants are often induced senescence under ABA treatment (1). Sacher (21) recently suggested that fruit ripening is regarded as a senescent process, and that in fruit tissue a decline in endogenous auxin (cytokinin or GA) and an increase in ABA could lead to the onset of ripening. Ethylene accelerates the ripening of all fruits on which it has been tested (2, 3), and there is strong evidence that ethylene is a fruit-ripening hormone (3, 12).

In grapes it is reported that auxin delayed the ripening (10, 26), and ethylene or CEPA hastened the ripening when applied at a proper stage of development, but the effect of ethylene or CEPA was much smaller than other fruits (11). Little is known about the effects of GA, cytokinin and ABA on the ripening of grape berries.

The authors have been studying the mechanism of hormonal regulation of the ripening of grape berries, and consider that ABA hastens fruit ripening, if auxin (GA or cytokinin) delays it. This paper describes the effects of auxin, GA, BA, CEPA and ABA on the ripening of grape berries.

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Abbreviations : CEPA : 2-chloroethylphosphonic acid ; IAA : indoleacetic acid ; NAA : naphthalene-acetic acid ; 2,4-D : 2,4-dichlorophenoxyacetic acid ; 2,4,5-T : 2,4,5—trichlorophenoxyacetic acid ; BA : benzyladenine ; GA : gibberellin ; ABA : abscisic acid.

Materials and Methods

GA-induced seedless mature Delaware vines, growing in the Kyoto Prefectural University orchard were used in the experiment. Delaware is one of the most important table grapes in Japan and the use of GA is standard commercial practice. GA induces completely seedless berries which are somewhat smaller than untreated but color and ripen two to three weeks earlier. For the induction of seedless berries, clusters were dipped in GA_3 at a concentration of 100 ppm 10 days before anthesis and again 1 week after bloom.

Clusters were treated with IAA, NAA, 2,4-D at 100ppm, 2,4,5-T at 40 ppm, CEPA at 500 ppm, GA_3, BA at 1000 ppm, and ABA[1] at 500 or 1000 ppm, at the stages indicated below, by immersing them in 70% ethanol solution containing 0.05% Tween 20. The wettability of grape berries for aqueous solution is so low that ethanol solution was used in all treatments which has no injury to berries. Futhermore, ethanol is very effective to make a high concentrated hormone solution.

Clusters were dipped once in 2,4-D at weekly intervals after 2 weeks of anthesis, in IAA, NAA, 2,4,5-T immediately before veraison, CEPA at 3, 5 or 6 weeks after anthesis, GA, BA at 3, 4, 5 or 6 weeks after anthesis, ABA at 3, 2, 1 week before or at veraison. The cluster was also dipped in ABA at 1, 3, or 5 consecutive days. Some of ABA treated clusters received an additional 2,4-D application at 50 ppm after 1 week.

Berries were sampled at weekly intervals and held at -20°C until analysis. Fructose content in the berries was determined as a measure of ripeness by the method of Helbert and Brown (13).

Results

Figure 1 shows the diagram of the growth curve of the seedless Delaware berries, and indicates shorter growing period from anthesis to ripeness and much shorter duration of stage II than other grapes.

Figure 2 shows the fructose content of the berries from CEPA treatment at stages I, II and III of berry development. It indicates that the ripening was delayed by the treatment with CEPA at stages I and II, by approximately 2 and 3 weeks respectively, but CEPA had almost no influence on the ripening when treated at stage III though many cracked berries were observed. CEPA treatments at stages

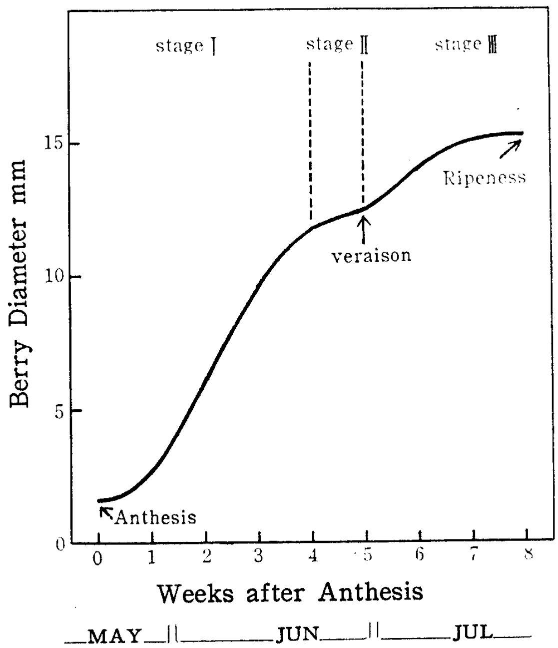

Fig. 1. Diagram of the growth curve of a GA-induced seedless Delaware berry.

[1] (±)-ABA

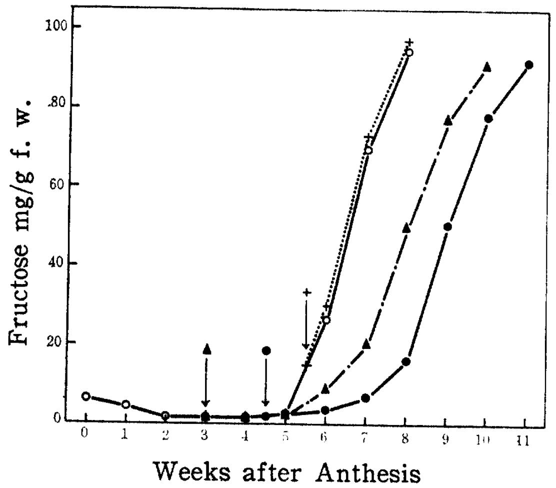

Fig. 2. Fructose content of untreated berries (○) and of berries treated with CEPA at stages I (▲), II (●) and III (+). Arrows indicate treatment times.

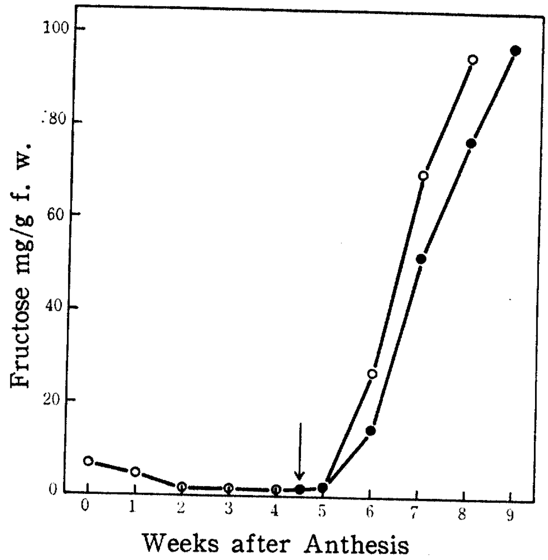

Fig. 3. Fructose content of untreated berries (○) and of berries treated with GA immediately before veraison (●). Arrow indicates treatment time.

I and II were toxic, and many berries shriveled and dropped.

The effect of GA treatment at stage II on the ripening of berries is shown in Figure 3. GA application at stage II delayed ripening slightly, while it had almost no effect on the ripening when applied at stages I and III.

Figure 4 shows the effect of BA treatment at stage II on the ripening of the berries. BA treatment at stage II retarded the ripening slightly, but had almost no influence on the ripening at stages I and III.

Figure 5 shows the fructose content of grapes from 2,4-D treatment at various

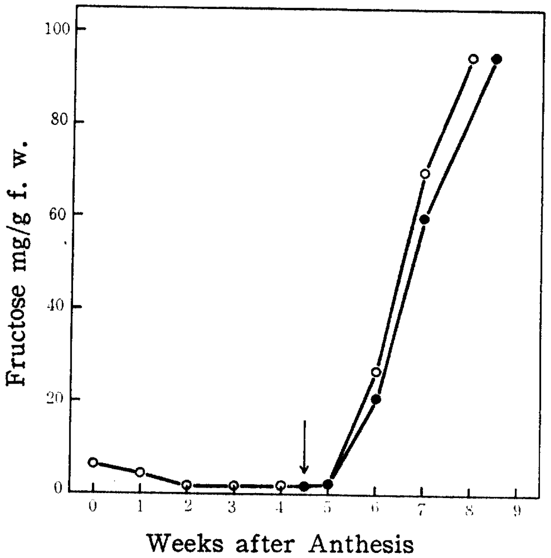

Fig. 4. Fructose content of untreated berries (○) and of berries treated with BA immediately before veraison (●). Arrow indicates treatment time.

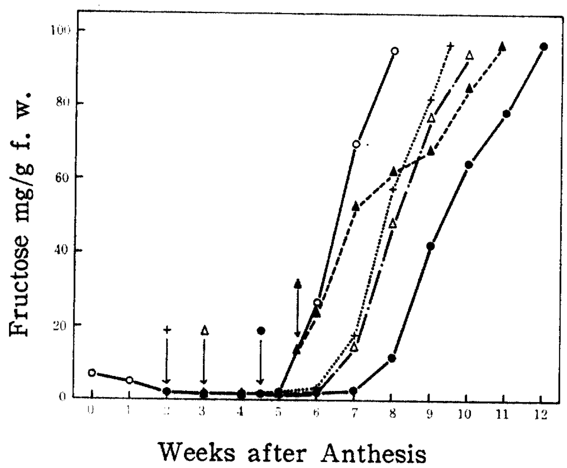

Fig. 5. Fructose content of untreated berries (○) and of berries treated with 2,4-D 2 weeks (+), 3 weeks (△) after anthesis, immediately before (●) and after (▲) veraison. Arrows indicate treatment times.

stages of development. At all stages of development, 2, 4-D retarded the ripening of berries more or less and the greatest delay was induced at stage II. It was observed that 2, 4-D inhibited the berry development so much when treated 2 weeks after anthesis that the berry diameter at maturity was considerably smaller than that of control. However, no difference was observed in diameter between treated and untreated, when clusters were dipped after 4 weeks from anthesis.

The effects of various auxin treatments at stage II on ripening of the berries are shown in Figure 6. It is noticed that auxin, especially NAA, 2, 4-D and 2, 4, 5-T, delayed the ripenng remarkably. The delaying duration was 3 weeks with NAA, 4 weeks with 2, 4-D, and 5 weeks with 2, 4, 5-T, but IAA application delayed ripening only slightly. Thus the ripening-retarding effects of the treated auxin were in the order of the activity strength of auxins themselves.

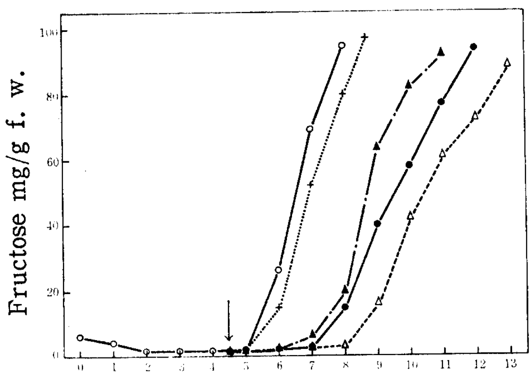

Fig. 6. Fructose content of untreated berries (○)and of berries treated immediately before veraison with IAA (+), NAA (▲), 2,4-D (●), and 2, 4, 5-T (△). Arrow indicates treatment time.

Figure 7 shows the effects of ABA treatment and additional 2, 4-D application to the ABA treated clusters on the ripening of grape berries. It is evident that ABA clearly hastened the ripening of grape berries when applied to clusters within 2 weeks before veraison, but the treatment 3 weeks before veraison had almost no influence on the ripening. The hastening duration was 10 days in the treatment 2 weeks before veraison and 7 days in the treatment 1 week before veraison. Although the greatest hastening effect was obtained by the treatment 2 weeks before veraison, some unaffected berries in the cluster were observed at this stage. The berries in the cluster, however, were uniformly hastened the ripening by the treatment with ABA 1

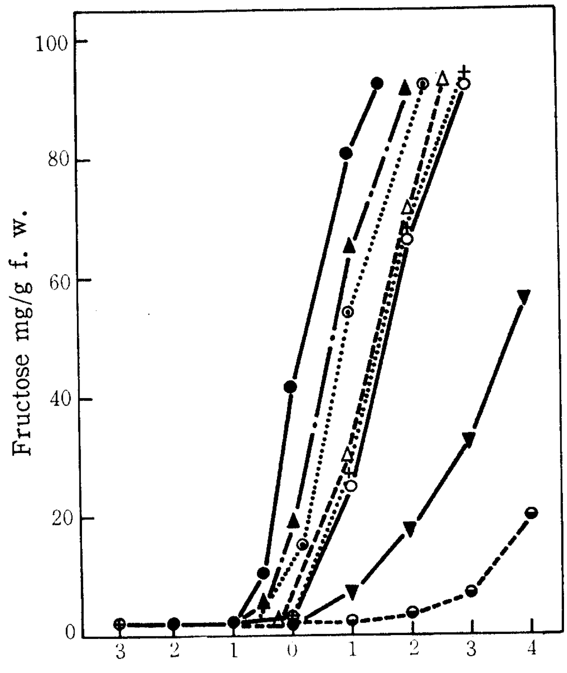

Fig. 7. Effects of ABA and 2,4-D treatments on the fructose content of berries. Untreated (○), ABA (1000 ppm, 5 consecutive days) 3 weeks(+), 2 weeks (●), 1 week (▲) before, and at (△) veraison ; 2, 4-D after 1 week of ABA (1000 ppm, 5 consecutive days) 2 weeks (◒) and 1 week (⊙) before veraison ; 2, 4-D after 1 week of single ABA application(500 ppm) 1 week (▼) before veraison.

week before veraison. 2, 4-D reversed the hastening effect of ABA in all concentrations used when applied after 1 week of ABA treatment dipped 2 weeks before veraison, while this reversal effect of 2, 4-D was not observed in the clusters which were treated with ABA in the higher concentration 1 week before veraison. It was also observed that the consecutive ABA applications more hastened the ripening than the single applications, and the higher concentration had the greater effect than the lower.

Discussion

There have been many reports evidencing that ethylene or CEPA can accelerate ripening of fruit, while in the present experiment, CEPA delayed the ripening of grape berries when applied during stages I and II. These results are identical to those obtained with fig (15) and grape (11). Marei and Crane (15) reported that exogenous ethylene inhibited the growth of fig fruit when applied in stage I, but stimulated the climacteric and other aspects of ripening when applied in or preceding stage III. Hale et al. (11) observed that early treatments with CEPA delayed ripening, while both ethylene and CEPA hastened the onset of ripening of grape berries if applied at a restricted period immediately before the normal time of the start of ripening. However, in the experiment reported here CEPA had almost no effect on the ripening even when applied at stage III. The cause of the difference is probably due to that GA-induced seedless Delaware berries have so much shorter growing period that the untreated berries ripen before the appearance of the effects of treated CEPA. Sacher (21) suggests from another point that continuous application of ethylene is neccessary in order for ripening to proceed after being initiated. It appears, however, that ethylene has almost no effect on the ripening of grape berries though it acts as ripening hormone in other fruits.

Many investigations were undertaken on the effects of treatment of GA and cytokinin on the ripening of fruit. GA retarded the accumulation of rind carotenoids and simultaneously slowed the net loss of chlorophylls in navel orange (14). Dostal and Leopold (6) showed that treatment with GA retarded color changes of tomato fruit. Wade and Brady (25) reported that infiltration of kinetin into fresh banana slices markedly retarded the peel degreening, whereas normal ripening occured in the pulp. BA delayed degreening of at least one variety of apple (22), and color changes in mature green oranges (7). The results presented in this paper, however, show that GA and BA have almost no effect on the ripening of grape berries.

Auxins considerably delayed the ripening of grape berries unlike above mentioned hormones, and the effects of treated auxins were in the order of the activity strength of auxins themselves. These results suggest that auxin is a prominent factor as a ripening retardant in grape berries. Similar results were obtained with grapes (10, 26), namely auxin delayed ripening if applied during the lag phase of development. Frenkel and Raymond (8) reported that softening and degreening of pear fruit were inhibited greatly in response to increased concentrations of infiltrated auxins. Vendrell (23, 24) found that auxin infiltrated into banana slices delayed ripening and when intact fruit was dipped into auxin solution, peel ripening was delayed. He attributed the auxin retardation of ripening to maintaining the tissue in a juvenile state. It seems from the above considerations that auxin, GA and cytokinin have a ripening-retarding effect depending on the kind of fruit, and that in grape berries auxin is a prominent factor. Therefore, it is assumed that endogenous auxin in grape berries represents a resistant factor in ripening and must be inactivated before ripening initiates. These concepts agree well with the observation that the endogenous auxin activity in grape berries is declining or has fallen to a low level when the second rapid growth stage begins (4. 16).

It is considered reasonable from this concept that the inhibitors promote the ripening of fruit. Sacher (21) suggested that fruit ripening is regarded as a senescent process, and recent analyses indicated that senescence was correlated with increase in endogenous ABA content in plant tissue. Rudnicki et al. (18, 19, 20) showed that ABA increased during ripening of apples, pears and strawberries. Goldschmidt et al. (9) found that ABA and neutral inhibitor, which is probably xanthoxin, increased during ripening of citrus fruit. Coombe and Hale (5) showed that a large increase in ABA generally proceeded the onset of ripening stage in grapes, and that the treatment for delaying verison also delayed the increase in ABA content. They also stated that ABA application to grapes during stage II hastened ripening. On the basis of these concepts, the authors applied ABA to Delaware grapes during development, and the results clearly indicate that ABA hastened the ripening of grape berries when applied within 2 weeks before veraison. The response of grape berries to exogenous ABA varies delicateley with physiological age ; i. e., (a) no berries in the cluster responsible to ABA 3 weeks before veraison, (b) partly not, but many berries responsible 2 weeks before veraison, (c) every berries responsible 1 week before veraison. These evidences indicate that the effect of ABA is limited by the stage of berry development though ABA acts as a ripening hormone to grape berries. Furthermore, 2, 4-D is able to reverse completely the effect of ABA applications 2 weeks before veraison, but in higher ABA concentration 1 week before veraison, no reversal effect of 2,4-D was observed. This, together with the fact that the ripening delaying effect of 2, 4-D was weaker when applied after veraison, seems depending on that once the onset of ripening initiates physiologically auxin may be unable to reverse it. The authors also found that sudden increase in ABA content begins in seedless, seeded and 2, 4-D treated Delaware berries, which ripen at different times, as soon as berries passed their veraison stages (unpublished data).

In conclusion, it is reasonable to explain that for grape berries decline in auxin and increase in ABA could lead to the onset of ripening. Further investigations concerning the levels of endogenous hormones, especially auxins, during the berry development including internal level of applied auxins, will be needed to clarify this hypothesis.

References

1) Addicott, F. T. and J. J. Lyon(1969) : Ann. Rev. Plant Physiol. **20** : 139-164.

2) Burg, S. P. (1962) : Ann. Rev. Plant Physiol. **13** : 265-302.

3) Burg, S. P. and E. A. Burg (1965) : Science **148** : 1190-1196.

4) Coombe, B. G. (1960) : Plant Physiol. **35** : 241-250.

5) Coombe, B. G. and C. R. Hale (1973) : Plant Physiol. **51** : 629–634.

6) Dostal, H. C. and A. C. Leopold (1967) : Science **158** : 1579–1580.

7) Eilati, S. K., E. E. Goldschmidt and S. P. Monselise (1969) : Experimentia **25** : 209–210.

8) Frenkel, C. and N. F. Haard (1973) : Plant Physiol. **52** : 380–384.

9) Goldschmidt, E. E., R. Goren, Z. Even-Chen and S. Bittner (1973) : Plant Physiol. **51** : 879–882.

10) Hale, C. R. (1968) : Aust. J. agric. Res. **19** : 939–945.

11) Hale, C. R., B. G. Coombe and J. S. Hawker (1970) : Plant Physiol. **45** : 620–623.

12) Hansen, E. (1966) : Ann. Rev. Plant Physiol. **17** : 459–480.

13) Helbert, J. R. and K. D. Brown (1955) : Anal. Chem. **27** : 1791–1796.

14) Lewis, L. N., C. W. Coggins, Jr. and M. J. Garber (1964) : Proc. Amer. Soc. Hort. Sci. **84** : 177–180.

15) Marei, N. and C. Crane (1971) : Plant Physiol. **48** : 249–254.

16) Nitsch, J. P., C. Pratt, C. Nitsch and N. J. Shaulis (1960) : Amer. J. Bot. **47** : 566–576.

17) Pratt, H. K. and J. D. Goeschl (1969) : Ann. Rev. Plant Physiol. **20** : 541–584.

18) Rudnicki, R., J. Machnik and N. Pieniazek (1968) : Bull. Acad. Pol. Sci. **16** : 509–512.

19) Rudnicki, R., J. Pieniazek and N. Pieniazek (1968) : Bull. Acad. Pol. Sci. **16** : 127–130.

20) Rudnicki, R. and J. Pieniazek (1970) : Bull. Acad. Pol. Sci. **18** : 577–580.

21) Sacher, J. A. (1973) : Ann. Rev. Plant Physiol. **24** : 197–224.

22) Smock, R. M., D. Martin and C. S. A. Padfield (1962) : Proc. Amer. Soc. Hort. Sci. **81** : 51–56.

23) Vendrell, M. (1969) : Aust. J. biol. Sci. **22** : 601–610.

24) ——— (1970) : Aust. J. biol. Sci. **23** : 1133–1142.

25) Wade, N. L. and C. J. Brady (1971) : Aust. J. biol. Sci **24** : 165–167.

26) Weaver, R. J. (1962) : Amer. Jour. Enol. Vitic. **13** : 141–149.

27) Weaver, R. J. (1972) : Plant Growth Substances in Agriculture, W. H. Freeman.

要旨：GA処理による無核デラウエアブドウを用い，オーキシン (IAA, NAA, 2,4-D, 2,4,5-T), GA, BA, CEPA および ABA 処理が果実の成熟におよぼす影響について調査した。その結果，GA および BA 処理は発育第Ｉ期と第Ⅲ期では果実の成熟にほとんど影響をおよぼさなかったが，第Ⅱ期ではわずかに成熟を抑制することが認められた。オーキシン処理は果実の成熟を著しく抑制し，その効果は veraison の直前処理で最も大きいことが認められた。また，オーキシンによる成熟遅延効果は用いたオーキシンの作用性の順になっているように思われた。CEPA処理は第Ｉ期および第Ⅱ期では成熟を抑制することが認められ，第Ⅲ期でも成熟を促進することはなかった。いっぽう，ABA は veraison 前の２週間以内に処理した場合，明らかに果実の成熟を促進することが認められた。この ABA による成熟促進効果は 2, 4-D により部分的に打消された。これらのことから，ブドウ果実の成熟にはオーキシンと ABA のバランスが関与しているように思われた。