ブドウ熟枝挿しの発根と内生オーキシンに及ぼす外生BAP,GA3,ABAの影響

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Effects of Exogenous BAP, GA_3, and ABA on Endogenous Auxin and Rooting of Grapevine Hardwood Cuttings

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Summary

The effects of applications of 6-benzylaminopurine (BAP), gibberellic acid (GA_3), and abscisic acid (ABA) on the root formation of grapevine hardwood cuttings were investigated. BAP suppressed rooting of grapevine cuttings completely, whereas GA_3 and ABA inhibited it temporarily. Endogenous indoleacetic acid (IAA) in cuttings treated with BAP increased linearly to 12.6 and 19.4 times per cutting and per g fresh weight, respectively, over the control for 30 days after planting. In cuttings treated with GA_3, the level of diffusible IAA was higher than that of control cuttings through the experimental period; the fluctuation pattern of extractable IAA was similar to that of the control. In cuttings treated with ABA, the extractable IAA content was significantly lower than that in control cuttings 10 days after planting. The fluctuation pattern of endogenous IAA in BAP treatment was different from that in GA_3 and ABA treatments. Cuttings treated with BAP presumably failed to root because the level of endogenous IAA was unbalanced. GA_3 may inhibit root formation directly whereas ABA may cause inhibition of rooting by depressing the bud activity.

Introduction

Growth regulators play an important role in the organogenesis of plants. The plant growth regulator, auxin, affects rhizogenesis, i. e. promotes root formation. Other phytohormones are also known to affect root formation when applied exogenously. Cytokinins and gibberellins generally inhibit adventitious root formation (Ernsten and Hansen, 1986; Hansen, 1988; Van Staden and Harty, 1988). In hypocotyls of sunflower, however, a low level of cytokinin increased root primordia formation (Fabijan et al., 1981). Gibberellin enhances root formation in several species and under certain environmental conditions (Hansen, 1988). The application of abscisic acid (ABA) may induce a number of responses pertaining to adventitious root formation in cuttings whereas ethylene may stimulate root initiation.

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Grape cuttings often induce roots without exogenous auxin treatment. That endogenous auxin (IAA) increased in cuttings when the root initiation began and decreased when the root appeared was reported previously (Kawai, 1966). Few studies have investigated the effects of other hormones on endogenous auxin level of grapevine cuttings. This paper reports the results of 6-benzylaminopurine (BAP), gibberellic acid (GA_3), and ABA applications to grapevine hardwood cuttings and may explain the interaction between them and root formation.

Materials and Methods

Grapevine Cutting

Dormant canes of the grapevine cv. 'Muscat Bailey A' {Bailey (a hybrid of *Linsecumii* × *Labrusca* × *Vinifera*) × Muscat Hamburg} were collected and stored at 5 °C. After breaking dormancy, 7 cm-length, 1-bud cuttings were prepared, treated with growth regulators (see below) and planted in a mixture of vermiculite and perlite in the ratio of 1: 1 (v/v). The cuttings were then set under intermittent mist in a greenhouse.

BAP, GA_3 and ABA Application Experiment

The cuttings were dipped in 100 ppm BAP, GA_3, and ABA solutions overnight; the control was dipped into distilled water prior to planting. BAP, GA_3, ABA experiments were conducted from 13 May to 22 June, 1993; form 18 March to 27 April, 1994; and from 20 May to 24 June, 1994, respectively. In the BAP and GA_3 experiments 25 cuttings were sampled at random at 10-day intervals for 40 days after planting; in the ABA experiment, the last sample was collected 35 days after treatment. The rooting percentages were recorded and the cuttings analyzed for endogenous IAA.

Diffusible IAA and Extractable IAA

Two types of endogenous auxins, the diffusible IAA and extractable IAA, were analyzed.

The cuttings, collected on each sampling day, were divided two parts: upper 6-cm (upper part) and basal 1-cm part (basal part). The basal part is where roots form. The upper part was placed upright in a 5-ml vial containing 1.5 ml agar, which was set in a dark chamber kept at 25 °C. After diffusing for 24 h, the upper part was removed and 2 ml of 100% methanol were added to the vial to extract the diffusible IAA in the agar. After setting overnight at 5 °C, 5 extracts per sampling date were combined and analyzed for diffusible IAA.

The 5 basal parts were chopped and soaked in 50 ml of 80% methanol at 5 °C for 3 days. The MeOH extract was filtered through 5B filter paper (TOYO) and analyzed for extractable IAA.

IAA Analysis

The IAA sample was partially purified using aminopropyl bonded phase column as described previously (Kawai, 1996).

The diffusible IAA and extractable IAA were analyzed using the Jasco 980 series HPLC system equipped with a 821-FP fluorescent detector (Jasco Corporation, Tokyo, Japan), a 4.6 × 35 mm CrestPak C18T-5P precolumn, and a 4.6 × 150 mm CrestPak C18 column (Jasco Corporation). The column temperature was 40 °C. The elution condition of HPLC was programmed to a binary gradient from 15% to 40% acetonitrile in 0.05 M sodium acetate buffer (pH 4) for 15 minutes with a flow rate of 1 ml/min. Fluorescence detection was carried out at 280 nm for excitation and at 350 nm for emission.

Diffusible IAA is expressed per unit cross sectional area ($ng \cdot cm^{-2}$) and per cutting ($ng \cdot cutting^{-1}$); the extractable IAA is expressed per unit fresh weight ($ng \cdot g^{-1}FW$) and per cutting ($ng \cdot cutting^{-1}$).

Results

Cytokinin Application Experiment

The root formation of grapevine hardwood cuttings was inhibited completely by the application of 100 ppm BAP (Fig. 1) whereas in the control cuttings rooting was visible 30 days after planting. The rooting percentages were 64% and 92% at 30 and 40 days after planting, respectively. The level of diffusible IAA in BAP treatment increased linearly from 10 days to 30 days after planting and then decreased (Fig. 2). The level of diffusible IAA in control cuttings remained low; the differences in diffusible IAA level between control and BAP treatments became significant 20 and 30 days after planting. The extractable IAA content of cuttings treated with BAP also increased linearly from 0 day to 30 days after planting and thereafter decreased (Fig. 3). Likewise, the extractable IAA content of control cuttings increased 10 to 20 days after planting and then decreased 10 days

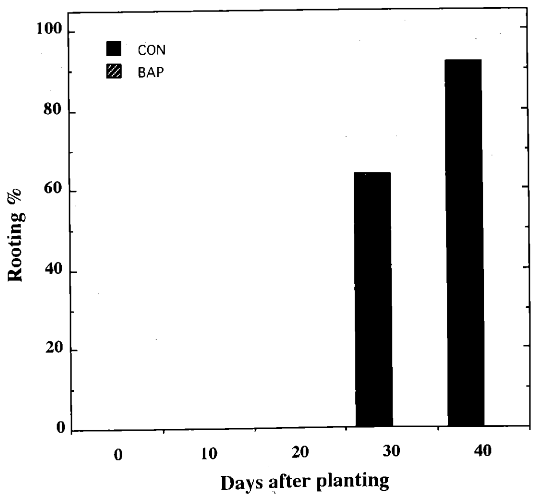

Fig. 1. Rooting percentage of 'Muscat Bailey A' grapevine cuttings treated with distilled water (CON) and 100 ppm BAP (BAP).

later, when roots became visible. The extractable IAA content in BAP treatment was significantly higher than that of the control throughout the experimental period, attaining levels of 12.6 and 19.4 times more per cutting and per g fresh weight, respectively, over the control 30 days after planting.

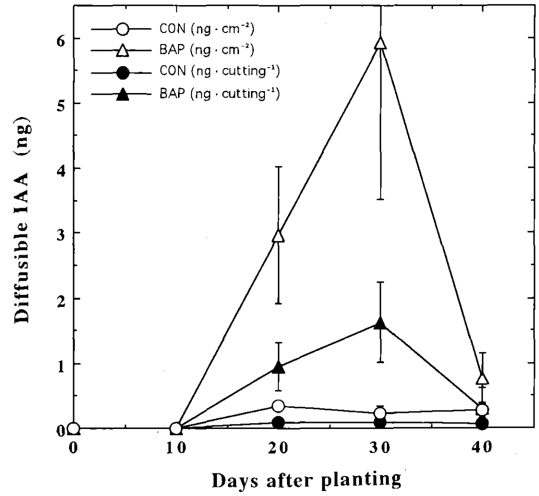

Fig. 2. Changes in the amounts of diffusible IAA ($ng \cdot cm^{-2}$ and $ng \cdot cutting^{-1}$) in 'Muscat Bailey A' grapevine cuttings treated with distilled water (CON) and 100 ppm BAP (BAP). Vertical bars represent SE (n=5).

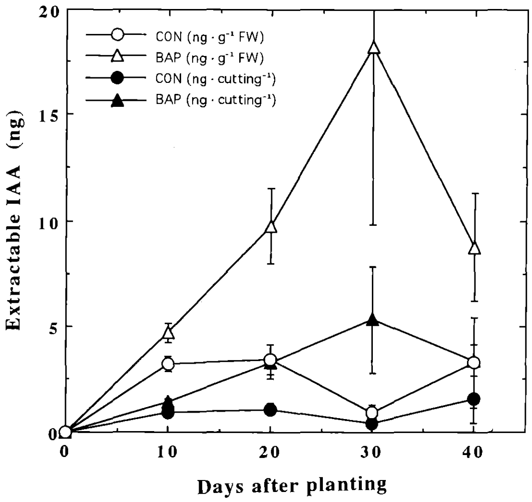

Fig. 3. Changes in the amounts of extractable IAA ($ng \cdot g^{-1}FW$ and $ng \cdot cutting^{-1}$) in 'Muscat Bailey A' grapevine cuttings treated with distilled water (CON) and 100 ppm BAP (BAP). Vertical bars represent SE (n=5).

GA_3 Application Experiment

GA_3 application delayed root fromation of cuttings (fig. 4), the final rooting percentage reaching

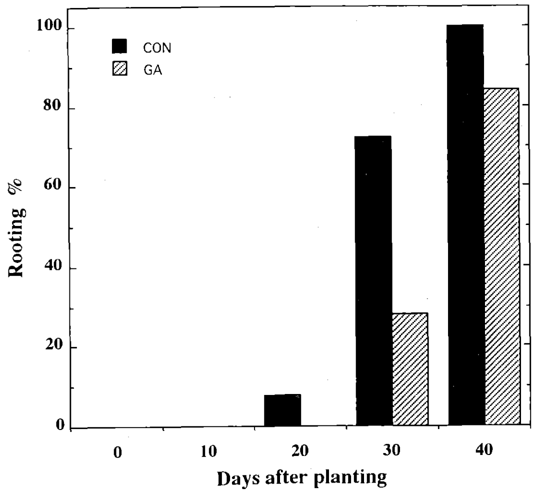

Fig. 4. Rooting percentage of 'Muscat Bailey A' grapevine cuttings treated with distilled water (CON) and 100 ppm GA_3 (GA).

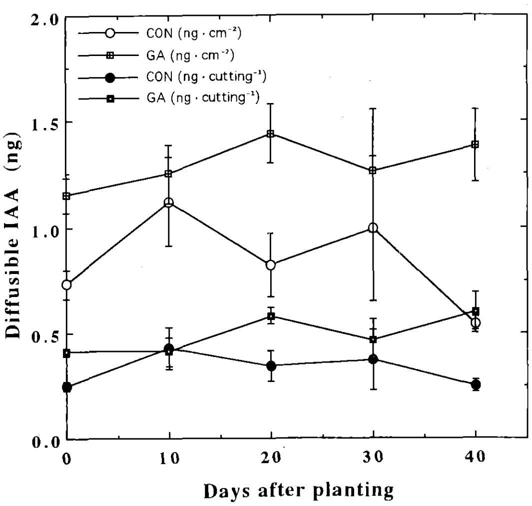

Fig. 5. Changes in the amounts of diffusible IAA ($ng \cdot cm^{-2}$ and $ng \cdot cutting^{-1}$) in 'Muscat Bailey A' grapevine cuttings treated with distilled water (CON) and 100 ppm GA_3 (GA). Vertical bars represent SE (n=5).

84% 40 days after planting. Roots on the control cutting appeared within 20 days after planting, reaching 100% within 40 days after planting. The level of diffusible IAA (ng·cm^{-2}) was higher than that in the control throughout the experimental period (Fig. 5). The fluctuation patterns of extractable IAA were similar between control and GA_3 treatments (Fig. 6). Their extractable IAA contents decreased for 20 days and then increased slightly for 10 days and then decreased again.

ABA Application Experiment

ABA application also inhibited root formation on cuttings (Fig. 7), the percentages being lower 30 and 35 days after treatment than those in the control. The level of diffusible IAA in the control and ABA treatment increased for 20 days after treatment and then decreased for 10 days (Fig. 8). The extractable IAA content in the treated cuttings was the same as in the control throughout the experimental period except for 10 days after planting during which the IAA content of the control cuttings was significantly higher than that of the ABA treated ones (Fig. 9).

Discussion

Exogenous BAP, GA_3, and ABA treatments completely suppressed, or delayed rooting of grapevine hardwood cuttings. Fujii and Nakano (1974) showed that basal soaking of 'Delaware' grapevine cuttings with cytokinin (BAP and kinetin) and GA_3 inhibited rooting, whereas ABA promoted it. Experiments in which cytokinin promoted adventitious root formation of cuttings are rare. In my ex-

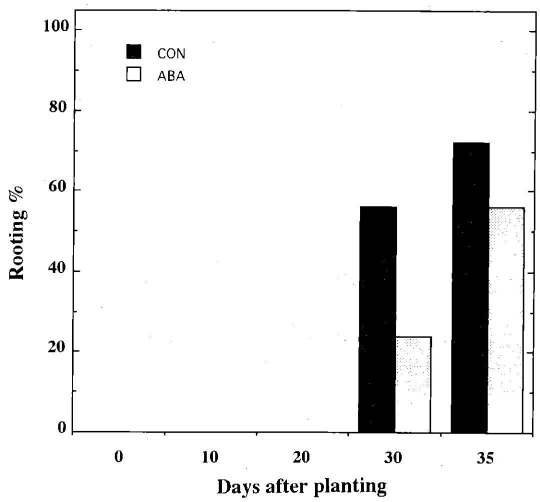

Fig. 7. Rooting percentage of 'Muscat Bailey A' grapevine cuttings treated with distilled water (CON) and 100 ppm ABA (ABA).

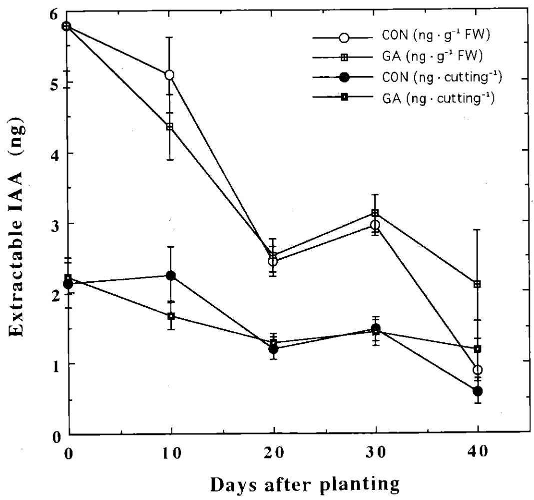

Fig. 6. Changes in the amounts of extractable IAA (ng·g^{-1}FW and ng·cutting^{-1}) in 'Muscat Bailey A' grapevine cuttings treated with distilled water (CON) and 100 ppm GA_3 (GA). Vertical bars represent SE (n=5).

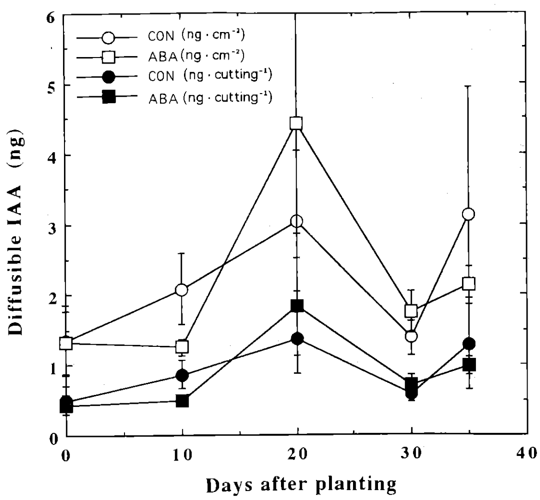

Fig. 8. Changes in the amounts of diffusible IAA (ng·cm^{-2} and ng·cutting^{-1}) in 'Muscat Bailey A' grapevine cuttings treated with distilled water (CON) and 100 ppm ABA (ABA). Vertical bars represent SE (n=5).

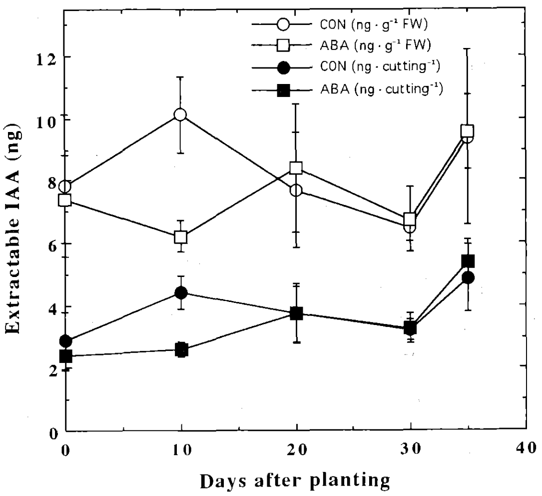

Fig. 9. Changes in the amounts of extractable IAA ($ng \cdot g^{-1}FW$ and $ng \cdot cutting^{-1}$) in 'Muscat Bailey A' grapevine cuttings treated with distilled water (CON) and 100 ppm ABA (ABA). Vertical bars represent SE (n=5).

periment, the application of BAP greatly increased the extractable IAA of cuttings but GA_3 did not. Skoog and Miller (1957) discovered that root and shoot initiation *in vitro* is regulated by interactions between auxin and cytokinin; the high auxin concentrations in the presence of low cytokinin levels promote root formation. Although the increase in endogenous IAA result in high concentration of auxin relative to cytokinin, the grapevine cuttings failed to root. I assume that the BAP-treated cuttings failed to control endogenous IAA metabolism or to keep suitable IAA levels for root formation. Fabijian et al. (1981) reported that hypocotyls of sunflower which had been treated with BAP did not undergo cell division, typical of the earliest stage of root formation. Hence, high concentration of cytokinin may inhibit root primordia formation.

The application of GA_3 to cuttings inhibited root formation temporarily. Because cuttings treated with GA_3 did not significantly reduce endogenous auxin levels compared with those of the control. This inhibition of rooting by GA_3 may be a direct, local effect (Brian et al., 1960). Haissig (1972) concluded that in brittle willow, applied gibberellic acid blocks the action of auxin in primordium development, subsequent to the initiation phase. My data show that diffusible IAA is higher in cuttings treated with GA_3 than that in control cuttings. In savoy cabbage, apical treatment with gibberellins increased diffusible auxin levels (Andersen and Muir, 1969).

That exogenous ABA promoted the rooting of cuttings and partially overcame GA_3-induced inhibition of adventitious rooting was reported by Chin et al. (1969), Coleman and Greyson (1976), and Hartung et al. (1980). Chin et al. (1969) have suggested that ABA might be the 'rooting cofactor', whereas Fujii and Nakano (1974) postulated that auxins in the buds may promote rooting synergistically with a suitable amount of endogenous ABA. In my experiment, basal application of ABA to cuttings inhibited rooting temporarily, the level of extractable IAA decreasing to a level lower than that in the control 10 days after planting. Disbudding grapevine cuttings reduced the level of extractable IAA relative to control cuttings and simultaneously inhibited rooting (Kawai, 1996). Because the application of ABA inhibited budbreak of hardwood cuttings (not shown data), ABA may alter the activity of buds, thus inhibiting root formation.

The difference with respect to the inhibition of rooting between BAP and GA_3 or ABA may be attributed to changing physiological condition of the cuttings. In cuttings treated with BAP, the physiological process of rooting was completely blocked, whereas in cuttings treated with GA_3 and ABA, the rooting process was temporarily delayed for some unknown reason(s).

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ブドウ熟枝挿しの発根と内生オーキシンに及ぼす外生 BAP，GA_3，ABA の影響

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摘　要

ブドウの挿し穂にベンジルアミノプリン（BAP），ジベレリン酸（GA_3），アブシジン酸（ABA）を処理した場合の発根に及ぼす影響について調査した．その結果，BAP は挿し木からの発根を完全に抑制した．GA_3 と ABA は一時的に発根を抑制した．内生のインドール酢酸（IAA）は BAP 処理により挿し木後 30 日まで直線的に増加し，30 日後のその量は対照区に比べ，挿し木 1 本当たりでは 12.6 倍，新鮮重当たりでは 19.4 倍になった．GA_3 処理の挿し木においては，拡散性 IAA が実験期間中対照区に比べ高かった．また，抽出性 IAA の変動は対照区の挿し木と同様な変動パターンを示した．ABA 処理の挿し木においては，抽出性 IAA の量が挿し木後 10 日に対照区に比べ有意に低くなった．BAP 処理区における内生 IAA の変動パターンは，GA_3 と ABA 処理区とは異なっていた．BAP 処理により内生 IAA が大量に増加したためにブドウの挿し穂は発根できなかったものと思われる．一方，GA_3 は直接，不定根形成の抑制に作用し，ABA は挿し穂の芽の働きを抑えることにより発根を抑制したものと考えられる．