ブドウの芽の休眠打破に伴う生理的変化

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Physiological Aspects of Bud Associated with Breaking Dormancy in Grapevines

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(Course of Applied Plant Science)

Changes in CO_2 and C_2H_4 production and water content of bud associated with breaking in 'Pione' grapevine (*Vitis labrusca* × *V. vinifera*) were investigated throughout dormancy. Buds were collected monthly from August to December, during dormancy induction and maintenance, and CO_2 and C_2H_4 production were determined by GC after incubation. Both CO_2 and C_2H_4 production, especially for the latter, were low throughout the experiment. Water content of bud gradually increased until October ; thereafter it was constant. When CO_2 and C_2H_4 production was determined from December to April, during dormancy maintenance to release, CO_2 production was low from beginning of experiment to early April, prior to bursting, then rapidly increased to April 13, the bursting date. C_2H_4 production was almost undetectable throughout the experiment. Cuttings obtained at 3 different stages of dormancy were applied with 2% H_2CN_2 or distilled water (control), and budbreak was monitored in a plastic house kept at 20°C or more. The CO_2 and C_2H_4 production of bud were also determined weekly until budbreak. Regardless of treatment time H_2CN_2 significantly promoted budbreak compared to the control. Significantly higher production of CO_2 was observed in cuttings treated with H_2CN_2 at 3 to 9 days before bursting for all the treatment times. C_2H_4 production was very low throughout the experiment for all the treatments. Irrespective of chemical application and treatment time, water content of bud decreased to the bursting stage, H_2CN_2 treatment especially showing a large decline. When dormant cuttings were treated with ACC, GSH (reduced glutathione) and GSSG (oxidized glutathione), only ACC promoted budbreak. Budbreak in cuttings treated with cyanamides such as $CaCN_2$ and H_2CN_2 and cyanides such as KCN and NaCN was significantly accelerated except for H_2CN_2. Based on these results, the relationship between budbreak of grapevine buds and physiological changes in buds, and the roles of substances related to ethylene biosynthesis on breaking bud dormancy are discussed.

Key words : grapevine, breaking bud dormancy, respiration rate, ethylene production, substances related to ethylene biosynthesis

Introduction

Many studies have been done on bud dormancy of temperate fruit trees, including grapevine with the focus on issues such as physiology of dormancy[2,5,16,19], and artificial termination of dormancy[6,8,9,12,17,18,24], among others. Generally, bud dormancy is divided into various stages or phases based on physiological and ecological aspects. Faust et al.[2] divided dormancy of perennial fruit trees into three phases, such as induction, maintenance and release based on their physiological aspects. Horiuchi et al.[5] reported that bud dormancy of 'Delaware' grapevines was deep at the beginning of autumn, but its intensity gradually decreased from late autumn to early winter. We found that each period of July to September, October to December, and January to March could correspond to paradormancy, endodormancy and ecodormancy in 'Pione' grapevine buds, respectively (unpublished data). Available evidence on the factors and mechanisms involved in these phases has been reviewed by many researchers[1,2,3,16]. Most research has focused on breaking of dormancy, and on the physiological involvement of hormones, nutrients and water[2,3,7,13,16]. However, there is little information about physiological aspects such as changes in respiration rate and ethylene production and water content of grapevine bud during dormancy induction and release.

It has been reported that various substances related to ethylene biosynthesis closely relate to breaking bud dormancy of grapevine. Tohbe et al.[20,22] have reported

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that substances such as 1-aminocyclopropane-1-carboxylic acid (ACC), oxidized glutathione (GSSG) and reduced glutathione (GSH) closely relate to budbreak of 'Delaware' grapevines. Glutathione especially is very important, because GSSG operates dormancy induction, while GSH operates dormancy release. Tohbe et al.[21] also reported that cyanamides and cyanides, which concern with biosynthesis of ethylene in plants, are closely related to breaking bud dormancy of 'Delaware' grapevines. Effects of cyanide on breaking bud dormancy of trees have been reported by Fuchigami and Nee[3] and Mizutani et al.[10] Nevertheless, our information about the possible factors or substances inducing release from dormancy is limited.

The objective of this study was to investigate the changes in respiration rate (CO_2), ethylene production (C_2H_4) and water content of grapevine bud during dormancy induction and release, and the effects of substances related to ethylene biosynthesis on breaking bud dormancy.

Materials and Methods

All plant materials were obtained during dormancy from four mature 'Pione' grapevines (*Vitis labrusca* × *V. vinifera*) grown at the Research Farm of the Faculty of Agriculture, Okayama University.

Changes in CO_2 and C_2H_4 production and water content of bud during dormancy in outdoor-grown grapevines

'Pione' buds were collected monthly throughout dormancy induction from August 25 to December 20, 2006, and the fresh weights were measured immediately. Three buds were put into a vial of 14 ml in volume, sealed, and kept at 25°C to incubate for two hours. One ml of head space gas from the vial was injected into gas chromatograph (GC-3BT, Shimazdu, Kyoto) to determine CO_2 production. The contributions for gas chromatography were as follows: Detector, thermal conductivity detector (TCD); column, Porapak Q 60/80 mesh; temperature, 80°C; carrier gas, He at 20 ml/min. Respiration rate of buds was expressed as $\mu l\ CO_2 \cdot g^{-1}FW \cdot hr^{-1}$. In addition, one ml of head space gas was injected into gas chromatograph (GC-4CM, Shimadzu, Kyoto) to determine C_2H_4 production. The contributions for gas chromatography as follows: Detector, flame ionization detector (FID); column, activated alumina 60/80 mesh; temperature, 80°C; carrier gas, N_2 at 40 ml/min. Ethylene production of buds was expressed as $nL\ C_2H_4 \cdot g^{-1}FW \cdot h^{-1} \times 10^{-4}$. Four replications were allocated each time. After determining CO_2 and C_2H_4 production, buds were dried in an oven at 60°C for 24 hours and weighed. Water content of buds was calculated dividing dry weight by fresh weight.

'Pione' buds were collected at two week intervals from December 5, 2005, corresponding to the deep dormant stage, until April 13, 2006, corresponding to the bursting stage, respectively. After incubation at 25°C for two hours, CO_2 and C_2H_4 were determined in the same way as mentioned above.

Budbreak in cuttings treated with H_2CN_2 and changes in CO_2 and C_2H_4 production and water content of bud during breaking process

'Pione' canes were obtained at three different stages of dormancy, December 7, 2006, and January 11 and February 15, 2007. Cuttings with a single bud were prepared and their apical parts, including bud, were treated with 2% H_2CN_2 or distilled water for the control, and mounted on a style foam plate, floated in a water bath. They were put into a plastic house heated at 25°C or more. Ten sets consisting of six cuttings were allocated in each plot for each treatment. The number of cuttings broken was monitored using 4 of 10 sets treated at 2-day intervals for 60 days after treatment. Buds were regarded as broken when the buds tips became green. The CO_2 and C_2H_4 production of buds in each plot were also determined using the remaining 6 sets at 5 to 7 days intervals from start of treatment to bursting in each plot in the same ways as described above. Water content of bud was also determined as mentioned above. Throughout the experiment water in the bath was renewed weekly to prevent decomposition.

Effect of ACC, glutathiones, cyanamides and cyanides on budbreak of cuttings

The canes were obtained from 'Pione' grapevines on October 27, 2004, at the deepest dormant stage, and cuttings with a single bud were prepared. Cuttings were treated with 5 mM 1-aminocyclopropane-1-carboxylic acid (ACC), reduced glutathione (GSH) and oxidized glutathione (GSSG), and were treated with cyanamides (20% $CaCN_2$, 10% H_2CN_2) and cyanides (10% KCN, 10% NaCN), which induce budbreak in grapevine[3,14,15,21]. All chemicals were applied to the upper part of cuttings, including the bud, whereas untreated control cuttings were treated with distilled water. Immediately after treatment, the cuttings were mounted on a plastic foam plate, floated in a water bath, and placed in a plastic house maintained at 20°C or more. Each treatment consisted of four replications. The number of cuttings broken was monitored at 2-day intervals for 60 days after treatment. Four sets of six cuttings were allocated to each treatment. Throughout the experiment water in the bath was renewed weekly to prevent decomposition.

Statistical analysis

An analysis of variance was applied to the results of

the determinations to test for significant differences among the chemicals. Statistical methods employed were LSD and t-test.

Results

Changes in CO_2 and C_2H_4 production and water content of bud during dormancy in outdoor-grown grapevines

Throughout the induction of dormancy, both CO_2 and C_2H_4 production of 'Pione' buds were markedly low, especially for C_2H_4 production, which was almost undetectable (Fig. 1). Water content of bud gradually increased from August to October, thereafter it was constant until December.

In dormancy release from early December to middle April, on the other hand, CO_2 production was low from early December to early April, then rapidly increased just before bursting, and it reached maximum level at bursting date of April 13 (Fig. 2). C_2H_4 was not detected throughout the experiment.

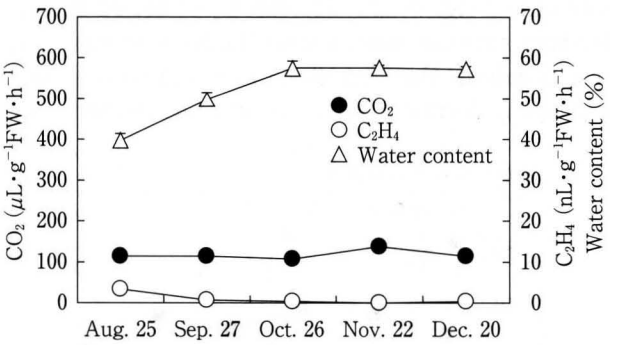

Fig. 1 Changes in respiration rate (closed circle), ethylene production (open circle) and water content (open triangle) of 'Pione' grapevine buds during dormancy induction.
Buds were kept at 25°C for 2 hours before determining.
Vertical bars are the SE (n = 4).

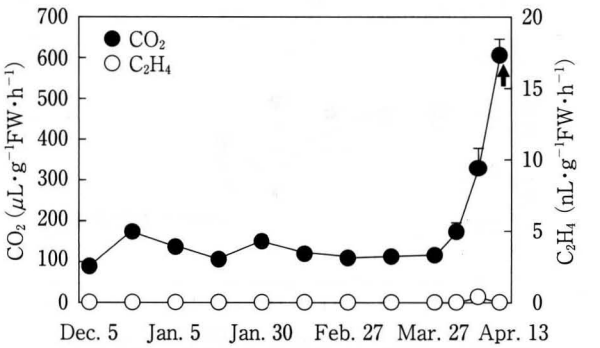

Fig. 2 Changes in respiration rate (closed) and ethylene production (open) of 'Pione' grapevine buds during dormancy release.
Buds were kept at 25°C for 2 hours before determining.
Arrow indicates budbreak.
Vertical bars are the SE (n = 4).

Budbreak in cuttings treated with H_2CN_2 and changes in CO_2 and C_2H_4 production and water content of buds during breaking process

Irrespective of treatment time, namely different stages of dormancy, budbreak was significantly accelerated in the cuttings treated with 2% H_2CN_2 compared to the control ones (Fig. 3, data for January treatment is not shown). The earlier treatment resulted in the larger difference in budbreak between two treatments.

When CO_2 and C_2H_4 production of bud in cuttings with H_2CN_2 and distilled water were compared during breaking for each treatment time, CO_2 production rapidly increased just before bursting for all plots regardless of treatment time (Fig. 4). Increase of CO_2 production of bud at bursting stage was larger in the later treatment than in the earlier one. The C_2H_4 production of bud was very low throughout the experiment regardless of treatment time.

No change in water content of bud was observed until just before bursting, but it decreased at the bursting stage regardless of treatment (Fig. 5). The H_2CN_2 treatment showed marked decrease at bursting stage compared to the control for all treatment times.

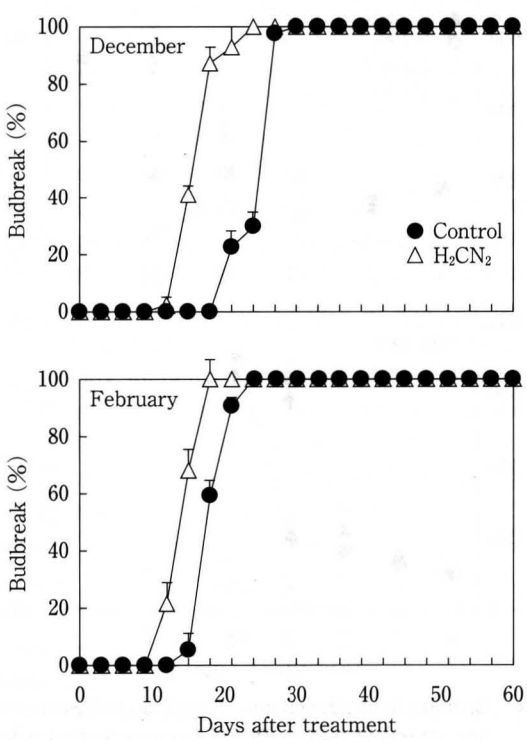

Fig. 3 Effects of painting with 2% H_2CN_2 on budbreak in 'Pione' cuttings at 2 different stages of dormancy (upper: December treatment, lower: February treatment).
Vertical bars are the SE (n = 4).

Effects of ACC, glutathiones, cyanamides and cyanides on budbreak of cuttings

When dormant 'Pione' cuttings were treated with ACC, GSH and GSSG at 5mM, only ACC was effective in budbreak, judging from promotion and the uniformity of budbreak. Both GSH and GSSG had no effect on budbreak (Fig. 6). When effects of 20% $CaCN_2$, 10% H_2CN_2, 10% KCN and 10% NaCN on breaking bud dormancy of cuttings were compared, all chemicals except for H_2CN_2, which markedly inhibited budbreak, resulted in significantly earlier and uniform budbreak compared to the control.

Discussion

Physiological aspects of bud associated with breaking dormancy in grapevines

Although the level of CO_2 production during dormancy release was similar to the report of Horiuchi et al.[5], its seasonal change did not agree. Horiuchi et al.[5] reported that respiration rate determined by two ways of oxygen uptake and carbon production in 'Delaware' grapevine buds gradually decreased from mid-September corresponding to dormancy induction, and reached the lowest level at mid-October corresponding to early stage of ecodormancy, then it was constant during the subsequent ecodormancy. The reasons for different CO_2 productions of grapevine bud between two experiments during dormancy induction are not known, although both cultivars tested and methods of determination were different. In this experiment CO_2 production of bud in grapevine grown outdoors was low throughout dormancy induction and release, but it increased markedly just before bursting. It seems that CO_2 production in grapevine buds rapidly increases only just before bursting stage, although Horiuchi et al.[5] did not investigate respiration rate at bursting stage. Hatch and Walker[4] have previously reported that respiration rates of peach and apricot buds are low during winter, but rapidly increase toward spring. In this experiment, marked increase of respiration rate toward bursting was observed in buds not only of grapevine grown outdoors but also of cuttings induced breaking by H_2CN_2, an effective substance on budbreak in grapevines[12,14,15,18]. Accordingly, irrespective of treatment time, respiration rate of bud significantly increased just before bursting for both cuttings treated with H_2CN_2 or without. Our results indicate that respiration rate of grapevine bud is low during dormancy induction and release, and mark-

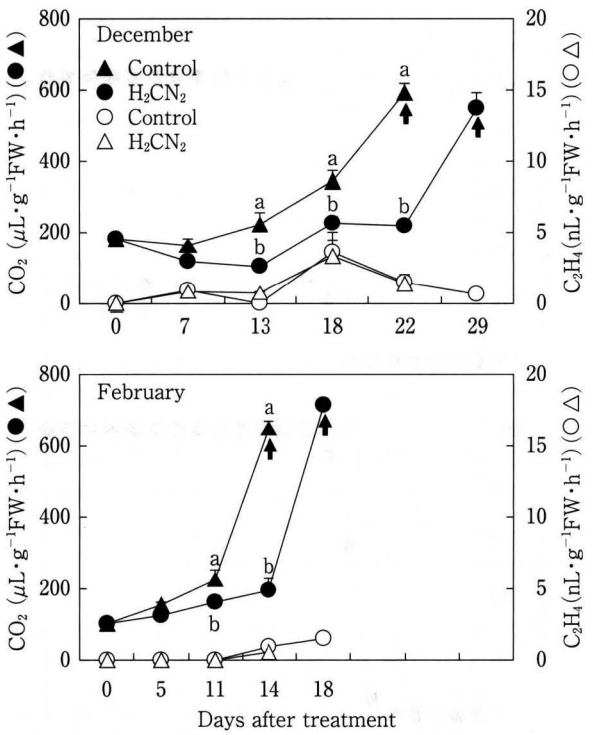

Fig. 4 Changes in respiration rate (closed) and ethylene production (open) of bud in 'Pione' cuttings treated with 2% H_2CN_2 at 2 different stages of dormancy.
Refer to Fig. 3. Buds were kept at 25°C for 2 hours before determining. Arrows indicate budbreak in each plot. Vertical bars are the SE (n = 4).

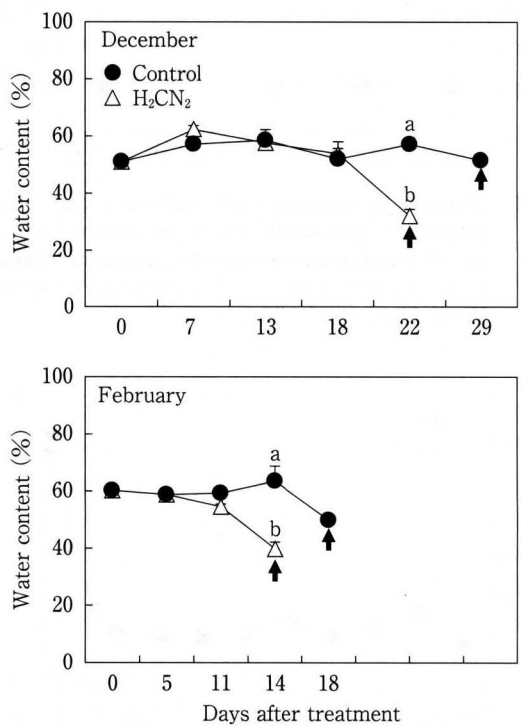

Fig. 5 Changes in water content of bud in 'Pione' cuttings treated with 2% H_2CN_2 at 2 different stages of dormancy.
Refer to Fig. 3. Arrows indicate budbreak in each plot. Vertical bars are the SE (n = 4).

edly increases just before bursting stage. Tamura et al.[19] have found that respiration rate and ethylene evolution of Japanese pear buds gradually increase after 1,100 hr and 1,250 hr of chilling, respectively. However, the mode of action of breaking dormancy is not yet known.

In this experiment, water content of bud increased from 40% to 60% during dormancy induction; thereafter it was constant until before bursting. When cuttings were treated with 2% H_2CN_2, water content of bud was kept constant, ranging from 50 to 60% after treatment, then it markedly decreased at bursting stage; the degree of decline was larger in cuttings treated with H_2CN_2 than in the control. Our results agreed with the report of Nakagawa[11].

Roles of substances related to ethylene biosynthesis on breaking bud dormancy

When various substances related to ethylene biosynthesis were applied to dormant 'Pione' cuttings, 2% H_2CN_2 was most effective in budbreak. Effectiveness of H_2CN_2 on budbreak of grapevines has been reported by many researchers[12,14,15,18,21]. In this experiment, 2% H_2CN_2 was effective in budbreak regardless of treatment time, but 10% H_2CN_2 markedly inhibited budbreak. Our result does not agree with the report of Tohbe et al.[21] in which 10% H_2CN_2 was effective in budbreak of 'Delaware' grapevines as well as 20% $CaCN_2$. Reasons for the different responses of grapevine buds to 10% H_2CN_2 are unclear. It is well known that ethylene or ethephon had no effect on budbreak of grapevines[6,10,20], but ACC, a precursor of ethylene, which is closely related to ethylene biosynthesis[10,20,21] is effective in budbreak of dormant grapevine. In this experiment, ACC was effective in budbreak of dormant 'Pione' cuttings as in the reports of Mizutani et al.[10] and Tohbe et al.[20]. Tohbe et al.[21] also found that high temperature stimulated budbreak of dormant 'Delaware' grapevines, and increased ACC content and ethylene production of cuttings. These results may suggest that ethylene itself has little or no effect on budbreak of dormancy in grapevines and that HCN, which is produced during ethylene biosynthesis, may break bud dormancy.

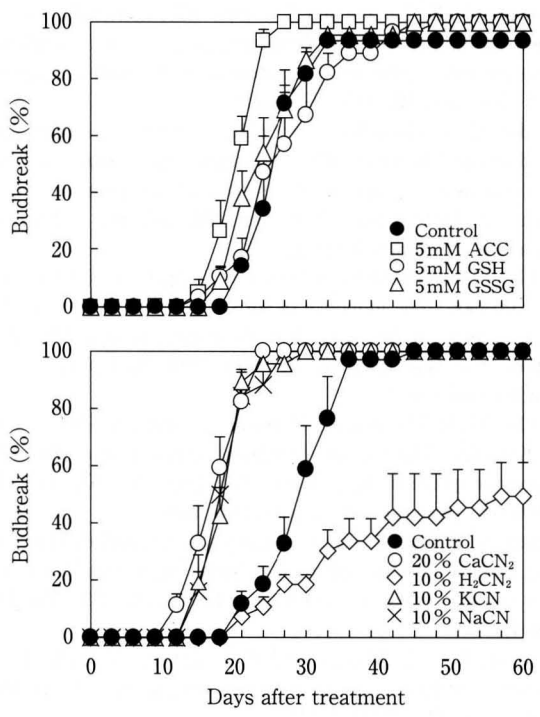

Fig. 6 Effects of application of ACC (1-aminocyclopropane-1-carboxylic acid), GSH (glutathione, reduced form) and GSSG (glutathione, oxidized form) (upper) and of cyanamides ($CaCN_2$, H_2CN_2) and cyanides (KCN, NaCN) (lower) on budbreak of dormant 'Pione' grapevine cuttings.
Vertical bars indicate the SE (n = 4).

Tohbe et al.[20,22] investigated the changes in glutathione content of grapevine bud and the effects on breaking bud dormancy of high temperature. They reported that reduced glutathione (GSH) in cuttings exposed to high temperature was higher than that of the control cuttings throughout the experiment, but the situation was reversed by oxidized glutathione (GSSG). Namely, GSH broke bud dormancy, but GSSG inhibited it. Tohbe et al.[20] suggested that dormancy of grapevine buds is broken when GSSG converts to GSH, and that cyanide produced during ethylene biosynthesis may stimulate this conversion. Tohbe et al.[20] also found that GSH content in the shoot with high temperature increased, whereas in shoots treated with low temperature it decreased gradually, and also GSSG content increased in shoots induced to dormancy by the ABA treatment. Therefore, Tohbe et al.[22] concluded that glutathione is one of the most important factors in controlling dormancy of grapevine buds. However, neither GSH nor GSSG had any influence on budbreak of 'Pione' grapevine cuttings. The reasons for the different responses by buds of different cultivars to GSH are not known. The role of glutathione, especially of GSH, in breaking bud dormancy has been reported by Fuchigami and Nee[3]. Moreover, Wang and Faust[23] reported that breaking induced by cold, heat and allyl disulfide in apple buds is associated with the removal of free radicals through activated peroxide-scavenging systems.

Conclusions

Respiration rate of bud in grapevines grown outdoors was low during dormancy induction and release, but it rapidly increased just before bursting stage. A similar trend of change in CO_2 production was observed in cuttings, in which budbreak was stimulated by H_2CN_2

treatment. The ethylene production was not associated with budbreak in grapevines because there was no change throughout the experiment. Water content of bud markedly decreased at bursting stage. Of substances related to controlling dormancy, ACC and cyanides were effective in budbreak of 'Pione' grapevines, but both GSH and GSSG had no effect. Further investigation is needed to clarity the effects of substances related to ethylene biosynthesis on breaking bud dormancy of grapevines.

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References

1) Dennis Jr., F. G. : Dormancy-what we know (and don't know). HortScience, **29**, 1249-1255 (1994)
2) Faust, M., A. Erez, L. J. Rowland, S. Y. Wang and H. A. Norman : Bud dormancy in perennial fruit trees: Physiological basis for dormancy induction, maintenance, and release. HortScience, **32**, 623-629 (1997)
3) Fuchigami, L. H. and C. C. Nee : Degree growth stage model and rest-breaking mechanisms in temperate woody perennials. HortScience, **22**, 836-845 (1987)
4) Hatch, A. H. and D. R. Walker : Rest intensity of dormant peach and apricot leaf buds as influenced by temperature, cold hardiness and respiration. J. Amer. Soc. Hort. Sci., **94**, 304-307 (1969)
5) Horiuchi, S., S. Nakagawa and A. Kato : General characteristics of bud dormancy in the vine. J. Japan. Soc. Hort. Sci., **50**, 176-184 (1981) (In Japanese with English Summary)
6) Iwasaki, K. : Effects of bud scale removal, calcium cyanamide, GA_3 and ethephon on bud break of 'Muscat of Alexandria' grape (*Vitis vinifera* L.). J. Japan. Soc. Hort. Sci., **48**, 395-398 (1980)
7) Iwasaki, K. and R. J. Weaver. : Effects of chilling, calcium cyanamide, and bud scale removal on bud break, rooting and inhibitor content of buds of 'Zinfandel' grapes (*Vitis vinifera* L.). J. Amer. Soc. Hort. Sci., **102**, 584-587 (1977)
8) Kubota, N. and M. Miyamuki : Breaking bud dormancy in grapevines with garlic paste. J. Amer. Soc. Hor. Sci., **117**, 898-901 (1992)
9) Kuroi, I., Y. Shiraishi and S. Imano : Studies on breaking the dormancy of grapevines. I. Effect of lime nitrogen treatment for shortening the rest period of glasshouse-grown grape vines. J. Japan. Soc. Hort. Sci., **32**, 175-180 (1963) (In Japanese with English summary)
10) Mizutani, F., A. Hino, S. Amano, K. Kadoya, J. Watanabe and H. Akiyoshi : Effect of calcium cyanamide, GA_3 and scale removal on bud break, ethylene production and ACC content in grapevine buds. Mem. Coll. Agr. Ehime Univ., **40**, 91-97 (1995)
11) Nakagawa, S. : Principles of Pomology. pp. 442-466. Yokendo, Tokyo (1978) (In Japanese)
12) Nir, G., I. Klein, S. Lavee, G. Spieler and U. Barak : Improving grapevine budbreak and yields by evaporative cooling. J. Amer. Soc. Hort. Sci., **113**, 512-517 (1988)
13) Ndung'u, C. K., M. Shimizu, G. Okamoto and K. Hirano : Abscisic acid, carbohydrates, and nitrogen contents of Kyoho grapevines in relation to budbreak induction by water stress. Amer. J. Enol. Vitic., **48**, 115-120 (1997)
14) Potjanapimon, C., F. Fukuda and N. Kubota : Effects of various chemical compounds and their concentrations on breaking bud dormancy in grapevines. Sci. Rep. Fac. Agr. Okayama Univ., **96**, 19-24 (2007)
15) Potjanapimon, C., F. Fukuda and N. Kubota : Effects of chilling exposures and chemicals on breaking bud dormancy in seven tetraploid grape cultivars. Hort. Res. (Japan), (in press) (In Japanese with English Summary)
16) Powell, L. E. : Hormonal aspects of bud and seed dormancy in temperate-zone woody plants. HortScience, **22**, 92-109 (1987)
17) Samish, R. M. : Dormancy in woody plants. Ann. Rev. Plant. Physiol., **5**, 183-204 (1954)
18) Shulman, Y., G. Nir, L. Fanberstein and S. Lavee : The effect of cyanamide on the release from dormancy of grapevine buds. Scientia Hortic., **19**, 97-104 (1983)
19) Tamura, F., K. Tanabe and K. Banno : Effect of chilling treatment on intensity of bud dormancy, respiration and endogenous growth regulators in Japanese pear 'Nijisseiki'. J. Japan. Soc. Hort. Sci., **60**, 763-769 (1992)
20) Tohbe, M., R. Mochioka, S. Horiuchi, T. Ogata, S. Shiozaki and H. Kurooka : Roles of ACC, and glutathione during breaking of dormancy in grapevine buds by high temperature treatment. J. Japan. Soc. Hort. Sci., **67**, 897-901 (1998) (In Japanese with English Summary)
21) Tohbe, M., R. Mochioka, S. Horiuchi, T. Ogata, S. Shiozaki and H. Kurooka : The influence of substances related to ethylene biosynthesis on breaking bud dormancy in grapevines. J. Japan. Soc, Hort. Sci., **67**, 902-906 (1998) (In Japanese with English Summary)
22) Tohbe, M., R. Mochioka, S. Horiuchi, T. Ogata, S. Shiozaki and H. Kurooka : The role of glutathione on the onset of endodormancy of grape buds. J. Japan. Soc, Hort. Sci., **67**, 912-916 (1998) (In Japanese with English Summary)
23) Wang, S. Y. and M. Faust : Changes in the antioxidant system associated with budbreak in 'Anna' apple (*Malus domestica* Borkh.) buds. J. Amer. Soc. Hort. Sci., **119**, 735-741 (1994)
24) Weaver, R. J., L. Manivel and F. L. Jensen : The effects of growth regulators, temperature and drying on *Vitis vinifera* buds. Vitis, **13**, 23-29 (1974)

ブドウの芽の休眠打破に伴う生理的変化

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ブドウ‘ピオーネ’について，休眠の導入と覚醒の過程における芽の生理的変化を調査した．休眠導入期の８月から覚醒初期の12月まで露地で栽培されている個体から芽を採取し，呼吸量，エチレン生成量および含水率を測定した．調査期間を通して呼吸量は低く，エチレンもほとんど検出されなかった．芽の含水率は８月から10月まで僅かに上昇し，その後は変化がみられなかった．休眠覚醒初期の12月から発芽期の４月中旬まで，芽の呼吸量とエチレン生成量を測定した．呼吸量は４月上旬までは低く推移し，発芽（４月13日）の直前に急上昇した．エチレンは測定期間を通して低かった．休眠期の12月，１月および２月に採取した穂木を２％ H_2CN_2 または蒸留水（対照）で処理し，25℃以上に保ったプラスチックハウスに入れて発芽を調査するとともに，経時的に芽の呼吸量，エチレン生成量および含水率を測定した．両時期とも対照区よりも H_2CN_2 処理区の発芽が早く，しかも休眠の深い12月処理で区による差が大きかった．両区いずれの時期とも，芽の呼吸量は発芽直前に急上昇したのに対し，エチレン生成量は調査期間を通して低いままであった．芽の含水率は，いずれの時期および処理区とも発芽期に低下し，特に H_2CN_2 処理区の低下が大きかった．休眠最深期の10月に採取した穂木に ACC，GSH（還元型グルタチオン）および GSSG（酸化型グルタチオン）を処理し，発芽に及ぼす影響を調査したところ，ACC だけが発芽を促進した．同様に，４種のシアン化合物（$CaCN_2$，H_2CN_2，KCN，NaCN）を処理したところ，H_2CN_2を除き有意に発芽を促した．これらの結果を基に，ブドウの発芽と生理的変化との関係および休眠覚醒に及ぼすエチレン生合成関連物質の作用性について考察した．