Effect of Hydrogen Peroxide on Breaking Endodormancy in Flower Buds of Japanese Pear (*Pyrus pyrifolia* Nakai)

Haruyuki Kuroda*, Toshihiko Sugiura** and Hiroyoshi Sugiura

National Institute of Fruit Tree Science, NARO, Tsukuba 305–8605

Summary

The effect of hydrogen peroxide (H_2O_2) on the breaking of endodormancy was studied by using flower buds of Japanese pear (*Pyrus pyrifolia* Nakai 'Kosui') in the latter stage of endodormancy. Applications of 0.25, 1.0, 2.5, and 10% H_2O_2 promoted budbreak in flower buds; the most effective concentration was 2.5% applied in late November and 10% in early December. No phytotoxicity with H_2O_2 was observed in any treatment concentration or time. These results indicate that H_2O_2 is an effective compound for breaking endodormancy in flower buds of the Japanese pear.

Key Words: breaking endodormancy, endodormancy-breaking compound, flower bud, hydrogen peroxide, Japanese pear.

Introduction

For commercial cultivation of deciduous fruit trees in a zone in which the low temperature requirement is not sufficiently met, it is necessary to break endodormancy artificially. Much work has been conducted to induce termination of endodormancy in fruit trees by applying chemicals, such as mineral oil and dinitro-*o*-cresol (Samish, 1954), calcium cyanamide (Iwasaki, 1980; Kuroi et al., 1963; Shulman et al., 1983), and hydrogen cyanamide (H_2CN_2) (Shulman et al., 1983). Because they are toxic, most of these chemicals adversely affect the grower's health. Moreover, when large amounts of these chemicals remain, the environment becomes polluted.

Recently, the development of endodormancy-breaking compounds benign to the environment and human beings has progressed; their active substances for the breaking of endodormancy have been identified as diallyl sulfides in garlic (Kubota et al., 1999), methyl mercaptan and allyl mercaptan in the Chinese chive (Kubota et al., 2003), and dimethyl disulfide in rakkyo (Kubota et al., 2003).

On the other hand, previous studies (Kuroda et al., 2002) have shown that the endogenous hydrogen peroxide (H_2O_2) content in flower buds of the Japanese

pear increased gradually as the breaking of endodormancy advanced; it peaked in late December when endodormancy was broken and then decreased. However, in unchilled flower buds held in a phytotron kept at 25°C, the endogenous H_2O_2 content did not change significantly, remaining at low levels. In addition, the endogenous H_2O_2 content increased in H_2CN_2 -treated flower buds in which the end of endodormancy was advanced (Kuroda et al., 2002). These results suggest that endogenous H_2O_2 may substitute for the chilling requirement so that exogenous applications of H_2O_2 may hasten the breaking of endodormancy. This study was undertaken to test this hypothesis that H_2O_2 controls endodormancy in flower buds of the Japanese pear.

Materials and Methods

Two-year-old branches with flower buds on mature Japanese pear trees (*Pyrus pyrifolia* Nakai 'Kosui'), grown at the National Institute of Fruit Tree Science (Tsukuba), located at 36°3'N and 140°8'E, were used.

Seasonal changes of endodormancy status in flower buds

The branches were collected at approximately 2-week intervals from October 4 to December 27, 2001. They were placed in a container of tap water in a phytotron kept at $25 \pm 1.0^\circ\text{C}$. After 22 days, the percent budbreak was evaluated on three branches, each with 6–8 flower buds. Budbreak occurs when the basal part of the bud scales appears green.

Effect of H_2O_2 treatment on breaking of endodormancy in flower buds

The branches, collected on November 22 and December 7, 2001, during the latter stage of endodormancy were sprayed to run-off with distilled water (control), or

Received; July 28, 2004. Accepted; November 22, 2004.

This work was supported by a Grant-in-Aid from the Ministry of Agriculture, Forestry and Fisheries of Japan, and is contribution No. 1354 of the National Institute of Fruit Tree Science, NARO.

* Corresponding author (E-mail: kurodah@agri.kagoshima-u.ac.jp).

* Present address: Experimental Farm, Faculty of Agriculture, Kagoshima University, Korimoto, Kagoshima 890–0065.

** Present address: National Agriculture and Bio-oriented Research Organization Headquarters.

0.25, 1.0, 2.5, or 10% H_2O_2 containing 0.01% (v/v) Tween 20. After treatment, the cuttings were placed in a container with tap water in a phytotron kept at $25 \pm 1.0^\circ C$. The percent budbreak was evaluated at 2-day intervals on three branches with 6–8 flower buds each.

Results

Seasonal changes of endodormancy status in flower buds

In flower buds of 'Kosui' Japanese pear under natural conditions, the percent budbreak decreased from 26.3% to 0% throughout October, which indicated that endodormancy deepened the most in late October. The deep endodormancy was maintained until early November. Thereafter, endodormancy was released gradually as manifested by the percent budbreak that increased to 37.7% on November 20 and 55.0% on December 7; rest requirement was completely broken by mid-December (Fig. 1).

Effect of H_2O_2 treatment on breaking of endodormancy in flower buds

In flower buds treated with water or H_2O_2 on November 22, no difference in days to the first budbreak occurred within two weeks. However, budbreak of H_2O_2 -treated flower buds increased rapidly thereafter compared to the control. The final percent budbreak was highest in the 2.5% H_2O_2 -treated flower buds, followed by the 10% H_2O_2 treatment (Fig. 2).

In flower buds treated with water or H_2O_2 on December 7, the onset of budbreak occurred earlier than in that on November 22. In particular, budbreak in flower buds treated with H_2O_2 at 2.5 and 10% occurred 8 days after treatment, which was 8 days earlier than that on November 22. The percent budbreak in all the plots treated on December 7 increased more gradually than that on November 22. However, H_2O_2 treatment promoted budbreak that attained the final percent budbreak of 96% in the 10% H_2O_2 -treated flower buds (Fig. 3).

No phytotoxicity with H_2O_2 was observed in any

Fig. 1. Seasonal changes of endodormancy status in flower buds of 'Kosui' Japanese pear (2001). Bars indicate SE ($n=3$).

Fig. 2. Effect of hydrogen peroxide on budbreak in flower buds of 'Kosui' Japanese pear (treatment on November 22). Different letters within columns represent significant differences at 5% level (Duncan's multiple range test).

Fig. 3. Effect of hydrogen peroxide on budbreak in flower buds of 'Kosui' Japanese pear (treatment on December 7). NS and different letters within columns represent non-significance and significant differences at 5% level, respectively (Duncan's multiple range test).

treatment, concentration, or time.

Discussion

The endodormancy in flower buds of 'Kosui' Japanese pear that began in early November was completely broken by mid-December, which indicated that the latter stage of endodormancy extends between late November and early December. In this study, the efficacy of H_2O_2 on the breaking of endodormancy was tested on flower buds during this stage of endodormancy. The results indicate that H_2O_2 treatment at 0.25, 1.0, 2.5, and 10% concentrations promoted budbreak in flower buds; the most effective H_2O_2 concentration for budbreak was 2.5% in late November and 10% in early December.

In a previous study, endogenous H_2O_2 was proposed as a substitute for the chilling requirement in flower buds of the Japanese pear (Kuroda et al., 2002). Al-

though H_2O_2 provided exogenously and produced endogenously may not necessarily be the same in efficiency in breaking endodormancy, our results indicate that exogenous H_2O_2 can play an important role in the process. Matsuda et al. (1994) reported that treatment of the crowns of winter wheat with H_2O_2 in the presence of a specific inhibitor of catalase, 3-amino-1, 2, 4-triazole, induced or stimulated the synthesis of 25 polypeptides. Lang (1994) reported that breaking of endodormancy induced by low temperatures is gene(s)-controlled. Therefore, exogenous H_2O_2 seems to be one of many factors that can be involved in the modification of gene expression for breaking endodormancy.

In general, the effective concentration of endodormancy-breaking compounds differs depending on the treatment time (Iwasaki et al., 1980; Kuroi et al., 1963). In this study, the effective H_2O_2 concentration for budbreak was 2.5% on November 22 and 10% on December 7, which agrees with the above notion. In apple trees, the activities of H_2O_2 -scavenging systems coupled with the pentose phosphate cycle and catalase increase from autumn to winter (Kuroda et al., 1990). Thus, the different responses of flower buds to the exogenous application of H_2O_2 seen in late November and early December may be related to seasonal changes in activities of H_2O_2 -scavenging systems coupled with the pentose phosphate cycle and catalase. However, this parallel remains uncertain.

No phytotoxicity from H_2O_2 was observed at concentrations of 0.25 to 10% in late November or early December. H_2O_2 is derived from intracellular production which originates mainly from superoxide (O_2^-) via the monovalent pathway of the electron transfer reaction (Azzi et al., 1975; Cadenas et al., 1977). Moreover, H_2O_2 is a material without an environmental burden because it is metabolized to oxygen and water, although it is a strong oxidant. Therefore, H_2O_2 is thought to be an environmentally friendly endodormancy-breaking compound similar to diallyl sulfides (Kubota et al., 1999), methyl mercaptan, allyl mercaptan, and dimethyl disulfide (Kubota et al., 2003). Further investigations at molecular levels are required to clarify details of the effect of H_2O_2 on breaking endodormancy.

Literature Cited

- Azzi, A., C. Montecucco and C. Richter. 1975. The use of acetylated ferricytochrome *c* for the detection of superoxide radicals produced in biological membranes. *Biochem. Biophys. Res. Commun.* 65: 597–603.
- Cadenas, E., A. Boveris, C. I. Ragan and A. O. M. Stoppani. 1977. Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinol-cytochrome *c* reductase from beef-heart mitochondria. *Arch. Biochem. Biophys.* 180: 248–257.
- Iwasaki, K. 1980. 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.
- Kubota, N., K. Toriu, Y. Yamane, K. Kawazu, T. Higuchi and S. Nishimura. 2003. Identification of active substances in Chinese chive and rakkyo plants responsible for breaking bud dormancy in grape cutting. *J. Japan. Soc. Hort. Sci.* 72: 268–274.
- Kubota, N., Y. Yamane, K. Toriu, K. Kawazu, T. Higuchi and S. Nishimura. 1999. Identification of active substances in garlic responsible for breaking bud dormancy in grapevines. *J. Japan. Soc. Hort. Sci.* 68: 1111–1117.
- Kuroda, H., S. Sagisaka and K. Chiba. 1990. Seasonal changes in peroxide-scavenging systems of apple trees in relation to cold hardiness. *J. Japan. Soc. Hort. Sci.* 59: 399–408.
- Kuroda, H., T. Sugiura and H. Sugiura. 2002. Changes in hydrogen peroxide content in flower buds of Japanese pear (*Pyrus pyrifolia* Nakai) in relation to breaking of endodormancy. *J. Japan. Soc. Hort. Sci.* 71: 610–616.
- Kuroi, I., Y. Shiraishi and S. Imano. 1963. Studies on breaking the dormancy of grape vines. I. Effects of lime nitrogen treatment for shortening the rest period of glasshouse-grown grape vines. *J. Japan. Soc. Hort. Sci.* 32: 175–180 (In Japanese with English summary).
- Lang, G. A. 1994. Dormancy – The missing links: molecular studies and integration of regulatory plant and environmental interactions. *HortScience* 29: 1255–1263.
- Matsuda, Y., T. Okuda and S. Sagisaka. 1994. Regulation of protein synthesis by hydrogen peroxide in crowns of winter wheat. *Biosci. Biotech. Biochem.* 58: 906–909.
- Samish, R. M. 1954. Dormancy in woody plants. *Ann. Rev. Plant Physiol.* 5: 183–204.
- Shulman, Y., G. Nir, L. Fanberstein and S. Lavee. 1983. The effect of cyanamide on the release from dormancy of grapevine buds. *Scientia Hort.* 19: 97–104.

ニホンナシ花芽の自発休眠覚醒に及ぼす過酸化水素の影響

黒田治之*・杉浦俊彦**・杉浦裕義

農業・生物系特定産業技術研究機構果樹研究所
305-8605 つくば市藤本

摘 要

自発休眠後期におけるニホンナシ「幸水」の花芽を用いて、自発休眠覚醒に及ぼす過酸化水素(H_2O_2)の影響を調査した。0.25%, 1.0%, 2.5%, 10% 濃度での H_2O_2 処理は、花芽の発芽を促進させた。発芽促進に効果的な H_2O_2 の濃度は、11 月下旬では 2.5%, 12 月上旬では 10% であった。 H_2O_2 の害作用は、処理濃度、処理時期のすべてにおいて認められなかった。これらの結果から、 H_2O_2 はニホンナシ花芽の自発休眠覚醒に有効な物質であると考えられた。

* 現在: 鹿児島大学農学部附属農場。

** 現在: 農業・生物系特定産業技術研究機構本部。