[Research Note]

Effect of Rare Sugars, Methionine, and Sea Water on 'Kyoho' Grape Bud Dormancy Release

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The chilling requirement for breaking grape bud dormancy can be substituted with chemical treatment. Calcium cyanamide or cyanamide is frequently used for grape bud dormancy release. However, those chemicals are potentially harmful to humans. In this study, we used rare sugars that are less toxic to the environment and grape growers, such as L-fructose, D-psicose, and D-allose; methionine; and sea water to determine their effects on 'Kyoho' grape budbreak. The rare sugars improved percentage budbreak when applied at 1% concentration, but the improvement was insignificant. When the concentration was increased to 3%, percentage budbreak was increased. Among the rare sugars used, L-fructose at 3% gave the highest percentage budbreak and initiated earlier budbreak than the other rare sugars. On the other hand, methionine induced budbreak and slightly improved budbreak at 3% concentration compared to control. Sea water at either concentration did not improve budbreak.

Key words: bud dormancy, methionine, rare sugar

Introduction

Deciduous fruit species usually cease vegetative growth as buds enter dormancy. The intensity of dormancy varies depending upon the species and even the cultivar (Kovacs et al. 2003). Dormant buds must be exposed to a low-temperature or chilling period for growth resumption. Budbreaking is usually dependent upon chilling accumulation (below 7ºC or 10ºC) for a certain duration. However, the precise temperature and duration may vary depending upon the species and cultivar (Phivnil et al. 2004, Poudel 2008, Chavarria et al. 2009). More rapid and uniform budbreak was achieved when chilling duration was increased (Dokoozlian 1999). It is believed that bud dormancy is dependent on a number of internal and external factors that determine the onset and termination of bud dormancy (Weaver and Iwasaki 1977, Erez and Levee 1971).

The chilling requirement for bud dormancy release of several fruit trees, including grapevines, has been substituted with the treatment with chemicals and growth regulators. Examples include mineral oil, dinitro-*O*-cresol (DNOC), thiourea, calcium cyanamide, potassium cyanamide, hydrogen cyanamide, and garlic paste (Botelho et al. 2007, Iwasaki 1980, Mizutani et al. 1994, Kubota and Miyamuki 1992, Zelleke and Kliewer 1989). In peach, such growth regulators as gibberellic acid have also been used to increase percentage budbreak (Donoho and Walker 1957). The effects of calcium cyanamide and hydrogen cyanamide on grape bud dormancy release have been well documented (Weaver et al. 1961, Iwasaki 1980, Kuroi 1985, Lin and Wang 1985, Shulman et al. 1983) and thus, those chemicals are used commercially in many wine-producing countries. However, those cyanamides are known to have a negative impact on grape growers' health and the environment (U.S. Environmental Protection Agency 2000). Hence, there is an urgent need to develop dormancy release agents that pose no health risks to humans. Rare sugars, methionine, and sea water may be utilized as budbreak agents as they are safer than cyanamides. The rare sugar D-psicose shows no toxic

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effects and mutagenicity and is a component of human food (Matsuo and Izumori 2006). Methionine has been found to have no adverse effect on the human body (Garlick 2006). Similarly, sea water is known to be useful for certain diseases, such as atopic eczema/dermatitis syndrome (AEDS) (Kimata et al. 2002). However, there is little information on the effects of those chemicals on budbreak. It has been reported that some rare sugars stimulated the budbreak of some grape cultivars (Mochioka et al. 2007) Methionine and sea water may induce budbreak through the ethylene biosynthesis pathway by which such substances as hydrogen cyanamide (HCN) are produced, which is responsible for budbreak (Tohbe et al. 1998). It would be a great achievement if those chemicals were proven to be a suitable substitute for hazardous cyanamides as budbreak agents. Moreover, the practice of growing table grape cultivars under a plastic cover or the greenhouse condition and in comparatively warm areas has led to erratic budbreak due to insufficient chilling (0-7ºC) accumulation. The erratic budbreak consequently results in poor yield and low berry quality (Corrales-Maldonado et al. 2010). Hence, the objective of this study was to examine the effects of environmentally friendly compounds, such as rare sugars methionine, and sea water, on the bud dormancy release of ‘Kyoho’, a tetraploid table grape cultivar.

Materials and Methods

Single-node cuttings were collected on the 17th and 12th of November in 2008 and 2009 respectively, from ‘Kyoho’ (*Vitis vinifera* × *V. labrusca*) grapevines grown under a plastic cover at the University Farm of Kagawa University, Sanuki, Kagawa, Japan. The cuttings were approximately 15 cm long. To reduce heterogeneity of the plant materials, the cuttings were taken from the middle portion of the vine, i.e., above the 5th node from the base and below the 5th node from the tip. The substances used for treatment were D-psicose, L-fructose, D-allose, methionine, and sea water. Sea water was obtained from Shido Bay in Kagawa, Japan and its sodium salt concentration was 3.21%. In 2008, all the chemicals were used at 1% concentration whereas sea water was used after diluting three times. The control was treated with deionized water. Because the role of calcium cyanamide in bud dormancy release is known (Iwasaki 1980, Shulman et al. 1983, Kuroi 1985), it was employed for comparison with the chemicals and sea water.

The upper cut ends, including the buds of the cuttings, were dipped in a solution of chemical with the above-mentioned concentration for approximately 5 seconds. The treated cuttings were immediately placed in a tray (35 cm × 25 cm × 8 cm) containing akadamatsuchi soil (medium size; GI Company Ltd., Aichi, Japan). Akadamatsuchi soil particles have dihedral angles and are porous. Thus, akadamatsuchi has good water retention ability and air circulation. The trays containing the cuttings were placed in a growth chamber with a 16-hour photoperiod and whose temperature was maintained at 25 ± 1º C. Light source was provided by cool fluorescent tubes at 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Watering was done regularly at an interval of 2-3 days to maintain a sufficient moisture level. Each treatment was replicated three times with 10 cuttings per replication. Budbreak was considered to have occurred when green tissue appeared beneath the bud scale (Eichhorn and Lorenz 1977). Budbreak was recorded every week until eight weeks after treatment.

Results

Effect of rare sugars on ‘Kyoho’ grape bud dormancy release

Budbreak response differed depending upon the type and concentration of rare sugar used. In 2008, percentage budbreak with rare sugar treatment was higher than that with control from five to seven weeks of treatment (Fig. 1A). Percentage budbreak with D-allose treatment was higher than that with control and the other rare sugar treatments at the end of the experiment, i.e., on the eighth week. D-psicose and L-fructose treatment showed similar percentage budbreak to control on the eighth week. Fewer days were required to initiate budbreak with rare sugar treatment than with control. Moreover, cuttings treated with calcium cyanamide yielded earlier and higher percentage budbreak than cuttings treated with the different chemicals. Percentage budbreak with D-psicose treatment was similar to that with fructose treatment. Meanwhile, D-allose and L-fructose treatment yielded higher percentage budbreak

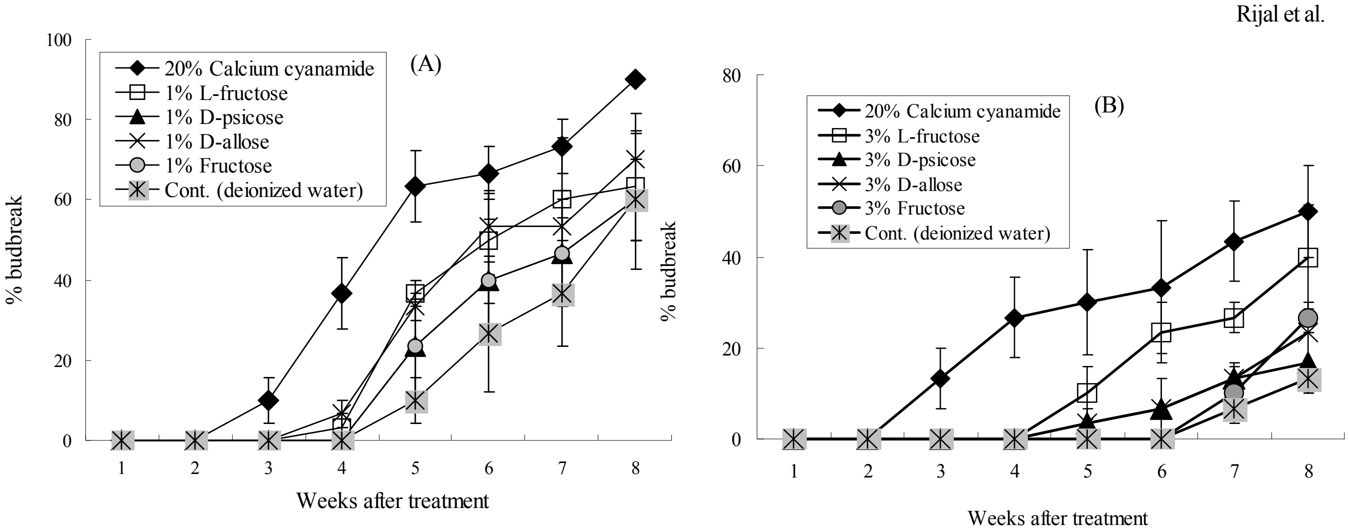

Fig. 1 Budbreak patterns of 'Kyoho' grape cuttings treated with rare sugars and fructose in 2008 (A) and 2009 (B). Data are means of 10 cuttings per treatment replicated three times. Vertical bars represent standard error.

than fructose treatment at the end of the experiment. In 2009, L-fructose treatment led to higher percentage budbreak than control and the percentage budbreak was similar to that of calcium cyanamide treated cuttings (Fig. 1B). D-allose and D-psicose also gave higher percentage budbreak than control at the end of the experiment. L-fructose treatment yielded higher percentage budbreak than fructose treatment. However, the percentage budbreak with D-allose and D-psicose treatment was similar to that with fructose treatment, although budbreak initiation took place earlier than the case of fructose treatment.

Effect of methionine and sea water on 'Kyoho' grape bud dormancy release

In 2008, percentage budbreak with both methionine and sea water treatment was almost similar to that of control and lower than that with calcium cyanamide treatment (Fig. 2A). However, methionine induced earlier budbreak than control. In 2009, percentage budbreak of cuttings treated with methionine was higher than that of control (Fig. 2B). Sea water treatment had little or no effect on percentage budbreak.

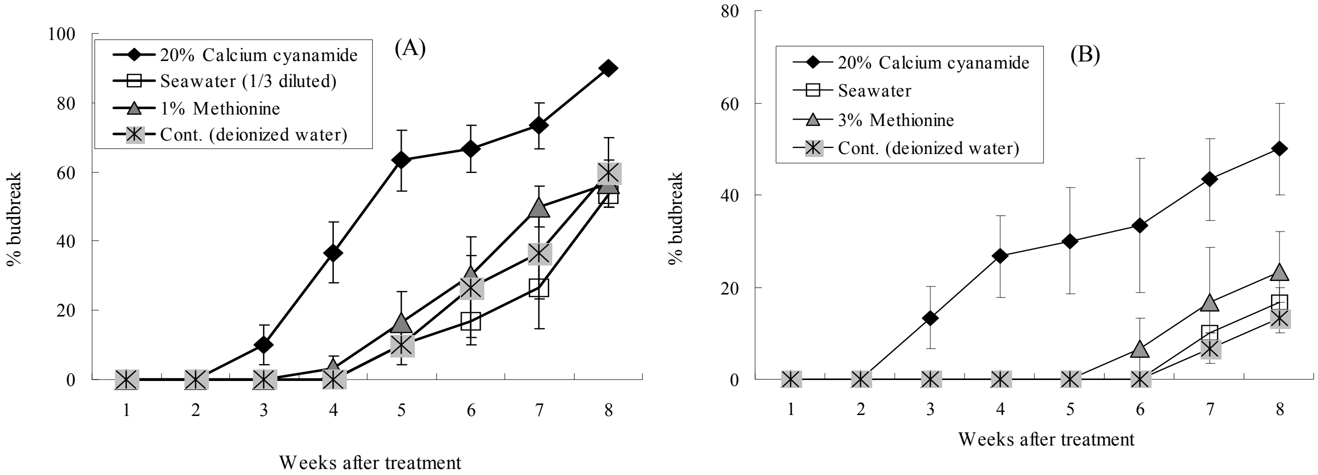

Fig. 2 Budbreak patterns of 'Kyoho' grape cuttings treated with methionine and sea water in 2008 (A) and 2009 (B). Data are means of 10 cuttings per treatment replicated three times. Vertical bars represent standard error.

Discussion

The rare sugars improved the percentage budbreak of 'Kyoho' cuttings compared to control in 2008. Cuttings treated with D-allose had higher percentage budbreak than those treated with the other rare sugars. As rare sugar treatment at 1% concentration in 2008 did not influence budbreak markedly, we increased the concentration of all the rare sugars used in 2009. This improved percentage budbreak relative to control, and the improvement was notable compared to the data for 2008 (Fig. 1 A, B). There is little information of the effects of rare sugars on bud dormancy release of plant species. The results of our experiments in 2009 are in agreement with the findings of Mochioka et al. (2007) who also reported that grape cuttings treated with 3% L-fructose, D-psicose or D-allose had higher percentage budbreak and earlier budbreak initiation than control cuttings.

The results of our experiments in 2009 revealed that the treatment with rare sugars stimulated budbreak of 'Kyoho' cuttings. However, the average percentage budbreak for all the treatments at the end of the experiments was lower than that of the previous year (2008). Daily temperatures in late September to October, 2009 were higher than those in the same period in 2008 (Fig. 3). The higher autumn temperature might have delayed defoliation and caused deep dormancy. Chandler (1960) noted that apple trees with delayed autumn leaf abscission due to higher autumn temperature appeared to have greater chilling requirement for budbreak. Mohamed (2008) reported that late defoliation had led to greater chilling requirement for budbreak in 'Anna' apple. Similarly, Walser et al. (1981) noted that warm field temperature delayed defoliation and extended the rest period in peach. Hence, it is likely that the high temperature in 2009 resulted in the low percentage budbreak compared to that in 2008.

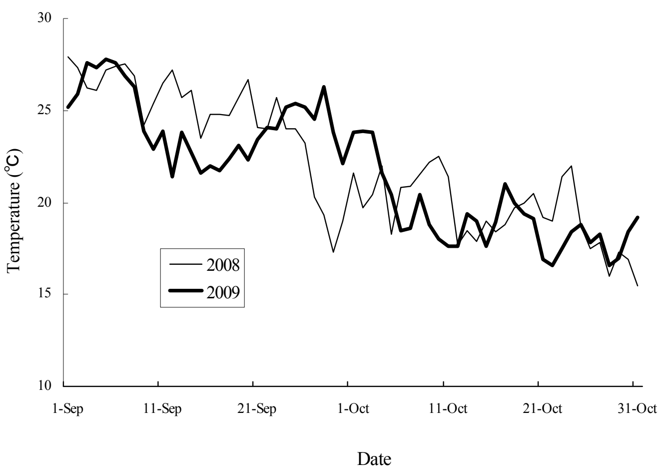

Fig. 3 Changes of average daily temperature from September through October, 2008 and 2009.

The mechanism by which budbreak of 'Kyoho' cuttings is induced by rare sugars is unknown. In general, abscisic acid (ABA) has been implicated with rest in *Vitis* species (Emmerson and Powell 1978). Mochioka et al. (1996) reported that the ABA-like substances in 'Muscat Bailey A' grape increased as dormancy deepened and decreased with dormancy release. Similarly, Poudel (2008) reported a decrease in ABA level as the chilling accumulation increased in some grape cultivars. Hence, it is likely that the rare sugars might have influenced ABA metabolism, which subsequently affected bud dormancy release. Detailed studies are needed to confirm the relationship between ABA and the external application of rare sugars.

Methionine at 1% concentration did not break bud dormancy of 'Kyoho' cuttings. At 3% concentration, it influenced budbreak, but the effect was minimal. Methionine may induce budbreak through the production of ethylene that is considered to affect dormancy release in plant species (Keegan et al.1989). On the other hand, seawater had no effect on bud dormancy break at either of the concentrations used.

The results revealed that rare sugars may be used as budbreak agents for breaking grape bud dormancy. Among the rare sugars tested, 3% L-fructose solution had the greatest budbreak inducing ability. Hence, 3% L-fructose solution shows potential for use as a commercial budbreak agent in the future. Further investigations are needed to elucidate the effects of these rare sugars and determine suitable methods for application and the amounts required for breaking bud dormancy.

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［Research Note］

希少糖、メチオニン、海水がブドウ‘巨峰’の芽の休眠打破に及ぼす影響

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要　約

ブドウ芽の休眠打破のための低温要求はいくつかの薬剤により代替される。それら薬剤のうち、石灰窒素やシアナミドは休眠打破に広く用いられているが、使用条件によっては人体に有害な場合もある。そこで、環境および人体に対する毒性の低い希少糖（L-フルクトース、D-プシコース、D-アロース）、エチレンの前駆物質メチオニン（アミノ酸）、海水がブドウ‘巨峰’の芽の休眠打破に及ぼす影響を調査した。希少糖水溶液は 1%より 3%で休眠打破効果が高かった。その中で、3%L-フルクトース水溶液が休眠打破効果は高かった。一方、3%メチオニン水溶液はわずかに休眠打破効果があったが、海水には休眠打破効果が認められなかった。

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