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Changes in β-Cyanoalanine Synthase Activity and Prunasin Content in Peach Flower Buds during Dormant Period

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Summary

Changes in β-cyanoalanine synthase (CAS) activity and prunasin content in peach buds were investigated during dormant period. Bud weight remained unchanged or only slightly increased before early February but rapidly increased with bud swelling. The β-CAS activity was very low before it began to rise in early December and increased greatly toward bud burst. The content of cyanogenic glucoside, prunasin, tended to decline slightly from middle October to middle December but then increased concomitantly with β-CAS activity. Fructose was consistently lowest among sugars before early December, followed by a gradual increase. Glucose decreased from middle October to middle November and thereafter increased. On the other hand, sucrose started to increase in middle October and peaked in early January and then declined. Sorbitol, the largest constituent among sugars, began to increase sharply in early December to reach a maximum in early February. It seems sugar metabolism operates even in dormant flower buds and that cyanide metabolism begins to be activated long before noticeable swelling of buds.

Introduction

Prunus species are cyanogenic and contain cyanogenic glycosides, prunasin and amygdalin. Prunasin distributes in whole plant parts but amygdalin is localized in seeds at late developmental stages[13,17]. When prunasin of the root residues is broken down in replant sites, its hydrolytic products and derivatives such as hydrogen cyanide, mandelonitrile, benzaldehyde and benzoic acid cause replant injuries although they may not be only causes for the soil sickness[13]. β-CAS is the enzyme which catalyzes the formation β-cyanoalanine from hydrogen cyanide and L-cysteine and plays a role for detoxification of hydrogen cyanide in the tissues[6,8]. Miller and Conn[12] reported that in cyanogenic plants there is a general trend between the activity of β-CAS and HCN potential : the higher the HCN potential, the greater the cyanide metabolizing activity of the enzyme. β-CAS activity increases concomitantly with the increase of amygdalin content in plum seeds at late developmental stages[14]. Furthermore, β-CAS synthase is activated in relation to ethylene production because cyanide is produced

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from the breakdown of ACC to ethylene[11,14,20]. Exogenous ethylene application induces β-CAS activity but hydrogen cyanide not[5]. Here we report the changes in β-CAS activity and prunasin content in peach flower buds in relation to bud dormancy and break.

Materials and Methods

Plants

Flower buds were collected at periodic intervals from four-year-old peach trees(*Prunus persica* Batsch, wild from) growing in the Experimental Farm, College of Agriculture, Ehime University. Samples were immediately used for enzyme assay or freeze-dried for the analysis of prunasin and sugar content.

Assay for β-CAS activity

Fifty flower buds were weighed and homogenized with a cold mortar and pestle which had previously stored in a freezer. The homogenate was taken in 12.5ml cold Tris-HCl buffer(50mM, pH8.5) and 300 ㎎ insoluble PVP was added. After centrifugation at 12,000 x g at 4℃ for 10min, the resultant supernatant was used for enzyme assay. NaCN and L-cysteine were dissolved in 100mM Tris-HCl buffer (pH8.5) to a final concentration of 50 and 100mM, respectively. To the enzyme extract(1.0ml) was added 0.5ml of the buffered NaCN, followed immediately by 0.5ml of the buffered L-cysteine solution. After one-hour incubation at 30℃, the reaction was stopped by the addition of 0.5ml of 0.02M *N*, *N*-dimethyl-*p*-phenylenediamine sulfate in 7.2N HCl and 0.5ml of 0.03M ferric chloride in 0.2N HCl. The samples were then centrifuged at 1,050 x g for 5min to remove precipitated proteins and the absorbance at 650nm was recorded with a Hitachi spectrophotometer(EPU-2). Identical assays lacking substrates and containing boiled enzyme were used as control. Sodium sulfate was used as the standard reference.

Analysis of sugars

Freeze-dried flower buds were ground in a mill and a 50㎎ sample was taken in a 3-ml vial, to which 1 ml pyridine containing 1,3,5-triphenylbenzene(1㎎/ml) as internal standard was added. The vial was subjected to an ultrasonic generator for several times during a 48-hour extraction period at room temperature. A 20μl aliquot of the supernatant of extract was taken in a 1-ml reacti-vial and dried in air. To the vial, 20μl pyridine, 20μl hexamethyldisilasane and 10μl trimethylchlorosilane were added successively and the vial was heated at 60℃ for 30min. A 2.0μl of the reaction mixture was injected into a Hitachi 063 gas chromatograph equipped with FID. Gas chromatographic conditions were as follows : column, 2m×3㎜ ϕ glass column packed with 1.5% SE-30 coated on Chromosorb WAW DMCS(80-100 mesh) ; oven temperature, 125-265℃ at an increment rate of 10℃/min ; injection temperature, 240℃ ; carrier gas, N_2.

Analysis of prunasin

A 50㎎ of powdered sample was taken in a test tube and 1ml of 80% aqueous ethanol was added. The test tube was subjected to the ultrasonic generator for several times during 48-hr extraction period at room temperature. Since no cyanide was released from the precipitated residue fraction at the end of the

extraction period, prunasin in the samples was completely transferred the supernatant phase. A 0.5ml of the supernatant was placed in the outer well of the Conway microdifusion dish and dried in a stream of air. A 1 ml of 0.1N KOH was placed in the center well, then added 2ml of 0.1% β-glucosidase (Sigma, 5.7units/㎎ protein) to the outer well and the dish was immediately sealed. After 5-hr incubation at 30℃, during which period the dish was gently rocked several times, a 0.5ml of the KOH solution was taken and diluted up to 2.0ml with 0.1N KOH. One ml of 0.05% KOH, 0.5ml of 1% phenolphtalin and 1 ml of 0.01% $CuSO_4 \cdot 5H_2O$ were added, and the absorbance at 548nm was recorded with Hitachi spectrophotometer(EPU-2). Sodium cyanide was used as the standard reference.

Results and Discussion

The changes in fresh weight of flower buds from early October to middle March are shown in Fig. 1. No pronounced change in the weight was observed before February but a rapid increase began in early February as the buds swelled.

The β-CAS activity was low from early October to early December. But thereafter it started to rise and continued to increase until bud burst(Fig. 1). The prunasin content decreased slightly from middle October to a minimum level in middle December and thereafter rose in parallel with the increase in the enzyme activity until bud burst(Fig. 1). The changes in the content of total sugars and each component suger are presented in Figs. 2 and 3. Fructose content showed no appreciable changes from early October to middle January but it rapidly increased thereafter. Glucose content declined from middle October to a minimum level in middle November, then gradually increased until middle January followed by a marked increased. In contrast, sucrose showed an increase from middle October to a peak in middle December, then decreased. Sorbitol showed a somewhat different pattern from the other sugars : the content increased from early December to a maximum level in late January, then declined until middle

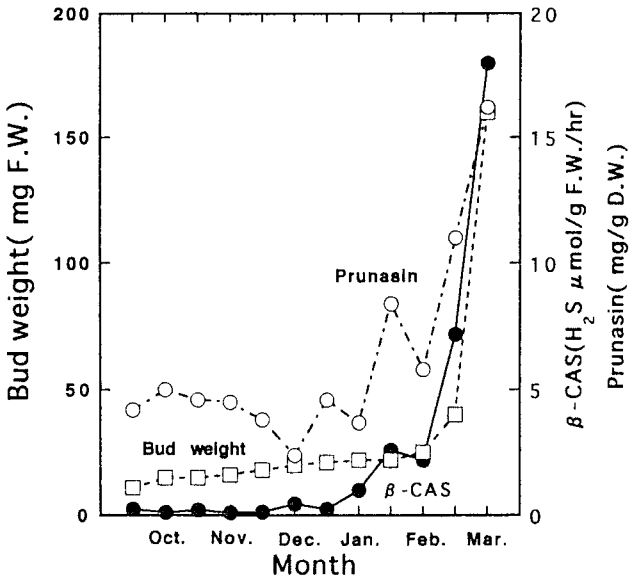

Fig. 1 Changes in bud weight, prunasin content and β-cyanoalanine synthase(CAS) activity in peach flower buds during dormant period.

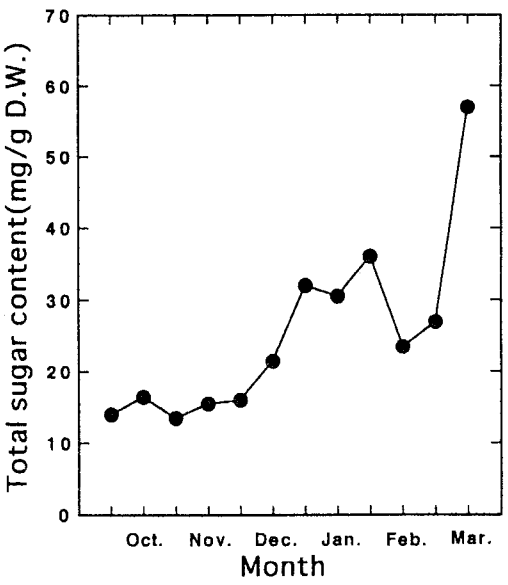

Fig. 2 Changes in total sugar content in peach flewer buds during dormant period.

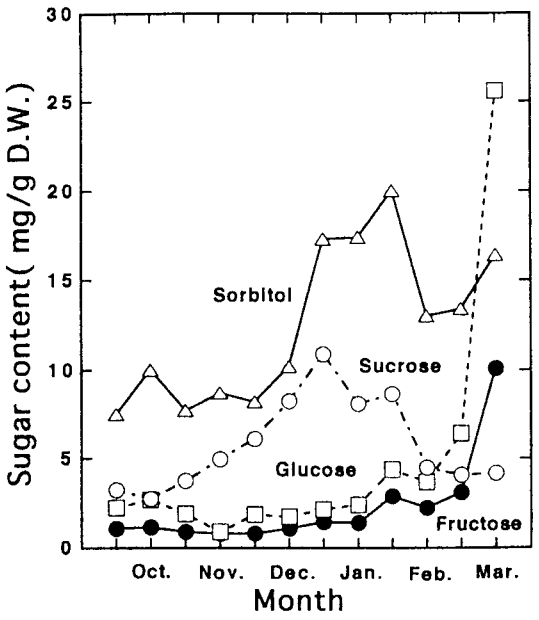

Fig. 3 Changes in sugar content in peach flower buds during dormant period.

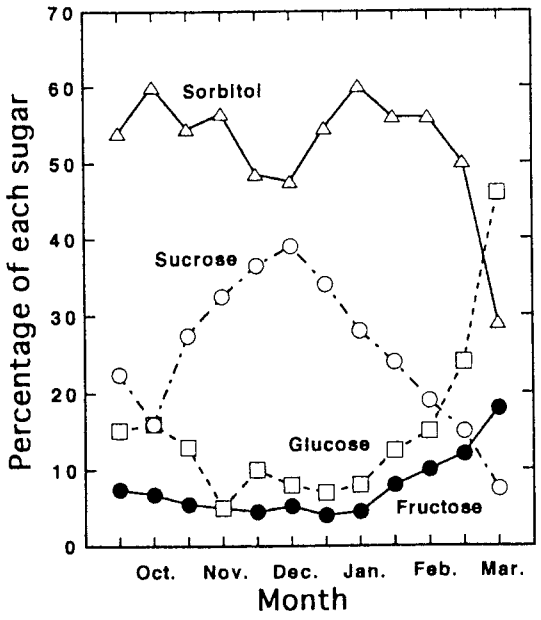

Fig. 4 Changes in percentage of each sugar component in peach flower buds during dormant period.

February and again increased thereafter. Total sugar content showed a trend similar to sorbitol(Fig. 2) This seems because sorbitol constitutes the largest part of total sugars during dormant period(Fig. 4). The percentage of sorbitol fluctuated between 48% and 60% from early October to early March but rapidly declined to 29% in middle March. Percentages of glucose and fructose showed a similar trend although the amplitude was greater in glucose. The percentage of sucrose in early October was only 20% but gradually increased to a peak(40%) in middle December and declined to 8% in middle March. Therefore, it seems that active sugar metabolism operates even during bud dormancy long before morphological bud swelling appears.

Jones[10] reported seasonal trend of cyanide content in peach flower buds in relation to bud dormancy. He found thet total cyanide content in the flower buds decreased from early October to early November, then remained constant until near the end of dormancy. It again rose rapidly before noticeable swelling of the buds. In the present study, seasonal changes in prunasin content was similar to his observation. Fig. 1showed the concomitant changes of β-CAS activity with prunasin content. This indicates that biosynthesis of prunasin and cyanide metabolism are closely related. It is known that prunasin is biosynthesized from phenylalanine via aldoxim and mandelonitrile[1,4,5,9,23]. A parallel increase in prunasin content and β-CAS activity with the release of dormancy indicates that the turnover of prunasin is accelerated in which the synthesis is superior to the degradation. Nahrstedt[19] reported that prunasin content in the pedicel of *Prunus avium* fruit was regulated by IAA. Since bud dormancy is regulated by plant hormones such as ABA, prunasin biosynthesis may also be under hormonal control.

Cyanide is known as an effective dormant-breaking agent for many plant species[7,16,21,24]. It is generally accepted that cyanide is a respiratory inhibitor that interferes an electron transfer through cytochrome oxidases. On the other hand, exogenously applied hydrogen cyanide is effectively incorporated to β-

cyanoalanine, which is further hydrolyzed to asparagine[2,3,12]. However, there is no evidence available that β-CAS is involved in such cyanide-induced bud break in fruit trees. Taylorson and Hendricks[21] reported with *Amaranthus albus* that effectiveness of cyanide in stimulating seed germination is fully indicated as resulting from protein synthesis through β-cyanoalanine as an intermediate. Therefore, the increase in β-CAS activity in peach flower buds may be an indication of amino acid metabolism leading to the active synthesis of proteins(enzymes) required for bud burst.

β-CAS activity is regulated by ethylene, not hydrogen cyanide[18]. It remains unknown whether ethylene is associated with the induction of β-CAS activity in peach flower buds in relation to the release of dormancy.

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休眠期間中のモモの花芽中のβ-シアノアラニン合成酵素活性とプルナシン含量の変化

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摘　要

休眠期間中のモモの花芽のβ-シアノアラニン合成酵素(CAS)活性とプルナシン含量の変化を調査した。モモの花芽の重さは2月まではほとんど変わらないか少し増加しただけで、その後芽が膨らむにつれて増大した。CASの活性は12月初旬まで低く推移し、その後増大し始め萌芽に向かって著しく高くなった。青酸配糖体であるプルナシンは10月中旬から12月中旬にかけて少し含量が減少したが、CAS活性の増大と共に増加した。果糖は12月初旬まで糖のうちで最も含量が低く推移し、その後次第に少し増加した。ブドウ糖は10月中旬から11月中旬にかけて減少し、その後増大した。いっぽうショ糖は10月中旬に増加し始め、1月初旬にピークに達し、その後減少した。糖の内で最も含量が高いソルビトールは12月初旬から急激に増加し始め、2月初旬に最高値に達した。休眠中の花芽でも糖代謝は機能しており、芽の外観的な膨らみが観察されるずっと前から青酸代謝の活性化が行われているように思われた。