Plant Omics Journal POJ

POJ 4(4):215-219 (2011) ISSN:1836-3644

Research Note

Influence of sucrose on rutin content and flavonoid biosynthetic gene expression in seedlings of common buckwheat (*Fagopyrum esculentum* Moench)

Xiaohua Li[1], Nam Il Park[1], Chul Ho Park[2], Su Gwan Kim[3], Sook Young Lee[3*], and Sang Un Park[1*]

[1]Department of Crop Science, College of Agriculture and Life Sciences, Chungnam National University, 79 Daehangno, Yuseong-gu, Daejeon, 305-764, Korea
[2]Department of Bio-Health Technology, College of Biomedical Science, Kangwon National University, Chuncheon, Gangwon 200-701, Korea
[3]Regional Innovation Center for Dental Science & Engineering, Chosun University, 375 Seosuk-Dong, Dong-Gu, Gwangju, 501-759, Korea

***Corresponding Author: koreanseedbank@gmail.com, supark@cnu.ac.kr**

Abstract

Rutin is the main flavonoid compound in common buckwheat (*Fagopyrum esculentum* Moench) and sucrose is a major enhancer of both phenolic production and organ development in plants. In this work, we measured the effect of sucrose on on the growth of buckwheat seedlings. The accumulation of rutin in these seedlings and the expression pattern of the structural genes that are involved in the flavonoid biosynthetic pathway were also investigated. The growth of buckwheat was inhibited when the concentration of sucrose was increased to 50 g/L; however, the expression of most flavonoid biosynthetic genes were increased after 1 or 2 days of treatment and rutin content showed a marked increase when the concentration of sucrose was increased from 10 g/L to 50 g/L.

Keywords: Sucrose, *Fagopyrum esculentum*, flavonoid biosynthetic pathway, rutin, gene expression.
Abbreviations: HPLC, high-performance liquid chromatography; PAL, phenylalanine ammonium lyase; C4H, cinnamic acid 4-hydroxylase; CHI, chalcone isomerase; CHS, chalcone synthase; 4CL, 4-coumarate-CoA ligase; DFR, dihydroflavonol reductase; F3H, flavanone-3-hydroxylase; F3′H, flavonoid-3′-hydroxylase; FLS, flavonol synthase; ANS, anthocyanin synthase.

Introduction

Buckwheat, which belongs to the Polygonaceae family, has received great interest mostly because of its antioxidant components, namely, polyphenols, including rutin, catechins, orientin, vitexin, quercetin, isovitexin, and isoorientin (Kalinova et al., 2006; Oomah and Mazza, 1996; Watanabe, 1998). Buckwheat contains more rutin than most other grain crops, fruits, and vegetables. Rutin has been shown to have many functions, including antioxidative, anti-inflammatory, and anti-hypertensive activities (Afanas'ev et al., 1989; Afanas'eva et al., 2001; Holasova et al., 2002; Matsubara et al., 1985) and has also been shown to act as a protectant against ultraviolet (UV) radiation or diseases in plants (Gaberscik et al., 2002). Flavonoids comprise a large family of low-molecular-weight polyphenolic secondary metabolites that are widespread throughout the plant kingdom, ranging from mosses to angiosperms (Koes et al., 1994). Flavonoids are involved in UV-scavenging, attracting pollinators and seed dispersal, forming pigments in flowers, fruit fertility, and disease resistance (Koes, et al., 1994). Recent evidence suggests that certain flavonoids reduce dental caries and cariogenic bacteria incidence and use as a promising natural agent for noninvasive root caries therapy (Wood, 2007; Wu, 2009). Almost all of the enzymes involved in the biosynthetic pathways of different flavonoid classes have been completely elucidated (Forkmann and Martens, 2001; Schijlen et al., 2004). With respect to buckwheat, we have obtained the basic genes information of the flavonoid biosynthetic pathway in common buckwheat ((Li et al., 2010). In the past decade, attempts to modify flavonoid biosynthesis have been made using different biotic or abiotic methods. The expression of flavonoid biosynthesis regulatory genes appears to be highly dependent on tissue type and/or response to internal or external signals such as hormones, light, microbial elicitors, UV radiation, sugars, phosphate limitation, or cold stress, which affect the signal transduction and gene expression involved in biosynthesis (Dixon and Paiva, 1995; Ferri et al., 2009; Laura et al., 2007; Leyva et al., 1995; Mol et al., 1996; Tsukaya et al., 1991). As an essential factor for plant growth and metabolism, sugars are not only energy sources and structural components, but also are physiologic signals regulating the expression of a variety of genes involved both in primary and secondary metabolism (Koch, 1996). It has been reported that sucrose and other sugars are involved in responses to many biotic and abiotic stresses, cross-talking with hormones (Gazzarrini and McCourt, 2003; Gibson, 2004), and modulating the expression of many genes implicated in photosynthesis, respiration, nitrogen metabolism, and defense processes (Jang et al., 1997). Ohto et al. (2001) reported that anthocyanin production in *Arabidopsis* was enhanced when plants were grown on a sucrose-containing medium (Ohto et al., 2001). Solfanelli et al. (2006) also reported that the induction of anthocyanin synthesis is sucrose-specific, as determined by testing the effects of a set of metabolic sugars and nonmetabolic sugars in *Arabidopsis* seedlings (Solfanelli et al., 2006). Specific polyphenol families such as anthocyanins,

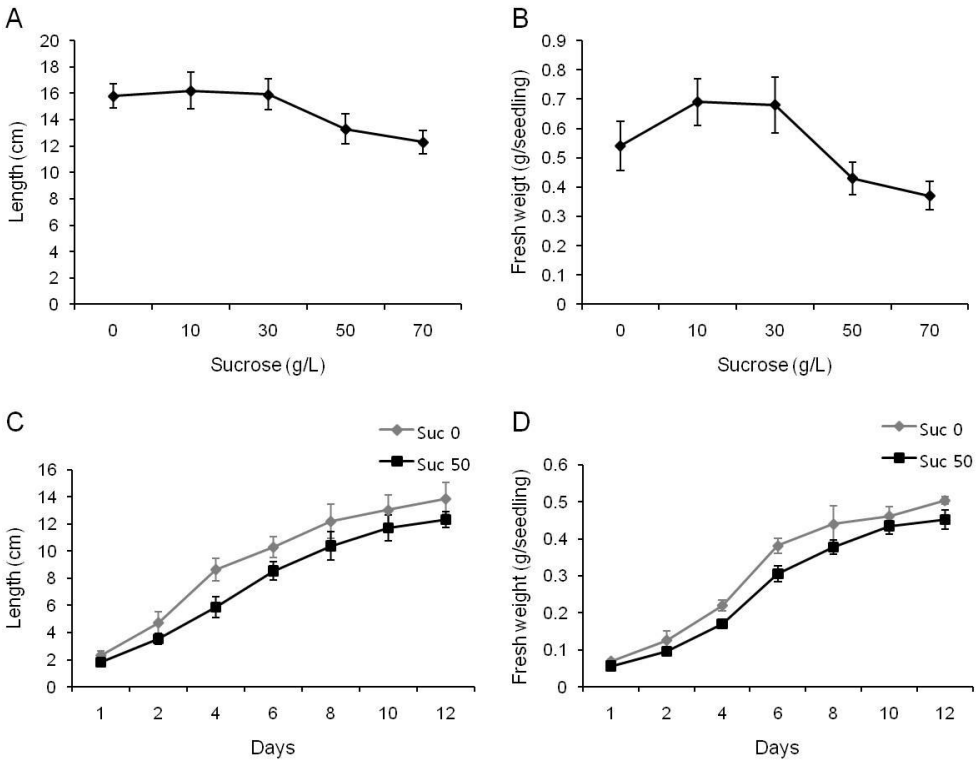

Fig 1. The growth of seedlings in different concentration of sucrose and time-course treatment. (A, B) Effects of different concentrations of sucrose on the length and fresh weight of buckwheat seedlings. The seedlings were treated for 10 days. (C, D) The time-course of 50 g/L sucrose treatment on the length and fresh weight of buckwheat seedlings. Data are presented as the mean ± SD of triplicate experiments.

catechins, and stilbenes in *Vitis vinifera* berries and cell cultures were also enhanced by increasing sucrose concentration (Ferri et al., 2011). Boss et al. reported that the expression of 7 genes involved in anthocyanin biosynthesis was enhanced during the development of berry skins in *V. vinifera* under the influence of sugars (Boss et al., 1996). In this study, Buckwheat (*Fagopyrum esculentum* Moench) seedlings were treated with different sucrose concentrations to investigate the influence and/or induction effect of sucrose on the synthesis of rutin in the culture of buckwheat. We also examined the transcription patterns and levels of a key set of enzymes involved in the flavonoid pathway.

Results and discussion

Effects of sucrose on the growth of buckwheat seedlings

After cultivation with different concentrations of sucrose, the length and fresh weight of buckwheat seedlings were compared. The length and fresh weight of seedlings were slightly increased by low-concentration treatment (less than 30 g/L sucrose), but they were obviously decreased when more concentration (from 50 to 70 g/L) of sucrose were used (Fig. 1 A and B). The length and fresh weight of seedlings treated with 50 g/L sucrose were lower compared to those of control seedlings throughout the time-course of sucrose treatment (Fig. 1 C and D). Previously, it has been reported that high sugar levels inhibited the development seedlings, repressed photosynthetic gene expression, and induced storage metabolism genes (Rook et al., 2006). High concentrations of exogenous sugars also have been shown to delay germination and arrest early growth, preventing seedlings from expanding cotyledons and developing true leaves and an extensive root system (Rognoni et al., 2007). In this study, we showed that high concentrations of sucrose (more than 30 g/L) inhibited the growth of buckwheat seedlings in a dose-dependent manner.

Effects of sucrose on the expression of flavonoid biosynthetic genes in buckwheat seedlings

In order to elucidate the effects of sucrose on the flavonoid biosynthetic pathway, we examined the expression levels of flavonoid biosynthetic genes in seedlings of *F. esculentum*. The expression levels of *FePAL*, *FeC4H*, *Fe4CL1*, *Fe4CL2*, *FeCHS*, *FeCHI*, *FeF3H*, *FeF3'H*, *FeFLS1*, *FeFLS2*, *FeDFR*, and *FeANS* are shown in Fig. 2. Even though all of these genes were expressed throughout the course of treatment, the expression of most genes (*FeC4H*, *FeCHS*, *FeCHI*, *FeF3H*, *FeF3'H*, *FeFLS1*, *FeFLS2*, *FeDFR*, and *FeANS*) was greatest after 1 or 2 days of treatment. *FePAL* was an exception, as the expression of this gene was only slightly increased after 1 day of 50 g/L sucrose treatment and then markedly repressed after 2 days; *FePAL* expression remained repressed during almost all the sucrose time-course treatment compared to control treatment. Even more interesting is the finding that the expression of *FeCHS*, *FeF3H*, *FeFLS1*, *FeFLS2*, and *FeANS*, which are downstream genes of the flavonoid biosynthetic pathway, was greater with sucrose treatment than control treatment during most of the treatment course. Further, sucrose was only associated with up-regulation of the *FeDFR* gene after 1 day treatment, with gene expression not markedly changed during the course of treatment compared to control seedlings. This finding is not consist with a previous report that expression of the *DFR* gene was induced by sucrose in the grape; the reason for this discrepancy may be that expression of the *DFR* gene is also regulated by other factors such as

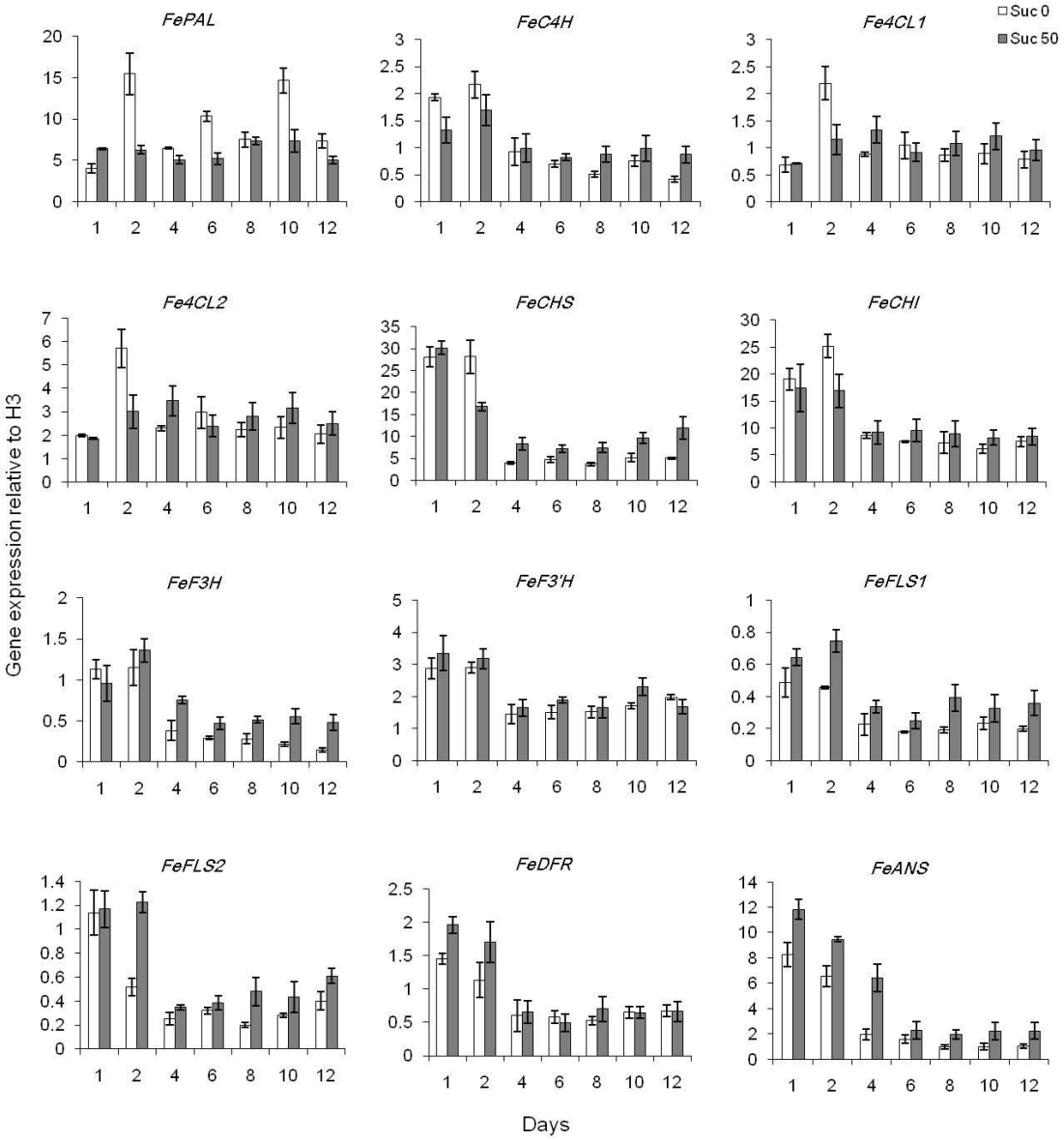

Fig 2. Expression levels of flavonoid biosynthesis genes in *F. esculentum* seedlings treated with 50 g/L sucrose. The expression level of each gene is relative to that of the constitutively expressed histone H3 gene. Each value is the mean of 3 replicate experiments ± SD.

light and developmental mechanisms of the plant (Gollop et al., 2002).

Effects of sucrose on rutin content of buckwheat seedlings

Rutin content of buckwheat seedlings was determined using HPLC (Fig. 3). The results showed that the production of rutin was increased slightly by more concentrations of sucrose (up to 50 g/L), however, it was decreased markedly by 70 g/L sucrose treatment (Fig. 3 A), this result indicated that too high concentration of sucrose was not contribute to rutin biosynthesis in buckwheat seedlings. During the time-course of 50 g/L sucrose treatment, compared to the control, rutin content was markedly increased with seedling age especially in the sucrose-treated seedlings after 8 days treatment (Fig. 3 B). According to our results, suitable sucrose concentration and harvest time are two important factors to affect the nutritional quality of buckwheat sprouts.
Solfanelli et al. (2006) revealed that the flavonoid and anthocyanin biosynthetic pathways are strongly up-regulated following sucrose treatment and that sucrose also affects both flavonoid and anthocyanin content in *Arabidopsis*. In this study, we found that a series of genes, namely, *FeC4H*, *FeCHS*, *FeF3H*, *FeFLS1*, *FeFLS2*, and *FeANS* was up-regulated during sucrose treatment. It has been reported that the transcription factor genes *MYB75/PAP1*and *PAP2/MYB90* play an essential role in the sucrose-induced anthocyanin biosynthesis pathway (Lloyd and Zakhleniuk, 2004; Teng et al., 2005); it is possible that such regulatory genes have a close relationship and contribute to the increase in rutin synthesis we observed in this study. Further work is needed to elucidate the possible relationship between sucrose signal and transcription factors

Materials and methods

Plant materials and culture conditions

Seeds of *F. esculentum* Moench were surface-sterilized with 70% ethanol for 30 s and 4% (*v:v*) bleach solution for 15

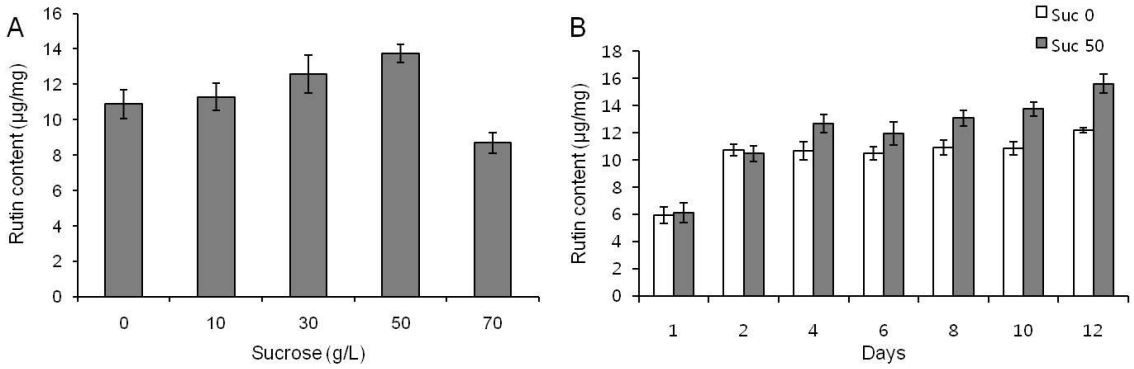

Fig 3. The production of rutin in buckwheat seedlings treated with sucrose. (A) Effects of different concentrations of sucrose on rutin production in buckwheat seedlings (10 days treatment) (B) Time course of 50 g/L sucrose treatment on the rutin production in buckwheat seedlings. Data are represented as the mean ± SD of triplicate experiments.

min, then rinsed several times in sterile water. The seeds were placed on sucrose-free half-strength sterilized Murashige-Skoog (1/2 MS) medium solidified with 0.8% agar. The seeds were germinated at 25°C in a growth chamber with approximately 60% humidity in the light/dark (16/8 h). After 2 days, the germinated seedlings were transferred to solid 1/2 MS medium containing 0, 10, 30, 50, or 70 g/L sucrose. Samples were harvested after 1, 2, 4, 6, 8, 10, and 12 days by cutting off the roots with scissors, rapidly measuring the length and fresh weight, freezing in liquid nitrogen, and storing at -80°C until analysis.

Total RNA extraction and quantification of gene expression

Total RNA was isolated from different *F. esculentum* seedlings using the RNeasy Plant Mini Kit (Qiagen; Valencia, CA, USA). For qRT-PCR, 1 μg of total RNA was reverse-transcribed using the Superscript II First Strand SynthesisKit (Invitrogen; Carlsbad, CA, USA) and an oligo $(dT)_{20}$ primer. Transcription levels were analyzed by real-time PCR. The gene-specific primer sets were designed for real-time PCR, as previously described (Li, et al., 2010). Gene expression was normalized to that of the histone H3 gene as a housekeeping gene (Timotijevic et al., 2010). Real-time PCR reactions were performed in triplicate on a MiniOpticon system (Bio-Rad Laboratories; Hercules, CA) with the Quantitect SYBR Green PCR Kit (Qiagen). The PCR protocol was as follows: denaturation for 5 min at 95°C, followed by 40 cycles of denaturation for 15 s at 95°C, annealing for 15 s at 56°C, and elongation for 20 s at 72°C. PCR results were calculated as the mean of 3 replicated treatments. Statistical differences between treatments were evaluated by standard deviation.

Quantitative analysis of rutin using high-performance liquid chromatography (HPLC)

Rutin concentration in buckwheat samples was determined using a Futecs model NS-4000 HPLC apparatus (Daejeon, Korea) with a UV-Vis detector and auto sampler. Rutin was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Acetic acid was purchased from Jun sei Chenical Co., Ltd. (Kyoto, Japan). For HPLC analysis, the above extraction was filtered through 0.45-μm poly filter and then diluted 2-fold with methanol prior to HPLC analysis. The analysis was monitored at 280 nm and performed using a C18 column (250 mm x 4.6 mm, 5 μm; RStech, Daejeon, Korea); the mobile phase was a gradient prepared from mixtures of 0.15% acetic acid and methanol, and the column was maintained at 30°C.The flow rate was set at 1.0 mL/min. The injection volume was 20 μL. The results were calculated using a standard curve.

Acknowledgments

This study was supported by Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry, and Fisheries, Republic of Korea and in part, this study was supported by the Regional Innovation Center for Dental Science & Egineering, Chosun University, Gwangju, Korea (B0008940).

References

Afanas'ev IB, Dcrozhko AI, Brodskii AV, Kostyuk VA, Potapovitch AI (1989) Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem Pharmacol 38:1763-1769

Afanas'eva IB, Ostrakhovitch EA, Mikhal'chik EV, Ibragimova GA, Korkina LG (2001) Enhancement of antioxidant and anti-inflammatory activities of bioflavonoid rutin by complexation with transition metals. Biochem Pharmacol 61:677-684

Boss PK, Davies C, Robinson SP (1996) Analysis of the expression of anthocyanin pathway genes in developing *Vitis vinifera* L. cv Shirazg grape berries and the implications for pathway regulation. Plant Physiol 111:1059-1066

Dixon R, Paiva N (1995) Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085 - 1097

Ferri M, Righetti L, Tassoni A (2011) Increasing sucrose concentrations promote phenylpropanoid biosynthesis in grapevine cell cultures. J Plant Physiol 168:189-195

Ferri M, Tassoni A, Franceschetti M, Righetti L, Naldrett MJ, Bagni N (2009) Chitosan treatment induces changes of protein expression profile and stilbene distribution in *Vitis vinifera* cell suspensions. Proteomics 9:610-624

Forkmann G, Martens S (2001) Metabolic engineering and applications of flavonoids. Curr Opin Biotech 12:155-160

Gaberscik A, Voncina M, Trost T, Germ M, Björn LO (2002) Growth and production of buckwheat (*Fagopyrum esculentum*) treated with reduced, ambient, and enhanced UV-B radiation. J Photochem Photobiol B: Biol 66:30-36

Gazzarrini S, McCourt P (2003) Cross-talk in plant hormone signalling: what Arabidopsis mutants are telling us. Ann Bot 91:605-612

Gibson SI (2004) Sugar and phytohormone response pathways: navigating a signalling network. J Exp Bot 55:253-264

Gollop R, Even S, Colova-Tsolova V, Perl A (2002) Expression of the grape dihydroflavonol reductase gene and analysis of its promoter region. J Exp Bot 53:1397-1409

Holasova M, Fiedlerova V, Smrcinova H, Orsak M, Lachman J, Vavreinova S (2002) Buckwheat-the source of antioxidant activity in functional foods. Food Res Int 35:207-211

Jang JC, Leon P, Zhou L, Sheen J (1997) Hexokinase as a sugar sensor in higher plants. Plant Cell 9:5-19

Kalinova J, Triska J, Vrchotova N (2006) Distribution of vitamin E, squalene, epicatechin, and rutin in common buckwheat plants (*Fagopyrum esculentum* Moench). J Agric Food Chem 54:5330-5335

Koch KE (1996) Carbohydrate-modulated gene expression in plants. Ann Rev Plant Physiol Plant Mol Biol 47:509-540

Koes RE, Quattrocchio F, Mol JNM (1994) The flavonoid biosynthetic pathway in plants: Function and evolution. BioEssays 16:123-132

Laura R, Franceschetti M, Ferri M, Tassoni A, Bagni N (2007) Resveratrol production in *Vitis vinifera* cell suspensions treated with several elicitors. Caryologia 60:169-171

Leyva A, Jarillo JA, Salinas J, Martinez-Zapater JM (1995) Low temperature induces the accumulation of phenylalanine ammonia-lyase and chalcone synthase mRNAs of *Arabidopsis thaliana* in a light-dependent manner. Plant Physiol 108:39-46

Li X, Park NI, Xu H, Woo S-H, Park CH, Park SU (2010) Differential expression of flavonoid biosynthesis genes and accumulation of phenolic compounds in common buckwheat (*Fagopyrum esculentum*). J Agric Food Chem 58:12176-12181

Lloyd JC, Zakhleniuk OV (2004) Responses of primary and secondary metabolism to sugar accumulation revealed by microarray expression analysis of the *Arabidopsis* mutant, *pho3*. J Exp Bot 55:1221-1230

Matsubara Y, Kumamoto H, Iizuka Y, Murakami T, Okamoto K, Miyake H, Yokoi K (1985) Structure and hypotensive effect of flavonoid glycosides in *Citrus unshiu* peelings. Agric Biol Chem 49:909-914

Mol J, Jenkins G, Schaefer E, Weiss D, Walbot V (1996) Signal perception, transduction, and gene expression involved in anthocyanin biosynthesis. Crit Rev Plant Sci 15:525-557

Ohto M-a, Onai K, Furukawa Y, Aoki E, Araki T, Nakamura K (2001) Effects of sugar on vegetative development and floral transition in Arabidopsis. Plant Physiol 127:252-261

Oomah BD, Mazza G (1996) Flavonoids and antioxidative activities in buckwheat. J Agric Food Chem 44:1746-1750

Rognoni S, Teng S, Arru L, Smeekens S, Perata P (2007) Sugar effects on early seedling development in Arabidopsis. Plant Growth Regul 52:217-228

Rook F, Hadingham SA, Li Y, Bevan MW (2006) Sugar and ABA response pathways and the control of gene expression. Plant Cell Environ 29:426-434

Schijlen EGWM, Ric de Vos CH, van Tunen AJ, Bovy AG (2004) Modification of flavonoid biosynthesis in crop plants. Phytochemistry 65:2631-2648

Solfanelli C, Poggi A, Loreti E, Alpi A, Perata P (2006) Sucrose-specific induction of the anthocyanin biosynthetic pathway in Arabidopsis. Plant Physiol 140:637-646

Teng S, Keurentjes J, Bentsink L, Koornneef M, Smeekens S (2005) Sucrose-specific induction of anthocyanin biosynthesis in Arabidopsis requires the MYB75/PAP1 gene. Plant Physiol 139:1840-1852

Timotijevic GS, Milisavljevic MD, Radovic SR, Konstantinovic MM, Maksimovic VR (2010) Ubiquitous aspartic proteinase as an actor in the stress response in buckwheat. J Plant Physiol 167:61-68

Tsukaya H, Ohshima T, Naito S, Chino M, Komeda Y (1991) Sugar-dependent expression of the CHS-A gene for chalcone synthase from petunia in transgenic Arabidopsis. Plant Physiol 97:1414-1421

Watanabe M (1998) Catechins as antioxidants from buckwheat (*Fagopyrum esculentum* Moench) groats. J Agric Food Chem 46:839-845

Wood N (2007) The effects of selected dietary bioflavonoid supplementation on dental caries in young rats fed a high-sucrose diet. J Med Food 10:694-701

Wu CD (2009) Grape Products and Oral Health. J Nutr 139:1818S-1823S