**RESEARCH ARTICLE**

*Arachis hypogaea* resveratrol synthase 3 alters the expression pattern of UDP-glycosyltransferase genes in developing rice seeds

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**Abstract**

The resveratrol-producing rice (*Oryza sativa* L.) inbred lines, Iksan 515 (I.515) and Iksan 526 (I.526), developed by the expression of the groundnut (*Arachis hypogaea*) resveratrol synthase 3 (AhRS3) gene in the *japonica* rice cultivar Dongjin, accumulated both resveratrol and its glucoside, piceid, in seeds. Here, we investigated the effect of the AhRS3 transgene on the expression of endogenous piceid biosynthesis genes (UGTs) in the developing seeds of the resveratrol-producing rice inbred lines. Ultra-performance liquid chromatography (UPLC) analysis revealed that I.526 accumulates significantly higher resveratrol and piceid in seeds than those in I.515 seeds and, in I.526 seeds, the biosynthesis of resveratrol and piceid reached peak levels at 41 days after heading (DAH) and 20 DAH, respectively. Furthermore, RNA-seq analysis showed that the expression patterns of UGT genes differed significantly between the 20 DAH seeds of I.526 and those of Dongjin. Quantitative real-time PCR (RT-qPCR) analyses confirmed the data from RNA-seq analysis in seeds of Dongjin, I.515 and I.526, respectively, at 9 DAH, and in seeds of Dongjin and I.526, respectively, at 20 DAH. A total of 245 UGTs, classified into 31 UGT families, showed differential expression between Dongjin and I.526 seeds at 20 DAH. Of these, 43 UGTs showed more than 2-fold higher expression in I.526 seeds than in Dongjin seeds. In addition, the expression of resveratrol biosynthesis genes (*PAL, C4H* and *4CL*) was also differentially expressed between Dongjin and I.526 developing seeds. Collectively, these data suggest that AhRS3 altered the expression pattern of UGT genes, and *PAL, C4H* and *4CL* in developing rice seeds.

**Introduction**

Resveratrol (3,5,4'-trihydroxystilbene), a stilbene compound, occurs in many plant species including grape (*Vitis vinifera*), groundnut (*Arachis hypogaea*), *Eucalyptus* spp., Texas fescue...
(Festuca versuta) and Japanese knotweed (Polygonon cuspidatum) [1–8]. The glucoside of resveratrol, piceid (3,5,4′-tri-hydroxystilbene-3-β-monoglucoside), has also been detected in several plant species, either with or without resveratrol, such as Eucalyptus spp., grape, Picea spp. and P. cuspidatum [3,5,6,9–13]. Both resveratrol and piceid play various physiological roles in animals [14–20]. Resveratrol exhibits antineoplastic, cardioprotective and antioxidant properties in activated blood platelets [14–16] and inhibits low-density lipoprotein (LDL) peroxidation [17]. Studies in mice and rats show that piceid is involved in the improvement of renal ischemia/reperfusion injury, neuroprotection, and cardiomyocyte protection [18–20]. In planta metabolic engineering of the resveratrol synthase (RS), a gene encoding stilbene synthase that catalyzes the biosynthesis of resveratrol from one molecule of p-coumaroyl-CoA and three molecules of malonyl-CoA [21–25], has been conducted in many plant species to develop cultivars with high resveratrol levels for human health benefits [26–30].

Iksan 515 (I.515) and Iksan 526 (I.526) are two resveratrol-producing inbred lines of rice (Oryza sativa L. subsp. japonica cv. Dongjin) carrying the groundnut resveratrol synthase 3 (AhRS3; DQ124938) gene [29,31,32]. These inbred lines were generated by Dr. Baek’s research team at the National Institute of Crop Science (NICS), Republic of Korea, using the Agrobacterium-mediated transformation method [29,31,32]. Both resveratrol and piceid were detected in the leaves and seeds of I.526 [29,32], although the quantity of piceid in leaves was significantly higher than that in seeds. Like I.526, tomato (Lycopersicon esculentum) fruits of transgenic lines overexpressing the grape stilbene synthase gene produced both resveratrol and piceid [26,28]. Interestingly, only piceid was detected in the transgenic lines of other plant species, such as alfalfa (Medicago sativa), apple (Malus domestica), poplar (Populus alba), Brassica napus and Arabidopsis thaliana, overexpressing the stilbene synthase gene of grape or the RS gene of groundnut or P. cuspidatum [33–37].

A schematic biosynthetic pathway of the resveratrol and piceid in the developing seeds of I.526 is shown in Fig 1. In this pathway, phenylalanine is converted to p-coumaroyl-CoA in a three-step process by the action of phenylalanine ammonia-lyase (PAL) [38], cinnamate 4-hydroxylase (C4H) [39] and 4-coumarate:CoA ligase (4CL) [40]. Then, p-coumaroyl-CoA is converted to resveratrol by AhRS3 upon the addition of malonyl-CoA [24,29]. Piceid is readily generated from resveratrol by the action of UDP-glycosyltransferase(s) (UGTs). This is consistent with previous reports on the metabolic engineering of resveratrol in other plant species [26,28,33–37]; however, the role of UGT genes in resveratrol biosynthesis has not been reported previously, except for the bi-functional resveratrol/hydroxycinnamic acid glucosyltransferase gene reported in Concord grape (Vitis labrusca) [41] (Fig 1).

In this study, we investigated the number of UGT genes whose expression levels were affected by the introduction of AhRS3, with the aim to identify candidate UGT gene(s) involved in piceid biosynthesis. In addition, we examined whether the expression patterns of genes acting upstream of AhRS3, including PAL, C4H and 4CL, were altered by the introduction of AhRS3 in the developing seeds of I.526.

Materials and methods

Growth of resveratrol-producing transgenic rice lines

To develop resveratrol-producing transgenic rice lines, AhRS3 (DQ124938) was cloned from groundnut (A. hypogaea) and inserted into pSB2220 vector carrying the maize Ubi1 promoter for overexpression of a target gene [29]. Agrobacterium tumefaciens strain LBA4404 carrying pSB2220 vector with AhRS3, was cocultivated with calli derived from seeds of Dongjin, a japonica rice cultivar (O. sativa L. subsp. japonica cv. Dongjin), to generate transgenic calli. Transgenic rice plants were regenerated from transgenic calli selected. These works were performed by Dr. Baek’s research team at the NICS, Republic of Korea [29].
Seedlings of wild-type rice (*Oryza sativa* subsp. *japonica* cv. Dongjin) and inbred lines of its transgenic counterpart, I.515 and I.526, were transplanted in the genetically modified organism (GMO) greenhouse (30 cm × 15 cm) of the National Institute of Crop Science (NICS), Republic of Korea, using the standard rice cultivation method of the NICS. A completely randomized design (CRD) was used, with three replications. Developing seeds were harvested from one panicle per plant (total four plants per replication) at 9 days after heading (DAH).

The seedlings of wild-type rice and I.526 were transplanted in the GMO paddy field (30 cm × 15 cm) of the NICS, Republic of Korea, using the standard rice cultivation method of the NICS. As mentioned above, a CRD was used, with three replications. Developing seeds were harvested from one panicle per plant (total four plants per replication) at 6, 13, 20, 31 and 41 days after heading (DAH).

**Quantification of resveratrol and piceid by UPLC**

Seed extracts were prepared from each sample, as described previously [29], and 1-μl aliquots of each sample were analyzed using the ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) with ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm; Waters Corporation, Milford, MA, USA). Resveratrol was analyzed over a total run time of 30 min using acetonitrile (solvent A) and water (solvent B) as the mobile phase. The column was initially equilibrated with 10% A and 90% B. The ratio of A:B was gradually changed to 25:75 by 20 min from the initial time with curve7 and then to 100:0 (curve6) by 21 min. The A:B ratio was...
kept at 100:0 by 25 min with curve6, changed to 10:90 (curve6) by 26 min and then kept at 10:90 by 30 min with curve6. To analyze piceid, the column was initially equilibrated with 10% A and 90% B. The A:B ratio was gradually changed to 50:50 with curve9 by 20 min and then transformed to 100:0 with curve6 by 21 min. The UPLC analytical method for piceid from 21–30 min was same as that used for resveratrol, as described above. Both resveratrol and piceid were detected at 308 nm using the ACQUITY UPLC Tunable UV detector (Waters Corporation, Milford, MA, USA), and the flow rate was maintained at 0.2 ml/min.

Resveratrol and piceid standards were purchased from Sigma-Aldrich (Saint Louis, MO, USA). The UPLC solvents, water and acetonitrile were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Total RNA extraction and RNA-seq
Total RNA was extracted from frozen and milled samples of developing seeds, including 9 DAH seeds of Dongjin, I.515 and I.526, and 6, 13, 20, 31 and 41 DAH seeds of Dongjin and I.526, using the RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer’s instructions. The total RNA samples of Dongjin and I.526 collected at 20 DAH seeds were sent to Macrogen, Inc. (Seoul, Republic of Korea) for RNA-seq using the Illumina technology.

Raw sequence reads were cleaned by removing low-quality nucleotides (Phred score < 20) and short sequence reads (read length < 20 nt). The cleaned reads were mapped onto the Nipponbare reference genome sequence retrieved from the Rice Genome Annotation Project database (http://rice.plantbiology.msu.edu, version 7.0) using the HISAT2 software with default parameters [42]. The featureCounts software [43] was used to quantify the raw read counts from the BAM file, and normalized read counts were calculated by dividing the read counts of all genes with those of the OsUBI1 gene. Raw data for RNA-seq are available at https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-9695.

Gene expression analysis by RT-qPCR
The isolated total RNA was quantified by NanoVue Plus (GE Healthcare Life Sciences, Chicago, IL, USA), and cDNA was synthesized from 1 μg of total RNA using the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). The primer sets used in the quantitative real time-polymerase chain reaction (RT-qPCR) are shown in S1 and S2 Tables. The rice ACTIN1 gene (OsACT1; LOC_Os03g50885) was chosen as a reference (S1 Table). The RT-qPCR was carried out on the CFX96™ Real-Time Detection System (Bio-Rad, Hercules, CA, USA) using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA). The relative expression of AhRS3, UGT genes, and genes acting upstream of AhRS3, including PAL, C4H and 4CL, was determined using the Pfaffl method [44].

Statistical analysis
All statistical analyses were performed using SAS 9.4 TS Level 1M5 (64-bit; SAS Institute Inc., Cary, NC, USA).

Results
Resveratrol and piceid biosynthesis in the developing seeds of resveratrol-producing rice inbred lines, Iksan 515 and Iksan526
The expression of AhRS3 was confirmed in seeds of both I.515 and I.526 at 9 DAH, and the expression level of AhRS3 was higher in 9 DAH seeds of I.515 than that in I.516 (Fig 2A).
However, significantly higher quantities of resveratrol and piceid, respectively, were detected in 9 DAH seeds of I.526, compared to those of I.515 (Fig 2B–2D). Based on biosynthesis of resveratrol and piceid in developing seeds, I.526 was chosen for paddy field experiments to investigate biosynthesis of resveratrol and piceid, and gene expression analysis.

The expression level of \textit{AhRS3} in I.526 seeds increased with development, reaching a peak at 41 days after heading (DAH) (Fig 3A). Ultra-performance liquid chromatography (UPLC; Waters Corp., Milford, MA, USA) analysis revealed that the biosynthesis of resveratrol and piceid in I.526 seeds increased initially and then decreased with development (Fig 3B–3E); however, both resveratrol and piceid reached maximal biosynthesis at different time points (41 and 20 DAH, respectively) (Fig 3B and 3C).

In addition, the expression of genes acting upstream of \textit{AhRS3} in the resveratrol biosynthesis pathway was investigated by quantitative real-time PCR (RT-qPCR) analysis (S1 Fig). The relative expression levels of \textit{PAL}, \textit{C4H} and \textit{4CL} were altered in developing seeds of I.526 compared with those in Dongjin. In I.526 seeds, all genes tested in this study, including \textit{PAL}, \textit{C4H} and \textit{4CL}, showed typical expression patterns with significantly higher expression at 20, 31 and 41 DAH than those in Dongjin (S1 Fig).
RNA-seq analysis of Dongjin and I.526 seeds

To analyze the transcriptome of the developing seeds of Dongjin and I.526, we performed RNA-seq analysis. Because UPLC analysis revealed that the glycosylation of resveratrol in I.526 seeds was the highest at 20 DAH (Fig 3C), we chose this time point for RNA-seq analysis. Raw sequence data of Dongjin and I.526 were mapped to the Nipponbare reference genome, and read counts of each gene were normalized relative to those of the rice *UBIQUITIN 1* (*OsUBI1*; LOC_Os03g13170) gene. A total of 245 UGT genes were selected from the normalized data (S2 Fig and S3 Table). Functional annotation of these genes using the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu) and the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/) databases revealed their putative roles in the glycosylation of several secondary metabolites, including cytokinin, anthocyanidin, indole-3-acetate, flavonoids, hydroquinone, *cis*-zeatin, limonoid, betanidin, *cyclo*-DOPA and *N*-hydroxythioamide. In I.526 seeds at 20 DAH, 43 out of 245 UGT genes were upregulated by more than 2-fold compared with Dongjin seeds, whereas 95 UGT genes showed extremely low expression (S2 Fig and S3 Table).
To verify the RNA-seq data, 15 out of 43 UGT genes upregulated in I.526 seeds were selected for RT-qPCR analysis (S2 and S3 Tables). The expression of all 15 UGT genes were first investigated by RT-qPCR analysis in seeds of Dongjin, I.515 and I.526 at 9 DAH, thereby resulting in consistency with the RNA-seq data, except for LOC_Os03g55010 (Fig 4). In seeds of Dongjin and I.526 at 20 DAH, the results of RT-qPCR analysis of all 15 UGT genes were consistent with the RNA-seq data (Fig 5). Taken together, these results indicate that our RNA-seq data were highly reliable (Figs 4 and 5).

**Classification of the differentially expressed UGT genes**

Of the 245 differentially expressed UGTs, 186 genes encoded proteins containing more than 300 amino acids. The nomenclature of these 186 UGTs was determined by the UDP-glycosyltransferase (UGT) Nomenclature Committee (https://prime.vetmed.wsu.edu/resources/udp-glucuronosyltransferase-homepage) (S3 Table). Based on the nomenclature of these 186 UGTs, the public data obtained from the UGT Nomenclature Committee website and data retrieved from the NCBI, phylogenetic analysis of 245 UGTs was carried out using ClustalX 2.1 [45] and MEGA X [46] (Fig 6 and S3 Table). Of the 245 UGTs, 195 were classified into 31 families,
while 50 remained unclassified. UGTs encoded by 43 UGTs, which showed >2-fold higher expression in I.526 seeds than in Dongjin seeds, were uniformly distributed among approximately 60% of the UGT families, including UGT72, UGT74, UGT75, UGT77, UGT79, UGT83, UGT84, UGT85, UGT90, UGT91, UGT93, UGT96, UGT97, UGT99, UGT703, UGT706, UGT707, UGT708, UGT709 and UGT710 (Fig 6 and S3 Table).

Discussion

The AhRS3 [21,24] gene, a missing link in the resveratrol biosynthesis pathway in rice, was successfully transformed into rice, resulting in the production of resveratrol [29] via the phenylpropanoid pathway, which produces p-coumaroyl CoA, a precursor of resveratrol [47] (Fig 1). In this study, RT-qPCR and UPLC analyses showed that AhRS3 expression and resveratrol and piceid biosynthesis, respectively, increased in I.526 seeds with maturation, indicating that the biosynthesis of resveratrol is closely related to seed development (Fig 3). However, the expression of AhRS3 and quantity of piceid in I.526 seeds were the highest at 41 and 20 DAH, respectively (Fig 3). This molecular phenotype might be related to expression patterns of UGTs (data not known) and availability of UDP-glucose in developing rice seeds [48]. Moreover, in seeds of black rice cultivars, the maximal biosynthesis
of anthocyanins including cyanidin 3-glucoside and peonidin 3-glucoside was detected at 20 or 26 DAH (Lee et al., unpublished), consistent with the biosynthesis of piceid in developing seeds of I.526 (Fig 3).
In previous studies conducted on the metabolic engineering of resveratrol in tomato, piceid was detected along with resveratrol [26,28]; however, in genetically engineered plants of alfalfa, Arabidopsis, apple, poplar and B. napus, only piceid was detected [33]. This difference is probably related to endogenous UGTs, which are involved in the modification of secondary metabolites (to improve their stability and water solubility), inactivation and detoxification of xenobiotics, and regulation of hormones, including auxin, abscisic acid, cytokinins, brassinosteroids and salicylic acid [49].

Genes acting upstream of AhRS3, including PAL, C4H and 4CL, were upregulated in the developing seeds of 1.526 (S1 Fig). The upregulation of genes acting upstream or downstream of the target gene has been previously reported in the metabolic engineering of secondary metabolites in planta [50–54]. In addition, 245 UGTs showed differences in expression levels between 1.526 and Dongjin seeds at 20 DAH (Fig 6 and S3 Table). All of these 245 UGTs were grouped into 31 UGT families. Of these, 43 UGT genes upregulated in 1.256 seeds compared with Dongjin seeds (FC > 2) were classified into 20 UGT families, including UGT72, UGT74, UGT75, UGT77, UGT79, UGT83, UGT84, UGT85, UGT90, UGT91, UGT93, UGT96, UGT97, UGT99, UGT703, UGT706, UGT707, UGT708, UGT709 and UGT710 (Fig 6 and S3 Table). The biological roles of several UGTs have been revealed in planta (reviewed in Paquette et al. [55], Bowles et al. [56] and Bock [57]). For example, UGTs belonging to UGT71 [58], UGT74 [58], UGT76 [59], UGT78 [60], UGT79 [61], UGT84 [62], UGT88 [63], UGT90 [64], UGT95 [64], UGT707 [65] and UGT708 [66] families are involved in the biosynthesis of flavonoids. However, of all the UGTs reported to date, only one UGT gene (DQ832169) belonging to V. labrusca has been identified and characterized; this gene, which encodes resveratrol 3-O-glucosyltransferase, has been classified under the UGT84 family based on the UDP-glycosyltransferase Nomenclature Committee Website (https://prime.vetmed.wsu.edu/resources/udp-glycosyltransferase-homepage) [41]. Furthermore, four differentially expressed UGT genes (LOC_01g49240, LOC_Os01g49230, LOC_Os02g09510 and LOC_Os05g47950) were classified under the UGT84 family, one of which (LOC_Os01g49240) showed 2-fold higher expression in 1.526 seeds than in Dongjin seeds (Fig 6 and S3 Table). However, the biological functions of these UGTs remain unknown. Therefore, biochemical and genetic analyses are needed to characterize the biological roles of these UGTs in planta and to determine whether other UGTs upregulated in 1.526 seeds are involved in the glycosylation of resveratrol.

Overall, we showed that in the developing seeds of 1.526, the time points of maximal biosynthesis of resveratrol and piceid were distinct, and AhRS3 altered the expression pattern of PAL, C4H, 4CL, and UGTs, resulting in the biosynthesis of resveratrol and piceid. Based on the expression data of target genes obtained by RNA-seq and RT-qPCR, we propose a model showing the positive regulation of upstream and downstream genes by AhRS3 (S3 Fig).

Supporting information

S1 Fig. Relative expression of PAL, C4H and 4CL in developing seeds of Dongjin and 1.526 analyzed by RT-qPCR. The expression of all genes was normalized relative to that of OsACT1. Data represent mean ± standard deviation (SD). Asterisks indicate significant differences (*: 0.01 < p < 0.05; **: 0.001 < p < 0.01; ***: p < 0.001). ■: Dongjin, ●: 1.526. N.A.: not applicable.

(TIF)

S2 Fig. Identification of UGT genes differentially expressed between Dongjin and 1.526 seeds by RNA-seq.

(TIF)
S3 Fig. Model showing AhRS3-mediated positive regulation of upstream genes (PAL, C4H and 4CL) and downstream genes (UGTs).

(TIF)

S1 Table. Primer sets for quantitative real time PCR (RT-qPCR).

(DOCX)

S2 Table. List of primers of UGTs used for RT-qPCR.

(DOCX)

S3 Table. List of 245 UGTs differentially expressed between Dongjin and I.526 seeds at 20 DAH.

(XLSX)

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References

1. Langcake P, Pryce RJ. The production of resveratrol by Vitis vinifera and other members of the Vitaceae as a response to infection or injury. Physiological Plant Pathology. 1976; 9(1):77–86. https://doi.org/10.1016/0048-4059(76)90077-1

2. Ingham JL. 3,5,4’-trihydroxystilbene as a phytoalexin from groundnuts (Arachis hypogaea). Phytochemistry. 1976; 15(11):1791–3. https://doi.org/10.1016/S0031-9422(00)97494-6

3. Hillis WE, Carle A. The origin of the wood and bark polyphenols of Eucalyptus species. Biochemical Journal. 1962; 82(3):435–9. https://doi.org/10.1042/bj0820435 PMID: 13907456
4. Powell RG, TePaske MR, Plattner RD, White JF, Clement SL. Isolation of resveratrol from Festuca versuta and evidence for the widespread occurrence of this stilbene in the poaceae. Phytochemistry. 1994; 35(2):335–8. https://doi.org/10.1016/S0031-9422(00)94759-9

5. Kiselev KV, Grigorchuk VP, Ogneva ZV, Suprun AR, Dubrovin AS. Stilbene biosynthesis in the needles of spruce Picea jezoensis. Phytochemistry. 2016; 131:57–67. https://doi.org/10.1016/j.phytochem.2016.08.011 PMID: 27576046

6. Chen L, Han Y, Yang F, Zhang T. High-speed counter-current chromatography separation and purification of resveratrol and piceid from Polygonum cuspidatum. Journal of Chromatography A. 2001; 907 (1):343–6. https://doi.org/10.1016/s0021-9673(00)00960-2 PMID: 11217042

7. Jeandet P, Sobarzo-Sánchez E, Sanches-Silva A, Clément C, Nabavi SF, Battino M, et al. Whole-cell biocatalytic, enzymatic and green chemistry methods for the production of resveratrol and its derivatives. Biotechnology Advances. 2020; 39:107461. https://doi.org/10.1016/j.biotechadv.2019.107461 PMID: 31678221

8. Jeandet P, Vannozzi A, Sobarzo-Sánchez E, Uddin MS, Bru R, Martinez-Marquez A, et al. Phytostilbenes as agrochemicals: biosynthesis, bioactivity, metabolic engineering and biotechnology. Natural Product Reports. 2021. https://doi.org/10.1039/D0NP00030B PMID: 33351014

9. Hillis WE. Polyphenols in the leaves of eucalyptus hirt: A chemotaxonomic survey—I. Phytochemistry. 1966; 5(6):1075–90. https://doi.org/10.1016/S0031-9422(00)86101-4

10. Romero-Pérez AI, Lamuela-Raventós RM, Andrés-Lacueva C, de la Torre-Boronnat MC. Method for the Quantitative Extraction of Resveratrol and Piceid Isomers in Grape Berry Skins. Effect of Powdery Mildew on the Stilbene Content. Journal of Agricultural and Food Chemistry. 2001; 49(1):210–5. https://doi.org/10.1021/jf000745o PMID: 11170579

11. Duan D, Halter D, Baltenweck R, Tisch C, Tröster V, Kortekamp A, et al. Genetic diversity of stilbene biosynthesis in the needles of Spruce Picea abies. Plant Science. 2016; 131:57–67. https://doi.org/10.1016/j.phytochem.2016.08.011 PMID: 27576046

12. Wang L, Xu M, Liu C, Wang J, Xi H, Wu B, et al. Resveratrols in Grape Berry Skins and Leaves in Vitis. Journal of Agricultural and Food Chemistry. 2013; 61(4):161–9. https://doi.org/10.1021/jf000745o PMID: 23637874

13. Jyske T, Kuroda K, Suuronen J-P, Pranovich A, Roig-Juán S, Aoki D, et al. In Plant Localization of Stilbenes within Picea abies Phloem. Plant Physiology. 2016; 172(2):913–28. https://doi.org/10.1104/pp.16.00990 PMID: 27531441

14. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CWW, et al. Cancer Chemopreventive Activity of Resveratrol, a Natural Product Derived from Grapes. Science. 1997; 275(5297):218–20. https://doi.org/10.1126/science.275.5297.218 PMID: 8985016

15. Hung L-M, Chen J-K, Huang S-S, Lee R-S, Su M-J. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. Cardiovascular Research. 2000; 47(3):549–55. https://doi.org/10.1016/s0008-6363(00)00102-4 PMID: 10963727

16. Olas B, Wachowicz B, Holmsen H, Fukami MH. Resveratrol inhibits polyphosphoinositide metabolism in activated platelets. Biochimica et Biophysica Acta (BBA)—Biomembranes. 2005; 1714(2):585–90. https://doi.org/10.1016/j.bbamem.2005.06.008 PMID: 16051184

17. Serafini M, Laranjinha JAN, Almeida LM, Maiani G. Inhibition of human LDL lipid peroxidation by phenol-rich beverages and their impact on plasma total antioxidant capacity in humans. The Journal of Nutrition and Metabolism. 2000; 11:585–90. https://doi.org/10.1016/s0955-2863(00)00124-8 PMID: 11137897

18. Meng Q-H, Liu H-B, Wang J-B. Polydatin ameliorates renal ischemia/reperfusion injury by decreasing apoptosis and oxidative stress through activating sonic hedgehog signaling pathway. Food and Chemical Toxicology. 2016; 96:215–25. https://doi.org/10.1016/j.fct.2016.07.032 PMID: 27481074

19. Gao Y, Chen T, Lei X, Li Y, Dai X, Cao Y, et al. Neuroprotective effects of polydatin against mitochondrial-dependent apoptosis in the rat cerebral cortex following ischemia/reperfusion injury. Molecular Medicine Reports. 2016; 14:5461–8. https://doi.org/10.3892/mmr.2016.5936 PMID: 27840959

20. Zhang M, Zhao Z, Shen M, Zhang Y, Duan J, Guo Y, et al. Polydatin protects cardiomyocytes against myocardial infarction injury by activating Sirt3. Biochimica et Biophysica Acta (BBA)—Molecular Basis of Disease. 2017; 1863:792–99. https://doi.org/10.1016/j.bbadis.2016.09.003 PMID: 27613967

21. Ruprich N, Kindl H. Stilbene synthases and Stilbene-carboxylate Synthases, I. Enzymatic Synthesis of 3,5,4′-Trihydroxystilbene from p-Coumaroyl Coenzyme A and Malonyl Coenzyme A. Hoppe Seylers Z Physiol Chem. 1978; 359(2):165–72. PMID: 649051

22. Schröder G, Brown JWS, Schröder J. Molecular analysis of resveratrol synthase. European Journal of Biochemistry. 1988; 172(1):161–9. https://doi.org/10.1111/j.1432-1033.1988.tb13688.x PMID: 2450022
23. Lanz T, Schröder G, Schröder J. Differential regulation of genes for resveratrol synthase in cell cultures of Arachis hypogaea L. Planta. 1990; 181(2):169–75. https://doi.org/10.1007/BF02411534 PMID: 24196732

24. Melchior F, Kindl H. Grapevine stilbene synthase cDNA only slightly differing from chalcone synthase cDNA is expressed in Escherichia coli into a catalytically active enzyme. FEBS Letters. 1990; 268(1):17–20. https://doi.org/10.1016/0014-5793(90)80961-f PMID: 2200709

25. Lanz T, Tropf S, Marner FJ, Schröder J, Schröder G. The role of cysteines in polyketide synthases. Site-directed mutagenesis of resveratrol and chalcone synthases, two key enzymes in different plant-specific pathways. Journal of Biological Chemistry. 1991; 266(15):9971–6. PMID: 2033084

26. Giovinazzo G, D’Amico L, Paradiso A, Bollini R, Sparvoli F, DeGara L. Antioxidant metabolite profiles in tomato fruit constitutively expressing the grapevine stilbene synthase gene. Plant Biotechnology Journal. 2005; 3(1):57–69. https://doi.org/10.1111/j.1467-7652.2004.00099.x PMID: 17168899

27. Morelli R, Das S, Bertelli A, Bollini R, Scalzo RL, Das DK, et al. The introduction of the stilbene synthase gene enhances the natural antiradical activity of Lycopersicon esculentum mill. Mol Cell Biochem. 2006; 282(1–2):65–73. https://doi.org/10.1007/s11010-006-1260-7 PMID: 16317513

28. D’Introno A, Paradiso A, Scoditti E, D’Amico L, De Paolis A, Carluccio MA, et al. Antioxidant and anti-inflammatory properties of tomato fruits synthesizing different amounts of stilbenes. Plant Biotechnology Journal. 2009; 7(5):422–9. https://doi.org/10.1111/j.1467-7652.2009.00409.x PMID: 19490505

29. Baek S-H, Shin W-C, Ryu H-S, Lee D-W, Moon E, Seo C-S, et al. Creation of Resveratrol-Enriched Rice for the Treatment of Metabolic Syndrome and Related Diseases. PLoS ONE. 2013; 8(3):e57930. https://doi.org/10.1371/journal.pone.0057930 PMID: 23483945

30. Delaunois B, Cordelier S, Conreux A, Clément C, Jeandet P. Molecular engineering of resveratrol in plants. Plant Biotechnology Journal. 2009; 7(1):2–12. https://doi.org/10.1111/j.1467-7652.2008.00377.x PMID: 19021877

31. RDA. Main Research Achievement in Development Project of Agricultural Science and Technology on Rice for the Treatment of Metabolic Syndrome and Related Diseases. PLoS ONE. 2013; 8(3):e57930. https://doi.org/10.1007/s11010-006-1260-7 PMID: 16317513

32. Qin Y, Ahn H-I, Kweon S-J, Baek S-H, Shin K-S, Woo H-J, et al. Molecular Characterization of Transgenic Rice Producing Resveratrol. Plant Breeding and Biotechnology. 2013; 1(4):406–15. https://doi.org/10.9787/PBB.2013.1.4.406

33. Hipskind JD, Paiva NL. Constitutive Accumulation of a Resveratrol-Glucoside in Transgenic Alfalfa Increases Resistance to Phoma medicaginis. Molecular Plant-Microbe Interactions. 2000; 13(5):551–62. https://doi.org/10.1094/MPMI.2000.13.5.551 PMID: 10796021

34. Szankowski I, Briviba K, Fleschhut J, Schoenherr J, Jacobsen H-J, Kiesecker H. Transformation of apple (Malus domestica Borkh.) with the stilbene synthase gene from grapevine (Vitis vinifera L.) and a PGIP gene from kiwi (Actinidia delicosa). Plant Cell Reports. 2003; 22(2):141–9. https://doi.org/10.1007/s00299-003-0668-8 PMID: 14504909

35. Giordelli A, Sparvoli F, Mattivi F, Tava A, Balestrazzi A, Vrhovsek U, et al. Expression of the Stilbene Synthase (StSy) Gene from Grapevine in Transgenic White Poplar Results in High Accumulation of the Antioxidant Resveratrol Glucosides. Transgenic Research. 2004; 13(3):203–14. https://doi.org/10.1023/b:trag.0000034658.64990.7f PMID: 15359598

36. Hüsken A, Baumert A, Milkowski C, Becker H, Strack D, Möllers C. Resveratrol glucoside (Piceid) synthesis in seeds of transgenic oilseed rape (Brassica napus L.). Theoretical and Applied Genetics. 2005; 111(8):1553–62. https://doi.org/10.1007/s00122-005-0085-1 PMID: 16160820

37. Liu Z, Zhuang C, Sheng S, Shao L, Zhao W, Zhao S. Overexpression of a resveratrol synthase gene (PcRS) from Polygonum cuspidatum in transgenic Arabidopsis causes the accumulation of trans-piceid with antifungal activity. Plant Cell Reports. 2011; 30(11):2027–36. https://doi.org/10.1007/s00299-011-1110-2 PMID: 21717185

38. Appert C, Logemann E, Hahlbrock K, Schmid J, Arnheim N. Structural and Catalytic Properties of the Four Phenylalanine Ammonia-Lyase Isoenzymes from Parsley (Petroselinum Crispum Nym.). European Journal of Biochemistry. 1994; 225(1):491–9. https://doi.org/10.1111/j.1432-1323.1994.tb18931.x PMID: 7925471

39. Urban P, Werck-Reichhart D, Teutsch HG, Durst F, Regnier S, Kazmaier M, et al. Characterization of recombinant plant cinnamate 4-hydroxylase produced in yeast. European Journal of Biochemistry. 1994; 222(3):843–50. https://doi.org/10.1111/j.1432-1323.1994.tb18931.x PMID: 8026495

40. Lozoya E, Hoffmann H, Douglas C, Schulz W, Scheel D, Hahlbrock K. Primary structures and catalytic properties of isoenzymes encoded by the two 4-coumarate:CoA ligase genes in parsley. European Journal of Biochemistry. 1988; 176(3):661–7. https://doi.org/10.1111/j.1432-1323.1988.tb14328.x PMID: 3169018
AhRS3 alters the expression pattern of UDP-glycosyltransferase genes in developing rice seeds

41. Hall D, De Luca V. Mesocarp localization of a bi-functional resveratrol/hydroxycinnamic acid glucosyltransferase of Concord grape (Vitis labrusca). The Plant Journal. 2007; 49(4):579–91. https://doi.org/10.1111/j.1365-313X.2006.02987.x PMID: 17270014

42. Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Nature Protocols. 2016; 11(9):1650–67. https://doi.org/10.1038/nprot.2016.095 PMID: 27560171

43. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 2014; 30(7):923–30. https://doi.org/10.1093/bioinformatics/btt656 PMID: 24227677

44. Pfaffl MW. Quantification strategies in real-time PCR. In: Bustin SA, editor. A-Z of quantitative PCR. La Jolla, CA. U.S.A.: International University Line (IUL); 2004. p. 87–112.

45. Lijuan C, Huiming G, Yi L, Hongmei C. Chalcone synthase EaCHS1 from Eupatorium adenophorum. Phytochemistry. 2006; 67(15):1598–612. https://doi.org/10.1016/j.phytochem.2006.06.026 PMID: 16876834

46. Masada S, Terasaka K, Oguchi Y, Okazaki S, Mizushima T, Mizukami H. Functional and Structural Characterization of a Flavonoid Glucoside 1,6-Glucosyltransferase from Catharanthus roseus. Plant and Cell Physiology. 2009; 50(8):1401–15. https://doi.org/10.1093/pcp/pcp088 PMID: 19561332

47. Cui L, Yao S, Dai X, Yin Q, Liu Y, Jiang X, et al. Identification of UDP-glycosyltransferases involved in the biosynthesis of astringent taste compounds in tea (Camellia sinensis). Journal of Experimental Botany. 2016; 67(8):2285–97. https://doi.org/10.1093/jxb/erw053 PMID: 26941235
61. Koja E, Ohata S, Maruyama Y, Suzuki H, Shimosaka M, Taguchi G. Identification and characterization of a rhamnosyltransferase involved in rutin biosynthesis in Fagopyrum esculentum (common buckwheat). Bioscience, Biotechnology, and Biochemistry. 2018; 82(10):1790–802. https://doi.org/10.1080/09168451.2018.1491286 PMID: 29972345

62. Okitsu N, Matsui K, Horikawa M, Sugahara K, Tanaka Y. Identification and Characterization of Novel Nemophila menziesii Flavone Glucosyltransferases that Catalyze Biosynthesis of Flavone 7,4'-O-Diglucoside, a Key Component of Blue Metalloanthocyanins. Plant and Cell Physiology. 2018; 59(10):2075–85. https://doi.org/10.1093/pcp/pcy129 PMID: 29986079

63. Noguchi A, Horikawa M, Fukui Y, Fukuchi-Mizutani M, Iuchi-Okada A, Ishiguro M, et al. Local differentiation of sugar donor specificity of flavonoid glycosyltransferase in Lamiales. The Plant Cell. 2009; 21(5):1556–72. https://doi.org/10.1105/tpc.108.063826 PMID: 19454730

64. Witte S, Moco S, Vervoort J, Matern U, Martens S. Recombinant expression and functional characterization of regiospecific flavonoid glucosyltransferases from Hieracium pilosella L. Planta. 2009; 229(5):1135–46. https://doi.org/10.1007/s00425-009-0902-x PMID: 19238428

65. Trapero A, Ahrazem O, Rubio-Moraga A, Jimeno ML, Gómez MD, Gómez-Gómez L. Characterization of a glucosyltransferase enzyme involved in the formation of kaempferol and quercetin sophorosides in Crocus sativus. Plant Physiology. 2012; 159(4):1335–54. https://doi.org/10.1104/pp.112.198069 PMID: 22649274

66. Nagatomo Y, Usui S, Ito T, Kato A, Shimosaka M, Taguchi G. Purification, molecular cloning and functional characterization of flavonoid C-glucosyltransferases from Fagopyrum esculentum M. (buckwheat) cotyledon. The Plant Journal. 2014; 80(3):437–48. https://doi.org/10.1111/tpj.12645 PMID: 25142187