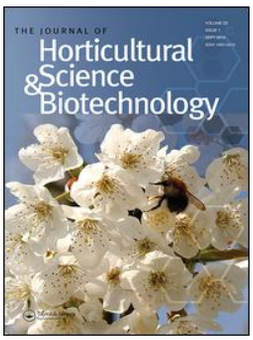

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Isolation, cloning, and expression of five genes related to nitrogen metabolism in peach (*Prunus persica* L. Batsch)

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ABSTRACT

An optimal dosage of nitrogen has long been known to play important roles in governing nitrogen-use efficiency and improving the yield of plants. However, there is limited information on the isolation and expression profiles of genes related to nitrogen metabolism in peach (*Prunus persica* L. Batsch). This study isolated five genes involved in nitrogen metabolism and evaluated the effects of a single foliar application of urea on levels of expression of these genes in the leaves of 'Dazhenbaochiyue' peach. Cloning resulted in the isolation of 1,767, 1,236, 1,074, 1,758, and 2,721 nt-long cDNAs with full-length open reading frames encoding 588, 411, 357, 585, and 906 amino acids representing the asparagine synthetase (*AS*), glutamate dehydrogenase (*GDH*), glutamine synthetase (*GS*), nitrite reductase (*NiR*), and nitrate reductase (*NR*) genes in peach, respectively. An alignment of multiple amino acid sequences revealed that the AS, GDH, GS, and NR proteins in peach shared high levels of sequence conservation or identity at the amino acid level with their homologues in *Arabidopsis thaliana* and grapevine (*Vitis vinifera*). Based on analyses of expression of the five genes in peach leaves, we demonstrated that genes related to nitrogen metabolism responded to a foliar application of urea within 2 d. This was consistent with previous reports which indicated that leaves rapidly absorbed urea-nitrogen after foliar application. Foliar application of 0.5% (w/v) urea inhibited expression of *GS* and *NiR*, but increased expression of *GDH, AS*, and *NR* compared to control leaves. The different patterns of expression of the five genes in this study suggested that their expression might reflect their various roles in nitrogen metabolism, plant N status, and responses to weather conditions such as high light intensity, temperature, or humidity, all of which affect photosynthesis. These findings provide a basis for future functional analyses of these five genes in peach.

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KEYWORDS
Peach; *Prunus persica*; nitrogen metabolism; urea; gene expression

Nitrogen is an essential mineral nutrient required by plants in comparatively larger amounts than other elements (Marschner, 1995). It is an important component of many compounds such as chlorophyll, nucleotides, proteins, alkaloids, enzymes, hormones, and vitamins in plants (Marschner, 1995). Kuykendall and Wallace (1953) reported that the assimilation of nitrogen by plants involved the combination of nitrogen with carbohydrates, ultimately to form proteins or other nitrogenous compounds. When plants absorb nitrate, nitrate reductase (NR; EC 1.6.6.1) catalyses the reduction of nitrate to nitrite, which is further reduced to ammonia by nitrite reductase (NiR; EC 1.7.1.1). Ammonium ions are then fixed onto glutamate to form glutamine by glutamine synthetase (GS; EC 6.3.1.2; Liseron-Monfils et al., 2013). Glutamate dehydrogenase (GDH; EC 1.4.1.2) also operates in the assimilation of ammonium ions, as a shunt to the glutamate synthase cycle to release carbon from amino compounds in the form of keto-acids and to enable the synthesis of compounds with low C/N ratios (Miflin and Habash, 2002). Asparagine synthetase (AS; EC 6.3.5.4) catalyses the formation of asparagine and glutamate from glutamine and aspartate in higher plants. These nitrogen-assimilation enzymes have long been proposed to play important roles in governing the nitrogen-use efficiency of plants (Tian et al., 2015).

Urea ($NH_2.CO.NH_2$) is the most commonly applied nitrogen fertiliser and has been widely used as a foliar spray to improve the nitrogen status of many plant species (Wang et al., 2008). Due to its small molecular size, non-ionic nature, and high solubility, urea is usually taken up rapidly through the leaf cuticle (Toselli et al., 2004). Theoretically, foliar-applied urea is more environmentally friendly, target oriented, rapidly absorbed, and translocated faster than soil-applied urea since it is delivered directly to plant tissues during critical stages of plant growth (Fernández et al., 2013). In deciduous fruit trees, early leaf growth and flowering predominantly use nitrogen remobilised from storage organs (Quartieri

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et al., 2002). During leaf growth and flowering, a foliar supply of nitrogen can be beneficial if root uptake and translocation of newly absorbed nitrogen are insufficient to meet plant requirements due to low soil temperature, inadequate or excess moisture, or poor root growth (Weinbaum and Van Kessel, 1998).

Peach (*Prunus persica* L. Batsch) is one of the most popular fruit in the world because of its high nutritional value and desirable flavour (Wan et al., 2009). Nitrogen fertiliser application is one of the most important horticultural practices during the production of peach and efforts have been made to study nitrogen metabolism in peach (Falguera et al., 2012; Pascuala et al., 2013). However, these studies focussed primarily on the physiological or biochemical aspects. Little information exists on genes related to nitrogen metabolism in peach, and the effect of a foliar application of urea on the levels of expression of these genes in leaves.

This study was undertaken (i) to isolate peach genes encoding AS, GDH, GS, NiR, and NR and (ii) to measure their levels of expression in leaves at 2 h intervals over 48 h following a single foliar spray application of urea. The findings from this study may contribute to our understanding the roles of these genes in nitrogen metabolism in peach.

Materials and methods

Plant material and treatments

Trees of a peach (*Prunus persica* L. Batsch) cultivar 'Dazhenbaochiyue', growing in the National Peach Germplasm Repository, Jiangsu Academy of Agricultural Sciences, Nanjing, P. R. China, were used as sources of leaf tissue. All the peach trees (n = 6) used in these experiments were 7 years old and subjected to the same field conditions.

The peach orchard was divided into two plots, from which three trees were selected per plot and were subjected to the treatments. Each treatment was replicated three times. Urea [0.5% (w/v)] or water (control) was sprayed evenly on each selected flowering tree (7 l tree^{-1}) at 07.00 h on 10 April 2014. The fifth leaf from the apex of each selected 1-year-old fruiting shoot, located on the outer southern canopy of each tree was picked at 2 h intervals during the daytime for 2 d, starting at the time of spray application. Leaves were immediately frozen in liquid nitrogen and stored at −70°C.

RNA extraction and cDNA synthesis

Total RNA was extracted from the sampled leaves using the Trizol™ method (Invitrogen, Carlsbad, CA, USA). The extracted RNA was treated with gDNA Eraser (Takara Biotechnology Co. Ltd., Dalian, P. R. China) to remove any genomic DNA contamination, then reverse transcribed into cDNA using the PrimeScript™ RT reagent kit (Takara Biotechnology Co. Ltd.). All cDNA samples were diluted to 100 ng µl^{-1} with RNase-free water and stored at −20°C before being used as templates for cloning or for real-time quantitative PCR (RT-qPCR).

Cloning of genes related to nitrogen metabolism

The full-length cDNA sequences of the *AS, GDH, GS, NiR*, and *NR* genes of *A. thaliana* (GenBank Accession Numbers AT3G47340, AT3G03910, AT1G66200, AT2G15620, and AT1G37130) were obtained from the TAIR database (http://www.arabidopsis.org/index.jsp). These sequences were used as queries to BLAST-search the peach genome database (https://www.rosaceae.org/peach/genome; Jung et al., 2014) to identify homologous genes in peach. The coding sequences (CDS) of the five genes identified in peach (Gene ID: Ppa003265m, Ppa006457m, Ppa007728m, Ppa003315m, and Ppa001113m) were obtained from the Genome Database for Rosaceae (GDR; https://www.rosaceae.org/peach/genome). Based on the predicted CDS of these genes in peach, specific primers (Table 1) were designed using Primer Premier 5.0 software (Premier Biosoft, Palo Alto, CA, USA) and synthesised by the Invitrogen Biotechnology Co. Ltd. (Shanghai, P. R. China).

Partial cDNAs of water-treated sample leaves (control) at each sampling time were mixed together and used as the initial template to clone the *AS, GDH, GS, NiR*, and *NR* genes. Each 25 µl PCR reaction contained 2.5 µl 10× PCR buffer, 2.5 µl dNTP mixture (2.5 mM each), 1.5 µl $MgCl_2$, 0.2 µl (5 Units µl^{-1}) high-fidelity PrimeSTAR® HS DNA polymerase (Takara Biotechnology Co. Ltd.), 2.0 µl cDNA, 1.0 µl of each gene-specific primer, and 14.3 µl of RNase-free water. PCR was performed in an Eppendorf-Authorised Thermal Cycler using the following programme: 94°C pre-denaturing for 5 min, followed by

Table 1. Primer sequences and sizes of the PCR-amplified products designed to clone genes related to nitrogen metabolism in the peach cultivar 'Dazhenbaochiyue'.

Gene‡	Sequence (5′ → 3′)	Size of amplified product (bp)	T_m (°C)
AS	ATGTGTGGAATTCTTGCTG TTAGCTGAGAATCGCGACC	1,767	53.4
GDH	ATGAACGCATTGGTGGCAACCAACA TTAGGCTTCCCACCCTCGGAGGACA	1,236	55.1
GS	ATGTCTCTGCTCCTCACTGATCTTC CTATGGTTTCCAGAGAACTGTAGTT	1,074	55.1
NiR	ATGTCATCATCACTATCTGTTC TTAATCTTCCCCTTCTTC	1,758	53.4
NR	ATGACGGCCTCCGTCCAGAA TCAAAACACCAGCAACGAATCTG	2,721	53.4

‡AS, asparagine synthetase; GDH, glutamate dehydrogenase; GS, glutamine synthetase; NiR, nitrite reductase; NR, nitrate reductase.

38 cycles of 94°C for 1 min, primer T_m for 30 s (see Table 1), 72°C for 3 min, and 72°C for 10 min. The PCR products were separated by electrophoresis in 2.0% (w/v) agarose gels and visualised by staining with 0.5 μg ml^{-1} ethidium bromide.

The target DNA fragments were purified using an AxyprepTM DNA Gel Extraction Kit (Aygen Biosciences, Union, CA, USA), then ligated into the pEASY-T3 vector (TransGen Biotech Co. Ltd., Beijing, P. R. China) and transformed into DH5α *E. coli* cells. Positive clones identified by PCR analysis were sequenced by the Invitrogen Biotechnology Co. Ltd.

The structures of all five genes, including the numbers of exons and introns and their locations, were predicted and displayed using the Gene Structure Display Server (GSDS; http://gsds.cbi.pku.edu.cn/). Similarities between the five nitrogen metabolism-related genes in peach and those in two other plant species were determined using NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The peach gene sequences obtained were submitted to the NCBI database with Accession Numbers KJ000548, KJ000549, KJ000550, KJ000551, and KT897469. The deduced amino acid sequences of the five genes in peach, and the corresponding sequences in *A. thaliana* and grapevine (*Vitis vinifera*) were imported into DNAMAN software and multiple sequence alignments was performed.

Expression analysis of the five nitrogen metabolism-related genes

Gene-specific primers for reverse-transcription quantitative PCR (RT-qPCR) (Table 2) were designed based on the cloned sequences using Primer Premier 5.0 software (Premier Biosoft). One of each pair of primers was designed within a 3′-untranslated region to guarantee primer specificity. Before RT-qPCR, each primer pair was tested using semi-quantitative RT-PCR and electrophoresis in 0.5 μg ml^{-1} ethidium bromide-stained 2.0% (w/v) agarose gels to verify the size specificity of the amplicons. The amplified bands were cloned and sequenced to confirm that these bands were fragments of the targeted genes. The genes for peach RNA polymerase II (*RP II*; peach EST database accession number TC1717) and translation elongation factor 2 (*TEF2*; peach EST database accession number TC3544) were used as internal reference genes for RT-qPCR.

Table 2. Primer sequences designed for RT-qPCR of genes in the peach cultivar 'Dazhenbaochiyue'.

Gene‡	Sequence (5′ → 3′)	Size of amplified product (bp)	T_m (°C)
AS	GGGCAAACAAATCAACAT GTGCTTTGGCAGATAAGG	188	60.0
GDH	TAGACATTCCAAGCCTACTTAA TTTCCCTGTTGATGACACCTC	150	60.0
GS	CCTGGTTTGGATTAGAGC TTGATGCCACTGATGTCG	199	60.0
NiR	GTGGGAGGCTTCTTTAGT TATGCCTAGTTCATCAATCA	177	60.0
NR	CTGAAGCGTGGTGGTAC CACGAGTTTATAGGCAGAA	103	60.0
TEF2	GGTGTGACGATGAAGAGTGATG TGAAGGAGAGGGAAGGTGAAAG	129	60.0
RP II	TGAAGCATACACCTATGATGATGAAG CTTTGACAGCACCAGTAGATTCC	128	60.0

‡AS, asparagine synthetase; GDH, glutamate dehydrogenase; GS, glutamine synthetase; NiR, nitrite reductase; NR, nitrate reductase; *TEF2*, translation elongation factor 2; *RP II*, RNA polymerase II.

RT-qPCR reactions were conducted using an Applied Biosystems 7500 Real-Time PCR System and a SYBER Premix Ex Taq Kit (Takara Biotechnology Co. Ltd.) according to the manufacturer's instructions. Each 20 μl reaction contained 2.0 μl of diluted cDNA, 0.4 μl of each primer, 10.0 μl of master mix, and 7.2 μl of RNase-free water. Thermal cycling conditions were an initial polymerase activation step for 30 s at 95°C, followed by 40 cycles of 5 s at 95°C for template denaturation, and 34 s at 60°C for annealing (fluorescence measurement). Stage 3 was set for 15 s at 95°C, followed by 60 s at 60°C and 15 s at 95°C. Each assay was repeated three times using each of the orchard treatment replicates. Relative levels of expression for each gene were calculated using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001).

Results

Cloning of genes related to nitrogen metabolism in peach

Cloning resulted in the isolation of 1,767, 1,236, 1,074, 1,758, and 2,721 nt-long cDNAs with full-length open reading frames (ORFs) encoding 588, 411, 357, 585, and 906 amino acids from the *AS, GDH, GS, NiR*, and *NR* genes in peach, respectively. The GenBank Accession Numbers for *AS, GDH, GS, NiR*, and *NR* were KJ000548, KJ000549, KJ000550, KJ000551, and KT897469, respectively.

BLAST searches using the nucleotide sequence of the peach *AS* gene against the NCBI database revealed the highest identity (83%) with the *AS* gene of *V. vinifera* (NM001281237.1). *GDH* shared 85% homology with the *GDH1* isoform in *Theobroma cacao* (XM007030662.1). *GS* shared 85% identity with the *GS* gene in *V. vinifera* (NM001281246.1). *NiR* was found to be 83% identical to the *NiR* sequence of *T. cacao* (XM007042368.1), while *NR* shared 84% homology with the *NR* gene of *Malus hupehensis* (JN632526.1). *NiR* and *NR* were each composed of four exons and three introns (Figure 1), while *AS, GDH*, and *GS* each contained 9–12 exons according to GSDS analysis.

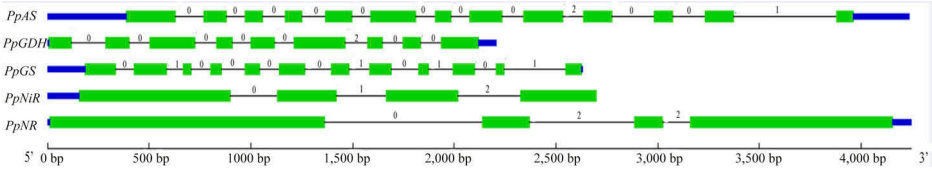

Figure 1. Exon/intron organisation of five genes related to nitrogen metabolism in peach. Green bars represent the exons, thin black lines represent introns, blue bars represent upstream/downstream regulating regions, and numbers represent the phase of the intron.

Using the DNAMAN program, multiple sequence alignments revealed that the AS, GDH, GS, and NR proteins in peach shared high levels of conservation at the amino acid level with their homologues in *A. thaliana* and grapevine (Figure 2A–D). The deduced amino acid sequence of peach NiR had higher levels of sequence conservation or identity with the NiR of grapevine than with *A. thaliana* (Figure 2E).

Expression analysis of genes encoding nitrogen metabolising enzymes

The level of expression of *GDH* in peach trees that did not receive a foliar spray of urea did not change over the 2 d of observations (Figure 3). *GDH* expression was characterised by a decrease between 0 h and 2 h, followed by an increase from 2 h to 4 h, then a gradual decrease until 8 h and an increase up to 10 h each day. The first peak of expression of *GDH* in 0.5% (w/v) urea-treated trees appeared at 6 h after spraying, followed by a second and higher peak at 26 h. This second peak persisted over a longer period (6 h) compared to 2 h in the control treatment. Although the pattern of expression of *GDH* over 34 h after the application of 0.5% (w/v) urea followed the same pattern as exhibited in the controls, expression was significantly higher than expression in the untreated trees. In addition, the first and second peaks of *GDH* expression in urea-treated trees appeared to lag 2 h behind on day-2 compared to *GDH* expression in control trees.

On day-1, the pattern of expression of *AS* exhibited the same trend in both treatments. The relative level of expression of *AS* after foliar urea treatment was characterised by a sharp increase from 26 h to 28 h, followed by a rapid decrease between 28 h and 30 h, before a gradual decrease until 34 h after treatment. At 28 h post-treatment, the relative level of expression of *AS* in leaves on urea-sprayed trees was 15.8-fold higher than in control trees. The peak in expression of *AS* in urea-treated leaves appeared to lag 2 h behind that in the control treatment, which peaked at 26 h post-treatment.

Expression of the *NiR* gene in urea-treated or untreated peach trees displayed similar characteristics throughout day-1, but the relative level of expression of the NiR gene exhibited a sharp increase from 24 h to 26 h post-application, followed by a rapid decrease from 26 h to 28 h, before a gradual decrease until 34 h. In general, expression of *NiR* was 2.56-fold higher in control than in urea-treated plants.

The level of expression of the *NR* gene over the total 48 h of observation in urea-treated trees followed a similar pattern to that in control trees. However, levels of *NR* expression were 2.33- to 5.91-fold higher in urea-treated trees than in control trees and the peak lasted for 8 h on day-2. Just as with the *AS* and *NiR* genes, urea caused a similar trend and level of expression of *NR* in control plants from 0 h to 10 h after application.

Two peaks of gene expression were observed for *GS*, at 8 h and 32 h post-treatment, in control plants, with the 32 h peak being higher than that at 8 h. When urea was applied, *GS* transcript levels did not change between 0 h and 2 h or 24 h and 26 h, but increased from 2 h to 4 h and from 26 h to 28 h, before declining at 4–6 h and 28–30 h. Only one peak of *GS* transcripts was observed on day-1, 8 h after urea treatment, before declining at 10 h in trees that had received foliar urea. However, the peak of *GS* expression at 8 h in the leaves of plants treated with urea was much lower than in control plants that received water on day-1.

Discussion

Five peach genes related to nitrogen metabolism were cloned and the levels of expression of these genes in the leaves of peach trees sprayed with 0.5% (w/v) urea were measured. The peach trees used were at the full-bloom stage. The results showed that *AS* and *NiR* began to function in the nitrogen metabolism pathway at 10 h and 24 h, respectively, after the foliar application of urea. Conversely, the *GDH* and *GS* genes responded to urea 2 h after spraying, while *NR* appeared to respond to urea immediately. These findings suggest that, within the first 34 h after the application of urea, all five genes were involved in nitrogen metabolism in peach leaves. This was consistent with previous reports which indicated that leaves rapidly absorbed nitrogen from urea after they had been sprayed (Lee and Rudge, 1986).

The highest foliar uptake of nitrogen occurred during the first 2 d after urea spraying in *Hydrangea*

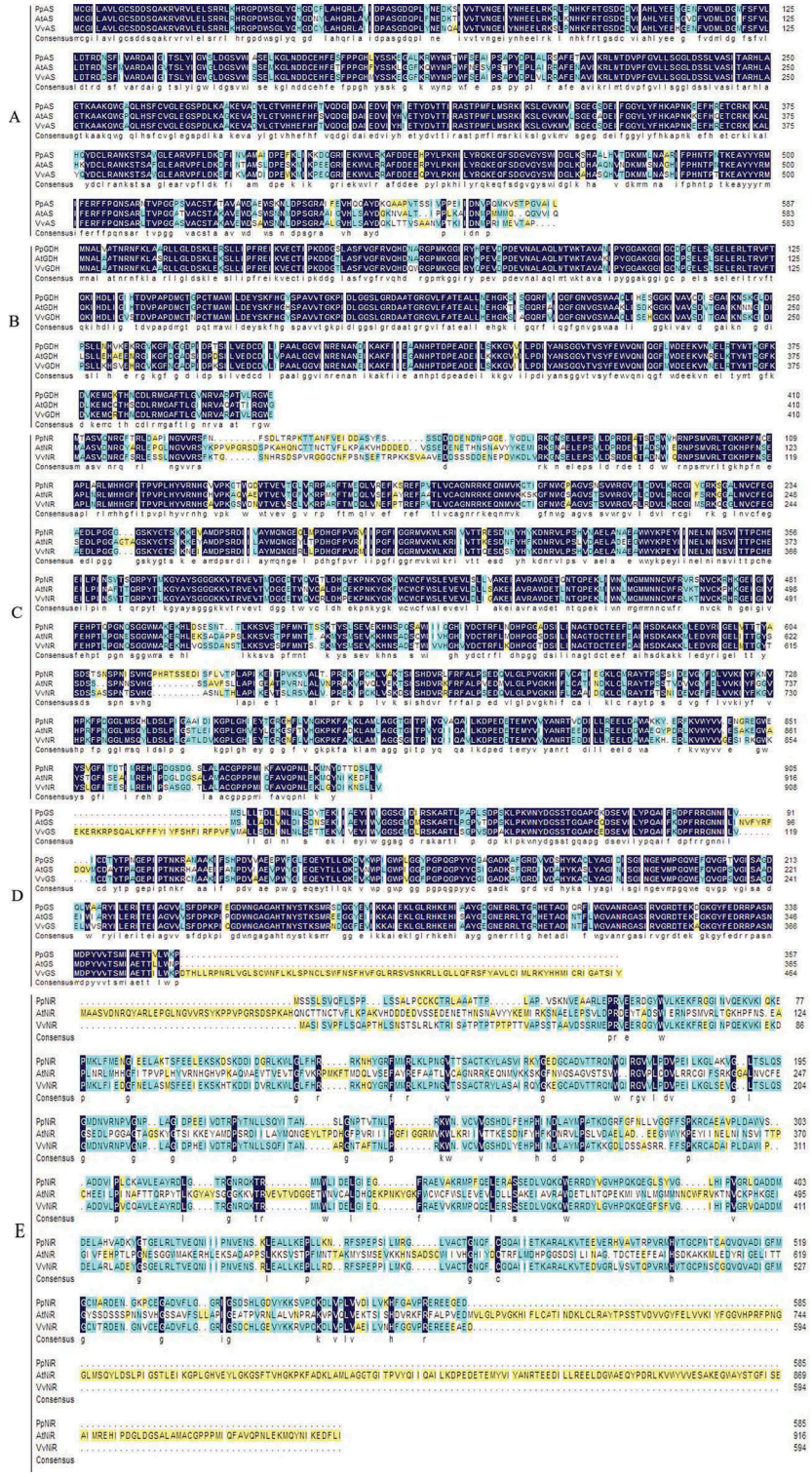

Figure 2. Alignment of the predicted amino acid sequences of asparagine synthetase (AS, Panel A), glutamate dehydrogenase (GDH, Panel B), nitrate reductase (NR, Panel C), glutamine synthetase (GS, Panel D), nitrite reductase (NiR, Panel E) proteins in peach (Pp), *Arabidopsis thaliana* (At), and grapevine (*Vitis vinifera*; Vv). GenBank Accession Numbers were JF796049.1, AT3G47340, JF796045.1, and AT3G03910 for VvAS, AtAS, VvGDH, and AtGDH, respectively. The GenBank Accession Numbers were JF796047.1, X94320.1, AT1G37130, and AT1G66200 for VvNR, VvGS, AtNR, and AtGS, respectively. The GenBank Accession Numbers were JF796046.1 and AT1G37130 for VvNiR and AtNiR, respectively. Identical amino acids among three or two plant species are shaded in dark blue or light blue, respectively. Amino acids that differed between all three plant species are shaded in yellow. Gaps are indicated by dots.

macrophylla or apple leaves (Bi, 2008; Dong et al., 2002). The speed and ability of leaves to take up urea differed depending on species, age or developmental stage, nitrogen status, concentration of urea sprayed, and weather conditions. For example, approx. 75% of applied urea was absorbed over 24 h by apple leaves (Shim et al., 1973). Boynton et al. (1953) also reported that, in apple, 50% of foliar-applied urea was absorbed

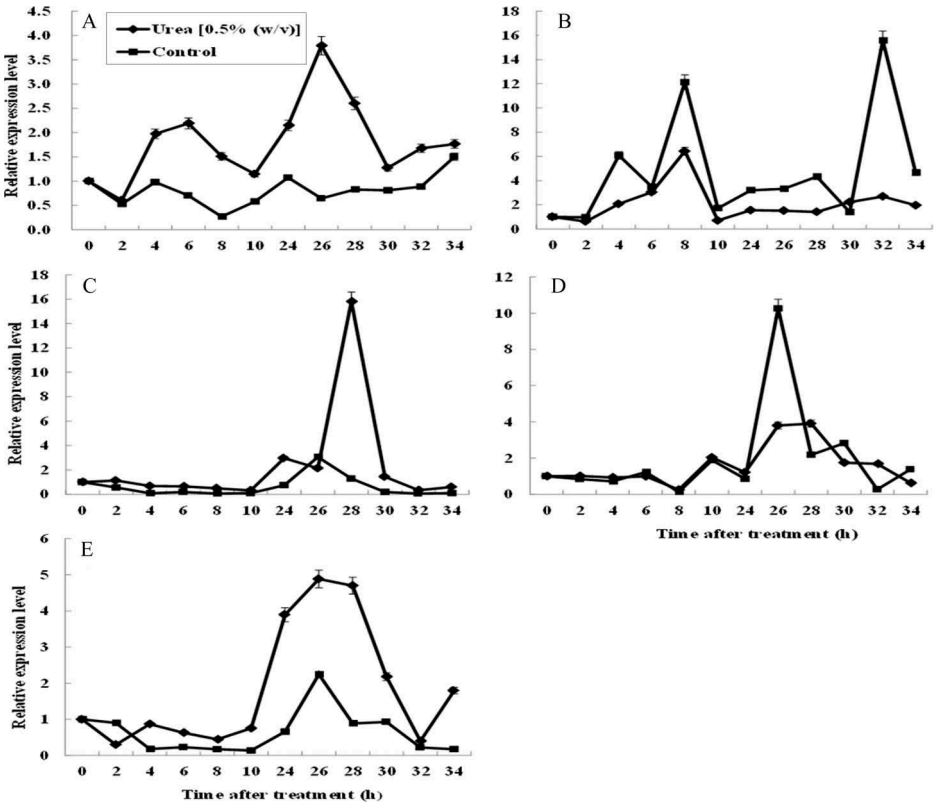

Figure 3. Relative levels of expression of five genes related to nitrogen metabolism in the leaves of peach trees after a single foliar spray with 0.5% (w/v) urea and in untreated control leaves. Levels of expression of genes were calculated by RT-qPCR using RNA polymerase II *(RP II)* and translation elongation factor 2 (*TEF2*) as reference genes. The *x*-axes show the 2 h intervals from 0 h to 34 h after foliar spraying. Vertical bars represent ±SE of each mean value (n = 9) unless smaller than the symbol. Panel A, glutamate dehydrogenase (GDH); Panel B, glutamine synthetase (GS); Panel C, asparagine synthetase (AS); Panel D, nitrite reductase (NiR); Panel E, nitrate reductase (NR).

within 8 h and 88% was absorbed within 4 d; while Cain (1956) found that nitrogen absorption was almost complete in <24 h in coffee, cacao, and banana. Foliar-applied nitrogen was rapidly absorbed by cotton leaves and transported into the closest bolls within 6–48 h (Oosterhuis and Weir, 2010). The efficient and rapid uptake of urea may due to its non-polar organic properties, plant nitrogen status, the absence of losses due to nitrogen leaching, and plant energy saving because urea assimilation in leaves can by-pass the reduction of nitrate.

The peak of *AS* gene expression appeared 28 h after urea application, with low levels of expression in both controls and urea-treated leaves at 32–34 h, which illustrates that the expression of *AS* might be affected simultaneously by light, temperature, and urea concentration. This observation confirmed previous findings in high-protein soybean cultivars, where the level of expression of *AS1* appeared higher under high light intensity conditions (Wan et al., 2006). However, these results contradicted other reports in which light was shown to repress genes encoding AS in corn, *Arabidopsis*, and asparagus (Brouquisse et al., 1992; Davis and King, 1993; Lam et al., 1994). A possible reason for the differences in results in this study and previous findings is that the *AS* gene expressed in this work may not be under the same regulation as the *AS* genes mentioned in previous reports. The *AS* gene family is reported to be a small gene family with several members; different members probably play different roles in nitrogen metabolism at different developmental stages or in different tissues. This can be exemplified by the expression of *AS2* in the dark in all soybean cultivars tested in a study by Wan et al. (2006), who also observed that, unlike *AS2*, expression of *AS1* did not increase in any soybean cultivar kept in the dark for 8 h or 48 h.

NR is considered to be the first, key rate-limiting enzyme in the nitrogen metabolism pathway. In this study, the level of expression of *NR* in leaves from 0.5% (w/v) urea-treated trees was much higher at 24–30 h and slightly higher at 4–10 h after spraying than in leaves from control trees. These observations confirmed earlier results where it was shown that the change of expression of *NR* was a complex process, regulated by the interplay of different factors such as substrate levels, carbon skeletons, nitrogen metabolites, CO_2, and light (Crawford, 1995). Plants must photosynthesise in order to provide the energy for nitrogen absorption and metabolism. Therefore, when photosynthesis is

repressed on cloudy days, or at night, the ability of plants to absorb nitrate may also be reduced or inhibited. Nitrogen metabolism thus depends heavily on conditions that affect photosynthesis, such as light intensity, temperature, humidity, and leaf area. Additionally, a biological clock may also be involved in controlling the level of *NR* transcript accumulation (Lopes et al., 1997).

In this work, 0.5% (w/v) urea reduced the level of expression of the *NiR* gene compared with that in the control treatment. The difference in expression of *NiR* in control and 0.5% (w/v) urea-treated trees at 24–34 h was probably due to variations in regulators of the enzyme such as light, and nitrate and nitrite concentrations (Galvan et al., 1992). All these factors can have effects on a plant's capacity for nitrogen metabolism. In addition, nitrogen metabolism must be coordinated with other metabolic processes, including carbohydrate metabolism, nitrogen allocation, and amino acid synthesis (Tian et al., 2015).

GS was the other gene whose level of expression in urea-treated leaves was reduced in this study. The highest peaks of expression of the *GS* gene in urea-treated and control trees appeared at 8 h and 32 h after spraying, respectively. This illustrated that the level of expression of the *GS* gene was affected by light and nitrogen concentration. This agreed with studies performed in pea (Edwards and Coruzzi, 1989) and in *Arabidopsis* (Peterman and Goodman, 1991), which demonstrated that the level of expression of the *GS2* gene was tightly regulated by light and, in several cases, this has been shown to be mediated, at least in part, by phytochromes.

GDH transcripts have been shown to exist in the leaves of tea (Wang et al., 2012) and maize (Sakakibara et al., 1995) plants. In this study, t *GDH* transcripts were also found to be abundant in peach leaves. Unlike the *GS* and *NR* genes, the level of expression of *GDH* increased with the application of 0.5% (w/v) urea. The pattern of expression of *GDH* in the leaves of control peach trees over the 2 d illustrated that weather conditions affected *GDH* gene expression. Furthermore, the pattern of expression of *GDH* after urea treatment varied over the 2 d. This was consistent with previous findings that the patterns of GDH isoenzymes varied most depending on the source of nitrogen (Kretovich et al., 1973). In addition, our observation differed from a previous finding in which the accumulation of *GDH1* mRNA was repressed by light (Lam et al., 1996). The *GDH* gene in this study was only one gene among members in the peach *GDH* gene family, which may be different from the *GDH* gene reported above. Further studies are needed in order to provide a more comprehensive understanding of the functions of the *AS, GDH, GS, NiR*, and *NR* genes in the nitrogen metabolism pathway in peach trees.

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