African Journal of Biotechnology Vol. 10(34), pp. 6481-6491, 11 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2483
ISSN 1684-5315 © 2011 Academic Journals

Full Length Research Paper

Glucosinolate content and related gene expression in response to enhanced UV-B radiation in *Arabidopsis*

Yin Wang, Wen-Jia Xu, Xiu-Feng Yan and Yang Wang*

Alkali Soil Natural Environmental Science Center, Northeast Forestry University/Key Laboratory of Saline-alkali Vegetation Ecology Restoration in Oil Field, Ministry of Education, Harbin 150040, China.

Accepted 29 April, 2011

Increasing UV-B radiation reaching the earth's surface can affect the growth and development of plants. Glucosinolate metabolism is evolved through plant interactions with the environment and constantly regulated by different environmental factors. We investigated the contents of glucosinolates and the expression of related genes in response to enhanced UV-B radiation (1.55 W·m^{-2}) and the succeeding dark recovery process in *Arabidopsis* (*Arabidopsis thaliana*) rosette leaves. At the initial 1 h of enhanced UV-B radiation, UV-B radiation induced the production of glucosinolates; however, after continuous UV-B exposure for 12 h the expression of glucosinolate metabolism related genes was significantly inhibited and the glucosinolate content was declined, especially that of indolic glucosinolates. Additional analyses indicated that UV-B exposure also led to the cell membrane damage and the decrease of relative water content. Then, the plants irradiated by UV-B radiation were kept in darkness for 12 h so as to the physiological status of the leaves could be partially recovered. As a result, both glucosinolate gene expression and the content returned to the control levels.

Key words: UV-B, *Arabidopsis*, glucosinolates, gene expression.

INTRODUCTION

Recently, depletion of the stratospheric ozone layer catalyzed by chlorofluorocarbons and other anthropogenic pollutants are leading to an increase in solar ultraviolet-B (UV-B: 280 to 320 nm) radiation reaching the earth's surface. UV-B radiation acts as an environmental stress and triggers various responses in plants, including changes in growth, development, morphology and physiological aspects (A-H-Mackerness et al., 1999; Andrady et al., 2009; Hollosy, 2002). Many researches focus on the effect of UV-B radiation on regulating the biosynthesis of secondary metabolites such as flavonoids and other phenolic compounds (A-H-Mackerness et al., 1999; Casati and Walbot, 2003; Hectors et al., 2007).

Glucosinolates are plant secondary metabolites mainly found in species of Cruciferae family including the model plant *Arabidopsis* (*Arabidopsis thaliana*). It had been proved that glucosinolates play an important role in the plants defense against generalist herbivores and pathogens and also affect growth and development (Clay et al., 2009; Yan and Chen, 2007; Zhao et al., 2002). Furthermore, people have noticed their roles in cancer prevention (Halkier and Gershenzon, 2006). In *Arabidopsis* rosette leaves, glucosinolates were mostly with aliphatic or indolic side-chains, the indolic glucosinolates are derived from tryptophan, while aliphatic glucosinolates are derived from methionine (Beekwilder et al., 2008; Brown et al., 2003), their biosynthetic pathway (Figure 1) has been reviewed (Halkier and Gershenzon, 2006; Yan and Chen, 2007). Future research needs to focus on questions related to the regulation and control of glucosinolate metabolism. It has been reported *Arabidopsis* glucosinolate production is induced in response to insect feeding (Wittstock et al., 2003) or mechanical damage (Mikkelsen et al., 2003). Analysis of *Arabidopsis* signal transduction mutants and treatments with the inducers methyl jasmonate and 2, 6-dichloro-isonicotinic acid showed that the jasmonate- and salicylate-mediated defense pathways are involved in the induction of different glucosinolates (Kliebenstein et al., 2002; Mikkelsen et al., 2003).

*Corresponding author. E-mail: ywang@nefu.edu.cn Tel: +86 451 8219 2185. Fax: +86 451 8219 2185.

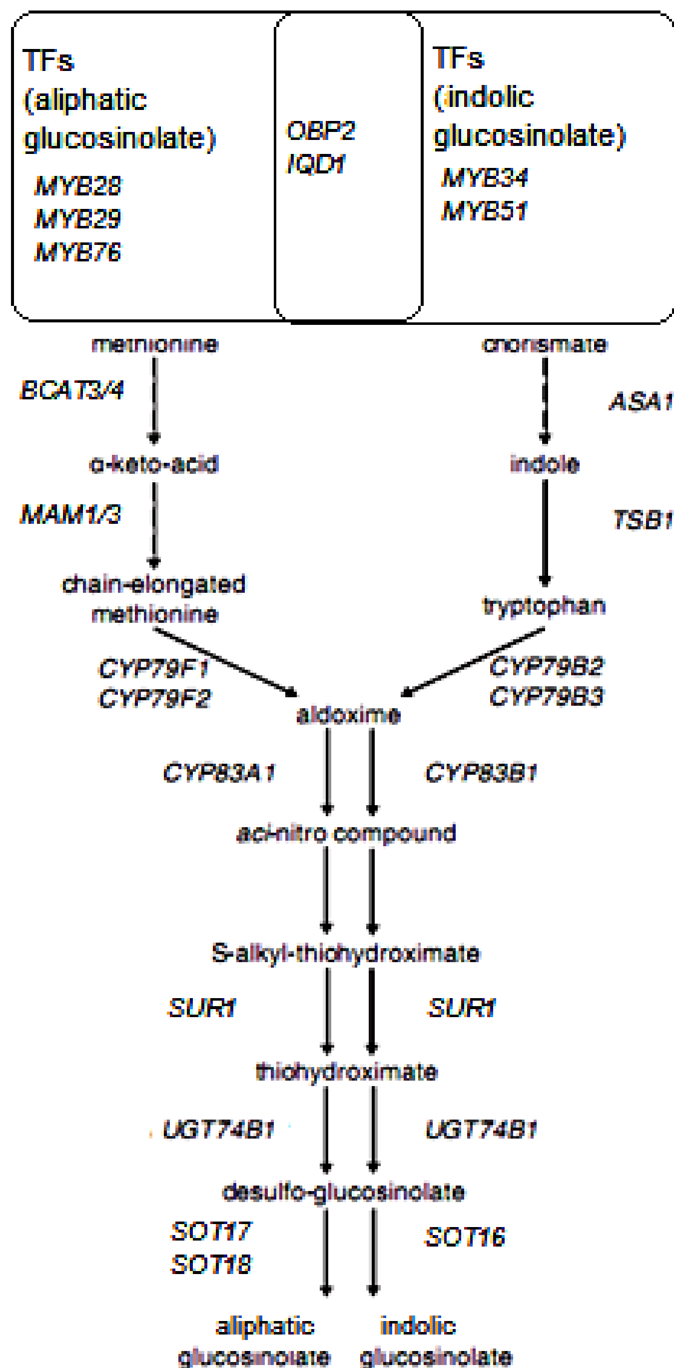


Figure 1. Glucosinolate biosynthetic pathway in *Arabidopsis* rosette leaves (Halkier and Gershenzon, 2006; Yan and Chen, 2007).

In recent years, some researches had already noticed that glucosinolate metabolism was affected by UV-B radiation. Microarray data showed that the genes related to the biosynthesis of flavonoids, glucosinolates and terpenoids were differently expressed after UV-B radiation (Hectors et al., 2007). The study on *Tropaeolum majus* demonstrated that appropriate UV-B dosage could increase the glucotropaeolin concentration (Schreiner et al., 2009). However, there were also some researches which supported glucosinolate contents were unaffected by UV-B radiation (Kuhlmann and Müller, 2009a, b; Reifenrath and Müller, 2007). In this study, our goal was to investigate how glucosinolate metabolism (the contents of glucosinolates and the expression of related genes)

responded to enhanced UV-B radiation and the succeeding dark recovery in *Arabidopsis*.

MATERIALS AND METHODS

Plant material and UV-B treatment

Seeds of *Arabidopsis* (ecotype Col-0) were sterilized with 1% NaClO and 0.1% Tween-20, cold-treated at 4°C for 3 days in the dark, then sown on fertilized soil: vermiculite mixture (1:2, w/w) in polystyrene trays and watered from the bottom, grown in a culture chamber with photosynthetic flux of 100 µmol $m^{-2} s^{-1}$, a photoperiod of 12 h light at 26°C and 12 h dark at 20°C and a relative humidity of approximately 60%. After germinating, plants were irrigated with 1/4 Hoagland nutrient solution every 3 days and were not subjected to attack by aphids or any other visible pests. Following growth for 6 to 8 weeks (18 to 20 rosette leaves with a diameter of ca. 55 mm and no visual symptoms of flowering), the plants were transferred to exposure chamber fitted with fluorescent lamps and UV-B lamps (TL20W/01RS, Philips, Holland) to provide enhanced UV-B radiation, with the identical temperature and humidity of that in growth chamber. The control plants were cultured under fluorescent lamps. The output of UV-B lamps was filtered through a 0.1 mm thick cellulose acetate film to filter out UV-C radiation (<290 nm) emitted by the tubes and the cellulose acetate film was replaced every 2 days to ensure the radiation similarity. The lamps were suspended at about 30 cm above the plants. UV-B fluence rates were routinely measured using an UV power meter (TN2340, Taina, Taiwan). The UV-B fluence rate used in our experiments (1.55 W·m^{-2}) represents the highest fluence rate received at noon in summer in North China Plain (Ma et al., 2007). The mature rosette leaves were harvested after 1, 3 and 12 h of treatment and 2 and 12 h of dark recovery following 12 h of UV-B exposure. Samples were quickly frozen in liquid nitrogen and stored at -80°C until analysis. Three independent biological replicates were prepared for each sample.

Relative water content and relative electrical conductivity analysis

About 1 g leaf samples were used for the determination of relative water content (RWC) with the method described by Reiss (1994). Plant fresh weight (FW) was measured immediately after harvesting and then dried at 103°C for 30 min and at 65°C for 72 h dry weight (DW) was measured. The RWC was calculated as follows: RWC (%) = (FW-DW)/FW × 100 %. The levels of damage to rosette leaves caused by enhanced UV-B radiation were evaluated by measuring changes in relative electrical conductivity (REC) using the method described by Zhou et al. (1994) with several modifications. Two rosette leaves were washed in deioned water and then cut into halves along the mid-vein. The four halves were immediately put into a small glass vessel filled with 15 ml deioned water. The vessel was then capped, shaken, vacuum-infiltrated for 30 min and rocked at room temperature for 30 min. Electric conductivity (C1) was measured with a conductivity meter (Conductivity cell 011050, Orion Research Inc., USA). After electric conductivity being measured, samples were heated in boiling water for 15 min, rocked at room temperature for 30 min and electric conductivity was measured again (C2). The REC was calculated using the formula C1/C2 × 100%.

Glucosinolate analysis by HPLC mass spectrometry

Glucosinolates were extracted, converted to desulfo-glucosinolates and identified using HPLC online with a quadrupole ion trap mass spectrometer (4000QTRAP MS/MS system, Applied Biosystems Inc., USA) as previously described (Petersen et al., 2001; Chen et al., 2003; Pang et al., 2009). The MS/MS fragmentation pattern of each individual peak was searched against an internal metabolite database constructed from the MS/MS spectra of glucosinolates in *Arabidopsis* and *Brassica napus* using Analyst 1.4.1 software (Applied Biosystems Inc.). Based on retention time, UV and MS spectra, a total of 8 major peaks (including internal standard desulfo-benzylglucosinolate) could be confirmed (Supplementary Figure 1). Quantification was based on integrative peak areas (Waters Breeze™ software) and normalized against an internal standard, benzylglucosinolate (0.1 µmolg^{-1}). The relative response factors of different glucosinolates were also taken into consideration (Petersen et al., 2001; Brown et al., 2003; Chen et al., 2003). Data presented are means ± standard errors of three samples. The glucosinolate contents in rosette leaves under PAR served as control. The statistical analysis of differences between the treatment and the control at the same time point was performed by 2-tailed T-test, *: $P < 0.05$.

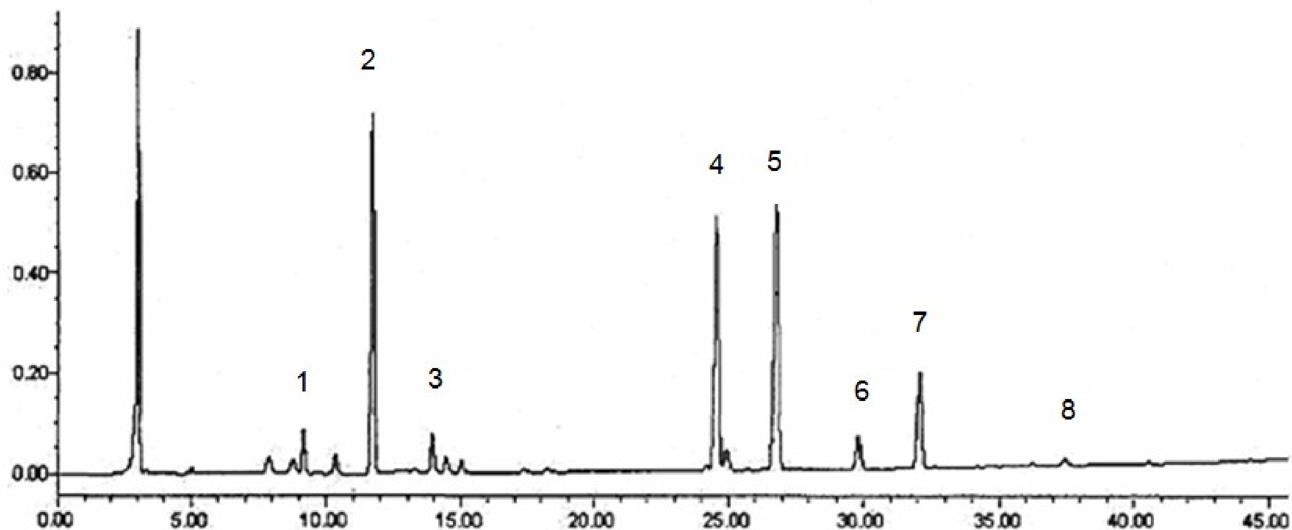
Isolation of RNA and real-time quantitative RT-PCR

Total RNA was extracted and purified with the Qiagen plant RNAeasy kit (Qiagen Inc., USA). First-strand cDNA was synthesized using the transcriptor first strand cDNA synthesis kit (TaKaRa Inc., Japan). RNA integrity was checked on denaturating 37% formaldehyde agarose gel and RNA quality was measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., USA). Sequences provided in the TAIR database (http://www.*arabidopsis*.org) were used for designing the primers (Supplementary Table 1). The absence of genomic contamination was confirmed by designing one primer pair in the intron-exon over-spanning region. Quantitative real-time PCR preformed on the opticon monitor 2 (MJ Research Inc., USA) using SYBR green I reagents (TaKaRa Inc., Japan) and PCR reacting condition was performed according to the manufacturer's instructions. At the end of each PCR program, a melting curve was generated and analyzed with dissociation curves software (MJ Research Inc., USA). PCR product lengths were verified by agarose gel electrophoresis to confirm the specificity of PCR products. Gene expression levels were normalized relatively to the expression of *Actin-2* (At3g18780) gene. Each quantification assay included three replicates. The mean normalized expression was calculated using Q-Gene software (http://www.biotechniques.com/softlib/qgene.html) based on the formula of Muller et al. (2002). The relative expression ratio of the gene was calculated as the ratio of the treatment to the control at the same time. The statistical analysis of differences was performed by 2-tailed T-test, *: $P < 0.05$.

RESULTS

Relative electrical conductivity and relative water content of *Arabidopsis* rosette leaves affected by enhanced UV-B radiation

High fluence rates of UV-B produce reactive oxygen species and may cause plant membrane degradation and lipid peroxidation. The relative electrical conductivity (REC) reflects the damage degree of cell membrane in plant leaves exposed to enhanced UV-B radiation (A-H-Mackerness et al., 2001; Ulm and Nagy, 2005). The result showed that after 1 h of UV-B radiation (1.55 W·m^{-2}), there was no significant difference in REC of rosette



Supplementary Figure 1. HPLC chromatogram (229 nm) of desulfo-glucosinolates identified in rosette leaves of *Arabidopsis*. (1) 3-methylsulfinylpropyl desulfo-glucosinolate, 3MSOP; (2) 4-methylsulfinylbutyl desulfo-glucosinolate, 4MSOB; (3) 5-methylsulfinylpentyl desulfo-glucosinolate, 5MSOP; (4) desulfo-benzylglucosinolate (internal standard); (5) indole-3-ylmethyl desulfo-glucosinolate, I3M; (6) 8-methysulfinyloctyl desulfo-glucosinolate, 8MSOO; (7) 4-methoxyindol-3-ylmethyl desulfo-glucosinolate, 4MOI3M; (8) 1-methoxyindol-3-ylmethyl desulfo-glucosinolate, 1MOI3M.

Supplementary Table 1. List of primers used in this study.

Gene	Locus	Primer (5' --- 3')
MYB51	At1g18570	CTACAAGTGTTTCCGTTGACTCTGAA ACGAAATTATCGCAGTACATTAGAGGA
MYB34	At5g60890	CACGACTGTCGATAATTTTGGGTTT CATATTGTCATCTTCGTTCCAGGAA
OBP2	At1g07640	CGTTGTTCCCGATATTACCGTCGT TTTGAGCCCCTTTGCTCTCTTCG
MYB28	At5g61420	CCAAGGCGTGTTTATTACGA CCAATTGATTCGCGAGGTTA
MYB76	At5g07700	AGTGGTGAGACGCAGATAGA TGGGAGTCCTGAAGATGATG
MYB29	At5g07690	GTTTAGTAACAACGAAGGGG GAAATCGGAATGGTCAAGGA
IQD1	At3g09710	TCCTACAACAAAATCAGCAC TACACAATCTCATAACTCCA
MAM1	At5g23010	CGATGAAATCTCTTTGGAGA AATTGATATTACTGTGGTAC
MAM3	At5g23020	ATCTGAAGGCATTAGTGGTGAACG ATACAACAGCGGAAATCTGAGGG

Supplementary Table 1 Contd.

BCAT3	At3g49680	TGGATGAATTGTTAGAAGCAGACG GAAAAGAAAGCAAAACCAAGCAG
CYP79F1	At1g16410	AAGAAGGTGGTAAGGCTGCTGTT AATGTGGCTACCTTTGGGAATGA
CYP79F2	At1g16400	GTCGGGACAATTATGATGGC CACAGAGAAAAAACAAGGCG
CYP83A1	At4g13770	GATTCCTCTCCTTATCCCTC TAAACTCGTAGTCCGTGCCT
CYP83B1	At4g31500	GGCAACAAACCATGTCGTATCAAG CGTTGACACTCTTCTTCTCTAACCG
CYP79B2	At4g39950	GTGTGTTGGTTTCTTGGTTC GGCAGATTTGTTAATTTGAT
CYP79B3	At2g22330	GCACTCTTTGCGTCAAGACCACT GCATTTCCACAATAATGCCTCGTA
SUR1	At2g20610	ACAATCCCTGTGGAAATGTCTACTC ACAACCCATCCCTTAGATATGCC
TGG1	At5g26000	AGACCTCAAAGCATCTGGCA CTCCTTATCTATGGAGCAAG
SOT16	At1g74100	CCATCTTCCACAACTCTAAC GCCACCAGTGTCCACCGTAT
SOT17	At1g18590	TCAGCTTACGGTCCTTATCTTGATC CCTTTCCTGAAATACGCACTGTTGG
SOT18	At1g74090	ACGACGAGACCAAGACAGAATCAAC GAGAACATCAACTTCAGGGAAGAAA
UGT74B1	At1g24100	CACCACTACCTACACCGCCTCCTCA GCTCAAAGACGGTAAGCCACGGATA
NIT1	At3g44310	GTCCAATCCTCCACCGTCTA AACTCATCACGCCCTTCTTC
CYP81F2	At5g57220	AAAGGCAAAAGCTGAGATAGACG CAACGAACCTAAAGCCAACAATAC
ACTIN2	At3g18780	TCCAGGAATCGTTCACAGAA GCTACAAAACAATGGGACTAAAA

leaves between the treatment and the control (without suffering any UV-B radiation). At 3 h after UV-B radiation, REC began to rise slightly. Enhanced UV-B radiation significantly increased the REC up to 37.9±2.4% for 12 h of treatment. After 12 h of recovery in darkness, the REC of plants irradiated by UV-B decreased, but could not recover to the control level (Figure 2a).

Relative water content (RWC) is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit (Barr and Weatherley, 1962). During the process of enhanced UV-B radiation treatment, the RWC in rosette leaves (Figure 2b) was showing continued decrease which started from 3 till 12 h, however, after 12 h of dark recovery, the RWC returned to the control level and without showing significantly wilted.

The variation of glucosinolate profiles in response to enhanced UV-B radiation in *Arabidopsis*

The glucosinolate profiles have been well characterized in *Arabidopsis* rosette leaves (Petersen et al., 2001; Reintanz et al., 2001; Chen et al., 2003). In this study,

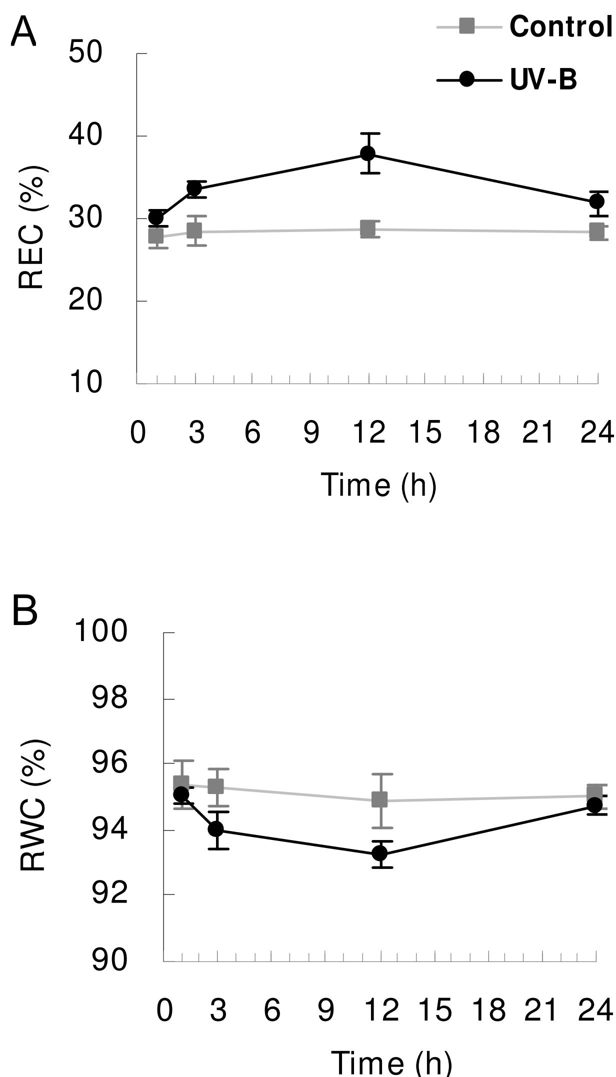


Figure 2. A: The REC of *Arabidopsis* rosette leaves affected by enhanced UV-B radiation; B: the RWC of *Arabidopsis* rosette leaves affected by enhanced UV-B radiation. 1, 3 and 12 h represent the time points of enhanced UV-B radiation; 24 h represents the time point of 12 h of dark recovery following 12 h of UV-B radiation. The REC and RWC of rosette leaves under PAR was as control. The data represent the average of three independent biological replicates and vertical bars indicate standard errors.

four aliphatic glucosinolates; namely 3-methylsulfinylpropyl glucosinolate (3MSOP), 4-methylsulfinylbutyl glucosinolate (4MSOB), 5-methylsulfinylpentyl glucosinolate (5MSOP) and 8-methysulfinyloctyl glucosinolate (8MSOO) and three indolic glucosinolates; indole-3-ylmethyl glucosinolate (I3M), 4-methoxyindol-3-ylmethyl glucosinolate (4MOI3M) and 1-methoxyindol-3-ylmethyl glucosinolate (1MOI3M) were identified in *Arabidopsis* rosette leaves. Total glucosinolate content was calculated by the summation of the contents of identified aliphatic and indolic glucosinolates.

The contents of different side-chain glucosinolates varied discriminatively during 12 h of UV-B exposure and the dark recovery process. At the initial 1 h of treatment, enhanced UV-B radiation induced 4MSOB and I3M contents to slightly increase ($p < 0.05$), but no effect to other glucosinolates; after 3 h, there were no significant differences in total glucosinolate content between the treatment and the control, although, the content of 4MOI3M decreased (Figure 3); after 12 h, total glucosinolate content, especially the indolic glucosinolate content significantly decreased (Table 1). In natural environment, the plants are only irradiated by sunshine in the day, so we stopped applying the UV-B radiation in the dark cycle in order to investigate how did the glucosinolate metabolism in the plants irradiated by enhanced UV-B radiation respond during the dark cycle. So the plants which had been irradiated by 12 h of UV-B radiation were kept in the darkness for another 12 h. After 12 h of dark recovery, we found that the content of total glucosinolate increased up to the level of control (Table 1); aliphatic glucosinolate (especially 4MSOB and 3MSOP) content became slightly higher than control; however, there were no significant differences in indolic glucosinolate content (Figure 3).

The expression profiles of glucosinolate metabolism related genes in response to enhanced UV-B radiation

The expression profiles of glucosinolate metabolism related genes in response to enhanced UV-B radiation were analyzed by real-time PCR technology. We compared the gene expression levels of the plants irradiated by enhanced UV-B with that in the control plants at the same time point. The genes of glucosinolate biosynthetic pathway determined (Figure 1) were according to the information of the TAIR database (http://www.*arabidopsis*.org) and the previous researches (Chen et al., 2003; Dombrecht et al., 2007; Gigolashvili et al., 2008; Skirycz et al., 2006; Mikkelsen et al., 2003; Kliebenstein et al., 2002). The expression levels of 24 genes in glucosinolate biosynthetic pathway were determined, including the transcription factors (*MYB28*, *MYB29* and *MYB76* for aliphatic glucosinolates, *MYB51*, *MYB34* for indolic glucosinolates, *OBP2* and *IQD1* for both), the structural genes encoding enzymes of aliphatic glucosinolate biosynthesis (*MAM1*, *MAM3*, *BCAT3*, *CYP79F1*, *CYP79F2*, *CYP83A1*, *SOT17* and *SOT18*); indolic glucosinolate biosynthesis (*CYP79B2*, *CYP79B3*, *CYP83B1*, *SOT16*, *CYP81F2*) and genes involved in both (*SUR1* and *UGT74B1*) and the genes encoding enzymes of glucosinolate degradition *(NIT1* for indolic glucosinolates and *TGG1* for aliphatic glucosinolates). The results showed that several transcription factor

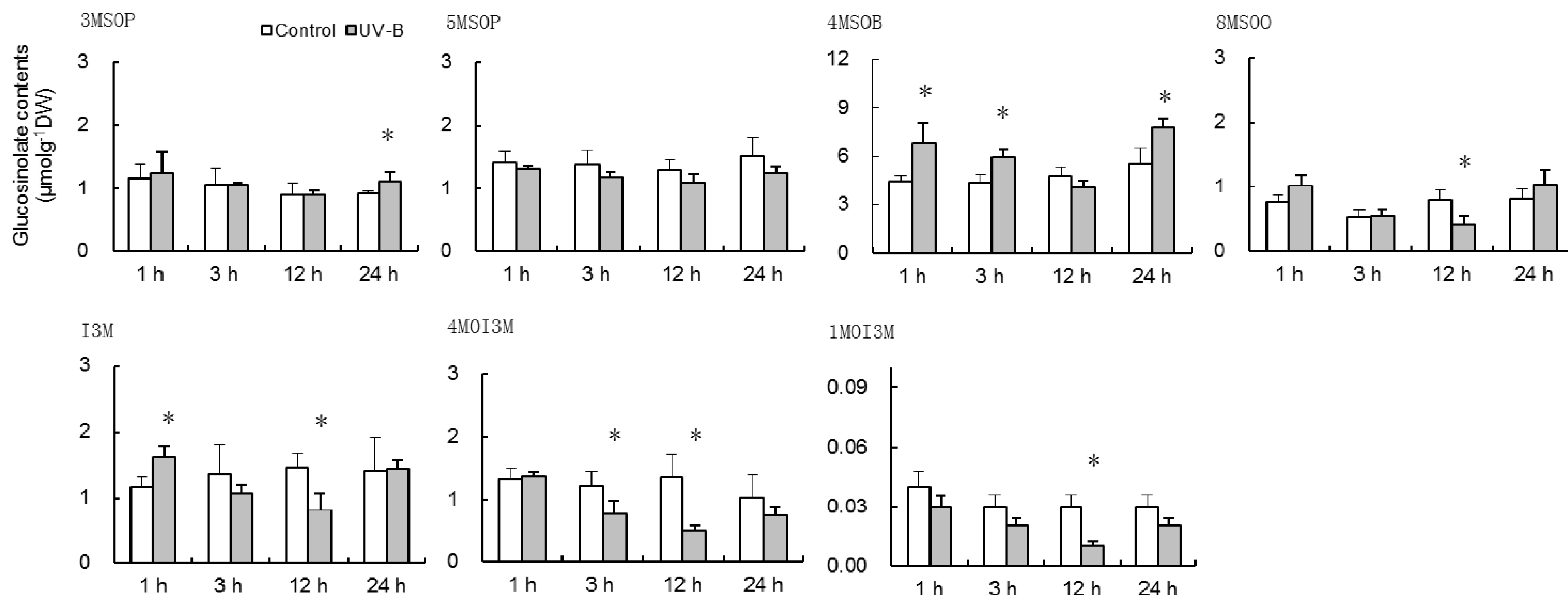


Figure 3. The variation of glucosinolate profiles response to enhanced UV-B radiation. 1, 3 and 12 h represent the time points of enhanced UV-B exposure; 24 h represents the time point of 12 h of dark recovery following 12 h of UV-B exposure. The glucosinolate content of rosette leaves under PAR was as control. The data represent the average of three independent biological replicates and vertical bars indicate standard errors. The statistical analysis of differences was performed by 2-tailed t-test, *: P < 0.05.

Table 1. The changes of glucosinolate content during enhanced UV-B radiation and dark recovery.

Time (h)	Total glucosinolate content (μmolg^{-1}DW)		Aliphatic glucosinolate content (μmolg^{-1}DW)		Indolic glucosinolate content (μmolg^{-1}DW)	
	Control	UV-B	Control	UV-B	Control	UV-B
1	10.35±1.21	13.43±2.03	7.82±0.87	10.41±1.78	2.53±0.34	3.02±0.25
3	9.97±1.77	10.60±1.03	7.36±1.08	8.74±0.67	2.61±0.69	1.85±0.36
12	10.68±1.63	7.80±1.11*	7.81±1.04	6.48±0.78	2.85±0.59	1.32±0.33*
24	11.29±2.37	13.45±1.24	8.81±1.49	11.25±0.96*	2.48±0.88	2.20±0.28

1, 3 and 12 h represent the time points exposed to enhanced UV-B radiation; 24 h represents the time point of 12 h of dark recovery following 12 h of UV-B radiation. The glucosinolate content of rosette leaves under PAR was as control. The statistical analysis of differences between the treatment and the control at the same time point were performed by 2-tailed t-test, *: P ≤ 0.05.

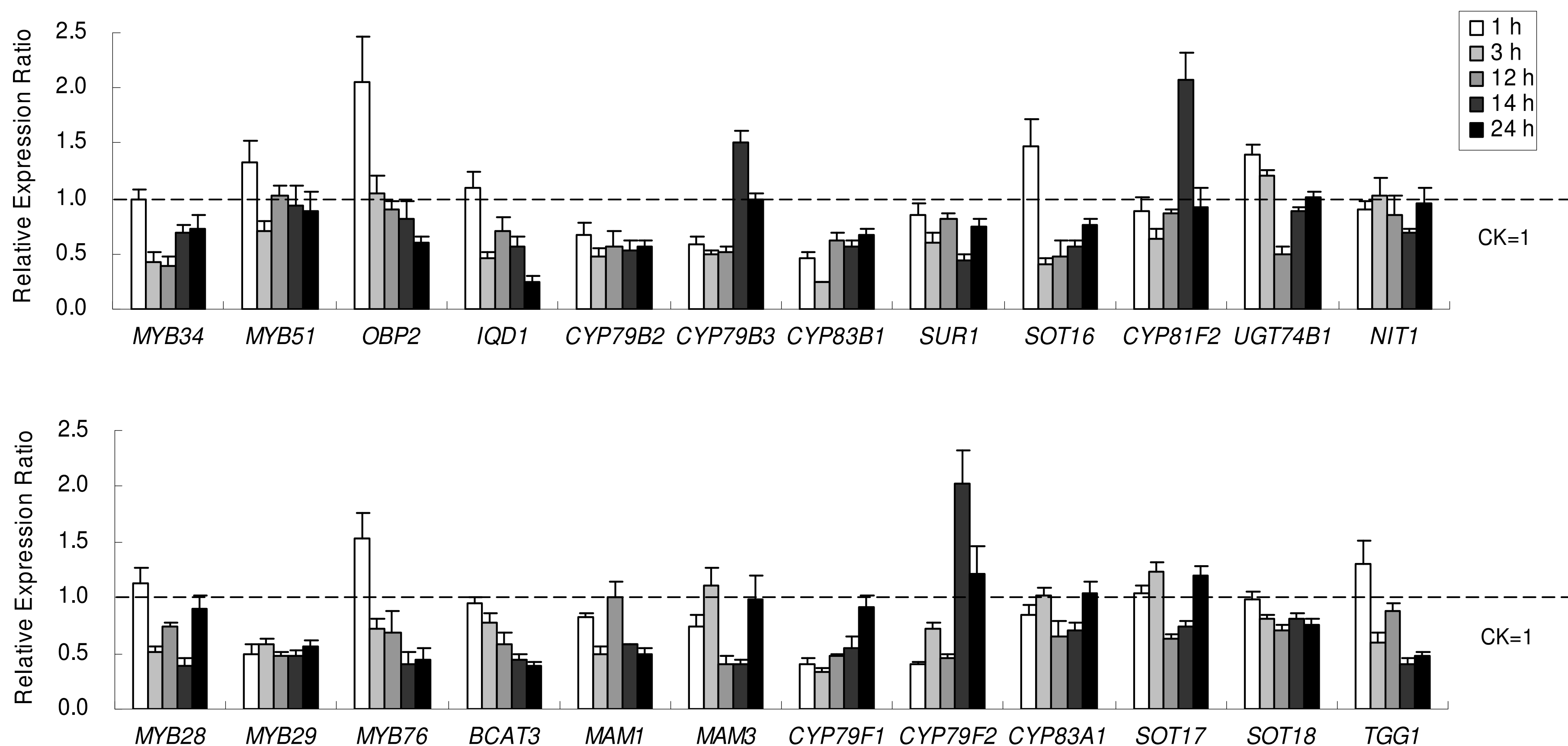


Figure 4. The expression profiles of glucosinolate metabolism related genes in response to enhanced UV-B radiation. The top panel showed the expression profiles of indolic glucosinolate genes and genes (*OBP2*, *IQD1*, *SUR1* and *UGT74B1*) involved in both kinds of glucosinolates biosynthesis; the bottom panel showed the expression profiles of genes involved in aliphatic glucosinolate metabolic pathway. 1, 3 and 12 h represent the time points exposed to enhanced UV-B radiation; 24 h represents the time point of 12 h of dark recovery following 12 h of UV-B radiation. The gene relative expression ratio is calculated as the ratio of the treatment to the control at the same time. The data represent the average of three independent biological replicates and vertical bars indicate standard errors.

genes (*MYB51*, *MYB76* and *OBP2*) and structural genes (*SOT16* and *UGT74B1*) were up-regulated within 1 h of treatment. But in general, during the 12 h of enhanced UV-B radiation, the majority of genes were inhibited. Interestingly, after 2 h of dark recovery, several genes (*CYP79B3*, *CYP79F2 and CYP81F2*) expression up-regulated and 12 h of dark recovery later, more transcription factor gene (*MYB51* and *MYB28*) and structural gene (*UGT74B1*, *MAM3*, *CYP79F1*, *CYP83A1* and *SOT17*) expression levels came back to the levels of the control (Figure 4).

DISCUSSION

The effects of enhanced UV-B radiation on the physiological status of *Arabidopsis* leaves

In plants, water is as an essential ingredient for

physiological activities. The measurement of RWC in tissue is essential to assess the extent of cellular water deficit (Barr and Weatherley, 1962). Additionally, REC could reflect the permeability of cell membrane. The changes of these two aspects could affect a series physiological of processes. Through, the measurement of REC and RWC could generally evaluate the damage level of the physiological satus of leaves which were under UV-B exposure (A-H-Mackerness et al., 2001; Ulm and Nagy, 2005).

The results (Figure 2) showed that the cell membrane of leaves was gradually damaged and the RWC was continuiously decreased during the UV-B treatment. Cell membrane degradation and lipid peroxidation by enhanced UV-B radiation was mainly caused by accumulated ROS which could lead to oxidative stress (Dai et al., 1997). After 12 h of recovery in darkness, the RWC of plants irradiated by UV-B returned to the control level and the REC decreased but could not recover to the control level (Figure 2). It supported that 12 h of enhanced UV-B radiation (1.55 $W·m^{-2}$) had negative effects to the physiological status of leaves, which could cause a series of physiological activities to respond, however, the damage could be partially recovered after recovery in the darkness for 12 h.

The biosynthesis of glucosinolates affected by enhanced UV-B radiation

Plants respond to UV-B irradiation by activating a large variety of defense responses. Multiple elements, including ROS, ethylene, jasmonates, salicylic acid, NO, CDPK and MAPK have frequently been discussed as putative components involved in the UV-irradiation signal-transduction chains (A-H-Mackerness et al., 2001; Caputo et al., 2006). These signaling molecules may act as an alert signal to induce protective responses, such as pathogenesis-related genes expression, secondary metabolites biosynthesis and so on. Glucosinolates are usually regarded as defense compounds against pathogens and insects and their biosynthesis were regulated by defense related signal transduction pathway (Dombrecht et al., 2007; Staswick, 2008). Some studies report that glucosinolate accumulation occurs due to biotic and abiotic stress, including herbivores and pathogen attack, mechanical wound (Beekwilder et al., 2008; Halkier and Gershenzon, 2006) and CO_2 enrichment (Schonhof et al., 2007). Schreiner et al. (2009) reported enhanced UV-B radiation led to an increase of glucosinolate concentrations in *T. majus* L. and regarded the increase of glucosinolate as a stress response.

Our results (Figure 4) showed that in the initial 1 h of UV-B exposure, some genes (*MYB51*, *OBP2*, *MYB76, SOT16* and *TGG1*) up-regulated, which were also induced by jasmonic acid and wounding (Dombrecht et al., 2007; Skirycz et al., 2006; Staswick, 2008). These transcription factors (*MYB51*, *OBP2* and *MYB76*) can positively mediate glucosinolate biosynthesis (Dombrecht et al., 2007; Gigolashvili et al., 2008; Skirycz et al., 2006). MYB51 and MYB76 specifically activate indolic and aliphatic glucosinolate biosynthesis, respectively, which might be responsible for the temporary and initiative increase of 4MSOB and I3M. After 3 h of treatment, *MYB28* and *MYB29,* the genes encoding the transcription factors that regulate aliphatic glucosinolate biosynthesis and *MYB34* that regulates indolic glucosinolate biosynthesis were down-regulated and structural genes (*CYP79F1*, *CYP83B1*, *MAM1*, *CYP79B2* and *CYP79B3*) encoding enzymes catalyse the synthesis of aliphatic glucosinolates were also down-regulated. Afterwards, the majority of genes were inhibited and the glucosinolate contents decreased. So the glucosinolate biosynthesis could be inhibited by continued 12 h of UV-B exposure since the expression levels of the related genes were declined. After 12 h of treatment, the plants irradiated by UV-B radiation were kept in the darkness for 12 h. Some of the genes that encode the key enzymes of the glucosinolate biosynthesis expressed differently during the dark recovery. The expression profiles of two homologous genes, *CYP79F1* and *CYP79F2*, were distinct. The up-regulation of *CYP79F2* expression might be responsible for the increase of 4MSOB, 3MSOP and the recovery of 8MSOO content. Similarly, *CYP79B3* rather than *CYP79B2* up-regulated at 2 h of the dark recovery*,* both of them encoded the enzymes converting tryptophan to indole-3-acetaldoxime (Zhao et al., 2002), which might be helpful to the recovery of the indolic glucosinolate content in the dark recovery process.

In recent years, some researches had already noticed that glucosinolate metabolism was affected by the UV-B radiation. Microarray data showed that the genes related to the biosynthesis of flavonoids, glucosinolates and terpenoids were differently expressed after UV-B radiation (Hectors et al., 2007). The study on *T. majus* demonstrated that the plant response to UV-B exposure is organ- and plant tissue age-specific, appropriate UV-B dosage could increased the glucotropaeolin concentration (Schreiner et al., 2009). However, some researches supported glucosinolate concentrations were unaffected by UV-B radiation in *Brassica oleracea*, *Nasturtium officinale* and *Sinapis alba* (Kuhlmann and Müller, 2009a, b; Reifenrath and Müller 2007). But these experiments were executed in greenhouse filtered or unfiltered the UV-B range, rather than using the additional UV-B tube to provide the enhanced UV-B radiation as the experiments previously described. So the controversies between the conclusions of these experiments probably caused by the differences of UV-B treatment methods. Our results provided evidence that enhanced UV-B radiation could affect the biosynthesis of glucosinolates.

In summary, we supposed that the increase of glucosinolate (GS) might be a stress response when

Arabidopsis leaves were irradiated by acute UV-B (1.55 $W{\cdot}m^{-2}$) for a short time (1 h). However, continuously UV-B radiation caused *Arabidopsis* leaves damaged, accumulated ROS could lead to oxidative stress (Dai et al., 1997), REC ascended and water content declined (Figure 2). As the deterioration of leaf physiological status, the expression of glucosinolate biosynthetic genes was inhibited and glucosinolate contents decreased. After the recovery in darkness, the extent of leaves damage was alleviated, as a result, the level of glusosinolate genes expression were partially recovered and total glucosinolate content came back to normal. The upregulation of several genes at early time-point in recovery period might be a kind of response to the recovery of physiological status. Further studies of related gene mutants will be helpful to understand the potential effects of glucosinolates in response to the enhanced UV-B radiation on *Arabidopsis*.

ACKNOWLEDGEMENTS

This work was supported by National Natural Science Foundation of China (No. 30670325 and No. 30528013) and the Program for New Century Excellent Talents in University (NCET-05-0328).

REFERENCES

A-H-Mackerness S, John CF, Jordan B, Thomas B (2001). Early signaling components in ultraviolet-B responses: distinct roles for different reactive oxygen species and nitric oxide. FEBS Letters, 489: 237-242.

A-H-Mackerness S, Surplus SL, Blake P, John CF, Buchanan-Wollaston V, Jordan BR, Thomas B (1999). UV-B induced stress and changes in gene expression in *Arabidopsis thalaina*: role of signaling pathways controlled by jasmonic acid, ethylene and reactive oxygen species. Plant Cell Environ., 22: 1413-1424.

Andrady A, Aucamp PJ, Bais A, Ballaré CL, Björn LO, Bornman JF, Caldwell M, Cullen AP, Erickson DJ, de Gruijl FR, Häder DP, Ilyas M, Kulandaivelu G, Kumar HD, Longstreth J, McKenzie RL, Norval M, Paul N, Redhwi HH, Smith RC, Solomon KR, Sulzberger B, Takizawa Y, Tang X, Teramura AH, Torikai A, van der Leun JC, Wilson SR, Worrest RC, Zepp RG (2009). Environmental effects of ozone depletion and its interaction with climate change: progress report, 2008. Photochem. Photobiol. Sci., 8: 13-22.

Ávila V, Bertolotti SG, Criado S, Pappano N, Debattista N, García NA (2001). Antioxidant properties of natural flavonoids: quenching and generation of singlet molecular oxygen. Int. J. Food Sci. Tech., 36: 25-33.

Barr HD and Weatherley PE (1962). A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust. J. Biol. Sci., 15:413-428.

Beekwilder J, van Leeuwen W, van Dam NM, Bertossi M, Grandi V, et al. (2008). The Impact of the Absence of Aliphatic Glucosinolates on Insect Herbivory in *Arabidopsis*. PLoS ONE, 3(4): e2068.

Brown PD, Tokuhisa JG, Reichelt M, Gershenzon J (2003). Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. Phytochemistry, 62: 471-481.

Casati P, Walbot V (2003). Gene expression profiling in response to ultraviolet radiation in maize genotypes with varying flavonoid content. Plant Physiol., 132: 1739-1754.

Chen S, Glawischnig E, Jørgensen K, Naur P, Jørgensen B, Olsen CE (2003). CYP79F1 and CYP79F2 have distinct functions in the biosynthesis of aliphatic glucosinolates in *Arabidopsis*. Plant J., 33: 923-937.

Clay NK, Adio AM, Denoux C, Jander G, Ausubel FM (2009). Glucosinolate metabolites required for an *Arabidopsis* innate immune response. Science, 323: 95-101.

Dai Q , Yan B, Huang S, Liu X, Peng S, Ma LL, Miranda, Chavez AQ, Vergara BS, Olszyk DM (1997). Response of oxidative stress defense systems in rice (*Oryza sativa*) leaves with supplemental UV-B radiation. Physiol. Plantarum, 101: 301-308.

Dombrecht B, Xue GP, Sprague SJ, Kirkegaard JA, Ross JJ, Reid JB, Fitt GP, Sewelam N, Schenk PM, Manners JM, Kazan K (2007). MYC2 differentially modulates diverse jasmonate-dependent functions in *Arabidopsis*. Plant Cell, 19: 2225-2245.

Gigolashvili T, Berger B, Mock HP, Müller C, Weisshaar B, Flügge UI (2007). The transcription factor *HIG1/MYB51* regulates indolic glucosinolate biosynthesis in *Arabidopsis thaliana*. Plant J., 50: 886-901.

Gigolashvili T, Engqvist M, Yatusevich R, Müller C, Flügge UI (2008). HAG2/MYB76, HAG3/MYB29 exert a specific and coordinated control on the regulation of aliphatic glucosinolate biosynthesis in *Arabidopsis thaliana*. New Phytol., 177: 627-642.

Halkier BA, Gershenzon J (2006). Biology and biochemistry of glucosinolates. Ann. Rev. Plant Biol., 57: 303-333.

Hectors K, Prinsen E, De Coen W, Jansen MA, Guisez Y (2007). *Arabidopsis thaliana* plants acclimated to low dose rates of ultraviolet B radiation show specific changes in morphology and gene expression in the absence of stress symptoms. New Phytol., 175: 255-270.

Hollosy F (2002). Effects of ultraviolet radiation on plant cells. Micron, 33: 179-197.

Kuhlmann F, Müller C (2009a). Development-dependent effects of UV radiation exposure on broccoli plants and interactions with herbivorous insects. Environ. Exp. Bot., 66: 61-68.

Kuhlmann F, Müller C (2009b). Independent responses to ultraviolet radiation and herbivore attack in broccoli. J. Exp. Bot., 60: 3467-3475.

Kuhlmann F, Müller C (2010). UV-B impact on aphid performance mediated by plant quality and plant changes induced by aphids. Plant Biol., 12: 676-684.

Ma JY, Liu JM, Li SK, Liang H, Liao RW, Ren SX, Bai YM (2007). UV-B Radiation and its ecological effects in North China Plain: a case study in the Gucheng Experimental Station. Res. Sci., 29: 32-39.

Mikkelsen MD, Petersen BL, Glawischnig E, Jensen AB, Andreasson E, Halkier BA (2003). Modulation of CYP79 genes and glucosinolate profiles in *Arabidopsis* by defense signaling pathways. Plant Physiol., 131: 298-308.

Müller PY, Janovjak H, Miserez AR, Dobbie Z (2002). Processing of gene expression data generated by quantitative realtime RT-PCR. Biotechniques, 32: 1372-1379.

Pang QY, Chen SX, Li LX, Yan XF (2009). Characterization of glucosinolate-myrosinase system in developing salt cress *Thellungiella halophila*. Physiol. Plant, 136: 1-9.

Petersen BL, Andreasson E, Bak S, Agerbirk N, Halkier BA (2001). Characterization of transgenic *Arabidopsis thaliana* with metabolically engineered high levels of *p*-hydroxybenzylglucosinolate. Planta, 212: 612-618.

Reifenrath K, Müller C (2007). Species-specific and leaf-age dependent effects of ultraviolet radiation on two Brassicaceae. Phytochemistry, 68: 875-885.

Reintanz B, Lehnen M, Reichelt M, Gershenzon J, Kowalczyk M, Sandberg G, Godde M, Uhl R, Palme K (2001). Bus, a bushy *Arabidopsis CYP79F1* knockout mutant with abolished synthesis of short-chain aliphatic glucosinolates. Plant Cell, 13: 351-367.

Reiss C (1994). Experiments in plant physiology. Prentice Hall Press, New Jersey USA, pp. 456.

Reymond P, Farmer EE (1998). Jasmonate and salicylate as global signals for defense gene expression. Curr. Opin. Plant Biol., 1: 404-411.

Schonhof HP, Kläring A, Krumbein, Schreiner M (2007). Interaction between atmospheric CO_2 and glucosinolates in broccoli. J. Chem. Ecol., 33: 105–114.

Schreiner M, Krumbein A, Mewis I, Ulrichs C, Huyskens-Keil S (2009). Short-term and moderate UV-B radiation effects on secondary plant metabolism in Different organs of nasturtium (*Tropaeolum majus* L.). Innovative Food Sci. Emerging Technol., 10: 93-96.

Skirycz A, Reichelt M, Burow M, Birkemeyer C, Rolcik J, Kopka J, Zanor MI, Gershenzon J, Strnad M, Szopa J, Mueller-Roeber B, Witt I (2006). DOF transcription factor AtDof1.1 (OBP2) is part of a regulatory network controlling glucosinolate biosynthesis in *Arabidopsis*. Plant J., 47: 10-24.

Staswick PE (2008). JAZing up jasmonate signaling. Trends Plant Sci., 13: 66-71.

Ulm R, Nagy F (2005). Signaling and gene regulation in response to ultraviolet light. Curr Opin Plant Biol 8: 477-482.

Yan X, Chen S (2007). Regulation of plant glucosinolate metabolism. Planta, 226: 1343-1352.

Zhao Y, Hull AK, Gupta NR, Goss KA, Alonso J, Ecker JR, Normanly J, Chory J, Celenza JL (2002). Trp-dependent auxin biosynthesis in *Arabidopsis*: involvement of cytochrome P450s *CYP79B2* and *CYP79B3*. Genes Dev., 16: 3100-3112.

Zhou BL, Arakawa K, Fujikawa S, Yoshida S (1994). Cold-induced alterations in plasma membrane proteins that are specifically related to the development of freezing tolerance in cold-hardy winter wheat. Plant Cell Physiol., 35: 175-182.