African Journal of Biotechnology Vol. 11(44), pp. 10161-10169, 31 May, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3603
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Arabidopsis rad23-4 gene is required for pollen development under UV-B light

Xiushan Li , Xinhong Guo, Lijian Zhao, Jihong Zhang, Dongying Tang, Xiaoying Zhao, Xuanming Liu*

Bioenergy and Biomaterial Research Center, College of Biology; Hunan University, Changsha, Hunan, P. R. China, 410082.

Accepted 20 January, 2012

Nucleotide excision repair (NER) is a highly conserved DNA repair pathway for correcting DNA lesions that cause distortion of the double helical structure. The protein heterodimer Rad23 is involved in recognition and binding to such lesions. Here, we showed that *rad23-4* (AT5g38470) was expressed in the roots, mature leaves, floral buds and developing siliques. The collapsed pollen grains were observed in ultraviolet (UV)-B–treated *rad23-4* mutants. Compared with the wild type (*WT*), the *rad23-4* mutants has decreased pollen germination efficiency (11.7-17.3%) and increased seeds abortion rate (12.5-18.9%). Furthermore, the *rad23-4* mutants has decreased anthocyanin production and showed changes in the expression of several NER homologous genes, including *Rad4*, *Rad10*, *and Rad16*. Our studies identified a previously uncharacterized role of *rad23-4* gene in regulating the pollen development and seed abortion, which is important for understanding the precise processes of NER in plant.

Key words: Nucleotide excision repair (NER), *rad23-4* gene, anthocyanin, pollen grains, seeds abortion.

INTRODUCTION

Plants are exposed to different physical, pharmacological or environmental factors that affect genome integrity (Hoeijmakers, 2001). Organisms have developed a number of highly conserved DNA repair systems to maintain the integrity of the genome, such as nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR) and double-strand break (DSB) repair (Puchta and Hohn, 1996; Vonarx et al.,1998; Britt, 1999). NER is a versatile DNA repair mechanism that can remove various types of DNA lesions (Reed and Gillette, 2007; Klimyuk and Jones, 1997; Doutriaux et al., 1998; Gallego et al., 2000; Hartung and Puchta, 2000; Hays, 2002).

The various NER proteins exist *in vivo* as part of multiprotein subassemblies named *NEFs* (nucleotide excision repair factors). For instance, *Rad14* and *Rad1–Rad10* form a subassembly *NEF1*; *Rad2* and *TFIIH* constitute *NEF3*; and *Rad7-Rad16* and *Rad23-Rad4* complexes are named *NEF4* and *NEF2,* respectively (Prakash and Prakash, 2000). Among these *NEFs*, *Rad23*, the ortholog of human *hHR23A* and *hHR23B*, is involved in the recognition of photolesions in NER (Masutani et al., 1994; Min and Pavletich, 2007). *Rad23* was first discovered in yeast (Guzder et al., 1998). Recent studies showed that *Rad23* contributed to diverse cellular functions including DNA repair, stress response and development (Smalle and Vierstra, 2004; Dreher and Callis, 2007; Vierstra, 2009; Lisa-Farmer and Vierstra, 2010). Experiments with mice also showed that similar effects occurred in mammalian cells (Ng et al., 2003; Okuda et al., 2004). These studies have markedly improved our understanding of NER in eukaryotes (Guzder et al., 1998; Aboussekhra et al., 1995; Guzder et al., 1995), but our knowledge about the exact processes is rather limited in plant kingdom.

Enhanced exposure to UV light is potentially detrimental to all above ground parts of higher plants, including reproductive organs (Demchik and Day, 1996; Van de Staaij et al., 1997). It affects plants by modifying both their biological and chemical environment. UV-B irradiation can induce accumulations of anthocyanin in the plants (Tahara et al., 2007). The anthocyanin compounds func-

*Corresponding author. Email: sw_xml@hnu.cn.

tion as defense against phytopathogens and ultraviolet light (Sapir et al., 2008). UV-B can cause serious damage to DNA, membrane, and proteins (Hollosy, 2002). Damage induced by UV-B can be diverse to morphogenetic changes in plants: Decrease of height, altered emergence, seed production (Hollosy, 2002; Jansen et al., 2008), and its consequences include a reduction in the growth yield, changes in levels of plant hormones and alteration of periods of flowering (Kakani et al., 2003).

With a view to study the role of *Rad23* protein in plant development, we adopted a reverse genetic approach to obtain mutants for each of these genes. Our experimental results showed that *Rad23-4* played an important role in pollen development, and pollen from plants of *Rad23-4* under UV-B-treatments has lower fertility. Therefore, it is important to investigate the exact function of NER genes on plants development.

MATERIALS AND METHODS

Plant materials and growth conditions

The T-DNA insertion mutants (SALK_014137, SALK_120646) of *Rad23* gene in *Arabidopsis* were purchased from the ABRC (Arabidopsis Biological Resource Center, OH, USA). Plants were grown in a culture chamber under a 16-h photoperiod (22°C). As for the soil cultured plants, seeds were sown in pots at a density of 20 seeds per pot. The pots were kept in the dark at 4°C for 3 days, and then transferred to a phytotron kept under a regime of a 16 h photoperiod (22°C; 70% humidity).

Isolation of Rad23 knock-out allele

To identify individuals homozygous for the T-DNA insertion, the primers specific for the T-DNA left border and *AtRad23-4* (AT5g38470) gene were used. The T-DNA insertion site in the allele was described in the Salk Institute website (http://signal.salk.edu) and confirmed by PCR-based genotyping. The PCR amplification profile consisted of an initial denaturation step at 94°C for 5 min, followed by twenty-two cycles of 94°C for 20 s, 55°C for 30s, and 72°C for 20 s. The sense and antisense primers of *AtRad23-4-1* (SALK_014137) are 5'-TCCCTAAACGATGAAGAT-3' and 5'-GTGTCACGGTCCCAACTA-3', respectively. The sense and antisense primers of *AtRad23-4-2* (SALK_120646) are 5'-GATCCACCAAGGAAAGGT-3' and 5'-TGAAGCATAGGCTGTAGAAT-3', respectively. The left border primer used to amplify the T-DNA insertions is 5'-TGGTTCACGTAGTGGGCCATCG- 3'.

UV-B treatment and phenotypic analysis

Fluorescence sunlamps (30-W, Baoji Lamp Factory, China) were suspended above and perpendicular to the plates and filtered with 0.13 mm thick cellulose diacetate (transmission down to 290 nm) for UV-B irradiance or 0.13 nm polyester plastic films (absorbs all radiation below 320 nm) as a control (He et al., 2006). Plants were exposed to enhanced UV-B for 0-6 h daily for one week. After UV-B treatment, the mature flowers from *rad23-4* mutant and *WT* were isolated. Pollen germination was assayed by suspending pollen in medium containing 5 mM MES (pH 5.8), 1 mM KCl, 10 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1.5 mM boric acid, 2% sucrose, and 24% polyethylene glycol, which was modified from the basic germination medium reported by (Fan et al., 2001). Pollen was germinated for 4 h at room temperature prior to the assessment of germination efficiency, and then observed with light microscopy (Nikon PE2000). The other half of the mature flowers was immediately frozen in liquid nitrogen for future RNA isolation. Each experiment was repeated three times. All the reported values were expressed as the means of nine replicates ± SE (standard error).

Anthocyanin production estimation

Anthocyanin production of plant leaf discs was determined in 95% ethanol, as described by (Mancinelli et al., 1975). All experiments were repeated at least three times.

Polyclonal antibody preparation and western-blotting analysis

Standard immunization protocols were used to generate polyclonal antibodies to *Arabidopsis* Rad23 protein. Briefly, recombinant pCold-Rad23-4 protein (Li et al., 2011) was mixed with equal volume of Freund's incomplete adjuvant and injected subcutaneously into two mice (1-month-old). Initial immunization was followed by three booster injections at 2-week intervals. Antiserum was collected after 10 weeks of killing the animals.

Western blotting analysis was performed using the antiserum to visualize the plant Rad23-4 protein. The tissue protein was transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore) followed by separation on a 10% (v/v) SDS-PAGE under 20 mA current for 1 h in the transfer buffer (CAPS). The membrane was blocked for 1 h at the room temperature, and subsequently washed five times in Phosphate Buffered Saline Tween (PBS-T). After 1 h incubation with the antiserum at 1:3000 dilution, the membrane was washed with PBS-T and incubated for 1 h at the room temperature with the secondary antibody (anti-mouse-IgG conjugated with HRP) at 1/3000 dilution. Finally, the band was revealed by the peroxidase activity on 4-chloro-1-naphthol (Sigma) as the chromogenic substrate.

The slides preparation and immunohistochemistry analysis

The slides were kept in a moist chamber during all incubations. Sections were first blocked with 10% (v/v) normal goat serum in PBST buffer (10 mM Na-phosphate, pH 7.2, 0.15 M NaCl, 0.1% (v/v) Tween 20) for 1 h followed by incubation with the antiserum at 1/3000 dilution in PBST, overnight at 4°C. The slides were rinsed four times in PBST (5 min per wash), and incubated with the secondary antibody (anti-mouse-IgG conjugated with HRP) at 1:3000 in PBST for 1 h and finally rinsed four times in PBST (5 min per wash), and six times in sterile water (5 min per wash). Sections were then treated with 3, 3′-diaminobenzidine (DAB) for 5 min according to the supplier's instructions. The positive pollens were stained brown. Each slide was enhanced in the dark for 20 min at room temperature, and rinsed for 4 min under a gentle stream of deionized water. The sections were examined under a Nikon microscope.

Gene expression analysis

Total RNAs were extracted from the 4-week-old *in vitro* germinated *Rad23-4* mutants and *Col-0* plants which were irradiated with a dose of 30 Kergs/cm^2 of UV-B usingTrizol reagent (Invitrogen Corporation, USA), and then treated with RNase-free DNase I (Promega Biotech Corporation, USA). The Dnase-digested RNA sample was used for

Table 1. the sense and antisense primers for *AtRad1*, *AtRad2*, *AtRad7*, At*Rad10*, *AtRad16,* and *AtRad4.*

Gene	Sense primer	Antisense primer
AtRad1:	5'- CAACAGGGAAGAGGAAGC-3'	5'- TTGACGCATAGCCAAACT-3'
AtRad2:	5'- TGACCGAATCTACAACGA-3'	5'- TTCTGAGTAATGCCCTAT-3'
AtRad7:	5'- GCAACTTCGAGCTACCCG-3'	5'-CAGGCTTCAATAACCAACCA-3'
AtRad10:	5'-AGATACAACAACGGCGTCAG-3'	5'-CAGGCTTCAATAACCAACCA-3'
AtRad16:	5'- TTTCACGGCTAAACCAT-3'	5'- CATACAACCGCTTCACCT-3'
AtRad4:	5'- GGAACAAGGAGGAAAGGA-3'	5'- CGAGATGTACTAACGGTGC-3'

reverse transcription by Superscript III reverse transcriptase (Invitrogen Corporation, USA). Samples which served as cDNA stocks for PCR analysis were stored at -80°C. A semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was used for gene expression analysis. RT-PCR was performed in a 20 µL solution containing 1µL cDNA stock using rTaq DNA polymerase (TaKaRa Dalian, China) on a programmable thermal cycler (Biometra T-gradient 96050-801, Germany). The PCR amplification profile consisted of an initial denaturation step at 94°C for 5 min, followed by twenty-two cycles at 94°C for 20 s, 60°C for 30 s, and 72°C for 20 s. The sense and antisense primers for *AtRad1*, *AtRad2*, *AtRad7*, At*Rad10*, *AtRad16,* and *AtRad4* are listed in Table 1.

RESULTS

Isolation and analysis of T-DNA insertion mutant of *Rad23-4*

Homozygous individuals were identified by PCR and RT-PCR analyses. The SALK _120646 has a T-DNA insertion in its first exon, and the SALK_014137 has a T-DNA insertion in its second exon (Figure 1A). The homozygous was identified by the three-primer method. The first column has no band suggesting negative insertion of T-DNA, while the second and the third columns show significant bands revealing the positive insertion of T-DNA (Figure 1B). Three homozygous and two heterozygous individuals were found to have SALK_120646 (Figure 1B), SALK_014137 was identified in four individuals homozygous and one heterozygous. The homozygous A, B, and C of *rad23-4* mutants were selected for further studies. Semi-quantitative RT-PCR results demonstrated that the insertion of T-DNA severely impaired the expression of *rad23-4* (Figure 1C). However, the *rad23-4* homozygous mutants did not show any obvious phenotypic defects when grown under normal conditions (data not shown).

Rad23-4 gene expression analysis

With a view to investigate the tissue expression levels of the *rad23-4* gene, RNA was isolated from roots, mature leaves, floral buds and developing siliques. It was found that *rad23-4* gene was expressed at differential levels in all tissues examined with the highest level in floral buds (Figure 2A). Further examination by dissecting developing floral buds into several developmental stages (Smyth et al., 1990), revealed a high-level expression of the *rad23-4* gene in mature floral buds, but a low-level expression in early floral buds (Figure 2B).

Expression analysis of *Rad23-4* protein

To investigate the expression patterns of *Rad23-4* protein, we constructed the recombinant protein of *pCold-Rad23-4*. The purified recombinant protein was used to generate polyclonal antibodies. Extracts from various plant tissues were tested for *Rad23-4* protein by western blotting. Antibodies recognized one band representing the expected size of *Rad23-4* proteins (Li et al., 2011). The *Rad23-4* protein was detected in mature leaves, floral buds, and roots, with the highest expression level in the floral buds (Figure 2C), which was consistent with the RT-PCR results.

Immunolocalisation studies were performed on sections of *WT* and *rad23-4* in parallel to gain insight into the spatial expression pattern of *Rad23-4* protein. At the pollen mother cell stage, the *Rad23-4* protein was not detected at the control (Figure 2E) and *rad23-4* floral buds (Figure 2D). However, the *Rad23-4* protein was expressed abundantly in the mature pollen grains of *WT* floral buds (Figure 2F), indicating that pollen is the main organ in floral bud for the gene expression.

Effects of *rad23-4* mutant on pollen morphology

The development of pollen grains from WT and *rad23-4* mutants was investigated under white light and UV-B–treated condition. Many pollen grains were collapsed, and incapable of forming pollen tubes or plugs after UV-B–treated. We scored percentage of the collapsed pollen grains from the *WT* (Figure 3A, B, C and D) and the *rad23-4* (Figure 3E, F, G and H) plants. Compared with the *WT,* the *rad23-4* mutant number of collapsed pollen grains decreased by 11.7-17.3% after UV-B–treated for 6 h (Figure 3I).

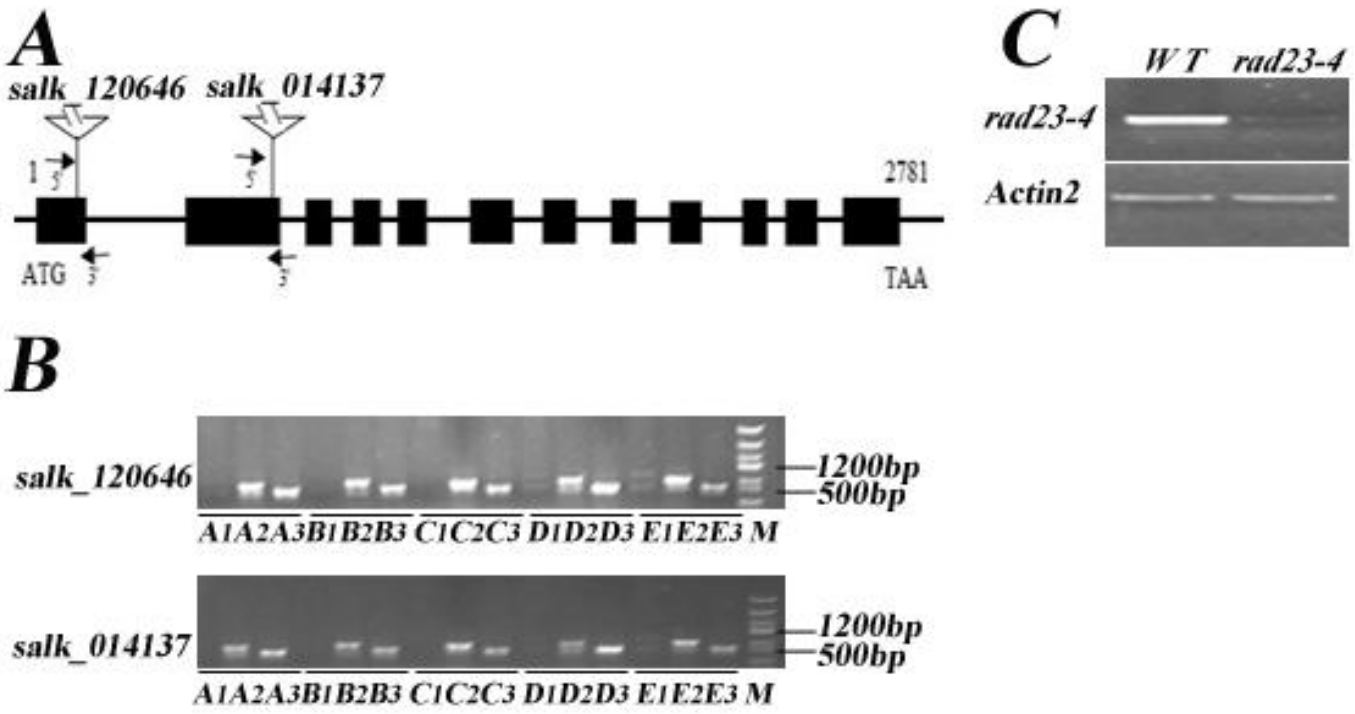

Figure 1. Isolation and identification of *Rad23-4* T-DNA insertion mutant. A. Scheme of the *rdad23-4* gene and localization of the T-DNA insertions of SALK_014137 and SALK_120646. B. Genomic DNA PCR products generated from *rad23-4* with various primer combinations. Lanes A1, B1, C1, D1, E1 correspond to PCR products using the primers F-R. Lanes A2, B2, C2, D2 and E2 correspond to PCR products using the primers F-LBpROK2; lanes A3, B3, C3, D3 and E3 correspond to PCR products using the primer LBpROK2 + R; lane M, DNA Marker. C. RT-PCR result shows absence of *Rad23-4* transcript in mutant genotype. Actin2 is used as an internal control.

Effects of *rad23-4* mutant on seeds development and anthocyanin production

The seed were harvested from 30 siliques in adult plants, and the rates of seed abortion were calculated. A number of ovules were aborted by UV-B light treatment in *rad23-4* plants whereas ovule development was less affected in wilt plants (Figure 4A and B). The rate of seed abortion of *rad23-4* plants at the ripening phase increased by 12.5-18.9% compared with *WT* (Figure 4C).

We also investigated the anthocyanin content of *WT* and *rad23-4* mutants. Compared to *WT*, the anthocyanin production of the *rad23-4* mutants decreased after UV light treatment (Figure 4D). The increased anthocyanin production of *WT* plants was mainly induced by the UV light (Figure 4D). However, the UV light treatment could not enhance the anthocyanin production in *rad23-4* mutants.

Expression analysis of a series of NER genes

Compared to the *WT*, the viability of pollen grains of the *rad23-4* mutants decreased under UV light condition (Figure 3I). As we know, the plant cell utilizes nucleotide excision repair (NER) to remove UV-induced DNA damage and various other bulky DNA lesions from the genome (Hoeijmakers, 2001). To test whether the *rad23-4* plays role in NER genes, we investigated the NER genes levels in the transgenic plants and the *WT* under UV-B treatment. The transcript level of the *Rad4* gene involved in the early step of recognition of UV DNA damage was found to be down-regulated in the *rad23-4* mutants after UV-B exposure (Figure 5A and B). Moreover, genes, *Rad2* and *Rad10*, encoding components involved in damage recognition were found to be slightly up-regulated in the UV-B treated *rad23-4* mutants (Figure 5A and B). There were no measurable differences in the expression levels of *Rad1, Rad7, Rad16* genes (Figure 5A and B). Therefore, it can be concluded that the reduced transcript level of the *Rad4* gene was the primary reason resulting in the high sensitivity of the *rad23-4* mutant to the UV-B light.

DISCUSSION

In this study, UV-B–treated *rad23-4* mutant plants were analyzed and compared with the control plants. Experimental results show that the predominant phenotype decreased efficiency of the pollen grains

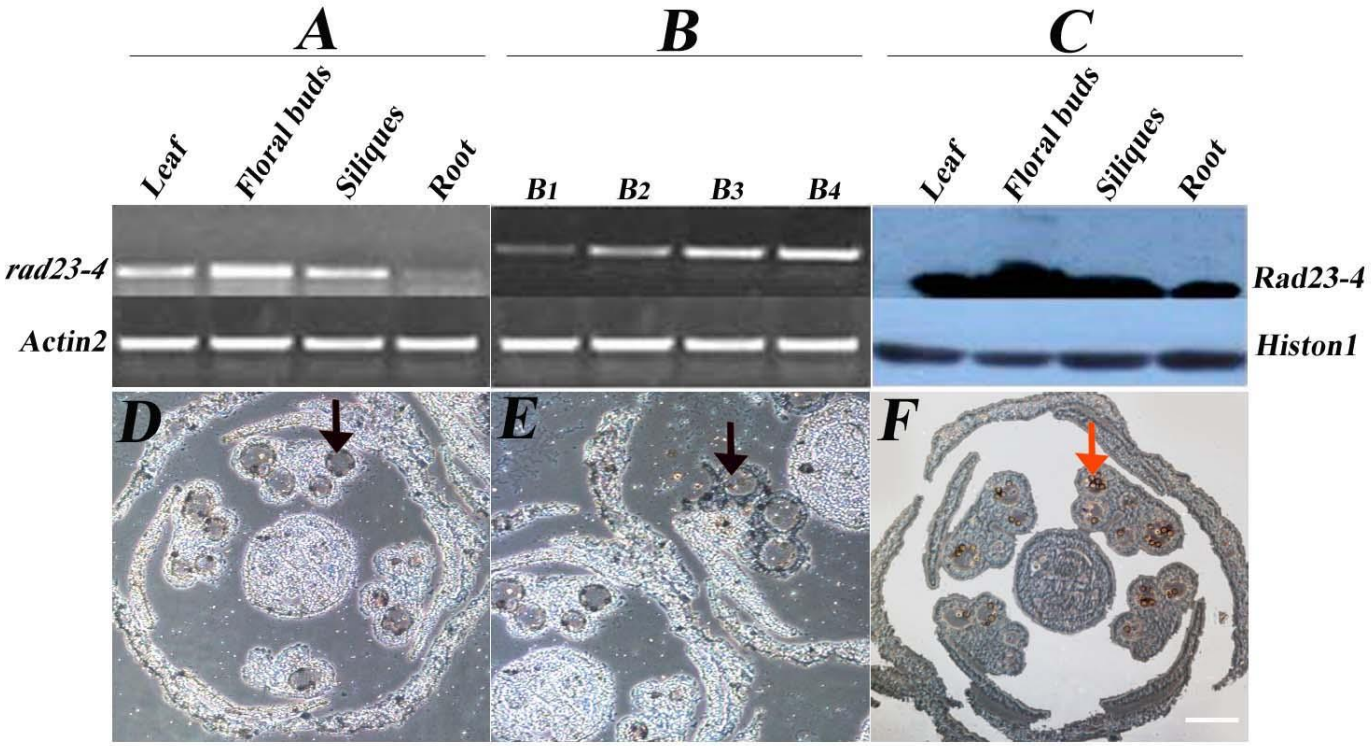

Figure 2. Temporal and spatial expression patterns of Rad23-4. (A). The RT-PCR analysis of *Rad23-4* gene in mature leaves, floral buds, developing siliques and roots of *col-0* wild type plants. Actin2 is used as an internal control. These experiments were repeated at least three times. A representative result is given. (B). Expression of the *Rad23-4* gene in floral buds. Sample B1 is the collection of the floral buds belonging to flower development stages 1-8 (flower development stages according to Smyth et al., 1990). Samples B2 and B3 include the floral buds of flower development stages 9-10 and 11-13, respectively. (C). Total protein was collected from mature leaves, floral buds, developing siliques and roots of *col-0* plants for western blot. Antibody against *Rad23-4* fusion protein were used as first antibody, HRP conjugated antibody against mouse IgG were used as the second antibody, and Histon 1 was used as internal control. (D, E, and F). Immunolocalisation analysis revealed specific *rad23-4* protein activity in the anther. The immunostaining is not detectable in the in r*ad23-4* floral buds (D). Control section from the same block in which the antiserum was replaced by pre-immun serum (E) Strong staining is seen in the *col-0* pollen grains (F). The r*ad23-4* protein expressions are indicated by red arrow heads. Control is indicated by black arrowheads, the scale bars are 50 μm.

germination and increased rate of seeds abortion. In addition, the contents of transcription expression of *Rad4* gene and the anthocyanin production was markedly reduced.

UV-B radiation (280-320 nm) has long been known to cause damage to life. NER is the main mechanism responsible for removing various types of DNA lesions (McCready et al., 2000). Previous studies showed that *Rad4* and *Rad23* formed a complex which is essential in a reconstituted NER reaction *in vitro*. Moreover, the *Rad4-Rad23* protein complex was found to be involved in DNA damage recognition and the assembly and disassembly of NER complexes (Guzder et al., 1995). Our experiments showed that compared with the *WT*, the viability of the mutant pollen grains decreased significantly after UV-B treatment (Figure 3I). The abortion rate of the seeds of mutant plants was about 12.5-18.9% higher than the corresponding ones of the *WT* (Figure 4C). These results indicate that the mutant has a high sensitivity to UV-B treatment. More interestingly, our experiments revealed that the level of *Rad4* mRNA decreased obviously in the *rad23-4* after UV-B treatment for 0-6 h (Figure 5A and B), the steady-state mRNA level of *Rad2*, *Rad10* encoding gene slightly increased in the *rad23-4* floral buds (Figure 5A and B). In contrast, the steady-state mRNA levels of *Rad1*, *Rad7*, and *Rad14* genes showed significant changes in the UV-B-treated floral buds (Figure 5A and B). Based on these facts, we speculated that the decreased level of *Rad4* mRNA might be the major reason leading to the high sensitivity of the *rad23-4* mutant pollen grains to UV light.

Previous study indicated that the anthocyanin compounds play roles in a variety of functions in flower pigmentation, maintaining the redox homeostasis, defending against phytopathogens, as well as protection against ultraviolet light (Gonza´lez-Gallego et al., 2007). It is interesting to note that the UV light can induce the anthocyanin accumulated in leaf epidermal cells, where

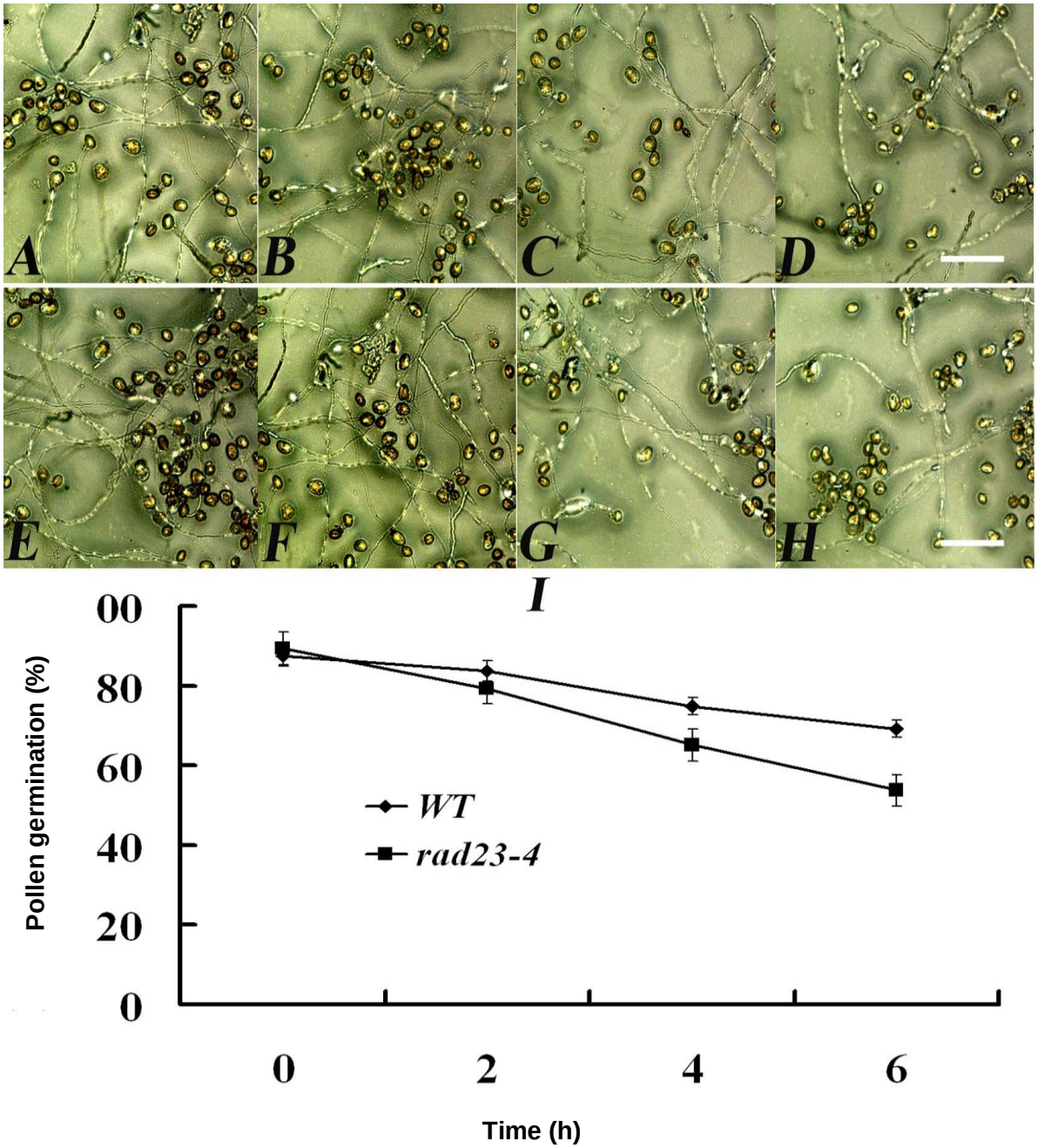

Figure 3. Germination analysis of pollen grains from WT and *rad23-4* mutant plants.Pollen germination for 4 h on the medium prepared according to Thorsness et al. (1993). A. Wild-type pollen germination without UV-B light treated; B,C,D wild-type pollen germination with UV-B light treated for 2, 4, and 6 h; E. *rad23-4* pollen germination without UV-B light treated; F, G, H. *rad23-4* pollen germination with UV-B light treated for 2, 4, and 6 h. I. The pollen grains germination rate of *col-0* and *rad23-4* mutant plants after UV-B light treated and each sample was tested in triplicate. The scale bars are 50 μm.

they may protect the inner cell layers from UV-B damage (Caldwell et al., 1983). In this study, our observation confirmed that the pollen grains of mutants showed a higher sensitivity to UV light and a decreased anthocyanin production than the *WT* plants after UV light treatment (Figure 4C). These results indicate that the decreased level of anthocyanin production in the *rad23-4* mutant might be another reason accounting for the high sensitivity of the *rad23-4* mutant to UV light.

In conclusion, we have proved for the first time that *Rad23-4* protein acts as a regulator of pollen development and seeds abortion under UV light. Our future studies will

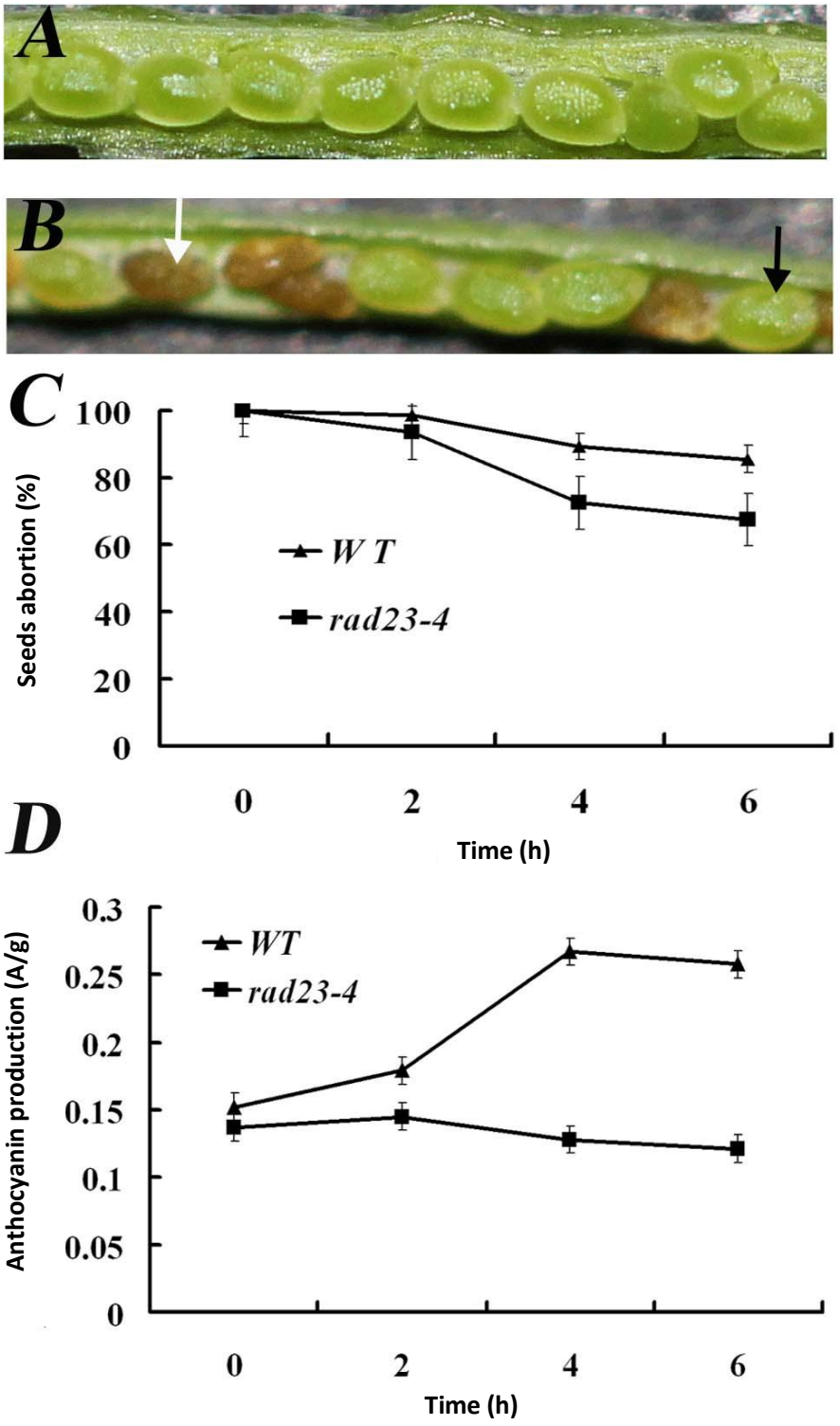

Figure 4. Seed development and anthocyanin production in *WT* and *rad23-4* mutant plants. The *rad23-4* plants are semi-sterile. Pictures are made of siliques from self-fertilized wild-type (A) and homozygous *rad23-4* plants (B). Aborted ovules are indicated by white arrowheads, normal ovules are indicated by black arrowheads. C. The seed abortion frequency of *col-0* and *rad23-4* mutant plants after UV-B light treated, and each sample was tested in triplicate. D. Effects of UV-B exposure on anthocyanin accumulation in the *col-0* and *rad23-4* mutant plants, sample was harvested at the same time points, anthocyanin accumulation was evaluated and each sample was tested in triplicate.

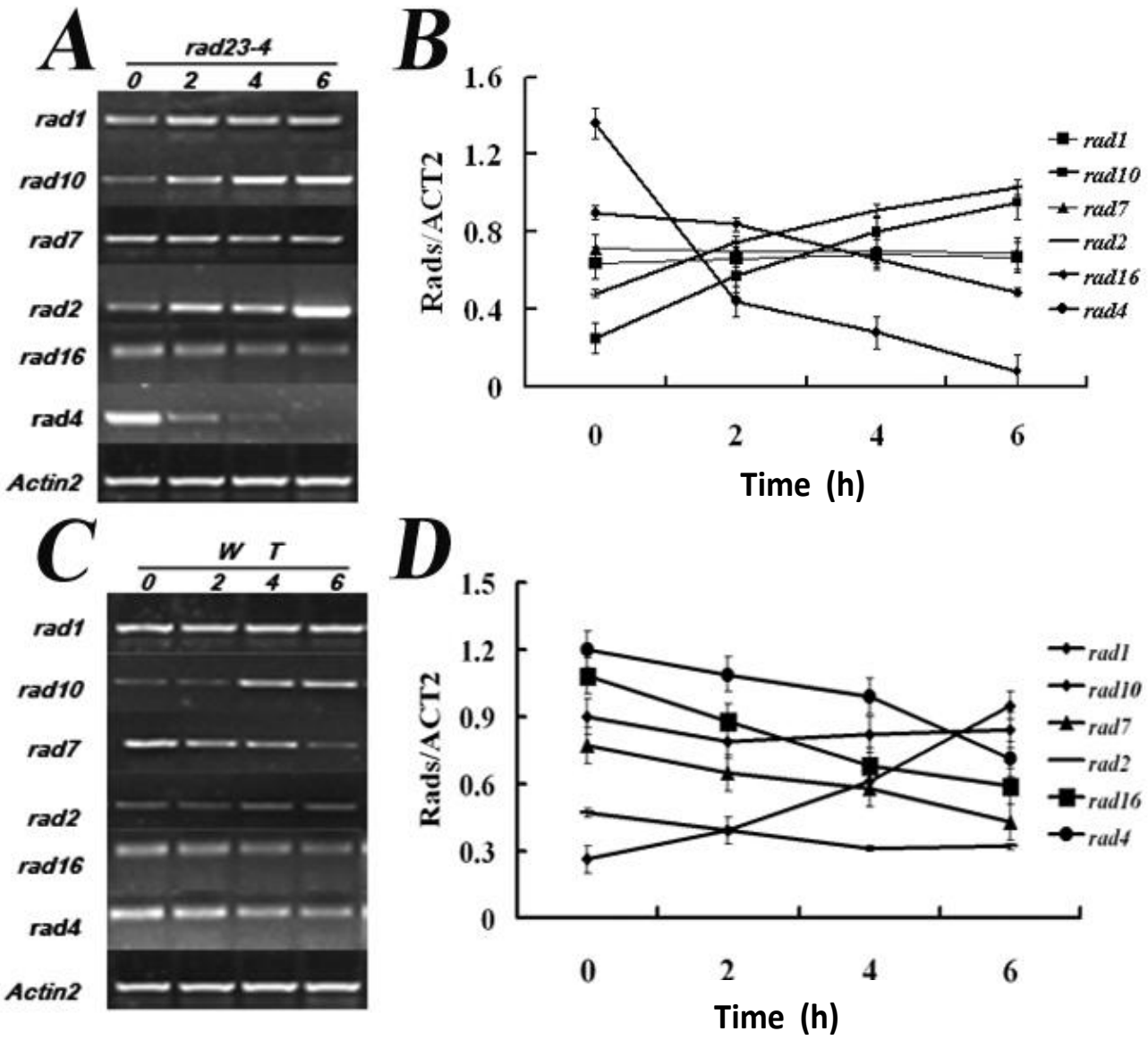

Figure 5. RT-PCR expression analysis of the NER homeotic genes in *rad23-4* and *WT* plants. The level of mRNAs in each case was normalized to that of actin2. The data were derived from three biological independent sets from *col-0* (A)and *rad23-4* mutant plants(B), and each sample was tested in triplicate. The relative amount of NER homeotic genes were determined by calculating the mean of the median values for the RNA accumulation in each three biological replicates.The relative signal intensities of the NER homeotic genes in *rad23-4* plants(C).The relative signal intensities of the NER homeotic genes in *col-0* plants(D).

focus more on the relationship between *Rad23* protein and the damage repair caused by UV-B.

ACKNOWLEDGMENTS

The authors acknowledge the final support of National Natural Science Foundation of China (Grant No. 30770200, 30871325, and 31071076), Program for New Century Excellent Talents in University (2010), and Science Foundation for Distinguished Young Scholars of Hunan Province (Grant No. 11CB004).

REFERENCES

Aboussekhra AM, Biggerstaff Shivji JA, Vilpo MKK, Moncollin VN, Produst M, Proti C, Hiibscher U, Egly JM, Wood RD (1995). Mammalian DNA nucleotide excision repair reconstituted with purified protein components. Cell, 80: 859-868.

Britt AB (1999). Molecular genetics of DNA repair in higher plants.Trends Plant Sci. 4: 20-24.

Caldwell MM, Robberecht R, Flint SD (1983). Interna1 filters: Prospects for UV-acclimation in higher plants. Physiol. Plant. 58: 445-450.

Doutriaux MP, Couteau F, Bergounioux C, White C (1998). Isolation and characterisation of the RAD51 and DMC1 homologs from Arabidopsis thaliana. Mol. Gen. Genet. 257: 283-291.

Dreher K, Callis J (2007). Ubiquitin, hormones and biotic stress in plants. Ann. Bot. (Lond). 99: 787-822.

Demchik SM, Day T (1996). Effects of enhanced UV-B radiation on pollen quantity, quality and seed yield in *Brassica rapa* (Brassicaceae). Am. J. Bot. 83: 573-579.
Fan LM, Wang YF, Wang H, Wu WH (2001). *In vitro* Arabidopsis pollen germination and characterization of the inward potassium currents in Arabidopsis pollen grain protoplasts. J. Exp. Bot. 52: 1603-1614.
Gallego F, Fleck O, Li A, Wyrzykowska J, Tinland B (2000). AtRAD1, a plant homologue of human and yeast nucleotide excision repair endonucleases, is involved in dark repair of UV damages and recombination. Plant J. 21: 507-518.
Gonza´lez-Gallego J, Sa´nchez-Campos S, Tun MJ (2007). Antiinflammatory properties of dietary flavonoids. Nutr. Hosp. 22: 287-293.
Guzder SN, Sung P, Prakash L, Prakash S (1998). Affinity of yeast nucleotide excision repair factor 2, consisting of the Rad4 and Rad23 proteins, for ultraviolet damaged DNA. J. Biol. Chem. 273: 31541-31546.
Guzder SN, Habraken Y, Sung P, Prakash L, Prakash S (1995b). Reconstitution of yeast nucleotide excision repair with purified Rad proteins, replication protein A, and transcription factor TFIIH. J. Biol. Chem. 370: 12973-12976.
Hartung F, Puchta H (2000). Molecular characterisation of two paralogous SPO11 homologues in Arabidopsis thaliana. Nucleic Acids Res. 28: 1548-1554.
Hays JB (2002).Arabidopsis thaliana, a versatile model system for study of eukaryotic genome-maintenance functions. DNA Repair, 1: 579-600.
He JM, Liu ZH, Xu H, She XP, Chen H (2006).The involvement of hydrogen peroxide in UV-B-inhibited pollen germination and tube growth of Paeonia suffruticosa and Paulownia tomentosa in vitro. Plant Growth Regul. 49: 199-208.
Hoeijmakers JH (2001). Genome maintenance mechanisms for preventing cancer. Nature, 411: 366-374.
Hollosy F (2002) Effects of ultraviolet radiation on plant cells. Micron. 33: 179-197.
Jansen LET, Verhage RA, Brouwer J (1998). Preferential binding of yeast Rad4-Rad23 complex to damaged DNA. J. Biol. Chem. 273: 33111-33114.
Jansen MAK, Hectors K, O'Brien NM, Guisez Y, Potters G (2008). Plant stress and human health: Do human consumers benefit from UV-B acclimated crops? Plant Sci. 175: 449-458.
Kakani VG, Reddy KR, Zhao D, Sailaja K (2003). Field crop responses to ultraviolet-B radiation: a review. Agric. Forest Meteorol. 120: 191-218.
Klimyuk VI, Jones JD (1997). AtDMC1, the Arabidopsis homologue of the yeast DMC1 gene: Characterization, transposoninduced allelic variation and meiosis-assiociated expression. Plant J. 11: 1-14.
Lisa Farmer M, Vierstra RD (2010). The RAD23 Family Provides an Essential Connection between the 26S Proteasome and Ubiquitylated Proteins in Arabidopsis. Plant Cell, 22: 124-142.
Li X, Zhao L, Tang D-y, Zhao X, Liu B, Liu X (2011). Purification of Arabidopsis Rad23 Recombinant Protein in Engineering Bacteria and Preparation of Its Polyclonal Antibody. J. Hunan University Sci. 11: 66-69.
Masutani C, Sugasawa K, Yanagisawa J, Sonoyama T, Ui M, Enomoto T, Takio K, Tanaka K, van der Spek PJ, Bootsma D (1994). Purification and cloning of a nucleotide excision repair complex involving the xeroderma pigmentosum group C protein and a human homolog of yeast RAD23. EMBO J. 13: 1831-1843.
Mancinelli A, Yang C, Lindquist P, Anderson OR, Rabino I (1975). Photocontrol of anthocyanin synthesis. III. The action of streptomycin on the synthesis of chlorophyll and anthocyanin. Plant Physiol. 55: 251-257.
McCready SJ, Osman F, Yasui A (2000). Repair of UV damage in the fission yeast *Schizosaccharomyces* pombe. Mutat. 451: 197-210.
Min JH, Pavletich NP (2007). Recognition of DNA damage by the Rad4 nucleotide excision repair protein. Nature, 449: 570-575.
Ng JM, Vermeulen W, van der Horst GT, Bergink S, Sugasawa K, Vrieling H, Hoeijmakers JH (2003). A novel regulation mechanism of DNA repair by damage-induced and RAD23-dependent stabilization of xeroderma pigmentosum group C protein. Genes Dev. 17: 1630-1645.
Okuda Y, Nishi R, Ng JM, Vermeulen W, van der Horst GT, Mori T, Hoeijmakers JH, Hanaoka F, Sugasawa K (2004). Relative levels of the two mammalian Rad23 homologs determine composition and stability of the xeroderma pigmentosum group C protein complex. DNA Repair. 3: 1285-1295.
Puchta H, Hohn B (1996). From centimorgans to base pairs: Homologous recombination in plants. Trends Plant Sci. 1: 340-348.
Prakash S, Prakash L (2000). Nucleotide excision repair in yeast. Mutat. Res. 451: 13-24.
Reed SH, Gillette TG (2007). Nucleotide excision repair and the ubiquitin proteasome pathway-do all roads lead to Rome? DNA Repair. 6: 149-156.
Sapir M, Oren-Shamir M, Ovadia R, Reuveni M, Evenor D, Tadmor Y, Nahon S, Shlomo H, Chen L, Meir A, Levin I (2008). Molecular aspects of anthocyanin fruit tomato in relation to high pigment-1. J. Hered. 99: 292-303.
Smalle J, Vierstra RD (2004). The ubiquitin 26S proteasome proteolytic pathway. Annu. Rev. Plant Biol. 55: 555-590.
Smyth DR, Bowman JL, Meyerowitz EM (1990). Early Xower development in Arabidopsis. Plant Cell, 2: 755-767.
Tahara S (2007). A journey of twenty-five years through the ecological biochemistry of flavonoids. Biosci. Biotechnol. Biochem. 71: 1387-1404.
Vonarx EJ, Mitchell HL, Karthikeyan R, Chatterjee I, Kunz BA (1998). DNA repair in higher plants. Mutat. Res. 400: 187-200.
Vierstra RD (2009). The ubiquitin-26S proteasome system at the nexus of plant biology. Nat. Rev. Mol. Cell Biol. 10: 385-397.
Van de Staaij JWM, Bolink E, Rozema J, Ernst WHO (1997). The impact of elevated UV-B (280-320 nm)radiation levels on the reproduction biology of a highland and a lowland population of Silene vulgaris. Plant Ecol. 128: 172-179.