Universität für Bodenkultur

University of Natural Resources and Applied Life Sciences


Department of Biotechnology

Institute for Applied Microbiology

# Natural variation in tolerance of UV-C induced DNA damage in *Arabidopsis thaliana*

**Diploma Thesis**

**Johannes Rungger**

**Supervisors:**
**Prof. Dr. Ortrun Mittelsten Scheid**
**Dr. Ales Pecinka**
**Prof. Dr. Margit Laimer**

Vienna, November 2010

# Table of Contents

# Abstract

DNA is the carrier of the genetic information from cell to daughter cell and from one generation to the next. However, DNA gets damaged by various endogenous and exogenous factors, leading to a large spectrum of lesions. Absorption of UV by DNA leads to the photoproducts cyclobutane pyrimidine dimer (CPD) and 6-4 photoproducts, inhibiting DNA replication and transcription and causing mutations. Plants developed strategies to repair such lesions by direct reversal through photolyases, excision repair, lesion bypass and recombinational repair. This thesis asks for natural variation of UV-C tolerance among different accessions of *Arabidopsis thaliana* and for potential loci underlying the variation. Indeed, there is a high degree of variation among Arabidopsis collected from different locations with regard to their ability to recover from UV-C irradiation. Phenotypes ranged from extreme tolerance to hypersensitivity. QTL mapping of a Ler-1/Cvi-0 RIL population revealed two major independent QTLs at the top arm of chromosome 3. Linkage-based fine mapping defined the intervals of QTL1 and QTL2 to 500 and 123 kb, respectively. Unexpectedly, sensitivity to UV-C is inherited as a dominant trait for both QTLs. This suggests, together with other data, that QTL1 contains a suppressor influencing directly or indirectly the expression of *UVR2,* a photolyase repairing cyclobutane pyrimidine dimers. QTL2 most likely contains a novel gene not described previously in connection with UV response. Possible candidate genes are *AtSABP2* (At3g29770), *AtRALF27* (At3g29780) and *NFI* (AT3g29760). Further research based on these results may provide means in genetic engineering or breeding of UV-tolerant lines as a basis for potential crop improvement by increasing UV-tolerance.

# 1 Introduction

## 1.1 *Arabidopsis thaliana* as a model for studying plant biology

*Arabidopsis thaliana* (Arabidopsis) emerged as the model organism of choice for researcher in plant biology already decades ago (reviewed in Koornneef and Meinke 2010, Van Lijsebettens and Van Montagu 2005). It was originally adopted as representative higher plant because of its usefulness for genetic experiments: short generation time, small size and prolific seed production through self pollination. Further advantages that could not have been foreseen in the pre-molecular era have allowed Arabidopsis to stay the premier model for plant biologists. Such advantages are the relative small genome, the easiness of transformation, the abundance of natural variation and close relatedness to several thousands of *Brassicaceae* species (reviewed in Somerville and Koornneef 2002). In addition, a wealth of genomics resources exists, such as the completely sequenced genome, a near saturation insertion mutant collections or genome arrays that contain the entire transcriptome. This makes *Arabidopsis* an excellent model plant for the studying, among other fields, abiotic stress responses (reviewed in Rounsley and Last 2010, Zhang *et al.* 2004).

## 1.2 Natural variation in *Arabidopsis thaliana*

The naturally occurring genetic variation is one of the most important basic resources for biology. In addition to the variation generated artificially by mutagenesis, naturally occurring genetic variation is extensively found in Arabidopsis (reviewed in Alonso-Blanco *et al.* 2009, Alonso-Blanco and Koornneef 2000). Motivations for studying natural variation range from simply exploiting it in order to find new genes involved in specific aspects of plant physiology or development, trying to understand the molecular basis of adaptation to the local environment or general trends in evolution (Weigel and Nordborg 2005). Natural variation present among accessions is usually multigenic which has hindered its analysis. However, the exploitation of this resource down to the molecular level has now become feasible. According to Koornneef et al. (2004), analyzing natural genetic variation involves three main steps:

1. phenotypic identification of genetic variation for traits of interest
2. determination of the genetic basis underlying this variation
3. finding the molecular nature of the allelic differences that account for the genetic differences

Dissecting the natural variation requires first a quantitative trait locus (QTL) analysis, which, in case of well-defined and robust phenotypes, in *Arabidopsis* can be effectively carried out by using F2 hybrid populations or recombinant inbred lines (RILs). The major challenges are the second and third step, identifying the specific gene and the nucleotide polymorphism underlying the QTL and the proof of causal relationship. The analysis of natural genetic variation is providing unique knowledge from functional, ecological, and evolutionary perspectives. Just one example: the analysis of natural variation for Arabidopsis flowering time revealed several genes, some of which correspond to genes with previously unknown function (reviewed in Koornneef *et al.* 1998).

## 1.3 Ultraviolet radiation

As plants use sunlight for photosynthesis, they are exposed to all its components, including ultraviolet (UV) radiation. UV radiation is formally divided into three classes: UV-A, UV-B, and UV-C. The classification is based on the different range of the wavelength and specified in table 1 (Cockell and Knowland 1999).

**Table 1: Wavelength spectrum of UV radiation (Cockell and Knowland 1999).**

| Name | Wavelength (nm) |
|---|---|
| UV-A | 315-400 |
| UV-B | 280-315 |
| UV-C | 100-280 |

The high energetic UV-C spectrum is effectively absorbed by the ozone layer in the stratosphere and therefore not present in the sunlight at Earth's surface. Approximately 5% of the UV-B portion of sunlight reach the ground level and has received much attention because of the ozone depletion, which leads to a higher amount of UV-B radiation and may cause health problems of animals and plants. The UV-A fraction is not attenuated by the ozone layer. Hence the ozone depletion has no influence on the amount of UV-A on Earth's surface (McKenzie *et al.* 2003).

Although UV-C damage is physiologically not relevant on Earth's surface, it is commonly used as a mutagenic agent, and the DNA photoproducts are the same as those obtained with UV-B

radiation (Brash 1997). UV-C has been used because DNA has an absorption maximum at 260 nm. In addition UV-C photons are highly energetic and thus a high level of damage can be created in a time-efficient manner (Stapleton 1992). When exposed to elevated UV-B radiation, plants display a wide variety of physiological and morphological responses (Jansen *et al.* 1998). In case of UV-C radiation the DNA damage is dominant (reviewed in Tyrrell 1996), and therefore UV-C radiation is frequently used to study DNA damage repair processes.

## 1.4 Importance of DNA repair

The deoxyribonucleic acid (DNA) is the carrier of the genetic information. DNA is responsible for passing the genetic information to the progeny. However, DNA gets damaged by various endogenous and exogenous factors which can produce a large spectrum of lesions (reviewed in Tuteja *et al.* 2001). DNA damage can affect replication (Painter 1985) as well as transcription (Proticsabljic *et al.* 1986). The DNA lesions can be removed by repair or recombination, or retained, leading to genome instability often connected with mutations, carcinogenesis and/or cell death. Organisms respond to genome damage by activating a DNA damage response pathway that regulates DNA repair pathways, temporarily arrests cell-cycle and may induce apoptosis. An unrepaired damage can also lead to the general deterioration of cellular functions and cell death (reviewed in Tuteja *et al.* 2001).

## 1.5 DNA damage products

According to Britt (review in 1996), the most common naturally occurring DNA damage products and their immediate biological effects can be classified as: 1) hydrolytic damage, 2) alkylation damage, 3) oxidative damage, 4) damage induced by ionizing radiation and 5) damage-induced by UV radiation.

**Hydrolytic damage:** Most common is the hydrolysis of the glycosylic bond between purine bases and the DNA backbone. In organisms with large genomes, like humans or maize, this depurination is responsible for a loss of several thousand purine bases per day per cell (Lindahl and Nyberg 1972). However, these abasic sites are rapidly recognized and repaired and, as a consequence, the spontaneously generated abasic sites do not play a relevant role in mutagenesis in microbes or mammals and most likely also not in plants.

Another type of hydrolysis reaction is the deamination of cytosine and 5-methylcytosine, resulting in the formation of uracil and thymine, respectively. Uracil is rapidly recognized and excised by uracil glycosylase in plants and in animals. Thymine, however is highly mutagenic because it is not recognized as faulty base and triggers a base pair change from C:G to T:A. Thymine as deamination product may be the most important cause of spontaneous point mutations in mammalian cells. Due to a high rate of cytosine methylation in plants (CpG and CpNpG) it is likely that 5-methylcytosine to thymine transitions are also frequent in plants. The underrepresentation of CpG vs. GpC (Setlow 1976) gives evidence that CpG was frequently mutated in evolutionary time scales.

**Alkylation Damage:** DNA experiences a biologically significant level of spontaneous DNA methylation (Rebeck and Samson 1991). Some of the methylation products are premutagenic or lethal if unrepaired. Although a variety of bonds in all four bases are susceptible to methylation, most of the modifications occur at purine bases. The most frequently generated alkylation product, 7-methyladenine, pairs still preferentially with thymine and is therefore not mutagenic or toxic. However, 3-methyladenine cannot serve as a template for DNA synthesis and stops DNA replication as well as DNA transcription and is lethal for cells. A third lesion, O-6-methylguanine, pairs with thymine and creates a very potent premutagenic lesion, which ends up in the transition from G to A (Dolferus *et al.* 1990, Orozco *et al.* 1993). A variety of alkylation damage-inducing agents is known, and some of them, e.g. EMS (ethylmethane sulfonate) are used in laboratory mutagenesis experiments. Plants, animals and microbes have developed repair pathways to reverse or excise methylation damage (reviewed in Britt 1996).

**Oxidative damage:** A variety of oxidative damage products are caused by hydroxyl radicals, superoxide, and nitric oxide (Demple and Harrison 1994). Some of these damages block DNA replication or transcription. The most important premutagenic oxidized base is 8-hydroxyguanine, which pairs to A and C equally. The nucleotide 8-hydroxyGTP could potentially be used by DNA polymerase for DNA synthesis but gets degraded enzymatically before incorporation. Due to the easy oxidation of bases *in vitro* and inherent instability of some oxidation products, the spontaneous rates at which bases are modified or repaired in the genome are difficult to determine. Since bases are quite protected from hydroxyl radicals by the intact double helix, a larger fraction of oxidation damage occurs at the sugar backbone, leading to DNA single strand breaks. These breaks are generally repaired in an efficient and error-free manner. Sources of oxygen damage are ozone (Kanofsky and Sima 1991) or eventually radicals

from neighboring cells, produced during hypersensitive response (Levine *et al.* 1994). Also very high levels of UV-B radiation can lead to oxidative damage in DNA (Hariharan and Cerutti 1977, Kanofsky and Sima 1991).

**Damage induced by ionizing radiation:** Ionizing radiation is the least specific mutagen. The probability of any component of the cell directly affected by ionizing radiation depends simply on the mass fraction of the cell that it represents. Therefore, the primary absorbant of ionizing radiation in plant cells is water, leading to formation of hydroxyl radicals, which can interact with DNA (Ward 1975). Direct absorption of radiation may induce nicks as well as double-strand breaks that can lead to chromosomal rearrangements such as inversions, duplications and translocations.

**UV-induced damage:** UV radiation induces predominantly oxidative damage and crosslinked lesions (reviewed in Britt 2004). The main products of UV-induced DNA damage are cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidinone dimers also called 6-4 photoproducts. They make up to 75% and 25%, respectively, of the UV-induced DNA damage products (Mitchell and Nairn 1989). These dimers are induced most efficiently by radiation at approximately 260 nm (UV-C light), congruent to the absorbance spectrum of the DNA. Since the UV-C portion of the sunlight does not reach the earth's surface (McKenzie *et al.* 2003), most naturally occurring UV-induced DNA damage in plants or animals originates from the small amount of UV-B and the much greater flux of UV-A. Dimer formation occurs up to wavelengths as long as 365 nm. For wavelength shorter than 310 nm, there is a difference between DNA in solution or 'shielded' DNA, arranged in protective complexes or contained in pigmented cells: unshielded DNA is markedly more sensitive to ultraviolet damage, differing by a factor of more than 100 at 280 nm (Quaite *et al.* 1992). Plants have evolved protection to some extend by developing multiple cell layers and pigments such as flavonoids, which absorb photons in the UV-A and UV-B range but not in the UV-C range (Chappell and Hahlbrock 1984). The pyrimidine dimers are not directly mutagenic but inhibit the progress of the DNA polymerase. Mammalian RNA polymerase II was shown to be blocked at CPDs as wells as 6-4 photoproducts. If no repair occurs, a single pyrimidine dimer is sufficient to stop DNA transcription and DNA replication (Mitchell *et al.* 1989). Furthermore, the mammalian DNA-dependent RNA polymerase remains bound to the site of obstruction, which leads to a reduction of free RNA polymerase and can therefore also influence the DNA transcription of genes which are located near to the lesion (Donahue *et al.* 1994). Both types of dimers are also

premutagenic lesions. Due to a specialized polymerase all organism are able to bypass a dimer to some extent. However, this polymerase has a substantially reduced accuracy, which leads to the formation of point mutations (reviewed in Britt 2004). In summary, every dimer leads to a block of transcription and replication, whereas only a small fraction of dimers results in a mutation. Thus cells need to repair UV-induced dimers in order to restore transcription and to restore error-free DNA replication. Mutagenesis is undoubtedly an important consequence of the induction of DNA damage, but the effects on transcription are more critical to the survival of the organism. Nevertheless, UV-induced mutagenesis and deamination of methylated cytosins can also occur and result in predominantly G:C to A:T transition, as observed in the spontaneous mutation rate in *Arabidopsis* (Ossowski *et al.* 2010).

## 1.6 Repair of UV-induced DNA damage

UV-induced DNA damage may be repaired by several mechanisms (reviewed in Britt 2004, Sinha and Hader 2002) which can be classified as follows: 1) direct reversal by photolyases, 2) excision repair, 3) lesion bypass, 4) recombinational repair and 5) alternative repair pathways. Additional repair mechanisms exist (e.g. non-homologous end joining) but are not activated by UV- induced damages.

**Direct reversal by photolyases**

Most of DNA damage products are repaired by a variety of 'remove and replace' excision repair mechanism. However, pyrimidine dimers are one of the few lesions which are repaired by direct reversal through the action of specialized enzymes - photolyases. This mechanism, called photoreactivation, is triggered by exposure to visible light. Arabidopsis possesses two photolyases, *UVR2* and *UVR3*, specialized in the repair of CPDs and 6-4 photoproducts, respectively. Many organism do have only a photolyase pathway for CPD repair, and placental mammals have no photolyase pathway at all and rely on the nucleotide excision repair (NER) to repair bulky adducts (Sancar 1994a). All photolyases are monomeric and carry two chromophores. The flavin cofactor (FADH-) acts as an electron donor to reverse the crosslink between the bases, and the second chromophore (methenyltetrahydrofolate or 8-deazaflavin) acts as an antenna pigment to excite the electron donor. Photolyases are able to recognize lesions also in the absence of light, but the lesion is reversed and the enzyme dissociates from the DNA only if a photon (UV-A to blue range) is absorbed (Sancar 1994b). Photolyases are sorted into two classes based on their structure: i) class-I comprise the photolyases of fungi and

some bacteria and ii) class-II photolyases include the CPD enzymes of plants, vertebrates, insects and some bacteria and the 6-4 photolyases (Todo 1999).

Some plant photolyases are regulated by visible light and by UV-B. Arabidopsis CPD photolyases are only active when plants have been exposed to visible light before and during the period of repair because the transcription of the gene is regulated by white light and UV-B (Chen *et al.* 1994). The gene is not only induced by light, but requires a diurnal cycle for high-level of mRNA (Waterworth *et al.* 2002). However, 6-4 photolyase is constitutively expressed (Tanaka *et al.* 2002).

Photoreactivation plays a main role in removing CPD and 6-4 photoproducts of plants and eliminates the majority of dimers typically within hours, or even minutes in some cases. In the absence of photoreactivation, the dimers are also reduced but have a much longer half live. If organellar DNA (mitochondrion, plastid) is also subject to photoreactivation is a matter of discussion.

**Excision repair**

Excision repair does not directly reverse DNA damage but instead replaces damaged components with new ones. There are two major pathways: base excision repair (BER) and nucleotide excision repair (NER) (reviewed in Sinha and Hader 2002).

Base excision repair (BER) protects the cells from endogenous DNA damage caused by hydrolysis, reactive oxygen species, alkylating agents, ionizing radiation and intracellular metabolic processes that modify DNA base structure. DNA glycosylases remove different types of modified or damaged bases by cleavage of the N-glycosidic bond between the base and the deoxyribose. Once the base is removed, an endonuclease or a lyase nicks the DNA strand 5'-3' to the site. The remaining deoxyribose phosphate residue is removed by a phospodiesterase. The gap is filled by a repair DNA polymerase, and the strand is sealed by DNA ligase (reviewed in Seeberg *et al.* 1995). Many different DNA glycosylases are present in a variety of organism (reviewed in Sinha and Hader 2002). Eukaryotic endonucleases have been described in fungi (Bowman *et al.* 1994). The characterization of endonuclease activities in plants suggests some specificity for repair of UV-radiation-induced damage (Doetsch *et al.* 1989).

Nucleotide excision repair (NER) fixes a wide range of DNA lesions, including CPDs and 6-4 photoproducts. The NER pathway is dependent on around 30 genes, is highly conserved in

eukaryotes and present in most organisms. The NER pathway starts with the recognition of the DNA distortion by a protein complex. Subsequently, a DNA helicase unwinds the DNA. In a further step, a protein with nuclease activity removes the nucleotide. The gap is filled in by DNA synthesis und closed by a DNA ligase (reviewed in Gillet and Scharer 2006). In plants, the rate of repair of CPDs varies widely between species. High rates of repair have been reported for carrot suspension culture (Howland 1975), petunia and tobacco (Howland and Hart 1977), whereas NER was not observed in cultured soybean cells (Reilly and Klarman 1980).

**Lesion bypass**

If repair of the lesion is not possible, the cell can survive only by bypassing the lesion. The stalled DNA polymerase will be rolled back or removed from the side of the lesion. A second set of less fastidious polymerases, able to bypass the lesion, will be up-regulated and recruited to the site of damage. These bypass polymerases are less accurate than normal polymerases. Most of the mutagenesis resulting from damage by UV radiation, ionizing radiation or various chemicals seems to be due to the incorrect insertion of nucleotides during translesion synthesis (reviewed in Goodman 2002). However, some bypass polymerase families are still able to interpret many common lesions correctly (Yu *et al.* 2001). Dimer bypass was also described in humans (Lin *et al.* 1999, Tissier *et al.* 2000). Arabidopsis mutants defective in *ARABIDOPSIS THALIANA RECOVERY PROTEIN 3 (AtREV3*, At1g67500) exhibit hypersensitivity to UV radiation, in the dark and under photoreactivating conditions. *AtREV3* encodes the catalytic subunit of DNA polymerase zeta and is thus involved in damage-tolerance mechanisms through translesion synthesis (www.arabidopsis.org). Hypersensitivity was also observed to the crosslinking agent mitomycin C, suggesting a role of *AtREV3* in the tolerance and/or repair of closely opposed or crosslinked lesions. These lesions cannot be repaired by NER, because there is no undamaged strand available to act as a template for repair (Sakamoto *et al.* 2003).

**Recombinational repair**

Somatic homologous recombination is an important process in DNA repair. Mainly double strand breaks and single strand gaps are efficiently repaired by mechanism associated with recombination. Recombinational repair fills a gap by transferring and copying a complementary strand from a region homologous to the damaged DNA. In bacteria, the RecA protein forms a right-handed helical nucleoprotein filament on the DNA and carries out the homology search and the strand-exchange reaction (reviewed in Kowalczykowski *et al.* 1994). As a consequence,

repair by homologous recombination is precise but may cause genomic rearrangements like inversions, translocations etc, depending on the resolution of the recombination intermediates. UV-C induced homologous intrachromosomal rearrangements have been observed in Arabidopsis (Puchta *et al.* 1995), but the enzymatic components are not well characterized.

## 1.7 Aim of the thesis

In this thesis, I have addressed two major objectives. First, I have investigated the natural variation of UV-C tolerance among 96 Arabidopsis accessions. Subsequently, I have analyzed the genetic segregation of UV-C tolerance upon crosses between one sensitive and one resistant accession, in order to identify genetic loci underlying the variation.

# 2 Results

## 2.1 Arabidopsis accessions differ in sensitivity to UV-C irradiation

The screen for sensitivity of Arabidopsis accessions to UV irradiation was planned to be performed with all 96 different natural accessions contained in the 'Nordborg collection' (NASC set N22660). However, due to problems with germination, only 83 accessions could be evaluated. Four days-old seedlings were treated with a single dose of UV-C radiation (254 nm, 8000J/m²), and their phenotypes were evaluated after 12 days of recovery. The phenotypes ranged from extreme UV-C tolerance (no difference to the non-treated control) to hypersensitivity (no survival after radiation), with intermediate phenotypes (Figure 2). They were classified into four main categories based on plant phenotypes (Figure 1). Each class was assigned with a relative survival rate value (figures in bracket): a) green, well surviving (1), b) chlorotic (yellow), mildly reduced in growth but surviving (0.66), c) dwarfish (0.33) and d) nearly or fully dead (0). An average survival rate was calculated for each accession based on the individual plants. A relative survival rate of 1 indicated that all the individuals of a given accession were unaffected by radiation, and a survival rate of 0 denoted that all individuals could not survive the given UV-C dose.

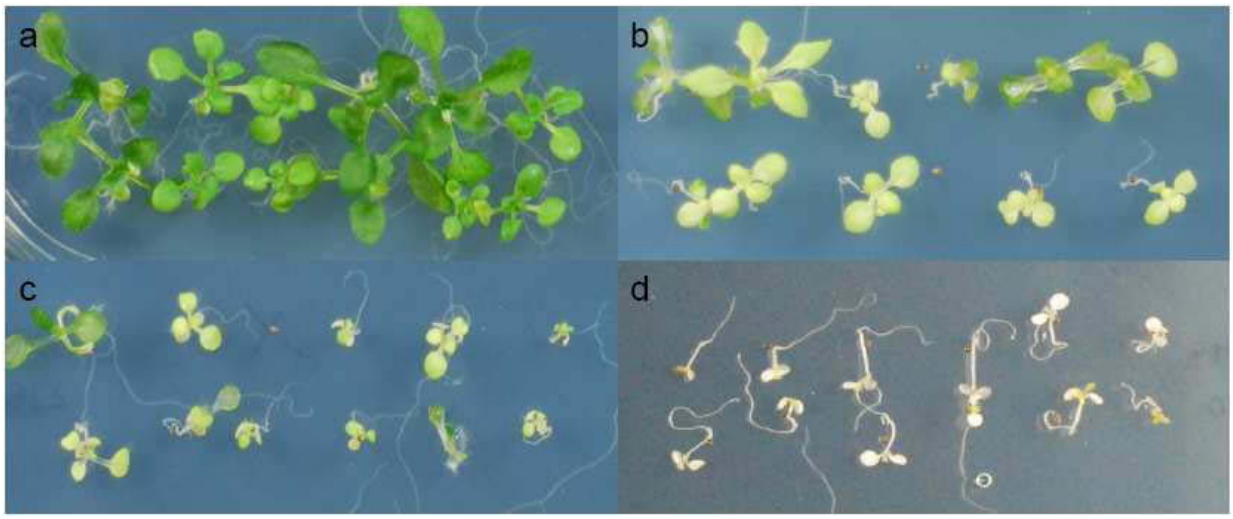


**Figure 1: Phenotypes after UV-C irradiation:** a) green, well surviving, b) chlorotic and mildly reduced growth, c) chlorotic and strongly reduced growth (dwarfish), d) nearly or fully dead.

In order to minimize possible false positive or negative results, a biological replicate of the screen was carried out under the same conditions, with the exception that the UV-C treatment was performed with freshly amplified seed material (progeny of sibling plants from the first screen) on 5 days-old seedlings and after 13 days of recovery. For the biological replicate, 85 out of the 96 accessions were evaluated (Figure 3). Due to germination differences, only 60 accessions could be used for a direct comparison of the two replicates (Figure 4). The comparison revealed that several accessions hypersensitive in the first replicate were relatively tolerant in the second one. Additional experiments are required to clarify sensitivity of these accessions. The most likely source of variation is in the plant material, due to the different seed batches.

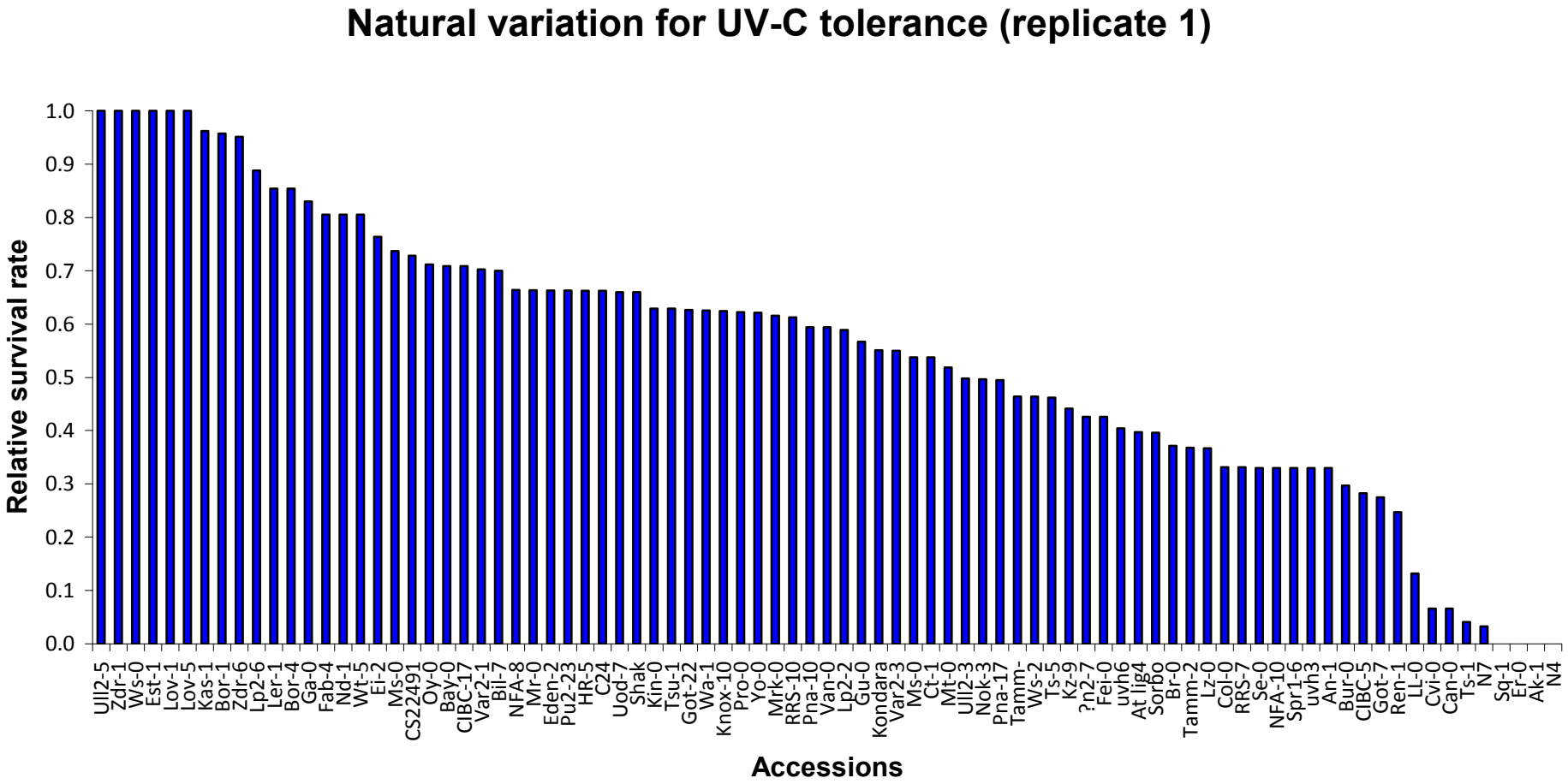


**Figure 2: Natural variation among 83 Arabidopsis accessions for UV-C tolerance (replicate 1)**

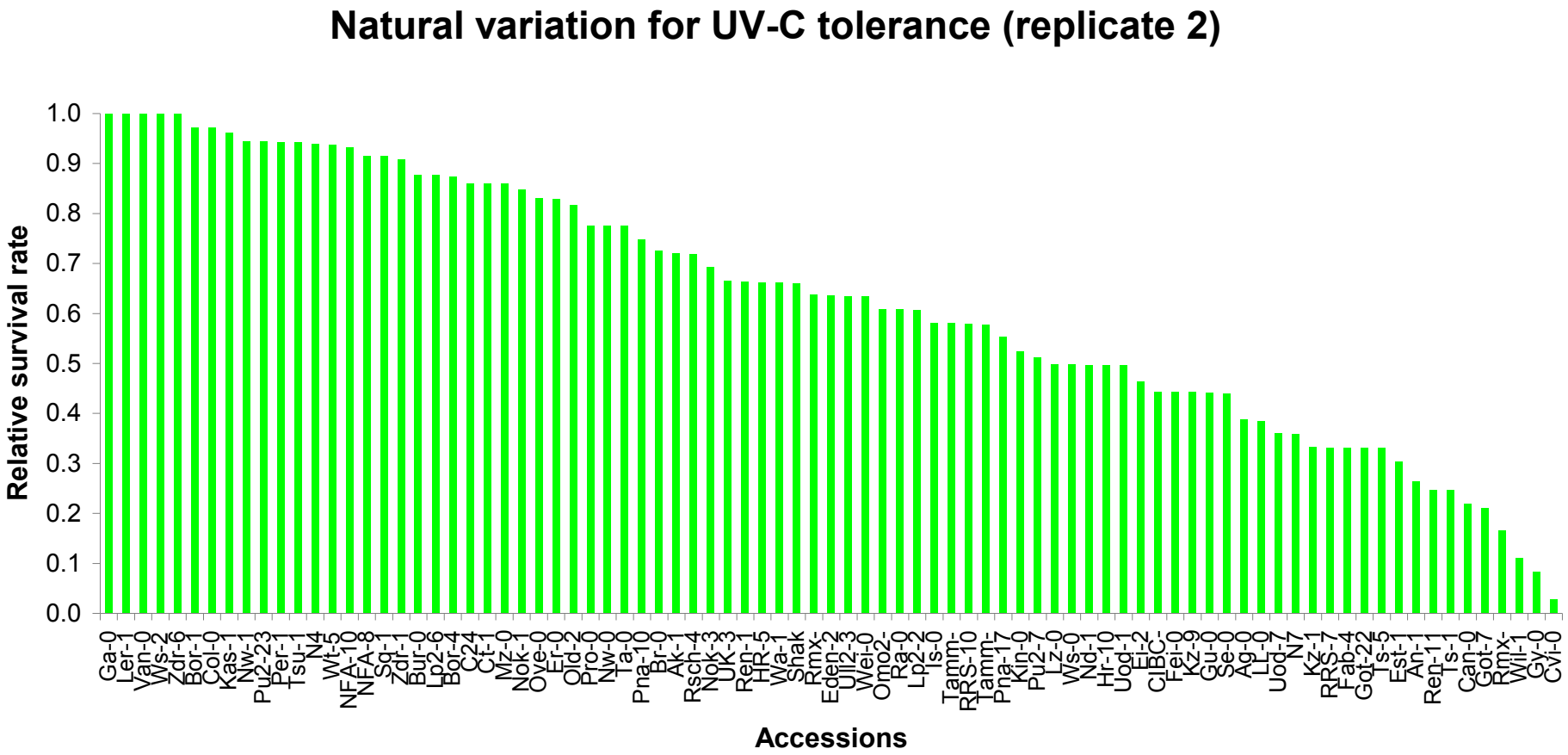


**Figure 3: Natural variation among 85 Arabidopsis accessions for UV-C tolerance (replicate 2)**

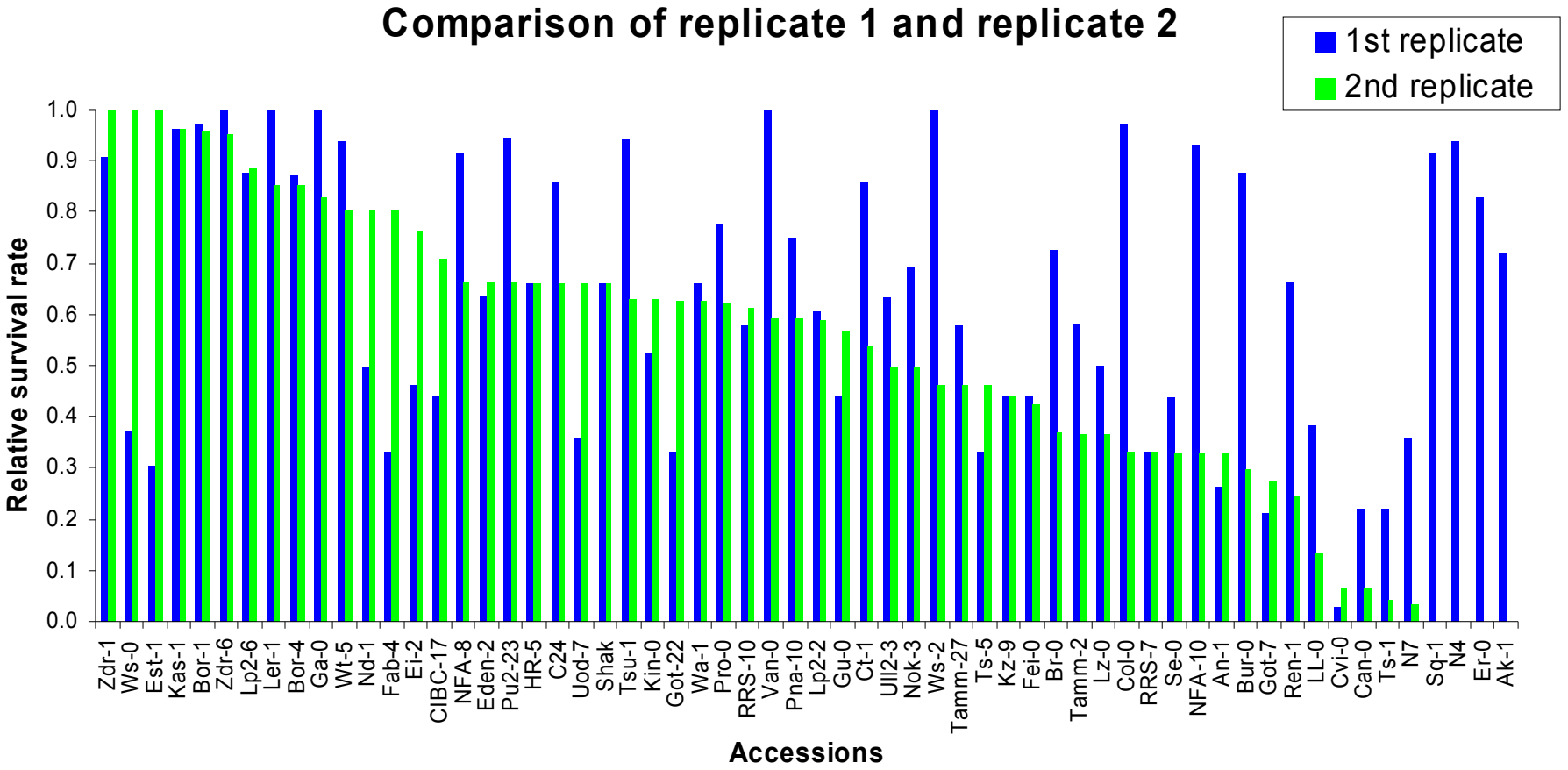


**Figure 4: Natural variation among 60 Arabidopsis accessions for UV-C tolerance.** Blue and green bars indicate results of the first and the second biological replicate, respectively. The accessions are ordered according to the decreasing average survival rate of the second biological replicate.

In summary, the assay showed a strong natural variation between the investigated Arabidopsis accessions in tolerance of UV-C induced DNA damage, from full resistance to full sensitivity. However, the results were not identical in both replicates for all accessions. It is likely that differences in the plant material used for both assays are responsible for the divergence. Nevertheless, reproducible results for some Arabidopsis accessions allowed to move on to the next steps, in order to elucidate loci responsible for the observed variation.

## 2.2 Phenotypes after UV-B radiation are similar to that after UV-C radiation

As mentioned above, UV-C does not occur under natural conditions; however, it was shown to induce the same type of damage as irradiation with UV of shorter wavelength. Therefore, I expected the set of accessions to show sensitivity and resistance, respectively, to both types of UV. In order to test this, 10 accessions with reproducible and clear phenotypes under UV-C and two reference accessions (Col-0 and Ws-0) were treated with UV-B (assays done by M. A. Gonzalez Besteiro, laboratory of Dr. R. Ulm, Albert-Ludwigs-University, Freiburg). Seedlings (7 days old) were radiated with acute UV-B dose (1.5 mW/cm² for 3.5 hours), returned back to previous growth conditions (24°C, 12 hours day length) and phenotyped after 8 days of recovery. The accessions Ler-1, Zdr-6, Kas-1, Zdr-1, and Bor-1 showed a resistant phenotype whereas Got-7, Ts-1, Cvi-0, An-1, and Can-0 showed hypersensitivity. This is congruent with the result of the UV-C radiation (Figure 4). The reference accession Col-0 which showed a different response to UV-C treatment in the two biological replicates, exhibited resistance after UV-B treatment, whereas the reference accession Ws-0, also ambiguous after UV-C radiation, was rather sensitive to UV-B treatment.

The result of UV-C and UV-B treatment indicates that UV-C and UV-B radiation causes similar phenotype in most of the cases. However, it remains to be analyzed whether the similar phenotypes are conferred by identical molecular factors.

## 2.3 Genome-wide association studies (GWASs) did not indicate clear candidate loci

Recent development of new mapping techniques allows finding of causal loci in a time- and labour-saving manner. To take advantage of this, I tried to identify loci responsible for the natural variation in UV-C sensitivity in a series of GWA studies (the statistical analysis was done by Dr. B. Vilhjalmsson, laboratory of Dr. M. Nordborg, GMI, Vienna). The GWA

analysis was based on 216,130 single nucleotide polymorphisms (SNPs) with an estimated error rate of 1.6 %. Given the *A. thaliana* genome size of around 150 Mbp, the resulting density is approximately one SNP per 500 bp. This is considerably higher than is commonly used for instance in human studies (Atwell *et al.* 2010).

GWASs were carried out for replicate 1 (83 accessions) and replicate 2 (85 accessions) with the phenotype of 'average survival rate' (described above). Using the Kruskal-Wallis (KW) test for replicate 1, the phenotype 'average survival rate' yielded a single significant association peak located on chromosome 2 at nucleotide position 7,209,466 (Figure 5).

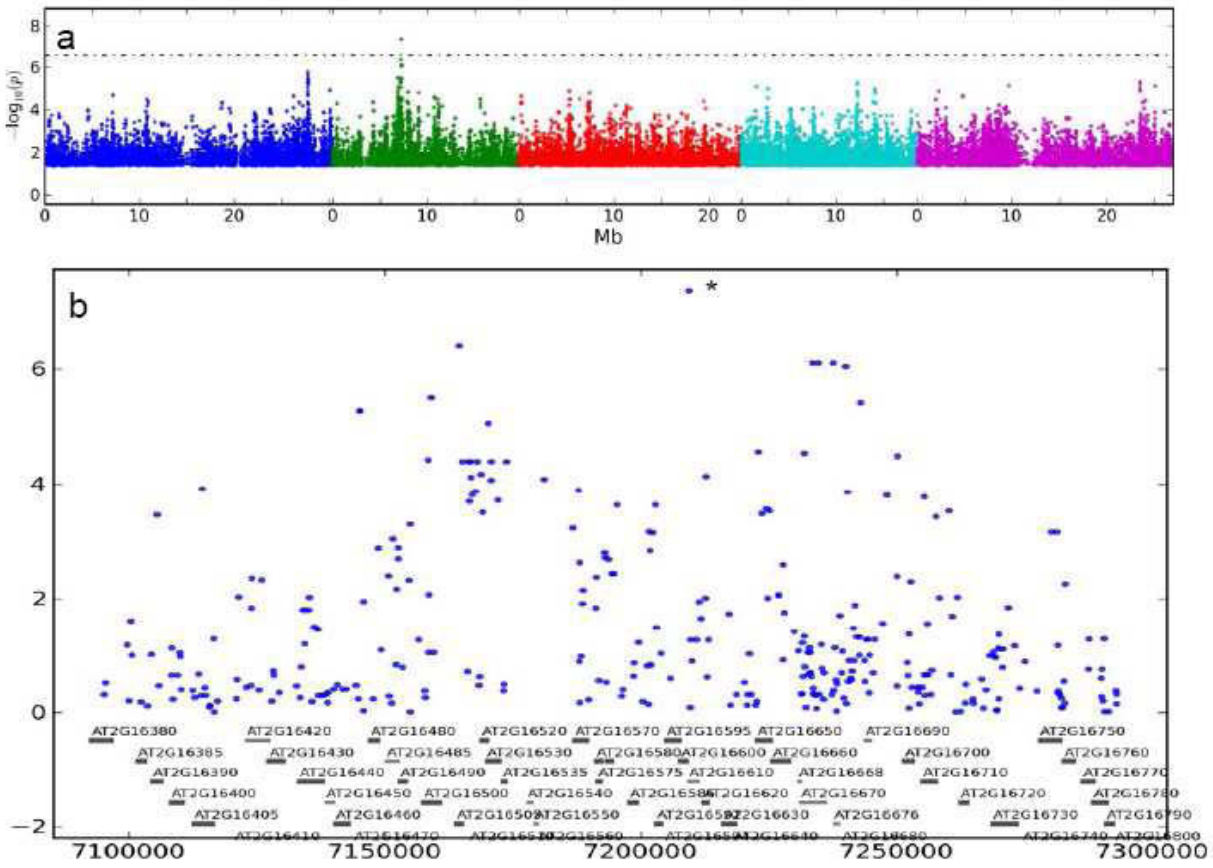


**Figure 5:** GWASs of the phenotype 'average survival rate' of replicate 1 using the KW test yielded a single association peak. b) Physical position of the significant peak (indicated by an asterisk) on chromosome 2 and the correlated genes.

At this position (Figure 5b) a transposable element is located (At2g16610). The first gene upstream is *ROC3* (At2g16600), a member of the cyclophilin gene family; the first gene downstream is At2g16620 which is annotated as a kinase-related gene with unknown function (www.arabidopsis.org). However, none of these genes, and no other gene located in the region,

corresponds to any known component responsible for DNA damage, DNA repair or UV resistance. Nevertheless, a possible association between *ROC3* or the kinase-related gene and UV resistance remains to be tested.

The GWASs using KW test for replicate 2 as well as the GWA based on efficient mixed-model association (EMMA) for both replicates did not show any significant association between trait and genotype (Figure 6).

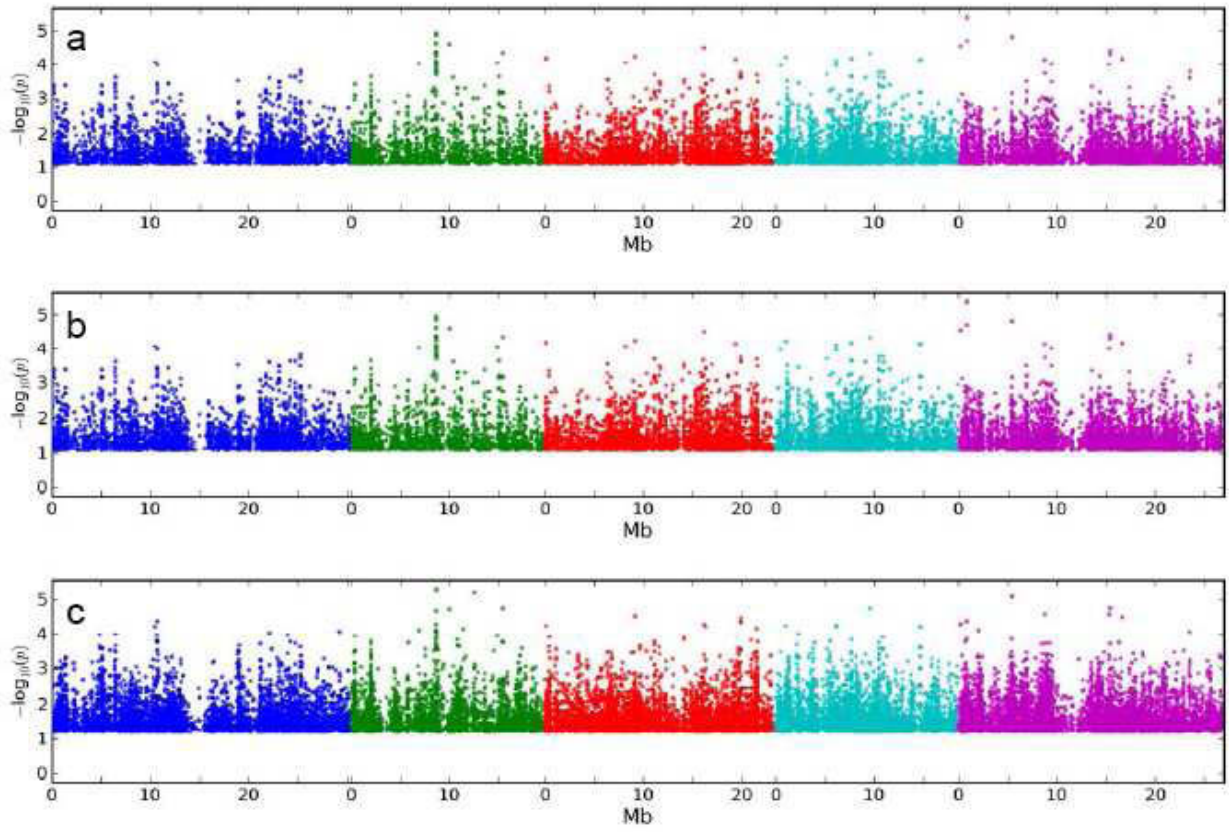


**Figure 6: GWASs for the phenotype 'average survival rate':** a) replicate 1 (EMMA) b) replicate 2 (EMMA) c) replicate 2 (KW test). None showed a significant peak (threshold at 6.5, outlying the visible scale).

In addition, GWASs with KW test and EMMA for both replicates was tested, using only the two extreme phenotypes: i) the percentage of plants unaffected and ii) the percentage of dead plants. These two phenotypes rely on the evaluation scheme described above (Figure 1). None of these GWASs showed any significant association (data not shown) and could therefore not

confirm the position on chromosome 2 nor identify any other locus responsible for the trait of interest.

In addition, GWASs with EMMA and KW tests were performed for a selected sample of 41 accessions. These accessions were selected for congruence between replicate 1 and 2 of the UV-C experiment, with less than 0.3 difference in the relative survival rates. Both association studies did not reveal any significant association peak (Figure 7).

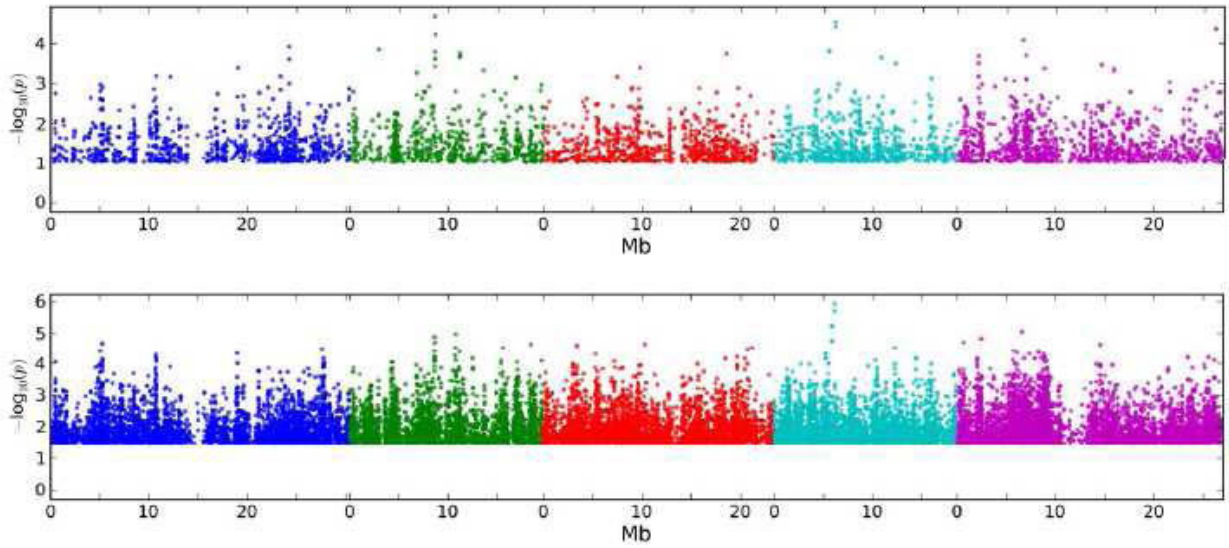


**Figure 7:** GWASs for accessions selected for consistent response to UV-C and the phenotype 'average survival rate': using a) the model EMMA and b) KW-test. None showed a significant peak (threshold at 6.5, outlying the visible scale).

In summary, only one out of 14 GWASs revealed a significant peak indicating an association between genotype and trait. However, no obvious candidate gene was located at the respective locus. Therefore, GWASs did not provide a clear answer to the genetic basis of variability in sensitivity to UV radiation in different Arabidopsis accessions.

## 2.4 Selection of accessions for classical mapping

As GWASs did not indicate clear candidates, QTL mapping followed by a linkage-based fine mapping was chosen as alternative strategy to find candidate polymorphisms. A suitable mapping population should be generated between one resistant and one sensitive accession with the most reproducible phenotypes. Because of the response variation between experiments, 10 additional biological replicates were set up with accessions Can-0, Cvi-0, Col-0, Est-1, Ler-1

and Ws-0, to select reproducibly sensitive or resistant accessions. These accessions were chosen based on the amount of mapping resources, availability of whole genome sequence and performance in both initial screens. I performed UV-C assays with 8000, 9000 and 10000 J/m² on the selected accessions, to reveal possible variability and optimal irradiation dose leading to the clearest phenotypes.

Among all accessions, Ler-1 and Cvi-0 showed the most reproducible resistance and hypersensitivity to UV-C radiation, respectively. This was a fortunate combination since these two accessions offer a unique advantage of many publicly available mapping resources such as recombinant inbreed lines (RILs) (Alonso-Blanco *et al.* 1998), near isogenic lines (NILs) (Keurentjes *et al.* 2007) and the complete genome sequence (http://signal.salk.edu/atg1001/2.0/gebrowser.php).

## 2.5 QTL mapping identified two QTLs at chromosome 3

A Ler-1/Cvi-0 RIL population was used for QTL mapping. The RIL set N22000 (NASC, http://arabidopsis.info/) contained 162 lines in F8 generation. An amplified fragment length polymorphism (AFLP)-based linkage map was already generated for the RIL population (Alonso-Blanco *et al.* 1998), and genotype data for the whole set were hence publicly available (ftp://ftp.arabidopsis.org/home/tair/Maps/Ler_Cvi_RIdata/). In total, 321 PCR-based markers were used to construct a genetic map. From the 162 RILs, a basic set of 50 RILs showing the highest recombination rate was used for statistical analysis.

The UV-C radiation of RILs was carried out on 5 days-old seedlings with intensities of 10000 and 12000 J/m². These Two UV-C doses were applied to elucidate a potential influence on the result of the QTL-mapping. The plants were evaluated 10 days after the UV treatment as described above (2.1).

QTL Mapping was done with the 'qtl' package of R (www.r-project.org). The genome scan with the single QTL model as well as the two-dimensional scan was performed using the standard model for QTL mapping (Lander and Botstein 1989). The residual phenotypic variation is assumed to follow normal distribution, and analysis is analogous to that of variance. The maximum likelihood was calculated via the expectation-maximization (EM) algorithm (Dempster *et al.* 1977).

The logarithm of the odds (LOD) scores of both UV-C doses, that can be considered also as biological replicates, were calculated and revealed two significant QTLs (named QTL1 and QTL2) at the top arm of chromosome 3 (Figure 8). The threshold of 3.09 for QTL-identification was determined by permutation testing (α=0.05) based on 1000 permutations. QTL1 could be located between the markers CC.110L/127C and GH.390L referring to 19 and 22 cM, respectively, of the AFLP linkage map (Alonso-Blanco *et al.* 1998). QTL2 was mapped between the markers BF.134C Col and BH.225C Col referring to 40 and 44 cM, respectively. Hence, using a linkage map containing 321 markers, QTL mapping could narrow down two loci responsible for the trait described to 3 and 4 cM, respectively.

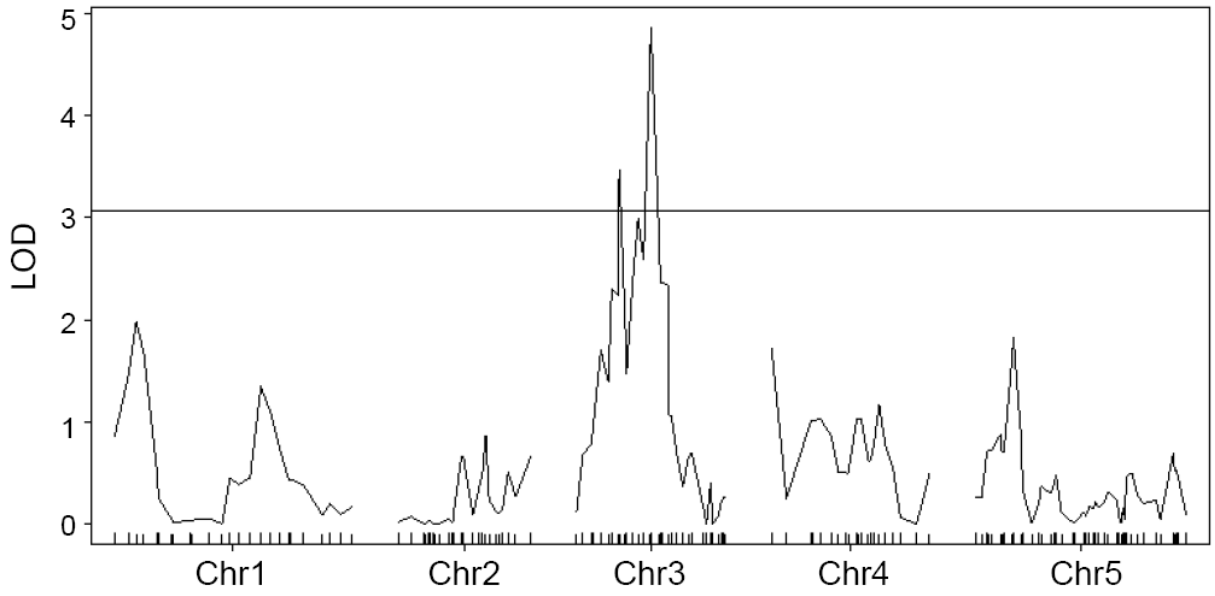


**Figure 8: QTL-map of sensitivity to UV-C induced DNA damage.** The y-axis is indicating the LOD and the x-axis individual AFLP markers (bars heading up) on all 5 chromosomes. The two peaks above the threshold correspond to QTL1 (left) and QTL2 (right).

In addition to the genome-wide scan with a single QTL model, I performed also a two-dimensional scan to test all possible pairs of QTL locations for association with the phenotype. This test showed no epistasis between QTL1 and QTL2 (Figure 9). The analysis showed epistatic interaction between two loci on chromosome 1. However, no locus on chromosome 1 showed a major effect on resistance to UV-C induced DNA damage.

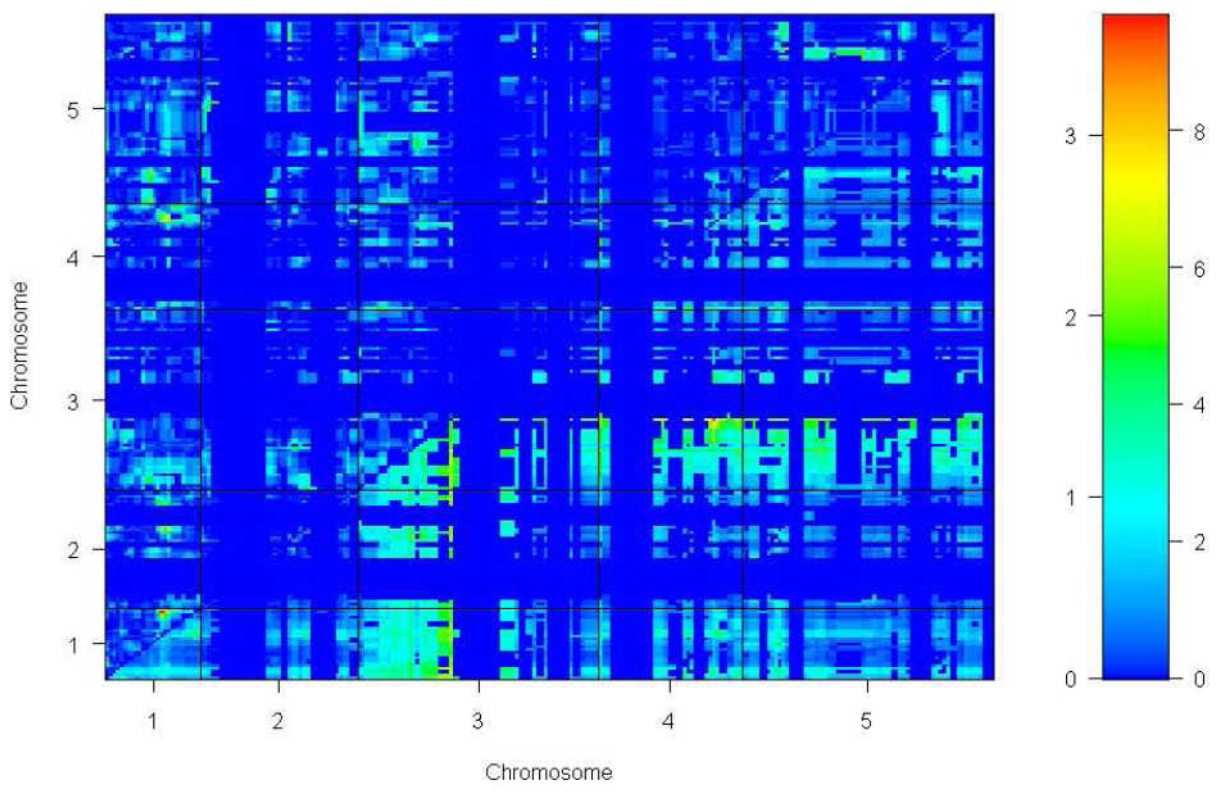


**Figure 9: Test for interactions between QTLs.** The triangle above the diagonal shows the LOD scores for epistatic interaction and the triangle below contains the LOD scores for the full model considering two QTL and their interaction together. Color scale indicates LOD scores for epistasis (scale on the left side) and additive interactions (scale on the right side).

In summary, QTL analysis revealed 2 independent QTLs with major effects located on chromosome 3. The AFLP-linkage map (Alonso-Blanco *et al.* 1998) indicated position of the two QTLs and genetic distance of the markers.

## 2.6 The trait 'sensitivity' is dominant

RILs do not allow estimating the mode of inheritance due to their highly homozygous genotypes. To test, whether the sensitivity and resistance to UV-C radiation were recessive or dominant traits, F1 and F2 hybrids of Ler-1 x Cvi-0 were tested. In F1, 100 % (10 out of 10) of seedling showed the same sensitive phenotype as Cvi-0, indicating that the sensitivity to UV-C irradiation is dominant, at least for one of the QTLs. In the F2 generation, 93.2 % (69 out of 74) of seedlings showed the same phenotype as Cvi-0. This indicates that, for both QTLs, sensitivity to UV-C is inherited as a dominant Mendelian trait. This correlates well with the result of the two-dimensional genome-scan, indicating that both QTLs are independent.

## 2.7 Linkage-based fine mapping

An estimation of mapped intervals using genetic distance is useful for low-resolution mapping and in species where the whole genome sequence is not available. Since there are high quality whole genome sequences available for several Arabidopsis accessions, Dr. Pecinka and I decided to anchor the genetic map at the position of QTLs 1 and 2 to the physical map. A physical map with relative low resolution generated *in silico* served as a basis (Peters *et al.* 2001). The closest AFLP marker determined by physical position to QTL1 was upstream BF.270L Col/271C located at 5.2 Mbp and downstream GH.321L/323C Col located at 7.2 Mbp, to QTL2 it was upstream BF.134C Col located at 11.5 Mbp and downstream GD.113C Col located at 12.9 Mbp. The resulting region of 2.0 Mbp for QTL1 and 1.4 Mbp for QTL2 was subsequently narrowed down by fine mapping with self-designed cleaved amplified polymorphisms (CAPS) markers. In a first step, the markers were applied to those 50 RILs with the highest recombination rates used for QTL mapping. With these 50 RILs, the interval containing Cvi-0 sequence and being associated with UV-C sensitivity was narrowed down to 800 kb for QTL 1 and 230 kb for QTL 2. In order to further reduce the interval, mapping was extended to the remaining 112 RILs, and this reduced the interval of QTL1 down to 500 kb and QTL2 down to 123 kb. The left and the right border of QTL1 were determined by the marker JR25 at 5.7 Mbp and JR30 at 6.2 Mbp, respectively. The left and the right border of QTL2 were determined by the marker JR7 at 11.593 Mbp and JR6 at 11.716 Mbp, respectively. As no more RILs are available, the fine mapping can not be extended at the moment and requires more Cvi-0 x Ler-1 populations.

## 2.8 Candidate genes for sensitivity to UV-C induced DNA repair

The interval of QTL1 (500kb) is still too large to select a single potentially causal gene, especially due to the high gene density in this interval. However, a suppressor gene may explain the dominant inheritance of the sensitivity. *HOMOLOG OF HY5* (*HYH*, At3g17609), a basic leucine zipper transcription factor mediates photomorphogenesis, triggers expression of light-inducible genes and promotes transcription of *UVR2*, a photolyase repairing CPDs. (Castells *et al.* 2010). The expression of *HYH* is controlled transcriptionally and also by posttranslational degradation in darkness, and it becomes stabilized soon after light exposure to promote the expression of photomorphogenesis-related genes (Holm *et al.* 2002, Osterlund *et al.* 2000).

To check if different expression pattern of *HYH* influence the activation of *UVR2*, a quantitative reverse transcription PCR (qRT-PCR) was performed. Seedlings (16 days old) were treated with 8000 J/m² and tissue were harvested immediately (R0), 1 hour (R1), and 6 hours (R6) after UV-C radiation. The qRT-PCR showed that Cvi-0 does not exhibit statistically significant differences (p=0.05) after treatment compared to the untreated control (Mock) (Figure 10). However, Ler-1 showed a significant increase of *HYH* immediately and 1 hour after UV treatment compared to control. The data are based on only one biological replicate and needs further repetitions to confirm the result.

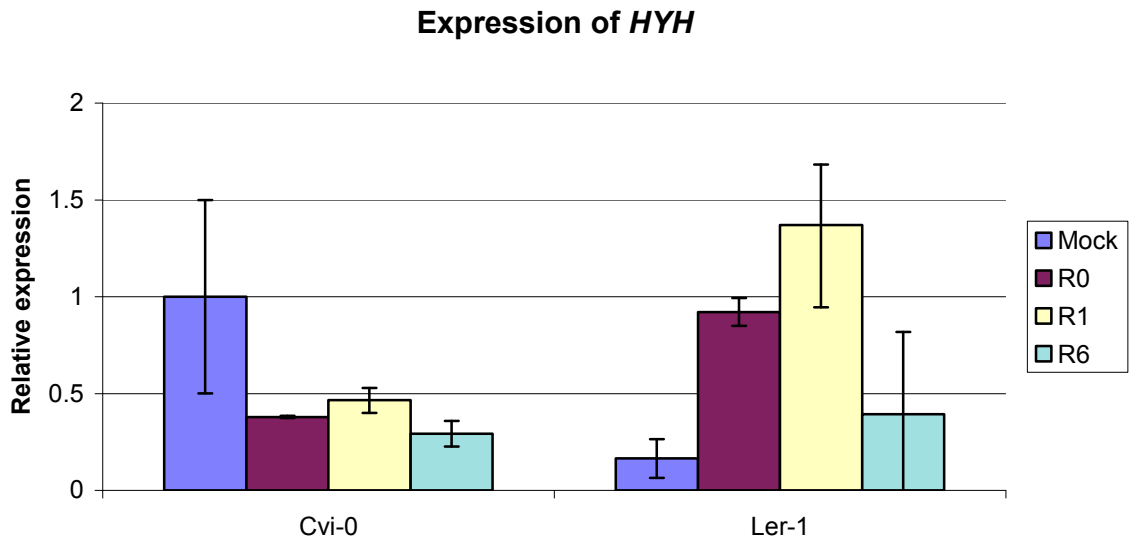


**Figure 10: Expression of *HYH*.** Tissue was harvested immediately (R0), 1 hour (R1) and 6 hours (R6) after radiation. Expression is shown relative to the expression of untreated Cvi-0. Error bars are showing standard deviation.

With just 123 kb, the interval of QTL2 had a better suitable size to look for candidate genes. As this QTL is close to the pericentromeric region, there are only a relative small number of genes within the mapped interval. The region contains 79 genes, 44 of them are transposable gene elements and 8 are pseudogenes (TAIR). Based on the TAIR database annotation, the best candidate genes at this interval are *AtSABP2* (At3g29770), *AtRALFL27* (At3g29780) and *NIF* (AT3G29760).

*AtSABP2* encodes a protein predicted to act as carboxylesterase and shows similarity to the methyl salicylate esterase *SABP2* from tobacco. *AtRALFL27* is a member of a diversely expressed predicted peptide family showing sequence similarity to tobacco rapid alkalinization

factor (*RALF)* (www.arabidopsis.org). Also the function of *NIF* is unknown to large extent. It contains the NLI interacting factor *(NFI)* domain found in proteins of diverse functions, these include phosphatases in which the domain is often present N-terminally to the BRCT domain, in proteins involved in cell cycle checkpoint functions responsive to DNA damage (www.ebi.ac.uk).

An *in silico* comparison of the DNA sequences was performed with these 3 genes (Figure 11). The coding region of *NFI* shows 2 non-synonymous amino acids changes between the coding region of Ler-1 and Cvi-0. *AtSABP2* shows a higher degree of polymorphism between Ler-1 and Cvi-0. However, most of the polymorphisms are at the end of the coding region and in an intron. Only one non-synonymous amino acid change is in the coding region. *AtRALFL27* shows a high degree of amino acid changes equally distributed throughout the coding region, and 3 of them are non-synonymous changes.


**Figure 11:** DNA sequence comparison at the genes a) *NFI* (AT3G29760), b) *AtSABP2* (At3g29770) and c) *AtRALFL27* (At3g29780). Colored bars indicate sequence changes between Ler-1 and the reference genome of Col-0 and between Cvi-0 and Col-0. Green bars with a letter in a green square indicate a synonymous change; red bars with a letter in a red square a non-synonymous change. For simplicity, the amino-acid code was used also outside of the translated regions. Bars without square indicate amino-acid changes in non-coding regions.

To summarize, a qRT-PCR analysis revealed a significant difference in the expression level of *HYH* in response to UV-C between Ler-1 and Cvi-0. *In silico* comparison of three candidate genes of QTL2 revealed that all three genes show amino acid divergence, thus leaving them as possible causal genes for natural variation of the trait. Because of the limited functional description of the 3 genes in the literature, no gene can be favored at this point. However, *AtRALFL27* and *NFI* show the highest degree of polymorphism which makes them more likely as candidate genes so far. More biological tests are needed to confirm their responsibility for natural variation in resistance of UV-C induced DNA damage.

## 2.9 Expression of Arabidopsis photolyases

As described earlier, the two photolyases *UVR2* and *UVR3* of Arabidopsis are specialized in the repair of CPDs and 6-4 photoproducts, respectively. Both photolyases are the most downstream components of the repair pathway. In the darkness, the nuclear-localized *CONSTITUTIVE PHOTOMORPHOGENIC 1* (*COP1*, AT2G32950) and *DE-ETIOLATED 1* (*DET1,* AT4G10180) contribute to repression of *UVR2* and *UVR3* expression through degradation of *ELONGATED HYPOCOTYL 5* (*HY5,* AT5G11260) and *HYH* transcription factors. Upon illumination, COP1 is excluded from the nucleus and *UVR2* and *UVR3* expression levels are balanced through opposing actions of *DET1* and *HY5* and *HYH* (Castells *et al.* 2010). Genes for both photolyases are located outside of the currently mapped QTL intervals, however, their activity may well indicate defects in upstream signaling processes. Therefore, a qRT-PCR was carried out to test for possible expression differences between Cvi-0 and Ler-1. Seedlings were treated with UV-C radiation (8000J/m²) and tissue was harvested immediately (R0), 1 hour (R1), and 6 hour (R6) after treatment. Expression levels were normalized to the housekeeping gene *EUKARYOTIC TRANSLATION INITIATION FACTOR 4A1* (*EIF4A1,* At3G13920).

The expression of *UVR2* in Cvi-0 was not significantly changed (p=0.05) by UV-C radiation at any time point (Figure 12). In contrast, the accession Ler-1 showed a significant increase of *UVR2* expression 1 hour after UV-C treatment. This suggests that a possible failure in the signaling pathway that activates *UVR2* and consequently a lack in repair of the CPDs is responsible for the hypersensitivity of Cvi-0 to UV radiation.

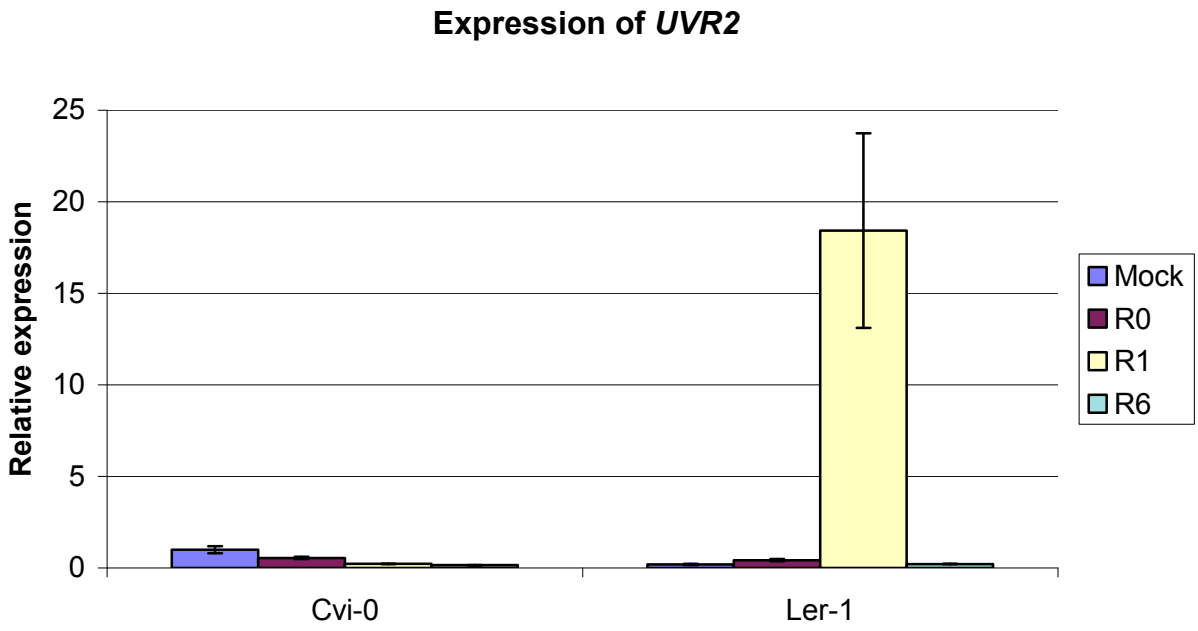


**Figure 12: Expression of *UVR2*.** Tissue was harvested immediately (R0), 1 hour (R1) and 6 hours (R6) after radiation. Expression is shown relative to the expression of untreated Cvi-0. Error bars indicate standard deviation.

Expression of *UVR3* showed a slight tendency to be up-regulated in Ler-1 immediately and 1 hour after treatment, whereas in Cvi-1 the expression is even down-regulated after UV-C exposure (Figure 13). However, the differences are not statistically significant.

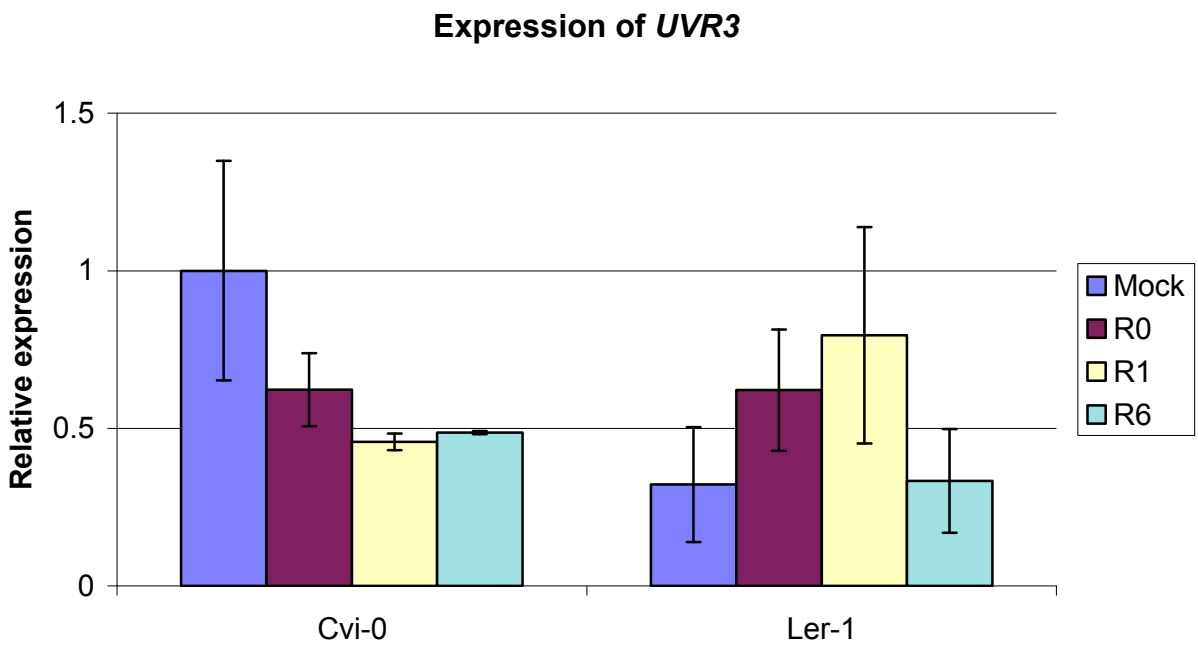


**Figure 13: Expression of *UVR3*.** Tissue was harvested immediately (R0), 1 hour (R1) and 6 hours (R6) after radiation. Expression is shown relative to the expression of untreated Cvi-0. Error bars indicate standard deviation.

In summary, after UV radiation, transcripts for both photolyase genes are elevated in Ler-1 but not in Cvi-0. The different extent makes *UVR2* the more plausible candidate but additional functional proof is required to postulate a causal relationship with UV resistance.

# 3 Discussion

**DNA damage by UV-radiation**

DNA is the carrier of the genetic information and responsible for passing the genetic information to progeny. However, DNA gets damaged by various endogenous and exogenous factors, leading to a large spectrum of lesions (reviewed in Tuteja *et al.* 2001).

Absorption of UV by DNA leads to photoproducts which inhibit DNA replication and transcription and, in addition, cause mutations (reviewed in Britt 1996). The photoproducts are CPD and 6-4 photoproducts, which make up 75% and 25% respectively, of the UV-induced DNA damage products (Mitchell and Nairn 1989). Plants developed strategies to repair such lesions by direct reversal through photolyases, excision repair, lesion bypass and recombinational repair (reviewed in Britt 2004). In this study, I analyzed variation in resistance to UV-induced DNA damage among natural accessions of the model plant *Arabidopsis thaliana*. I used UV-C radiation (100-280 nm) to induce lesions, because, in contrast to UV-B radiation, it induced mainly photoproducts, and DNA has an absorption maximum at 260 nm. Thus, the project aimed to address more directly DNA damage responses while avoiding physiological and morphological responses, like deposition of UV-absorbing phenolic compounds in epidermal tissues which are associated with UV-B (Jansen et al. 1998, Rozema *et al.* 1997).

**Arabidopsis accessions showed high degree of natural variation in sensitivity to UV-C irradiation**

As a response to UV-C treatment, the 96 Arabidopsis accessions exhibited great differences in relative survival. Accession phenotypes ranged from extreme tolerance to hypersensitivity. Highly resistant accessions did not show any differences compared to untreated controls, whereas the same dose of UV-C was lethal for sensitive ones. Comparison of two biological replicates revealed variability for some accessions. The reason for this variation is unclear. However, it may be due to different batches or age of seeds. The first replicate was done with seeds obtained from the European Arabidopsis Stock Centre (NASC), whereas the second one analyzed amplified seeds from plants grown at the GMI. Since some of the NASC seed stocks showed lower germination rates, we can not exclude age of received seeds as a potential factor causing this variation.

**UV-B and UV-C radiation caused similar phenotype**

A radiation of selected accessions with high doses of UV-B led to similar phenotypes as after UV-C treatment. Since the type of damage at the DNA level is similar, it is likely that resistance to both wavelengths are based on the same molecular responses. Nevertheless, UV-B is known for triggering a variety of responses beside photoproducts (Jansen, Gaba and Greenberg 1998), and it cannot be excluded that they contribute to the phenotype more indirectly.

**GWASs did not indicate clear candidate loci**

The performance of GWASs in order to detect loci responsible for natural variation in a time and labour efficient manner (reviewed in Hirschhorn and Daly 2005) did not indicate clear candidate loci. Only one out of 14 different GWASs revealed a significant peak, indicating association between trait and SNP marker position. The significant peak pointed to *ROC3*, a kinase-related gene at chromosome 2 (www.arabidopsis.org). However, neither *ROC3* nor other genes close to the pointed locus were described to be associated with repair of UV induced DNA damage. Possible association between *ROC3* and UV damage resistance remains to be tested. *In silico* comparison showed that the coding region of *ROC3* does not differ between Ler-1 and Cvi-0. An increased number of accessions may provide clearer and statistically more significant results. In addition, if the trait would depend on several independent causal nucleotide changes, this would cause problems for detection by a GWAS, as it was observed for example for the FLC locus (Atwell *et al.* 2010).

**Linkage-based mapping identified two QTLs**

Since GWASs did not allow identification of candidate gene(s), a classical QTL mapping approach using two parental accessions with extreme phenotypes was started within the frame of this diploma project. As suitable parental lines, I selected Ler-1 showing resistance and Cvi-0 showing hypersensitivity towards UV-C radiation in several additional biological replicates. These accessions were extensively used in other mapping projects, and therefore many mapping resources such as markers, RILs, NILs were publicly available (Alonso-Blanco *et al.* 2007) and could immediately be used for this study.

The QTL mapping using F8 (biological replicate 1) and F9 (biological replicate 2) populations of 162 Ler-1/Cvi-0 RILs revealed two QTLs at the top of chromosome 3 with significant

effects on survival after exposure to high dose of UV-C radiation. Linkage-based fine mapping narrowed the intervals of QTL1 and QTL2 down to 500 and 123kb, respectively. Borders of QTL1 are 5.7 and 6.2 Mbp and of QTL2 11.593 and 11.716 Mbp.

For future fine mapping of causal genes, development of additional mapping resources would be needed. Within this work, I performed crossing between Cvi-0 and Ler-1 to obtain a segregating F2 population of approximately 100 plants. In a follow-up project, individual plants are further selfed to obtain recombinant inbred lines and to generate additional several hundreds of F3 and F4 lines.

**Sensitivity to UV-C is inherited as a dominant trait**

Sensitivity to DNA damage is generally inherited as a recessive Mendelian trait, as demonstrated by many forward and reverse genetic experiments (Friesner and Britt 2003, Garcia *et al.* 2003, Landry *et al.* 1997). Therefore, it was surprising that, for both QTLs, sensitivity to UV-C is inherited as a dominant Mendelian trait. In F1 (Ler-1 x Cvi-0), 100% of progeny showed the sensitive phenotype and 93.2% in F2. A two-dimensional genome scan indicated that both QTLs are independent. Dominant sensitivity would be plausible assuming that the causal genes underlying the two dominant QTLs may act as suppressors of one or more genes in the UV DNA response pathway.

**Possible candidate genes of QTL1 and QTL2**

The interval of QTL1 is a gene-rich region and still relatively large for empirical identification of candidate genes. However, the transcription factor *HYH* triggering expression of light inducible genes such as *UVR2* (Castells *et al*. 2010) and the photolyase *UVR2* itself were up-regulated in the resistant accession Ler-1 after UV-C treatment. Considering that sensitivity is inherited in a dominant way, these data suggest that a suppressor influencing *UVR2* directly or indirectly (e.g. via *HYH*) may be a candidate for determining tolerance to UV-C.

The relatively narrow region of QTL2 made a systematic search for candidate genes more appropriate. Among 79 genes within this region, the best candidates were the *NLI INTERACTING FACTOR (NIF)* protein (AT3g29760), *AtSABP2* (At3g29770) and *AtRALFL27* (At3g29780). None of these genes was assigned to a defined molecular function in Arabidopsis. However, *NIF* contains the NLI interacting factor domain that is often found in proteins of diverse function, including phosphatases in which the domain is often present together with the

BRCA1 C-terminus (BRCT) domain of *BREAST CANCER GENE 1* (*BRCA1*) domain. This domain is present in proteins involved in cell cycle checkpoint control in response to DNA damage (www.ebi.ac.uk). *AtSABP2* encodes a protein predicted to act as carboxylesterase and shows similarity to the methyl salicylate esterase *SABP2* from tobacco. *AtRALFL27* is a member of a diversely expressed predicted peptide family showing sequence similarity to tobacco *RAPID ALKALINIZATION FACTOR* (*RALF*). *AtRALFL27* is believed to play an essential role in Arabidopsis physiology (www.arabidopsis.org). All three genes contain sequence differences between Cvi-0 and Ler-1. The coding region of *NFI* shows 3 non-synonymous amino acid changes, one of them in an intron. *AtSABP2* shows a high degree of polymorphism, but only one non-synonymous amino acid change. *AtRALFL27* shows the highest degree of amino acid changes distributed throughout the cis-regulatory region and protein coding region (3 of them are non-synonymous changes).

**Outlook**

As outlined above, the currently mapped intervals may be too large to unambiguously identify the causal genes. Therefore, further fine mapping is needed. A new mapping population has been started in this thesis and is currently propagated within the scope of a follow-up PhD project.

A common strategy for confirming the biological relevance of candidate genes is the screen of mutations causing defects in these genes. As the trait of sensitivity to UV-C radiation is dominant, such a test has its limitations in this case. A further, in this case better suited method for confirming the candidate gene is the cloning and transformation of the putative gene from the sensitive (Cvi-0) into the resistant accession (Ler-1). Should the trait be transferred to the recipient accession, this method should finally confirm that the gene is responsible for the natural variation observed in this study.

Furthermore, additional experiments should verify the effects of the UV treatment applied in this study on the plants at the molecular level. Literature suggests the DNA lesions CPD and 6-4 photoproducts already described above (reviewed in Britt 2004). The accessions might vary in the amount or type of these products in response to UV and thereby offer a hint to the molecular differences. Such an analysis could be done by an ELISA test or immunoblotting assays as demonstrated in other studies (Castells *et al.* 2010, Klar *et al.* 2006).

## Concluding remarks

The main objective of this research was to gain further insight into the natural variation of resistance mechanism coping with UV-induced DNA damage. The high degree of natural variation found is an example of the adaptation of local populations to their environment. Within this project, phenotypic screens and QTL mapping were performed, and fine mapping was initiated. QTL1 and QTL2 most likely contain novel genes not described previously in connection with UV response. Hence this work is the basis for further research to identify molecular factors responsible for the natural variation of *Arabidopsis* in DNA repair of lesions caused by UV-C radiation. The depletion of the ozone layer increased UV-B radiation on earth surface (Paul 2000). The harmful effects of UV irradiation on plants include suppression of growth, browning and chlorosis of the leaf or DNA damage. As a consequence, yield of crops as well as food quality are decreasing (Mohammed and Tarpley 2009, Teramura and Sullivan 1994). As UV-C radiation has similar effects on plants as UV-B (Brash 1997), further research based on this work will elucidate genes responsible for UV tolerance and hence provide means in genetic engineering or breeding of UV-tolerant lines. Therefore, this work provides a basis for potential crop improvement by increasing UV-tolerance.

# 4 Materials and Methods

## 4.1 Plant material

### 4.1.1 Accessions

The collection of 96 *Arabidopsis thaliana* accessions (set N22660, listed in the appendix) and the F8 generation of 162 RILs of Cvi-1 x Ler-0 cross (set N22000) as well as the mutant lines for *AtRALFL27* (N575170) and *AtSABP2* (N511783) were obtained from the Nottingham Arabidopsis Stock Centre (NASC, http://arabidopsis.info/). The seeds used for all experiments were harvested from plants grown on soil (Frux Einheitserde ED 63, Werkverband Einheitserde Germany) in a greenhouse under long day conditions (16:8 hours light:dark).

For trait inheritance tests and fine mapping, the different accessions were crossed in order to obtain segregating populations. To avoid contamination by own pollen, flowers were emasculated by removing the immature anthers with surface-sterilized forceps. Two to three days later, plants were pollinated with pollen from the selected paternal accession. Flowers were labeled and seeds were harvested approximately three weeks later.

### 4.1.2 Seed sterilization

Seeds were surface-sterilized by incubating them for 6 min. in a bleach solution (5 % sodium hypochlorite and 0.01 % Tween 20 as a detergent to lower surface tension in sterile water), followed by a washing step with sterile water through a Miracloth filter paper. After drying, seeds were stored under sterile, dry and dark conditions until use.

## 4.2 Growth media

Stock solutions

MS macro (1 liter): $KNO_3$ (19 g), $NH_4NO_3$ (16.5 g), $CaCl_2$ x $H_20$ (4.4 g), $MgSO_4$ x 7 $H_2O$ (3.7 g), $KH_2PO_4$ (1.7 g).

B5 micro (100 ml): $MNSO_4$ x $H_20$ (1 g), $H_3B0_3$ (300 mg), $ZnSO_4$ x 7 $H_2O$ (200 mg), KJ (75 mg), $Na_2MoO_4$ (25 mg), $CuSO_4$ x 5 $H_20$ (2.5 mg), $CoCl_2$ x $H_2O$ (2.5 mg).

Ferric citrate (500 ml): ammonium ferric citrate (5 g)

MES (100 ml): MES (14 g) dissolved in $H_20$ (80 ml), pH 6 with 2 M NaOH and final volume brought to 100 ml with $H_20$

GM (germination medium):

Solution 1: MS macro (25 ml), B5 micro (0.5 ml), ferric citrate (2.5 ml), sucrose (5 g); adjusting pH 5.4 with 2 M NaOH, filling up with H20 to a final volume of 50 ml, filter-sterilization.

Solution 2: Merck agar (4 g), $H_20$ (450 ml) adjusting pH 6 with MES, autoclaving.

Final GM: Mixing solution 1 (50 ml) and solution 2 (450 ml).

## 4.3 UV assay

For UV-C assay twelve seeds per accession were plated in regular intervals on the surface of GM media, stratified at 4°C for 4 days and then transferred to a growth chamber (16 h day length, 22°C, light bulbs: Osram L36W/840). Four days later, seedlings were irradiated with UV-C (254 nm) in a Stratalinker 2400 (Stratagene, La Jolla, USA) at different doses (see Results), while the covers of the plates were removed. The UV-C doses were validated with a UV-meter equipped with a detector for 254 nm, (VLX-3.W, Vilber Lourmat, Marne-la-Vallée, France). Plants were placed back to the growth chamber under the same conditions as before. Phenotypes were evaluated 7-13 days after radiation, depending on the assay. For UV-B assays, 7 days old seedlings were radiated with acute UV-B dose (1.5 mW/cm² for 3.5 hours). The growth conditions were 24°C and 12 hours day length. Phenotypes were evaluated 8 days after radiation.

## 4.4 QTL mapping

QTL Mapping was done with the 'qtl' package of R (www.r-project.org). The genome scan with a single QTL model was performed using the standard model for QTL mapping (Lander and Botstein 1989). The residual phenotypic variation is assumed to follow a normal distribution, and analysis is analogous to analysis of variance. The maximum likelihood was calculated via the expectation-maximization (EM) algorithm (Dempster *et al.* 1977). QTL-

thresholds were calculated based on 1000 permutations and a confidence interval of 0.05%. The two-dimensional genome scan with a two-QTL model was done also by standard interval mapping (Lander and Botstein 1989) by EM algorithm (Dempster *et al.* 1977).

## 4.5 Genome Wide Association Studies (GWAS)

The GWA analysis was based on 216,130 SNPs with an estimated error rate of 1.6 %. GWA analysis was done with and without correction for confounding. For analysis with confounding, the efficient mixed-model association (EMMA) was applied (Kang *et al.* 2008) in order to correct a wide range of sample structures by accounting for pair wise relatedness between individuals. Without confounding, the Kruskal-Wallis (KW) test was used (Atwell *et al.* 2010). The KW test can be considered as the non-parametric counterpart to ANOVA for testing equality among groups. The test does not assume normal distribution of the traits and is therefore more robust to outliers and violations (Hao *et al.* 2010).

## 4.6 Nucleic acids extraction

### 4.6.1 DNA extraction

Plant tissue was shock-frozen in liquid nitrogen, and the DNA was isolated using the PhytoPure Genomic DNA Extraction Kit (GE Healthcare, Little Chalfont, United Kingdom) as described. In brief, 0.05 g of frozen young leaves was milled in a pre-cooled ball mill (Retsch MM2000, Retsch GmbH & Co. K.G., Haan Germany) for 4 min at 300 rpm. Reagent 1 (300 µl) with 20 µg/ml RNase A (Fermentas, Burlington, Canada) were added, and the samples were incubated at 37°C for 30 min. Subsequently, 200 µl Reagent 2 was added, and the samples were incubated at 65°C for 10 min and inverted every 2 min. Chloroform (250 µl) and 50 µl PhytoPure Resin Solution were added, and the samples were rotated for 10 min on a tilt shaker at room temperature. Samples were centrifuged at 3700 rpm for 10 min at room temperature, and the upper phase of supernatant was transferred (without disturbing the protein layer) into fresh tube and mixed with 360 µl isopropanol. After full speed centrifugation at 4°C for 10 min, the supernatant was discarded and the pellet was washed in 0.5 ml of ice-cold 70 % ethanol, followed by full speed centrifugation for 7 min at 4°C. The supernatant was decanted, the pellet air-dried and resuspended in 25 µl $H_20$ and stored at 4°C.

### 4.6.2 RNA extraction

Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Venlo, The Netherlands) following manual specifications. In brief, 0.1 g of young leaves was bead-milled as described for preparation of DNA and 450 µl RLT Buffer containing 5 µl β-mercaptoethanol was added. The sample was transferred to a QIAshredder spin column placed in a 2 ml collection tube and centrifuged for 2 min at full speed. The supernatant of the flow-through was transferred into a new centrifuge tube without disturbing the cell-debris. Ethanol (96-100 %) corresponding to the half volume of the sample was added. The sample was transferred into an RNeasy spin column placed in a 2 ml collection tube and centrifuged for 15 s at 10000 rpm. The flow-through was discarded. RW1 buffer (350 µl) was added to the column, centrifuged for 15 s at 10000 rpm and the flow-through discarded. RDD buffer (80 µl) containing 10 µl DNaseI (1 u/µl, Fermentas) was added directly to the spin column and incubated for 15 min at room temperature. RW1 buffer (350 µl) was added to the column and centrifuged for 15 s at 10000 rpm and the flow-trough discarded. The column was washed twice with 500 µl RPE buffer by centrifugation for 15 s at 10000 rpm. The column was placed into a 1.5 ml collection tube, 35 µl of RNase-free water was added and the column was centrifuged for 1 min at 10000 rpm to elute the RNA.

## 4.7 PCR

PCR reactions were done using a standardized protocol, adjusted to the primer melting temperatures and to the expected fragment length. Each reaction was set up with 10.8 µl $H_2O$, 2 µl Buffer, 2 µl dNTPs (2.5 mM each), 2 µl forward and 2 µl reverse primer (10 µM), 0.2 µl Taq Polymerase (5 u/µl, 5 Prime, Hamburg Germany) and 1 µl template DNA (30 ng/µl). PCR cycle protocol: 94°C for 5 min, 34-38 cycles of 94°C for 30 sec followed by 53-60°C (depending on primer melting temperature) for 30 sec followed by 72°C for 30 sec.

## 4.8 Restriction digest

The restriction digestions of the PCR fragments was done as recommended by the supplier (Fermentas): the reaction was set up with 2 µl buffer, 0.2 µl enzyme (10 u/µl), 10.8 µl $H_20$ and 7 µl template and performed over night at a temperature specified for each enzyme by the supplier.

## 4.9 Gel electrophoresis

The detection of PCR and restriction fragments was performed via a gel doc system. DNA was separated on 1.2-2 % agarose gels stained with ethidium bromide under 75 V for around 1h. A UV light transilluminator was used for image capturing.

## 4.10 cDNA Synthesis

At first, possible residual DNA contamination was cleaned by one more round of DNase I digestion. For this, total RNA concentration was measured with a UV/Vis Spectrophotometer (Nanodrop ND-1000, Peqlab Biotechnologie GmbH, Erlangen, Germany) and 2.5 µg were mixed with 2.5 µl of 10 x DNase I buffer with $MgCl_2$ (Fermentas), 1.25 µl RNase inhibitor (Fermentas), 2.5 µl DNase I (1 u/µl, Fermentas) and filled up to 25 µl with RNase-free water. Then the samples were incubated at 37°C for 30 min. Afterwards 2.5 µl (25 mM) EDTA (Fermentas) was added and the samples were incubated at 65°C for 10 min to inactivate DNase I.

After DNase I treatment, the cDNA was synthesized with oligo dT primers (Fermentas) by adding 2.5 µl oligo dT primer, 10 µl 5 x M-MuLV RT buffer (Fermentas), 5 µl dNTPs (10 mM), 4 µl RNase-free water and 1 µl of RevertAid M-MuLV Reverse Transcriptase (Fermentas). Control samples without Reverse Transcriptase were prepared in the same way, just by adding 1 µl of RNase-free water instead of this enzyme. Reverse transcription reaction was carried out at 25°C for 10 min, followed by incubation at 42°C for 90 min and by 70°C for 10 min.

The control PCR for approving cDNA transcription was set up with the common PCR protocol (see 4.7) using the primer UBC28q. The negative control was set up with the reaction without reverse transcriptase and overall control with Col-0 and $H_20$. PCR cycle protocol for positive control: 94°C for 4 min, 30 cycles of 94°C for 30 sec followed by 60°C for 30 sec followed by 72°C for 15 sec and a last step of 72°C for 5 min. PCR cycle protocol for negative control: 94°C for  min, 40 cycles of 94°C for 30 sec followed by 60°C for 30 sec followed by 72°C for 1 min and a last step of 72°C for 5 min. The amplicons obtained were analyzed by gel electrophoresis and the gel-doc system.

## 4.11 Quantitative PCR (qPCR) Analysis

The qPCR analysis was performed in technical triplicates using the SensiMix Plus SYBR Kit (Peqlab Biotechnologie GmbH, Erlangen Germany) and iQ5 equipment (Bio-Rad, Hercules, USA). Each PCR reaction was set up with 7.5 µl SensiMix, 0.4 µl forward primer, 0.4 µl reverse primer, 1.7 µl $H_2O$ and 5 µl cDNA (diluted 1:5 in $H_2O$). The expression was normalized to that of the housekeeping gene EIF4A1. For statistical analysis, the unpaired 2-sided t-test was applied.

## 4.12 Primers

SNP data of the two accessions Cvi-0 and Ler-1 were available, thus CAPS markers were used for genotyping, and the following primers were used.

**Table 2: Primer sequences for genotyping.**

| Primer Name | Primer Sequence (5' to 3') |
|---|---|
| JR1-F | GGTGAAAAATCTTGCTAAGCAGAAT |
| JR1-R | GATGATTTGGTCATATCAGCAAGT |
| JR2-F | AACATCAGTTATCAAAGTAACGT |
| JR2-R | TGTCTCGATTCGAGGCCTACT |
| JR3-F | TTCCAACCCATGAGAAACGATCA |
| JR3-R | GCGAGGCAAGTGCTGTGAGT |
| JR4-F | CTTATCTGTATTAATAGTGGTTGTGT |
| JR4-R | GGAAGCTTGAGACTTCCGAGA |
| JR5-F | CTTATCTGTATTAATAGTGGTTGTGT |
| JR5-R | GGAAGCTTGAGACTTCCGAGA |
| JR6-F | CTCTTCTACGGTCAATACATTCT |
| JR6-R | TCCATTGATGAGTCTTCTGTATCA |
| JR7-F | ATCCGCTGGAACATCCTTGAGA |
| JR7-R | ATCGGCTGTTACGAGTAATGATGA |
| JR9-F | GGTTTGTTTAGTTATATGCCTAGT |
| JR9-R | GAGCTCGATGCACACTGAGA |
| JR10-F | ACCCTCCAACCGCAATTGACT |

| | |
|---|---|
| JR10-R | GGGCTAATTCTGGCGATCTCA |
| JR11-F | GACTAACTAGTGACCAAACCACT |
| JR11-R | GTTGCAAACAAGGTTGTTACGACT |
| JR20-F | GTGCTATTCGTAGGCGATTCTCT |
| JR20-R | CTTATTCCATCAGGGACAATACATA |
| JR21-F | TGGGCTTCTACTACGGCTTCTA |
| JR21-R | GGAAATGAGTGGTCTGGGAAGAA |
| JR22-F | CTTCTATTTCTCACGCTTCAAAC |
| JR22-R | TCTTCAAGGTATTAATGGCGTAA |
| JR23-F | TAGACCCAAGACTATTTGATTGAC |
| JR23-R | TTCCCAAGAGTAGACATCCACCT |
| JR24-F | GGATCAGTTAGTCAGCCCTGAT |
| JR24-R | AAACCCAAAAATGCGACCACGTT |
| JR25-F | ACGAGTTAGAAATACTGTGCACGT |
| JR25-R | TCTCGTATAACAGTGCTCGTTGA |
| JR26-F | GTAAATCAGAAAGGAGGTTGATGT |
| JR26-R | CGAATCATCACTACGGTAACGTT |
| JR27-F | TTGTTATACGTACTCGGATGCTGA |
| JR27-R | CGGAGAACAAGCCGTTCATATTC |
| JR28-F | ACTTCGCAATTCTTAGCAATTAG |
| JR28-R | TTCACCTGCATGATGGCAGAAA |
| JR29-F | ATACTCAATCCTGCCGCTTCCAT |
| JR29-R | ATCCCGCTCTGTAGTAGTGCAA |
| JR30-F | ATTAGGTGGGATTCAGGATCAAA |
| JR30-R | GGAATCAGGCAAGAGTTGAGAA |
| JR31-F | AATAGCCACCGCTGAATACGGAA |
| JR31-R | CCGGAAACGGAAAGGGGATATT |
| JR32-F | ATAAAACCCCTAGACCAGAAGAA |
| JR32-R | GATGTTACAGGAGTCCTTTTCAT |
| JR33-F | AAAAATCCGACAGGCCTGGAGA |
| JR33-R | CTCTCTGTTACTGTAAGTTTTGTGA |

The qRT-PCR was performed with the following primers:

**Table 3: Primer sequences for qRT-PCR.**

| **Primer Name** | **Primer Sequence (5' to 3')** |
|---|---|
| UVR2qF | CAGTTAGAGAAGGGACTGACAGCAGAT |
| UVR2qR | TTCAGGTCCCTTGGTCCATTCTAGAAT |
| UVR3qF | GGCTGCTTGGAGGGACGGTAAGACAGG |
| UVR3qR | TAGATGGTGCATCCAACCCCATTTCA |
| HYHqF | AAGCAGCAGCGCCGACGATGGAGTCAA |
| HYHqR | GTTGCGCTGATACTCTGTTCCTCAATAA |
| EIF4A1qF | ATCCAAGTTGGTGTGTTCTCC |
| EIF4A1qR | GAGTGTCTCGAGCTTCCACTC |

# 5 Acknowledgements

Special thank goes to Ales Pecinka who was my first address in carrying out the experiments, for scientific discussions, showing me all the new techniques in the lab, for his proofreading and the introduction into the scientific world.

I would like to thank my supervisor Ortrun Mittelsten Scheid for the opportunity to join her group at the Gregor Mendel Institute of Molecular Plant Biology, for her support and providing the interesting research topic.

It would like to thank also Nicole Lettner and Bonnie Wohlrab for technical assistance, taking care of the plants, making reliable media and showing me all the basic methods for looking at least a bit professional in a molecular biology laboratory.

Thanks go also to the other group members for their help, discussion and creating a nice lab environment: Andrea Förster, Laura Sedman, Marisa Rosa, Huy Dinh and Vanja Cavrak.

I would also like to acknowledge my supervisor Margit Laimer for her support and readiness to review this thesis for submission at my home university, the University of Natural Resources and Life Sciences (BOKU).

I am grateful to Bjarni Vilhjalmsson (Lab of Magnus Nordborg, Vienna) for performing the statistical part of the genome-wide association study and to Marina Gonzalez (Lab of Roman Ulm, Freiburg/Geneva) for carrying out the UV-B assays.

# 6 References


**Alonso-Blanco, C., Aarts, M.G.M., Bentsink, L., Keurentjes, J.J.B., Reymond, M., Vreugdenhil, D. and Koornneef, M.** (2009) What Has Natural Variation Taught Us about Plant Development, Physiology, and Adaptation? *Plant Cell*, **21**, 1877-1896.

**Alonso-Blanco, C. and Koornneef, M.** (2000) Naturally occurring variation in Arabidopsis: an underexploited resource for plant genetics. *Trends in Plant Science*, **5**, 22-29.

**Alonso-Blanco, C., Peeters, A.J.M., Koornneef, M., Lister, C., Dean, C., van den Bosch, N., Pot, J. and Kuiper, M.T.R.** (1998) Development of an AFLP based linkage map of Ler, Col and Cvi Arabidopsis thaliana ecotypes and construction of a Ler/Cvi recombinant inbred line population. *Plant Journal*, **14**, 259-271.

**Atwell, S., Huang, Y.S., Vilhjalmsson, B.J., Willems, G., Horton, M., Li, Y., Meng, D.Z., Platt, A., Tarone, A.M., Hu, T.T., Jiang, R., Muliyati, N.W., Zhang, X., Amer, M.A., Baxter, I., Brachi, B., Chory, J., Dean, C., Debieu, M., de Meaux, J., Ecker, J.R., Faure, N., Kniskern, J.M., Jones, J.D.G., Michael, T., Nemri, A., Roux, F., Salt, D.E., Tang, C.L., Todesco, M., Traw, M.B., Weigel, D., Marjoram, P., Borevitz, J.O., Bergelson, J. and Nordborg, M.** (2010) Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. *Nature*, **465**, 627-631.

**Bowman, K.K., Sidik, K., Smith, C.A., Taylor, J.S., Doetsch, P.W. and Freyer, G.A.** (1994) A New Atp-Independent DNA Endonuclease from Schizosaccharomyces-Pombe That Recognizes Cyclobutane Pyrimidine Dimers and 6-4-Photoproducts. *Nucleic Acids Research*, **22**, 3026-3032.

**Brash, D.E.** (1997) Sunlight and the onset of skin cancer. *Trends in Genetics*, **13**, 410-414.

**Britt, A.B.** (1996) DNA damage and repair in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **47**, 75-100.

**Britt, A.B.** (2004) Repair of DNA damage induced by solar UV. *Photosynthesis Research*, **81**, 105-112.

**Castells, E., Molinier, J., Drevensek, S., Genschik, P., Barneche, F. and Bowler, C.** (2010) det1-1-induced UV-C hyposensitivity through UVR3 and PHR1 photolyase gene over-expression. *Plant Journal*, **63**, 392-404.

**Chappell, J. and Hahlbrock, K.** (1984) Transcription of Plant Defense Genes in Response to Uv-Light or Fungal Elicitor. *Nature*, **311**, 76-78.

**Chen, J.J., Mitchell, D.L. and Britt, A.B.** (1994) Light-Dependent Pathway for the Elimination of Uv-Induced Pyrimidine-(6-4) Pyrimidinone Photoproducts in Arabidopsis. *Plant Cell*, **6**, 1311-1317.

**Cockell, C.S. and Knowland, J.** (1999) Ultraviolet radiation screening compounds. *Biological Reviews*, **74**, 311-345.

**Demple, B. and Harrison, L.** (1994) Repair of Oxidative Damage to DNA - Enzymology and Biology. *Annual Review of Biochemistry*, **63**, 915-948.

**Dempster, A.P., Laird, N.M. and Rubin, D.B.** (1977) Maximum Likelihood from Incomplete Data Via Em Algorithm. *Journal of the Royal Statistical Society Series B-Methodological*, **39**, 1-38.

**Doetsch, P.W., McCray, W.H. and Valenzuela, M.R.L.** (1989) Partial-Purification and Characterization of an Endonuclease from Spinach That Cleaves Ultraviolet Light-Damaged Duplex DNA. *Biochimica Et Biophysica Acta*, **1007**, 309-317.

**Dolferus, R., Vandenbossche, D. and Jacobs, M.** (1990) Sequence-Analysis of 2 Null-Mutant Alleles of the Single Arabidopsis Adh Locus. *Molecular & General Genetics*, **224**, 297-302.

**Donahue, B.A., Yin, S., Taylor, J.S., Reines, D. and Hanawalt, P.C.** (1994) Transcript Cleavage by Rna-Polymerase-Ii Arrested by a Cyclobutane Pyrimidine Dimer in the DNA-Template. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 8502-8506.

**Friesner, J. and Britt, A.B.** (2003) Ku80- and DNA ligase IV-deficient plants are sensitive to ionizing radiation and defective in T-DNA integration. *Plant Journal*, **34**, 427-440.

**Garcia, V., Bruchet, H., Camescasse, D., Granier, F., Bouchez, D. and Tissier, A.** (2003) AtATM is essential for meiosis and the somatic response to DNA damage in plants. *Plant Cell*, **15**, 119-132.

**Gillet, L.C.J. and Scharer, O.D.** (2006) Molecular mechanisms of mammalian global genome nucleotide excision repair. *Chemical Reviews*, **106**, 253-276.

**Goodman, M.F.** (2002) Error-prone repair DNA polymerases in prokaryotes and eukaryotes. *Annual Review of Biochemistry*, **71**, 17-50.

**Hao, K., Chudin, E., Greenawalt, D. and Schadt, E.E.** (2010) Magnitude of Stratification in Human Populations and Impacts on Genome Wide Association Studies. *Plos One*, **5**.

**Hariharan, P.V. and Cerutti, P.A.** (1977) Formation of Products of 5,6-Dihydroxydihydrothymine Type by Uv Light in Hela-Cells. *Biochemistry*, **16**, 2791-2795.

**Hirschhorn, J.N. and Daly, M.J.** (2005) Genome-wide association studies for common diseases and complex traits. *Nature Reviews Genetics*, **6**, 95-108.

**Holm, M., Ma, L.G., Qu, L.J. and Deng, X.W.** (2002) Two interacting bZIP proteins are direct targets of COP1-mediated control of light-dependent gene expression in Arabidopsis. *Genes & Development*, **16**, 1247-1259.

**Howland, G.P.** (1975) Dark-Repair of Ultraviolet-Induced Pyrimidine Dimers in DNA of Wild Carrot Protoplasts. *Nature*, **254**, 160-161.

**Howland, G.P. and Hart, R.W.** (1977) Radiation Biology of Cultured Plant Cells. *REINERT, J. AND Y. P. S. BAJAJ (ED.n. APPLIED AND FUNDAMENTAL ASPECTS OF PLANT CELL, TISSUE, AND ORGAN CULTURE. XVIII+803P. ILLUS. SPRINGER-VERLAG: BERLIN, WEST GERMANY; NEW YORK, N.Y., USA. ISBN 3-540-07677-8; ISBN 0-387-07677-8*, 731-756.

**Jansen, M.A.K., Gaba, V. and Greenberg, B.M.** (1998) Higher plants and UV-B radiation: Balancing damage, repair and acclimation. *Trends in Plant Science*, **3**, 131-135.

**Kang, H.M., Zaitlen, N.A., Wade, C.M., Kirby, A., Heckerman, D., Daly, M.J. and Eskin, E.** (2008) Efficient control of population structure in model organism association mapping. *Genetics*, **178**, 1709-1723.

**Kanofsky, J.R. and Sima, P.** (1991) Singlet Oxygen Production from the Reactions of Ozone with Biological Molecules. *Journal of Biological Chemistry*, **266**, 9039-9042.

**Keurentjes, J.J.B., Bentsink, L., Alonso-Blanco, C., Hanhart, C.J., Vries, H.B.D., Effgen, S., Vreugdenhil, D. and Koornneef, M.** (2007) Development of a near-isogenic line population of Arabidopsis thaliana and comparison of mapping power with a recombinant inbred line population. *Genetics*, **175**, 891-905.

**Klar, T., Kaiser, G., Hennecke, U., Carell, T., Batschauer, A. and Essen, L.O.** (2006) Natural and non-natural antenna chromophores in the DNA photolyase from Thermus thermophilus. *Chembiochem*, **7**, 1798-1806.

**Koornneef, M., Alonso-Blanco, C., Peeters, A.J.M. and Soppe, W.** (1998) Genetic control of flowering time in arabidopsis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **49**, 345-370.

**Koornneef, M., Alonso-Blanco, C. and Vreugdenhil, D.** (2004) Naturally occurring genetic variation in Arabidopsis thaliana. *Annual Review of Plant Biology*, **55**, 141-172.

**Koornneef, M. and Meinke, D.** (2010) The development of Arabidopsis as a model plant. *Plant Journal*, **61**, 909-921.

**Kowalczykowski, S.C., Dixon, D.A., Eggleston, A.K., Lauder, S.D. and Rehrauer, W.M.** (1994) Biochemistry of Homologous Recombination in Escherichia-Coli. *Microbiological Reviews*, **58**, 401-465.

**Lander, E.S. and Botstein, D.** (1989) Mapping Mendelian Factors Underlying Quantitative Traits Using Rflp Linkage Maps. *Genetics*, **121**, 185-199.

**Landry, L.G., Stapleton, A.E., Lim, J., Hoffman, P., Hays, J.B., Walbot, V. and Last, R.L.** (1997) An Arabidopsis photolyase mutant is hypersensitive to ultraviolet-B radiation. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 328-332.

**Levine, A., Tenhaken, R., Dixon, R. and Lamb, C.** (1994) H2o2 from the Oxidative Burst Orchestrates the Plant Hypersensitive Disease Resistance Response. *Cell*, **79**, 583-593.

**Lin, W.S., Wu, X.H. and Wang, Z.G.** (1999) A full-length cDNA of hREV3 is predicted to encode DNA polymerase xi for damage-induced mutagenesis in humans. *Mutation Research-DNA Repair*, **433**, 89-98.

**Lindahl, T. and Nyberg, B.** (1972) Rate of Depurination of Native Deoxyribonucleic Acid. *Biochemistry*, **11**, 3610-&.

**McKenzie, R.L., Bjorn, L.O., Bais, A. and Ilyasd, M.** (2003) Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Photochemical & Photobiological Sciences*, **2**, 5-15.

**Mitchell, D.L. and Nairn, R.S.** (1989) The Biology of the (6-4) Photoproduct. *Photochemistry and Photobiology*, **49**, 805-819.

**Mitchell, D.L., Vaughan, J.E. and Nairn, R.S.** (1989) Inhibition of Transient Gene-Expression in Chinese-Hamster Ovary Cells by Cyclobutane Dimers and (6-4) Photoproducts in Transfected Ultraviolet-Irradiated Plasmid DNA. *Plasmid*, **21**, 21-30.

**Mohammed, A.R. and Tarpley, L.** (2009) Effects of Elevated Ultraviolet-B Radiation on Productive Tillers, Spikelet Sterility and Grain Characteristics of Southern US Rice (Oryza sativa L.) Cultivars. *Journal of Agronomy and Crop Science*, **195**, 292-300.

**Orozco, B.M., McClung, C.R., Werneke, J.M. and Ogren, W.L.** (1993) Molecular-Basis of the Ribulose-1,5-Bisphosphate Carboxylase Oxygenase Activase Mutation in Arabidopsis-Thaliana Is a Guanine-to-Adenine Transition at the 5'-Splice Junction of Intron 3. *Plant Physiology*, **102**, 227-232.

**Ossowski, S., Schneeberger, K., Lucas-Lledo, J.I., Warthmann, N., Clark, R.M., Shaw, R.G., Weigel, D. and Lynch, M.** (2010) The Rate and Molecular Spectrum of Spontaneous Mutations in Arabidopsis thaliana. *Science*, **327**, 92-94.

**Osterlund, M.T., Hardtke, C.S., Wei, N. and Deng, X.W.** (2000) Targeted destabilization of HY5 during light-regulated development of Arabidopsis. *Nature*, **405**, 462-466.

**Painter, R.B.** (1985) Inhibition and Recovery of DNA-Synthesis in Human-Cells after Exposure to Ultraviolet-Light. *Mutation Research*, **145**, 63-69.

**Paul, N.D.** (2000) Stratospheric ozone depletion, UV-B radiation and crop disease. *Environmental Pollution*, **108**, 343-355.

**Peters, J.L., Constandt, H., Neyt, P., Cnops, G., Zethof, J., Zabeau, M. and Gerats, T.** (2001) A physical amplified fragment-length polymorphism map of Arabidopsis. *Plant Physiology*, **127**, 1579-1589.

**Proticsabljic, M., Tuteja, N., Munson, P.J., Hauser, J., Kraemer, K.H. and Dixon, K.** (1986) Uv Light-Induced Cyclobutane Pyrimidine Dimers Are Mutagenic in Mammalian-Cells. *Molecular and Cellular Biology*, **6**, 3349-3356.

**Puchta, H., Swoboda, P. and Hohn, B.** (1995) Induction of Intrachromosomal Homologous Recombination in Whole Plants. *Plant Journal*, **7**, 203-210.

**Quaite, F.E., Sutherland, B.M. and Sutherland, J.C.** (1992) Action Spectrum for DNA Damage in Alfalfa Lowers Predicted Impact of Ozone Depletion. *Nature*, **358**, 576-578.

**Rebeck, G.W. and Samson, L.** (1991) Increased Spontaneous Mutation and Alkylation Sensitivity of Escherichia-Coli Strains Lacking the Ogt O6-Methylguanine DNA-Repair Methyltransferase. *Journal of Bacteriology*, **173**, 2068-2076.

**Reilly, J.J. and Klarman, W.L.** (1980) Thymine Dimer and Glyceollin Accumulation in Uv-Irradiated Soybean Suspension-Cultures. *Environmental and Experimental Botany*, **20**, 131-134.

**Rounsley, S.D. and Last, R.L.** (2010) Shotguns and SNPs: how fast and cheap sequencing is revolutionizing plant biology. *Plant Journal*, **61**, 922-927.

**Rozema, J., vandeStaaij, J., Bjorn, L.O. and Caldwell, M.** (1997) UV-B as an environmental factor in plant life: Stress and regulation. *Trends in Ecology & Evolution*, **12**, 22-28.

**Sakamoto, A., Lan, V.T.T., Hase, Y., Shikazono, N., Matsunaga, T. and Tanaka, A.** (2003) Disruption of the AtREV3 gene causes hypersensitivity to ultraviolet B light and gamma-rays in Arabidopsis: Implication of the presence of a translesion synthesis mechanism in plants. *Plant Cell*, **15**, 2042-2057.

**Sancar, A.** (1994a) Mechanisms of DNA Excision-Repair. *Science*, **266**, 1954-1956.

**Sancar, A.** (1994b) Structure and Function of DNA Photolyase. *Biochemistry*, **33**, 2-9.

**Seeberg, E., Eide, L. and Bjoras, M.** (1995) The Base Excision-Repair Pathway. *Trends in Biochemical Sciences*, **20**, 391-397.

**Setlow, P.** ed (1976) Nearest neighbor frequencies in deoxyribonucleic acids. Cleveland: CRC Press.

**Sinha, R.P. and Hader, D.P.** (2002) UV-induced DNA damage and repair: a review. *Photochemical & Photobiological Sciences*, **1**, 225-236.

**Somerville, C. and Koornneef, M.** (2002) Timeline - A fortunate choice: the history of Arabidopsis as a model plant. *Nature Reviews Genetics*, **3**, 883-889.

**Stapleton, A.E.** (1992) Ultraviolet-Radiation and Plants - Burning Questions. *Plant Cell*, **4**, 1353-1358.

**Tanaka, A., Sakamoto, A., Ishigaki, Y., Nikaido, O., Sun, G., Hase, Y., Shikazono, N., Tano, S. and Watanabe, H.** (2002) An ultraviolet-B-resistant mutant with enhanced DNA repair in Arabidopsis. *Plant Physiology*, **129**, 64-71.

**Teramura, A.H. and Sullivan, J.H.** (1994) Effects of Uv-B Radiation on Photosynthesis and Growth of Terrestrial Plants. *Photosynthesis Research*, **39**, 463-473.

**Tissier, A., Frank, E.G., McDonald, J.P., Iwai, S., Hanaoka, F. and Woodgate, R.** (2000) Misinsertion and bypass of thymine-thymine dimers by human DNA polymerase iota. *Embo Journal*, **19**, 5259-5266.

**Todo, T.** (1999) Functional diversity of the DNA photolyase blue light receptor family. *Mutation Research-DNA Repair*, **434**, 89-97.

**Tuteja, N., Singh, M.B., Misra, M.K., Bhalla, P.L. and Tuteja, R.** (2001) Molecular mechanisms of DNA damage and repair: Progress in plants. *Critical Reviews in Biochemistry and Molecular Biology*, **36**, 337-397.

**Tyrrell, R.M.** (1996) Activation of mammalian gene expression by the UV component of sunlight - From models to reality. *Bioessays*, **18**, 139-148.

**Van Lijsebettens, M. and Van Montagu, M.** (2005) Historical perspectives on plant developmental biology. *International Journal of Developmental Biology*, **49**, 453-465.

**Ward, J.F.** (1975) Molecular Mechanisms of Radiation Induced Damage to Nucleic-Acids. *Lett, John T. and Howard Adler (Ed.). Advances in Radiation Biology, Vol. 5. X+277p. Illus. Adacemic Press, Inc.: New York, N.Y., U.S.a.; London, England. Isbn 0-12-035405-5*, 181-239.

**Waterworth, W.M., Jiang, O., West, C.E., Nikaido, M. and Bray, C.M.** (2002) Characterization of Arabidopsis photolyase enzymes and analysis of their role in protection from ultraviolet-B radiation. *Journal of Experimental Botany*, **53**, 1005-1015.

**Weigel, D. and Nordborg, M.** (2005) Natural variation in arabidopsis. How do we find the causal genes? *Plant Physiology*, **138**, 567-568.

**Yu, S.L., Johnson, R.E., Prakash, S. and Prakash, L.** (2001) Requirement of DNA polymerase eta for error-free bypass of UV-induced CC and TC photoproducts. *Molecular and Cellular Biology*, **21**, 185-188.

**Zhang, J.Z., Creelman, R.A. and Zhu, J.K.** (2004) From laboratory to field. Using information from Arabidopsis to engineer salt, cold, and drought tolerance in crops. *Plant Physiology*, **135**, 615-621.