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Transgenerational stress memory in *Arabidopsis thaliana* (L.) Heynh.: antioxidative enzymes and HSP70

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Transgenerational transmission of information about stress exposure is manifested as an increase in the somatic homologous recombination frequency in plants. Our aim was to investigate whether information about changes of antioxidative enzyme activities and protein HSP70 induction are also transmitted in response to stress caused by UV-C irradiation. These stress indicators were investigated in *Arabidopsis thaliana* plants exposed to UV-C irradiation (6 and 600 J m^{-2}) and its non-irradiated progeny. The activity of catalase was significantly decreased in the irradiated plants in comparison to the non-irradiated control plants, while the activity of guaiacol peroxidase was increased. The ascorbate peroxidase activity was not significantly changed. In irradiated plants there was an induction of a new HSP70 protein isoform. In the non-irradiated progeny of irradiated plants, a significant decrease in catalase and ascorbate peroxidase activity was noticed in comparison to plants whose parents were not irradiated. There was no significant change in guaiacol peroxidase activity or induction of HSP70 isoforms in the progeny. The obtained results indicate that, besides the already known increase in frequency of somatic homologous recombination, transmission of information about stress exposure can also include changes in activities of antioxidative enzymes catalase and ascorbate peroxidase. The explanations for the observed changes and the mechanism by which they occur have to be established in further research.

Keywords: *Arabidopsis thaliana*, UV-C, stress memory, antioxidative enzyme, catalase, ascorbate peroxidise, guaiacol peroxidise, HSP70

Abbreviations: APX – ascorbate peroxidase, **CAT** – catalase, **POD** – peroxidase, **HSP** – heat shock protein, **PAGE** – polyacrylamide gel electrophoresis, **ROS** – reactive oxygen species, **SHR** – somatic homologous recombination

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Introduction

As sessile organisms, plants have to cope with various environmental stresses such as drought, soil salinity, low or high temperature, excess light, toxic substances and pathogen attack. Plant response to stress conditions includes not only changes in physiological processes, regulation of cellular metabolism and gene expression but also alterations of the dynamics of the genome. In the last decade several studies demonstrated an increased rate of somatic homologous recombination (SHR) in response to various stress factors, e. g. UV irradiation, herbicides, osmotic stress and high temperature (LEBEL et al. 1993; RIES et al. 2000a, b; LUCHT et al. 2002; MOLINIER et al. 2006; PECINKA et al. 2009).

UV irradiation affects plants at several levels. DNA damage causes heritable mutations, while disturbances of physiological processes (STAPLETON 1992) reduce growth, development, photosynthesis, flowering, pollination and transpiration (ROZEMA et al. 1997, JANSEN et al. 1998). In order to minimize the effects of UV irradiation plants activate different physiological responses, such as the biosynthesis of protecting compounds (STAPLETON 1992), the reactive oxygen species (ROS) scavenging system (XU et al. 2008) and DNA repair mechanisms (MOLINIER et al. 2005).

One of the most important DNA repair mechanism is SHR (RIES et al. 2000b). MOLINIER et al. (2006) noticed an elevation of its frequency in *Arabidopsis thaliana* (ecotype Columbia) not only in plants exposed to UV-C irradiation and flagellin (an elicitor of defence mechanisms to pathogen attack) but also in four generations of their progeny, which were not exposed to irradiation themselves. For this transgeneration »stress memory« the authors suggested an epigenetic mechanism. Epigenetic changes are mitotically and/or meiotically heritable alterations in the chromatin structure, which occur without a change in the DNA sequence. They are mediated by changes in DNA methylation, histone modifications and by non-coding RNAs, which can cause changes in the chromatin architecture or DNA methylation patterns (BOND and FINNEGAN 2007). Some physiological effects related to epigenetic regulation in plants have already been investigated and described, like the process of vernalization (BURN et al. 1993, BOND and FINNEGAN 2007, SCHMITZ and AMASINO 2007).

Most of the stress-induced epigenetic modifications are reset to the basal level once the stress is relieved, while some of them may be stable, that is, may be inherited as »stress memory« across mitotic and even meiotic cell divisions (CHINNUSAMY and ZHU 2009).

Oxidative stress in plant cells, as a consequence of the accumulation of reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, hydrogen peroxide, singlet oxygen and organic hydroperoxides is one of the earliest responses to most, if not all, biotic and abiotic environmental stress conditions, among them UV irradiation. If not scavenged, ROS can damage plants by oxidizing the proteins, nucleic acids, photosynthetic pigments and membrane lipids. However, ROS can also act as signalling molecules and trigger a range of cellular responses important for stress tolerance. Tight control of ROS levels in cells is achieved by the antioxidative defence system including various antioxidants (MITTLER 2002). Superoxide dismutase catalyses the dismutation of superoxide into H_2O_2 and molecular oxygen. Catalases (CAT; E.C.1.11. 1.6.), the main H_2O_2-scavenging enzymes in plants, convert H_2O_2 to water and molecular oxygen, but have low substrate affinity. In addition to having a role in lignin biosynthesis and indole-3-acetic acid (IAA) degradation,

peroxidases (POD; E.C.1.11.1.7) convert H_2O_2 to water using different organic cellular substrates. Under in vitro conditions they can use a wide range of artificial electron donors, e.g. guaiacol or pyrogallol, so they are usually referred as guaiacol peroxidases. Ascorbate peroxidase (APX; E.C.1.11.1.11.) is the key enzyme of the ascorbate cycle, as it eliminates H_2O_2 by converting ascorbic acid to monodehydroascorbate. In addition to antioxidative enzymes, the antioxidative system also includes non-enzymatic antioxidants such as ascorbic acid, glutathione, α-tocopherol and carotenoids. There is much evidence showing that the activities of antioxidative enzymes, their isoenzyme patterns and amounts of antioxidants are changed by various stress conditions (GASPAR et al. 1991, ALSCHER et al. 1997, DAT et al. 2000, ARORA et al. 2002, MITTLER 2002).

All organisms respond to excess heat by inducing the synthesis of a group of evolutionarily conserved proteins known as the heat shock proteins (HSPs). Most HSPs are expressed constitutively, but in plants their expression can also be additionally induced and/or enhanced by various environmental stress conditions. The correlations between HSP synthesis and stress response led to the assumption that these proteins protect cells from the destructive effects of stress conditions. They act as molecular chaperones and are involved in protein folding, unfolding, assembly and disassembly (VIERLING 1991).

As already mentioned, the mechanism of the transfer of epigenetic information between generations could provide a »memory« of environmental stresses experienced in earlier generations and has an important ecological role in preparing the progeny for potentially harmful conditions (MOLINIER et al. 2006). Therefore, we wanted to investigate whether there is a possibility that the transmission of information about exposure to UV-C stress from irradiated plants to their non-irradiated first generation progeny includes changes in antioxidative enzyme activities (CAT, APX and POD) and the induction of the heat shock protein HSP70. For this purpose *Arabidopsis thaliana* plants were exposed to UV-C irradiation doses of 6 and 600 J m^{-2} and the antioxidative enzyme activities and HSP70 protein induction were investigated in treated plants as well as in their non-irradiated progeny.

Materials and methods

Plant material, growth conditions and UV-C treatments

Arabidopsis thaliana seeds (ecotype Columbia) were germinated in the soil and incubated in a growth chamber at 24 ± 2 °C with a photoperiod of 16 h light/8 h darkness under fluorescent white light (35–40 μmol m^{-2} s^{-1}) for a period of five weeks.

Prior to entering the flowering phase the plants were divided into three groups. The first one was the non-irradiated control group, while the second and third were irradiated with UV-C doses of 6 J m^{-2} and 600 J m^{-2}, respectively. The plants were irradiated with UV-C lamps (Osram, Germany), λ = 254 nm, for time intervals previously determined with an UV radiometer (VLX-3W, France) to achieve irradiation doses of 6 and 600 J m^{-2}. Half of the plants of each group were allowed to continue growth, pass the flowering phase and produce seeds. The rest of the plants of each group were used for the preparation of protein extracts. Plants exposed to a 600 J m^{-2} dose were immediately taken for protein extraction, while the plants exposed to a 6 J m^{-2} dose were first kept in the dark for 30 minutes before protein extraction to elicit a response, since the UV-C treatment itself was very short.

Seeds produced by the first, second and third group were collected and planted. Five week old progeny of irradiated as well as control plants were used for protein extraction, without ever being irradiated themselves.

Enzyme assays and isoenzyme analysis

Plant extracts were prepared for the determination of ascorbate peroxidase (APX), guaiacol peroxidase (POD) and catalase (CAT) activities as well as isoenzyme analyses by gel electrophoresis. The plants (100 mg) were homogenized in 1 ml of a cold 50 mM potassium phosphate buffer (pH 7.0), containing 1 mM ascorbate and 5% (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 20,000 *g* for 30 minutes at 4 °C and the supernatant was used for enzyme assays. APX activity was determined by the decrease in absorbance at $\lambda = 290$ nm ($\varepsilon = 2.8$ mM^{-1} cm^{-1}), as described by NAKANO and ASADA (1981) and expressed as μmol of oxidised ascorbate per minute per gram of total soluble proteins. The reaction mixture consisted of a 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 5 mM H_2O_2 and 120 μL of an enzyme extract. CAT activity was assayed by measuring the decrease in absorbance at $\lambda = 240$ nm ($\varepsilon = 36$ mM^{-1} cm^{-1}) according to AEBI (1984) and expressed as μmol of decomposed H_2O_2 per minute per gram of total soluble proteins. The reaction mixture consisted of a 50 mM potassium phosphate buffer (pH 7.0), 20 mM H_2O_2 and 50 μL of an enzyme extract. POD activity was determined by the increase in absorbance at $\lambda = 470$ nm ($\varepsilon = 26.6$ mM^{-1} cm^{-1}) according to CHANCE and MAEHLY (1955) and expressed as μmol of oxidised guaiacol per minute per gram of total soluble proteins. The reaction mixture consisted of a 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2, 18 mM guaiacol and 20 μL of an enzyme extract. The protein content in the enzyme extracts was determined by a dye-binding technique (BRADFORD 1976) using bovine serum albumin as a protein standard.

For isoenzyme analysis, vertical PAGE according to LAEMMLI (1970) without sodium dodecylsulfate (SDS) was performed on 12% (w/v) polyacrylamide gels at 4 °C. Approximately equal amounts of proteins, 30–35 μg per well, were loaded. For APX, the gel was pre-run for 30 min before the samples were loaded and ascorbate was added to the electrode buffer. For APX detection the gels were stained by the protocol according to MITTLER and ZILINSKAS (1993), for CAT detection according to WOODBURY et al. (1971) and for POD according to CHANCE and MAEHLY (1955).

Protein extraction and HSP70 expression

The plants (50 mg) were homogenized in 1 mL of a cold 0.1 M Tris–HCl buffer (pH 8.0) (STAPLES and STAHMANN 1964) containing 5% (w/v) PVP and centrifuged at 20,000 *g* for 30 minutes, at 4 °C. The soluble protein content was determined according to BRADFORD (1976). Protein extracts were mixed with an equal volume of sample loading buffer (LAEMMLI 1970) and separated by 10% (w/v) SDS-PAGE.

After electrophoresis, one gel was electro-transferred to a nitrocellulose membrane in a Mini Trans-Blot cell (BioRad, Germany). The other one was stained with Coomassie Blue and served as an internal control of equal loading. After overnight incubation with primary antibodies (1:1000) against pea HSP70 (raised in rabbits), the protein blots were probed

with alkaline phosphatase-conjugated anti-rabbit IgG antibodies (1:2000) and detected with bromo-4-chloro-3-indolyl phosphate/nitrotetrazolium blue chloride (BCIP/NBT) as a substrate.

Statistics

The results were calculated as the mean value of at least six replicates ± standard error. Statistical analysis was performed using the STATISTICA 7.1 (StatSoft, Inc., USA) software package. Significant differences between mean values were established by one-way ANOVA followed by Duncan's multiple range test. Differences were considered to be significant at $P < 0.05$. Enzyme activities staining after PAGE and immunoblotting after SDS-PAGE were repeated at least two times.

Results

Antioxidative enzyme activities and HSP70 induction were determined in plants irradiated with UV-C doses of 6 and 600 J m^{-2} and corresponding non-irradiated control plants (P generation), as well as in their non-irradiated progeny (F_1 generation).

Besides the changes in antioxidative enzyme activities and the occurrence of a new HSP70 isoform, irradiated plants showed symptoms of tissue damage and stunted growth, which were more prominent in plants irradiated with a higher UV-C dose (600 J m^{-2}).

In the progeny of plants irradiated with a 6 J m^{-2} dose we observed an earlier progression to the flowering phase than in the progeny of plants exposed to an irradiation dose of 600 J m^{-2} and the non-irradiated (control) plants.

Antioxidative enzymes

When CAT activity was monitored, a statistically significant decrease of enzyme activity in the plants exposed to UV-C irradiation was observed. CAT activity of the parental plants exposed to an UV-C dose of 6 J m^{-2} was 3.5 times lower, while plants exposed to an UV-C dose of 600 J m^{-2} showed a 5.5 times lower activity in comparison to the control plants. The progeny of the control plants did not show a significantly decreased catalase activity in comparison to the parental generation, while the progeny of plants exposed to an UV-C dose of 6 J m^{-2} showed a significantly decreased catalase activity in comparison to the progeny of the control plants. Exposure of plants to a dose of 600 J m^{-2} did not cause a significant change in catalase activity in their progeny (Figure 1a). Results obtained from the detection of the catalase activity in gel after separation of proteins by gel electrophoresis in native conditions were in accordance with the activities measured spectrophotometrically: one catalase isoenzyme (marked K1) was present in extracts of both irradiated and non-irradiated parental plants. It is also evident that its activity was strongly decreased in plants irradiated with UV-C doses of 6 and 600 J m^{-2}. The same band of particularly similar activity as in the corresponding control plants was detected in plant extracts of the progeny of plants exposed to both investigated irradiation doses (Fig. 1b).

Although APX activities of parental plants exposed to UV-C irradiation doses of 6 and 600 J m^{-2} were not significantly decreased in comparison to control plants, their progeny

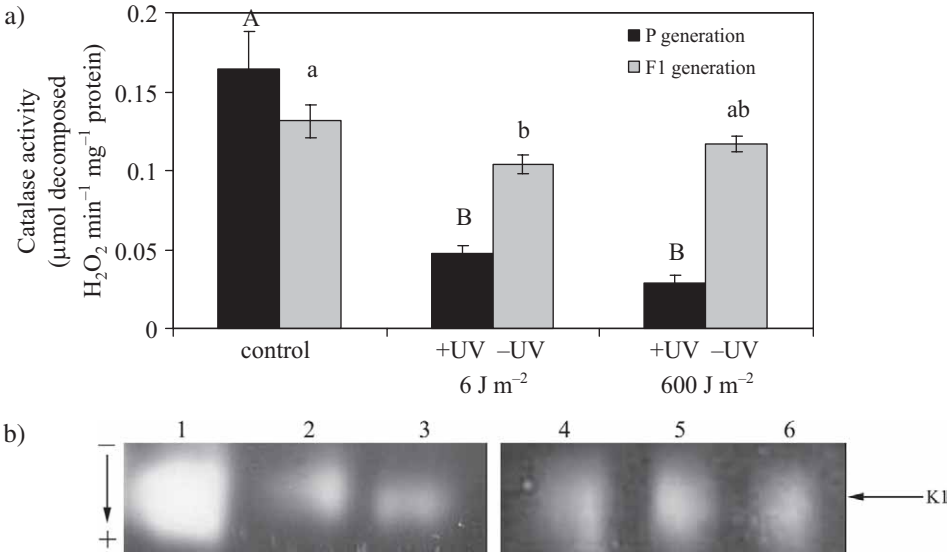

Fig. 1. Activity of CAT (a) and isoenzyme pattern (b) in parental plants (P generation) exposed to UV-C radiation and their non-irradiated progeny (F1 generation). Letters denote a statistically significant difference ($P < 0.05$) between P generation (uppercase letters) and F1 generation (lowercase letters).
1 – nonirradiated (control) plants (P-generation)
2 – parental plants exposed to 6 J m^{-2} UV-C
3 – parental plants exposed to 600 J m^{-2} UV-C
4 – progeny (F1 generation) of control plants
5 – nonirradiated progeny (F1 generation) of plants exposed to 6 J m^{-2} UV-C
6 – nonirradiated progeny (F1 generation) of plants exposed to 600 J m^{-2} UV-C

showed significantly decreased activities. This decrease was more evident in the progeny of plants exposed to a UV-C dose of 6 J m^{-2} (Fig. 2a). In plant extracts of non-irradiated and irradiated parental plants two distinct ascorbate peroxidase isoenzymes (A1 and A2) were detected. The isoenzyme A1 was present in extracts of both, non-irradiated and irradiated plants, whereas A2 was seen only in non-irradiated plants and plants irradiated with a lower UV-C dose (6 J m^{-2}). Furthermore, the A2 isoenzyme band was evidently weaker in irradiated plants. The progeny of non-irradiated (control) and irradiated plants also showed two ascorbate peroxidase isoenzymes (A1 and A2). The progeny of the exposed plants showed activity of the isoenzyme A1 and A2 less prominent than in the extracts from the corresponding control plants (Fig. 2b).

The POD activity was significantly increased in the parental plants exposed to UV-C irradiation in comparison to the non-irradiated control plants. The plants exposed to a dose of 6 J m^{-2} showed a 1.8 times higher and the ones exposed to a 600 J m^{-2} dose a 2.4 times higher activity. In the progeny of irradiated plants, the POD activity did not differ significantly from the activity of the corresponding control plants (Fig. 3a). By gel-electrophoresis two guaiacol peroxidase isoenzymes (G1 and G2) were resolved. The isoenzyme G1 was detected in both the extracts of non-irradiated (control) and irradiated plants, its band being more prominent in irradiated plants, especially in those exposed to a higher UV-C

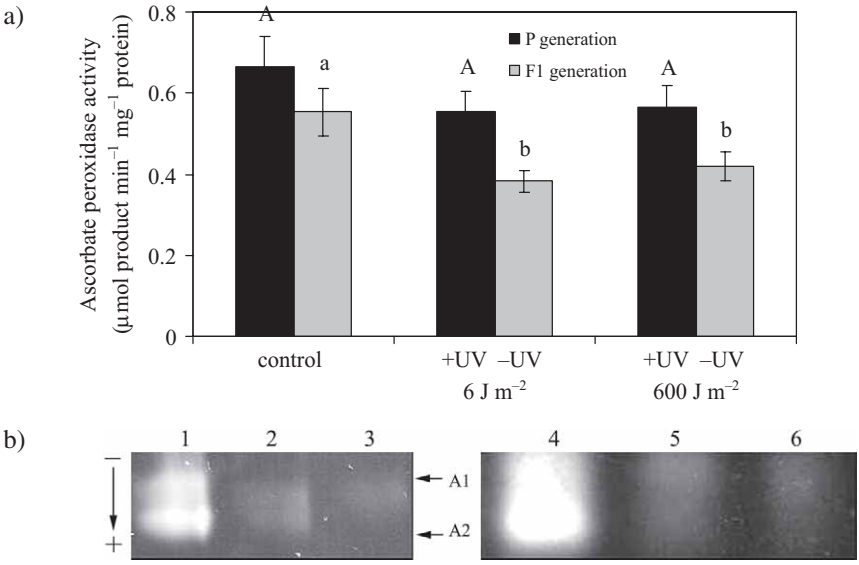

Fig. 2. Activity of APX (a) and isoenzyme pattern (b) in parental plants (P generation) exposed to UV-C radiation and their non-irradiated progeny (F1 generation). Letters denote a statistically significant difference ($P < 0.05$) between P generation (uppercase letters) and F1 generation (lowercase letters). Samples 1–6 are the same as in figure 1b.

dose (600 J m^{-2}). The isoenzyme G2 was found only in extracts of irradiated plants. In the progeny plants only one POD isoenzyme (G1) was observed in both plant groups – the progeny of non-irradiated (control) and the progeny of irradiated plants. The progeny of control plants had an isoenzyme band slightly weaker than the parental generation, whereas the progeny of exposed plants had an isoenzyme G1 activity decreased even more than the extracts of corresponding parental plants (Fig. 3b).

HSP70 expression

We detected two isoforms of the protein HSP70 (marked H1 and H2) in the extracts of parental plants. The H1 isoform was present in both the non-irradiated (control) and the irradiated plants, whereas we only observed H2 in plants exposed to UV-C irradiation doses of 6 and 600 J m^{-2}.

In plant extracts of the progeny we observed only the isoform H1. It was present in both the progeny of the control and the irradiated plants (Fig. 4).

Discussion

Since rearrangements between homologous DNA sequences in somatic cells are strongly stimulated by DNA damage (RIES et al. 2000a), the elevated frequency of SHR found in *Arabidopsis thaliana* plants exposed to UV-C irradiation in research done by MOLINIER et al. (2006) was not a surprise. Nevertheless, it was surprising that the information about stress exposure was transmitted to four generations of their non-irradiated prog-

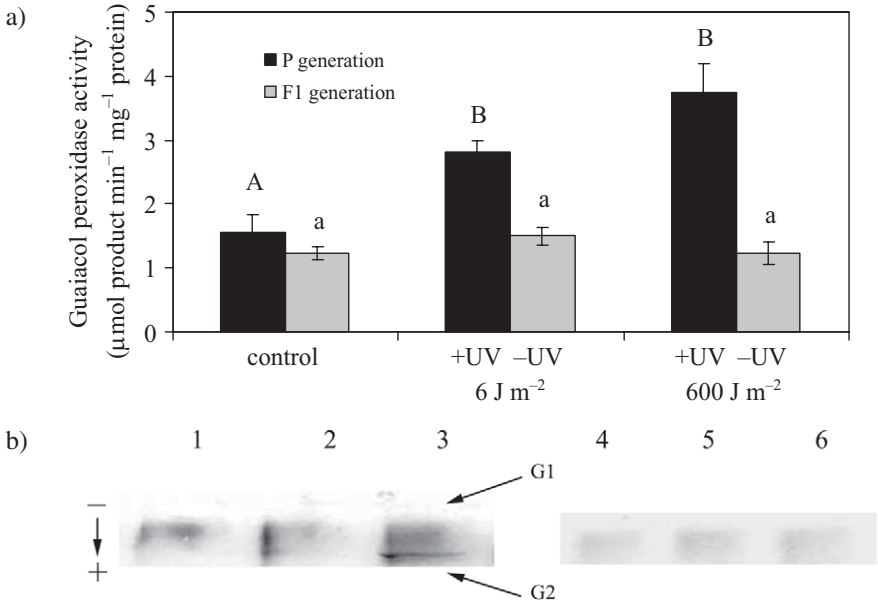

Fig. 3. Activity of POD (a) and isoenzyme pattern (b) in parental plants (P generation) exposed to UV-C radiation and their non-irradiated progeny (F1 generation). Letters denote a statistically significant difference ($P < 0.05$) between P generation (uppercase letters) and F1 generation (lowercase letters). Samples 1–6 are the same as in figure 1b.

eny plants. The authors suggested the basis for this transgeneration »memory« to be epigenetic. PECINKA et al. (2009) tested the effect of ten abiotic stress factors, among them UV-C irradiation, on parental and two non-exposed subsequent generations of *Arabidopsis thaliana* plants. Results confirmed the enhanced frequency of SHR in the treated generation, while the two subsequent generations showed a low and stochastic increase in SHR. Since the subject of our investigation was antioxidative enzymes, an interesting result for our work was the effect of the herbicide paraquat, a well-known ROS producer. This treatment, as a rare exception, showed a significantly increased frequency of SHR not only in the treated parental plants, but also in the first non-treated progeny generation.

The mechanism of the transgenerational stress memory is not fully elucidated yet, so our aim was to investigate whether this process could also include other stress responses, not only genetic material repair mechanisms. For this purpose we investigated (1) the activities of antioxidative enzymes (catalase, ascorbate peroxidase and guaiacol peroxidase), which have an important role in the process of plant adaptation to stress and (2) the induction of the heat shock protein HSP70, which catalyzes the correct folding of proteins denatured as a consequence of stress conditions.

Symptoms of tissue damage and stunted growth were observed in the plants irradiated with a higher UV-C dose (600 J m^{-2}), while the plants exposed to the lower irradiation dose (6 J m^{-2}) showed milder symptoms. That was expected, because the severity of UV-C irradiation symptoms increases with the irradiation dose, since a higher dose represents a more pronounced stress for the plant. In previous reports it was shown that an UV-C irradiation

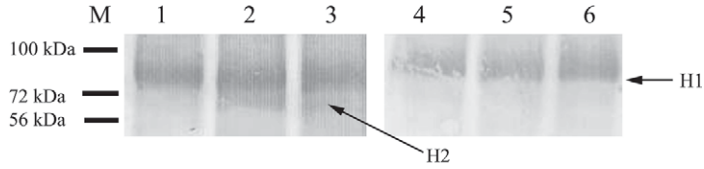

Fig. 4. Immunoblot probed with antibody against HSP70. Isoforms of HSP70 are marked H1 and H2. M – molecular mass markers. Samples 1–6 are the same as in figure 1b.

dose of 500-2000 J m^{-2} can trigger a stress response in *Arabidopsis thaliana* plants (MARTÍNEZ et al. 2004) and that a dose of 10 kJ m^{-2} caused severe symptoms of damage at the level of the whole plant (DANON et al. 2004). For even higher UV-C doses (10–50 kJ m^{-2}) DNA fragmentation was confirmed (DANON and GALLOIS 1998).

The exposure to stress conditions which cause oxidative stress usually increases antioxidative enzyme activities (DAT et al. 2000, ARORA et al. 2002, MITTLER 2002). Nevertheless, in our research a decreased activity of CAT in parental plants exposed to UV-C irradiation in comparison to non-irradiated plants was observed (Fig. 1a). The decrease was slightly more pronounced in the plants exposed to an UV-C dose of 600 J m^{-2} than in the plants exposed to a dose of 6 J m^{-2}. The obtained results are in accordance with the results of WILLEKENS et al. (1994), which confirmed the decrease of some CAT isoenzyme activities as a consequence of stress induced by UV-B irradiation. Also, CHO and SEO (2005) have shown that other forms of abiotic stress (e.g. exposure to cadmium) decreased CAT activity.

APX activity in the extracts of plants exposed to UV-C irradiation did not differ significantly from the activity in the non-irradiated (control) plant extracts (Fig. 2a). WILLEKENS et al. (1994) and YANNARELLI et al. (2006) monitored the APX transcripts in plants after exposure to UV-B irradiation and observed only insignificant differences. Similarly, SHIGEOKA et al. (2002) and DAVLETOVA et al. (2005) showed that the levels of the APX isoenzyme transcripts increased in the cytosol under conditions of high light intensities, but did not increase in the chloroplasts. Such results support the hypothesis that the free radical detoxifying system in chloroplasts is strong enough to monitor ROS production even in stressful conditions and does not need to be induced additionally (DAVLETOVA et al. 2005).

In contrast to CAT and APX, the activity of POD was significantly increased in parental plants exposed to UV-C irradiation, especially those exposed to a higher irradiation dose, 600 J m^{-2} (Fig. 3a). An explanation for the higher POD activity, when compared to the CAT and APX activities, is their different intracellular function. Although all the three enzymes use H_2O_2 as a substrate, the regulation of its intracellular levels is primarily the function of APX, but not of all the other classes of peroxidases (MITTLER 2002, SHIGEOKA et al. 2002). YANNARELLI et al. (2006) studied the effect of UV-B irradiation on the isoforms and activities of peroxidases in sunflower cotyledons. They concluded that some types of peroxidases act as ROS scavengers in the acclimation to UV-B but others rather play a role either in the metabolism of polyphenols by increasing the antioxidative capacity of the cell or in the cross-linking of UV-absorbing phenolic compounds. Furthermore, POD in apoplastic space participates in the generation of ROS (KAWANO 2003). ZACCHINI and DE AGAZIO (2004) reported that the activities of both APX and POD were enhanced 24 hours after

UV-C irradiation (50 kJ mol^{-1}) of tobacco calluses, whereas the activity of CAT remained unchanged. Research done by WILLEKENS et al. (1994) showed that UV-B irradiation also causes increased POD activity.

MOLINIER et al. (2005) studied the expression and regulation of genes involved in abiotic and biotic stress response. Immediately after UV-C exposure (6 J m^{-2}), a rapid but transient increase of enzymes responsible for the production of ROS (NADPH oxidase, peroxidase) was detected, while a few hours later genes encoding ROS scavengers (including APX) as well as other elements involved in the stress response (e.g. resistance proteins and proteins involved in signalling pathways) were up-regulated. There was thus reason to expect the detection of a higher APX activity, but this was not the case with the irradiated parental plants in our experiment. This could be explained by the difference in time period between treatment and sampling. In our work plant extracts were made 30 minutes after exposure to 6 J m^{-2}, while MOLINIER et al. (2005) took plants few hours after treatment.

The isoenzyme pattern of CAT, APX and POD after PAGE in native conditions is in line with the enzyme activity levels determined spectrophotometrically for the individual enzymes. UV-C irradiation induced the G2 isoenzyme of POD in the parental generation which explains the increased guaiacol peroxidase activity seen in the extracts of irradiated plants.

Concerning the reaction of progeny of irradiated plants, our first observation was the earlier progression to flowering phase. A variety of stressful conditions, e.g. a pathogen infection, extreme temperatures and even UV-C irradiation, can promote flowering (MARTÍNEZ et al. 2004). In our experiment the plants were irradiated prior to entering the flowering phase to avoid the possibility of exposing the gametophyte and the germ cells from which progeny plants will develop, to UV-C irradiation. In other words, if irradiation had affected the gametophyte itself, there would have been a possibility of direct effect on the progeny, which would be an explanation for the observed changes in the progeny of irradiated plants. An earlier progression to the flowering phase was observed in the progeny of plants exposed to an UV-C dose of 6 J m^{-2}, while the progeny of plants exposed to a higher UV-C dose (600 J m^{-2}) did not show this effect. This phenomenon could potentially have an important role for plants in their native environment, since it increases the chance of survival in periods of unfavourable conditions (TROYER and BROWN 1976, MARTÍNEZ et al. 2004). It is known that salicylic acid is a stress signal molecule involved in non-photoperiodic flowering mechanisms (ENDO et al. 2009) and could cause earlier flowering. Furthermore, salicylic acid binds to and inactivates CAT (CHEN et al. 1993, CONRATH et al. 1995), resulting in increased levels of ROS, including H_2O_2. In our work significantly lower CAT activity was seen in the progeny of those plants that were exposed to an irradiation dose of 6 J m^{-2} in comparison to the corresponding control plants (progeny of non-irradiated plants) (Fig. 1a). VRANOVÁ et al. (2002) showed that transgenic plants with constitutively increased levels of H_2O_2 posses a better pathogen resistance and produce more salicylic acid. Therefore, the decrease of CAT activity observed in the progeny of irradiated plants may be explained in the context of the role of H_2O_2 as a signalling molecule. The progeny of irradiated plants also showed a significant reduction in APX activity (Fig. 1b) when compared to the corresponding control group (progeny of non-irradiated plants). As for CAT, this would result in increased level of ROS molecules, which could then be involved in signalling processes. As described earlier, salicylic acid and nitrogen oxide, both

synthesized in stress conditions, can lead to reduction of CAT and APX activities (WILLEKENS et al. 1994), whereby CAT is repressed on a transcriptional and APX on a post-transcriptional level (MITTLER 2002). Reduced activities of both enzymes in the progeny demonstrate that the information about stress exposure could be obtained from the parental generation in order to achieve better response to stress conditions. However, it remains unclear how the information about the need for increased H_2O_2 levels and thus decreased activity of antioxidative enzymes was transmitted to the progeny.

The progeny of stressed plants does not show a significantly different POD activity when compared to corresponding control plants (the progeny of non-stressed plants). Furthermore, the POD activity in the progeny of all groups was similar to the activity observed in the parental generation of control plants (Fig. 3a). It could be concluded that the information about increased POD activity in the parental generation was not transferred to the progeny. An explanation could be a more important role of POD in polyphenol metabolism than in ROS scavenging.

In irradiated plants immunodetection revealed an induction of an additional isoform of HSP70, which was not observed in the control plants. Since HSP70 protein family members play a role in the correct re-folding or controlled degradation of denatured proteins, the induction of a new isoform of HSP70 in plants exposed to UV-C irradiation probably contributes to the minimization of the harmful effects of irradiation damage. The HSP70 family in *A. thaliana* has 14 members, three of which are constitutively expressed at low levels while the rest of them are induced during thermal stress, viral infections, etc. (SUNG et al. 2001, APARICIO et al. 2005). Furthermore, as mentioned earlier, the elevated levels of H_2O_2 enhance the induction of HSPs during light stress in *Arabidopsis* plants deficient in APX (PNUELI et al. 2003). The decreased CAT activity observed in our plants exposed to UV-C could also cause higher amounts of H_2O_2 in the cell and be the reason for the induction of an additional HSP70 isoform. The lack of difference with respect to the number of the HSP70 isoforms in the progeny generations of stressed and non-stressed plants suggests that there was no transmission of the HSP70 induction from the parental generation to the progeny and that elevated levels of H_2O_2 are not the only signal for HSP induction.

The absence of a difference in the POD activities and HSP70 isoform induction between the progeny of irradiated plants and their corresponding control means that no information about the changes in their activity and pattern, respectively, was transmitted to the progeny. Since the activities of CAT and APX, the enzymes responsible for H_2O_2 scavenging, were reduced it seems that only the information about the need for increased amounts of ROS was transmitted to the progeny. The basis of the described transmission of information about the response to an UV-C induced stress across generations is probably of an epigenetic nature because the entire population of plants changes its behaviour in a relatively homogeneous manner. By contrast, when comparing the effect of ten abiotic stress factors on the DNA repair process by SHR, PECINKA et al. (2009) concluded that two subsequent non-treated generations showed a low and stochastic increase in SHR frequency and that transcripts coding for SHR enzymes returned to the pre-treatment levels in the progeny. In other words, an increased frequency of SHR as a transgenerational stress effect was not a general response to abiotic stress in *Arabidopsis thaliana* plants. The authors explained this stochastic response as part of an evolutionarily successful adaptation mechanism in irregularly changing environmental conditions.

Our results showed that, besides the already known increase in frequency of somatic homologous recombination, transmission of information about stress exposure to progeny can also include changes in activities of antioxidative enzymes catalase and ascorbate peroxidase while changes in guaiacol peroxidase activity and HSP70 isoforms were not confirmed. In both cases – the increased frequency of SHR as a part of a DNA repair system and the antioxidative stress response as a ROS scavenging system – the exact nature of the transmission of information about exposure to stress conditions from the parental plants to their progeny remains to be elucidated.

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