TÜBİTAK

Turkish Journal of Botany
http://journals.tubitak.gov.tr/botany

Research Article

Turk J Bot
(2021) 45: 671-680
© TÜBİTAK
doi: 10.3906/bot-2012-43

Evaluation of the effects of temperature, light, and UV-C radiation on HSP70A expression in *Chlamydomonas reinhardtii*

Tuba SEVGİ*, Elif DEMİRKAN
Department of Biology, Faculty of Arts and Sciences, Bursa Uludag University, Bursa, Turkey

Received: 03.01.2021 **Accepted/Published Online:** 20.08.2021 **Final Version:** 28.12.2021

Abstract: In this study, various physical parameters (temperature, light intensity and UV-C radiation) which could be effective in heat shock response on *C.reinhardtii* by using molecular tools were investigated. In total, 256 transformants were obtained, among them, 160 transformants had continuous expression while 96 of them had heat-inducible expression. In these transformants, arylsulfatase activities were detected qualitatively and quantitatively. The best two transformants were selected and used in studies. To determine the effect of temperature, the cells were shifted from 23 °C to 35 °C, 37 °C, 40 °C and 42 °C. The heat shock response was induced at all temperatures. In investigating the effect of light intensity, 0, 14, 28, 70, 140 µmol $E.m^{-2}s^{-1}$ were used. It was found that the light intensity of 28 µmol $E.m^{-2}s^{-1}$ and above increased ARS activity. On the other hand, ultraviolet C radiation application was carried out for periods of 2, 6 and 12 h, and no significant change in ARS activity was observed. In order to compare the selected arylsulfatase activity results in the study, real-time polymerase chain reaction trials were conducted at the transcript level, and parallel results were obtained. As a result of the study, it was determined that the heat shock response was triggered by temperature and light intensity. These might be also important for plant stress and ecological studies.

Key words: *C. reinhardtii*, physical parameters, heat shock response, arylsulfatase

1. Introduction

Heat shock response is conserved in all organisms and protects cells against dangerous conditions. To protect against heat damage, the expression of genes encoding heat shock proteins (HSPs) produces a cellular resistance mechanism (Sung et al., 2003). The heat shock response provides resistance to a wide range of environmental stress conditions such as bacterial and viral infections, heavy metals, oxidants, and toxic chemicals (Morimoto, 1998). The main product of the heat shock response is heat shock proteins (HSPs), usually containing molecular chaperones and proteases (Schmollinger, 2012). In the absence of heat stress, HSPs are in the form of oligomers, separate from each other, in response to heat shock they inhibit the formation of dangerous protein aggregates by binding their hydrophobic surfaces to misfolded proteins (Nakamoto and Vigh, 2007).

Combinations of environmental stresses are one of the major problems of sustainable agriculture. In order to solve these problems, instead of a complex system, a simpler, single-celled microalgae, the plant model organism *Chlamydomonas reinhardtii* is used. It has been used in plant biology studies as plant model organism since the 1950s (Harris, 2001). There are several advantages using *C. reinhardtii* as a model system to examine the basic mechanisms of plant heat stress response: (i) it can be produced under defined conditions; (ii) makes it possible to distinguish between heat stress and drought stress; (iii) homogeneous application of heat stress to each cell in culture; (iv) each cell in the culture are at the same type but might be in a different stage in terms of cell cycle, which may be eliminated by simultaneous production of cells; (v) in general, the *Chlamydomonas* gene family is relatively small and simple to study (Schroda et al., 2015). Since the simple cell cycle and the easy isolation of mutant strains, the production of recombinant strains of *C.reinhardtii* has accelerated. Today, with the advantage of efficient reporter genes, dominant selective markers and strong promoter systems recombinant *C.reinhardtii* strains are obtained and used.

Certain genes are chosen as reporters because the characteristics they confer on organisms expressing them are easily identified and measured, or as they are selectable markers. Several reporter genes have been expressed in *C reinhardtii*, including *aadA* (Goldschmidt-Clermont, 1991), *uidA* (Sakamoto et al., 1993), aminoglycoside phosphotransferase (*aphA*) 6 (Bateman and Purton, 2000) and *Renilla* luciferase (Minko et al., 1999) and nuclear *ARS2* gene, which encodes an extracellular arylsulfatase (Davies et al, 1992) and a reliable reporter with high signal intensity and a stability under changing environmental conditions (Ohresser et al., 1997). Therefore, ARS was utilized as a strong reporter for quantitative analysis of gene expression in this work.

In this study, the effects of some physical parameters on HSP70A expression of recombinant *C.reinhardtii* were investigated. Firstly, recombinant *C.reinhardtii* strains were obtained using inducible HSP70A promoter, continuously expressing β_2TUB promoter and the Arylsulfatase reporter. Temperature, light intensity and UV-C radiation as physical parameters were studied.

2. Materials and methods

2.1. Strains and culture conditions

* Correspondence: tubaavci@uludag.edu.tr

C.reinhardtii strain cw15-302 *(arg7-)*, c124 and plasmids pCB412 (*ARG7* [Arginynosuccinate lyase] marker gene containing plasmid), pJD55 (*β2TUB-ARS* containing plasmid), pCB803 (*HSP70A-ARS* containing plasmid) were kindly provided from M.Schroda (Technical University of Kaiserslautern, Germany) (Schroda et al. 1999, Schroda et al. 2000). *E.coli* DH5α (C2987I-NEB, USA) was used to amplify ligation products (Kostner et al., 2006).

C. reinhardtii strains were grown in Tris Acetate Phosphate (TAP) medium under continuous light (14 µmol $E.m^{-2}s^{-1}$) on a rotatory shaker at 23 °C and 150 rpm (Harris, 2001). The TAP medium was supplemented with 50 mg/L of arginine when required. *E.coli* was produced in Luria–Bertani (LB) medium at 37 °C and 150 rpm.

2.2. Transformation of plasmids into *C. reinhardtii*

Transformation of plasmids into compotent *E. coli* DH5α cells was performed (Kostner et al., 2006). cw15-302 was used as recipient strain for co-transformation with pJD55 and pCB803 constructs with control construct pCB412 (Schroda et al., 1999). *C.reinhardtii* nuclear transformation was performed using glass beads method (Kindle, 1990). Before transformation, all constructs were linearized with EcoRI; those with *ARS* fusions were linearized with KpnI. For the transformation, 1×10^8 cells, 100 ng of plasmid DNA was used for *ARG7* containing plasmids, 500 ng for the others. Immediately after vortexing glass beads, cells were spread onto TAP-agar plates, containing ampicilline. Plates were incubated at 23 °C under continuous light, and transformants were counted after 2–3 weeks.

2.3. Arylsulphatase enzyme activity assays

The selection of co-transformants expressing ARS was performed by streaking transformants onto nylone membrane placed on TAP agar plates and spraying the solution of 5 mM 5-bromo-4chloro-3-indolyl sulphate (X-SO4), 10 mM tris-HCl, pH 7.5, under the membrane (Davies et al., 1992). After 1–24 h, ARS-expressing transformants became blue. Quantitative ARS enzyme activity was determined according to Ohresser et al., 1997. Enzyme activity was standardized against chl concentration.

2.4. Effect of temperature

Heat stress studies were performed (Schroda et al., 2000). For heat shock kinetics, recombinant *C.reinhardtii* cells were continuously grown at 23 °C at continuous light (CL) and 150 rpm. Then, a total of $\sim10^8$ cells were centrifuged at 3500 rpm for 3 min, and the pellet was transferred to 30 mL TAP medium in 100 mL flasks which were adjusted before to desired temperatures. As soon as the pellet was added to the medium, the stopwatch was started, and kinetic experiments were performed. Heat stress was applied at 35, 37, 40, 42 °C in water bath for different periods (0, 1, 5, 10, 20, 30, 60, 120 min), and ARS activities of samples were determined for each sample. Zero minute was taken as control.

2.5. Effect of light

Light stress studies were performed (Nowicka and Kruk, 2012). In order to investigate the effect of light intensity, *C.reinhardtii* cells were grown at 23 °C, continuous light (CL), and 150 rpm. A total of 10^8 cells were then centrifuged at room temperature for 3 min at 3500 rpm, and the pellet was transferred to 30 mL TAP medium in 100 mL flasks, then exposed to light stress at different illuminations (0, 14, 28, 70, 140 µmol $E.m^{-2}s^{-1}$) for various periods (0, 2, 6, 12, 24 h). ARS activity was determined for each sample and compared with control (0 h).

2.6. Effect of UV-C radiation

UV-C radiation studies were applied (Liu et al., 2010). After growing *C.reinhardtii* at 23 °C , continuous light (CL) and 150 rpm, $\sim10^8$ cells were centrifuged at room temperature for 3 min at 3500 rpm, and the pellet was transferred to 30 mL TAP medium in 100 mL flasks. UV exposure experiments were performed using ultraviolet lamp (UltraViole 254 nm, Intensity [WW/cm^2], 60, Lamp-VL-130.G, 1x30 W, Germination Lamp-Vilber) for 0, 6, and 12 h from the distance of 10 cm at 23°C. In order to prevent photoreactivation during UV mutation, UV studies were performed in the dark. Activities at 6 and 12 h were compared with that of 0 h (control), and ARS activities were determined.

2.7. Detection of the expression at the transcription level by RT-PCR

Transformants and control strain were exposed to different stress conditions and ARS activities. In order to verify the results at molecular level, expression amount of HSP70A and CβLP (Chlamydomonas β-subunit-like polypeptide) were measured by RT-PCR and compared with ARS activities. For this purpose, RNA was extracted with NucleoSpin RNA Plant Kit (Macherey Nagel), and RT-PCR experiments was perfomed by using one-Step RT-PCR Kit (NEB) by Step One Plus RT-PCR instrument at the program of 10 min 48 °C, 10 min 95 °C; 95 °C; 60 s were set at 65 °C for a total of 40 cycles; as primers $HSP70A_{For}$ GATCGAGCGCATGGTGC,$HSP70A_{Rev}$TCCATCGACTCCTTGT CCG,$C\beta LP_{For}$GCCACACCGAGTGGGTGTCGTGCG,$C\beta LP_{Rev}$CC TTGCCGCCCGAGGCGCACAGCG were used. The relative quantification of gene expressions were analyzed by the $2^{-\Delta\Delta}$Ct method. The protocols of Livak and Schmittgen (2001) was used.

3. Results

3.1. Transformation of *C.reinhardtii*

The plasmids pCB412 containing the *ARG7* marker gene, plasmid pJD55 *βTUB-ARS*, and plasmid pCB803 containing *HSP70A-ARS* were transfered to competent *E.coli* cells, allowing the multiplication of plasmids within the cell. The plasmids were then isolated from *E.coli* with the plasmid isolation kit (Macherey Nagel NucleoSpin), and the purity control of the plasmids was performed on agarose gel electrophoresis.

The linearized plasmids were transferred to the *C.reinhardtii* cw15-302 strain, with glass bead transformation method. After 14 days pCB412, pJD55 plasmid containing *C. reinhardtii* strains and pCB412, pCB803 plasmid containing *C. reinhardtii* strains were appeared on the TAP agar medium as green colonies. Plasmids were used in 5:1 and 5:5 ratios (Figure 1). A total of 256 transformants were obtained after 14 days of incubation.

After 14 days, a total of 256 transformants were obtained. Among these, 160 transformant were observed with continuous expression while 96 transformants showed heat-inducible expression. However, they may include either pCB412 only, pCB412 and pCB803, or pCB412 and pJD55. Therefore, ARS enzyme activity was assayed for all clones to eliminate only pCB412 containing clones. In total, 10 ARS (+) clones were identified. Among these, 7 transformant had positive results for continuous expression, while only 3 of them had positive results for inducible expression (Figure 2).

Ten ARS positive transformants included in the experiment were exposed to heat shock (HS) for 30 min at 40°C, and ARS activities were determined. The enzyme activities of βTub II-32, a continuously expressed transformant, were found close to each other in CL and after heat shock for 30min. Therefore, this transformant was selected as control strain. On the other hand, the ARS activities of HSP70A III-1, an inducible transformant, was far from each other for CL and after 30min of heat shock. Continuously expressing tranformant (βTub II-32, Control) and inducible transformant (HSP70A III-1) were selected, studies were continued with them (Figure 3).

3.2. The effect of temperature on heat shock response

HSP70A III-1 and βTub II-32 (control) transformants reaching ~ 10^6 cells/mL at 23 °C under CL were exposed to heat shock at temperatures of 35 °C, 37 °C, 40 °C, 43 °C for different periods of 0 (control), 1 min, 5 min, 10 min, 20 min, 30 min, 1 h, 2 h, and arylsulfatase activities were determined.

At 35°C , the inducible (HSP70A III-1) transformant responded to heat stress starting from 10th min and continued. Compared to the control, the enzyme activity was increased approximately 2.5 times. On the other hand, the highest arylsulfatase activity of HSP70A III-1 transformant at 37 °C and 40 °C were achieved at 120 min (951 µg-αnaftol/µgchl and 2260 µg-αnaftol/µgchl respectively). At 37°C , 3.5-fold increase in ARS activity was achieved compared to 0 min. However, at 40°C 8-fold increase was observed compared to the initial enzyme activity. In studies conducted at 42 °C , HSP70A III-1 transformant showed ARS activity at 1st min and reached the highest value at 60 min (1278 µg-αnaftol/µgchl). ARS activity increased approximately 4.5 times compared to the baseline. In the continuesly expressing gene containing transformant (control) (βTub II-32), arylsulfatase activity remained constant in all periods as expected (Figure 4).

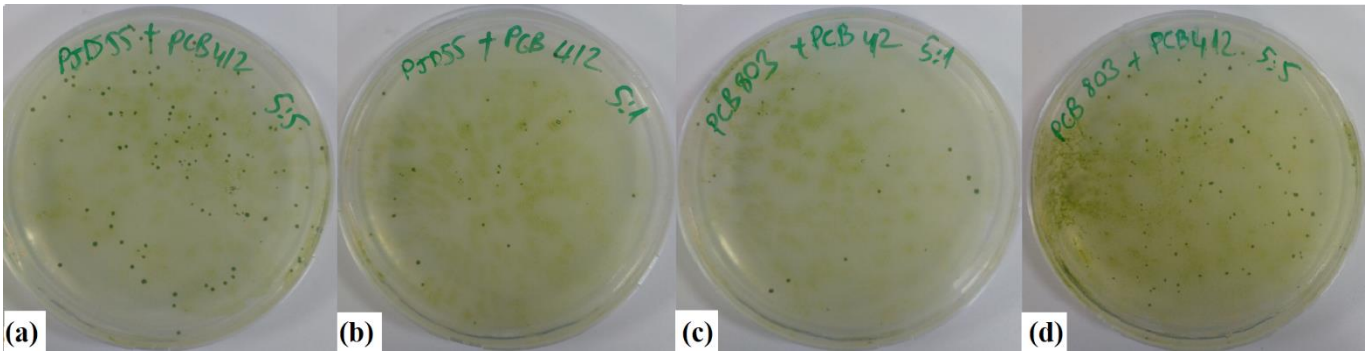

Figure 1. Colonies obtained by cotransformation of plasmid pCB412, pJD55 (A,B) and pCB412, pCB803 (C, D) at a ratio of 5:1 and 5:5 (v/v) at 23°C . It was formed as a result of 14 days of incubation at CL.

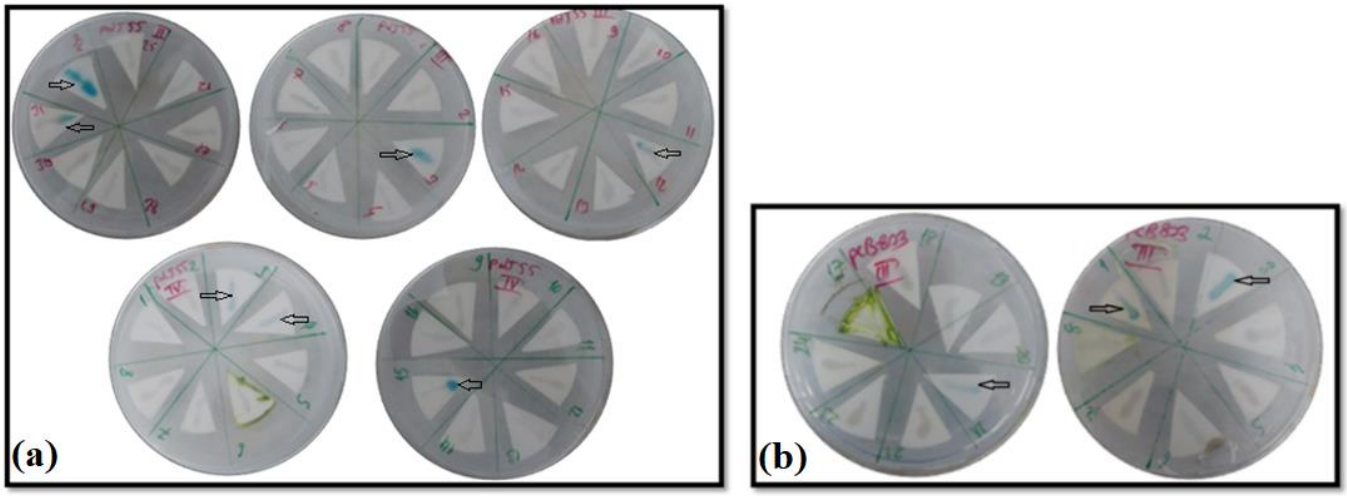

Figure 2. Qualitative screening of ARS activity in 7 clones with plasmids pCB412, pJD55 (a) and 3 clones with pCB412, pCB803 (b). The blue color was evaluated as ARS (+).

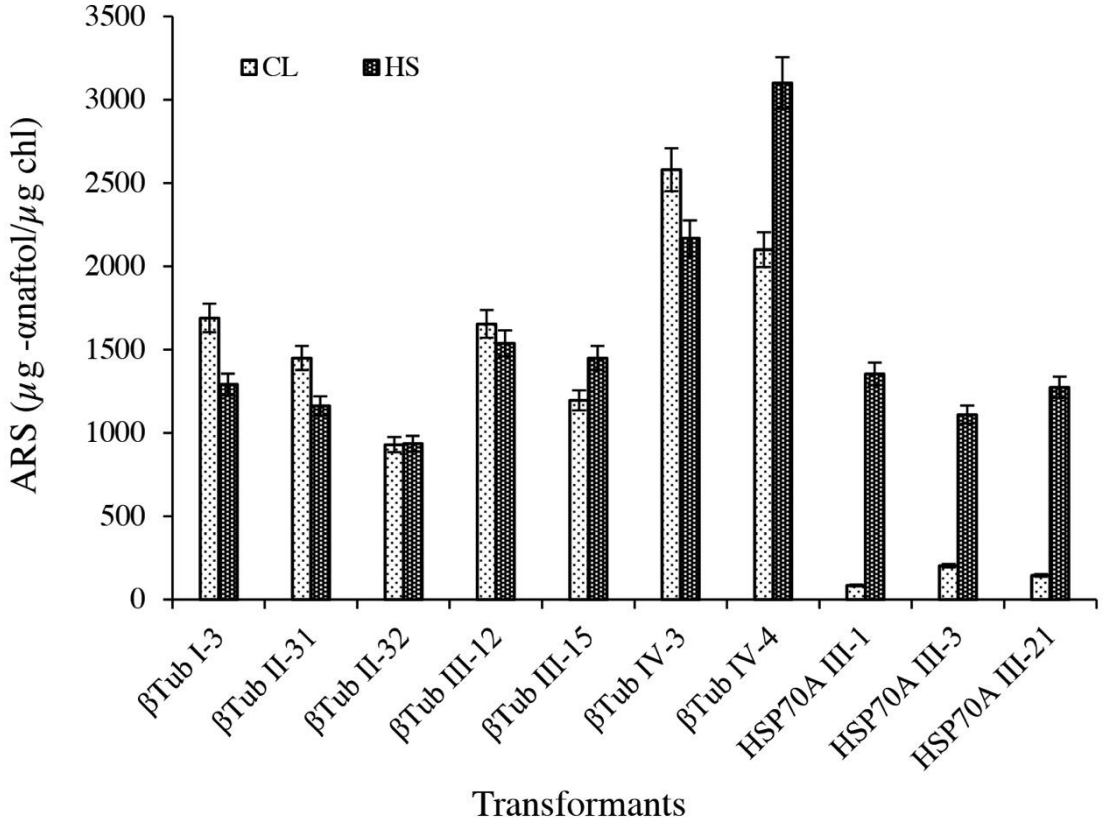

Figure 3. ARS activity values of ARS (+) transformants at 23°C and 40°C . Transformants were shifted from 23°C to 40°C under continuous light (CL) and exposed to 30 min heat shock (HS), and ARS activities were compared (µg-αnaftol / µgchl). Each point represents the average of three independent experiments. Error bars represent one standard deviation on each side of the mean.

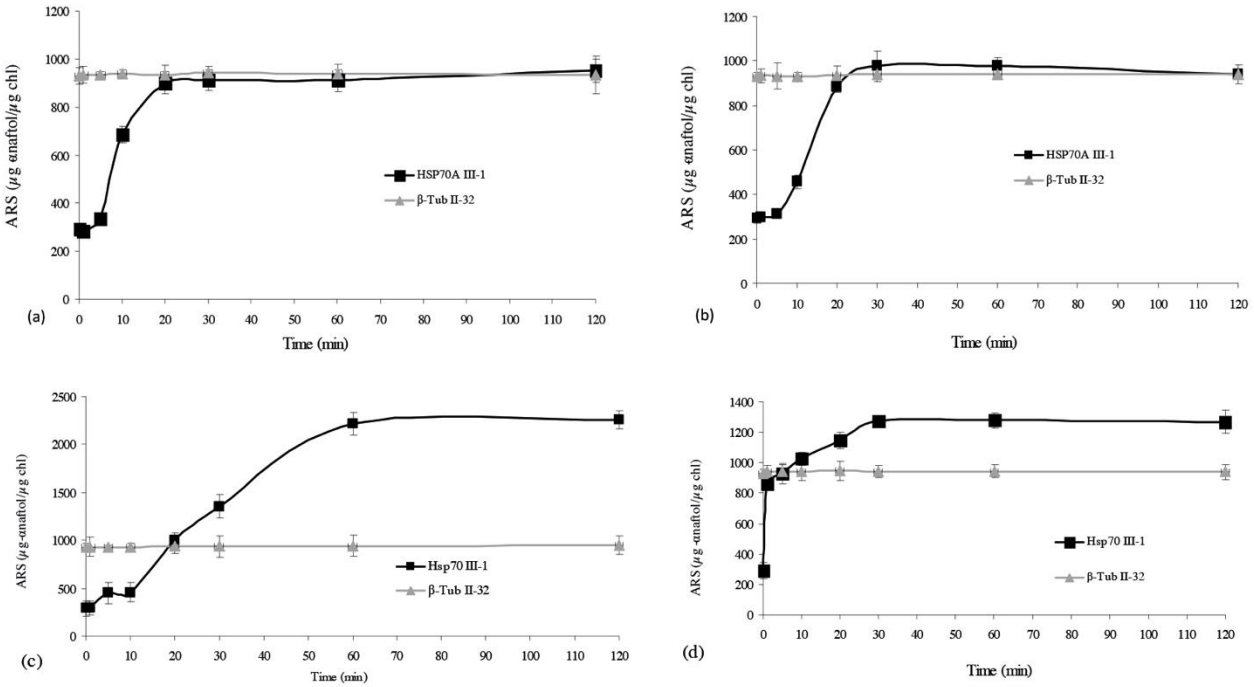

Figure 4. ARS activities of HSP70A III-1 and βTub II-32 transformants at 35°C (a), 37°C (b), 40°C (c), 42°C (d). Each point represents the average of three independent experiments. Error bars represent one standard deviation on each side of the mean.

Considering all the kinetics, shifts from 23 °C to 35, 37, 40 and 42 °C were found to cause a significant increase in ARS activity. This increase in ARS is thought to be caused by the cell's exposure to heat, expressing the HSP70A gene in the promoter region in the plasmid and other related heat shock proteins in the pathways linked to HSP70A.

3.3. The effect of light intensity on heat shock response

HSP70A III-1 and βTub II-32 (control) transformants reaching ~ 10^6 cells/mL under CL at 23 °C were exposed to different light intensities for different periods such as 2, 6, 12, 24 h conditions, and ARS activities were determined.

ARS activity remained constant in the HSP70A III-1 transformant at 0 µmol $E.m^{-2}s^{-1}$, and there was a slight increase in ARS activity at 14 µmol $E.m^{-2}s^{-1}$ (6 and 12 h), but it reached an initial value after 1 day. It was found that the activity increased gradually in high illumination of 28 µmol $E.m^{-2}s^{-1}$ and above. The maximum activity increase at 28 µmol $E.m^{-2}s^{-1}$ was determined after 6 h. At the end of 12 and 24 h, enzyme activity has continued to increase. After 2h at 70 µmol $E.m^{-2}s^{-1}$, ARS activity increased approximately 3.5 times. 140 µmol $E.m^{-2}s^{-1}$ is the light intensity with the highest ARS increase. Four times increase was observed after 2 h, and this value was almost preserved at the end of the 6th h. βTub II-32 transformant has the same ARS value at all light intensities (Figure 5).

3.4. The effect of UV-C radiation on heat shock response

HSP70A III-1 and βTub II-32 (control) transformants reach ~10^6 cells/mL under CL at 23 °C to detect the effects of UV-C radiation at a distance of 10 cm between the UV lamp and the culture. It was exposed to UV light at different periods such as 2, 6 and 12 h, and arylsulfatase activities were detected at the end of each period. There was a small decrease in ARS activity in both transformants. After 12 h, activity values were 203 for HSP70A III-1 and 916 µg-αnaftol/µgchl for βTub II-32 (Figure 6).

3.5. Transcript level analysis with RT-PCR

In order to compare the enzymatic activity results obtained from HSP70A III-1 at the transcript level, some parameters were applied, and RT-PCR trials were performed. As a result, transcript expressions were in parallel with ARS activity values in samples exposed to 40 °C for 120 min, at 140 µmol $E.m^{-2}s^{-1}$ for 2 h (Figure 7).

4. Discussion

As a result of global warming, plants are more exposed to heat stress, a condition that can seriously reduce crop yields. Understanding how plants react to such stress is crucial. Generally, when exposed to high temperatures, organisms provide a heat shock response (HSR) that adapts to a certain extent to the new situation. Green microalgae *C.reinhardtii* is a widely studied microorganism, which is widely used as a plant model organism.

It has been suggested that HSR is triggered by the accumulation of false or unfolded proteins and leads the activation of heat shock transcription through series of sensor and signal events. This continues with the synthesis of proteins, which stimulate the expression of HSPs and chaperones that are responsible for re-folding the degenerated proteins into their correct three-dimensional structures. The production of heat shock proteins (HSPs) is a key adaptation of the cell to stress and has an important role in determining the response of plants to climate change.

In this study, various physical parameters thought to be effective in heat shock response were investigated on *C.reinhardtii* by using molecular tools. Finding powerful efficient promoters for transformation studies is not an easy task. Heterologous promoters have the great advantage of being universal, but transformation efficiencies are generally low and transgenes are mostly unstable. The best transformation effectiveness and the most stable transformants are achieved with endogenous promoters (Davies et al., 1992). Among the most popular endogenous promoters frequently used in *C.reinhardtii* for many years are β_2TUB and HSP70A (Lee et al., 2018). In this study, the β_2TUB promotor for the acquisition of continuous expression and the HSP70A promoter for heat inducible expression were used. Also ARG7 gene is a popular selective marker for nuclear transformation of *C.reinhardtii*. In this study, the rate of cotransformation was determined as 4%.

The temperature at which the HSR is triggered has been tested with the help of ARS enzyme. For this purpose, strains were grown at 23 °C and then shifted to 35, 37, 40 and 42 °C. With the increase in temperature, also significant increase in the ARS activities of the cells were determined; however, it was found that this increase was quite high at 40 °C. The cell showed the maximum heat shock response at 40 °C. A decrease in ARS activity was observed at 42 °C, which may be due to the degradation of the structure of either the arylsulfatase enzyme or HSP70A at high temperatures. This may have an indirect negative effect on cell viability. A similar situation was reported by Schroda et al., 2015.

Many studies have been conducted on the perception and response of heat stress in *Chlamydomonas*, plants and other organisms (Morimoto, 1998; Schroda et al., 2000; Schmollinger et al., 2013). It was reported that when the ambient temperature of *Chlamydomonas* cells varies from 20 °C to 39–41 °C, increased heat shock protein expression creates the heat shock response and induction of HSPs starts between 35–40°C, and accumulation continues up to 41 °C (Tanaka et al., 2000). On the other hand, it was found that when the temperature was increased from 24 °C to 36 °C, the heat shock response of *Chlamydomonas* was induced, and the cells survived when they were raised up to 42.4 °C (Kobayashi et al., 2014).

Under heat stress conditions, unfolded proteins exceed the capacity of chaperones to cope with folded proteins, which leads to HSFs activation with the help of stress kinases.

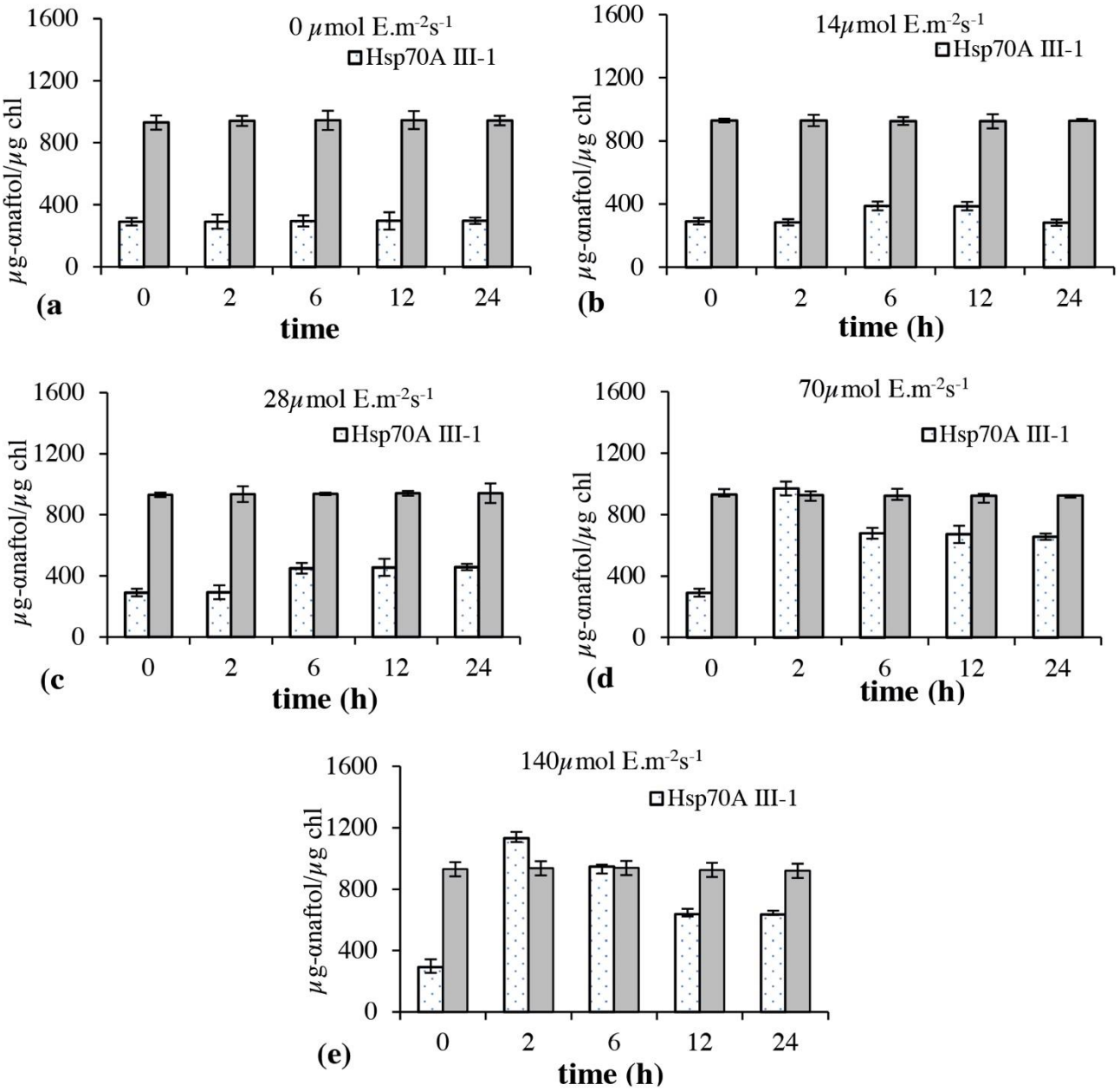

Figure 5. ARS activities at different light intensities (0–140 μmol E.m^{-2}s^{-1}). 0(a), 14(b), 28(c), 70(d), 140(e). Each point represents the average of three independent experiments. Error bars represent one standard deviation on each side of the mean.

Since HSF1 controls the transcription of heat shock genes, it stimulates the production of new chaperones and attempts to restore the heat shock-disrupted protein homeostasis (Schmollinger et al., 2013). In a study on *Physcomitrella patens* moss, a model showing the plasma membrane as the primary temperature sensor under heat shock conditions was reported (Saidi et al., 2010). This model suggests that the temperature difference in plants rather than absolute temperature determines the plant heat shock response (HSR) and when the membrane fluidity increases, the membrane lipid composition opens the calcium channels and regulates the calcium dependent heat signal pathway. On the other hand, it was showed as second model that the heat stress response was triggered by the accumulation of unfolded proteins. In this model, unfolded proteins accumulated under HS are detected and act together to reconstruct protein homeostasis by secreting a signaling cascade to trigger the production of suitable solutes and the expression of molecular chaperones. The first proposed model supports that the heat shock response is triggered by increased membrane fluidity, while the second supports that the model is realized by the accumulation of denatured proteins. It has been shown in several studies that the accumulation of unfolded proteins in land plants and *Chlamydomonas* triggers HSP gene expression (Schmollinger et al., 2013).

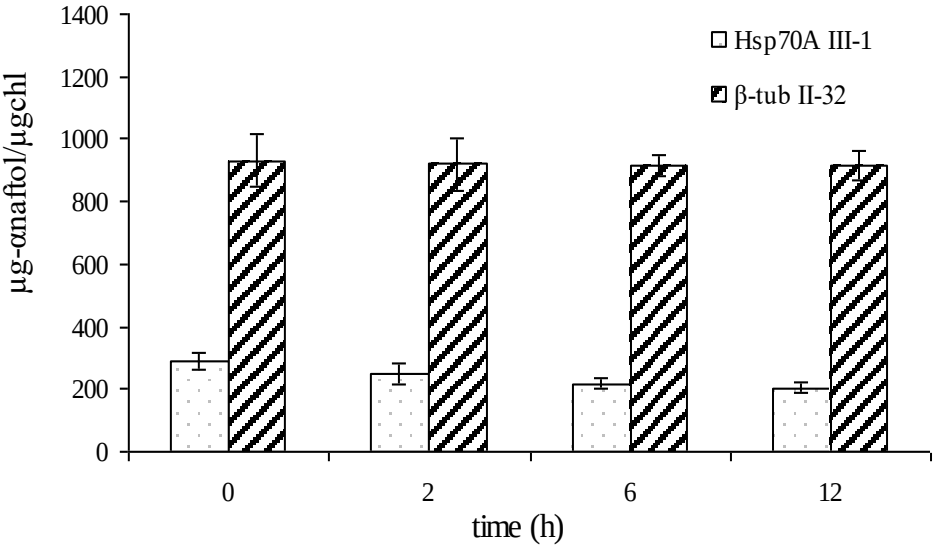

Figure 6. ARS activities at different periods (0, 2, 6, 12 h) after UV-C radiation treatment. Each point represents the average of three independent experiments. Error bars represent one standard deviation on each side of the mean.

In this study, it was also observed that the heat shock response was induced when the cells were even increased from 23 °C to 35 °C. Thus, the heat shock response has been shown to be related to absolute temperature rather than temperature differences, which supports the "accumulation of denatured proteins" model. The observed variations can be explained by differences in optimum growth conditions and / or differences between strains used (Schroda et al., 2015).

Light intensity is very effective against heat shock response. The expression of heat shock genes in *Chlamydomonas* is regulated by thermal stress, light and the expression of HSP genes through light can be controlled by a signal chain containing a photoreceptor. Molecular analysis of *Chlamydomonas* HSP genes, combined with photobiological studies on their expression, will provide information about the mechanisms of light regulating genes. In the study conducted, the values of 40 µEm^{-2}s^{-1} and 23 °C were evaluated as continuous light value, the values above was evaluated as high light intensity, and the values below was evaluated as low light intensity similar to recent studies (Mayfield and Schultz, 2004; Zhang et al., 2017). For light intensity studies, the cells were exposed to light stress between 0–140 µmol E.m^{-2}s^{-1}, for different periods (0 [control], 2, 6, 12, 24 h), and ARS activity was determined. Mayfield and Schultz (2004) carried out a research showing the usability of the luxCt (chloroplast optimized luciferase) gene as a reporter, by using 56 µmol E.m^{-2}s^{-1} high light intensity. It was examined the effect of light intensity on cell density using 8020 lux (112 µmol E.m^{-2}s^{-1}), 5700 lux (80 µmol E.m^{-2}s^{-1}), 3180 lux (44,52 µmol E.m^{-2}s^{-1}) and 0 lux (Argumedo et al., 2013). Fourty-two µmol E.m^{-2}s^{-1} was used as high light intensity and conducted various physiological researches (Gomes and Juneau, 2017). In this study, especially in low illumination conditions, no change in arylsulfatase activity was observed in HSP70A III-1 transformant, and, in high light intensity 28 µmol E.m^{-2}s^{-1} and above, it was found that the activity increased by 2–4 times. The maximum ARS increase at 140 µmol E.m^{-2}s^{-1} light intensity was achieved after 2 h. It has been observed that the effect of light intensity is not parallel with the increase in the period, and ARS activity decreases with the increase of the period. Therefore, this situation can be explained as the light intensity directly constitutes the stress response. Similarly, it was found that high light induces HSPs, like other stresses, by exhibiting photoinhibitory effects (Wang et al., 2004). Photoinhibition is a major limitation for photosynthesis and occurs when excessive radiation leads to a decrease in photosynthetic electron transport components. This leads to deterioration of electron transport and eventually irreversible damage to photosynthetic reaction centers. HSPs can be induced by high light to protect the cell against photoinhibition and reduces the harmful effects of light (Schroda et al., 1999).

The effect of light on induction of HSPs has been demonstrated in cyanobacteria, algae, and higher plants. When the exposure to light intensity increased, enzyme structure may break, new collagen bonds might form or metal ions might seperate and affect the activity of the enzyme. High light can catalyze many processes that can damage enzymes (Rossel et al., 2012).

UV-C overdose is associated with disruption of biological membranes, increases in ROS levels, disruption of lipid and phospholipid metabolism, damage of DNA itself, synthesis of proteins, photoinhibition, photosystem II degredation and respiration (Mittler, 2017; Sharma et al., 2014; Waszczak et al., 2018). Although UV-C shares these with UV-A/B, at much lower doses, the mechanisms

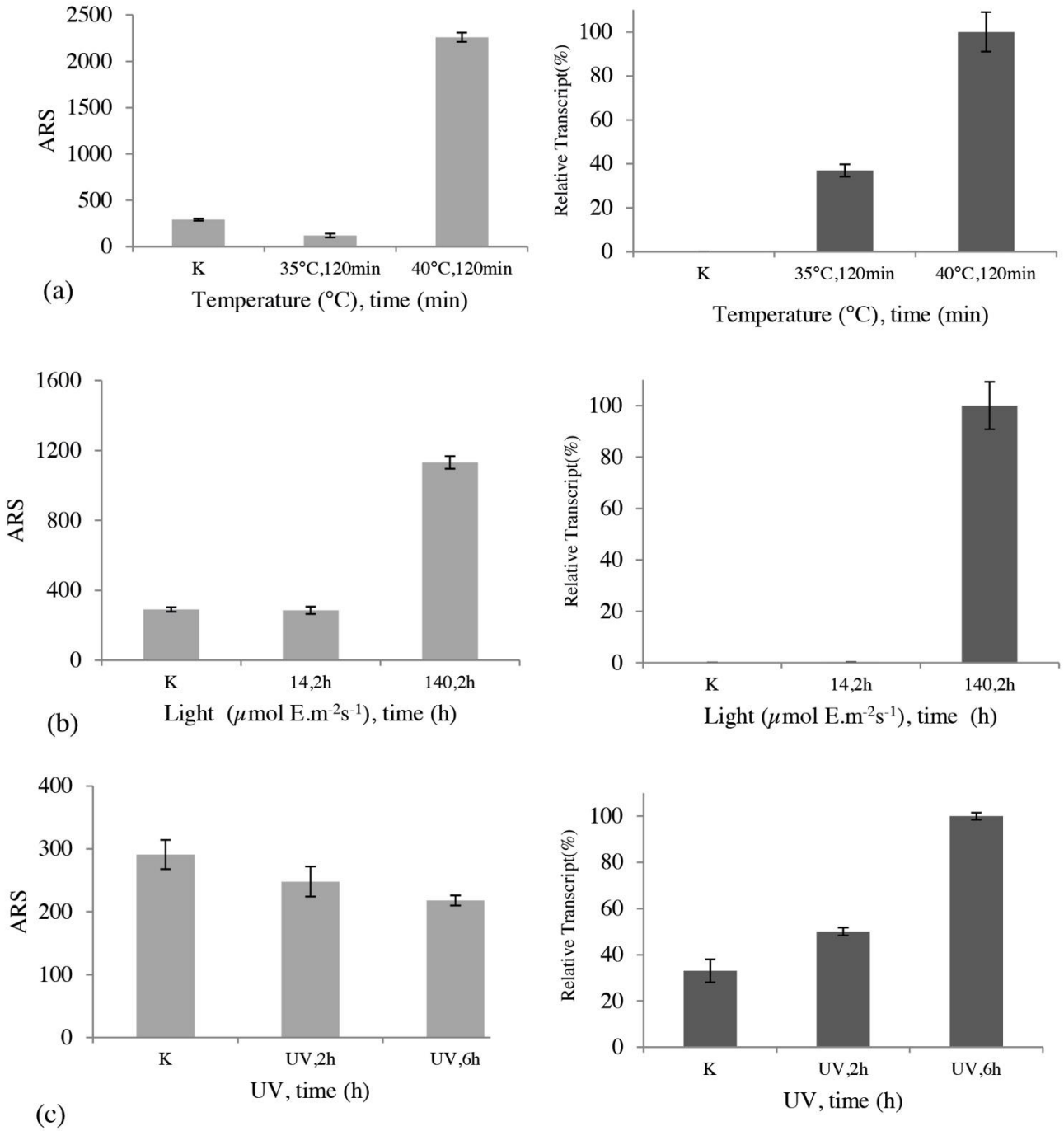

Figure 7. ARS activities and relative transcript levels of HSP70A after temperature change (a), light intensity increase (b), UV exposure determined by qPCR. The results show the mean of the data from three qPCR replicates and two biological replicates. The highest expression level of the respective transcript was set to 100%.

of UV-C detection and signaling are still mostly unknown (Colina et al., 2020). In our study, UV-C application generates no significant change in ARS activity. The reason might be the breakage and damage in DNA or RNA coming from overdose expoure of UV-C. Since UV-C radiation induces changes in the specific amino acid composition in proteins, it might stimulate the enzyme to change function and, thus, might stop the activity completely. In this case, the transformant may not have been able to provide an adaptive cellular response to UV-C radiation. Niu et al. (2006) suggest that overexpressed Hsp70 might play a role in protecting A549 cells from DNA damage caused by UV-C irradiation, with a threshold of protection from UV-C irradiation-induced DNA damage by Hsp70. On the other hand, it was proved that the exposure to low UV-C radiation dosages can improve the stress

tolerance of microalgae and plants (Tilbrook et al.,2016; Liang et al., 2018). The reason for the unchanged ARS activity and constant HSP70A transcript level in our study may be that low-dose UV-C leaded the *Chlamydomonas* resistant to UV-C stress.

All results were evaluated comparatively with RT-PCR results. They show parallel results with ARS activities. The samples exposed to 40 °C, 120 min heat, and shifted to 140 µmol $E.m^{-2}s^{-1}$ for 2 h showed maximum ARS activity. Thus, it can be concluded that, increases in the relative transcript level support the increases in analytically measured enzymatic analysis. The selective ARS reporter gene used in the study is favorable. In this study, using recombinant *C.reinhardtii* HSP70A III-1 clone, various parameters thought to be effective in heat shock response were studied, and data that would contribute to the literature were obtained.

Acknowledgments

This study is supported by the Scientific and Technological Research Council of Turkey, under project number 116Z892. We thank to Prof. Dr. Michael Schroda (Technische Universität Kaiserslautern) for the supply of the strains and the constructs.

References

Argumedo GJA, Choi SE, Hendricks S, Wang A (2013). Measuring the effect of decreasing light intensity on the cell density of the unicellular algae *Chlamydomonas reinhardtii* at 17°C. The Expedition 2.

Bateman JM, Purton S (2000). Tools for chloroplast transformation in *Chlamydomonas*: expression vectors and a new dominant selectable marker. Mol. Gen. Genet. 263: 404-410.

Colina F, Carbó M, Meijón M, Cañal MJ, Valledor L (2020). Low UV-C stress modulates *Chlamydomonas reinhardtii* biomass composition and oxidative stress response through proteomic and metabolomic changes involving novel signalers and effectors. Biotechnol. Biofuels 13 (1): 1-19.

Davies JP, Weeks DP, Grossman AR (1992). Expression of the arylsulfatase gene from the beta-2-tubulin promoter in *Chlamydomonas reinhardtii*. Nucleic Acids Research 20: 2959-2965.

Goldschmidt- Clermont M (1991). Transgenic expression of aminoglycoside adenine transferase in the chloroplast: a selectable marker for site-directed transformation of *Chlamydomonas*. Nucl. Acids Res. 19: 4083-4089.

Gomes MP, Juneau P (2017). Temperature and light modulation of herbicide toxicity on algal and cyanobacterial physiology. Frontiers in Environmental Science 5: 50.

Harris EH (2001). *Chlamydomonas* as a model organism. Annual Review of Plant Biology 52: 363-406.

Harris EH (2008). The Chlamydomonas Sourcebook: Introduction to *Chlamydomonas* and its Laboratory Use, Second ed. Elsevier/Academic Press, San Diego, CA.

Kindle KL (1990). High-frequency nuclear transformation of *Chlamydomonas reinhardtii.* Proceedings of the National Academy of Sciences 87 (3): 1228-1232.

Kobayashi Y, Harada N, Nishimura Y, Saito T, Nakamura M et al. (2014). Algae sense exact temperatures: small heat shock proteins are expressed at the survival threshold temperature in *Cyanidioschyzon merolae* and *Chlamydomonas reinhardtii*. Genome Biology and Evolution 6 (10): 2731-2740.

Kostner M, Schmidt B, Bertram R, Hillen W (2006). Generating tetracycline-inducible auxotrophy in *Escherichia coli* and *Salmonella enterica serovar Typhimurium* by using an insertion element and a hyperactive transposase. Applied and Environmental Microbiology 72 (7): 4717-4725.

Lee S, Lee YJ, Choi S, Park SB, Tran QG et al. (2018). Development of an alcohol-inducible gene expression system for recombinant protein expression in *Chlamydomonas reinhardtii.* Journal of Applied Phycology 30 (4): 2297-2304.

Liang T, Yang Y, Liu H (2018). Signal transduction mediated by the plant UV-B photoreceptor UVR8. New Phytol. 221 (3): 1247-52.

Liu S, Zhang P, Cong B, Liu C, Lin X et al. (2010). Molecular cloning and expression analysis of a cytosolic Hsp70 gene from antarctic ice algae *Chlamydomonas* sp. ICE-L. Extremophiles 14 (3): 329-337.

Livak KJ, Schmittgen TD. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25 (4): 402-408.

Mayfield SP, Schultz J. (2004). Development of a luciferase reporter gene, luxCt, for *Chlamydomonas reinhardtii* chloroplast. Plant Journal 37 (3): 449-458.

Minko I, Holloway SP, Nikaido S, Carter M, Odom OW, Johnson CH and Herrin DL (1999). *Renilla* luciferase as a vital reporter for chloroplast gene expression in *Chlamydomonas*. Mol. Gen. Genet. 262: 421-425.

Mittler, R (2017). ROS are good. Trends Plant Sci. 22, 11-19.

Morimoto RI (1998). Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Development 12: 3788-3796.

Nakamoto H, Vigh L (2007). The small heat shock proteins and their clients. Cellular and Molecular Life Sciences 64: 294-306.

Niu P, Liu L, Gong Z, Tan H, Wang F et al. (2006). Overexpressed heat shock protein 70 protects cells against DNA damage caused by ultraviolet C in a dose-dependent manner. Cell Stress & Chaperones 11 (2): 162.

Nowicka B, Kruk J (2012). Plastoquinol is more active than α-tocopherol in singlet oxygen scavenging during high light stress of *Chlamydomonas reinhardtii*. Biochimica et Biophysica Acta -Bioenergetics 1817 (3): 389-394.

Ohresser M, Matagne RF, Loppes R (1997). Expression of the arylsulfatase reporter gene under the control of the nit 1 promoter in *Chlamydomonas reinhardtii*. Current Genetics 31: 264-271.

Rossel JB, Wilson IW, Pogson BJ (2012). Global changes in gene expression in response to high light in *Arabidopsis*. Plant Physiology 130 (3): 1109-1120.

Saidi Y, Peter M, Finka A, Cicekli C, Vigh L (2010). Membrane lipid composition affects plant heat sensing and modulates Ca^{+2} dependent heat shock response. *Plant Signaling and Behavior* 5: 1530-1533.

Sakamoto W, Kindle K ,Stern DB (1993). In vivo analysis of *Chlamydomonas* chloroplast petD gene expression using stable transformation of β-glucuronidase translational fusions. Proc. Natl. Acad. Sci. USA 90: 497-501.

Schmollinger S (2012). Dissection of the HSF1-dependent stress response with a special focus on the chloroplast HSP70 system in *Chlamydomonas reinhardtii*. PhD, Kaiserslautern University of Technology, Kaiserslautern, Germany.

Schmollinger S, Schulz-Raffelt M, Strenkert D, Veyel D, Vallon O et al. (2013). Dissecting the heat stress response in *Chlamydomonas* by pharmaceutical and RNAi approaches reveals conserved and novel aspects. Molecular Plant 6: 1795-1813.

Schroda M, Vallon O, Wollman FA, Beck CF (1999). A chloroplast-targeted heat shock protein 70 (HSP70) contributes to the photoprotection and repair of photosystem II during and after photoinhibition. Plant Cell 11: 1165-1178.

Schroda M, Blocker D, Beck CF (2000). The HSP70A promoter as a tool for the improved expression of transgenes in *Chlamydomonas.* Plant Journal 21: 121-131.

Schroda M, Hemme D, Mühlhaus T (2015). The *Chlamydomonas* heat stress response. Plant Journal 82, 466-480.

Sharma K, Li Y, Schenk PM (2014). UV-C-mediated lipid induction and settling, a step change towards economical microalgal biodiesel production. Green Chem. 16, 3539-3548.

Sung DY, Kaplan F, Lee KJ, Guy CL (2003). Acquired tolerance to temperature extremes. Trends Plant Science 8: 179-187.

Tanaka Y, Nishiyama Y, Murata N (2000). Acclimation of the photosynthetic machinery to high temperature in *Chlamydomonas reinhardtii* requires synthesis de novo of proteins encoded by the nuclear and chloroplast genomes. Plant Physiology 124 (1): 441-450.

Thimijan RW, Heins RD (1983). Photometric, radiometric, and quantum light units of measure: a review of procedures for interconversion. HortScience 18 (6):818-822.

Tilbrook K, Dubois M, Crocco CD, Yin R, Chappuis R et al. (2016). UV-B perception and acclimation in *Chlamydomonas reinhardtii*. Plant Cell. 28 (4): 966-83.

Wang W, Vinocur B, Shoseyov O, Altman A (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Science 9: 244-252.

Waszczak C, Carmody M, Kangasjarvi J (2018). Reactive oxygen species in plant signaling. Annu. Rev. Plant Biol. 69, 209-236.

Zhang JG, Zhang F, Thakur K, Hu F, Wei ZJ (2017). Valorization of Spent *Escherichia coli* media using green microalgae *Chlamydomonas reinhardtii* and feedstock production. Frontiers in Microbiology 8: 1026.