UNIVERSIDADE DE SÃO PAULO

INSTITUTO DE QUÍMICA

Programa de Pós-Graduação em Ciências Biológicas

(Bioquímica)

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The Circadian Oscillator and Thermopriming Interaction in the Heat Stress Response in *Arabidopsis thaliana*

A Interação entre o Oscilador Circadiano e o Termocondicionamento na Resposta ao Estresse por Calor em *Arabidopsis thaliana*

Versão Corrigida da Dissertação Defendida

São Paulo

Data do Depósito na SPG:

8 de março de 2022

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Dissertação de Mestrado apresentada ao Instituto de Química da Universidade de São Paulo como requerimento para obtenção do Título de Mestre em Ciências Biológicas (Bioquímica)

Master's Dissertation presented to the Chemistry Institute of the University of São Paulo as part of the requirements to obtain the title of Master of Science in Biological Sciences (Biochemistry)

Supervisor: Prof. Dr. Carlos Takeshi Hotta

São Paulo

2022

Ficha Catalográfica elaborada eletronicamente pelo autor, utilizando o programa desenvolvido pela Seção Técnica de Informática do ICMC/USP e adaptado para a Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP

Bibliotecária responsável pela orientação de catalogação da publicação:

Marlene Aparecida Vieira - CRB - 8/5562

M434t

Matsukura, Bruno Fernandes
The circadian oscillator and thermopriming interaction in the heat stress response in Arabidopsis thaliana / Bruno Fernandes Matsukura. - São Paulo, 2022.
86 p.

Dissertação (mestrado) - Instituto de Química da Universidade de São Paulo. Departamento de Bioquímica.
Orientador: Hotta, Carlos Takeshi

1. oscilador circadiano. 2. relógio circadiano. 3. termocondicionamento. 4. termomemória. 5. Arabidopsis. I. T. II. Hotta, Carlos Takeshi, orientador.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor, Dr. Carlos Takeshi Hotta, for his guidance during the development of this work through all of its stages and for accepting me as a part of his research group when I was still an undergraduate student.

I would also like to thank all of my laboratory friends, particularly Ana Paula, Felipe, Flávia, João and Maira who helped me many times throughout these years with laboratory techniques, my presentations and my studies. Additionally, I also thank them for their friendship and comprehension of the many problems I faced during my Master's degree.

My gratitude to our laboratory technicians, Erica and Alessandra, who made the work in our laboratory possible and our ambience more pleasant.

Furthermore, I would like to thank my family, including my dogs Luke and Francisco, for always being around when I needed them and for all the support they gave me.

I would also like to thank my friends David, Juliana and Felipe for their help with the English language and statistical analysis.

Finally, I would like to thank the Chemistry Institute of the University of Sao Paulo, CAPES and all the other institutions that contributed to make this project possible.

AGRADECIMENTOS

Primeiramente, gostaria de agradecer ao meu orientador Dr. Carlos Takeshi Hotta por ter me guiado durante o desenvolvimento de todo este trabalho e por ter me aceitado em seu grupo de pesquisa quando ainda era aluno de iniciação científica.

Gostaria de agradecer também aos meus amigos de laboratório, em especial a Ana Paula, ao Felipe, a Flávia, ao João e a Maira, que muitas vezes me ajudaram com as técnicas de laboratório, nas minhas apresentações e nos meus estudos. Além disso, agradeço-os também pela amizade e pela compreensão dos problemas cotidianos que enfrentamos ao longo da pós-graduação.

Minha gratidão também às técnicas de laboratório Erica e Alessandra por tornarem o nosso trabalho em laboratório possível e mais agradável.

Agradeço também à minha família, incluindo meus cachorros Luke e Francisco que estiveram comigo e me apoiaram durante toda a minha formação.

Gostaria de agradecer também aos meus amigos David e Felipe pela contribuição com a escrita na língua inglesa e com as análises estatísticas, respectivamente.

Por fim, agradeço ao Instituto de Química da Universidade de São Paulo, à CAPES e às outras instituições que financiaram este trabalho.

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ABSTRACT

(MATSUKURA, B. F.) **The Circadian Oscillator and Thermopriming Interaction in the Heat Stress Response in Arabidopsis thaliana.** 2022. Número de páginas do trabalho (ex: 86p). Master Thesis - Graduate Program in Biological Sciences (Biochemistry). Instituto de Química, Universidade de São Paulo, São Paulo.

Heat stress is one of the critical problems faced by many crops. Heat waves are likely to become more frequent in the following years due to climate changes, affecting food production and nutritional values. Two mechanisms present in plants allow them to predict and prepare for upcoming events: priming and the circadian oscillator, or circadian clock. The first allows organisms to respond better to a stress condition if they have been previously exposed to a milder stress. The second allows organisms to prepare for diurnal and seasonal rhythmic events. The aim of this dissertation was to investigate how these two mechanisms are associated with the response to heat stress by comparing the thermoresistance and the gene expression between primed and non-primed Arabidopsis seedlings at different times of the day. We showed that both the circadian oscillator and the photoperiod influence thermopriming in Arabidopsis, affecting the acquired thermotolerance. In turn, thermopriming is capable of altering the expression of circadian oscillator components. Additionally, we observed that distinct sets of components appear to be involved in each time of the day thermopriming was more effective.

Keywords: Circadian oscillator, biological clock, thermopriming, thermomemory, Arabidopsis, heat-stress

RESUMO

(MATSUKURA, B. F.) **A Interação entre o Oscilador Circadiano e o Termocondicionamento na Resposta ao Estresse em Arabidopsis thaliana.** 2022. Número de páginas do trabalho (ex: 86p). Dissertação (Mestrado) - Programa de Pós-Graduação em Ciências biológicas (Bioquímica). Instituto de Química, Universidade de São Paulo, São Paulo.

O estresse de calor é um dos mais importantes problemas enfrentados por muitas plantações de importância agrícola. Nos próximos anos, é esperado que ondas de calor se tornem mais frequentes devido às mudanças climáticas, o que afetará a produção e o valor nutricional dos alimentos. Dois mecanismos presentes em plantas permitem que estas respondam mais efetivamente às mudanças nas condições ambientais: o condicionamento e o relógio biológico. O primeiro, confere uma melhor resistência do organismo a um determinado estresse se este tiver sido previamente exposto a um estresse de menor intensidade. O segundo, permite que seres vivos se preparem para as mudanças que ocorrem durante o dia e no decorrer do ano. Neste estudo, foi elaborado um estudo que permitisse a investigação de como estes dois mecanismos estão relacionados à resposta ao estresse de calor. Para isso, a termoresistência e a expressão de genes associados a estes mecanismos foram comparadas entre plantas condicionadas e não-condicionadas em diferentes horários do dia. Os resultados mostraram que tanto o relógio biológico como o fotoperíodo influenciam a termoresistência adquirida. Em contrapartida, o termocondicionamento é capaz de alterar a expressão de componentes do relógio biológico. Além disso, também foi observado que grupos distintos de componentes aparentam ser mais importantes em cada um dos horários em que o termocondicionamento foi mais efetivo.

Keywords: Oscilador circadiano, relógio biológico, termocondicionamento, termomemória, Arabidopsis, estresse de calor

1 INTRODUCTION

1.1 The effects of heat on Agriculture

Climate change, and the subsequent extreme weather, is predicted to be a critical threat to agriculture and food safety. Short periods of heat may lead to a reduction in the productivity of many crops, especially if they happen during their reproductive or grain filling phases, or during sensitive periods, such as during nighttime (DESAI et al., 2021; PORTER; A SEMENOV, 2005; PRASAD et al., 2003; PRASAD et al., 2011). Moreover, the commodity types that show the most significant losses associated with heat waves and severe drought are the cereals, followed by the oil crops and the pulses; all of them being essential sources of food and feed (POPELKA; TERRYN; HIGGINS, 2004; MEHRABI; RAMANKUTTY, 2017). For example, an increase of 2°C on the average temperature of Earth is expected to reduce wheat production up to 30% in certain areas (ASSENG et al., 2014). Similarly, heat stress has been reported to reduce the yield of many legumes consumed by humans, which contribute alone to 33% of human protein nutrition (GUILIONI; WERY; TARDIEU, 1997; PRASAD et al., 2002; PRASAD et al., 2003; KUMAR et al., 2013; SITA et al., 2017). Oil crops show the second-biggest loss in production due to heat events, being important not only for food production but also for other activities of great economic relevance, such as the production of biofuels and many compounds used in the chemistry industry (METZGER; BORNSCHEUER, 2006). The impact of high temperatures on these crops may affect not only the amount of oil they produce, as seen in sunflowers, but also their fatty acids profile, raising the ratio between monounsaturated fats over polyunsaturated fats, and hence decreasing their nutritional value (RONDANINI; SAVIN; HALL, 2003; SCHULTE et al., 2013). Financially, considering all crops, it is

estimated that agriculture lost 237 billion USD due to heat and drought events only between 1961 and 2014 (MEHRABI; RAMANKUTTY, 2017).

1.2 The circadian oscillator

The changes in environmental conditions that occur throughout the day and across the year may impose great challenges to some living beings. For this reason, in evolutionary history, organisms capable of anticipating these shifts had significant advantages, as they were able to prepare for upcoming events. One crucial mechanism that makes it possible is the circadian oscillator, present in most living beings (PARANJPE; SHARMA, 2005). Even though these circadian oscillators have appeared multiple times independently in evolution, they work in a very similar fashion across the many different phylogenetic groups in which they are present (ROSBASH, 2009). Structurally, they are composed of genes that encode transcription factors that regulate one another, creating feedback loops, leading to oscillations in their expression across the day (DUNLAP, 1999). Simultaneously, these transcription factors also interact in the transcription of many other genes, involved in various processes, making their expression also rhythmic and, consequently, creating rhythmic processes (MCCLUNG, 2006). A rhythm is usually considered to be regulated by the circadian oscillator when the organism keeps them under constant environmental conditions, where there are no environmental cues (MCCLUNG, 2011). For instance, in the study of photosynthetic beings, light and temperature are usually kept constant. However, in nature the circadian oscillator needs to continuously receive environmental cues to keep synchronised with the external environment for long periods. Otherwise, it will be slightly shifted each day, leading to a free-running period (CALWÉ et al., 2016). Therefore, this mechanism is not fully

independent from the environment, and a better analogy to it would be an oscillator rather than a clock, given that the latter should not be influenced by the exterior environment (WEBB et al., 2019). Additionally, it is important to note that the circadian oscillator does not regulate all repeated behaviours, once they might be induced in direct response to recurrent environmental stimuli such as the photoperiod.

The role of circadian oscillator is of extreme importance for plants, since they are sessile organisms and need to prepare without moving for all seasonal and daily shifts in temperature, water availability, attacks from predators and parasites, light intensity, photoperiod, as well as changes in many other biotic and abiotic factors (DODD et al., 2005; MODY et al., 2020; MOGHADDAM; ENDE, 2013; SIMON et al., 2020). In order to deal with these changes, processes such as the opening of stomata, flowering, the senescence of leaves and the production of metabolites related to UV-light protection or defence against insects are all influenced by the circadian oscillator (FRANCISCO; RODRÍGUEZ, 2021; GREENHAM; MCCLUNG, 2015). Moreover, since this mechanism maintains differences in the state of plant cells throughout the day, a given stimulus of the same intensity may lead to different responses depending on the time of day it is applied, a phenomenon known as *gating* (COVINGTON; HARMER, 2007; HOTTA et al., 2007). In agriculture, understanding the circadian oscillator is crucial, as it may be useful to decide the best time to harvest, irrigate or apply fertilisers (BENDIX et al., 2015; HOTTA et al., 2021; STEED et al., 2021).

The circadian oscillator of plants is mainly studied in only a few model species, especially *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae), which serves as a reference for the circadian oscillator of land plants in general. The green algae species *Chlamydomonas reinhardtii* and *Ostreococcus tauri*, are also important for understanding the evolutionary history of the oscillator in plants as well as in studies of this mechanism in

algae (LINDE et al., 2017). Additionally, species of significant economic value, such as wheat, rice, sugarcane, tomatoes and many others have their circadian oscillators well studied (BENDIX et al., 2015). The current models of the circadian oscillator of *Arabidopsis thaliana* are composed of transcriptional regulators that interact with each other creating a series of interlocked feedback loops. Each shows a phased expression, peaking at different times of day (MCCLUNG, 2019; WEBB et al., 2019) (Figure 1). Functionally, they can be split into five groups: a group of MYB-like transcription repressors, a group of MYB-like transcription activators, a group of Pseudo Response Regulators (PRRs), a group of nocturnal regulators and a group of proteins involved in protein stability (WEBB et al., 2019).

The core oscillator components CIRCADIAN CLOCK-ASSOCIATED (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) are MYB-like transcription factors that show their highest transcriptional and protein levels in the morning, being part of what is known as the morning phase components (HSU; HARMER, 2014). These transcriptional factors form homo or heterodimers, acting as regulators of genes that bear a sequence known as *EVENING ELEMENT* in their promoter region, which is mainly present in genes highly transcribed at night (LU et al., 2009; YAKIR et al., 2009). One of them is the *TIMING OF CAB EXPRESSION 1* (*TOC1*), a component of the core oscillator that represses the transcription of *CCA1* and *LHY*, closing the first feedback loop to be described for the Arabidopsis oscillator (ALABADÍ et al., 2001). Even though CCA1 and LHY function as repressors in most cases, it is suggested that they may act as well as activators of specific genes, including *PSEUDO-RESPONSE REGULATOR 7* (*PRR7*) and 9 (*PRR9*), that are also part of the core oscillator (NAGEL & KAY, 2012).

The peak of expression of the gene family PRR (*TOC1*, also known as *PRR1*, *PRR3*, *PRR5*, *PRR7* and *PRR9*) ranges from morning to evening, with each one of them

showing their highest levels at different times of day in sequence (GENDRON et al., 2012; MATSUSHIKA et al., 2000; NAKAMISHI et al., 2010). At the beginning of the photoperiod, *PRR9* increases its expression, reaching its maximum quantity of transcripts during the morning. Following the same pattern, *PRR7* and *PRR5* show their highest levels of transcription around noon. These three transcripts encode pseudo-response regulators that act together in the repression of *CCA1* and *LHY*. In the opposite direction, CCA1 and LHY proteins have been reported to repress both *PRR9* and *PRR7*, closing a negative feedback loop between these genes (KAMIOKA et al., 2016; NAKAMICHI et al., 2010). Additionally, *PRR3* and *TOC1* exhibit their highest expression levels in the evening, with the latter also acting as a repressor of *CCA1* after interaction with CCA1 HIKING EXPEDITION (CHE), another transcription factor (PRUNEDA-PAZ et al., 2019). The combined repressive action of all these PRRs restricts *CCA1* and *LHY* transcriptions to a brief period during the morning (MCCLUNG, 2019). Moreover, TOC1 is also a repressor of some components of the EVENING COMPLEX (EC), a transcriptional repressor of *PRR7* and *PRR9* (DIXON et al., 2011; HELFER et al., 2011). In addition to the interactions described, each of the PRRs act as a repressor of the preceding PRR and many other output genes involved in various physiological processes (LIU et al., 2013; NAKAMICHI et al., 2010).

The evening phased genes include TOC1 and PRR3 as previously described, as well as the ones that are part of the EC: LUX ARRHYTHMO (LUX) or BROTHER OF LUX ARRHYTHMO (BOA), EARLY FLOWERING 3 (ELF3) and ELF4; this protein complex acts repressing itself as a repressor of *LUX* and many other genes repressed at night, including *PRR7* and *PRR9* as described above (DAI et al., 2011; HERRERO et al., 2012; NUNISOW et al., 2011). Two types of EC may be formed depending on which homologue, LUX or BOA, is associated with ELF3 and ELF4. Therefore, two sets of genes might be

targeted and downregulated by this complex, allowing a more nuanced regulation of output genes (MCCLUNG, 2019).

Even though most of the first core oscillator components discovered were transcriptional repressors, later on, a variety of studies have shown that many activators play essential roles in this mechanism and are now considered as parts of it (FOGELMARK & TROEIN, 2014). For example, LIGHT-REGULATED WD1 (LWD1) and LIGHT-REGULATED WD2 (LWD2) are two coactivators reported to be involved in the recruitment of activators to the promoter region of *CCA1*, *PRR5*, *PRR9* and *TOC1* (WU; WANG; WU, 2008; WU et al., 2016). Moreover, as opposed to their homologs CCA1 and LHY, the REVEILLE family (REVEILLE 4, REVEILLE 6 and REVEILLE 8) function as activators rather than repressors, having their peak of expression later in the day when compared to LWD1 and LWD2 (FARINAS & MAS, 2011; HSU et al., 2013; RAWAT et al., 2011). These transcriptional factors interact with coactivators NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED1 (LNK1) and NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED2 (LNK2) in the upregulation of the noon-phased *PRR5* and the evening-phased *TOC1* and *ELF4*; therefore, performing an important task in keeping the circadian oscillator running properly (RUGNONE et al., 2013; XIE et al., 2014).

Besides transcriptional regulation, many core oscillator proteins have their stability and degradation regulated by other proteins. For example, ZEITLUPE (ZTL) and GIGANTEA (GI) are involved in the post-translational regulation of TOC1 and PRR5 (KIM et al., 2007; MÁS et al., 2003). The former is an F-box protein that interacts with GI, acting as a mediator of ubiquitination and consequently controlling the degradation of its targets (SOMERS et al., 2000). In turn, the expression of *GI* is regulated by other circadian oscillator components, such as the EC, CCA1 and LHY that repress its transcription, or REV8, which acts as an activator (BERNS et al., 2014).

The many levels of regulation and the variety of circadian oscillator components highlight the complexity of this mechanism, its robustness and plasticity, allowing it to synchronise with the environment without deregulating with every external cue (WEBB et al, 2019). This accuracy also suggests the great importance of this oscillator to plants.

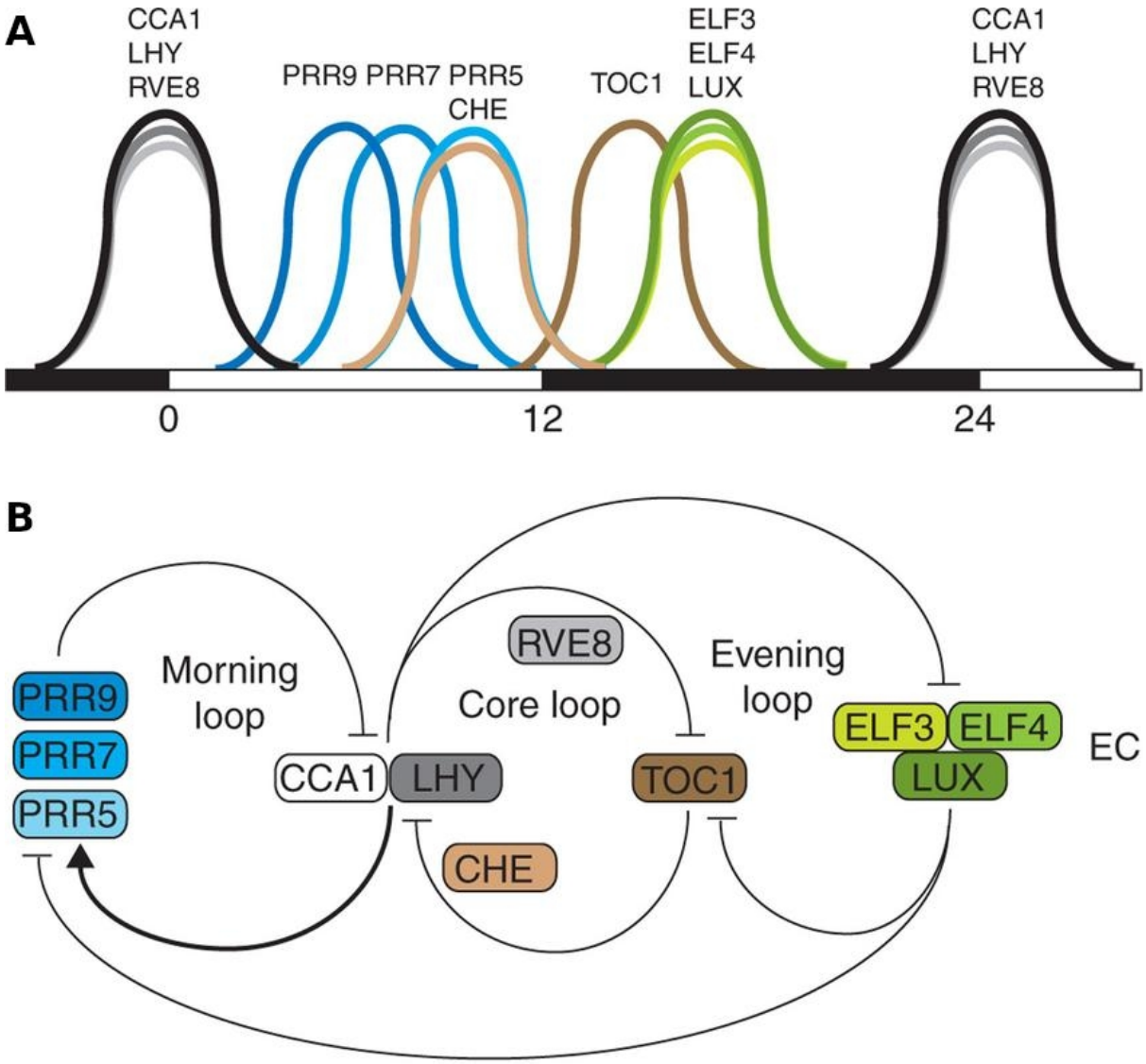

Figure 1. (A) Outline of the transcriptional levels of the *Arabidopsis thaliana* circadian oscillator components across the day (12L 12D). The open bar represents the day, the dark bar represents the night. Note that the amplitude of the oscillations were arbitrarily adjusted to be equal for all transcripts. (B) Blueprint of the circadian oscillator in Arabidopsis *thaliana* subdivided into three main loops (morning, core and evening). Adapted from STAIGER et al., 2013

1.3 Heat stress response and the circadian oscillator

In order to keep the plant´s rhythms synchronised with the rhythm of the environment, the circadian oscillator needs to receive environmental cues, a process known as entrainment. In plants, it relies on two main abiotic factors: light and temperature. Temperature influences the expression of core oscillator components, but the signalling pathway involved has not been clarified yet (GIL; PARK, 2018; WEBB et al., 2019). It is suggested that the transcription factor HSFB2b (Heat-Shock Factor B2b), a repressor that is highly expressed following exposure to heat, might have some influence in this process, since it downregulates the expression of *PRR7* (KOLMOS et al., 2014). Despite the thermal influence, the core oscillator is able to maintain its period across a broad range of physiological temperatures, allowing a more accurate perception of the passage of time regardless of the fluctuations in the environmental conditions, a feature known as temperature compensation (EDWARDS et al., 2005; SALOMÉ; WEIGEL; MCCLUNG, 2010).

In the opposite direction, the circadian oscillator also plays a vital role in response to abiotic stresses, including heat. For example, Arabidopsis *prr5 prr7 prr9* triple mutants (*prr579*) are more resistant to cold, drought and show an elevated ABA content when compared to wild-type individuals (FUKUSHIMA et al., 2009; NAKAMICHI et al., 2009). Additionally, PRR7 modulates ABA-regulated gene expression and sensitivity against oxidative stress (LIU et al., 2013). As a response to heat stress specifically, REVEILLE4 (RVE4) and RVE8 have been reported as essential transcription factors that mediate early

heat stress-induced genes, along with HSFA1s (LI et al., 2019). Another example of the influence of the circadian oscillator in response to heat is that thermoresistance changes when a heat stress of the same intensity and duration is applied at different times of the day. However, the environmental changes between dark and light cycles might be more relevant in this case (DICKINSON et al., 2018). Furthermore, transcriptome analysis showed that the time of day the heat stress is applied leads to distinct patterns of differentially expressed genes, and that CCA1 and LHY more relevant to the heat-response in the morning and PRR7 and PRR9 more important later in the day (BLAIR et al., 2019). Moreover, the core oscillator regulates thermomorphogenesis, an increase in the elongation of the hypocotyl in seedlings exposed to moderate heat that shows a gated response, meaning that it differs according to the time of day the heat is applied (ZHU et al., 2016).

1.3 Priming and molecular memory

Another important factor that plays a role in preparing the organism for upcoming changes in environmental conditions is priming, a process that happens when a stimulus triggers a response in the organism that leads to a more efficient response to upcoming stress, and consequently improves the fitness of the organism (Figure 2) (BECKERS; CONRATH, 2007; HILKER et al., 2015; MARTÍNEZ-MEDINA et al., 2016). The mechanism behind this process consists of a series of modifications that happen in response to the mild stimulus that are maintained for a certain amount of time, conferring to the organism what can be considered as molecular memory (HILKER et al., 2015). The known priming mechanisms are diverse, as an example: it may be generated by RNA polymerases that get stuck at the promoter region of genes that are highly transcribed

under certain stress conditions after exposure to a mild stimulus (WU; SNYDER, 2008). Similarly, upregulation of transcription of specific genes upon upcoming stress may also be induced by accumulation of transcription (co)factors or through chromatin modifications (CHARNG et al., 2006, JASKIEWICZ; CONRATH; PETERHÄNSEL, 2010, LÄMKE et al., 2015; MOORE; LOAKE; SPOEL, 2011). On the post-transcription level, siRNAs might regulate these epigenetic modifications, and the expression of transcription factors. Additionally, molecular memory might be generated by transcription upregulation followed by intron retention, since transcripts with retained introns tend to be stored and can readily leave the nucleus upon splicing activation. (LING et al., 2018; STIEF et al., 2014a; STIEF et al., 2014b). Finally, on the post-translational level, modifications of peptides, including transcription factors, may also be a mechanism of information storage (HILKER et al., 2015; MOORE; LOAKE; SPOEL, 2011).

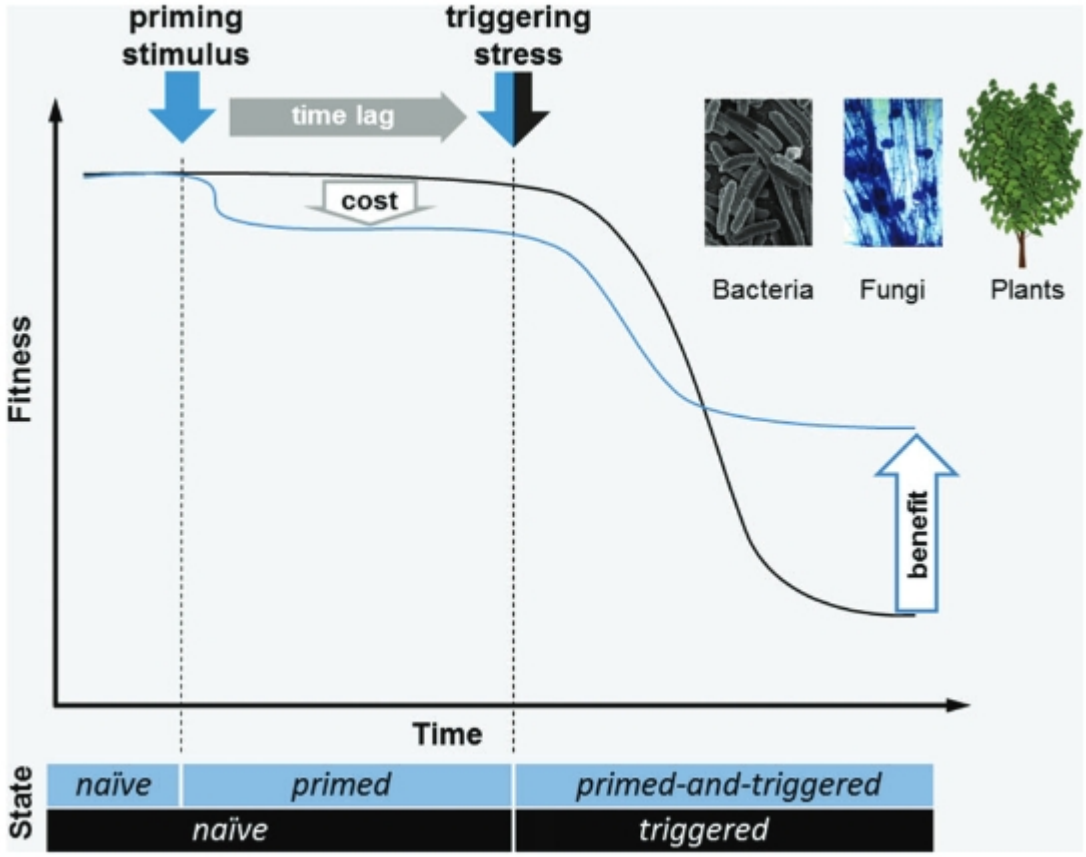

Figure 2. Generalised scheme of the relationship between fitness and priming of a stress response by bacteria, fungi or plants over time. Adapted from HILKER et al., 2015.

1.4 Thermotolerance and thermomemory in plants

Heat stress response has been well conserved throughout the evolutionary history of eukaryotes, being activated by the expression of Heat-Shock Factors (HSFs), which are transcription factors that upregulate the expression of Heat-Shock Proteins (HSPs) and other HSFs (SWINDELL; HUEBNER; WEBER, 2007; RICHTER; HASLBECK; BUCHNER, 2010; NISHIZAWA-YOKOI et al., 2011). Structurally, HSFs can be split into two main groups based on their motifs: HSFAs that are mostly activators and HSFBs that are mostly repressors. Some of HSFs (HSFA2, HSFB1 and HSFB2b) are crucial for thermomemory acquisition and maintenance in primed plants (CHARNG et al., 2007; IKEDA; MITSUDA;

OHME-TAKAGI, 2011). The proteins associated with thermotolerance are usually chaperones that function assisting in the correct folding and maintenance of other proteins considered to be essential to cell survival, protecting protein from denaturation under heat stress conditions (ZHANG et al., 2010; WENG et al., 2014; TANG et al., 2016).

The transcriptional patterns of HSFs and importance for thermoresistance and thermomemory usually follow three distinct patterns (LIU et al., 2018). In the first, transcription rises when heat stress is applied but subsequently declines when stress is relieved, as it has been observed for HSP70 and HSP101 that are usually associated with thermoresistance but not thermomemory (LÄMKE et al., 2015; LIN et al., 2014; WENG et al., 2014; ; WU et al., 2013; ZHANG et al., 2010). In the second, transcription levels are kept high for a long time, similarly to what happens for HSP22.0, HSP18.2, HSP21, and its post-translational regulator FTSH6, usually associated with thermomemory (SEDAGHATMEHR; MUELLER-ROEBER; BALAZADEH, 2016; STIEF et al., 2014). Finally, in the third pattern, transcription is upregulated when heat stress is applied and ceases after it is relieved. However, when plants are exposed to a subsequent heat, transcription rises to even higher levels (LÄMKE et al., 2015).

Most studies regarding the influence of heat on the circadian oscillator include temperature stimuli only in naive plants. On the other hand, most thermomemory studies do not consider the time of day in the priming process. The two primary studies that took into account thermopriming and the circadian oscillator have been published in the past few years. Grinevich et al. (2019) demonstrated that priming is more effective around 12h after the beginning of the photoperiod. Additionally, Li et al. (2019) showed that basal thermotolerance in *reveille7/8* mutants is lower than in wild-type depending on the time of day. Moreover, these double mutants showed a less effective thermomemory than Wt plants, which was not observed for the single mutants *reveille7* or *reveille8*. However, the

relation between thermopriming and the circadian oscillator is still poorly understood. For this reason, we decided to investigate how these two mechanisms are related by analysing the survival of seedlings after exposure to heat treatments and the expression of genes that are important for each or both of them.

1.4 Hypothesis and Objectives

Hypothesis:

Thermopriming is influenced by the circadian oscillator and affects the expression of circadian oscillator components

Objectives:

1) Determine if the time of day and the circadian oscillator influences on survival of primed plants and how it compares to thermoresistance in naive plants
2) Analyse the expression of genes related to thermomemory and thermoresistance in both primed and non-primed plants prior to and after exposure to heat-shock
3) Analyse the expression of circadian oscillator genes under the same conditions
4) Determine if the results observed in the survival experiment are connected to the expression of the analysed genes.

2 METHODS

2.1 Plant Materials and Growth Conditions

Firstly, seeds of *Arabidopsis thaliana* ecotype Col-0 wild-type (WT), *prr7-11* mutants or *hsfb2b* mutants (T-DNA knockout mutants, *athsfb2b* (SALK_047291), KUMAR et al., 2009) were surface-sterilised in 1.5 ml tubes with ethanol 70% for 1 min, which was then removed; next, a solution of 50% bleach and a droplet of Tween® was added and removed after 7 min; after that, the same volume of distilled water was added and removed from the tube at least 5 times in order to wash the bleach away. The seeds were then put in plastic Petri dishes containing half-Murashige and Skoog (MS) medium and agar, and then left in the fridge (4 °C) for two days. Following that, they were transferred to a growth chamber under a 12 h light and 12 h dark at 22°C, where they remained for 8 days prior to exposure to heat stimuli on the following day. Each Petri dish used in the survival experiments had 30 to 36 seeds, whereas the ones used for the RT-PCR analysis had an indeterminate amount. Nonetheless, the seedlings were kept under constant light before the day they received the temperature treatments in the circadian experiment.

2.2 Thermopriming and heat-shock treatments

Both temperature treatments were done by transferring the plates to a second chamber at higher temperatures. The group that suffered the thermopriming treatment (also referred to as thermoconditioning treatment) was moved to a pre-heated chamber at 37 °C where they remained for 30 min before returning to their

original chamber, remaining there for 1 h 30 min prior to exposure to the heat-shock treatment, which was performed by moving the plants to a pre-heated chamber at 50 °C for 50 min. The groups of plants that did not receive the pre-treatment (non-primed), on the other hand, were directly exposed to heat-shock, along with the plants of the primed group (Figure 3). This assay was repeated at different times throughout the day. The temperatures and length of the treatments were based on previous tests performed in our chambers. In order to determine the survival rate, only the seedlings that produced a new pair of green leaves up to 2 weeks after being stressed were counted as survivors (Figure 3B). For RT-PCR analysis, we collected individuals of both groups (non-primed and primed) prior to the heat-shock treatment and immediately after its end at each timepoint. The samples were put in 1.5 ml tubes, readily frozen in liquid nitrogen and stored at -80 °C.

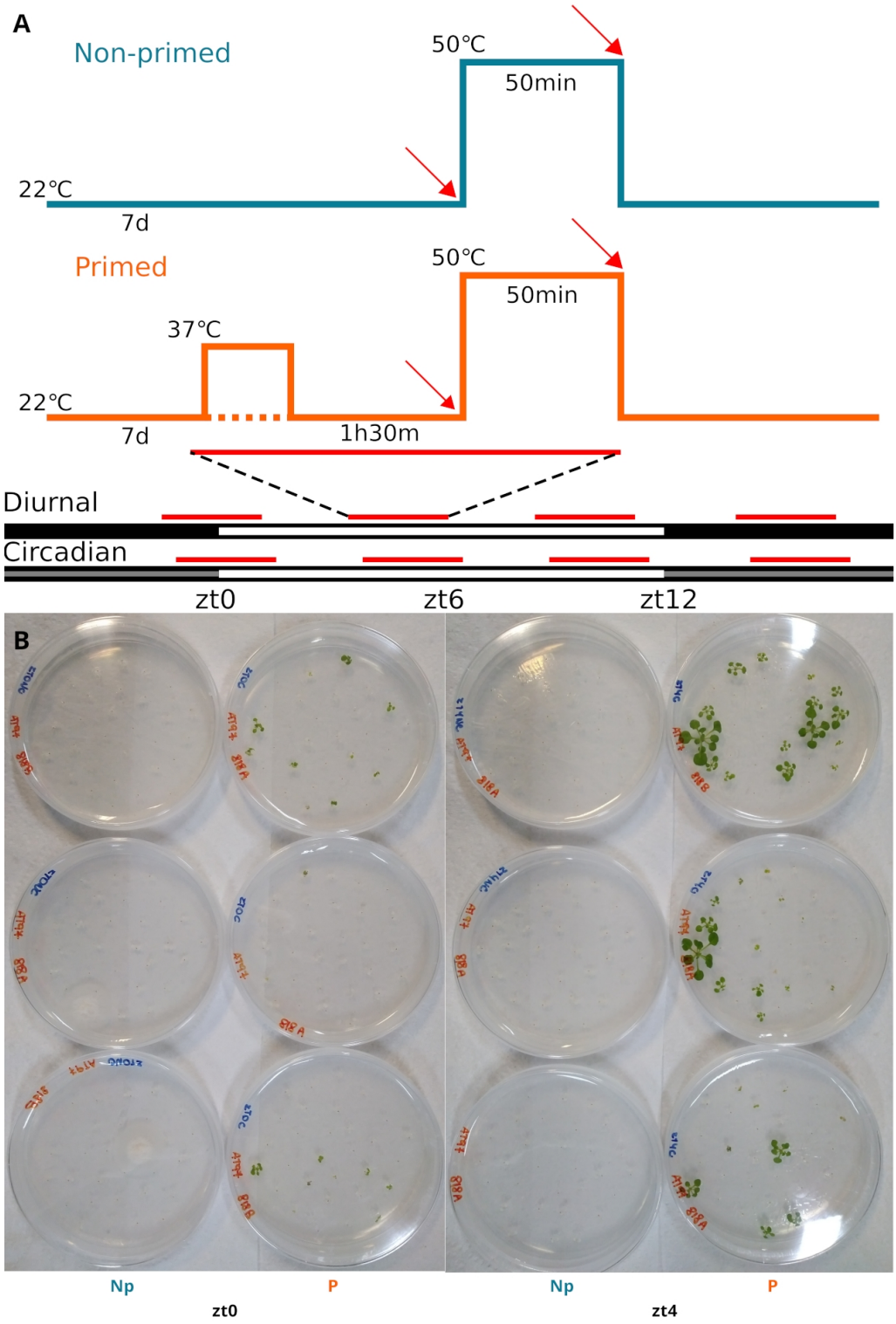

Figure 3. (A) Outline of the temperatures the two groups were exposed to throughout their development. The red arrows indicate the moment and the temperatures at which the samples were when they were collected. The bars at the bottom illustrate the light conditions the plants were exposed to when they suffered the stimuli; black represents they were in the

dark, white and grey represents they were under light, the latter indicating that on the previous day the plants were in the dark at that time of the day. (B) Aspect of some of the plants stressed at zt0 and zt4 on the day survivors were registered (up to 3 weeks after exposure to heat-shock), primed and non-primed sets of plants as well as the timepoint were they were stressed are indicated below.

2.3 RNA extraction and DNAse treatment

At least 30 mg of frozen Arabidopsis tissue was ground in the collection tubes using plastic pistils previously immersed in liquid nitrogen. Total RNA extraction of the content was then performed using the RNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. Afterwards, 1 µg of the obtained RNA was treated with 1 µL DNAse (Life Technologies) for 15 min at room temperature, followed by the addition of 1µL EDTA 25 mM and exposure to 65 °C for 10 min to stop the DNAse activity.

2.4 RT-qPCR

Transcriptional levels were measured by real-time quantitative PCR in the presence of SYBR green. Firstly, a total amount of 500 ng of the RNA previously treated were submitted to cDNA synthesis using the SuperScript™ III kit (ThermoFischer) following its standard protocol. For the RT-qPCR reactions, the cDNA samples were diluted in the 1:5 proportion and then 2 µL were added to wells containing 6 µL of SYBR, 0,72 µL of the primer of interest and 3,28 µL of DEPC water. The RT-qPCR reactions were performed in a Fast 7500/Real-Time PCR

System or a QuantStudio 3 (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). The primers used in the experiments are indicated in Table 1. Relative expressions of the targets were calculated using UBQ10 as an internal reference.

Gene	Locus	Forward sequence	Reverse sequence
PRR7	AT5G02810	TTGGAGAAGATGCCAAAGTTCT	GTTCCGCTCTCACTTCCACTAC
LHY	AT1G01060	AGCATGAGAGGTTTCTAGAAGCC	TGAGGGCATTTTGCTATATTCCC
HSP21	AT4G27670	TGGACGTCTCTCCTTTCGGATTG	TTTGTCGCATCGTCCTCATTGG
HSP70	AT3G12580	CCGTCTTCGATGCTAAGCGTCT	AACCACAATCATAGGCTTCTCACC
FTSH6	AT5G15250	GCCGGAATGGAAGGGACAAAGATG	ATCATGACCCTCCGTCAAAGTCG
HSFA1b	AT5G16820	CTCCAGCTTCGTCAGACAGTT	TGATTCTGCTGCACATGCGA
HSFA3	AT5G03720	GTTGATGACCCGACTCTTGAC	GAGGATCCCAAACTACGAAGCTA
HSFA6a	AT5G43840	AGCCATGGTGTTGAGGATAA	GTCCTTGATGCTCCATTGAA
HSFA7a	AT3G51910	TGCATTCTTTCTCCACGATTCTCC	CAAATTCCCATCTCTCTGCTTCTA
HSFB2a	AT5G62020	CTATCCCAACGCCGTTTCTC	CGAAATCTGTCGGATTCCATACGA
HSFB1	AT4G36990	AAAAGTTCGCCGGAGATGAC	ATGAAACGACGTCGTCTGTGCTA
HSFB2b	AT4G11660	TTGTTCGTCAGCTCAATACTTACG	TTTCCGCCGTTGAATATCCCGAA
UBQ10	AT4G05320	TCGACCCTTCACTTGGTGTTGC	GGGTGATGGTCTTTCCGGTCAAAG

Table 1. Primers used in the RT-qPCR experiments

2.5 Replicates and statistical analysis

In this study, biological replicates correspond to each group of seedlings growing in the same Petri dish. For the survival experiments with WT plants, 6 to 21 biological replicates were used for each group at every timepoint tested, some of them being treated and harvested on different days. In the experiments with *prr7*-11 and *hsfb2b* mutants, at least 4 replicates were used, and all replicates for the same ZT were collected on the same day. For the RT-qPCR analysis of plants grown in diurnal environments, two biological replicates were used for every treatment at each

ZT unless samples were missing or in low quality, in these cases, only a single replicate for each condition was used. Similarly, the levels of *LHY* and *HSFA7a* and every transcript in plants grown in circadian conditions were performed using single samples. Different replicates are represented in the graphs by columns of different shades.

Additionally, three RT-qPCR technical replicates were analysed, indicated in the graphs by the error bars. Statistical analysis was performed using the median of the technical measurements obtained for each replicate. In experiments with single samples, statistical analysis was performed using the values for technical replicates only. In these cases, results were analysed using two-way ANOVA with Tukey's significant difference test. For RT-qPCR results with biological replicates, two-way ANOVA using uncorrected Fisher's LSD significant differences tests were performed to evaluate the differences. The software GraphPad Prism® from ©GraphPad Software, Inc. was used for all the analyses.

3 RESULTS

3.1 Thermopriming is influenced by the core oscillator

The survival ratio observed for the circadian condition experiment (constant light starting on the day prior to treatments) showed that for primed plants, the time of day thermoconditioning and heat shock treatments were applied is important for their survival (Figure 4). Priming increased the survival of plants at all times. Pre-conditioned seedlings that received the heat stress (HS) 14 h after the start of the subjective photoperiod (and consequently, the thermopriming treatment at ZT12), showed the highest survival ratio, followed by the ones stressed at ZT2. On the opposite side, seedlings exposed to the same treatment at ZT7 showed the highest mortality, followed by those exposed to HS at ZT19. Differently, no variation was observed regarding the time of day between non-conditioned plants, which might have happened because they could not bear the high HS temperature that was necessary to analyse the primed group. Therefore, this circadian test did not show any influence of the clock in thermotolerance without priming. Nonetheless, it made possible to observe that the circadian oscillator influenced the process of thermopriming since the time of day the stimuli were applied was relevant for the heat stress response.

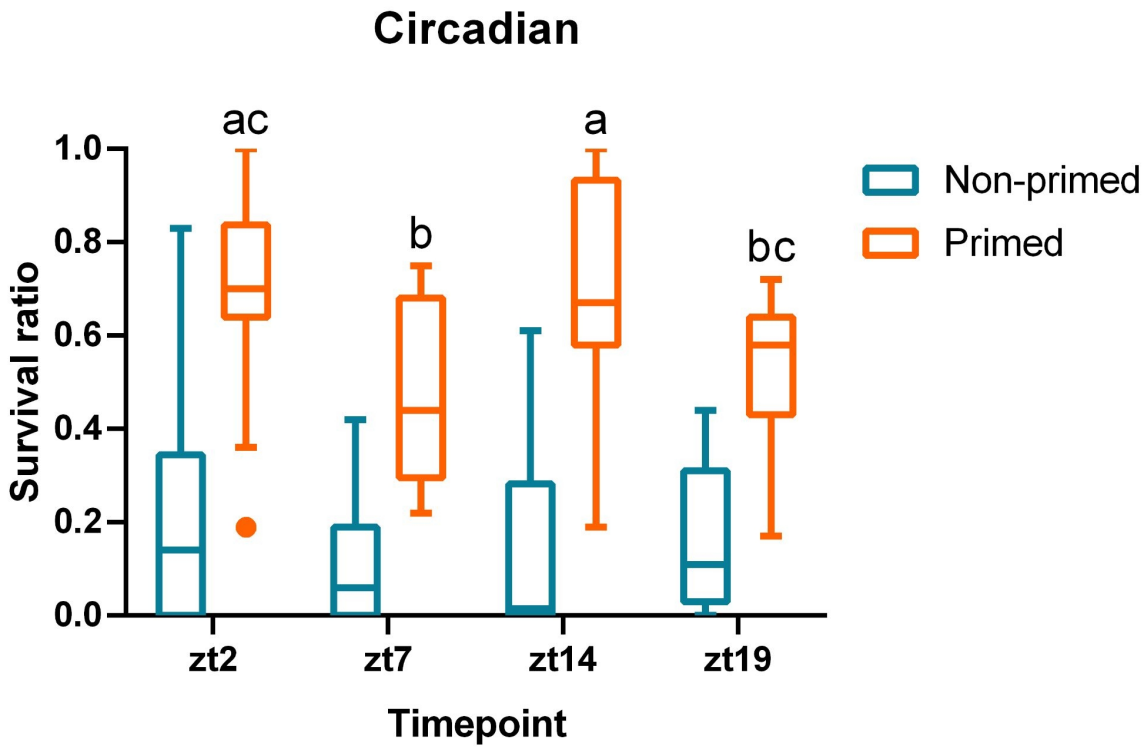

Figure 4. Survival ratio of Non-primed (Np) and Primed (P) plants two weeks after exposure to the temperature stimuli (thermopriming and heat-shock) on Circadian conditions at different times of the day. The timepoint numbers refer to the time Heat-shock was applied, meaning that thermopriming started 2 h earlier for primed plants. The subjective night started at ZT12. Statistically significant differences are indicated by different lower case letters.

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3.2.1 *HSP21* and *HSP70* transcriptional levels match the highest survival ratio observed when HS is applied at ZT14 but not at ZT2

Under constant environmental conditions, the highest levels of *HSP21* and *FTSH6* (which are co-transcribed) were measured for primed plants (P) stressed at ZT14 and ZT19 before exposure to HS (BHS) (Figure 5). *HSP21* transcription showed a very similar pattern for plants stressed at ZT2 and ZT7, whereas *FTSH6* levels in pre-conditioned plants were higher at ZT7 when compared to ZT2. Both

HSP21 and *FTSH6* were found in very low quantities prior to exposure to heat (BHS). However, in non-primed plants (Np), *HSP21* levels were slightly increased shortly after HS (AHS) (not statistically significant), whereas *FTSH6* levels remained the same. In primed plants, *HSP21* levels were kept high, remaining the same (ZT2 and ZT7) or showing a slight decrease just after the end of HS (ZT14 and ZT19). Similarly, *HSP21* levels were also maintained or decreased slightly (ZT19).

The highest amount of *HSP70* transcripts were measured for primed plants stressed at ZT14 before heat-shock, followed by the ones that received the same treatments at ZT7 or ZT19, with the lowest levels registered at ZT2. After exposure to HS, *HSP70* expression decreased in pre-conditioned plants at ZT14 but did not change at ZT2, ZT7 and ZT19. Similarly to what was observed for *HSP21* and *FTSH6*, *HSP70* levels were low for plants that were not exposed to any heat treatment and no significant differences were observed for its expression in naive seedlings.

The differences in transcriptional levels observed for these three genes indicate a gated response, suggesting that the core oscillator somehow influences them. Additionally, the peak of expression at ZT14 in primed plants coincided with the highest survival ratio observed for this group. Therefore, they might have been influential in conferring the thermoresistance observed when HS was applied at this timepoint. On the other hand, *HSP21*, *FTSH6* and *HSP70* showed their lowest expression at ZT2 (with the first showing its lowest levels at ZT7 as well) even though the survival ratio observed at this time was higher than at ZT7 and similar to ZT19. Moreover, ZT19 had a low survival ratio despite the high levels of *HSP21* and *FTSH6*. These results suggest that the circadian oscillator controls other genes

involved in the thermomemory generation process that peak their expression at the beginning of the day.

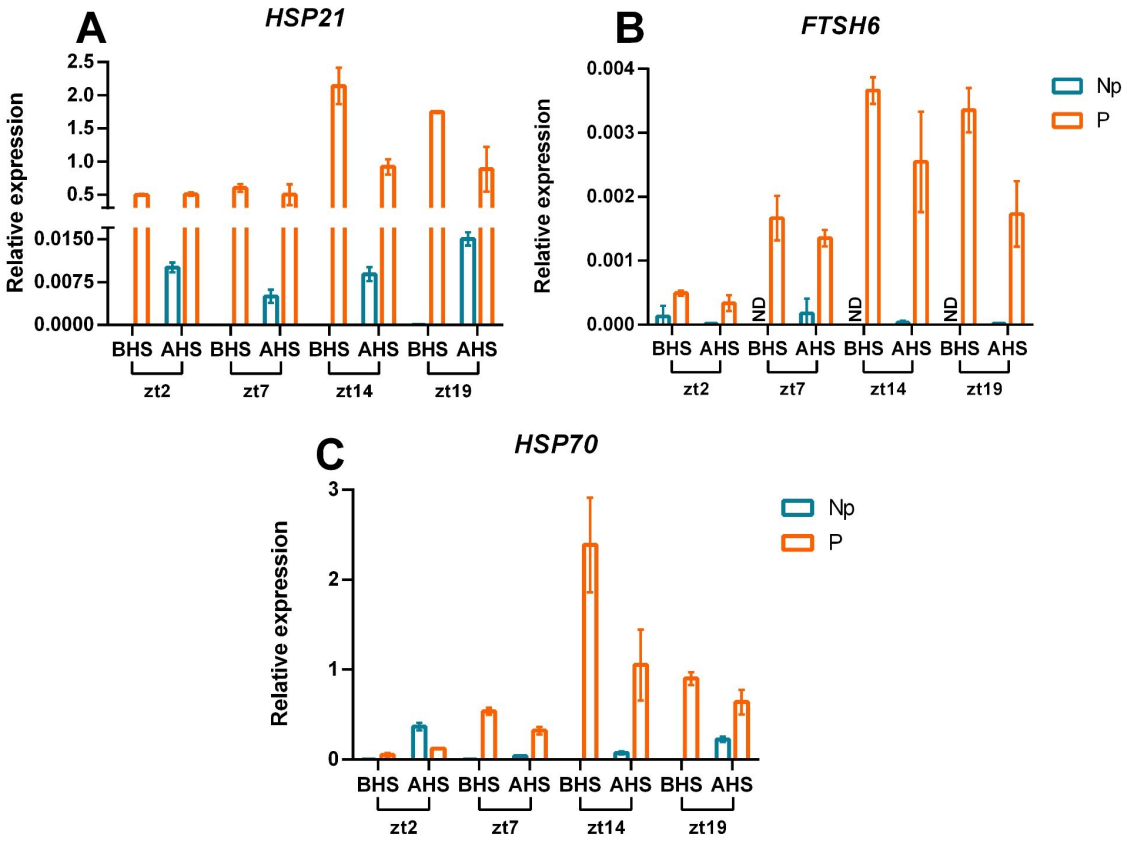

Figure 5. Relative expression of *HSP21* (A), *FTSH6* (B) and *HSP70* (C) in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in circadian conditions. Error bars indicate mean ± sd. ND = not detected

3.2.2 *HSFA1b* and *HSFB1* also showed the highest transcriptional levels for primed plants exposed to HS at ZT14

Similarly to what was observed for the expression of the *HSP*s and *FTSH6* previously described, the transcription factors *HSFA1b* and *HSFB1* had their peaks of expression in primed plants exposed to HS at ZT14 (Figure 6). The effect of priming on *HSFA1b* levels differed depending on the time of day it was applied, raising it in plants stressed at ZT7 and ZT14, and remaining the same at ZT2 and ZT19. Heat-shock either lowered (ZT7) or did not affect *HSFA1b* transcription levels immediately after exposure to it. The thermopriming treatment also raised *HSFB1* levels at ZT14 and ZT19 and remained low at ZT2 and ZT7. The exposure to 50c (HS) upregulated the low levels of this gene in non-primed plants at ZT19 and in both groups at ZT2, whereas when applied at other ZTs, it showed no effect (ZT7) or lowered its expression (ZT14).

The peak of expression of these two transcription regulators at ZT14 may also indicate that they are also playing an important role in regulating heat stress response at this time of day. Additionally, they in turn might be targets of other transcription factors that are active at the same moment. However, similarly to what has been seen for *HSP21*, *HSP70* and *FTSH6*, the low expression of *HSFA1b* and *HSFB1* at ZT2 do not explain the high survival observed at this time.

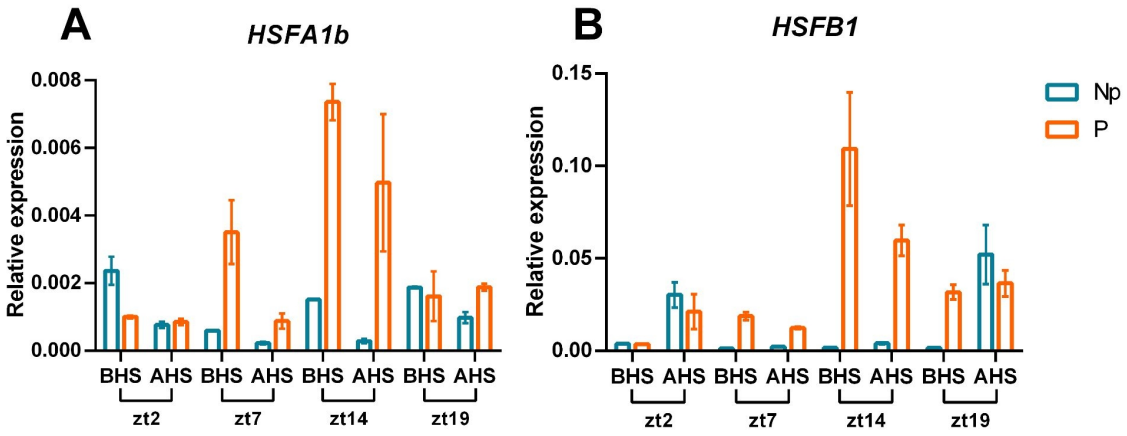

Figure 6. Relative expression of *HSFA1b* (A) and *HSFB1* (B) in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in circadian conditions. Error bars indicate mean ± sd.

3.2.3 *HSFB2a* peaks at ZT7 and is upregulated only after HS

The expression of *HSFB2a* was low before exposure to high temperatures, and there was no difference in its expression between primed and non-primed plants or across the different times prior to HS (Figure 7). In naive plants after HS, there was a considerably higher level of *HSFB2a* transcripts at ZT2 in comparison to ZT7 and ZT14. In conditioned seedlings, the peak of expression of this gene was registered for plants heat-shocked at ZT7. Additionally, after HS, differences between the groups that received distinct treatments were observed at ZT7 and ZT14.

Different from what was seen for the expression of genes analysed previously, *HSFB2a* peaked at ZT7 when heat tolerance was low, as indicated by the survival ratios. This result might indicate some influence of this transcriptional

repressor on the high mortality rate registered at this time of day. Additionally, a differential expression of this gene was also detected between plants stressed at different times, suggesting an influence in its regulation by the circadian oscillator.

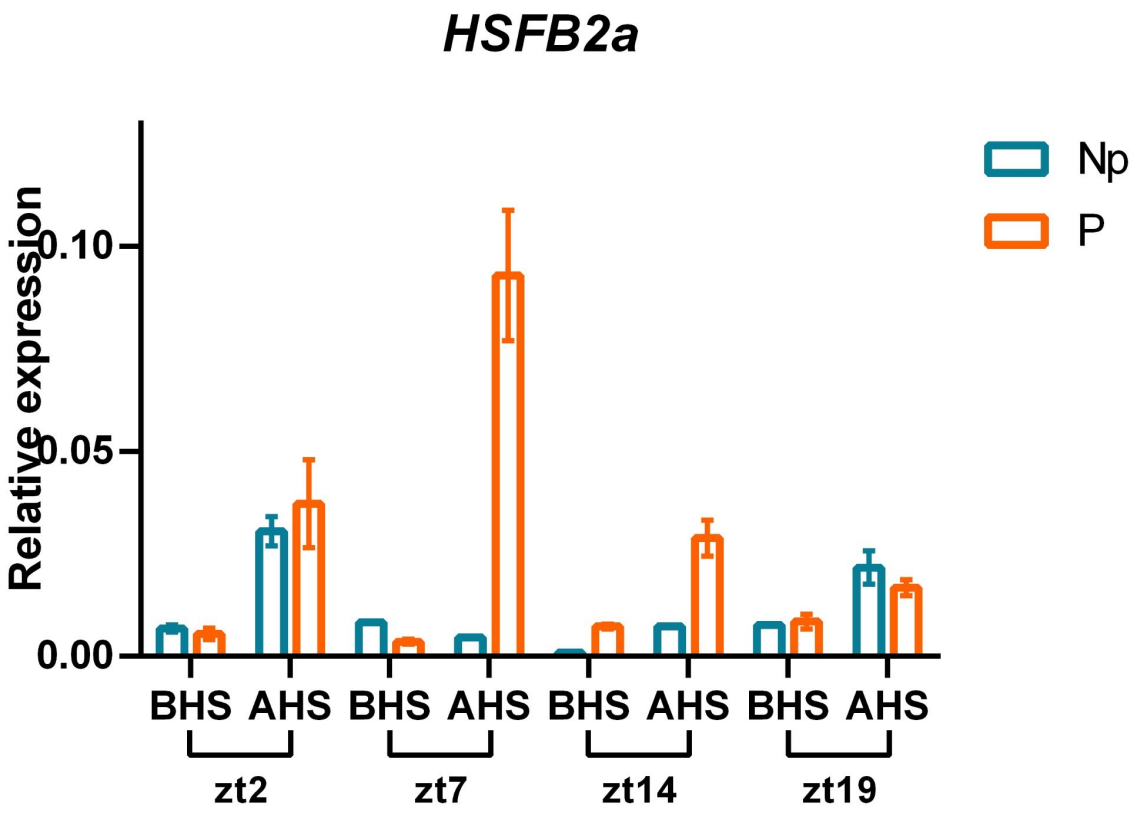

Figure 7. Relative expression of *HSFB2a* in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in circadian conditions. Error bars indicate mean ± sd.

3.2.4 HS lowers *PRR7* expression, whereas thermopriming rise it when applied later on the day

The number of transcripts of the core oscillator component *PRR7* in natural conditions peak around ZT7, similarly to what happened for the naive plants before exposure to HS in our experiments (Figure 8). Thermopriming led to different effects

in its regulation depending on the time of day it was applied. It drastically raised *PRR7* expression when applied in seedlings exposed to HS at ZT14 and ZT19, whereas no effect in its transcription was seen for plants that received this treatment at ZT2 and ZT7. However, it is possible that the high levels of *PRR7* that naturally occur at the latter made it impossible to observe an upregulation at this time. On the other hand, heat-shock showed the same effect on *PRR7* regardless of the time of day, decreasing its levels.

The drastic effect thermopriming had in upregulating this gene for plants stressed at ZT14 coincides with the increased expression observed for the previous transcripts analysed at the same timepoint, which might indicate that they are under control of the same transcription factors or transcription factors that share a co-transcriptional pattern.

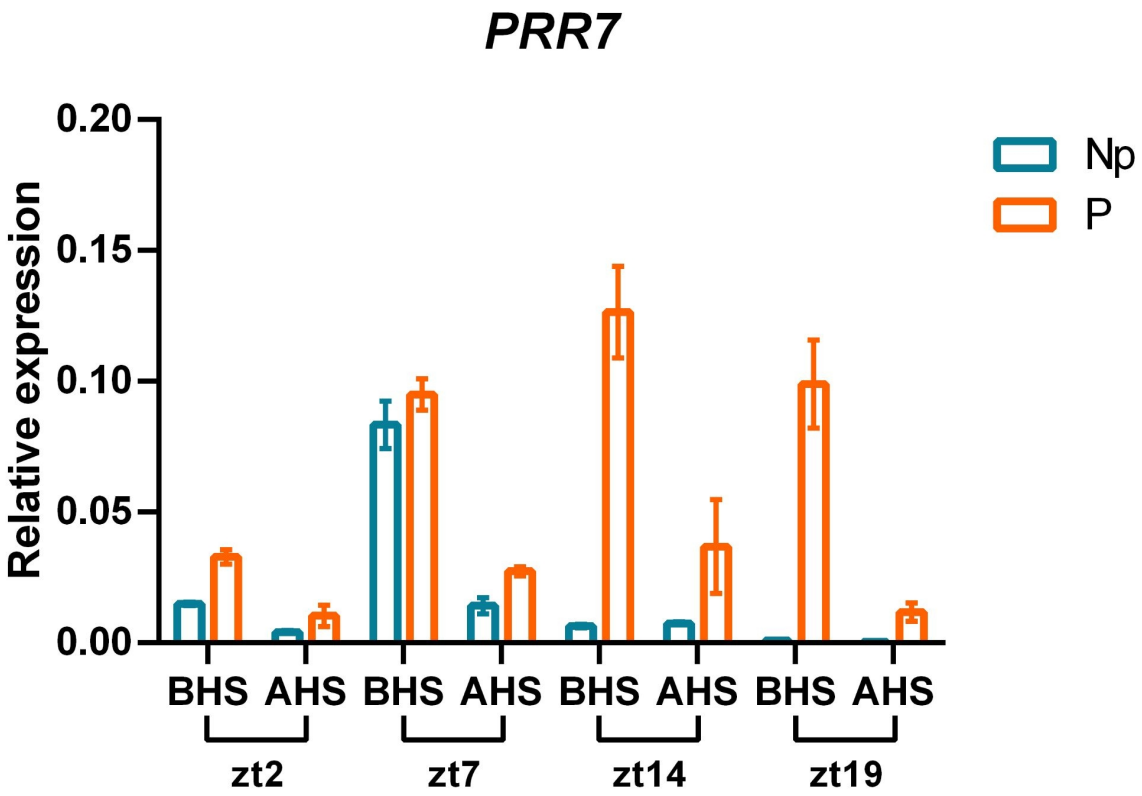

Figure 8. Relative expression of *PRR7* in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in circadian conditions. Error bars indicate mean ± sd.

3.2.5 *HSFB2b* transcription is higher after exposure to HS at ZT2 and ZT14 in primed plants,

Thermopriming had little effect on altering *HSFB2b* expression: primed and non-primed plants had similar quantities of its transcripts at ZT2, ZT7 and ZT14, whereas at and ZT19 pre-conditioned plants showed slightly lower levels of them ($p<0.05$) (Figure 9). After exposure to HS, the transcription levels rose at ZT2 and ZT19 for both groups, whereas at ZT7 and ZT14, that happened only for primed

seedlings. Overall, the highest levels of *HSFB2b* were registered at ZT2 and ZT14 for primed plants and at ZT2 and ZT19 for non-primed ones.

As opposed to what was observed for the previous genes related to heat response and *PRR7*, the expression of *HSFB2b*, that is a transcriptional factor, rose only after exposure to HS high temperatures, which might indicate it is partially responsible for the repression observed in these genes after this treatment. Additionally, in primed plants, the highest expression registered at ZT2 and ZT14 might indicate that this gene played a crucial role in the high survival ratio observed for plants stressed at these times.

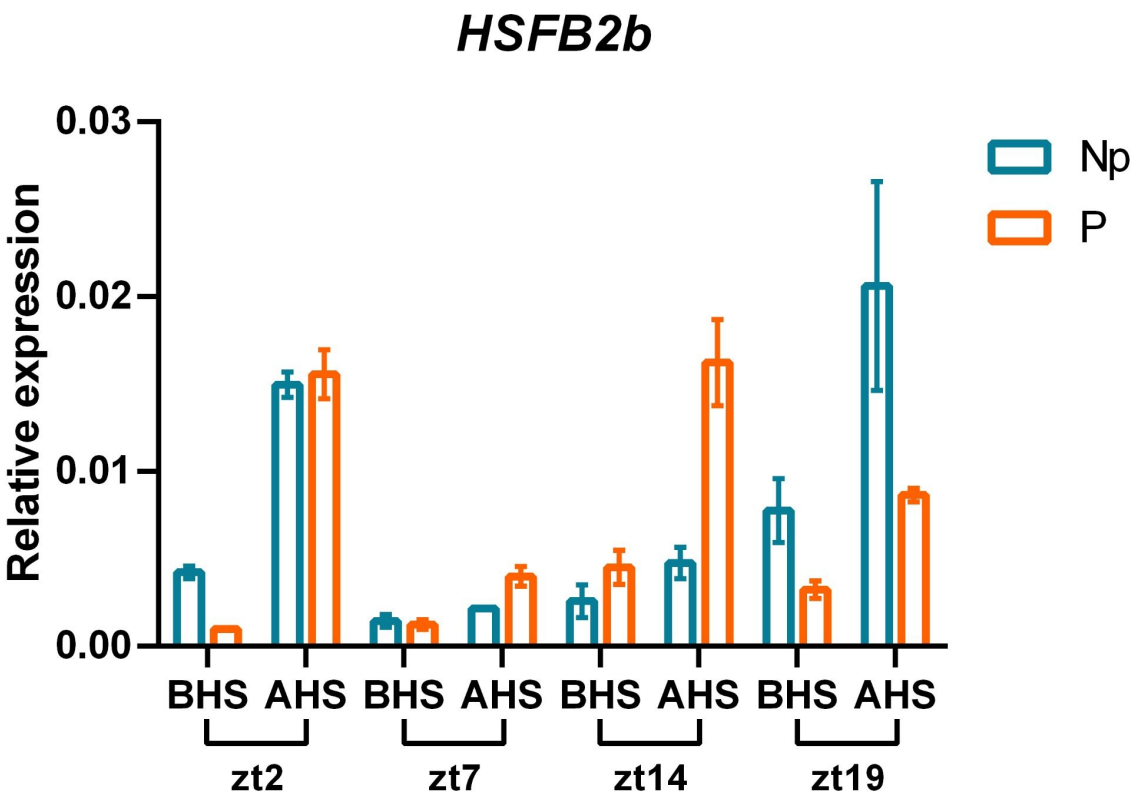

Figure 9. Relative expression of *HSFB2b* in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in circadian conditions. Error bars indicate mean ± sd.

3.3 Light and dark have a strong influence on thermotolerance and thermopriming

When the temperature treatments were applied in plants kept in the same 12h light: 12 h dark conditions they grew (diurnal conditions), the differences between timepoints were more pronounced (Figure 10). Plants that received the heatshock treatment 4h after the start of the photoperiod showed the greatest heat resistance, followed by the ones exposed to the same treatment at ZT0 and ZT12. Seedlings stressed at ZT8 and ZT16 had the lowest survival ratio. Moreover, similarly to what was observed in the circadian experiment, it was not possible to identify in our tests any significant difference among non-conditioned plants regarding the time of day the treatments were applied. Additionally, considering both experiments (diurnal and circadian), plants that received the heat shock treatment around ZT7-8 had low survival ratios, whereas high survival ratios were observed for plants stressed around ZT12-14 and ZT2-4.

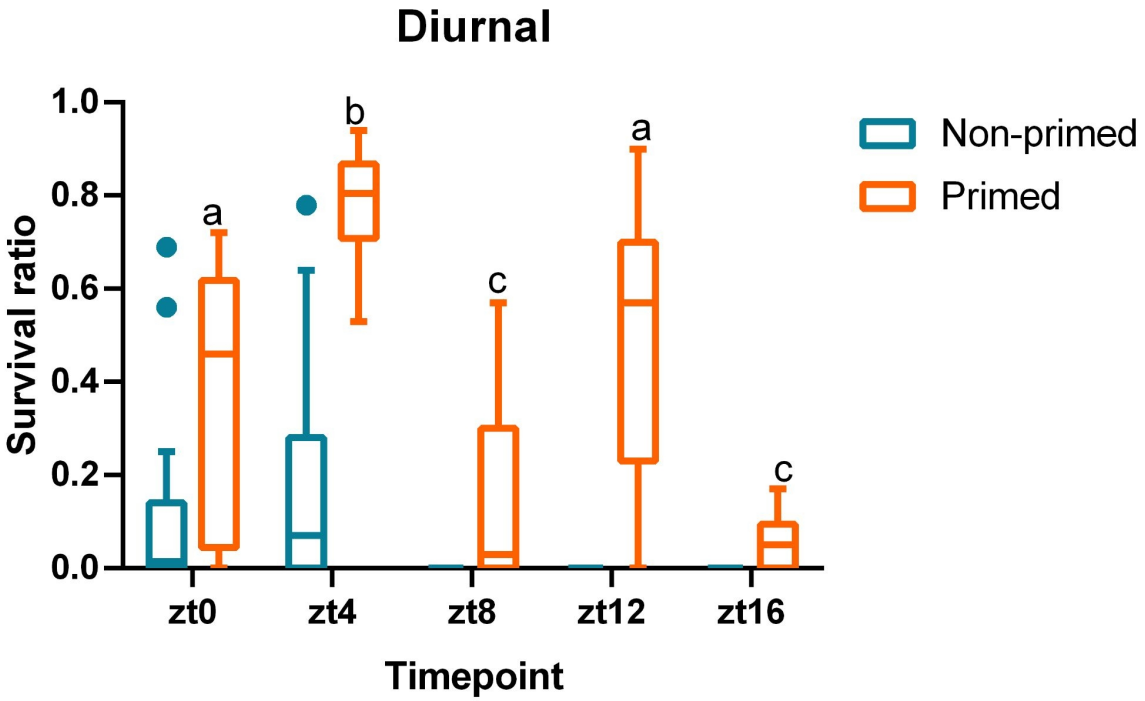

Figure 10. Survival ratio of Non-primed (NP) and Primed (P) plants two weeks after exposure to the temperature stimuli (thermopriming and heat-shock) on diurnal conditions at different times of the day. The photoperiod ended at ZT12. Statistically significant differences are indicated by different lowercase letters.

3.4.1 Thermopriming leads to *HSP21* upregulation in plants exposed to HS at ZT4 or at ZT12

The *HSP21* transcript levels were not detectable or extremely low for seedlings that were not exposed to any heat treatment, no matter the time they were stressed (Figure 11A). For this reason, no significant changes in its expression related to the time of day were seen in naive plants, neither BHS nor AHS. The thermopriming treatment only led to statistically relevant differences in the transcription of this gene in seedlings stressed at ZT4, ZT12 and ZT16. However,

only at the first two *HSP21* levels were higher than what was measured at the other ZTs.

The higher levels of this gene observed at ZT4 and ZT12 suggest that the circadian oscillator or the photoperiod influence the regulation of this gene. Moreover, this upregulation at these timepoints coincided with the high survival ratios observed for plants stressed at these times.

3.4.2 Primed plants show higher levels of *FTSH6* than naive plants

Even though the amount of *FTSH6* transcripts was much lower than the ones measured for *HSP21*, both showed a similar pattern (Figure 11B). *FTSH6* levels were relatively very low in naive plants, and there was no difference in its expression, neither between BHS and AHS nor across the different timepoints. Nonetheless, in primed plants, the highest transcription of this gene was measured at ZT4, followed by ZT16. Similarly to what was observed for naive seedlings, there was no difference in FTSH6 expression before or after HS.

Again, it was not possible to see a significant difference between plants stressed at different times, suggesting an influence from the circadian oscillator or the photoperiod. Moreover, the highest levels of this gene were also measured at ZT4 when heat stress response was higher.

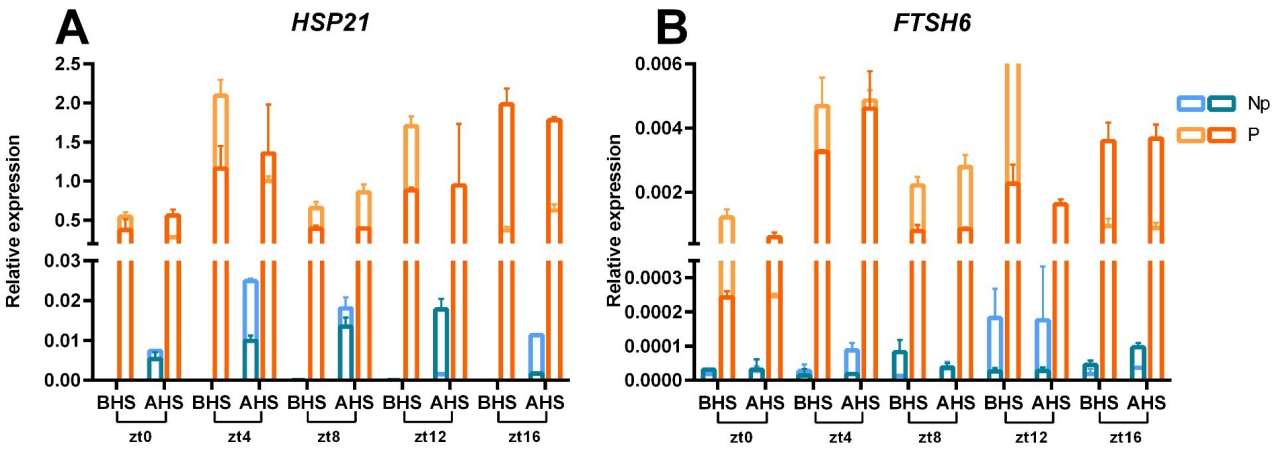

Figure 11. Relative expression of *HSP21* (A) and *FTSH6* (B) in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (NP) and Primed (P) plants in diurnal conditions. Error bars indicate mean ± SD. Different shades of blue and orange represent different biological replicates.

3.4.3 Subsequent heat stress leads to high expression of *HSP70* at ZT4

The highest levels of *HSP70* were measured for primed plants after exposure to HS at ZT4 (Figure 12). Similarly to *HSP21* and *FTSH6*, its levels were very low for non-treated plants and there were no differences in *HSP70* expression between them, neither BHS nor AHS at different timepoints. On the other hand, differences between its levels in primed and non-primed plants were registered for groups stressed at ZT4 after HS and at ZT12 before HS. Therefore, priming alone was not able to cause an upregulation of *HSP70* at ZT4, but when a subsequent stress was applied, it reached much higher levels than what was measured for non-conditioned plants.

The highest levels of *HSP70* measured for primed plants at ZT4 and ZT12 suggest an involvement of the circadian oscillator or the photoperiod in its regulation. Moreover, the higher levels of this transcript at these timepoints coincided with higher survival ratios. Nonetheless, plants stressed at ZT0, when survival ratio was also high, had low levels of this gene.

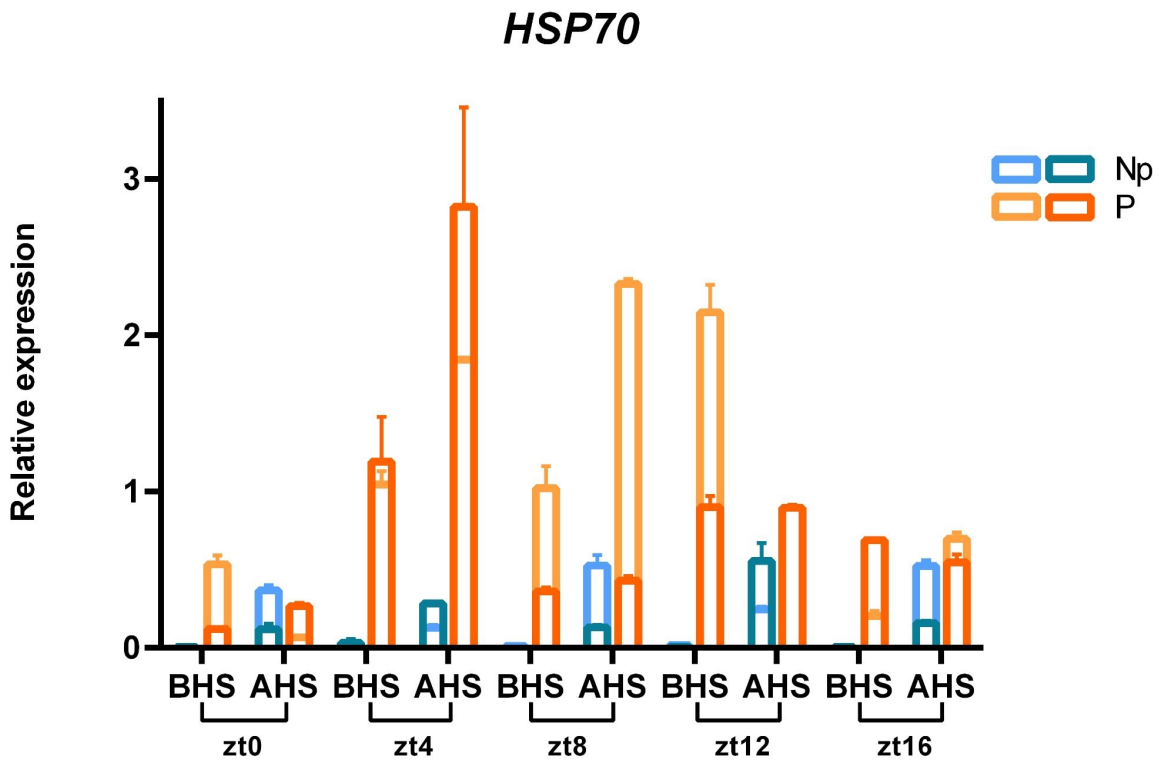

Figure 12. Relative expression of *HSP70* in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in diurnal conditions. Error bars indicate mean ± sd. The different shades of blue and orange represent different biological replicates.

3.4.4 In diurnal conditions, *HSFA1b* and *HSFB1* expression did not follow the same pattern observed in circadian conditions

The expression pattern observed for *HSFA1b* in circadian conditions (Figure 13A) was different from that observed in diurnal conditions (Figure 6A). First of all, no significant difference was seen between primed and non-primed plants ($p = 0.80$). Secondly, the time of day the heat treatments were applied had no influence on its transcription levels. Likewise, no relevant differences in *HSFB1* expression were registered between the different ZTs (Figure 13B). However, at ZT12, *HSFB1* levels were significantly higher in thermoprimed seedlings than in naive ones ($p < 0.05$). On the other hand, after HS exposure, the opposite was observed ($p < 0.0005$), indicating that at this time of the day HS led to *HSFB1* repression in conditioned seedlings and upregulation in non-conditioned ones.

Contrary to what was seen in circadian conditions, *HSFA1b* and *HSFB1* expressions were not influenced by the time of day the heat treatments were applied, suggesting that the differences observed in survival at different times were not caused by their action. On the other hand, the differences in *HSFB1* levels between primed and non-primed plants that happened only at ZT12 might indicate some influence of the core oscillator or the photoperiod on its expression. Additionally, it is noteworthy that the results obtained for each biological replica showed great variation in some cases, especially at ZT8 after HS and ZT12 before HS, which may have interfered in the data analysis.

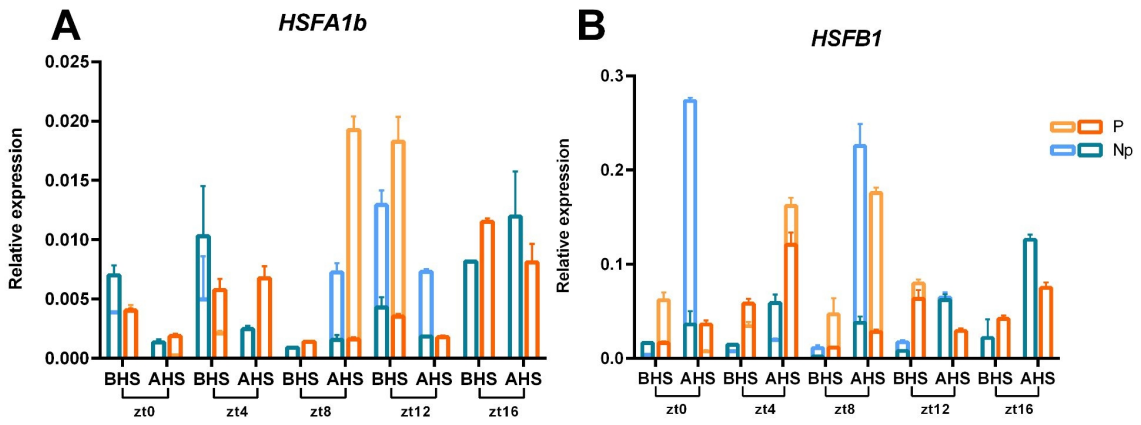

Figure 13. Relative expression of *HSFA1b* (A) and *HSFB1* (B) in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in diurnal conditions. Error bars indicate mean ± sd. Different shades of blue and orange represent different biological replicates.

3.4.4 *HSFB2a* expression is gated, and it is upregulated only after HS

Similarly to what was observed in circadian conditions (Figure 7), the expression of *HSFB2a* was low before exposure to high temperatures. No differences were registered before exposure HS between treated and untreated plants nor across plants stressed at different times (Figure 14). In naive seedlings, HS made the levels of *HSFB2a* rise to similar levels, no matter the time of day it was applied. On the other hand, in primed plants, when HS was applied, its highest levels were reached at ZT4, whereas its lowest levels were measured at ZT0.

As it was registered for the *HSFB2a* expression in circadian conditions, in diurnal conditions the time of day the treatments were applied was also relevant to its transcription, suggesting an influence of the circadian oscillator in its regulation.

However, when comparing *HSFB2a* levels to the survival ratios, the peak of expression in circadian conditions happened at a time where a higher mortality was registered, whereas the opposite was seen in diurnal conditions. This result shows that *HSFB2a* expression alone is not sufficient to explain the differences in thermotolerance observed across the day.

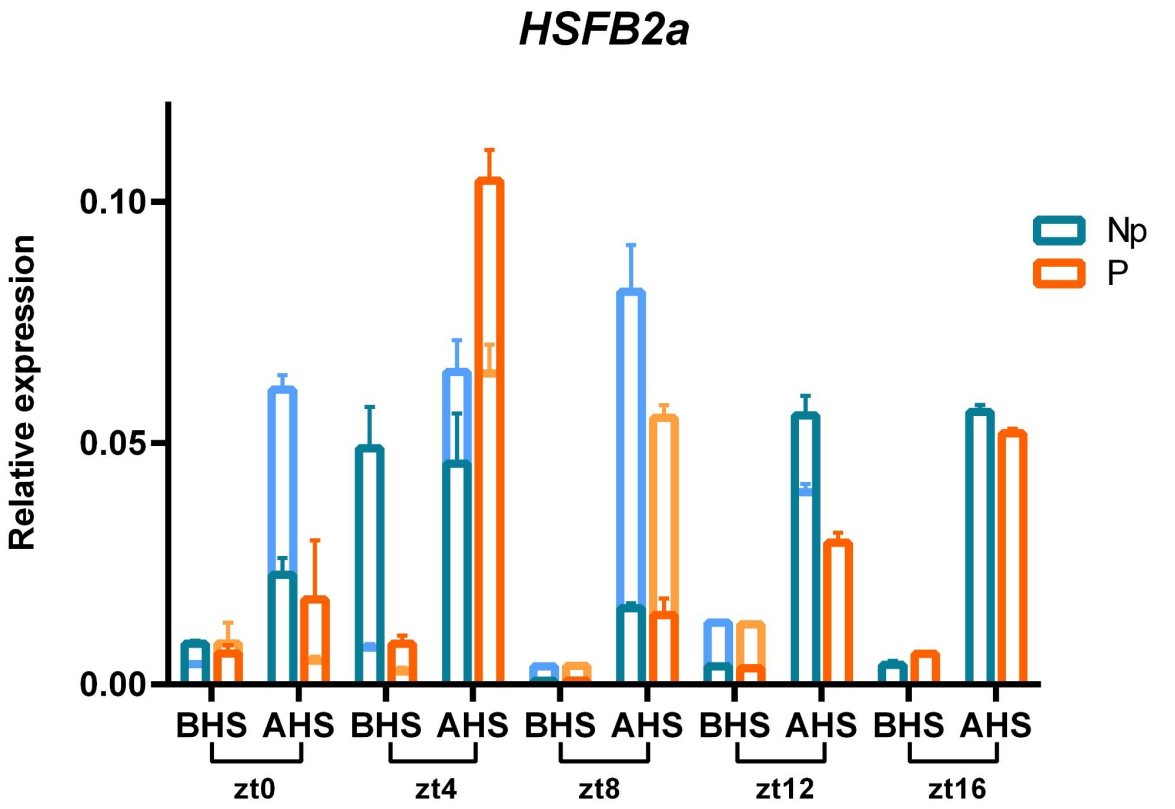

Figure 14. Relative expression of *HSFB2a* in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in diurnal conditions. Error bars indicate mean ± sd. Different shades of blue and orange represent different biological replicates.

3.4.5 Thermopriming resets *PRR7* levels whereas HS makes it decrease

Similarly to what was observed in circadian conditions, the levels of *PRR7* measured for naive plants followed what has been previously described for this transcript (Figures 8 and 15). They rose after ZT0, peaked between ZT4 and ZT8 and decreased after that, showing almost no expression in plants stressed at ZT16. On the other hand, in conditioned plants the relative expression of *PRR7* was similar at every ZT tested and no significant difference was registered before HS. Therefore, thermopriming had different effects on the transcription of this gene, depending on the time of day it was applied. It rose at ZT0, ZT12 and ZT16, when non-primed plants had low levels of it, and decreased at ZT4 and ZT12, when they had high levels of it. Finally, when HS was applied, *PRR7* levels either decreased or remained low at every time point tested for both groups of seedlings (primed and non-primed).

These results illustrate that *PRR7* transcription is influenced by thermopriming and HS in different ways. Additionally, they showed that the first has different effects on its expression depending on the time of day it was applied, whereas the latter showed only a repressive effect.

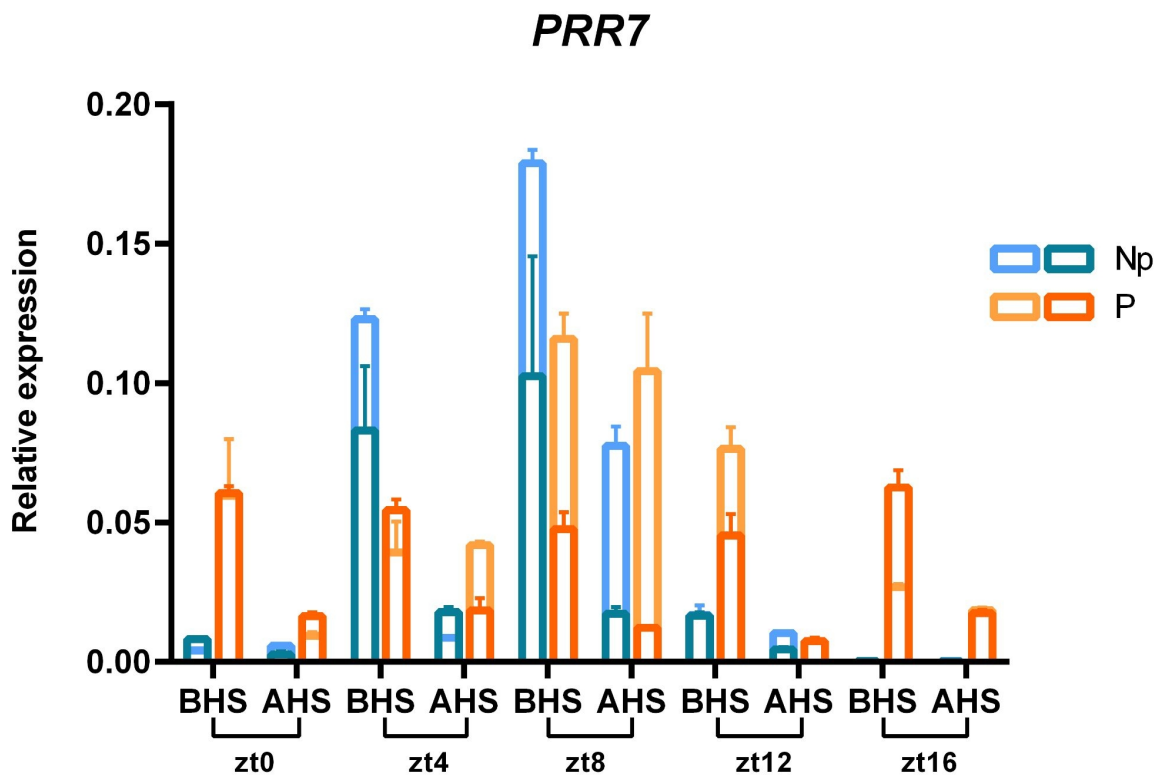

Figure 15. Relative expression of *PRR7* in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in diurnal conditions. Error bars indicate mean ± sd. Different shades of blue and orange represent different biological replicates.

3.4.6 *HSFB2b* upregulation only happens when HS is applied

The levels of *HSFB2b* transcripts were very low prior to exposure to HS, even for plants exposed to the mild temperatures of the pre-conditioned treatment; for this reason, no differences were observed before HS (Figure 16). Additionally, no significant differences were seen for primed plants after HS. In naive seedlings, the highest levels of transcripts were seen for plants stressed at ZT8, ZT12 and ZT16.

The differential expression at different times indicates that the core oscillator or the photoperiod also influences *HSFB2b* transcription. However, the higher levels

of expression of this gene occurred at timepoints where the survival ratio was either high (ZT12) or low (ZT8 and ZT16), suggesting that the influence of these factors on this gene expression is low or, most likely, that its regulation is complex and involves other factors.

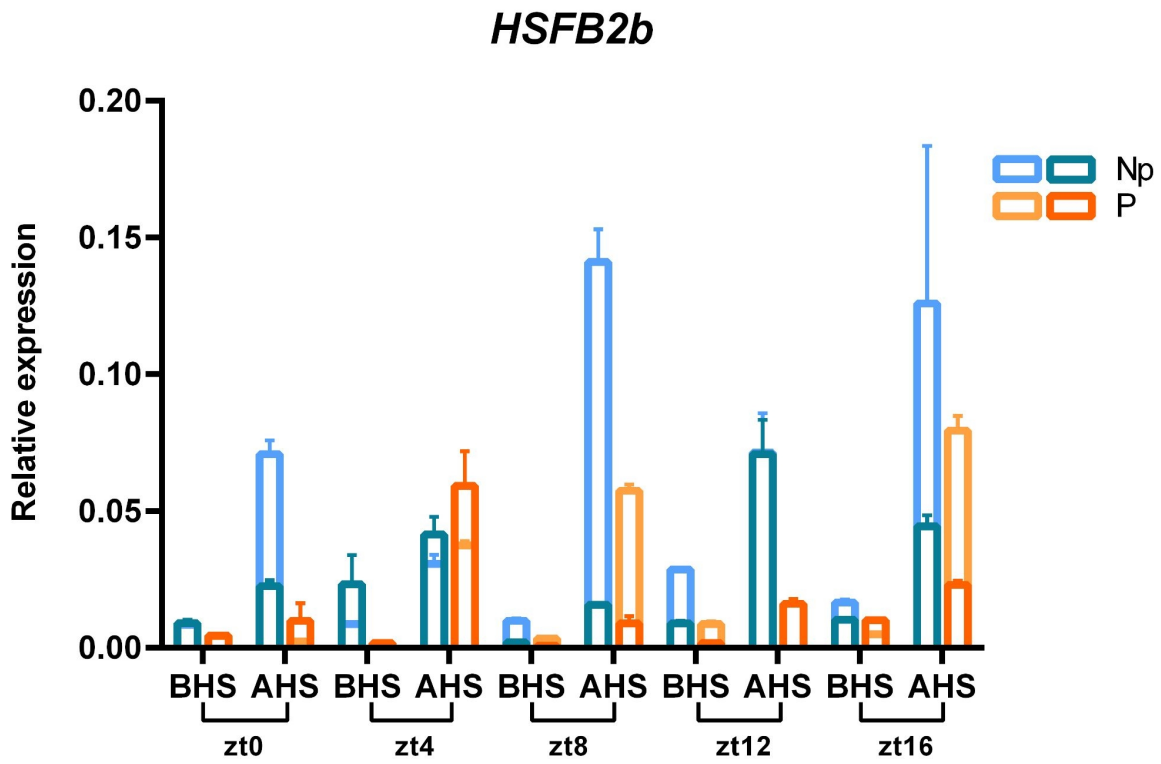

Figure 16. Relative expression of *HSB2b* in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in diurnal conditions. Error bars indicate mean ± sd. Different shades of blue and orange represent different biological replicates.

3.4.7 Themopriming decreases *LHY* expression at the beginning of the day

In normal conditions, *LHY* peaks at the beginning of the photoperiod, which was confirmed by the results obtained for non-primed plants in our experiments. The

highest levels of its transcripts were observed for seedlings exposed to HS at ZT0, followed by ZT4 (Figure 17). Additionally, *LHY* expression was very low at ZT8, ZT12 and ZT16 and remained roughly the same after the heat treatments were applied. Thermopriming decreased *LHY* levels for plants shocked at ZT0 ($p = 0.02$) and ZT4 ($p = 0.006$). On the other hand, heat-shock rose its levels at ZT0 and had no effect at ZT4 in non-conditioned seedlings, whereas in conditioned ones, HS had no effect in the *LHY* transcription at ZT0 ($p = 0.16$) but rose it at ZT4.

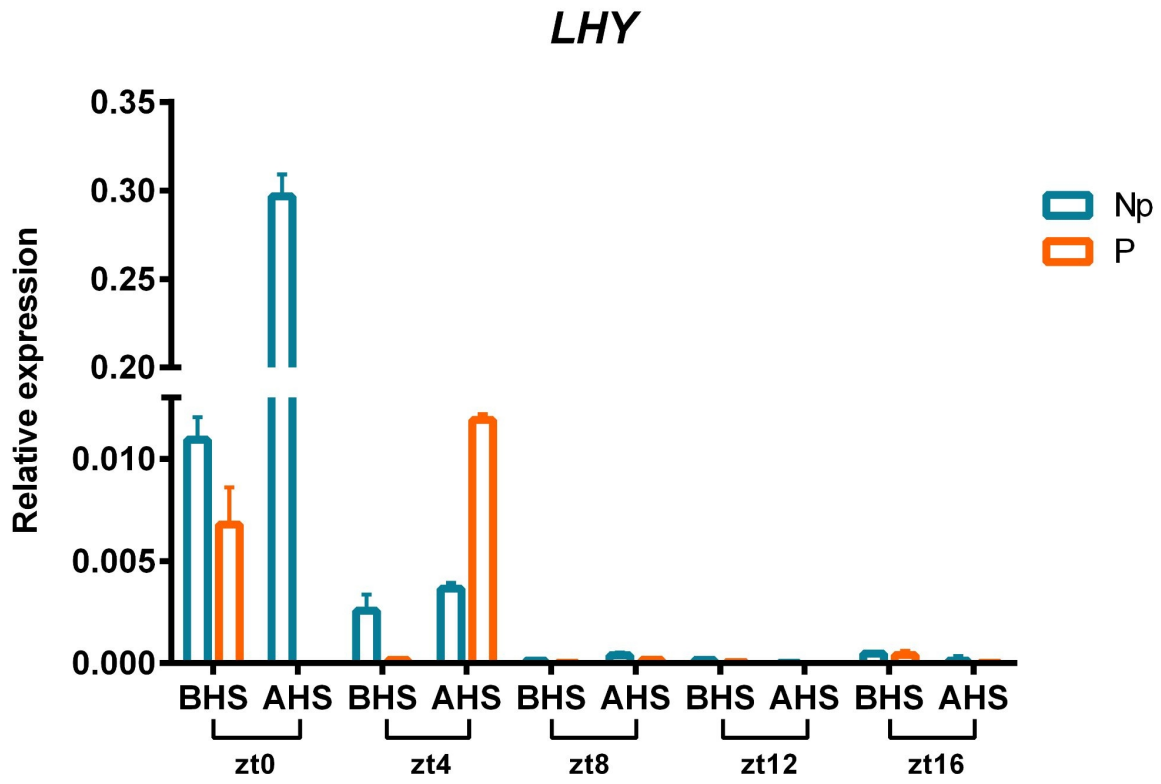

Figure 17. Relative expression of *LHY* in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in diurnal conditions. Error bars indicate mean ± sd.

3.4.8 The time of day had little influence on the expression of *HSFA7a*

The transcription of *HSFA7a* had a very similar pattern for every ZT analysed. In naive plants, its levels were very low, drastically rising when HS treatment was applied at every timepoint tested (Figure 18). In primed seedlings, the levels of *HSFA7a* transcripts were considerably higher prior to exposure to HS, indicating that thermopriming causes its upregulation. However, it remained roughly the same after HS was applied, showing lower levels than those registered for non-primed plants. Even though the same expression pattern was observed across the different timepoints, primed plants exposed to HS at ZT16 showed higher levels of *HSFA7a* whereas in non-primed ones it peaked at ZT12 and ZT16 after HS.

These results suggest that photoperiod and the core oscillator had little influence on *HSFA7a* expression, even though the highest levels measured for ZT16 and non-primed ZT12 might indicate otherwise.

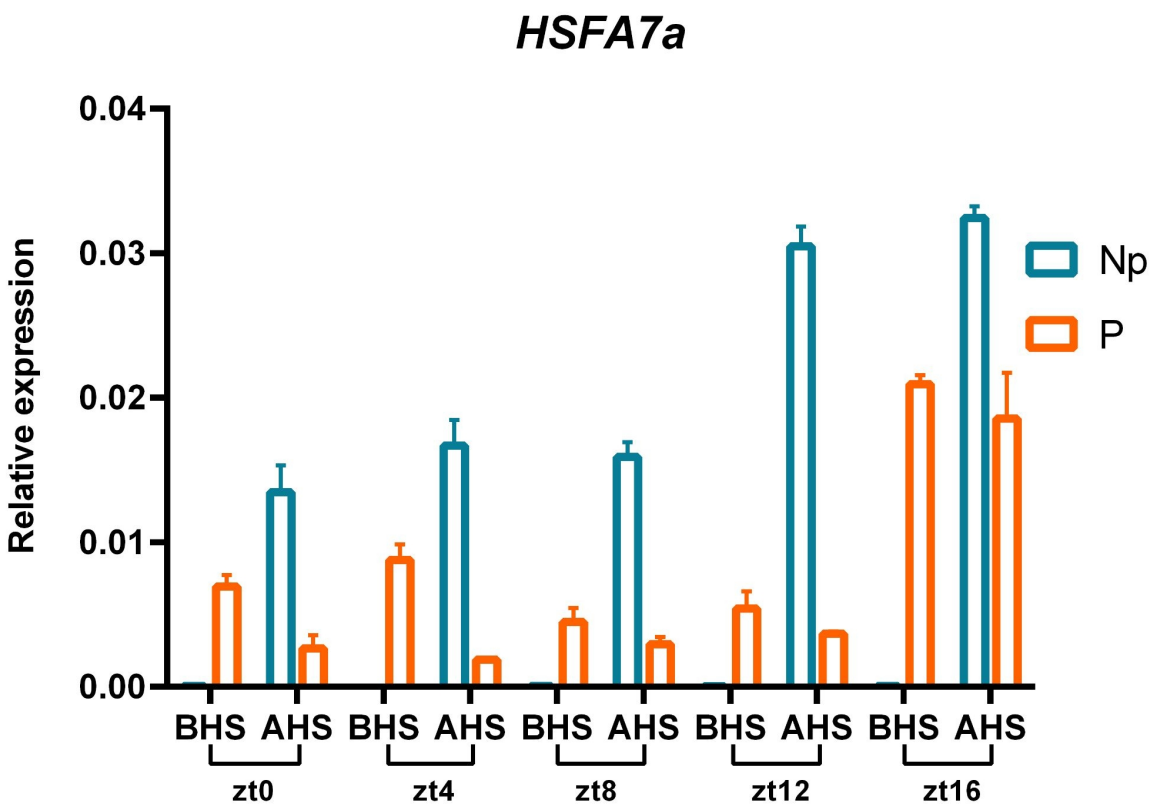

Figure 18. Relative expression of *HSFA7a* in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in diurnal conditions. Error bars indicate mean ± sd.

3.5.1 Thermopriming is affected by the time of day even in the absence of PRR7

The survival ratio observed for conditioned *prr7*-11 mutant plants were different between groups that received the HS in different timepoints, showing that PRR7 is not necessary for the rhythmic response to the heat stimuli (Figure 19). However, the overall survival ratio of *prr7-11* seedlings was significantly lower than what was observed for WT plants, suggesting that it is important for heat stress response. Moreover, the timepoints where heat resistance was higher differed from

that of wild-type (WT) seedlings. The only groups of *prr7*-11 mutants that survived HS were the primed ones exposed to it at ZT4, ZT8 and ZT12, with the first showing the highest death rate (among the ones that survived) and the latter the lowest. Unlike WT seedlings that showed a great resistance when HS happened at ZT0 and ZT4, the survival ratios of *prr7*-11 for the same timepoints were very low, with no seedlings surviving it at ZT0. The opposite happened at ZT8, where WT plants stressed at this time showed one of the lowest survival ratios. On the other hand, for both WT and prr7-11 plants, a high survival ratio was observed when heat-shocked at ZT12, followed by a low ratio when the plants were shocked at ZT16.

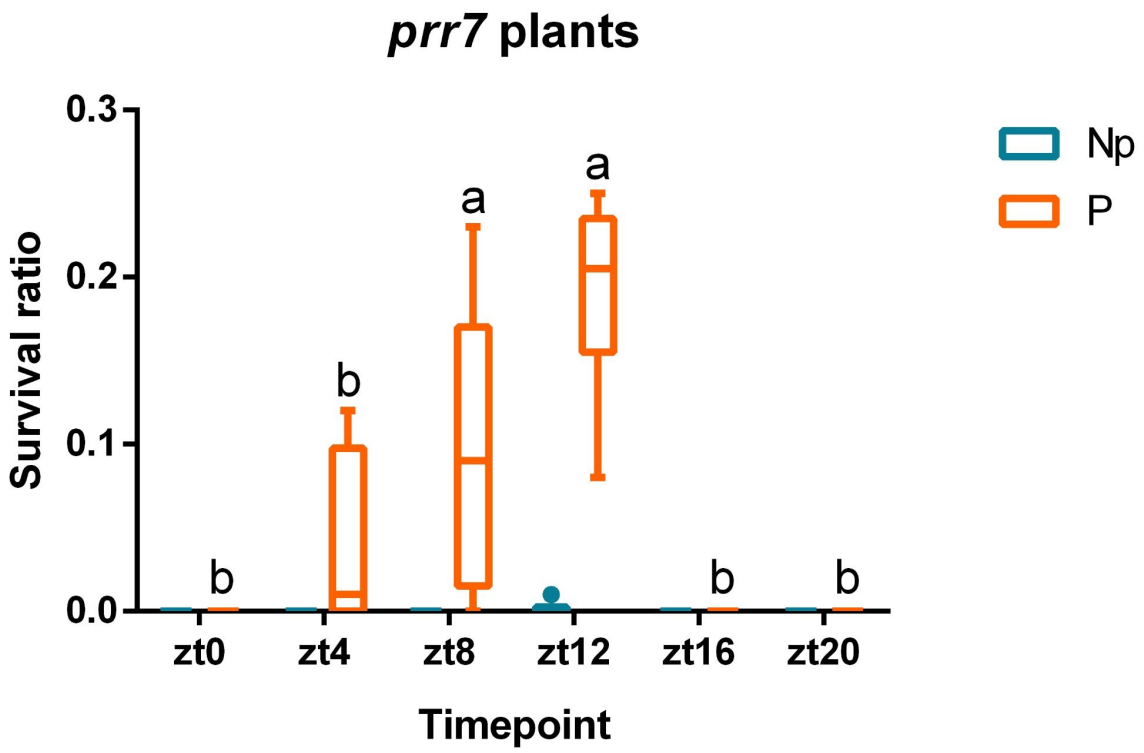

Figure 19. Survival ratio of Non-primed (Np) and Primed (P) *prr7* mutant plants two weeks after exposure to the temperature stimuli (thermopriming and heat-shock) on diurnal conditions at different times of the day. The photoperiod ended at ZT12.

3.5.2 Survival ratio of *hsfbB2b* mutants was similar to the one observed for *prr7* mutants

The survival ratio observed for *hsfB2b* plants across the different timepoints was similar to the results obtained for *prr7* plants (Figures 19 and 20). Both mutants had an overall survival ratio lower than wild-type seedlings and were not able to resist HS without thermopriming. In addition, *hsfB2b* conditioned plants also showed higher tolerance to HS at ZT4, ZT8 and ZT12, being the highest resistance registered for the latter. Moreover, none of the mutant seedlings survived the treatment at ZT0, as opposed to what was registered for Wt plants which showed a high survival ratio at this time. Uniformly, Wt and both mutants showed high tolerance to heat at ZT12 when the pre-conditioned treatment was applied.

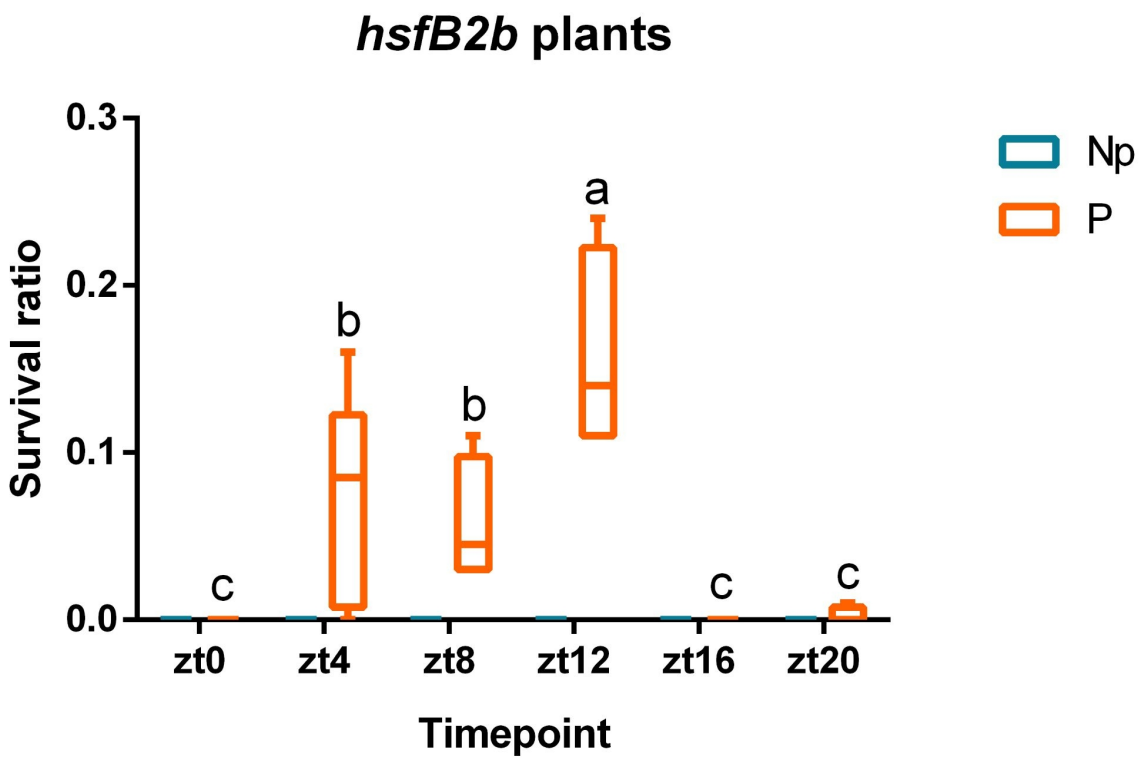

Figure 20. Survival ratio of Non-primed (NP) and Primed (P) *hsfB2b* mutant plants two weeks after exposure to the temperature stimuli (thermopriming and heat-shock) on diurnal conditions at different times of the day. The photoperiod ended at ZT12.

4 DISCUSSION

4.1 Circadian oscillator influences on thermopriming

The differences obtained in the survival experiment under circadian conditions for primed plants suggest that the core oscillator influences thermopriming process. The constant light that started on the previous day to the heat treatments indicates that the differences registered were not due to the changes between dark and light cycles alone. On the other hand, no significant differences were seen among plants exposed directly to high temperatures, similar to what was observed by DICKINSON et al. (2018). Additionally, the time of day in which the heat stimuli were applied also led to differences in the expression of many genes important for heat stress response and thermomemory. Again, these differences were in most cases more pronounced (*HSFA1b*, *HSFB1*, *HSFB2a*) or only present (*HSP21*, *FTSH6*, *HSP70*) among primed seedlings, highlighting that differences in thermoresistance might only be present depending on the pre-treatment or the time of day at which they are being analysed.

4.2 Dark and light are relevant for the differences in thermopriming at different times of day

In diurnal conditions, the time of day the heat stimuli were applied was also relevant for the survival ratio of primed plants; additionally, there were more significant differences between plants stressed at different times when compared to

plants under circadian conditions. Differently from what DICKINSON et al. (2018) observed, there was no difference in the survival ratio of non-primed plants, which might have been caused by the high temperatures of the HS treatment that kept the mortality ratios high no matter the time it was applied. Additionally, a high survival ratio was observed for conditioned plants stressed at ZT12, similar to the results of GRINEVICH et al. 2019. However, seedlings heat-shocked at ZT4 showed an even higher survival ratio, which may not have been seen in their work due to the timepoints they treated their plants (ZT0, ZT6, ZT12 and ZT18). Additionally, in diurnal conditions, there was also a difference in the expression of genes involved in thermoresistance and thermomemory across the time of the day (*HSP21*, *FTSH6*, *HSP70*, *HSFB2a*). Similarly to circadian conditions, these differences in expression were more prevalent in primed plants (*HSP21*, *FTSH6*, *HSP70*, *HSFB2a*), but in the case of *HSFB2b*, they were only present in naive plants. On the other hand, we did not observe an influence of the time in the expression of *HSFA1b* and *HSFB1* under diurnal conditions, which might indicate that the photoperiod plays a more relevant role in their regulation than the circadian oscillator. These results highlight that in natural conditions, not only the circadian oscillator but also the changes in environmental conditions (such as dark and light) are significant to the rhythm of biological processes.

4.3 Thermopriming and HS stimuli affect core oscillator components

In both conditions, *PRR7* expression was affected by thermopriming and HS treatments. However, the changes in its expression depended on the time of day it was applied and on the temperature. Thermopriming treatment seems to reset *PRR7* transcription to a similar level, no matter its expression levels before exposure to it. Heat-shock, on the other hand, decreases its levels. KOLMOS et al. (2016) showed that some Heat-Shock Factors interact with the promoter region of *PRR7*, including HSFB2b, which is also crucial in thermomemory development. Additionally, the expression of *LHY* between primed and non-primed plants was very different after exposure to HS at the beginning of the day. These results indicate that heat stress and thermomemory acquisition might influence the core oscillator.

4.4 Mild, high temperatures and subsequent heat stress might have different effects on the expression of genes

As seen for the expression of *PRR7*, many genes showed different patterns when exposed to thermopriming, to HS directly, or to both. Moreover, these differences were also dependent on the time of day the treatments were applied. One factor that might explain the distinct patterns is the action of HSFs. The transcription of all HSFAs analysed showed a difference in expression after exposure to themopriming treatment; however, some of them were only possible to be observed at specific timepoints. Differently, the HSFBs analysed only showed an upregulation of their expression after exposure to high temperatures and only at

certain times. These results highlight that the sensitivity of each HSFs to mild temperatures or high temperatures varies throughout the time of day, leading to differences in acquired thermotolerance. Therefore, the same stimulus might lead to different responses depending on its intensity, the time of day it is applied and on stimuli the organism has been exposed to previously.

4.5 Thermomemory at different times involve distinct pathways

In both circadian and diurnal conditions, a high thermoresistence for primed plants was registered at least at two different times throughout the day, one happening in the subjective morning (ZT2 in circadian conditions and ZT0 and ZT4 in diurnal conditions) and another around subjective dusk (ZT14 in circadian conditions and ZT12 in diurnal conditions). The transcription of the heat-shock proteins (*HSP21* and *HSP70*), *FTSH6* and some of the *HSF*s we analysed seem to be related to the heat resistance observed at some timepoints (Figure 21). For example, in circadian conditions, the highest survival ratio to HS exposure at ZT14 coincided with high levels of expression of *HSP21*, *FTSH6*, *HSP70*, *HSFA1b*, *HSFB1* and *HSFB2b*. However, out of these genes, the only one that had similar levels at ZT2 was *HSFB2b*. Similarly, in diurnal conditions, high expression of *HSP21* and *HSP70* were measured at ZT4 and ZT12; additionally, the peak of expression of *FTSH6* and *HSB2a* was also registered at ZT4. Nevertheless, none of these genes showed high transcription levels at ZT0, when thermoresistance was also high. These results and the survival ratio observed for prr7 and hsfb2b mutants, suggest that the thermopriming and themoresistance in the morning are different from those observed around dusk. Comparably to this idea, Li et al. (2019) demonstrated that acquired

themoresistance is influenced by REVEILLE 7 and REVEILLE 8 only at noon, when these core oscillator components are in high abundance and work as activators of heat-inducible genes that HSFAs do not regulate.

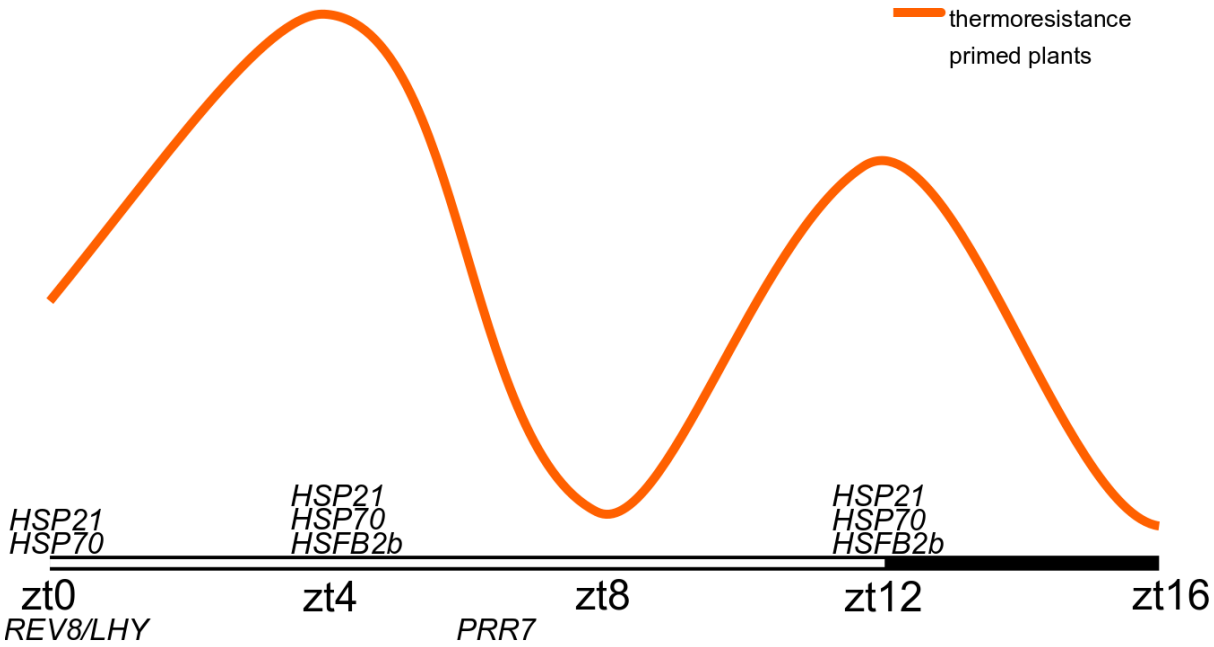

Figure 21. Outline of the thermoresistance in primed plants across the day. The open bar represents the day, the dark bar represents the night. HSPs and HSFs highly transcribed at timepoints where thermoresistance was high are shown above the bar. Under the bar are represented some molecular clock components at the time they show their peak of expression under regular conditions.

4.6 PRR7 and HSFB2b are important for thermopriming but are not necessary for short term memory

Compared to WT plants, both *prr7*-11 and *hsfB2b* mortality was higher and the pre-treatment and the time of the day they were stressed were crucial in their survival. Differently from what was demonstrated by IKEDA; MITSUDA; OHME-TAKAGI (2011), *hsfb2b* mutants did not raise the survival of naive plants, maybe due to the HS higher temperatures used in our experiment. On the other hand, in primed plants, we also registered a decrease in their survival in comparison to Wt ones. The effective heat stress response registered at ZT12 in preconditioned seedlings but not at ZT4 might indicate that in Wt plants, HSFB2b is more relevant to thermopriming in the morning than it is around dusk when there might be other factors responsible for the maintenance of the thermoresistance. The results observed for the transcription of this repressor and its absence in the analysed mutants might indicate that at certain times, a more restrained response to heat-stress pathways after HS is determinant to the plant survival. This might happen due to a faster and more effective return to a naive state. As for conditioned *prr7*-11 mutants, the survival ratio at ZT12 was considerably higher than ZT4, which was different from what was registered for Wt plants. In this case, the highest survival rate at ZT12 in *prr7*-11 might have been caused by the absence of PRR7 at this time, when its protein levels are the highest, which might lead to an increase in the expression of genes related to thermomemory and thermoresistance (FARRÉ; KAY, 2007).

5 CONCLUSION

The starting proposal of this work was to search for relations between the circadian oscillator and the thermopriming process. The results we obtained for both the survival experiment and the expression of essential genes related to these mechanisms pointed to a connection between them. This was possible to observe mainly due to the differences observed across different times of the day. Moreover, we observed differential expression of specific genes dependent on temperature intensity, highlighting that thermopriming and heat-shock responses are regulated differently. Furthermore, we were able to notice a decrease in thermoresistance in *hsfb2b* mutants, which lacked this important transcriptional factor responsive to heat-shock. Therefore, the outcome of a stressful heat event depends on its intensity, the time of day it happens, the previous expositions of the organism to a variety of stimuli and how efficient is the organism responds to restrain its stress response once the stressful event is over (Figure 22).

However, many questions about the relation between thermomemory and the circadian oscillator remain. In the future, additional analyses could be done to improve the understanding of the different factors and signalling pathways involved in this process. For example, proteomic and transcritptomic analyses might be useful in determining the signalling pathways that dictate thermomemory and thermoresistance at different times of the day. Moreover, it would also be important to design experiments to investigate the role of the circadian oscillator in long term thermomemory.

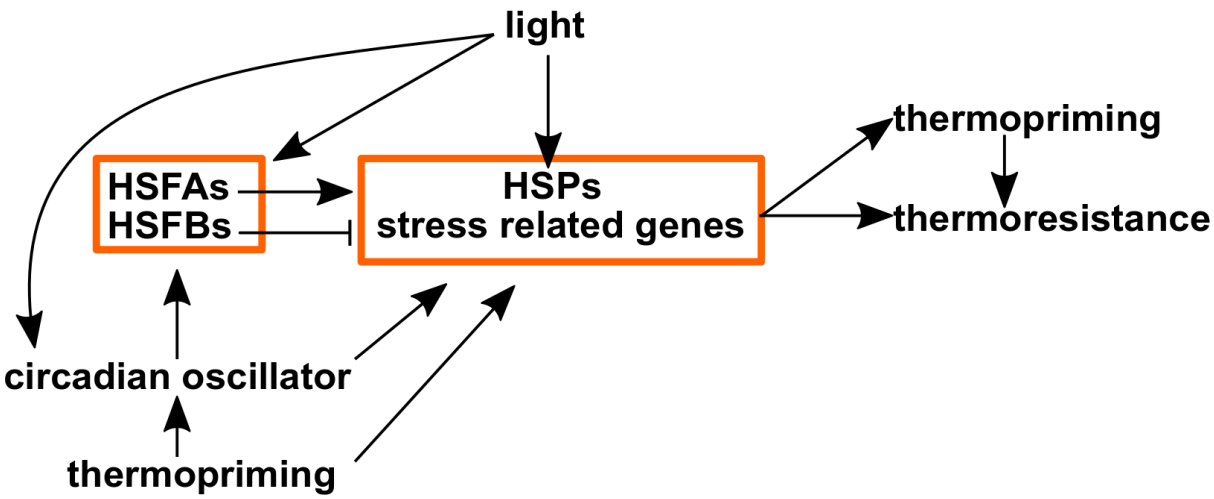

Figure 22. Blueprint of the environmental stimuli and possible molecular pathways that influence the differential thermoresistance of primed plants throughout the day.

6 SUPPLEMENTAL MATERIAL

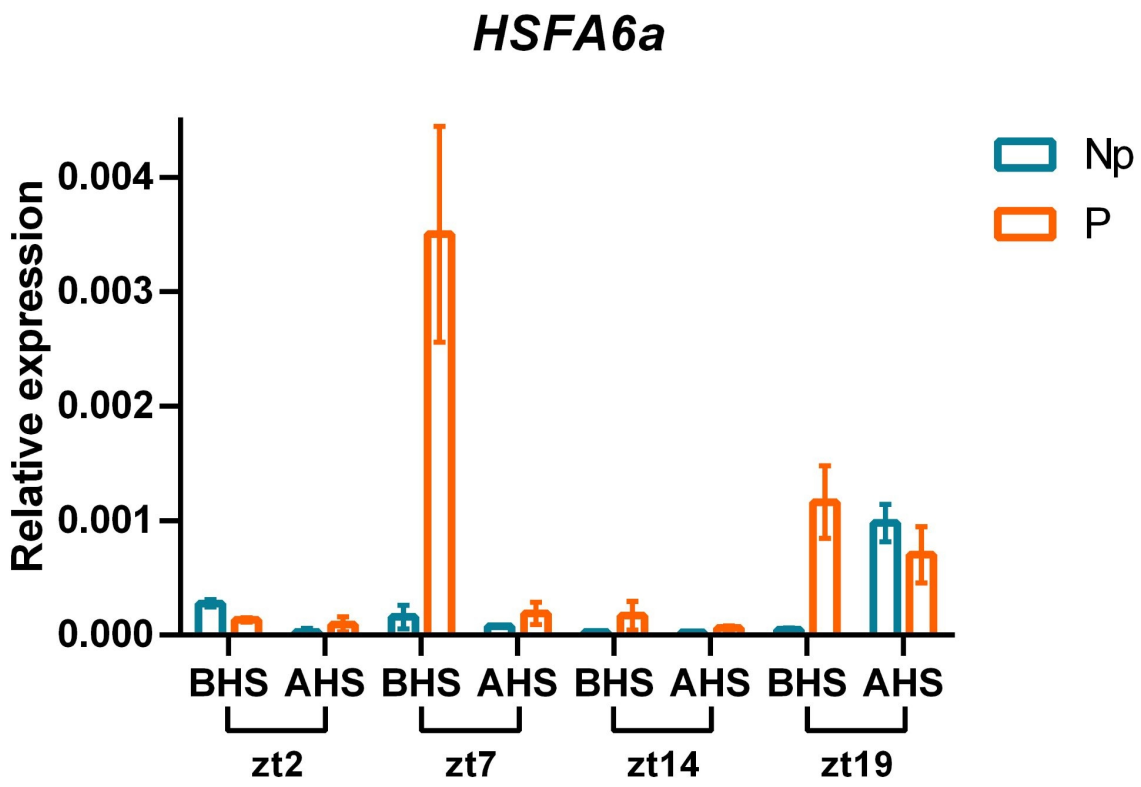

Figure S1. Relative expression of *HSFA6a* in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in circadian conditions. Error bars indicate mean ± sd.

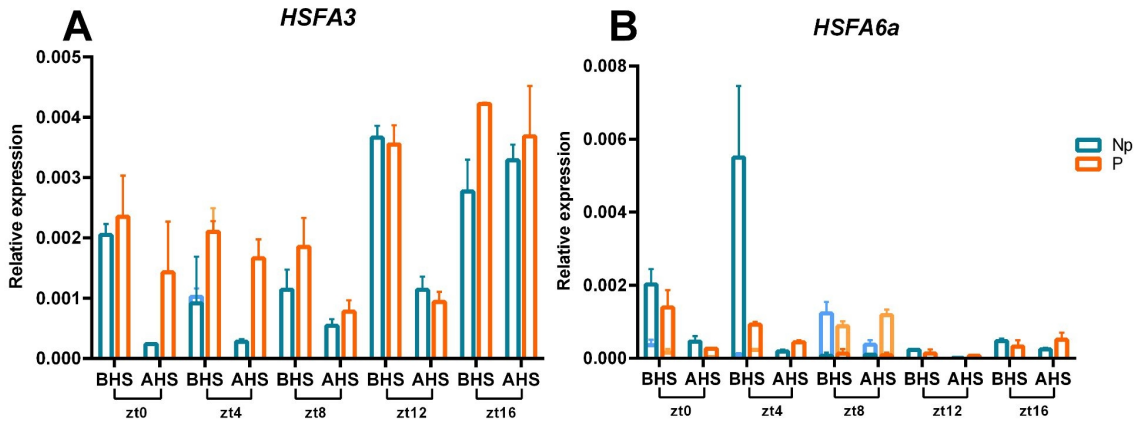

Figure S2. Relative expression of *HSFA3* (A) and *HSFA6a* (B) in relation to the internal control *UBQ10* Before Heat-Shock (BHS) and After Heat-Shock (AHS) at different times of day for Non-primed (Np) and Primed (P) plants in diurnal conditions. Error bars indicate mean ± sd.

Spreadsheets and other supplemental figures available at:

https://drive.google.com/drive/folders/1jUPvXtp8LmcUH4_YSvAQuW2Q9dwdS948?usp=share_link

7 REFERENCES

ALABADÍ, David; OYAMA, Tokitaka; YANOVSKY, Marcelo J.; HARMON, Franklin G.; MÁS, Paloma; KAY, Steve A.. Reciprocal Regulation Between TOC1 and LHY / CCA1 Within the Arabidopsis Circadian Clock. **Science**, [S.L.], v. 293, n. 5531, p. 880-883, 3 ago. 2001. American Association for the Advancement of Science (AAAS). http://dx.doi.org/10.1126/science.1061320.

ASSENG, S.; EWERT, F.; MARTRE, P.; RÖTTER, R. P.; LOBELL, D. B.; CAMMARANO, D.; KIMBALL, B. A.; OTTMAN, M. J.; WALL, G. W.; WHITE, J. W.. Rising temperatures reduce global wheat production. **Nature Climate Change**, [S.L.], v. 5, n. 2, p. 143-147, 22 dez. 2014. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/nclimate2470.

BANIWAL, S.K.; BHARTI, K.; CHAN, K.Y.; FAUTH, M.; GANGULI, A.; KOTAK, S.; MISHRA, S.K.; NOVER, L.; PORT, M.; SCHARF, K.D.; et al (2004) Heat stress response in plants: a complex game with chaperones and more than twenty heat stress transcription factors. **J Biosci** 29: 471–487.

BECKERS, Gerold Jm; CONRATH, Uwe. Priming for stress resistance: from the lab to the field. **Current Opinion In Plant Biology**, [S.L.], v. 10, n. 4, p. 425-431, ago. 2007. Elsevier BV. http://dx.doi.org/10.1016/j.pbi.2007.06.002.

BENDIX, Claire; MARSHALL, Carine M.; HARMON, Frank G.. Circadian Clock Genes Universally Control Key Agricultural Traits. **Molecular Plant**, [S.L.], v. 8, n. 8, p. 1135-1152, ago. 2015. Elsevier BV. http://dx.doi.org/10.1016/j.molp.2015.03.003.

BERNS, Markus C.; NORDSTRÖM, Karl; CREMER, Frédéric; TÓTH, Réka; HARTKE, Martin; SIMON, Samson; KLASEN, Jonas R.; BÜRSTEL, Ingmar; COUPLAND, George. Evening Expression of Arabidopsis GIGANTEA Is Controlled by Combinatorial Interactions among Evolutionarily Conserved Regulatory Motifs. **The Plant Cell**, [S.L.], v. 26, n. 10, p. 3999-4018, out. 2014. Oxford University Press (OUP). http://dx.doi.org/10.1105/tpc.114.129437.

BLAIR, Emily J.; BONNOT, Titouan; HUMMEL, Maureen; HAY, Erika; MARZOLINO, Jill M.; QUIJADA, Ivan A.; NAGEL, Dawn H.. Contribution of time of day and the circadian clock to the heat stress responsive transcriptome in Arabidopsis. **Scientific Reports**, [S.L.], v. 9, n. 1, p. 1-12, 18 mar. 2019. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/s41598-019-41234-w.

CALUWÉ, Joëlle de; XIAO, Qiying; HERMANS, Christian; VERBRUGGEN, Nathalie; LELOUP, Jean-Christophe; GONZE, Didier. A Compact Model for the Complex Plant Circadian Clock. **Frontiers In Plant Science**, [S.L.], v. 7, n. 0, p. 0-0, 5 fev. 2016. Frontiers Media SA. http://dx.doi.org/10.3389/fpls.2016.00074.

CHARNG, Yee-Yung. Arabidopsis Hsa32, a Novel Heat Shock Protein, Is Essential for Acquired Thermotolerance during Long Recovery after Acclimation. **Plant Physiology**, [s.l.], v. 140, n. 4, p.1297-1305, 1 abr. 2006. American Society of Plant Biologists (ASPB). http://dx.doi.org/10.1104/pp.105.074898.

CHARNG, Yee-Yung; LIU, Hsiang-Chin; LIU, Nai-Yu; CHI, Wen-Tzu; WANG, Chun-Neng; CHANG, Shih-Hsun; WANG, Tsu-Tsuen. A Heat-Inducible Transcription Factor, HsfA2, Is Required for Extension of Acquired Thermotolerance in Arabidopsis. **Plant Physiology**, [S.L.], v. 143, n. 1, p. 251-262, 3 nov. 2006. Oxford University Press (OUP). http://dx.doi.org/10.1104/pp.106.091322.

COVINGTON, Michael F; HARMER, Stacey L. The Circadian Clock Regulates Auxin Signaling and Responses in Arabidopsis. **Plos Biology**, [S.L.], v. 5, n. 8, p. 1773-1784, 7 ago. 2007. Public Library of Science (PLoS). http://dx.doi.org/10.1371/journal.pbio.0050222.

DAI, Shunhong; WEI, Xiaoping; PEI, Liping; THOMPSON, Rebecca L.; LIU, Yi; HEARD, Jacqueline E.; RUFF, Thomas G.; BEACHY, Roger N.. BROTHER OF LUX ARRHYTHMO Is a Component of the Arabidopsis Circadian Clock . **The Plant Cell**, [S.L.], v. 23, n. 3, p. 961-972, 1 mar. 2011. Oxford University Press (OUP). http://dx.doi.org/10.1105/tpc.111.084293.

DESAI, Jigar S.; LAWAS, Lovely Mae F.; VALENTE, Ashlee M.; *et al*. Warm nights disrupt transcriptome rhythms in field-grown rice panicles. **Proceedings of the National Academy of Sciences**, v. 118, n. 25, p. e2025899118, 2021. https://doi.org/10.1073/pnas.2025899118

DICKINSON, Patrick J.; KUMAR, Manoj; MARTINHO, Claudia; YOO, Seong Jeon; LAN, Hui; ARTAVANIS, George; CHAROENSAWAN, Varodom; SCHÖTTLER, Mark Aurel; BOCK, Ralph; JAEGER, Katja E.. Chloroplast Signaling Gates Thermotolerance in Arabidopsis. **Cell Reports**, [S.L.], v. 22, n. 7, p. 1657-1665, fev. 2018. Elsevier BV. http://dx.doi.org/10.1016/j.celrep.2018.01.054.

DIXON, Laura E.; KNOX, Kirsten; KOZMA-BOGNAR, Laszlo; SOUTHERN, Megan M.; POKHILKO, Alexandra; MILLAR, Andrew J.. Temporal Repression of Core Circadian Genes Is Mediated through EARLY FLOWERING 3 in Arabidopsis. **Current Biology**, [S.L.], v. 21, n. 2, p. 120-125, jan. 2011. Elsevier BV. http://dx.doi.org/10.1016/j.cub.2010.12.013.

DODD, Antony N.; SALATHIA, Neeraj; HALL, Anthony; KÉVEI, Eva; TÓTH, Réka; NAGY, Ferenc; HIBBERD, Julian M.; MILLAR, Andrew J.; WEBB, Alex A. R.. Plant Circadian Clocks Increase Photosynthesis, Growth, Survival, and Competitive Advantage. **Science**, [S.L.], v. 309, n. 5734, p. 630-633, 22 jul. 2005. American Association for the Advancement of Science (AAAS). http://dx.doi.org/10.1126/science.1115581.

DUNLAP, Jay C. Molecular Bases for Circadian Clocks. **Cell**, [S.L.], v. 96, n. 2, p. 271-290, jan. 1999. Elsevier BV. http://dx.doi.org/10.1016/s0092-8674(00)80566-8.

EDWARDS, Kieron D; LYNN, James R; GYULA, Péter; NAGY, Ferenc; MILLAR, Andrew J. Natural Allelic Variation in the Temperature-Compensation Mechanisms of the Arabidopsis thaliana Circadian ClockSequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession nos. AY685131 and AY685132. **Genetics**, [S.L.], v. 170, n. 1, p. 387-400, 1 maio 2005. Oxford University Press (OUP). http://dx.doi.org/10.1534/genetics.104.035238.

FARRÉ, Eva M.; KAY, Steve A.. PRR7 protein levels are regulated by light and the circadian clock in Arabidopsis. **The Plant Journal**, [S.L.], v. 52, n. 3, p. 548-560, 18 set. 2007. Wiley. http://dx.doi.org/10.1111/j.1365-313x.2007.03258.x.

FARINAS, Benoit; MAS, Paloma. Functional implication of the MYB transcription factor RVE8/LCL5 in the circadian control of histone acetylation. **The Plant Journal**, [S.L.], v. 66, n. 2, p. 318-329, 16 fev. 2011. Wiley. http://dx.doi.org/10.1111/j.1365-313x.2011.04484.x.

FRANCISCO, Marta; RODRÍGUEZ, Víctor M.. Importance of Daily Rhythms on Brassicaceae Phytochemicals. **Agronomy**, [S.L.], v. 11, n. 4, p. 639, 26 mar. 2021. MDPI AG. http://dx.doi.org/10.3390/agronomy11040639.

FOGELMARK, Karl; TROEIN, Carl. Rethinking Transcriptional Activation in the Arabidopsis Circadian Clock. **Plos Computational Biology**, [S.L.], v. 10, n. 7, p. 1-12, 17 jul. 2014. Public Library of Science (PLoS). http://dx.doi.org/10.1371/journal.pcbi.1003705.

FUKUSHIMA, Atsushi; KUSANO, Miyako; NAKAMICHI, Norihito; KOBAYASHI, Makoto; HAYASHI, Naomi; SAKAKIBARA, Hitoshi; MIZUNO, Takeshi; SAITO, Kazuki. Impact of clock-associated Arabidopsis pseudo-response regulators in metabolic coordination. **Proceedings Of The National Academy Of Sciences**, [S.L.], v. 106, n. 17, p. 7251-7256, 9 abr. 2009. Proceedings of the National Academy of Sciences. http://dx.doi.org/10.1073/pnas.0900952106.

GENDRON, J. M.; PRUNEDA-PAZ, J. L.; DOHERTY, C. J.; GROSS, A. M.; KANG, S. E.; KAY, S. A.. Arabidopsis circadian clock protein, TOC1, is a DNA-binding transcription factor. **Proceedings Of The National Academy Of Sciences**, [S.L.], v. 109, n. 8, p. 3167-3172, 6 fev. 2012. Proceedings of the National Academy of Sciences. http://dx.doi.org/10.1073/pnas.1200355109.

GIL, Kyung-Eun; PARK, Chung-Mo. Thermal adaptation and plasticity of the plant circadian clock. **New Phytologist**, [S.L.], v. 221, n. 3, p. 1215-1229, 29 out. 2018. Wiley. http://dx.doi.org/10.1111/nph.15518.

GREENHAM, Kathleen; MCCLUNG, C. Robertson. Integrating circadian dynamics with physiological processes in plants. **Nature Reviews Genetics**, [S.L.], v. 16, n. 10, p. 598-610, 15 set. 2015. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/nrg3976.

GRINEVICH, Dmitry O.; DESAI, Jigar S.; STROUP, Kevin P.; DUAN, Jiaqi; SLABAUGH, Erin; DOHERTY, Colleen J.. Novel transcriptional responses to heat revealed by turning up the heat at night. **Plant Molecular Biology**, [S.L.], v. 101, n. 1-2, p. 1-19, 6 maio 2019. Springer Science and Business Media LLC. http://dx.doi.org/10.1007/s11103-019-00873-3.

GUILIONI, Lydie; WERY, Jacques; TARDIEU, Francois. Heat Stress-induced Abortion of Buds and Flowers in Pea: is sensitivity linked to organ age or to relations between reproductive organs?. **Annals Of Botany**, [S.L.], v. 80, n. 2, p. 159-168, ago. 1997. Oxford University Press (OUP). http://dx.doi.org/10.1006/anbo.1997.0425.

HELFER, Anne; NUSINOW, Dmitri A.; CHOW, Brenda Y.; GEHRKE, Andrew R.; BULYK, Martha L.; KAY, Steve A.. LUX ARRHYTHMO Encodes a Nighttime Repressor of Circadian Gene Expression in the Arabidopsis Core Clock. **Current Biology**, [S.L.], v. 21, n. 2, p. 126-133, jan. 2011. Elsevier BV. http://dx.doi.org/10.1016/j.cub.2010.12.021.

HERRERO, Eva; KOLMOS, Elsebeth; BUJDOSO, Nora; YUAN, Ye; WANG, Mengmeng; BERNS, Markus C.; UHLWORM, Heike; COUPLAND, George; SAINI, Reena; JASKOLSKI, Mariusz. EARLY FLOWERING4 Recruitment of EARLY FLOWERING3 in the Nucleus Sustains the Arabidopsis Circadian Clock. **The Plant Cell**, [S.L.], v. 24, n. 2, p. 428-443, fev. 2012. Oxford University Press (OUP). http://dx.doi.org/10.1105/tpc.111.093807.

HILKER, Monika; SCHWACHTJE, Jens; BAIER, Margarete; BALAZADEH, Salma; BÄURLE, Isabel; GEISELHARDT, Sven; HINCHA, Dirk K.; KUNZE, Reinhard; MUELLER-ROEBER, Bernd; RILLIG, Matthias C.. Priming and memory of stress responses in organisms lacking a nervous system. **Biological Reviews**, [S.L.], v. 91, n. 4, p. 1118-1133, 20 ago. 2015. Wiley. http://dx.doi.org/10.1111/brv.12215.

HOTTA, Carlos Takeshi. From crops to shops: how agriculture can use circadian clocks. **Journal of Experimental Botany**, v. 72, n. 22, p. 7668–7679, 2021. https://doi.org/10.1093/jxb/erab371

HOTTA, Carlos T.; GARDNER, Michael J.; HUBBARD, Katharine E.; BAEK, Seong Jin; DALCHAU, Neil; SUHITA, Dontamala; DODD, Antony N.; WEBB, Alex A. R.. Modulation of environmental responses of plants by circadian clocks. **Plant, Cell & Environment**, [S.L.], v. 30, n. 3, p. 333-349, 29 jan. 2007. Wiley. http://dx.doi.org/10.1111/j.1365-3040.2006.01627.x.

HSU, Polly Yingshan; DEVISETTY, Upendra K; HARMER, Stacey L. Accurate timekeeping is controlled by a cycling activator in Arabidopsis. **Elife**, [S.L.], v. 2, 30 abr. 2013. ELife Sciences Publications, Ltd. http://dx.doi.org/10.7554/elife.00473.

HSU, Polly Yingshan; HARMER, Stacey L.. Wheels within wheels: the plant circadian system. **Trends In Plant Science**, [S.L.], v. 19, n. 4, p. 240-249, abr. 2014. Elsevier BV. http://dx.doi.org/10.1016/j.tplants.2013.11.007.

IKEDA, Miho; MITSUDA, Nobutaka; OHME-TAKAGI, Masaru. Arabidopsis HsfB1 and HsfB2b Act as Repressors of the Expression of Heat-Inducible Hsfs But Positively Regulate the Acquired Thermotolerance . **Plant Physiology**, [S.L.], v. 157, n. 3, p. 1243-1254, 9 set. 2011. Oxford University Press (OUP). http://dx.doi.org/10.1104/pp.111.179036.

JASKIEWICZ, Michal; CONRATH, Uwe; PETERHÄNSEL, Christoph. Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. **Embo Reports**, [S.L.], v. 12, n. 1, p. 50-55, 3 dez. 2010. EMBO. http://dx.doi.org/10.1038/embor.2010.186.

KAMIOKA, Mari; TAKAO, Saori; SUZUKI, Takamasa; TAKI, Kyomi; HIGASHIYAMA, Tetsuya; KINOSHITA, Toshinori; NAKAMICHI, Norihito. Direct Repression of Evening Genes by CIRCADIAN CLOCK-ASSOCIATED1 in the Arabidopsis Circadian Clock. **The Plant Cell**, [S.L.], v. 28, n. 3, p. 696-711, mar. 2016. Oxford University Press (OUP). http://dx.doi.org/10.1105/tpc.15.00737.

KIM, Woe-Yeon; FUJIWARA, Sumire; SUH, Sung-Suk; KIM, Jeongsik; KIM, Yumi; HAN, Linqu; DAVID, Karine; PUTTERILL, Joanna; NAM, Hong Gil; SOMERS, David E.. ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. **Nature**, [S.L.], v. 449, n. 7160, p. 356-360, 19 ago. 2007. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/nature06132.

KOLMOS, Elsebeth et al. Integrating ELF4 into the circadian system through combined structural and functional studies. **Hfsp Journal**, [s.l.], v. 3, n. 5, p.350-366, out. 2009. Informa UK Limited. http://dx.doi.org/10.2976/1.3218766.

KOLMOS, Elsebeth; CHOW, Brenda Y.; PRUNEDA-PAZ, Jose L.; *et al*. HsfB2b-mediated repression of PRR7 directs abiotic stress responses of the circadian clock. **Proceedings of the National Academy of Sciences**, v. 111, n. 45, p. 16172, 2014.

KUMAR, Mukesh; BUSCH, Wolfgang; BIRKE, Hannah; KEMMERLING, Birgit; NÜRNBERGER, Thorsten; SCHÖFFL, Friedrich. Heat Shock Factors HsfB1 and HsfB2b Are Involved in the Regulation of Pdf1.2 Expression and Pathogen Resistance in Arabidopsis. **Molecular Plant**, [S.L.], v. 2, n. 1, p. 152-165, jan. 2009. Elsevier BV. http://dx.doi.org/10.1093/mp/ssn095.

KUMAR, Sanjeev; THAKUR, Prince; KAUSHAL, Neeru; MALIK, Jahid A.; GAUR, P.; NAYYAR, Harsh. Effect of varying high temperatures during reproductive growth on reproductive function, oxidative stress and seed yield in chickpea genotypes differing in heat sensitivity. **Archives Of Agronomy And Soil Science**, [S.L.], v. 59, n. 6, p. 823-843, jun. 2013. Informa UK Limited. http://dx.doi.org/10.1080/03650340.2012.683424.

LÄMKE, Jörn et al. A hit-and-run heat shock factor governs sustained histone methylation and transcriptional stress memory. **The Embo Journal**, [s.l.], v. 35, n. 2, p.162-175, 9 dez. 2015. EMBO. http://dx.doi.org/10.15252/embj.201592593.

LI, Bingjie; GAO, Zhihua; LIU, Xinye; SUN, Daye; TANG, Wenqiang. Transcriptional Profiling Reveals a Time-of-Day-Specific Role of REVEILLE 4/8 in Regulating the First Wave of Heat Shock–Induced Gene Expression in Arabidopsis. **The Plant Cell**, [S.L.], v. 31, n. 10, p. 2353-2369, 29 jul. 2019. Oxford University Press (OUP). http://dx.doi.org/10.1105/tpc.19.00519.

LIN, Meng-Yi; CHAI, Kuo-Hsing; KO, Swee-Suak; KUANG, Lin-Yun; LUR, Huu-Sheng; CHARNG, Yee-Yung. A Positive Feedback Loop between HEAT SHOCK PROTEIN101 and HEAT STRESS-ASSOCIATED 32-KD PROTEIN Modulates Long-Term Acquired Thermotolerance Illustrating Diverse Heat Stress Responses in Rice Varieties. **Plant Physiology**, [S.L.], v. 164, n. 4, p. 2045-2053, 11 fev. 2014. Oxford University Press (OUP). http://dx.doi.org/10.1104/pp.113.229609.

LINDE, Anna-Malin; EKLUND, D. Magnus; KUBOTA, Akane; PEDERSON, Eric R. A.; HOLM, Karl; GYLLENSTRAND, Niclas; NISHIHAMA, Ryuichi; CRONBERG, Nils; MURANAKA, Tomoaki; OYAMA, Tokitaka. Early evolution of the land plant circadian clock. **New Phytologist**, [S.L.], v. 216, n. 2, p. 576-590, 28 fev. 2017. Wiley. http://dx.doi.org/10.1111/nph.14487.

LING, Yu et al. Thermopriming triggers splicing memory in Arabidopsis. **Journal Of Experimental Botany**, [s.l.], v. 69, n. 10, p.2659-2675, 27 fev. 2018. Oxford University Press (OUP). http://dx.doi.org/10.1093/jxb/ery062.

LIU, Tiffany; CARLSSON, Jenny; TAKEUCHI, Tomomi; NEWTON, Linsey; FARRÉ, Eva M.. Direct regulation of abiotic responses by the Arabidopsis circadian clock component PRR7. **The Plant Journal**, [S.L.], v. 76, p. 101-114, ago. 2013. Wiley. http://dx.doi.org/10.1111/tpj.12276.

LU, Sheen X.; KNOWLES, Stephen M.; ANDRONIS, Christos; ONG, May S.; TOBIN, Elaine M.. CIRCADIAN CLOCK ASSOCIATED1 and LATE ELONGATED HYPOCOTYL Function Synergistically in the Circadian Clock of Arabidopsis . **Plant Physiology**, [S.L.], v. 150, n. 2, p. 834-843, 13 fev. 2009. Oxford University Press (OUP). http://dx.doi.org/10.1104/pp.108.133272.

MARTINEZ-MEDINA, Ainhoa; FLORS, Victor; HEIL, Martin; MAUCH-MANI, Brigitte; PIETERSE, Corné M.J; POZO, Maria J.; TON, Jurriaan; VAN DAM, Nicole M.; CONRATH, Uwe. Recognizing Plant Defense Priming. **Trends In Plant Science**, [S.L.], v. 21, n. 10, p. 818-822, out. 2016. Elsevier BV. http://dx.doi.org/10.1016/j.tplants.2016.07.009.

MÁS, Paloma; KIM, Woe-Yeon; SOMERS, David E.; KAY, Steve A.. Targeted degradation of TOC1 by ZTL modulates circadian function in Arabidopsis thaliana. **Nature**, [S.L.], v. 426, n. 6966, p. 567-570, dez. 2003. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/nature02163.

MATSUSHIKA, Akinori; MAKINO, Seiya; KOJIMA, Masaya; MIZUNO, Takeshi. Circadian Waves of Expression of the APRR1/TOC1 Family of Pseudo-Response Regulators in Arabidopsis thaliana: insight into the plant circadian clock. **Plant And Cell Physiology**, [S.L.], v. 41, n. 9, p. 1002-1012, 15 set. 2000. Oxford University Press (OUP). http://dx.doi.org/10.1093/pcp/pcd043.

MCCLUNG, C. R.. Plant Circadian Rhythms. **The Plant Cell Online**, [s.l.], v. 18, n. 4, p.792-803, 10 mar. 2006. American Society of Plant Biologists (ASPB). http://dx.doi.org/10.1105/tpc.106.040980.

MEHRABI, Z.; RAMANKUTTY, N. The cost of heat waves and droughts for global crop production. **bioRxiv**, p. 188151, 1 jan. 2017.

METZGER, J. O.; BORNSCHEUER, U.. Lipids as renewable resources: current state of chemical and biotechnological conversion and diversification. **Applied Microbiology And Biotechnology**, [S.L.], v. 71, n. 1, p. 13-22, jun. 2006. Springer Science and Business Media LLC. http://dx.doi.org/10.1007/s00253-006-0335-4.

MODY, Tejasvinee; BONNOT, Titouan; NAGEL, Dawn H.. Interaction between the Circadian Clock and Regulators of Heat Stress Responses in Plants. **Genes**, [S.L.], v. 11, n. 2, p. 1-13, 1 fev. 2020. MDPI AG. http://dx.doi.org/10.3390/genes11020156.

MOGHADDAM, Mohammad Reza Bolouri; ENDE, Wim van Den. Sweet immunity in the plant circadian regulatory network. **Journal Of Experimental Botany**, [S.L.], v. 64, n. 6, p. 1439-1449, 11 fev. 2013. Oxford University Press (OUP). http://dx.doi.org/10.1093/jxb/ert046.

MOORE, John W.; LOAKE, Gary J.; SPOEL, Steven H.. Transcription Dynamics in Plant Immunity. **The Plant Cell**, [S.L.], v. 23, n. 8, p. 2809-2820, ago. 2011. Oxford University Press (OUP). http://dx.doi.org/10.1105/tpc.111.087346.

NAGEL, Dawn H.; KAY, Steve A.. Complexity in the Wiring and Regulation of Plant Circadian Networks. **Current Biology**, [S.L.], v. 22, n. 16, p. 648-657, ago. 2012. Elsevier BV. http://dx.doi.org/10.1016/j.cub.2012.07.025.

NAKAMICHI, Norihito; KIBA, Takatoshi; HENRIQUES, Rossana; MIZUNO, Takeshi; CHUA, Nam-Hai; SAKAKIBARA, Hitoshi. PSEUDO-RESPONSE REGULATORS 9, 7, and 5 Are Transcriptional Repressors in the Arabidopsis Circadian Clock . **The Plant Cell**, [S.L.], v. 22, n. 3, p. 594-605, 1 mar. 2010. Oxford University Press (OUP). http://dx.doi.org/10.1105/tpc.109.072892.

NAKAMICHI, Norihito; KUSANO, Miyako; FUKUSHIMA, Atsushi; KITA, Masanori; ITO, Shogo; YAMASHINO, Takafumi; SAITO, Kazuki; SAKAKIBARA, Hitoshi; MIZUNO, Takeshi. Transcript Profiling of an Arabidopsis PSEUDO RESPONSE REGULATOR Arrhythmic Triple Mutant Reveals a Role for the Circadian Clock in Cold Stress Response. **Plant And Cell Physiology**, [S.L.], v. 50, n. 3, p. 447-462, 8 jan. 2009. Oxford University Press (OUP). http://dx.doi.org/10.1093/pcp/pcp004.

NISHIZAWA-YOKOI, Ayako; NOSAKA, Ryota; HAYASHI, Hideki; TAINAKA, Hitoshi; MARUTA, Takanori; TAMOI, Masahiro; IKEDA, Miho; OHME-TAKAGI, Masaru; YOSHIMURA, Kazuya; YABUTA, Yukinori. HsfA1d and HsfA1e Involved in the Transcriptional Regulation of HsfA2 Function as Key Regulators for the Hsf Signaling Network in Response to Environmental Stress. **Plant And Cell Physiology**, [S.L.], v. 52, n. 5, p. 933-945, 6 abr. 2011. Oxford University Press (OUP). http://dx.doi.org/10.1093/pcp/pcr045.

NUSINOW, Dmitri A.; HELFER, Anne; HAMILTON, Elizabeth E.; KING, Jasmine J.; IMAIZUMI, Takato; SCHULTZ, Thomas F.; FARRÉ, Eva M.; KAY, Steve A.. The ELF4–ELF3–LUX complex links the circadian clock to diurnal control of hypocotyl growth. **Nature**, [S.L.], v. 475, n. 7356, p. 398-402, jul. 2011. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/nature10182.

PARANJPE, Dhanashree A.; SHARMA, Vijay. Evolution of temporal order in living organisms. **Journal Of Circadian Rhythms,** [s.l.], v. 3, n. 1, p.1-13, 2005. Ubiquity Press, Ltd.. http://dx.doi.org/10.1186/1740-3391-3-7.

POPELKA, J Carlos; TERRYN, Nancy; HIGGINS, T.J.V. Gene technology for grain legumes: can it contribute to the food challenge in developing countries?. **Plant Science**, [S.L.], v. 167, n. 2, p. 195-206, ago. 2004. Elsevier BV. http://dx.doi.org/10.1016/j.plantsci.2004.03.027.

PORTER, John R; A SEMENOV, Mikhail. Crop responses to climatic variation. **Philosophical Transactions Of The Royal Society B**: Biological Sciences, [S.L.], v. 360, n. 1463, p. 2021-2035, 24 out. 2005. The Royal Society. http://dx.doi.org/10.1098/rstb.2005.1752.

PRASAD, P. V. Vara; BOOTE, Kenneth J.; ALLEN, L. Hartwell; THOMAS, Jean M. G.. Effects of elevated temperature and carbon dioxide on seed-set and yield of kidney bean (Phaseolus vulgaris L.). **Global Change Biology**, [S.L.], v. 8, n. 8, p. 710-721, 11 jul. 2002. Wiley. http://dx.doi.org/10.1046/j.1365-2486.2002.00508.x.

PRASAD, P. V. Vara; BOOTE, Kenneth J.; ALLEN, L. Hartwell; THOMAS, Jean M. G.. Super-optimal temperatures are detrimental to peanut (Arachis hypogaea L.) reproductive processes and yield at both ambient and elevated carbon dioxide. **Global Change Biology**, [S.L.], v. 9, n. 12, p. 1775-1787, 20 out. 2003. Wiley. http://dx.doi.org/10.1046/j.1365-2486.2003.00708.x.

PRASAD, P. V. V.; PISIPATI, S. R.; MOMčILOVIć, I.; RISTIC, Z.. Independent and Combined Effects of High Temperature and Drought Stress During Grain Filling on Plant Yield and Chloroplast EF-Tu Expression in Spring Wheat. **Journal Of Agronomy And Crop Science**, [S.L.], v. 197, n. 6, p. 430-441, 18 jul. 2011. Wiley. http://dx.doi.org/10.1111/j.1439-037x.2011.00477.x.

PRUNEDA-PAZ, J. L.; BRETON, G.; PARA, A.; KAY, S. A.. A Functional Genomics Approach Reveals CHE as a Component of the Arabidopsis Circadian Clock. **Science**, [S.L.], v. 323, n. 5920, p. 1481-1485, 13 mar. 2009. American Association for the Advancement of Science (AAAS). http://dx.doi.org/10.1126/science.1167206.

RAWAT, Reetika; TAKAHASHI, Nozomu; HSU, Polly Yingshan; JONES, Matthew A.; SCHWARTZ, Jacob; SALEMI, Michelle R.; PHINNEY, Brett S.; HARMER, Stacey L.. REVEILLE8 and PSEUDO-REPONSE REGULATOR5 Form a Negative Feedback Loop within the Arabidopsis Circadian Clock. **Plos Genetics**, [S.L.], v. 7, n. 3, 31 mar. 2011. Public Library of Science (PLoS). http://dx.doi.org/10.1371/journal.pgen.1001350.

RICHTER, Klaus; HASLBECK, Martin; BUCHNER, Johannes. The Heat Shock Response: life on the verge of death. **Molecular Cell**, [S.L.], v. 40, n. 2, p. 253-266, out. 2010. Elsevier BV. http://dx.doi.org/10.1016/j.molcel.2010.10.006.

RONDANINI, D.; SAVIN, R.; HALL, A.J.. Dynamics of fruit growth and oil quality of sunflower (Helianthus annuus L.) exposed to brief intervals of high temperature during grain filling. **Field Crops Research**, [S.L.], v. 83, n. 1, p. 79-90, jun. 2003. Elsevier BV. http://dx.doi.org/10.1016/s0378-4290(03)00064-9.

ROSBASH, Michael. The Implications of Multiple Circadian Clock Origins. Plos Biology, [s.l.], v. 7, n. 3, p.1-5, 17 mar. 2009. **Public Library of Science (PLoS)**. http://dx.doi.org/10.1371/journal.pbio.1000062.

RUGNONE, M. L.; SOVERNA, A. Faigon; SANCHEZ, S. E.; SCHLAEN, R. G.; HERNANDO, C. E.; SEYMOUR, D. K.; MANCINI, E.; CHERNOMORETZ, A.; WEIGEL, D.; MAS, P.. LNK genes integrate light and clock signaling networks at the core of the Arabidopsis oscillator. **Proceedings Of The National Academy Of Sciences**, [S.L.], v. 110, n. 29, p. 12120-12125, 1 jul. 2013. Proceedings of the National Academy of Sciences. http://dx.doi.org/10.1073/pnas.1302170110.

SALOMÉ, Patrice A.; WEIGEL, Detlef; MCCLUNG, C. Robertson. The Role of the Arabidopsis Morning Loop Components CCA1, LHY, PRR7, and PRR9 in Temperature Compensation. **The Plant Cell**, [S.L.], v. 22, n. 11, p. 3650-3661, 1 nov. 2010. Oxford University Press (OUP). http://dx.doi.org/10.1105/tpc.110.079087.

SEDAGHATMEHR, Mastoureh; MUELLER-ROEBER, Bernd; BALAZADEH, Salma. The plastid metalloprotease FtsH6 and small heat shock protein HSP21 jointly regulate thermomemory in Arabidopsis. **Nature Communications**, [s.l.], v. 7, 26 ago. 2016. Springer Nature. http://dx.doi.org/10.1038/ncomms12439.

SCHULTE, L.R.; BALLARD, T.; SAMARAKOON, T.; YAO, L.; VADLANI, P.; STAGGENBORG, S.; REZAC, M.. Increased growing temperature reduces content of polyunsaturated fatty acids in four oilseed crops. **Industrial Crops And Products**, [S.L.], v. 51, p. 212-219, nov. 2013. Elsevier BV. http://dx.doi.org/10.1016/j.indcrop.2013.08.075.

SIMON, Noriane M. L.; GRAHAM, Calum A.; COMBEN, Nicholas E.; HETHERINGTON, Alistair M.; DODD, Antony N.. The Circadian Clock Influences the Long-Term Water Use Efficiency of Arabidopsis. **Plant Physiology**, [S.L.], v. 183, n. 1, p. 317-330, 16 mar. 2020. Oxford University Press (OUP). http://dx.doi.org/10.1104/pp.20.00030.

SITA, Kumari; SEHGAL, Akanksha; HANUMANTHARAO, Bindumadhava; NAIR, Ramakrishnan M.; PRASAD, P. V. Vara; KUMAR, Shiv; GAUR, Pooran M.; FAROOQ, Muhammad; SIDDIQUE, Kadambot H. M.; VARSHNEY, Rajeev K.. Food Legumes and Rising Temperatures: effects, adaptive functional mechanisms specific to reproductive growth stage and strategies to improve heat tolerance. **Frontiers In Plant Science**, [S.L.], v. 8, p. 1-30, 4 out. 2017. Frontiers Media SA. http://dx.doi.org/10.3389/fpls.2017.01658.

STAIGER, Dorothee; SHIN, Jieun; JOHANSSON, Mikael; DAVIS, Seth J. The circadian clock goes genomic. **Genome Biology**, [S.L.], v. 14, n. 6, p. 1-8, jun. 2013. Springer Science and Business Media LLC. http://dx.doi.org/10.1186/gb-2013-14-6-208.

STEED, Gareth; RAMIREZ, Dora Cano; HANNAH, Matthew A.; WEBB, Alex A. R.. Chronoculture, harnessing the circadian clock to improve crop yield and sustainability. **Science**, [S.L.], v. 372, n. 6541, abr. 2021. American Association for the Advancement of Science (AAAS). http://dx.doi.org/10.1126/science.abc9141.

STIEF, Anna; BRZEZINKA, Krzysztof; LÄMKE, Jörn; BÄURLE, Isabel. Epigenetic responses to heat stress at different time scales and the involvement of small RNAs. **Plant Signaling & Behavior**, [S.L.], v. 9, n. 10, 3 out. 2014. Informa UK Limited. http://dx.doi.org/10.4161/15592316.2014.970430.

SOMERS, David e; SCHULTZ, Thomas F; MILNAMOW, Maureen; A KAY, Steve. ZEITLUPE Encodes a Novel Clock-Associated PAS Protein from Arabidopsis. **Cell**, [S.L.], v. 101, n. 3, p. 319-329, abr. 2000. Elsevier BV. http://dx.doi.org/10.1016/s0092-8674(00)80841-7.

SWINDELL, W R; HUEBNER, M; WEBER, A P. Plastic and adaptive gene expression patterns associated with temperature stress in Arabidopsis thaliana. **Heredity**, [S.L.], v. 99, n. 2, p. 143-150, 2 maio 2007. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/sj.hdy.6800975.

TANG, Ting; YU, Anmin; LI, Ping; YANG, Hong; LIU, Gaojing; LIU, Li. Sequence analysis of the Hsp70 family in moss and evaluation of their functions in abiotic stress responses. **Scientific Reports**, [S.L.], v. 6, n. 1, 20 set. 2016. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/srep33650.

WEBB, Alex A. R.; SEKI, Motohide; SATAKE, Akiko; CALDANA, Camila. Continuous dynamic adjustment of the plant circadian oscillator. **Nature Communications**, [S.L.], v. 10, n. 1, p. 1-9, 1 fev. 2019. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/s41467-019-08398-5.

WENG, Minjie; YANG, Yue; FENG, Haiyang; PAN, Zongde; SHEN, Wen-Hui; ZHU, Yan; DONG, Aiwu. Histone chaperone ASF1 is involved in gene transcription activation in response to heat stress in Arabidopsis thaliana. **Plant, Cell & Environment**, [S.L.], v. 37, n. 9, p. 2128-2138, 19 mar. 2014. Wiley. http://dx.doi.org/10.1111/pce.12299.

WU, Jia; SNYDER, Michael. RNA polymerase II stalling: loading at the start prepares genes for a sprint. **Genome Biology**, [S.L.], v. 9, n. 5, p. 220, 2008. Springer Science and Business Media LLC. http://dx.doi.org/10.1186/gb-2008-9-5-220.

WU, Jing-Fen; WANG, Ying; WU, Shu-Hsing. Two New Clock Proteins, LWD1 and LWD2, Regulate Arabidopsis Photoperiodic Flowering . **Plant Physiology**, [S.L.], v. 148, n. 2, p. 948-959, 1 ago. 2008. Oxford University Press (OUP). http://dx.doi.org/10.1104/pp.108.124917.

WU, Jing-Fen; TSAI, Huang-Lung; JOANITO, Ignasius; WU, Yi-Chen; CHANG, Chin-Wen; LI, Yi-Hang; WANG, Ying; HONG, Jong Chan; CHU, Jhih-Wei; HSU, Chao-Ping. LWD–TCP complex activates the morning gene CCA1 in Arabidopsis. **Nature Communications**, [S.L.], v. 7, n. 1, p. 1-10, 13 out. 2016. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/ncomms13181.

WU, Ting-Ying; JUAN, Yu-Ting; HSU, Yang-Hsin; WU, Sze-Hsien; LIAO, Hsiu-Ting; FUNG, Raymond W.M.; CHARNG, Yee-Yung. Interplay between Heat Shock Proteins HSP101 and HSA32 Prolongs Heat Acclimation Memory Posttranscriptionally in Arabidopsis . **Plant Physiology**, [S.L.], v. 161, n. 4, p. 2075-2084, 25 fev. 2013. Oxford University Press (OUP). http://dx.doi.org/10.1104/pp.112.212589.

XIE, Qiguang *et al*. LNK1 and LNK2 Are Transcriptional Coactivators in the Arabidopsis Circadian Oscillator. **The Plant Cell**, [S.L.], v. 26, n. 7, p. 2843-2857, jul. 2014. Oxford University Press (OUP). http://dx.doi.org/10.1105/tpc.114.126573.

YAKIR, Esther; HILMAN, Dror; KRON, Ido; HASSIDIM, Miriam; MELAMED-BOOK, Naomi; GREEN, Rachel M.. Posttranslational Regulation of CIRCADIAN CLOCK ASSOCIATED1 in the Circadian Oscillator of Arabidopsis . **Plant Physiology**, [S.L.], v. 150, n. 2, p. 844-857, 1 abr. 2009. Oxford University Press (OUP). http://dx.doi.org/10.1104/pp.109.137414.

ZHANG, Jing-Xia; WANG, Cui; YANG, Chuan-Yan; WANG, Jia-Ying; CHEN, Lei; BAO, Xiao-Ming; ZHAO, Yan-Xiu; ZHANG, Hui; LIU, Jian. The role of arabidopsis AtFes1A in cytosolic Hsp70 stability and abiotic stress tolerance. **The Plant Journal**, [S.L.], v. 62, n. 4, p. 539-548, 18 fev. 2010. Wiley. http://dx.doi.org/10.1111/j.1365-313x.2010.04173.x.

ZHU, Jia-Ying; OH, Eunkyoo; WANG, Tina; WANG, Zhi-Yong. TOC1–PIF4 interaction mediates the circadian gating of thermoresponsive growth in Arabidopsis. **Nature Communications**, [S.L.], v. 7, n. 1, p. 1-10, dez. 2016. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/ncomms13692.