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Final report

Heat stress-induced changes in defense mechanisms of the model crop barley during fungal infections

Scientific report period: 01/12/2019 – 28/02/2025

Overview of project objectives

The general goal of the project was to gain a deeper insight on how heat stress influences defense responses of the model plant barley to different fungal pathogens.

Scientific background

Environmental factors such as temperature have a significant influence on plant pathogen interactions. Studies have demonstrated the differential effects of heat stress in distinct plant-pathogen interactions. However, these data on effects of long and short term heat stresses are difficult to interpret because each observation was made in a different type of plant pathogen interaction, making direct comparisons difficult. Therefore, a primary aim of the present project is to compare effects of heat stresses with differing parameters (e.g. temperature, duration) on the same plant-pathogen interaction. Moreover we hypothesized that high temperature pretreatments of barley affects plant defense against pathogens with different lifestyles (biotrophic, hemibiotrophic, necrotrophic) differently. Therefore we inoculated barley plants with the biotrophic barley powdery mildew (*Blumeria hordei*; Bh), the hemibiotrophic *Bipolaris sorokiniana* (Bs) and the necrotrophic *Pyrenophora teres* f. *teres* (Ptt).

Results

1. The influence of heat stress on barley – Bh interactions

For the experiments, ten different barley (*Hordeum vulgare*) cultivars and breeding lines were obtained which are as follows: cv. Ingrid, cv. GK-Stramm, cv. Antonella, cv. KWS-MERIDIAN, cv. Hanzi, cv. Mv Initium, MvHV 05-17, MvHV 07-17, MvHV 14-18, MvHV 118-17. In order to establish the plant response of the barley cultivars and breeding lines to Bh, we exposed the plants to artificial inoculation. Powdery mildew (*B. hordei* race A6; Bh A6) was maintained separately in infected *H. vulgare* cv. Ingrid plants in a versatile environmental test chamber at 20 °C and 16 h light/8 h dark photoperiod. Conidia from heavily infected barley were dusted equally on one-week-old barley seedlings of the cultivars and breeding lines listed above. Inoculation of barley primary leaves was performed in a versatile environmental chamber at 20 ºC. The formation of Bh A6 symptoms in leaves was evaluated visually 7 days after inoculation (DAI). Determination of disease severity was calculated on the percentage of area covered by powdery mildew (PM) symptoms per leaf. Based on these data the barley cultivars were classified to eight categories i.e., between 0 (0% no symptoms) and 7 (100% PM-covered). Our results showed that no visible Bh A6-elicited PM symptoms were detectable on GK-STRAMM, Antonella, MVHV 07-17, KWS-MERIDIAN and MvHV 05-17, however, KWS-MERIDIAN and MvHV 05-17 showed hypersensitive response (HR = localized necrotic lesions) during infection. MvHV 14-18, MV INITIUM, MVHV 118-17, Ingrid and Hanzi cultivars showed different levels of susceptibility to Bh A6. In order to detect how heat stress influences powdery mildew infection we selected one Bh A6 resistant (MvHV07-17) and one susceptible (MvHV118-17) line. We chose the susceptible line MvHV118-17 for our studies because powdery mildew coverage of leaves in this genotype was about 50%. This is important because if heat treatment increases susceptibility, this may be reflected in enhanced symptoms. In the fully susceptible varieties (cv. Ingrid, Hanzi), where leaf powdery mildew coverage is close to 100%, we could not detect the effect of heat treatment on powdery mildew symptoms. The selected Bh A6-resistant (MVHV 07-17) and susceptible (MVHV 118-17) barley lines were artificially exposed to heat stress prior to Bh A6 inoculation in versatile environmental chambers at 20 ºC (control) 28 and 35 ºC (heat stress). The duration of heat stress ranged from 30 seconds to 5 days (30 seconds, 1 minute, 1 hour, 2 hours, 6 hours, 24 hours, 48 hours and 120 hours). Powdery mildew

inoculation of heat stressed plants was performed as described above, immediately after heat treatment. Our results showed that the resistant barley line MVHV 07-17 retained its resistance to the pathogen even at high temperatures based on the extent of powdery mildew symptoms at 7 DAI. In the MVHV 118-17 susceptible line, there was a significant increase in the proportion of powdery mildew-covered area in plants exposed to 35 ° C for 24, 48, 120 hours and 28 ° C for 48 hours. In addition to the symptomatic assessment, the quantification of the pathogen was performed by quantitative real-time polymerase chain reaction (qPCR). For the qPCR method, samples were taken from plants after symptomatic evaluation and stored in liquid nitrogen. Based on literature data, two primer pairs (*Blumeria hordei glyceraldehyde-3-phosphate dehydrogenase*; Bh GAPDH and *B. hordei α-tubulin*; Bh TUBA) were selected and synthetized for the detection of powdery mildew. For barley reference genes, we also selected two primer pairs (*Hordeum vulgare* ubiquitin; Hv UBI and *H. vulgare* glyceraldehyde-3-phosphate dehydrogenase, Hv GAPDH). The different primers were tested how they react to heat shock and based on our experiments we selected Bh GAPDH for powdery mildew detection and Hv UBI as a barley reference gene which are optimal for our experiments. The qPCR results were almost identical to the results of the symptom assessment; however, we found minor differences. The resistant barley line MVHV 07-17 retained its resistance to Bh A6 even at high temperatures however, at a temperature of 35 ºC for up to 120 hours the qPCR showed a substantial, though comparatively negligible, rise in powdery mildew biomass as compared to plants held at 20 ºC. The Bh A6 biomass significantly increased in MVHV 118-17 susceptible line exposed to 35 ºC from 24 to 120 hours, however, at 28 ºC a *Bgh* A6 biomass increase was observed only from 48 to 120 hours. Interestingly, a short-term heat shock (30 seconds at 28 and 35 ºC) significantly reduced the Bh A6 biomass in MVHV 118-17. **In overall, based on our data the Bh A6 resistant MVHV 07-17 line retained its resistance even at high temperatures and the Bh A6 susceptible line MVHV 118-17 became even more susceptible following long term (24 – 120 h) heat stress.** These results are supported by our data from both the assessment of symptoms and fungal biomass. Furthermore, prolonged heat stress (24, 48 and 120 hours at 35 °C) significantly repressed the expression of several plant defense-related genes (*BAX inhibitor-1*, *Pathogenesis related-1b* and *Respiratory burst oxidase homologue F2*) in both resistant and susceptible barley lines. Remarkably, heat-suppressed defense gene expression returned to normal levels only in MvHV07-17, a possible reason why this barley line retains Bh resistance even at high temperatures. These results are published in the journal Genes (Schwarczinger et al., 2021).

In order to clarify if heat stable powdery mildew resistance in MvHV07-17 is also effective against a short-term heat shock at a higher temperature (49 C°) we exposed plants to either long-term heat stress (35 ºC to 120 h) or short-term heat-shock (49 ºC to 20 s) immediately before Bh A6 inoculation. Seven days after inoculation we evaluated powdery mildew symptoms in the heat pretreated and control barley plants (held at 20 ºC), respectively. Similar to our previous results MvHV07-17 plants held at 20 ºC or exposed to long-term heat stress (35 ºC to 120 h) did not show any visible powdery mildew symptoms 7 days after inoculation. However, as a result of short-term heat stress at a higher temperature (49 ºC for 20 s) local necrotic symptoms (hypersensitive response; HR) and powdery mildew symptoms were observed on MvHV07-17. On the other hand, enhanced powdery mildew symptoms in the susceptible barley line (MvHV118-17) following exposure to 35 ºC during 120 hours or 49 ºC for 20 s was detectable as compared to control plants held at 20 ºC. Taken together **long-term heat stress did not induce the appearance of powdery mildew symptoms in the resistant line MvHV07-17, however, a short-term heat stress at 49 C° (HS) suppressed powdery mildew resistance**, leading to the appearance of visible HR symptoms and powdery mildew colonies. In contrast, both short- and long-term heat stress enhanced powdery mildew coverage in the susceptible line (MvHV118-17).

In order to gain a deeper insight into how long-term and short-term heat stress affect plant defense we performed microscopic examinations 2 and 7 days after Bh inoculation in the resistant line and 2 DAI in the susceptible barley. The analysis of samples collected two DAI from plants grown at 20 ºC revealed that only 1-2 % of all conidia produced elongated secondary hyphae (ESH) (associated with successful fungal penetration and development of mature haustoria) in the resistant line MvHV07-17. However, 20 % of all conidia formed ESH in the susceptible line. Interestingly, the frequency of interaction sites where fungal penetration was prevented by papillae (PAP) was around 70 % of all attacked cells in both lines indicating a significant level of basal resistance in the susceptible line as well. A substantial difference

was observed between resistant and susceptible lines in the frequency of whole-cell DAB staining (indicating ongoing HR) in the epidermal cells. A larger proportion of attacked cells showed whole-cell DAB staining in the resistant plants (about 20%) than in the susceptible plants (about 10%). Both HS and long-term heat pretreatments led to a 2-3-fold increase in the rate of successful powdery mildew penetration attempts in susceptible plants. Furthermore, the proportion of effective papilla responses fell by around a third in the susceptible plants after either HS or long-term heat treatments, and at the same time, the number of DAB-stained cells decreased slightly but not significantly. The ESH production rate increased strongly in resistant barley line MvHV07-17 from 1% to 15% in response to long-term heat treatments and 40% in response to HS. The rate of papilla-based penetration resistance decreased significantly in the resistant plant (MvHV07-17) in response to both HS and long-term heat stress, however, the effect of HS was much stronger. Seven DAI the barley - Bh interaction was assessed microscopically only in the resistant barley line (MvHV07-17) because at that time point the primary leaves of susceptible plants were almost completely covered with powdery mildew especially in heat treated leaves, which made microscopic observations difficult. The results of heat untreated resistant MvHV07-17 plants indicated that very low numbers of conidia developed ESH, which is similar to the results obtained at two DAI. Most of these cases were accompanied by the induction of multi-cell death as indicated by an accumulation of autofluorescent material in adjacent mesophyll cells. Both HS and long-term heat pretreatments increased the production of ESH, however, the increase was more pronounced and statistically significant only as a result of HS in the resistant barley. Strikingly, the average lesion diameter was significantly larger in MvHV07-17 plants exposed to HS, as compared to plants exposed to long-term heat stress and in the control plants kept at 20 °C. Importantly, the sporulation of powdery mildew with or without cell death was observed only on those MvHV07-17 plants that were subjected to **HS (49 ºC for 30 sec)**. Therefore, it seems that **HS allows the fungus to complete the entire asexual infection cycle even in the Bh resistant barley line MvHV07-17.** We can conclude that HS mainly inhibits papilla formation while HR mediated resistance remains intact. Furthermore, we found that the accumulation of H_2O_2 in both resistant and susceptible barley was correlated with susceptibility induced by HS and long-term heat-stress. This study may contribute to a better understanding of plant defense responses to Bh in barley exposed to heat. These results are published in the journal Phytopathology (Fodor et al., 2024).

Glutathione peroxidases (GPXs) are catalyzing the reduction of H_2O_2 to water using reduced glutathione (GSH) as a reducing agent. Moreover glutathione is also known as a central regulator of plant signaling during plant–pathogen interactions. Therefore we determined reduced (GSH) and oxidized (GSSG) glutathione concentrations in infected powdery mildew resistant (MVHV07-17) and susceptible (MvHV118-17) barley previously exposed to long-term heat stress (35 °C for 5 days). Four treatments were tested on the plants (control, BH inoculated, heat treated and combined BH inoculated and heat treated). Glutathione concentrations were monitored with HPLC-MS at 0, 6, 9, 12, 16, 24, 72 and 168 hours after Bh inoculation (HAI). Our results showed that **neither long-term heat stress nor Bh inoculation has any effect on reduced glutathione contents**, only in 168 HAI susceptible plants did reduced glutathione levels increase non-significantly in response to infection or infection and heat treatment, as compared to untreated plants. However heat stress reduced GSSG levels in both lines at early time points but the effect of the reduction of GSSG due to heat stress was more significant and lasted longer in the susceptible line (Künstler et al., 2024). Furthermore, two review articles have been published on the subject of sulfur-containing compounds in plant defense, with a particular focus on glutathione as a master regulator in plant signaling. Glutathione plays a significant role in the resistance to both biotic and abiotic stressors, including pathogen infection and heat stress. (Künstler et al., 2020 a, b).

Between 2021 and 2024, we also carried out field trials with the above mentioned barley lines in Martonvásár. During the growing season of winter barley, from October to June, we monitored the level of plant infection with the three most important pathogens in Hungary. To determine the degree of Bh, *Pyrenophora teres* f. *teres* (Ptt) and leaf rust (*Puccinia hordei*) infection in barley, the Saari-Prescott scale the Tekauz scale and rust coverage on leaf surface was used, respectively. In addition, barley yields per hectare and the main weather characteristics of the growing season in 2021, 2022 and 2024 were recorded. According to our results, in 2022, when there were significant heat waves during the growing season, the **MvHV07-17 barley line maintained its resistance to Bh under field conditions and**

yielded approximately 20% more than the susceptible line MvHV118-17. Complete data from the field trails is still being analyzed.

Our next question was how heat stress affects different well characterized powdery mildew resistance genes in barley? *Hordeum vulgare* cv. Ingrid Mlo, Mla12, Mlg, mlo5 near isogenic lines were used to monitor Bh infection in response to heat stress. *Mla12* and *Mlg* resistance genes confer race-specific resistance to barley against Bh race A6. Ingrid barley lines containing *Mla12* show visible hypersensitive response (HR) when infected with A6 race of Bh, however, the resistance gene *Mlg* determines a single cell HR (no visible symptoms). Barley lines containing the recessive *mlo5* gene confer non-race-specific resistance showing strong papillae formation during infection, which inhibits Bh spread in infected plants without HR (no visible symptoms). Ingrid Mlo lines are susceptible to Bh. This experimental system allows us to investigate how different Bh resistance genes respond to heat stress.
Ingrid near isogenic lines (Mla 12, Mlg, mlo 5 and Mlo) were exposed to heat stresses (35 °C) for different time periods (30 sec to 120 hours) before Bh inoculation similar to our previous experiments (see above). Disease symptoms were visually estimated 7 days after inoculation. Because of heat stress, Bh symptoms appeared on resistant plants. Based on Bh coverage, the strongest symptoms appeared on Mla plants while the mildest symptoms were seen on plants carrying the Mlg R gene. Interestingly, as a result of short-term (30 seconds) heat stress Bh symptoms have appeared on plants. Along with the increase in the duration of heat stress, we also experienced an increase of Bh coverage in all three resistant lines. The Bh susceptible Mlo line became even more susceptible to long term (48 and 120 hours) heat stress. In addition to symptomatic assessment, quantification of Bh biomass was also performed by RT-qPCR, which supported our symptom evaluation data. Overall, we found that **Bh A6 resistant lines (cv. Ingrid Mla 12, Mlg, mlo 5) and the susceptible line cv. Ingrid Mlo become more susceptible to Bh after 35 °C heat stress**. The duration of heat stress increased Bh biomass in all examined lines. We also assessed expression of plant defense and stress genes in the plant pathogen interactions described above. For this purpose we chose one heat treatment (35 °C for 48 hours) because this treatment reduced the resistance of all 4 lines. Samples for gene expression analysis were collected 0, 2, 6, 24 and 48 hours after heat stress and inoculation. As controls, defense gene expression was also assayed in plants that received only heat treatment but no Bh and inoculated plants that were held at an optimal temperature (20 ºC). In overall heat stress significantly reduced and/or delayed the expression of the examined defense and stress related genes (*HvBI-1, HvPR1-b* and *HvRBOHF2*) in both susceptible and resistant genotypes. Furthermore we tested expression of a barley heat shock protein gene (*HvHSP90-1*) in heat stressed and inoculated nearly isogenic lines. Heat shock proteins are molecular chaperones that stabilize plant proteins during heat stress. It has been shown that *HSP 90-1* is not only activated during heat stress but also plays an important role in barley Bh resistance mediated by *Mla*. Our results show that increased expression is detectable in all three resistant lines (Mla 12, Mlg, mlo 5) 2 hours after inoculation (HAI) in plants held at 20 ºC. The increased expression in resistant lines might be necessary for Bh resistance. 48 hours heat stress at 35 ºC significantly induced the expression of Hsp 90-1 immediately after heat stress and before inoculation (0 h) but the expression crashed after 2 hours. The lack of 2 HAI induction of Hsp 90-1 is also likely to be associated with the impaired Bh resistance in resistant lines. These results are published in the journal Plants (Kolozsváriné Nagy et al., 2022). These results were disseminated also in Hungarian in the journal Növényvédelem (Künstler et al., 2022).

The findings of the study on the subject of heat stress and powdery mildew infection in barley have been disseminated in a book chapter, with the objective of making them accessible to a broader audience (Künstler et al., 2023a).

2. The influence of heat stress on barley – Bs interactions

To determine how HS influences the defense responses of barley cv. Ingrid susceptible to infection by the hemibiotrophic *Bipolaris sorokiniana* (BS) we pretreated the plants at 49 °C for 20 seconds, 2 hours before inoculation. The BS H-188 isolate cultured in potato-dextrose agar was used to inoculate barley. Conidial suspensions containing approximately 60,000 spores per ml water were used for artificial inoculation of one-week-old barley seedlings. Inoculated plants were held in a dark moist chamber for one day, after that they were transferred to a versatile environmental chamber at 20 °C and

16 h light/8 h dark photoperiod. The formation of spot blotch symptoms in barley leaves was evaluated visually seven days after inoculation. Determination of disease severity was calculated on the percentage of area covered by necrotic lesions per leaf at 7 DAI. BS biomass was assessed by quantitative PCR (qPCR) by quantifying the relative abundance of BS *glyceraldehyde 3-phosphate dehydrogenase* (*BsGAPDH*) gene. Reactive oxygen species (ROS) levels was determined by histochemical staining, while gene expression was assayed by RT-qPCR. Our results showed that HS suppressed the defense responses of barley to BS, resulting in more severe necrotic symptoms and increased fungal biomass, as compared to untreated plants. HS-induced increased susceptibility was accompanied by significant increases in ROS (superoxide, H_2O_2) production. Transient expression of plant defense-related antioxidant genes and a barley programmed cell death inhibitor gene (*HvBI-1*) were induced in response to HS. However, HS followed by BS infection caused further transient increases in expression of barley superoxide dismutase and Bax inhibitor genes (*HvSOD, HvBI-1*) correlated with enhanced susceptibility. Expression of the *HvPR-1b* gene encoding pathogenesis-related protein-1b increased several fold 24 h after BS infection, however, heat shock further increased transcript levels along with enhanced susceptibility. Collectively our results show that, **HS induced enhanced susceptibility of barley to BS, associated with elevated ROS production and enhanced expression of plant defense-related genes** encoding antioxidants, a cell death inhibitor, and PR-1b. It seems that **heat shock pretreatments in barley cv. Ingrid have a similar effect on infection by the hemibiotrophic BS and the biotrophic *Blumeria hordei*,** which cause barley powdery mildew, **probably because both pathogens behave as biotrophs in initial stages of pathogenesis when the effect of HS is still significant** in the treated barley plants. These results are now published in the journal Plant Biology (Künstler et al., 2023b).

3. The influence of heat stress on barley – Ptt interactions

We hypothesized that high temperature affects plant defense against pathogens with different lifestyles (biotrophic, hemibiotrophic, necrotrophic) differently. Therefore, we evaluated the effects of heat shock (HS, 49 °C for 20 s, 2 h before inoculation) in susceptible barley (*Hordeum vulgare* cvs. Ingrid and Himalaya) infected with the necrotrophic *Pyrenophora teres* f. *teres* (Ptt) by monitoring disease symptoms, Ptt biomass and plant defence components (ROS, antioxidants, and defence-related gene expression). Heat shock pretreatment was performed as we described previously in the case of Bh and *Bipolaris sorokiniana* infection by immersing barley leaves in a 49 °C water bath for 20 s, 2 h before Ptt inoculation. Ptt mycelium suspension was applied with a brush to the first leaves of 7-day-old barley plants, evenly covering their entire surfaces. Ptt symptoms were assessed on the inoculated leaves 4 days after inoculation by using the 10-point scale of Tekauz. Our results showed that heat shock significantly increased the intensity of Ptt-induced necrotic symptoms in leaves of both barley cultivars as compared to control plants kept at 20 °C. Himalaya plants showed an enhanced necrotization as compared to Ingrid regardless of the effect of HS. Importantly, in response to HS, Ptt-inoculated cv. Himalaya barley displayed far the most severe necrotization as compared to all other treatments. Heat shock itself did not cause any visible symptoms on the barley plants tested. Next, we estimated the levels of relative Ptt biomass in both barley cultivars at one and four days after inoculation (DAI) by detecting the levels of the Ptt *glyceraldehyde 3-phosphate dehydrogenase* (*PtGAPDH*) gene following fungal inoculation of HS-pre-treated and control plants. By 4 DAI the relative Ptt biomass in cv. Himalaya was about twice as high as that in cv. Ingrid when the plants were maintained at a constant temperature of 20 °C (Fig. 1c). Furthermore, HS pre-treatment significantly increased Ptt biomass in both barley cultivars one and four DAI. **In overall, cv. Himalaya exhibited greater susceptibility to Ptt as compared to cv. Ingrid and HS further increased the susceptibility in both cultivars.** This was evidenced by enhanced severity of necrotic symptoms and a corresponding increase in relative Ptt biomass.

Abiotic and biotic stresses often induce the production of ROS in plant tissues. Furthermore, ROS participate in plant defence signalling and in the development of tissue necrotization during a successful infection. Therefore, keeping an eye on ROS production in heat stressed and Ptt inoculated barley may provide valuable information on the pathophysiology of this plant-pathogen interaction. Therefore, hydrogen peroxide (H_2O_2) production was monitored in the inoculated leaves of both barley cultivars one and two days after HS and Ptt inoculation. Our results showed that HS itself did not induce the production of H_2O_2 in mock-inoculated barley leaves at the time points investigated. However, Ptt inoculation

slightly but significantly increased H_2O_2 production in both cultivars one and two DAI (Fig. 2). Interestingly, HS pre-treatment dramatically increased the Ptt-induced accumulation of H_2O_2 two days after inoculation. In overall, **HS-induced enhanced susceptibility to Ptt correlates with elevated levels of H_2O_2 in both barley cultivars but especially in Himalaya plants**. Moreover, the expression of selected plant stress/defense-related genes (encoding pathogenesis-related 1b, superoxide dismutase and glutathione reductase) and antioxidant levels/activites were evaluated in response to HS and Ptt infection. Our results showed that HS increased the expression of barley pathogenesis related gene 1b (*HvPR-1b*) in both barley cultivars at early time points after Ptt inoculation. On the other hand, Ptt inoculation led to a striking increase in *HvPR-1b* transcript levels 24 and 96 hours after inoculation (HAI), and combined stress (HS+Ptt) resulted in a further increase in the expression of *HvPR-1b* in the cv. Ingrid plants. Similar results were obtained in cv. Himalaya, except that at 96 HAI HS did not increase further the expression of *HvPR-1b*. ROS formation induced by abiotic and biotic stresses provoke an antioxidant response in plants. Therefore, we investigated the expression of genes encoding selected antioxidant enzymes in HS-treated and Ptt-inoculated barley plants. Superoxide dismutase (SOD) enzymes catalyse the dismutation of superoxide radicals ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) and oxygen. Our results showed that Ptt inoculation increased the expression of a barley superoxide dismutase gene (*HvSOD*) encoding a cytosolic CuZn-SOD in untreated plants of both barley cultivars from 3 to 9 HAI. Heat shock had different effects on *HvSOD* gene expression in the cultivars studied. In cv. Ingrid, HS further increased gene expression in inoculated leaves only at 3 and 24 HAI, whereas in cv. Himalaya, it increased the gene expression at all time points investigated. However, HS treatment alone did not increase *HvSOD* expression in non-infected plants. The HS-induced increase in *HvSOD* expression may be responsible for the elevated H_2O_2 levels in Ptt-infected Himalaya plants. Glutathione reductase (GR) is one of the enzymes of the plant antioxidant system, which sustains the reduced status of glutathione via the ascorbate–glutathione pathway. Regarding our results, neither HS nor Ptt infection resulted in an increase in expression of a barley cytoplasmic GR gene (*HvGRcyt*) in cv. Ingrid plants. In contrast, HS pre-treatment increased *HvGRcyt* expression in Ptt-inoculated Himalaya plants at 6 and 9 HAI. Glutathione is one of the most abundant non-enzymatic antioxidants in plants playing a principal role in the elimination of ROS, thus contributing to the maintenance of cellular redox homeostasis. Reduced (GSH) and oxidized (GSSG) glutathione contents were detected in both barley cultivars four DAI. Our results showed that Ptt itself and mock inoculation do not elevate neither GSH nor GSSG contents in both Ingrid and Himalaya plants. However, a combined stress (HS and Ptt) significantly increased GSH levels and with it the amount of GSSG also increased in cv. Ingrid. On the other hand, a combined stress treatment resulted in decreased GSH and drastically increased GSSG contents in cv. Himalaya. As a result, the ratio of GSH to GSSG decreased only slightly in the Ingrid cultivar; while a drastic decrease was observed in Himalaya plants indicating immense oxidative stress. The manuscript presenting these results is currently under review in the journal Plant Biology.

In summary, it can be concluded that both short-term heat shock and prolonged heat waves generally significantly inhibit the defense mechanisms of barley plants against fungal pathogens. The application of **short-term heat shock (HS, 49 °C for 20 sec) pretreatment led to a significant inhibition or delay in plant defense responses across all tested plant-pathogen interactions, regardless of the pathogen's lifestyle (biotrophic, hemibiotrophic or necrotrophic).** HS application resulted in enhanced pathogen induced symptoms and elevated pathogen biomass. HS pre-treatment led to a substantial augmentation in the accumulation of reactive oxygen species (ROS) H_2O_2 in inoculated barley. The elevated H_2O_2 levels, indicate heightened vulnerability in the affected plants as a biological marker. Moreover HS application resulted in reduction or delay in defense gene expression of barley. Usually prolonged heat waves also negatively affect the defense responses of barley to Bh infection, like HS. However **we have found a resistant barley line (MvHV07-17) able to maintain its resistance to Bh even if plants are exposed to a long-term high temperature of 35 °C for 120 h before Bh inoculation.** No Bh symptoms nor elevation in Bh biomass were detectable in the resistant line (MvHV07-17) exposed to prolonged high temperature (35 °C for 5 days). Prolonged heat stress significantly repressed the expression of several

defense-related genes in resistant line. During infection following prolonged heat stress, the expression of defense genes was rapidly restored in the resistant line compared to the susceptible line (MvHV118-17), a possible reason why this barley line retains Bh resistance even at high temperatures. Our results may participate to minimize yield reduction in barley caused by fungal pathogens in response to global warming conditions.

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