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The Participation of Trehalose Metabolism in Response to High-Humidity Stress in *Megoura crassicauda* (Hemiptera: Aphididae)

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Abstract: In the context of climate change, characterized by an increase in average precipitation, agricultural pests have demonstrated enhanced adaptability to high humidity and other challenging environmental conditions, thereby intensifying the need for effective prevention and control measures. Among these pests, *Megoura crassicauda* (Hemiptera: Aphididae) represents a significant threat to both crop yield and quality. The aim of this study was to investigate the physiological behavioral changes and the regulatory mechanisms of trehalose metabolism in *M. crassicauda* under conditions of high-humidity stress. Additionally, we sought to explore the survival strategies and water regulation mechanisms employed by this insect, with the goal of identifying new biological targets for its management. The findings indicated that, despite an increase in environmental humidity, there was no significant difference in the survival rate of *M. crassicauda*. However, a reduction in developmental duration and reproductive capacity was observed. Increased humidity correlated with elevated trehalose levels and decreased glycogen content. Notably, although the relative expression levels of trehalase (TRE) and Trehalose-6-phosphate synthase (TPS) were downregulated, Trehalose-6-phosphate phosphatase (TPP) expression was upregulated. These results suggest that high humidity environments significantly influence the growth, development, and trehalose metabolism of *M. crassicauda*. It appears that adaptations to high-humidity conditions in *M. crassicauda* are facilitated by modulations in the types and distribution of sugars within their bodies, achieved through alterations in the expression of genes associated with trehalose metabolism. In summary, the results of this study indicate that high humidity significantly affects the development and sugar metabolism of *M. crassicauda*. These changes may represent one of the potential mechanisms underlying its environmental adaptation and migration. This insight provides valuable assistance for predicting the occurrence and migration of the pest *M. crassicauda*.

Keywords: *Megoura crassicauda*; humidity; development; survival; trehalose metabolism

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1. Introduction

In recent years, the phenomenon of global climate change has led to an increase in the frequency of extreme weather events such as heatwaves, heavy rainfall, droughts, and tropical cyclones [1]. These events have had a substantial impact on agricultural production

and insect populations. The decline in food crop yields attributable to pest infestations poses a significant threat to global food production [2]. Climate change will have a vast impact on the fitness and ranges of current species. Increasing temperature is the most frequently considered variable, yet other attributes, such as changes in rainfall and the frequency and duration of extreme events, can lead to changes in insect populations, migration, and behavior [3,4]. Humidity is essential for the water necessary for normal physiological activities in insects, such as digestion, metabolism, and excretion [5]. However, the body structure of insects makes it challenging for them to retain water [6]. Consequently, they are highly sensitive to changes in humidity and seek out optimal microclimates to regulate their water balance [7].

Related studies have demonstrated that long-term exposure to high humidity can promote the proliferation and spread of insect pathogenic microorganisms, as well as disrupt the normal activities of their natural predators. This can significantly affect pest control efforts [8]. Furthermore, high humidity can negatively affect respiration by causing an accumulation of water in the tracheal system, which hinders efficient respiratory gas exchange [9]. High humidity is advantageous for extending the flight duration of *Sitobion avenae* (Homoptera: Aphididae), whereas low humidity negatively impacts its survival and flight speed [10]. Temperature and relative humidity have a marked effect on the egg development time and larval survival of *Dermacentor variabilis* (Ixodida: Ixodidae) [11]. Arthropods have gradually evolved a range of behavioral coping strategies as well as physiological and molecular regulatory mechanisms over time to adapt to the humidity of the surrounding environment [12]. Compared to drought conditions, the reproduction of *Bradysia odoriphaga* (Diptera: Sciaridae) adults and larvae significantly increase under high soil moisture levels [13]. Humidity can significantly influence the hibernation behavior of *Vespa magnifica* (Hymenoptera: Vespidae). As the relative humidity decreases, both the survival rate and hibernation rate of the queen bee decline [14]. Insects also use local variations in humidity as cues for foraging and oviposition site selection. For example, both nectar- and blood-feeding species use minute short-range local variations in humidity to guide them to food sources and optimal locations for laying eggs [15,16]. The humidity-sensing neurons in insects detect changes in humidity, enabling them to gather information about external humidity fluctuations and respond with appropriate behavioral and physiological adjustments [14]. Some insects can utilize their ability to perceive humidity to guide specific behavioral changes. For the biting insects of *Anopheles gambiae* (Diptera: Culicidae), increased humidity and temperature can enhance their attacking and biting behaviors [17]. Arthropods utilize their ability to perceive humidity for environmental navigation, while *Macroglossum stellatarum* (Lepidoptera: Sphingidae) uses humidity to assess the nectar content in flowers [18]. Related studies have demonstrated that humidity exhibits a strong nonlinear relationship with the intrinsic population growth rate of *Dermatophagoides farinae* (Acariformes: Pyroglyphidae), with peak values occurring at approximately 28 °C and 85% relative humidity [19].

Trehalose serves as a source of energy and sugar, and it is the primary carbohydrate found in insect hemolymph, commonly referred to as blood sugar [20]. Previous studies have indicated that trehalose metabolism is associated with the adaptation to changes in environmental water content [21]. This regulation of trehalose metabolism highlights the indispensability of its dynamics for overall insect fitness and survival [22]. As the most synthesized disaccharide in response to stress, the mixtures of trehalose with highly hydrophilic proteins enter a vitrification process to maintain the stability of cells and proteins under challenging conditions and acts as a stabilizer for biological proteins [23,24]. Like many organisms that produce trehalose, insects synthesize trehalose through the TPP/TPS pathway. The synthesis of trehalose in insect adipose tissues relies on the catalytic activity of Trehalose-6-phosphate synthase (TPS) and Trehalose-6-phosphate phosphatase (TPP). Trehalose is then hydrolyzed into two molecules of glucose by the specialized hydrolase, trehalase (TRE) [8,25]. Finally, it is transported to the hemolymph through

specific transmembrane transporters, thereby maintaining the balance of carbohydrate metabolism in the body [26]. There are two types of trehalase in insects: soluble trehalase (TRE1), which primarily decomposes trehalose within cells, and membrane-bound trehalase (TRE2), which features a transmembrane structure on the N-hydroxyl group and primarily hydrolyzes the trehalose in food [22].

Megoura crassicauda feeds selectively on a variety of fabaceous plants in the genus *Vicia* [27]. Their feeding is mainly focused on the inner side of plant leaves and fruit ears, and they suck out the sap through the sieve tube of the phloem [28]. In instances of substantial damage caused by aphids, soybean yields may experience reductions of up to 40% [29].

Trehalose is crucial for maintaining normal physiological activities of *M. crassicauda*, but there is currently limited research on the association between humidity and *M. crassicauda*, and the mechanism by which high humidity affects trehalose metabolism in *M. crassicauda* is not yet clear. Therefore, exploring the expression changes in trehalose metabolism in response to high-humidity stress is of great significance. In this study, we monitored the growth and development characteristics and the content of carbohydrates in the body of *M. crassicauda* under high-humidity stress, and conducted in-depth research on the expression of related genes in the trehalose metabolism pathway. This research provides a foundation for understanding the adaptive mechanisms of *M. crassicauda* to high-humidity environments and offers insights into the prediction and control of *M. crassicauda* occurrences.

2. Materials and Methods

2.1. Test Materials and Grouping

The broad bean (*Vicia faba*) variety used in this experiment was Qingcan No. 14, and the aphid species studied was *M. crassicauda*. Broad bean seedlings were cultivated to provide a food source for *M. crassicauda*. The feeding conditions for both the broad beans and aphids were maintained at 19 ± 1 °C with a photoperiod of 16 h of light and 8 h of darkness. Under this condition, *M. crassicauda* always remains in a state of viviparity and parthenogenesis. The control group was kept in an artificial climate chamber with a relative humidity (RH) of 60%, while two experimental groups were established with RH levels of 75% and 90% in incubators.

The newly born aphids produced by adult female aphids under RH65% were placed under conditions of RH60%, RH75%, and RH90% for cultivation; this generation of aphids is referred to as F1. The newly born aphids produced by the adult aphids of the F1 generation were then separated and further cultivated under their respective humidity conditions, with these aphids being the F2 generation. Similarly, the third-generation aphids produced by the adult aphids of the F2 generation were separated and continued to be cultivated under their corresponding humidity conditions, with these aphids forming the F3 generation.

2.2. Survival Rates, Development, and Reproductive Detection of M. crassicauda

One hundred newly hatched nymphs from each humidity condition of the F1 generation were isolated and reared in nylon net cages containing young broad bean seedlings, with their survival rates recorded every 24 h. The survival rate recording methods for the F2 and F3 generations were consistent with those for the F1 generation. The developmental period of the *M. crassicauda* refers to the time from newly born nymph to adult (considered an adult after completing the fourth molt). For the statistical analysis of the developmental period, 10 newly born nymphs from each humidity treatment were selected for each generation. For the fertility statistics, 100 female adults from each humidity condition were chosen for each generation, and the number of newly hatched aphids they produced was counted daily over a period of 10 days.

2.3. Determination of Carbohydrate Content and Trehalose Enzyme Activity in *M. crassicauda*

Adult aphids collected from three humidity groups were subjected to long-term stable high-humidity stress, using 15 aphids per biological replicate for a total of three biological replicates. Three biological replicates were established and three generations were continuously monitored. This portion of the experiment was conducted following the methodology of previous researchers [30].

2.4. Total RNA Extraction and Real-Time Fluorescence Quantitative PCR (qRT-PCR)

The samples used for RT-qPCR were derived from the F3 generation, with 45 adult aphids collected from each humidity treatment group. Each treatment group had three biological replicates, with each replicate consisting of 15 adult aphids.

According to the manufacturer's instructions, the total RNA was extracted using an RNAiso Plus kit (Takara, Kyoto, Japan). A NanoDrop™ 2000 (Waltham, MA, USA) was used to determine the concentration and purity of the extracted RNA. A PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara, Kyoto, Japan) was used for the reverse transcription of the cDNA.

The RT-qPCR reaction system (TaKaRa TB Green® Premix Ex Taq™, Takara, Kyoto, Japan) was used as follows: 1 µL of cDNA, 5 µL of TB Green, 0.4 µL of forward primer, 0.4 µL of reverse primer, and 3.2 µL of ddH_2O. The RT-qPCR reaction procedure was pre-denaturation at 95 °C for 30 s, 40 cycles of 95 °C for 5 s, and 55–60 °C for 30 s, using *M. crassicauda* β-*Actin* (GenBank accession number: AB627775.1) as an internal reference gene. The primer sequences are shown in Table 1. The RT-qPCR data were analyzed using the $2^{-\Delta\Delta CT}$ method [31].

Table 1. Primers used for real-time fluorescent quantitative PCR (qRT-PCR).

Gene ID	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Notes
AB627775.1	GATCATTGCCCCACCAGAAC	TTTACGGTGGACAATGCCTG	*Actin*
PB.1523.1	GTGACATGAAGGACACGGCT	TCAGGTAGTACACCCGTCCA	*TRE1-1*
PB.5448.1	GGTCCTTACCATTCACCGCA	TCACGTTTGAAGTTTGGACTGC	*TRE1-2*
PB.11546.1	TGGCGTATCAACTCTGGTGG	CGTCTTTGTTGCCTGTGCTG	*TRE1-3*
PB.13951.1	AGGTGGTCGATTCCGTGAAG	TGCTGATTACCCCTTTTGCTGT	*TRE1-4*
PB.14734.1	TGAAGACATACCAACTACTTTGAGC	ATATGTACTGAAGTGGGGGCCA	*TRE1-5*
PB.25140.1	GGGACAAACCAAATGCGTGG	TGTGAGCAATGTTTGACGCC	*TRE1-6*
PB.530.1	TGCCGCCTGATTTTGTTGAG	CCTTGCGGGCCAATCTTTTC	*TRE2-1*
PB.885.1	GTGAACAGTGGGACTACCCG	GCCCAGTTATCGCCTGAGTT	*TRE2-2*
PB.133.1	ACAGGCTTCCGTTCGTTCTC	AGTGACTAACCCACCAGCAC	*TPS-1*
PB.367.1	GCGCCCCTCATCGTAGTATC	GACCACCCGCACTTTGTTTC	*TPS-2*
PB.29534.1	TATCCACAGCACAGCGTCTC	ACAGGAGGTTTGGCCTCAAT	*TPP*

2.5. Statistical Analysis

The results were expressed as the standard error of the mean (SEM) of independent replicates ($n \geq 3$). The experimental data were analyzed using IBM SPSS Statistics version 20 statistical software. The survival curves were analyzed using GraphPad Prism 9 software with the Kaplan–Meier method to assess differences. Statistical significance was defined as $p < 0.05$. Tukey's post hoc test following one-way ANOVA was performed to assess the significance of differences among the treatments. All figures and tables were produced using Office 2021 and GraphPad Prism 9.0 software.

3. Results

3.1. Transcriptome Expression Analysis of Differential Enzyme Genes Related to Trehalose Metabolism

RNA sequencing (RNA-seq) was conducted on three humidity groups of *M. crassicauda* subjected to long-term stable stress. This was followed by transcriptome clustering and correction, as well as Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. Statistical analysis revealed that differentially expressed genes were primarily enriched in molecular function, metabolism, cellular processes, and genetic information processing. Differentially expressed enzyme gene sequences associated with trehalose metabolism were identified and compared using NCBI BLAST. As shown in Table 2, the analysis yielded differentially expressed genes, including six *TRE1* genes, two *TRE2* genes, two *TPS* genes, and one *TPP* gene. These genes were selected as target genes for quantitative detection under the three humidity treatments.

Table 2. Differentially expressed enzyme genes related to trehalose metabolism.

Gene	RH60/RH75		RH60/RH90		RH75/RH90	
	log_2Fold	***p*-Value**	**log_2Fold**	***p*-Value**	**log_2Fold**	***p*-Value**
TRE1-1	0.38783	**0.03301**	0.1186	0.451190	−0.25798	0.114360
TRE1-2	−0.54957	**0.01578**	−1.5048	**0.000158**	−0.94960	**0.030701**
TRE1-3	−0.10250	0.39999	−0.3395	**0.004930**	−0.22491	**0.089015**
TRE1-4	0.07676	0.69414	0.6637	**0.000272**	0.59825	**0.001318**
TRE1-5	1.15610	0.06738	1.4833	**0.024938**	0.33881	0.549570
TRE1-6	−0.13610	0.64496	0.6256	0.083270	0.76989	**0.042673**
TRE2-2	0.12484	0.33977	−0.2444	0.066887	−0.35637	**0.000320**
TRE2-2	−0.23049	0.24753	−1.4992	$\mathbf{1.06 \times 10^{-10}}$	−1.25620	$\mathbf{2.48 \times 10^{-11}}$
TPS1	0.19987	**0.02205**	−0.3434	$\mathbf{5.97 \times 10^{-5}}$	−0.53139	$\mathbf{1.80 \times 10^{-17}}$
TPS2	0.16273	0.19375	−0.8350	$\mathbf{1.36 \times 10^{-9}}$	−0.98539	$\mathbf{7.99 \times 10^{-11}}$
TPP	−1.54080	0.20991	1.3938	0.112010	2.95240	0.007052

Note: the bolded parts indicate genes that are significantly differentially expressed in the given comparison, i.e., with a p-value < 0.05.

3.2. Statistical Analysis of Survival Rate, Developmental Duration, and Reproductive Capacity of M. crassicauda

There was no significant difference ($p > 0.05$) among the groups across the three consecutive generations; however, it was observed that the survival rate decreased with increasing humidity (Figure 1A–C). The three generations exposed to 75% relative humidity (RH75%) exhibited the longest developmental period, while no significant difference was found in the developmental period of *M. crassicauda* between the F2 and F3 humidity groups. As the number of generations increased, the developmental period for each humidity group displayed a decreasing trend (Figure 1D). There was no significant difference in the reproductive capacity of *M. crassicauda* between RH60% and RH75%. However, under RH90% conditions, the offspring number of *M. crassicauda* was significantly lower compared to the other two groups (Figure 1E).

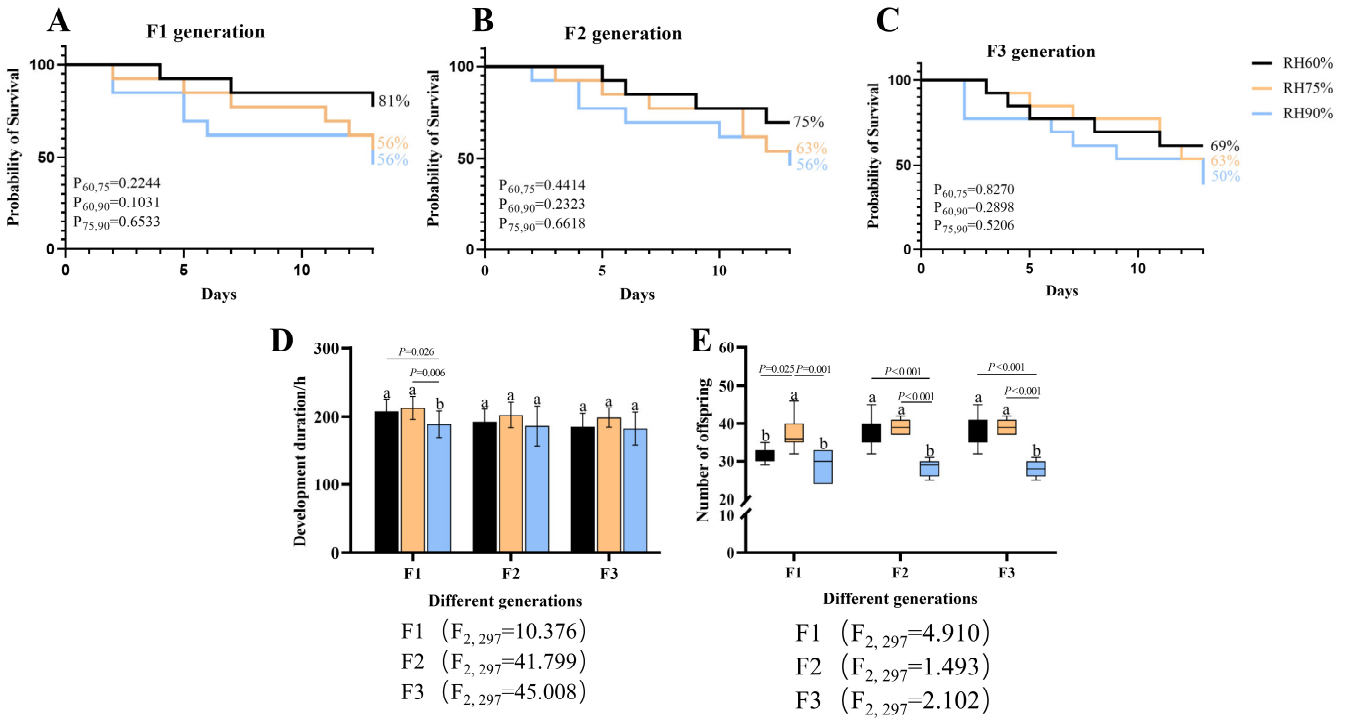

Figure 1. Growth and development of *M. crassicauda* under different humidity stress conditions. (**A**), (**B**) and (**C**) represent the survival rate of aphids in the first, second, and third generations, respectively. (**D**): developmental duration (in hours) of *M. crassicauda* in different generations. (**E**): number of offspring of *M. crassicauda* in different generations. Black, orange, and blue lines, columns, and boxes indicate treatments with 60%, 75%, and 90% relative humidity, respectively. The data are expressed as mean ± standard error. Kaplan–Meier survival curves were drawn using Prism software using the log rank test method ($p < 0.05$ level). Different letters indicate significant differences in aphids (Tukey test, $p < 0.05$ level).

3.3. Changes in Carbohydrate Content and Two Seaweed Enzyme Activities of M. crassicauda Under Different Humidity Stress Conditions

The research results indicated a significant difference in trehalose content between the control group at 60% relative humidity (RH60%) and the groups at 75% (RH75%) and 90% (RH90%). Furthermore, the trehalose content exhibited an upward trend with increasing humidity (Figure 2A). In contrast, there was no significant difference in glucose content among the three humidity groups (Figure 2B). A significant difference in glycogen content was observed between the RH75% and RH90% groups compared to the RH60% group. Additionally, as the humidity increased, the glycogen content demonstrated a decreasing trend (Figure 2C). There was a significant difference in soluble trehalase activity between the RH75% and RH90% groups (Figure 2D), while no significant difference was found in terms of membrane-bound trehalase activity among the three humidity groups. However, as the humidity increased, the membrane-bound trehalase activity showed a decreasing trend (Figure 2E).

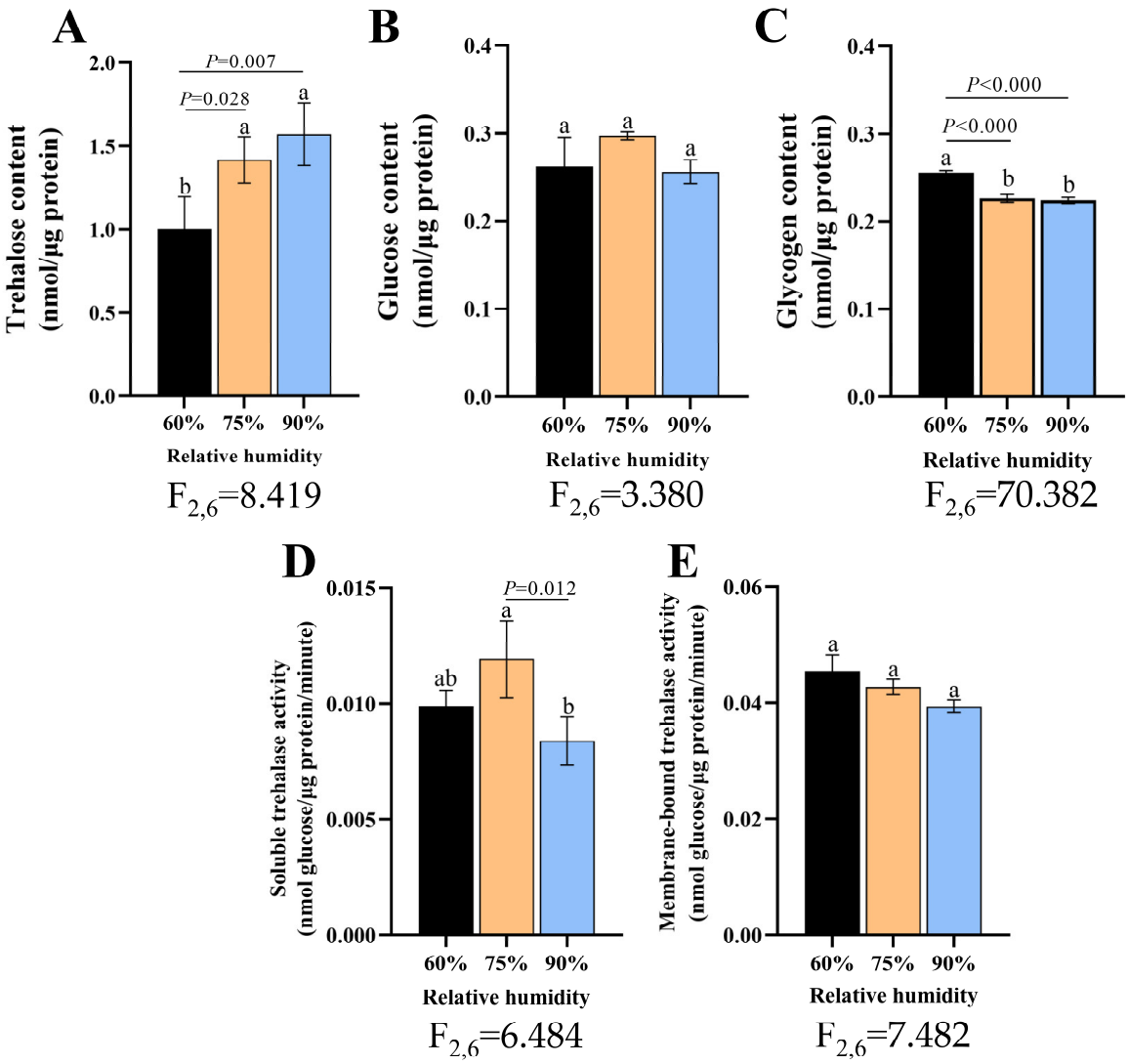

Figure 2. Under long-term stable high-humidity stress, the trehalose content (**A**), glucose content (**B**), glycogen content (**C**), soluble trehalase enzyme activity (**D**), and membrane-bound trehalase enzyme activity (**E**) of different groups of *M. crassicauda* were measured. RH—relative humidity. The data are expressed as mean ± standard error from three independent measurements. Different letters indicate significant differences in the developmental period or reproductive capacity of aphids, and the differences are all intragenerational comparisons. Bars with different letters indicate significant differences (Tukey test, $p < 0.05$ level).

3.4. Response of TRE Gene Expression in M. crassicauda Under Long-Term Stable High-Humidity Stress Conditions

According to the qRT-PCR results, the relative expression levels of *TRE1* genes in the RH90% group were lower than those in the RH60% and RH75% groups. With the exception of *TRE1-1* and *TRE1-5*, the relative expression levels of *TRE1* genes in the RH60% group were higher than those in the RH75% group. Overall, the *TRE1* gene group exhibited a trend of decreasing relative expression levels with increasing humidity, and significant differences were observed in the *TRE1-2*, *TRE1-3*, *TRE1-5*, and *TRE1-6* groups ($p < 0.05$) (Figure 3). Compared with the control group (RH60%), the relative expression level of *TRE2-1* in the RH75% and RH90% groups decreased significantly, while no significant difference was noted in the expression level of *TRE2-2* (Figure 3).

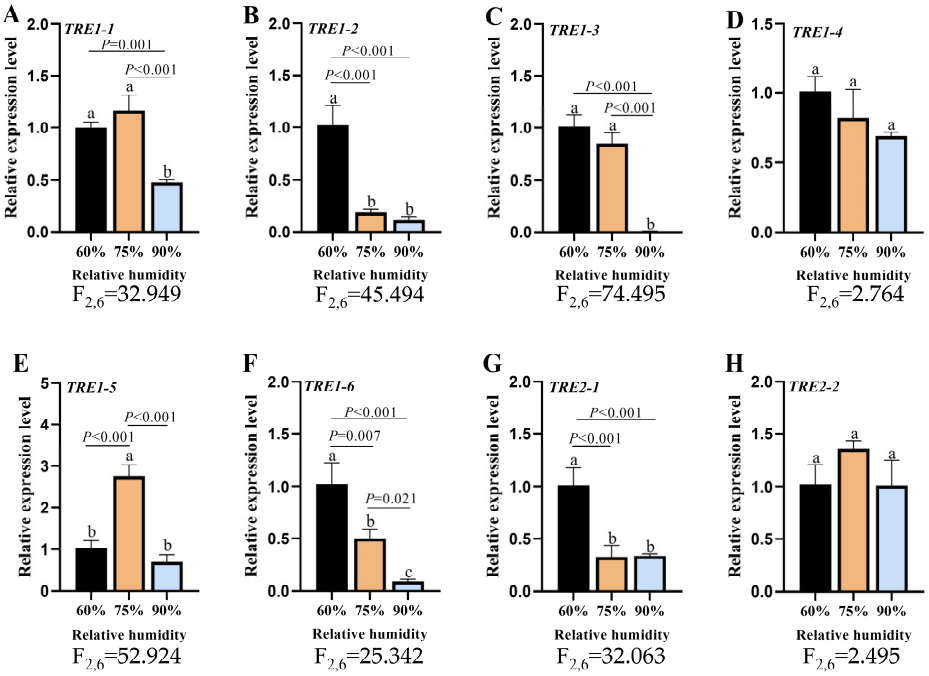

Figure 3. Relative expression levels of TRE1 (**A**–**F**) and TRE2 (**G**,**H**) genes under long-term stable high-humidity stress: TRE1, soluble trehalase; TRE2, membrane-bound trehalase. Expression levels were measured via quantitative real-time PCR, with 18S RNA as the internal control. Values are means ± standard error from three independent measurements. Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

3.5. Response of TPS and TPP Gene Expression in M. crassicauda Under Long-Term Stable High-Humidity Stress Conditions

The qPCR results indicated that as humidity increased, the expression level of *TPS* exhibited a trend of initially decreasing and then increasing. However, when compared to the control group, both the RH75% and RH90% groups demonstrated varying degrees of downregulation. The RH75% group showed a significant decrease in expression levels, while the RH90% group also exhibited a notable reduction in the *TPS1* expression level ($p < 0.05$; Figure 4). Additionally, the relative expression level of the *TPP* gene was positively correlated with humidity, revealing a significant difference between the RH90%, RH60%, and RH75% groups ($p < 0.05$; Figure 4).

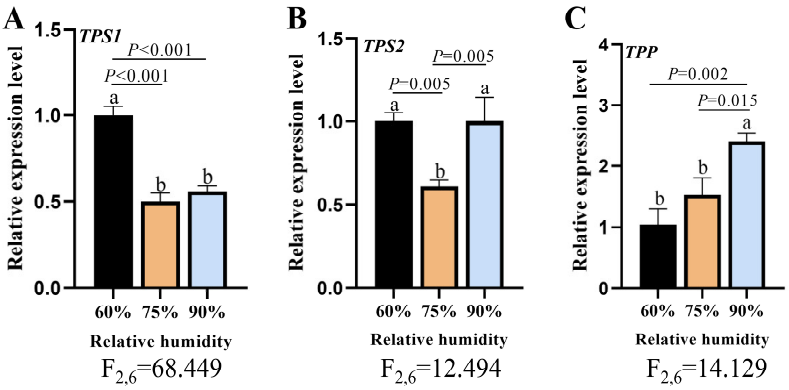

Figure 4. Relative expression levels of TPS (**A**,**B**) and TPP (**C**) genes under long-term stable high-humidity stress. TPS—Trehalose-6-phosphate synthase; TPP—Trehalose-6-phosphate phosphatase. Expression levels were measured via quantitative real-time PCR, with 18S RNA as the internal control. Values are means ± standard error from three independent measurements. Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

3.6. Response of Trehalose-Metabolizing Enzyme Gene Expression in M. crassicauda Under 24 H Emergency Stress Conditions

Under the conditions of 24 h emergency high-humidity stress, the expression levels at RH75% were significantly elevated ($p < 0.05$) in comparison to the control group at RH60%, with the exception of the *TRE1-1* group. In the *TRE1-1*, *TRE1-2*, and *TRE1-3* groups, the expression levels at RH90% were found to be lower than those of the control group, whereas the *TRE1-4*, *TRE1-5*, and *TRE1-6* groups exhibited higher expression levels than the control group at RH90%. Overall, the *TRE1* genome demonstrated an increase in expression levels at RH75%, while the response at RH 90% was variable (Figure 5). The *TRE2* genome showed the highest expression level at RH75%, with a statistically significant difference observed between RH75% and RH90%. Although no significant differences were found in the expression of the *TPS* gene, the *TPP* gene displayed a trend of a relative increase in expression levels as the humidity increased (Figure 5).

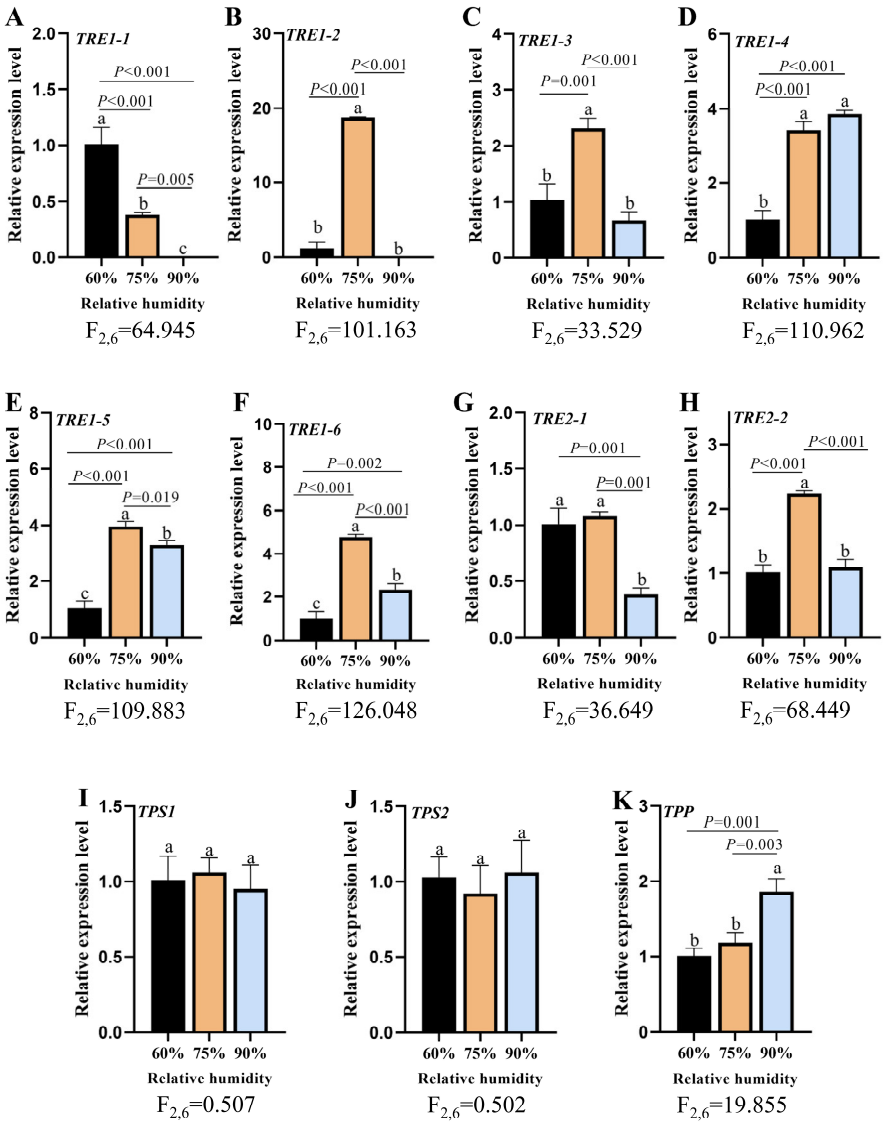

Figure 5. Relative expression levels of trehalose-metabolism-related enzyme genes under 24 h emergency stress. *TRE1* (**A**–**F**)—soluble trehalase; *TRE2* (**G**,**H**)—membrane-bound trehalase; *TPS* (**I**,**J**)—Trehalose-6-phosphate synthase; *TPP* (**K**)—Trehalose-6-phosphate phosphatase. Expression levels were measured via quantitative real-time PCR, with 18S RNA as the internal control. Values are means ± standard error from three independent measurements. Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

4. Discussion

Humidity is a critical climatic variable that exerts a significant influence on both crops and the arthropods that inhabit them. Relative humidity (RH) plays a pivotal role in modulating the physiological activities and other related parameters of arthropods. Arthropods have the ability to extract information about their surroundings via humidity sensation [32]. The experimental findings revealed that, while there were no statistically significant differences in survival rates across the various humidity groups, a trend was observed wherein the survival rates diminished with increasing humidity levels (Figure 1A–C). This observation implies that humidity has a discernible effect on the survival rates of *M. crassicauda*. This conclusion is consistent with prior research on *Halyomorpha halys* (Hemiptera: Pentatomidae), which indicated that exposure to low humidity (17% RH) significantly decreased *H. halys* survival compared with exposure to higher humidity at 40 °C [33]. In the present study, it was noted that the developmental duration of the F1 generation at 90% RH was significantly shortened (Figure 1D), further underscoring the substantial impact of high humidity on insect developmental timelines. Additionally, the previous literature demonstrated that the developmental periods of *H. halys* increased at temperatures above 30 °C [34]. The 90% RH condition exhibited a marked inhibitory effect on the reproductive capacity of *M. crassicauda*, which was particularly pronounced in the F2 and F3 generations (Figure 1E). Previous investigations have established that male insects suffer from decreased fertility in response to increased temperature [35], indicating that extreme humidity levels significantly disrupt the reproductive behaviors of some insects. As the number of generations progresses, the reproductive potential of *M. crassicauda* appears to converge under the two humidity environments of 60% RH and 75% RH. This observation is corroborated by evidence suggesting that cadmium (Cd) stress has negligible effects on the fourth and fifth generations of *M. crassicauda* [36]. This suggests that the diminution in reproductive capacity is contingent upon the specific type of environmental stressor, with conditions characterized by elevated humidity exerting a more pronounced influence on the reproductive performance of *M. crassicauda*.

M. crassicauda is classified as a hemimetabolous insect, wherein trehalose serves not only as an energy source, but also as a vital metabolite that enhances stress resistance during insect diapause and other stress-related processes [20]. In investigations focused on drought resistance in *Belgica antarctica* (Diptera: Chironomidae) and *Chironomus ramosus* (Diptera: Chironomidae), it was noted that the trehalose levels in the insect body significantly increase under drought stress, comprising a considerable portion of its overall body mass [37]. Furthermore, studies have demonstrated that insects can adapt to heavy metal stress by regulating their trehalose metabolism pathways [36]. *Drosophila melanogaster* (Diptera: Drosophilidae) also exhibits a marked rise in trehalose levels in its hemolymph when subjected to drought and water-deficient conditions, forming a protective layer on the cell surface that shields biomolecular structures from potential damage [38]. Experimental findings indicate a correlation between increased humidity and elevated trehalose content in *M. crassicauda* (Figure 2A), suggesting that insects can enhance their metabolic processes by augmenting trehalose levels to adapt to challenging environmental conditions. Additionally, research has established a close association between elevated trehalose levels and the molting process in *Periplaneta americana* (Blattodea: Blattidae) [39], which may also contribute to the observed reduction in developmental duration in *M. crassicauda*. Importantly, no significant differences were detected in the total glucose content of *M. crassicauda* across three humidity conditions during prolonged humidity exposure (Figure 2B). As a hemimetabolous insect, it is hypothesized that related hormones, such as 20-hydroxyecdysone [40], may also play a role in regulating glucose homeostasis. Notably, as humidity levels rise, the glycogen content in *M. crassicauda* exhibits a decreasing trend (Figure 2C). This suggests that insects can endure external stressors through the metabolic consumption of glycogen.

In this study, under long-term stable high-humidity stress, the relative expression level of the *TRE1* gene in the RH90% group decreased, the activity of soluble trehalose decreased,

and the content of trehalose increased (Figure 2D). Related studies have demonstrated that the pine wood nematode *Bursaphelenchus xylophilus* (Aphelenchida: Aphelenchoididae) responds to low-temperature stress, causing the downregulation of *Bx-tre-1* expression, a stress-induced increase in trehalose content, and the promotion of low-temperature stress resistance in *B. xylophilus* [41]. This suggests that the *TRE1* gene can modulate trehalose levels within the organism by altering its expression, thereby facilitating adaptation to growth and development. However, despite the significant downregulation of *TRE2-1* expression levels in the RH75% and RH90% groups, there was no significant change in *TRE2* activity (Figures 2E and 3). In a study on Conopomorpha sinensis (Lepidoptera: Gracillariidae), it was also demonstrated that TRE genes play a crucial role in trehalose metabolism [42]. Another study found that trehalose supplementation significantly enhanced the heat tolerance of *Ocymyrmex robustior* (Hymenoptera: Formicidae) workers and increased the metabolic levels of trehalose under conditions of heat stress [43]. Under long-term Cd stress, the trehalose content in *Aedes albopictus* (Diptera: Culicidae) increased [44]. In environments containing the heavy metals Cd and Pb, the content of trehalose in aphids increases to resist stress [36], indicating that, under adverse environmental conditions, insects can adapt to environmental changes by regulating intracellular trehalose content. At the same time, studies have shown that *TRE1* mainly decomposes intracellular trehalose, while *TRE2* mainly hydrolyzes trehalose in food [45]. It is speculated that, due to the fact that aphids under different treatments all feed on fava bean seedlings and have consistent food sources, there is no significant difference in trehalose content in the food. Therefore, there is no significant difference in *TRE2* content. In addition, previous studies have found that interference with *Tre2-like* and *Tre2* genes in *Harmonia axyridis* (Coleoptera: Coccinellidae) not only leads to their death, but also causes molting disorders [46]. Consequently, the membrane-bound trehalase enzyme TRE2 appears to exert a limited direct influence on the ability of *M. crassicauda* to withstand high-humidity stress and maintain internal water balance. Instead, it may regulate the metabolism of sugars, including trehalose, through the modulation of related gene expression within the insulin signaling pathway.

After rapid cold acclimation, the expression level of the *LoTPS* gene in *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae) increased. *LoTPS* can enhance survival ability by regulating trehalose metabolism [47]. A study demonstrated that *TPS1* increases the trehalose content in *D. melanogaster*, reduces protein aggregation caused by hypoxia, and increases tolerance to hypoxia [48]. The expression levels of *TPS* exhibited a pattern of initial decline followed by an increase as humidity levels rose, while the expression of Trehalose-6-phosphate phosphatase (*TPP*) consistently increased (Figure 4), ultimately resulting in an elevation of trehalose content (Figure 2A). Additionally, research indicates that *TPS* genes can regulate trehalose levels within the organism by modulating *TPS* expression, thereby facilitating adaptation during growth and development [20]. Transcriptomic and metabolomic analyses of *Arabidopsis thaliana* (*Brassicaceae*) have revealed a correlation between the expression levels of *TPS* and *TPP* genes and trehalose content under heat stress [49]. These findings suggest that the regulation of trehalose metabolism through the expression changes in *TPP*, *TPS*, and *TRE* is a relatively widespread strategy employed in nature for coping with environmental stress. This strategy is observed not only in higher organisms such as plants and insects, but also in lower organisms such as fungi.

The experiment concurrently demonstrated notable variations in the expression levels of different genes when comparing 24 h stress to long-term stable stress. This suggests that *M. crassicauda* experiences adaptive evolution as a response to high-humidity stress. These results align with prior research that has explored the intricate and dynamic natural and anthropogenic environments, illustrating that arthropods have developed a variety of adaptive strategies to rapidly acclimate to their local habitats [50].

Climate change has a significant impact on the prediction and early prevention of pest outbreaks. Changes in atmospheric humidity can affect the migration and reproduction of pests such as *M. crassicauda*. This study found that *M. crassicauda* can adapt to changes in environmental humidity by regulating its trehalose metabolism. Furthermore, changes

in humidity influence its survival, development, and reproduction. These findings are important for monitoring and controlling pests like *M. crassicauda* and also contribute to understanding the adaptive mechanisms of insects in response to increased humidity due to climate warming.

5. Conclusions

In this study, high humidity was found to significantly reduce the reproductive capacity of *M. crassicauda* and affect its trehalose metabolism, while having minimal impact on survival rate and developmental duration. These findings suggest that changes in trehalose metabolism may serve as one of the mechanisms by which *M. crassicauda* adapts to high-humidity environments. As humidity levels rise, there is an observed increase in the expression of *TPP*, accompanied by a decrease in the expression of *TRE1*. This intricate regulatory mechanism results in an elevated trehalose content, which aids in the organism's ability to endure high-humidity conditions.

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