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Direct Short-Time Exposure of Wheat Seeds of Cultivar AK58 to a High-Voltage Electrostatic Field at 5 kV/cm Positively Affects the Performance of *Sitobion miscanthi*

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Simple Summary

Directly exposing AK58 winter wheat seeds to a high-voltage electrostatic field at 5 kV/cm for either 5 or 50 min did not adversely affect the performance of *Sitobion miscanthi* Takahashi, but improved their population dynamic parameters. Further gene transcript analysis demonstrated that this physical treatment might trigger the extensive accumulation of auxin or serotonin in plant tissues; both compounds could directly or indirectly interfere plant immunity responses, consequently alleviating the plants' induced defense against aphids. The distinct expression profiles suggest that pre-exposure for 50 min might activate the indole-3-pyruvate-dependent auxin biosynthetic pathway and suppress auxin degradation, whereas the 5 min treatment triggered tryptamine-dependent auxin and serotonin accumulation in plant tissue.

Abstract

Our previous study revealed that directly exposing naturally stored or thermally treated seeds of cultivar AK58 to high-voltage electrostatic field (HVEF) for a short time improved their germination activity. However, whether the durations studied could trigger seedling resistance to *Sitobion miscanthi* Takahashi remained underdetermined. In the current study, an age-stage, two-sex life table was established to evaluate how directly exposing seeds to HVEFs at 5 kV/cm affected the performance of apterous *S. miscanthi* reared on this cultivar. The results revealed that the HVEF pre-exposure of seeds for either 5 or 50 min improved the population dynamic parameters of *S. miscanthi*. Subsequent gene transcript analysis demonstrated that HVEF treatment might trigger extensive auxin and/or serotonin accumulation. Under the 50 min treatment, auxin increased due to the activation of indole-3-pyruvate-dependent auxin biosynthetic pathways and the suppression of its degradation, whereas with the 5 min duration, tryptamine-dependent auxin and serotonin accumulation were triggered in plant tissue. The extensive accumulation of these compounds could directly or indirectly suppress plant immunity responses, that contributing to improving the performance of *S. miscanthi*. Overall, these findings will provide more experimental evidence to enable understanding of the antagonistic interaction between phytohormone signaling processes in cereals under HVEF exposure to seeds, and facilitate the applying of this strategy into agricultural productions with the aim of rescuing aged seeds and improving pest control.

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Keywords: high-voltage electrostatic field exposure; *Sitobion miscanthi*; life-table parameter; pest performance; auxin accumulation

1. Introduction

In recent decades, naturally aged seeds have been directly exposed to high-voltage electrostatic fields (HVEFs) for short times to rescue or improve their germination activity and sprout rate [1]. Studies on the underlying physiological basis revealed that HVEF exposure dramatically increases the activities of antioxidative enzymes, including superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and peroxidase (POD, EC 1.11.1.7), which enable the rapid scavenging of various reactive oxygen species (ROS) compounds that accumulate during the aging process [1–3]. Meanwhile, we previously found that directly exposing seeds of winter wheat cultivar (cv.) AK58 to HVEFs significantly increased the activities of SOD and CAT, and reduced the malondialdehyde (MDA) content in seedling tissue. It has also been reported that one of the coding genes of the CAT enzyme, *AtCATALASE2* (*AtCAT2*), exhibits H_2O_2-scavenging and ACX2/3-stimulating activity in *Arabidopsis thaliana* (L.) during the seedlings' post-germinative growth [4]. A further mechanism study revealed that AtCAT2 could directly interact with the ACX2 and ACX3 enzymes, and promote their activity [4]. ACX2/3 catalyzes the first step of the β-oxidation pathway in jasmonic acid (JA) biosynthesis and promotes JA accumulation in plant tissues [4,5]. This experimental evidence suggests that the direct short-time exposure of seeds to HVEFs to improve their germination activity probably induces JA accumulation and thereby triggers plant resistance.

In addition, our previous studies demonstrated that directly exposing seeds of winter wheat cv. XY22 to HVEF at 4 kV/cm for 20 min could trigger the plants' resistance to wheat aphids [6]. However, further antioxidant capacity assessment revealed that exposing seeds to HVEFs did not significantly increase CAT activity in plant tissue, and aphid infestation was also further decreased [7]. Meanwhile, biotrophic-pathogen-induced salicylic acid (SA) accumulation or direct SA treatments inhibit the activity of AtCAT2, one of the SA receptors, and can result in the upregulation of H_2O_2, which would further repress the level of the auxin biosynthetic precursor by sulfenylating tryptophan synthetase β subunit 1, eventually dampening both auxin accumulation and JA biosynthesis [8]. Accordingly, the inhibition of CAT activity in XY22 might be induced by SA accumulation, and could impair JA and auxin biosynthesis, eventually strengthening the plant's resistance to aphids. However, the molecular basis underlying how HVEF exposure altered CAT activity and auxin accumulation in wheat plants, and whether auxin-triggered plant physiological changes affected the performance of wheat aphids, remained to be determined. Moreover, empirical studies have revealed that aphid pre-infestation would trigger the upregulation of the transcripts of the marker genes associated with JA and SA signaling pathways [9–11]. However, our latest studies demonstrated that JA accumulation during aphid infestation might act antagonistically in direct or coordinated auxin mediation, alleviating SA-dependent plant responses, following the direct foliage spraying of methyl jasmonate (MeJA), a compound commonly used to simulate JA in artificial treatments [12,13]. Based on this experimental evidence, it is hard to comprehensibly characterize the wheat–aphid interactions observed under indirect HVEF exposure.

Therefore, the present study initially evaluated the effects of exposing AK58 seeds to HVEFs at 5 kV/cm for 5 and 50 min on the performance of the wheat aphid, *Sitobion miscanthi* Takahashi (Hemiptera: Aphididae), because these durations exhibited the smallest and largest positive effects on seed viability, respectively. Then, we determined the

underlying molecular basis based on alterations in the expression profiles of candidate genes contributing to phytohormone biosynthesis and defense responses. These results will provide more experimental evidence for applying HVEF exposure to seeds for short durations with the aim of rescuing aged seeds and improving pest control.

2. Materials and Methods

2.1. Test Insect Maintenance

A single colony of apterous *S. miscanthi* specimens originally collected from a field of wheat around Yangling (37°14′ N, 108°04′ E) was used for the entire experiment. After they were brought back to the Laboratory of Insect Physiology and Ecology at Yan'an University, the aphid specimens were reared on seedlings of wheat XN979 in a growth chamber under 16 h light and 8 h dark conditions with diurnal temperatures of 22 ± 1 °C and relative humidity of 70 ± 5%. To guarantee the vigor of aphids, approximately 10 adult apterous aphids were individually transferred to the newly cultured wheat seedlings in their three-leaf stage every two weeks and watered regularly as needed.

2.2. Plant Resources and Physical Treatments

For each treatment, more than 100 seeds of the wheat cv. AK58 (stored in a fridge at 4 °C for two years), of similar sizes and moisture contents, were exposed to HVEFs at 5 kV/cm for 5, 20, 30, 35, or 50 min, respectively. Seeds not exposed to HVEFs were used as the control. The HVEF generator (WJ-II, 0–100 kV output voltage) used in this study was purchased from Wuxi Boya Electronic Technology Co., Wuxi, China. Two parallel square aluminum plates (50×50 cm^2; distance between two plates, 8.0 cm) are installed in a wooden frame to form an electrical field. The output wires from the HVEF generator were connected to the top layer of aluminum plate, while a ground wire was connected to the bottom layer. A protecting earthing wire was connected the field to avoid electrostatic damage. Prior to HVEF exposure, a layer of wheat seeds was placed in uncovered Petri dishes for each treatment, and keep the ventral groove of seed faces upward. Subsequently these uncovered Petri dishes were placed into the bottom layer for HVEF exposure. HVEF exposure was conducted according to the method described by [14]. After that, some of the seeds were soaked in deionized–distilled (dd) H_2O, and the remaining seeds were cultivated in plastic pots ($9 \times 9 \times 10$ cm) filled with a soil and seedling substrate mixture (purchased from Zhonghe Agricultural, Changchun, China, N-P-K ≥ 2%, organic matter ≥ 40%, conductivity ≤ 3, pH = 6–7) and grown in the greenhouse conditions described previously. To avoid unnecessary pest infestation, each pot was immediately and individually covered with a transparent insect-rearing cage (height 30 cm, diameter 8 cm) with a fine mesh (200 nylon mesh sieve). Seedlings were watered regularly as needed from the base, and no further fertilizer was used during the experiment. Seedlings that had undergone three days of water cultivation were used for further gene transcription analysis, while plants at the three-leaf stage (BBCH 13, 12–13 days after germination) were used in the life table analysis [15].

2.3. Life Table of Aphids

An age-stage, two-sex life table was employed to assess alterations in the performance of *S. miscanthi* when feeding on the wheat plants grown from seeds pre-exposed to HVEF at 5 kV/cm for 5 and 50 min. Each treatment was set to 30 biological replicates. When the wheat plants reached the three-leaf stage, only one seedling, each of a similar plant size, was kept in each pot, and the other extra seedlings were individually removed from each pot. Newly born (within 24 h after birth) apterous first-instar *S. miscanthi* nymphs were individually transferred onto the first leaf of the wheat plant in each treatment using a fine-hair brush. Daily observations and raw data records were conducted according to the

method described by [12]. The life table parameters of the population growth, including net reproductive rate (R_0), intrinsic natural increase rate (r), mean generation time (T), and finite increase rate (λ), of *S. miscanthi* were considered in the current life table analysis. The definitions, explanations, and equations of the above parameters were described in [16], and the parameters of *S. miscanthi* were calculated and analyzed using the TWOSEX-MSChart software (Ver. 04/26/2022).

2.4. Gene Expression Profile Analysis

To elucidate whether the elevation in CAT activity improved auxin accumulation in plant tissues grown from seeds pre-exposed to HVEFs for 5, 20, 30, 35, and 50 min, the expression profiles of genes associated with auxin biosynthesis were analyzed. The genes considered in the current study included *TaTAA*, *TaYUC2*, *TaYUC9*, *TaTDC*, *TaALDO*, *TaFMO1a*, *TaAAO1*, *TaTSA1*, *TaTSB*, *TaCAD*, etc. Among them, *TaTAA*, *TaYUCs*, and *TaALDO* are involved in the indole-3-pyruvate (IPA) pathway, while *TaTDC*, *TaAAO1*, and *TaFMO1a* are involved in the tryptamine (TAM) pathway [16]. The *TaTSA1* and *TaTSB* genes were used as the marker genes for determining L-tryptophan (L-TRP) accumulation [17], a dominant building block for auxin biosynthesis, and the *TaCAD* gene was used as the marker gene for determining auxin accumulation because its transcript level is conversely affected by indole-3-acetic acid (IAA) level [18]. In addition, TAM could be converted into serotonin and melatonin in wheat plants, and we analyzed the transcript levels of related genes as well, including *TaT5H*, *TaCYP71A1*, *TaASMT*, *TaSNAT4*, etc. [19]. The primers were synthesized by Sangon Biological Technology (Shanghai, China) and are listed in Table S1. In order to exhibit the largest effects of direct HVEF exposure, wheat seedlings grown in water cultivation for three days were used in this experiment. Approximately 0.5 g of wheat leaves were collected from each HVEF-treated plant, and then immediately frozen in liquid nitrogen and stored in a −80 °C freezer for total RNA extraction. An Ultrapure RNA extraction kit purchased from Jiangsu Cowin Biotech Co., Ltd. (Taizhou, China) was employed to isolate the total shoot RNA, strictly following the instructions in the reference manual. After ascertaining the purity, quality, and integrity of the total RNA using a Nano-300 spectrophotometer (Hangzhou Allsheng Instruments Co., Ltd., Hangzhou, China) and agarose gel electrophoresis, approximately 2 μg of total RNA in each sample was reverse-synthesized to first-strand cDNA using the REALscript RT Master Mix (+gDNA Remover) (Belong Biotech Co., Ltd., Shanghai, China), and 20 μL reaction mixtures were prepared using the instructions in the reference manual. To prevent genomic DNA (gDNA) interference, we first extended the duration of the gDNA removal stage at 25 °C for 1 h, and the subsequent cDNA synthesis stage strictly followed the instructions in the reference manual. Then, after diluting the reverse transcription reaction mixture five times and adjusting the concentration of cDNA to around 100 ng/μL, the gene expression profiles were determined. The *TaGAPDH* gene was used as an endogenous reference gene. The Kit (SuperStar Universal SYBR Master Mix) purchased from Jiangsu Cowin Biotech Co., Ltd. (Taizhou, China) was employed to assess the expression profiles of these genes. Real-time quantitative PCR (RT-qPCR) was performed using the SLAN-96S real-time PCR system (Shanghai Hongshi Medical Technology Co., Ltd., Shanghai, China). The 20 μL reaction mixtures consisted of 1.0 μL of the first-strand cDNA reaction product, 10.0 μL of 2 × 2 × SuperStar Universal SYBR Master Mix, 0.4 μL of each primer pair (10 μM), and 8.2 μL of ddH_2O. The expression profiles of each gene were expressed with its relative expression levels, and calculated using the $2^{-\Delta\Delta Ct}$ method [20]. The relative expression level of the controls was arbitrarily set to 1. Three independent biological replicates were set to calculate the mean and standard error of the relative expression levels of each gene.

Because auxin might positively affect JA accumulation but adversely alleviate SA-mediated defenses, we analyzed the transcriptional profiles of the marker genes associated with JA and SA biosynthesis, as well as with signaling pathways. Among these genes, *TaLOX2*, *TaAOS2* and *TaAOC* are crucial for the biosynthesis of JA, and the *TaPDF1.2* and pathogenesis-related protein (*PR*)-*4* genes usually play important roles in JA-mediated defense responses, while the *TaPAL* and *TaICS2* genes are involved in SA synthesis and genes including *TaPR-1a* and the non-expressor of PR genes 1 (*TaNPR1*) are involved in SA-mediated defense responses. Moreover, to verify whether HVEF-triggered phytohormone accumulation affected the plant's defense against the aphid, we assessed the relative expression levels of some of the abovementioned genes in three-leaf-stage AK58 seedlings at 3 h following the foliage application of 2.5 mM MeJA, 0.5 mM SA, or 0.5 mM IAA, which were purchased from Shanghai Macklin Biochemical Technology Co., Ltd., Shanghai, China. The phytohormone treatments were conducted according to the method described by [12]. The expression profile for these genes was analyzed as described previously.

2.5. Data Analyses

The bootstrap technique with 100,000 resamplings was employed to estimate the standard error of mean (SEM) of the parameters involved in the performance experiment of *S. miscanthi*, and to compare the difference between different durations using the paired bootstrap test with the TWOSEX-MSChart software [21–23]. One-way analysis of variance (ANOVA) was employed to compare the relative gene expression levels of wheat plants grown from seeds either previously exposed to HVEFs or unexposed. The multiple comparisons between different treatments were performed using Tukey's multiple comparison test with GraphPad Prism (Ver. 11.0, GraphPad Software, Boston, MA, USA), as shown in figures and figure legends. Data are presented as mean ± SEM from at least three independent biological replicates. Differences were considered statistically significant at $p < 0.05$. All graphs were prepared using GraphPad Prism.

3. Results

3.1. Seed Pre-Exposure to HVEFs Positively Affected the Population Dynamic Parameters of S. miscanthi

The age-stage, two-sex life table of *S. miscanthi* revealed that when they fed on the wheat plants grown from cv. AK58 seeds pre-exposed to HVEFs at 5 kV/cm for 5 min and 50 min, a positive effect was seen on their population dynamic parameters (Figure 1). In comparison with controls, the pre-exposure of seeds to HVEFs significantly increased the r and λ, but shortened the T parameter of *S. miscanthi*. In addition, the 50 min HVEF exposure exhibited a more positive effect on these parameters than the 5 min treatment duration. However, while the R_0 parameter at the 50 min treatment duration exhibited the lowest value, and the largest R_0 value was present in the 5 min treatment, neither of them exhibited a significant difference from the controls.

The stage-specific survival rate (l_x) curve also supports the finding that AK58 seeds' pre-exposure to HVEFs improved the performance of *S. miscanthi* (Figure 2). In particular, although the survival rate of adult *S. miscanthi* in the 50 min treatment exhibited a similar profile to that of the controls at the ages of 9 to 21 days, the subsequent survival rate of the controls showed a rapid decline in comparison. Although the survival rate of aphids at 18–21 days during the 5 min treatment was lower than that of the controls, the curve showed a slower decline and longer life spans for these aphids than the controls. Moreover, the l_x curve of aphids in the 50 min HVEF treatment showed the longest life spans, and its value was 39 days.

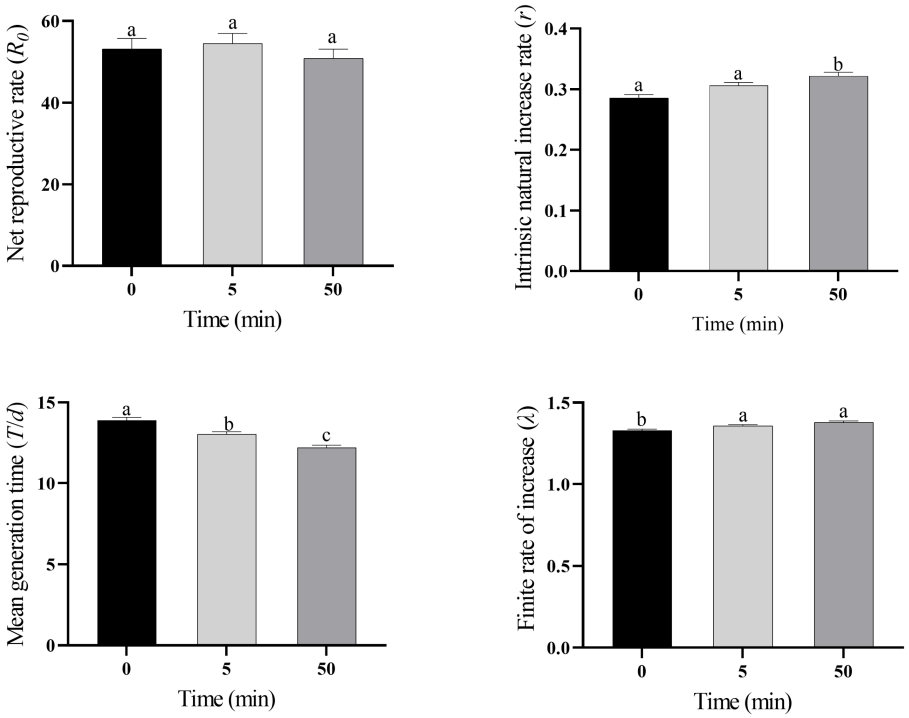


Figure 1. The population dynamic parameters of *S. miscanthi* when feeding on AK58 wheat seedlings grown from seeds with HVEF exposure at 5.0 kV/cm for 0, 5, and 50 min. Note: Different lowercase letters indicate significant differences at $p < 0.05$ between different durations. Differences were compared using a paired bootstrap test.

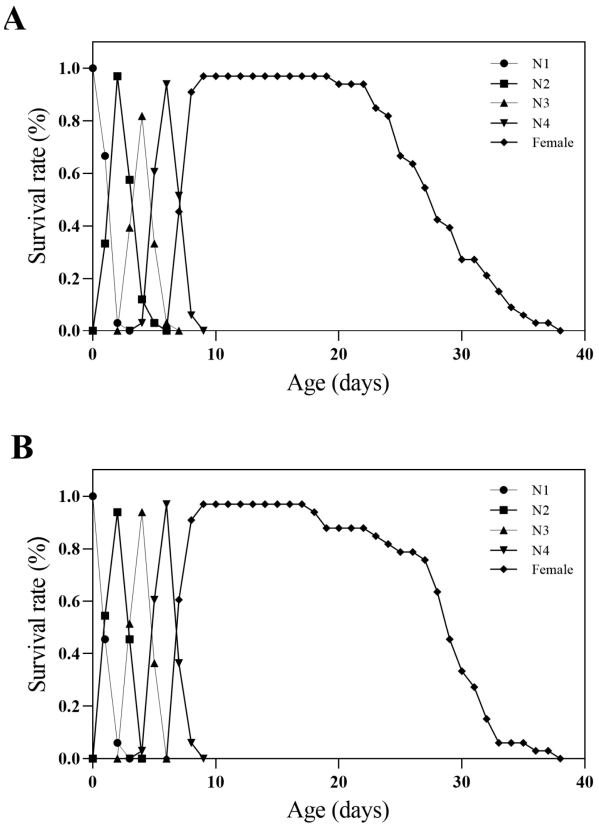


Figure 2. *Cont.*

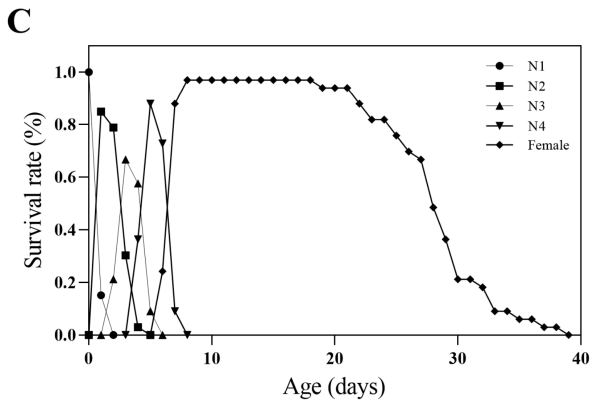


Figure 2. The stage-specific survival rate (l_x) of *S. miscanthi* when feeding on AK58 wheat seedlings grown from seeds with HVEF exposure at 5.0 kV/cm for 0, 5, and 50 min. (**A**) Control, 0 min; (**B**) 5 min; (**C**) 50 min.

3.2. Seed Pre-Exposure to HVEFs Did Not Trigger the Upregulation of the Genes Associated with Plant Immunity Responses in AK58 Seedlings

For JA-dependent signaling, seed pre-exposure to HVEFs significantly upregulated the transcription of JA biosynthesis genes, including *TaAOS2* and *TaAOC*, and slightly induced the transcription of the *TaLOX2* gene, while HVEF exposure for 5 min treatment slightly suppressed its transcription (Figure 3). When compared with the controls, HVEF exposure for the 50 min duration did not induce any significant upregulation of the above genes, while the 5 min treatment significantly upregulated the expression of the *TaAOS2* gene. Meanwhile, HVEF exposure for 35 min significantly upregulated the expressions of *TaAOS2* and *TaAOC* genes. As expected, the transcription of the JA-dependent response gene *TaPDF1.2* was also upregulated, and the 35 min treatment led to the greatest upregulation, while the expression of the *TaPR-4* gene was markedly suppressed under the other HVEF durations, except 35 min (Figure 3). Although the transcription of the SA biosynthesis gene *TaPAL* was adversely affected, *TaICS2* was significantly upregulated under HVEF exposure (Figure 4). Similarly, the *TaNPR1* gene was significantly induced under HVEF exposure at all set durations in the present study, whereas the relative expression level of the SA-mediated response gene *TaPR-1a* was not significantly induced in the AK58 seedling under HVEF treatment.

We further characterized whether seed pre-exposure to HVEF affected the plants' immunity responses, and found that the treatment didnot exhibit the adverse effect on the transcripts of *TaWRKY26* genes involved in the activation of the plant immunity responses, while its positive regulator protein-encoding gene *TaVQ22* was significantly suppressed (Figure 4). In particular, the relative expression levels of both genes under HVEF exposure at 5 min and 50 min exhibited the lowest and second lowest values, respectively, while the relative expression level of the *TaVQ22* gene at 20, 30, 35, and 50 min did not show significant differences from the controls. These results revealed that seed pre-exposure to HVEF might trigger defense-hormone-mediated responses in plant seedlings, but would not result in plant immunity responses.

3.3. Seed Pre-Exposure to HVEF Might Trigger Auxin Accumulation Through Biosynthesis of IAA in AK58 Seedlings

Initially, we found that transcription of the *TaCAD* gene was significantly downregulated in wheat shoot tissue grown from seeds pre-exposed to HVEF, which reveals that lignin biosynthesis might be suppressed as a result of IAA accumulation in plant tissue (Figure 5).

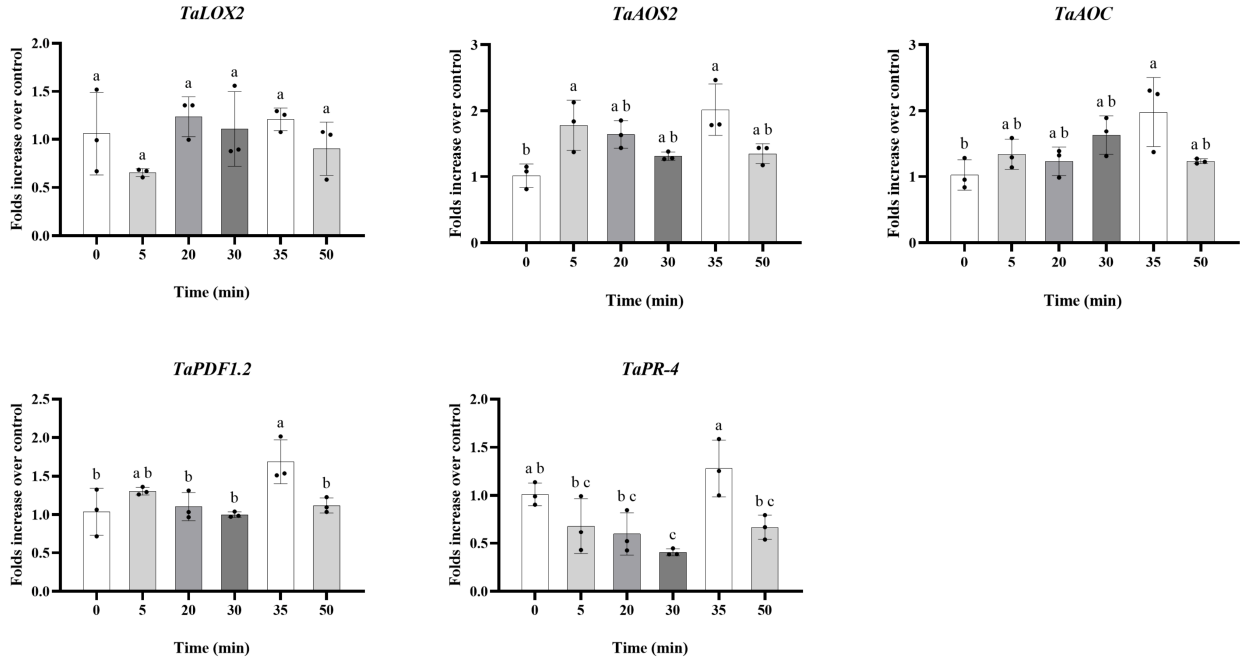


Figure 3. Effects of different durations of HVEF exposure on the relative expression level of genes associated with JA biosynthesis and responses in AK58 seedlings. Different lowercase letters indicate significant differences ($p < 0.05$) among different durations. The significant differences were determined by one-way ANOVA followed by Tukey's multiple comparison test (*TaLOX2*: $F_{5,12} = 1.826$, $p = 0.1821$; *TaAOS2*: $F_{5,12} = 5.898$, $p = 0.0056$; *TaAOC*: $F_{5,12} = 4.116$, $p = 0.0208$; *TaPDF1.2*: $F_{5,12} = 5.370$, $p = 0.0080$; *TaPR-4*: $F_{5,12} = 7.215$, $p = 0.0025$).

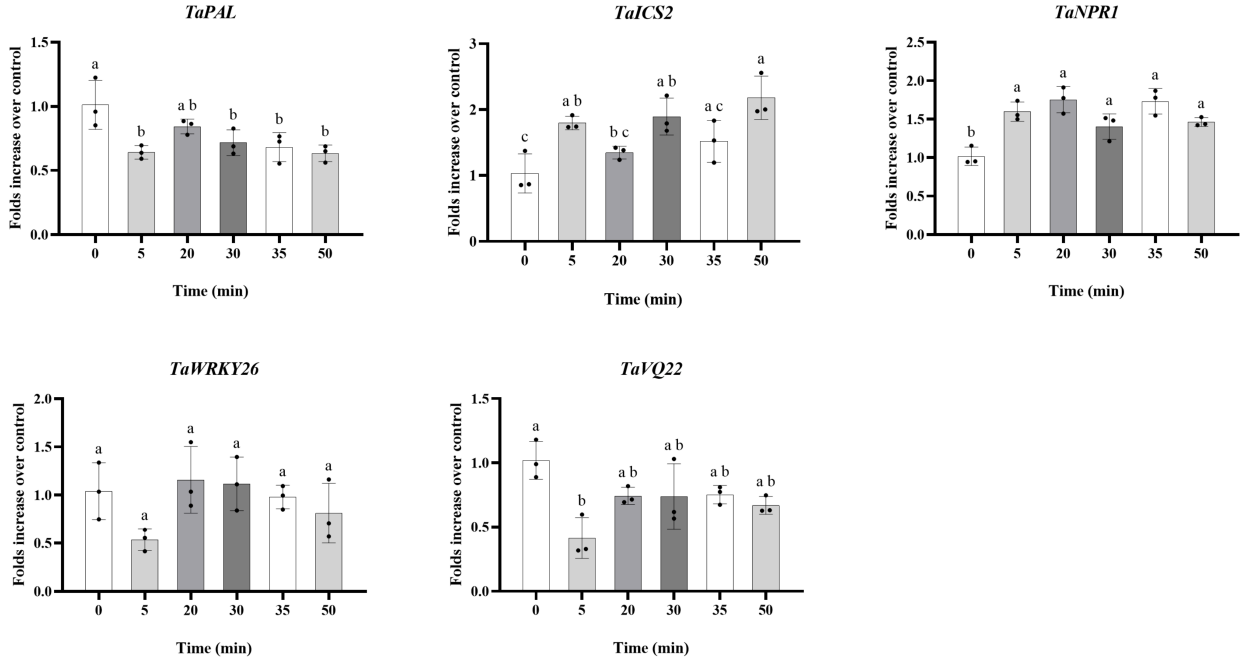


Figure 4. Effects of different durations of HVEF exposure on the relative expression level of genes associated with SA biosynthesis and responses and plant immunity in AK58 seedlings. Different lowercase letters indicate significant differences ($p < 0.05$) among different durations. The significant differences were determined by one-way ANOVA followed by Tukey's multiple comparison test (*TaPAL*: $F_{5,12} = 5.645$, $p = 0.0066$; *TaICS2*: $F_{5,12} = 7.749$, $p = 0.0018$; *TaNPR1*: $F_{5,12} = 11.410$, $p = 0.0003$; *TaWRKY26*: $F_{5,12} = 2.382$, $p = 0.1013$; *TaVQ22*: $F_{5,12} = 5.371$, $p = 0.0080$).

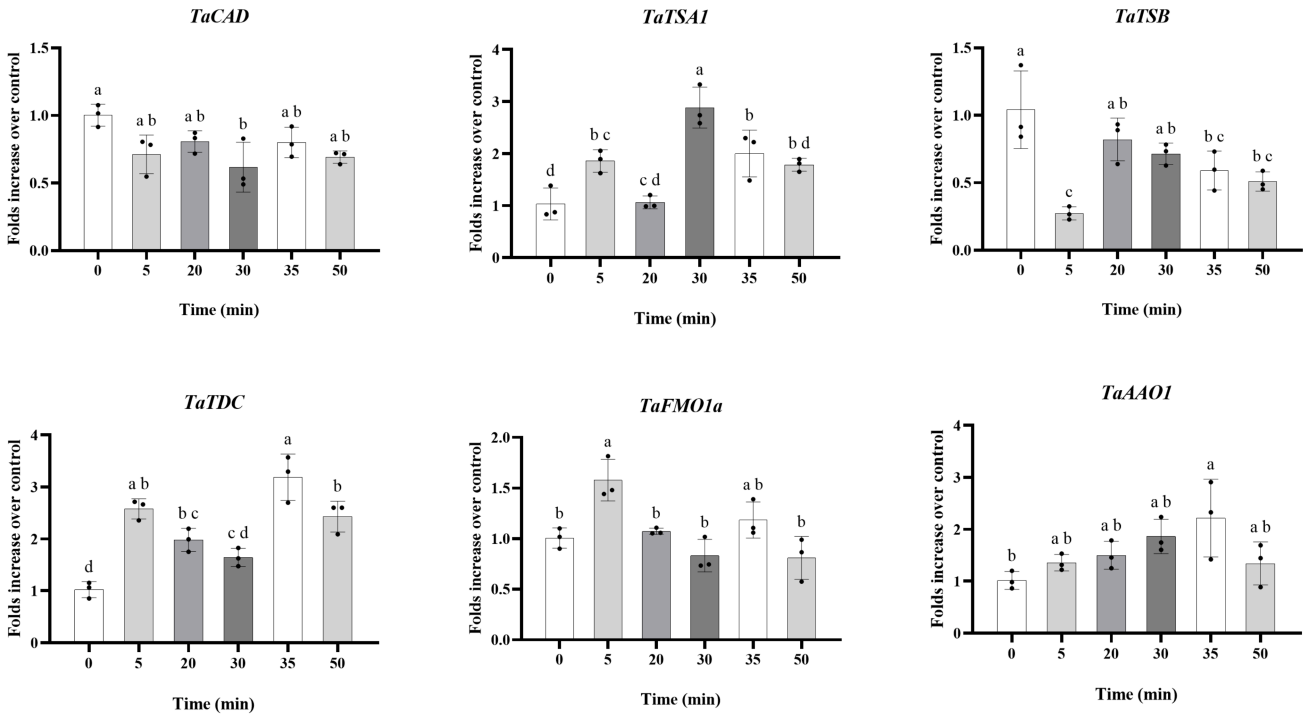


Figure 5. Effects of different durations of HVEF exposure on the relative expression level of genes associated with putative accumulation of IAA and TAM-IAA biosynthesis in AK58 seedlings. Different lowercase letters indicate significant differences ($p < 0.05$) among different durations. The significant differences were determined by one-way ANOVA followed by Tukey's multiple comparison test (*TaCAD*: $F_{5,12} = 3.913$, $p = 0.0245$; *TaTSA1*: $F_{5,12} = 15.980$, $p < 0.0001$; *TaTSB*: $F_{5,12} = 8.902$, $p = 0.0010$; *TaTDC*: $F_{5,12} = 24.430$, $p < 0.0001$; *TaFMO1a*: $F_{5,12} = 9.175$, $p = 0.0009$; *TaAAO1*: $F_{5,12} = 3.397$, $p = 0.0384$).

In addition, the transcription of the L-TRP synthase gene *TaTSA1* was significantly upregulated, whereas *TaTSB* was extensively suppressed under HVEF treatment (Figure 5). Similarly, the transcription of the *TaTDC* gene was also significantly elevated, which may have resulted in the accumulation of TAM (Figure 5). While the transcription of the *TaFMO1a* gene was significantly upregulated only in the 5 min treatment, the other treatments did not lead to a significant difference. Furthermore, the *TaAAO1* gene (putative encoding amino-oxidase to convert TAM to IAAld) was induced, and its relative expression level under HVEF exposure at 35 min exhibited significant differences from the control.

Moreover, the *TaTAA* gene was significantly promoted at other durations, but not during the 5 min treatment, and resulted in the accumulation of IPA (Figure 6). Subsequently, *TaYUC9* gene was slight induced for the conversion of IPA into IAAld, and *TaYUC2* was slight induced as well, except 20 and 50 min. Both genes was significantly induced in the 35 min treatment. However, the transcription of *TaALDO* (putative encoding IAAld dehydrogenase) did not exhibit a significant increase when compared with the control. Meanwhile, the transcription of *TaPAE3*, which probably negatively regulated the IPA-dependent auxin biosynthetic pathway, was significantly alleviated under HVEF exposure, and the 50 min treatment exhibited the lowest value. However, the transcription of its negative regulator *TaNAC73* gene did not show an increasing trend, but was significantly decreased under that treatment.

The distinct expression profiles for the *TaFMO1a*, *TaAAO1*, and *TaTAA* genes suggest that the HVEF pre-exposure of seeds might activate different auxin biosynthetic pathways, leading to IAA accumulation, under different durations of HVEF treatment (Figures 5 and 6).

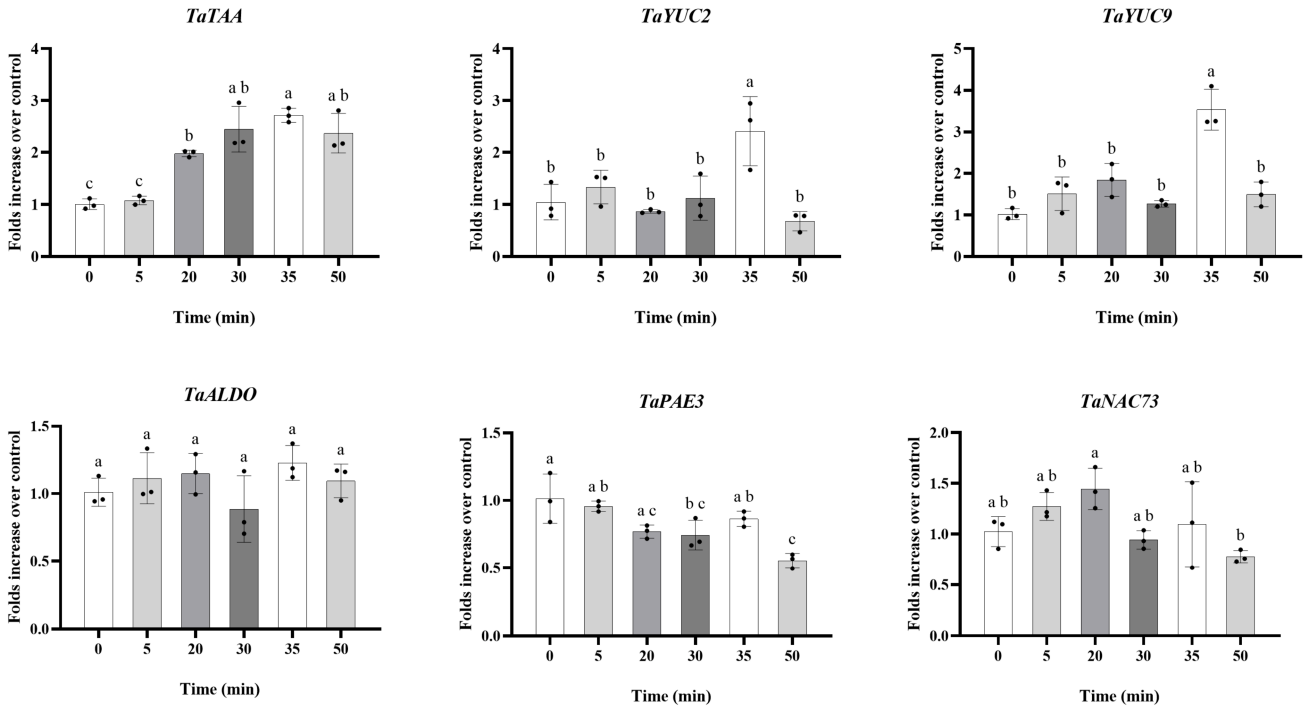


Figure 6. Effects of different durations of HVEF exposure on the relative expression level of marker genes associated with IPA-IAA biosynthesis and upstream regulation in AK58 seedlings. Different lowercase letters indicate significant differences ($p < 0.05$) among different durations. The significant differences were determined by one-way ANOVA followed by Tukey's multiple comparison test (*TaTAA*: $F_{5,12} = 25.360$, $p < 0.0001$; *TaYUC2*: $F_{5,12} = 7.717$, $p = 0.0019$; *TaYUC9*: $F_{5,12} = 21.990$, $p < 0.0001$; *TaALDO*: $F_{5,12} = 1.562$, $p = 0.2435$; *TaPAE3*: $F_{5,12} = 8.973$, $p = 0.0010$; *TaNAC73*: $F_{5,12} = 3.771$, $p = 0.0277$).

3.4. Pre-Exposure of Seeds to HVEFs for 50 min Might Suppress Auxin Degradation, Increasing IAA Levels

Auxin homeostasis has been reported to play an important role in promoting wheat defense [24–26], and we therefore evaluated the transcription alterations of crucial genes related to auxin degradation. When compared with controls, the transcription of genes associated with IAA degradation, including *TaGH3.8* and *TaILR1*, were seriously decreased, especially following HVEF exposure for 50 min; however, the transcript alterations of *TaGH3.8* and *TaILR1* under the 5 min treatment exhibited distinct changes and showed a similar level to the controls (Figure 7). Meanwhile, the transcripts of neither genes exhibited significant differences from the controls under treatment for other durations. Moreover, the transcription of the *TaGH3.8*-positive regulating transcription factor *TaWRKY75* gene was highly repressed, and HVEF exposure at 5 min and 50 min exhibited the lowest and second lowest values, respectively. However, these two durations did not exhibit significant differences. The transcription of its transcriptional coactivator *TaVQ16* showed similar alterations (Figure 7). These results suggest that seed pre-exposure to HVEF might suppress the TaVQ16-TaWRKY75-TaGH3.8 module's mediation of auxin homeostasis and result in the accumulation of auxin.

3.5. Seed Pre-Exposure to HVEF for 5 min Might Induce the Accumulation of Serotonin Levels in Seedling Tissue

Because the transcription of the genes involved in the TAM-dependent auxin biosynthesis pathway did not exhibit similar alterations to the *TaTDC* gene, we further assessed the transcription of genes involved in the conversion of TAM into serotonin and melatonin in plants. We found that the transcription of *TaT5H* and *TaCYP71A1* genes (both putative

encoding tryptamine 5-hydroxylase) exhibited the similar alternations under HVEF exposure, while the transcription of *TaT5H* did not exhibit a significant increase when compared with the control. The relative expression levels of both genes under HVEF exposure at 5 min showed the largest value, whereas the transcription of the 50 min treatment was not induced and exhibited the lowest value (Figure 8). Meanwhile, the relative expression of the genes involved in the conversion of serotonin to melatonin, including *TaASMT* and *TaSNAT4*, was notably induced. In particular, *TaASMT* genes were significantly induced under HVEF exposure at all set durations when compared with controls, while the relative gene expression level of *TaSNAT4* gene did not show significant differences under these treatments (Figure 8). Moreover, the transcription of both the *TaASMT* and *TaSNAT4* genes under the 35 min treatment was the highest.

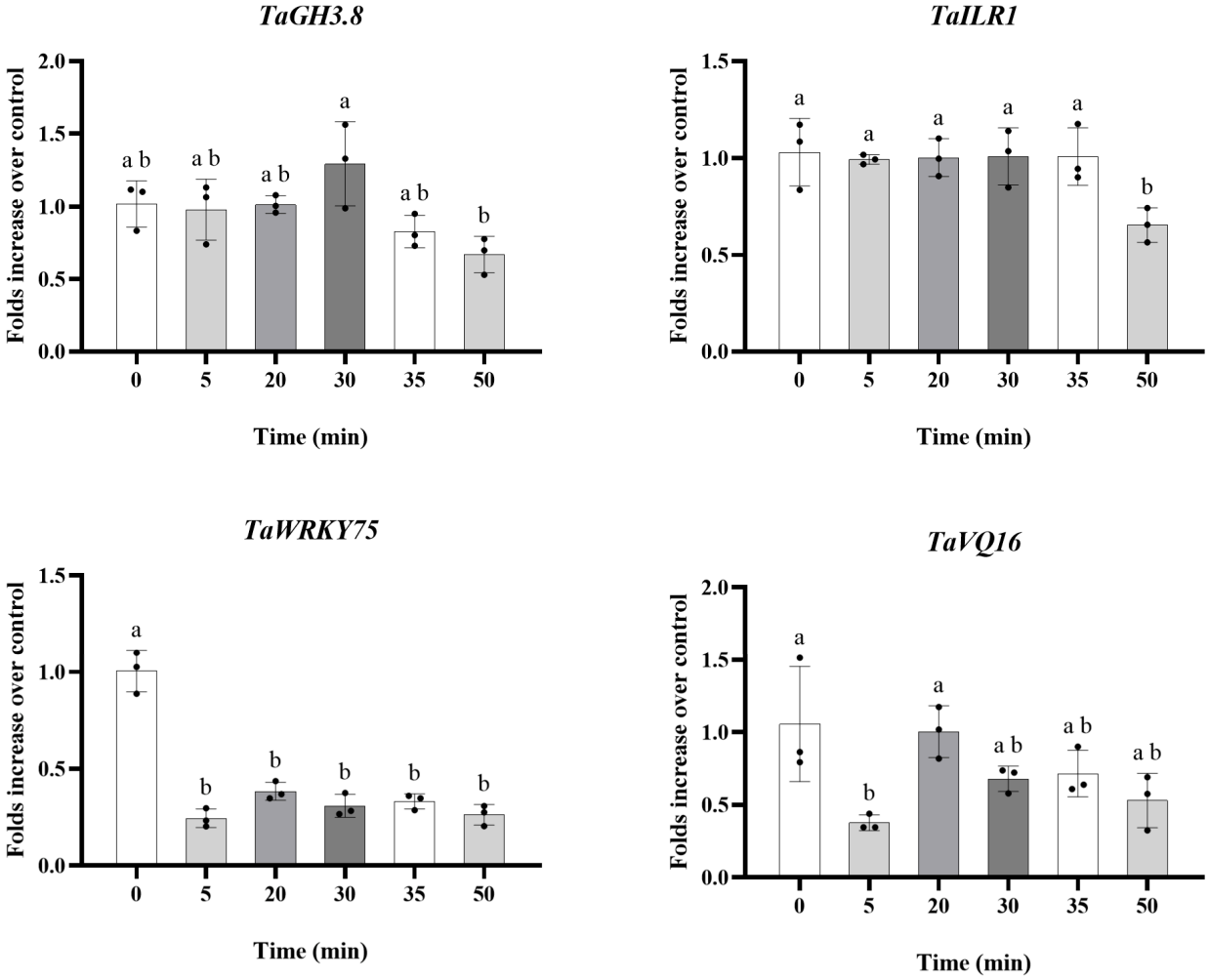


Figure 7. Effects of different durations of HVEF exposure on the relative expression level of genes associated with IAA degradation and upstream regulation in AK58 seedlings. Different lowercase letters indicate significant differences ($p < 0.05$) among different durations. The significant differences were determined by one-way ANOVA followed by Tukey's multiple comparison test (*TaGH3.8*: $F_{5,12} = 4.281$, $p = 0.0182$; *TaILR1*: $F_{5,12} = 4.136$, $p = 0.0204$; *TaWRKY75*: $F_{5,12} = 63.210$, $p < 0.0001$; *TaVQ16*: $F_{5,12} = 4.818$, $p = 0.0120$).

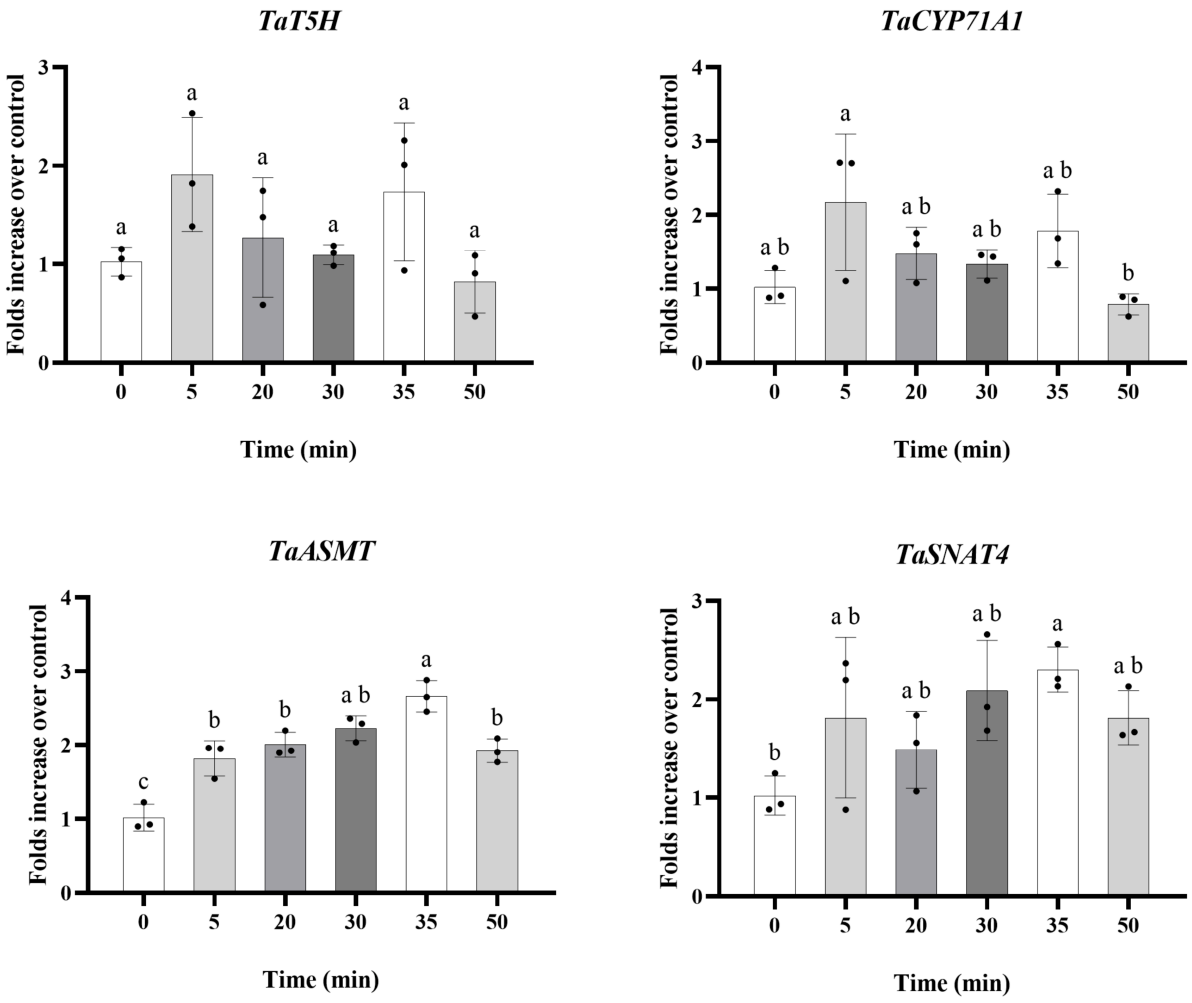


Figure 8. Effects of different durations of HVEF exposure on the relative expression level of genes associated with serotonin and melatonin via TAM in AK58 seedlings. Different lowercase letters indicate significant differences ($p < 0.05$) among different durations. The significant differences were determined by one-way ANOVA followed by Tukey's multiple comparison test (*TaT5H*: $F_{5,12} = 2.465$, $p = 0.0930$; *TaCYP71A1*: $F_{5,12} = 3.408$, $p = 0.0380$; *TaASMT*: $F_{5,12} = 24.500$, $p < 0.0001$; *TaSNAT4*: $F_{5,12} = 2.974$, $p = 0.0566$).

3.6. Pre-Exposure of Seeds to HVEFs Might Alleviate the Plant's Defenses Through Extensive Accumulation of IAA

The relative gene expression levels revealed that the direct foliage spraying of 0.5 mM IAA on AK58 seedlings seriously suppressed the transcripts of genes involved in SA biosynthesis and defense, including *TaICS2*, *TaNPR1*, and *TaPR-1a*, and the plant lignin biosynthesis gene *TaCAD* (Figures 9 and 10). Meanwhile, unlike MeJA and SA treatment, it slightly suppressed the expression of the *TaWRKY26* gene, and it did not seriously affect the JA biosynthesis gene, *TaLOX2*. In addition, IAA treatment significantly elevated L-TRP biosynthesis and conversion genes, including *TaTSA1*, *TaTDC*, and *TaTAA*; however, the relative expression of the *TaYUC9* and *TaTSB* genes was significantly suppressed at 3 h postspraying (Figure 10). Moreover, the direct foliage spraying of 2.5 mM MeJA or 0.5 mM SA notably induced the expression of the marker genes in their corresponding pathways, and MeJA treatment triggered a stronger expression of *TaTSA1* and *TaTDC* genes.

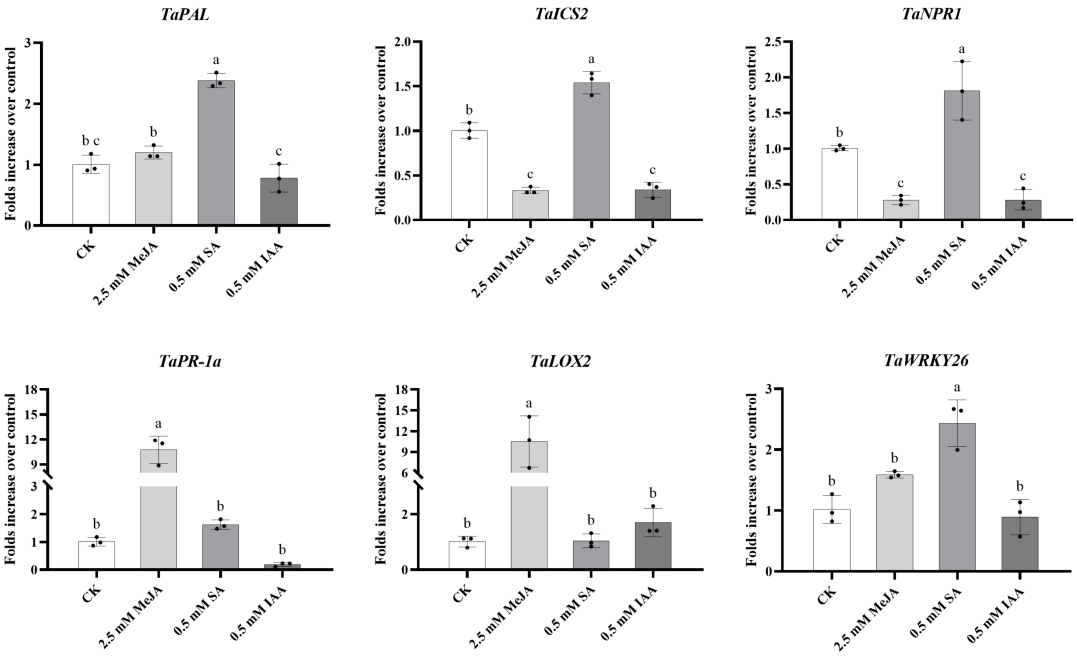


Figure 9. Effects of direct foliage spraying of phytohormones on the relative expression level of marker genes associated with JA and SA biosynthesis and responses in AK58 seedlings at 3 h postsparying. Different lowercase letters indicate significant differences ($p < 0.05$) among different phytohormone treatments. The significant differences were determined by one-way ANOVA followed by Tukey's multiple comparison test (*TaPAL*: $F_{3,8} = 61.030$, $p < 0.0001$; *TaICS2*: $F_{3,8} = 128.700$, $p < 0.0001$; *TaNPR1*: $F_{3,8} = 32.840$, $p < 0.0001$; *TaPR-1*: $F_{3,8} = 106.200$, $p < 0.0001$; *TaLOX2*: $F_{3,8} = 18.440$, $p = 0.0006$; *TaWRKY26*: $F_{3,8} = 20.760$, $p = 0.0004$).

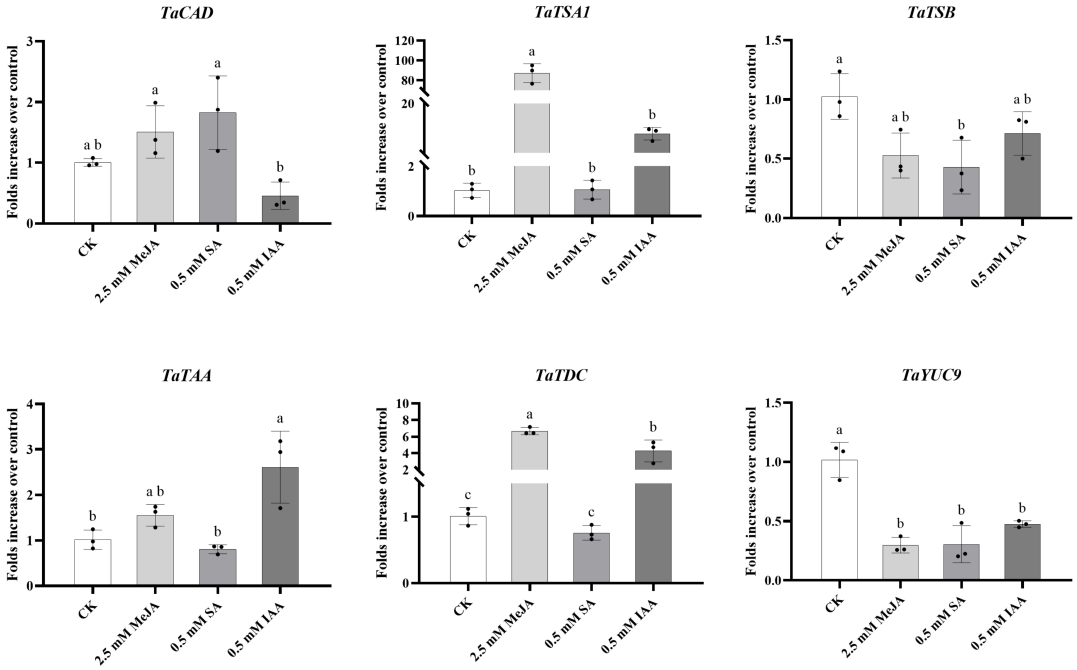


Figure 10. Effects of direct foliage spraying of phytohormones on the relative expression level of marker genes associated with IAA accumulation in AK58 seedlings at 3 h postsparying. Different lowercase letters indicate significant differences ($p < 0.05$) among different phytohormone treatments. The significant differences were determined by one-way ANOVA followed by Tukey's multiple comparison test (*TaCAD*: $F_{3,8} = 7.069$, $p = 0.0122$; *TaTSA1*: $F_{3,8} = 222.600$, $p < 0.0001$; *TaTSB*: $F_{3,8} = 5.224$, $p = 0.0274$; *TaTAA*: $F_{3,8} = 10.580$, $p = 0.0037$; *TaTDC*: $F_{3,8} = 48.520$, $p < 0.0001$; *TaYUC9*: $F_{3,8} = 26.660$, $p = 0.0002$).

4. Discussion

Our results revealed that the physiological alterations in AK58 seedlings triggered by seed pre-exposure to HVEF at 5 kV/cm did not exhibit resistance to *S. miscanthi*, but positively affected aphid performances. This is not consistent with our previous studies, where directly pre-exposing of wheat seeds of XY22 to HVEF at 4 kV/cm or vegetable seeds to HVEF at 5 kV/cm adversely affected aphid and moth performance, especially at a 20 min duration, respectively [6]. Regarding activity changes in antioxidant enzymes, both treatments induced negative effects on CAT activity, while our latest studies revealed that indirect HVEF treatment significantly elevated CAT activity in leaf tissue of AK58 seedlings, with a larger value with increased duration. The reverse-genetics experimental evidence demonstrated that the *AtCAT2* mutation plants exhibited lower amounts of L-TRP and IAA [8]. Similarly, HVEF pre-exposure for 35 min caused the largest CAT activity value in plant tissue, and the transcription of *TaTAA* and *TaTDC* genes showed the largest value as well, which might have been caused by the extensive accumulation of IAA through either IPA or TAM pathways [27,28]. In addition, the gene expression analysis in the present study revealed that HVEF treatment significantly elevated the *TaTSA1* gene, while the transcription of the *TaTSB* gene was significantly suppressed. This is not consistent with the results achieved by Yuan and colleagues, because the TSB activity was significantly alleviated in the *AtCAT2* mutant [8]. Our results revealed that the direct foliage spraying of SA significantly upregulated the transcription of the *TaTSB* gene, while JA treatment significantly elevated the *TaTSA1* gene's expression in AK58 seedlings. Meanwhile, we found that the crucial marker genes associated with JA-biosynthesis and signaling were also upregulated, especially under HVEF treatment for 35 min, where the largest values were seen. This suggests that the L-TRP level affected by the elevated CAT activity and the mediated JA accumulation are induced by TSA under HVEF exposure in AK58 seedlings, and not by TSB, which is probably because the SA level is suppressed. The upregulation of the transcript of the *TaTDC* gene in wheat shoot tissue is supported by seeds' pre-exposure to HVEFs triggering the conversion of L-TRP into TAM, as a result of JA accumulation during seedling post-germinative growth [12]. The downregulation of the transcript of the *TaPAL* gene in wheat shoot tissue also supports the previous suggestion. However, the transcription of the SA biosynthesis gene *TaICS2* was significantly elevated, especially in the 50 min treatment. The relative expression level of the *TaNPR1* gene was significantly upregulated as well. Interestingly, the transcript of the *TaNPR1* gene had been markedly induced, whereas the relative levels of the genes associated with either SA-mediated defenses or the plant immunity responses were not elevated [29]. It has been reported that NPR1 can stimulate SAUR63-EXPs-module-modulated hypocotyl elongation and growth in *A. thaliana* during early seedling growth in darkness [30]. Accordingly, HVEF-treatment-triggered SA accumulation derived from the isochorismate (ICS) pathway could positively affect the post-germination growth of AK58 seeds. Therefore, the elevation of CAT activity under pre-exposure of HVEF to AK58 seeds might adversely affect plant resistance by triggering L-TRP-dependent metabolites or JA accumulation [4,8,12,19,31–33].

In combination with the experimental evidence regarding the downregulation of the transcript of the *TaCAD* gene in wheat shoot tissue grown from seeds pre-exposed to HVEF, we proposed an effect on IAA accumulation in plant tissue [18]. The transcriptional profiles of corresponding auxin biosynthesis genes tested in the present study supported this speculation; however, the distinct auxin biosynthetic pathways were activated under HVEF treatments at different durations. For instance, the relative expression levels of *TaTAA* and *TaFMO1a* revealed that the 50 min treatment induced the IPA pathway, while the 5 min treatment triggered the TAM pathway to accumulate IAA. It demonstrated that the IPA pathway is the dominant route for auxin biosynthesis in most organisms [34], and the

experimental evidence in alfalfa revealed that the MsNAC73-MsPAE12 module modulated the IPA pathway for auxin accumulation [35]. Similarly, we found that the transcription of its homologous gene in wheat, *TaPAE3*, was significantly suppressed in the 50 min treatment, while 5 min exposure did not affect the relative expression of this gene. Surprisingly, the transcription of its negative regulator *TaNAC73* gene did not show the increasing trend seen in the alfalfa plant, but was significantly depressed. More work is required to unravel the underlying mechanisms of IAA accumulation through the IPA pathway in wheat plants. Furthermore, we found that HVEF at 5 min duration could trigger the accumulation of serotonin via notable elevation of the transcription of encoding genes, including *TaT5H* and *TaCYP71A1*. A previous study demonstrated that the mutation of *CYP71A1* in rice could strengthen its resistance to rice brown planthopper (BPH; *Nilaparvata lugens* Stål) by inhibiting serotonin accumulation, which could adversely affect SA accumulation, and direct serotonin treatment could stimulate the feeding behavior of BPH [31]. Accordingly, seeds pre-exposed to HVEF at 5 kV/cm for 5 min duration demonstrated positive effects on aphid performance by triggering TAM-dependent IAA accumulation and serotonin accumulation in plant tissue.

As described previously, indole-3-acetic acid–amido (IAA-AA) synthetase GH3.8-mediated auxin homeostasis had been reported as one of the important indicators for promoting plant defenses against diverse colonizers [24,25]. However, we found that the relative expression of the *TaGH3.8* gene was significantly suppressed following the 50 min treatment and showed the lowest value. Similarly, the downstream of the IAA-AA degradation enzyme coding gene *TaILR1* exhibited the lowest value in that duration as well [36]. The suppression of the transcription of *TaGH3.8* and *TaILR1* genes in that treatment might have resulted from the accumulation of IAA, derived from either IPA and/or TAM pathways. The latest study revealed that transcription factor SlWRKY75 positively maintains GH-enzyme-mediated auxin homeostasis for tomato defense against *Pseudomonas syringae* [25]. The subsequent protein–protein interaction study revealed that SlWRKY75 interacted with the tomato valine–glutamine (VQ) motif-containing protein 16 (SlVQ16) in vivo and in vitro, and serves as a transcriptional regulatory cofactor, enhancing the SlWRKY75-mediated transcriptional activation of SlGH3.3-dependent IAA degradation [25]. Furthermore, we found that the transcription of their homologous genes in wheat *TaWRKY75* and *TaVQ16* genes was significantly alleviated. Thus, seed pre-exposure to HVEF for 50 min might suppress TaVQ16-TaWRKY75-TaGH3.8-module-mediated auxin homeostasis and result in auxin accumulation. The extensive accumulation of IAA could directly or indirectly fine-tune the SA- or JA-dependent defense responses, or compensate for their negative effects to improve the fitness of plants to aphids.

Moreover, the apple leaf senescence study revealed that MdVQ10 could interact with the homologous genes of WRKY75 in apple *MdWRKY75*, and mediated wound-induced leaf senescence [37]. This suggests that the homologous genes of MdVQ10 in wheat *TaVQ22* could interact with *TaWRKY75* as well, and its relative expression level exhibited similar alteration trends to *TaWRKY75* and *TaVQ16* genes. The 5 min treatment showed the lowest value for both genes and the *TaWRKY26* gene. It was reported that the MdVQ10-MdWRKY75 module in healthy apple leaves is suppressed by the MdJAZ12/14 protein [37], and consequently, the lower level of JA under the 5 min duration (compared with the other durations) could suppress the TaVQ16-TaWRKY75, TaVQ22-TaWRKY75, or TaVQ22-TaWRKY26 modules' modulation of plant defenses.

In conclusion, seed pre-exposure to HVEFs positively affected the population dynamic parameters of *S. miscanthi* by activating the IPA auxin biosynthetic pathways and suppressing TaVQ16-TaWRKY75-TaGH3.8-module-mediated auxin degradation, eventually resulting in auxin accumulation in the 50 min treatment, whereas the 5 min treatment

triggered the accumulation of TAM-dependent IAA and serotonin in plant tissue. The extensive accumulation of IAA or serotonin could directly or/and indirectly alleviate plant immunity responses, eventually improving the performance of *S. miscanthi*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/insects17111208/s1, Table S1: Primer sequences used for quantification of gene expression by RT-qPCR.

Author Contributions: B.J.: Investigation, Data curation, Writing—original draft. J.G.: Conceptualization, Data curation, Writing—review and editing. J.Y.: Investigation, Data curation, Writing—review and editing. Z.W.: Writing—original draft, Data curation. G.-W.L.: Writing—original draft, Data curation. B.L.: Writing—original draft, Data curation. X.C.: Writing-review and editing, Data curation. K.L.: Conceptualization, Visualization, Writing—review and editing, Supervision, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

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