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Functional Characterization of *CaSpr2* in Jasmonate-Dependent Induced Defense Against Western Flower Thrips in *Capsicum annuum*

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Simple Summary

Insect pests, such as the western flower thrip (WFT), poses a significant threat to global agriculture by damaging crops and reducing yield. Although the jasmonic acid (JA) signaling pathway is known to be involved in plant defense against WFTs, the key molecular components of this pathway in non-model crops like pepper remain poorly understood. This study functionally characterizes the role of *suppressor of prosystemin-mediated responses2* (*Spr2*) in defense response against WFTs in pepper. We demonstrated that silencing *CaSpr2* accumulated lower levels of JA and jasmonoyl-isoleucine (JA-Ile), and exhibited enhanced susceptibility to WFTs. Moreover, WFT individuals on *CaSpr2*-silenced plants exhibited enhanced fitness, including prolonged adult longevity, increased fecundity, and accelerated population growth. Our findings establish *CaSpr2* as a crucial regulator within the JA-mediated signaling pathway that is essential for resistance to WFTs in pepper. This knowledge provides a valuable genetic target for breeding improved pepper cultivars with enhanced and sustainable pest resistance, potentially reducing reliance on chemical pesticides.

Abstract

Insect infestation poses a significant threat to global agriculture by impairing plant growth and reducing crop yields. The western flower thrip (WFT) causes substantial damage through both direct feeding and transmission of plant viruses. Although the jasmonic acid (JA) signaling pathway is known to participate in plant defense against WFTs, the underlying molecular mechanisms in non-model crops such as peppers, remain largely elusive. This study investigates the role of *suppressor of prosystemin-mediated responses2* (*Spr2*) within JA-mediated defense against WFTs in pepper. Through an integrated approach employing virus-induced gene silencing (VIGS), transcription analysis, phytohormone quantification, insect behavior assays and life history investigations, we demonstrated that silencing *CaSpr2* significantly reduced JA and JA-Ile accumulation, and led to a strong feeding preference of WFTs for *CaSpr2*-silenced plants. Furthermore, the adult lifespan, survival rate, female fecundity, oviposition rate, and population parameters of WFTs were significantly improved on *CaSpr2*-silenced plants. *Spr2* functions as an essential component within the JA signaling pathway, thereby playing a critical role in conferring resistance to WFTs in cultivated pepper. These findings provide profound insights and practical implications for breeding thrips-resistant cultivars in non-model plants, through genetic manipulation of JA signaling, offering a promising avenue for sustainable agricultural pest management.

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Keywords: western flower thrips (WFTs); *suppressor of prosystemin-mediated responses2* (*Spr2*); jasmonic acid (JA); life table study

1. Introduction

Insect infestation is one of the most paramount factors that significantly impedes plant growth, diminishes crop productivity, and causes a myriad of other agricultural challenges [1]. Among these pests, the western flower thrip (WFT), *Frankliniella occidentalis* (Pergande), belonging to the family Thripidae and order Thysanoptera, emerges as one of the most detrimental phytophagous insects worldwide [2]. As a polyphagous insect pest, it damages leaves, flowers, and fruits by piercing plant tissues and ingesting cellular contents [3]. In pepper crops, thrips feeding leads to leaf curling and deformation, flower drop, a reduced fruit set rate, and abnormal fruit development [4]. The WFT also acts as a vector for plant viruses such as tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV), with TSWV being a major contributor to pepper yield losses [5,6]. Furthermore, the WFT also prefers to hide in narrow crevices of plants, such as flower buds, developing fruits, or minute fissures in stems and bark. Its widespread insecticide resistance further complicates management [7]. Therefore, strategies based on plant defense responses are needed to mitigate WFT damage effectively.

Plants have evolved both constitutive and inducible defenses to protect themselves against herbivorous insects [8]. Constitutive defenses are inherently expressed, whereas induced defenses are triggered upon herbivore attack [9]. Inducible defenses often involve the synthesis of secondary metabolites that can directly deter or harm insects [10,11]. Phytohormones serve as crucial inducing factors that orchestrate plant defense responses, predominantly encompassing jasmonic acid (JA), salicylic acid (SA), ethylene (ET), and their related derivatives [12]. Among these, the JA signaling pathway stands out as the most pivotal pathway in regulating plant defense against phytophagous insects [13,14]. It is triggered by mechanical damage from insect feeding or by the exogenous application of JA or methyl jasmonate (MeJA) [15]. Activation of JA signaling induces secondary metabolites and volatiles that reduce insect feeding efficiency, slow growth, and limit reproduction [16–18]. The WFT is highly susceptible to JA-related induced defenses across various plant species, including *Arabidopsis thaliana* [19], kidney bean (*Phaseolus vulgaris*) [20], Chinese cabbage (*Brassica rapa*) [21], *Chrysanthemum* [22], cotton (*Aphis gossypii*) [23] and tomato (*Solanum lycopersicum*) [24]. Nevertheless, the mechanisms underlying the defense against phytophagous insects in pepper plants remain largely elusive.

This study focuses on the *suppressor of prosystemin-mediated responses2* (*Spr2*) gene, which encodes a chloroplast fatty acid desaturase (FAD7) involved in JA biosynthesis and systemic wound signaling [25]. FAD7 appears to modulate JA-dependent defenses against chewing insects and SA-dependent defenses against aphids via distinct effects on JA synthesis and SA signaling [26]. Arbuscular mycorrhizal fungi (AMF) colonization is significantly reduced in *spr2* mutant tomato plants, aligning with other phenotypic impacts of impaired FAD7 function [27]. In tomato, *spr2* mutant plants show reduced volatile organic compound levels and compromised resistance to herbivorous insects [28,29]. To investigate the role of *Spr2* in pepper defense against the WFT, and considering the difficulty in obtaining pepper mutant materials, the pTRV (tobacco rattle virus)-based VIGS vectors were employed to generate *Spr2*-silenced pepper plants (TRV-*CaSpr2*). Subsequently, a comprehensive analysis was conducted, wherein the endogenous levels of JA and JA-Ile were measured in both TRV-*GFP* (control) and TRV-*CaSpr2*-infected pepper plants. Moreover, selection preference experiments were performed, and the detailed lifespan

tables for WFTs were constructed to evaluate the ecological impact of *Spr2* silencing. The results demonstrated that TRV-*CaSpr2* plants had lower JA levels, were more attractive to WFTs and provided a more conducive environment for the survival and reproduction of the WFT population.

2. Materials and Methods

2.1. Insects and Plants

The initial population of *F. occidentalis* was generously donated by the Institute of Plant Protection, Hunan Academy of Agricultural Sciences, China. Subsequently, these insects were maintained on broad beans (*Vicia faba*) within an artificial climate incubator set at 25 °C, a relative humidity of 65 ± 5%, and a photoperiod consisting of 14 h of light followed by 10 h of darkness, with a light intensity of 4800 lx. Pepper plants (*Capsicum annuum*) of the edible hot pepper variety 'Bo La' were obtained from Hunan Xingshu Seed Company (http://www.xsseed.com, accessed on 29 March 2021). The plants were cultivated in a growth chamber under a daytime temperature of 25 °C and a nighttime temperature of 22 °C, with a light-dark cycle of 16 h and 8 h, respectively.

2.2. Sequence Comparisons and Phylogenetic Analyses

The full-length protein sequences employed for sequence comparisons and phylogenetic analyses were sourced from the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/, accessed on 15 May 2022). Pairwise sequence comparisons and calculation of sequence identities were conducted utilizing CLUSTALX (http://www.clustal.org, accessed on 20 May 2022) [30]. The average pairwise distances were determined using MEGA7.0.2 (https://www.megasoftware.net/, accessed on 21 May 2022). Phylogenetic trees were constructed via the Neighbor-Joining method, incorporating strict distance measures and employing randomized bootstrapping to evaluate the validity of branching patterns, all implemented within the MEGA7.0.2 framework. The robustness of the inferred evolutionary relationships was rigorously assessed by 1000 bootstrap replicates [31].

2.3. TRV-Mediated Silencing Assays

For TRV-based VIGS assay [32–34], DNA fragments corresponding to partial sequences of *CaPDS* (*phytoene desaturase*) and *CaSpr2* were independently amplified via PCR, employing specific primers (Table S1). The amplified fragments were cloned into the pTRV2-vector, thereby generating pTRV2-*PDS* and pTRV2-*Spr2*. These constructs were transformed into *Agrobacterium tumefaciens* strain GV3101 individually. Subsequently, *Agrobacterium* cultures harboring pTRV1, or one of the three plasmids (pTRV2-*GFP*, pTRV2-*CaPDS* and pTRV2-*CaSpr2*) were mixed at a 1:1 ratio to achieve a final OD_{600} of 1.0. This mixture was then infiltrated into the fifth to sixth leaves of three-week-old *N. benthamiana* plants to facilitate the proliferation of TRV, in order to achieve a higher inoculation efficiency. Four days post-infiltration, crude extracts were prepared by homogenizing the infiltrated leaf tissue in 0.01 M phosphate buffer (pH 7.0) at a 20% (*w*/*v*) ratio. The homogenate was centrifuged at 5000 rpm for 10 min at 4 °C, and the resulting supernatant was collected for mechanical inoculation onto the fifth to sixth leaves of four-week-old pepper plants. The pepper plants were cultivated in a growth chamber under a daytime temperature of 25 °C and a nighttime temperature of 22 °C, with a light-dark cycle of 16 h and 8 h, respectively.

2.4. RNA Isolation and RT-qPCR

Total RNA extraction was carried out using Trizol Reagent (Vazyme, Nanjing, China) adhering to the manufacturer's protocol. Subsequently, cDNA was synthesized from the extracted total RNA employing a cDNA synthesis kit (TransGen, Beijing, China). For the

quantitative real-time polymerase chain reaction (qPCR) analysis, SYBR Green Supermix (Vazyme, Nanjing, China) was utilized in accordance with the protocol. The reference gene *CaActin* served as an internal control to normalize and determine the relative expression levels of other genes. All primers employed in the qPCR experiments are detailed in Table S1. The relative gene expression was calculated by applying the geometric mean of threshold cycle (Ct) values for the reference gene *CaActin*, utilizing the $2^{-\Delta\Delta Ct}$ method. The statistical comparisons of gene expression levels were conducted using Student's *t*-test (data were presented as mean ± SE of three biological replicates, with the significance threshold set at $p < 0.05$), and the results were visualized using GraphPad Prism 9.0.0 software (https://www.graphpad.com/, accessed on 18 October 2022).

2.5. Hormone Quantification

Leaf samples, each weighing 100 mg, were collected from pTRV-*GFP* and pTRV-*CaSpr2* plants. These samples were then promptly flash-frozen in liquid nitrogen and subsequently stored at −80 °C. To quantify the contents of JA and JA-Ile, high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) was employed, following the previously described methodology [35]. The statistical comparisons of hormone contents between different groups were conducted using Student's *t*-test (data were presented as mean ± SE of three biological replicates, with the significance threshold set at $p < 0.05$), and the results were visualized using GraphPad Prism 9.0.0.

2.6. Selection Preference of WFTs for Host Plants

Preference tests to evaluate WFT responses to plants were conducted using a glass Y-tube olfactometer (Fujian Minbo Glass Co., Ltd., Fuzhou, China), following the method previously described [36]. The olfactometer featured a Y-shaped tube with a central stem branching into two arms, each connected to a distinct glass odor bottle. One bottle contained TRV-*GFP*-infected pepper leaves, while the other held TRV-*CaSpr2*-infected pepper leaves. To ensure sufficient odor dispersion, the tested odors from pepper leaves were sealed in vials 5 min prior to each trial, allowing the odors to permeate the arms. For each trial, individual 2-day-old thrips, starved for 1 h, were introduced into the central tube and permitted to choose between the two arms. Each treatment involved 30 WFTs, with five replicates conducted per treatment. The statistical comparisons of selection preference experiments were conducted using chi-squared (χ^2) test (data were presented as mean ± SE, with the significance threshold set at $p < 0.05$), and the results were visualized using GraphPad Prism 9.0.0.

2.7. The Life Tables of WFT Feeding on TRV-GFP and TRV-CaSpr2-Infected Pepper Plants

In alignment with the age-stage two-sex life table theory [37,38] and following the methodological framework of Li et al. [39], we carried out a systematic observational study on WFTs that were feeding on TRV-*GFP* or TRV-*CaSpr2*-infected pepper leaves. We precisely recorded the number of surviving thrips at each distinct developmental stage, closely monitored and documented their feeding conditions, and accurately tallied the egg-laying quantity of female thrips. Subsequently, leveraging the comprehensive dataset thus obtained, we constructed highly detailed life tables for the WFTs.

The life history and population parameters of the WFTs fed on TRV-*GFP* or TRV-*CaSpr2*-infected pepper plants were calculated and analyzed employing the two-sex life table analysis method, in conjunction with the paired bootstrap test in TWOSEX-MSChart software v.2020 [39,40], and the results were visualized using SigmaPlot v.12.0 (https://systat-sigmaplot.com/, accessed on 10 January 2023).

3. Results

3.1. Identification and Silencing of CaSpr2 in Pepper

To investigate the function of *CaSpr2*, the amino acid sequences of Spr2 from various solanaceous crops (pepper, tomato, potato, tobacco, etc.) were downloaded from the GenBank database, and further phylogenetic and homology analyses were conducted. The Spr2 proteins derived from three pepper species were clustered within the same sub-clade, distinctly separated from those of tobacco, tomato, and potato (Figure 1A). These Spr2 proteins have been previously implicated in JA biosynthesis.

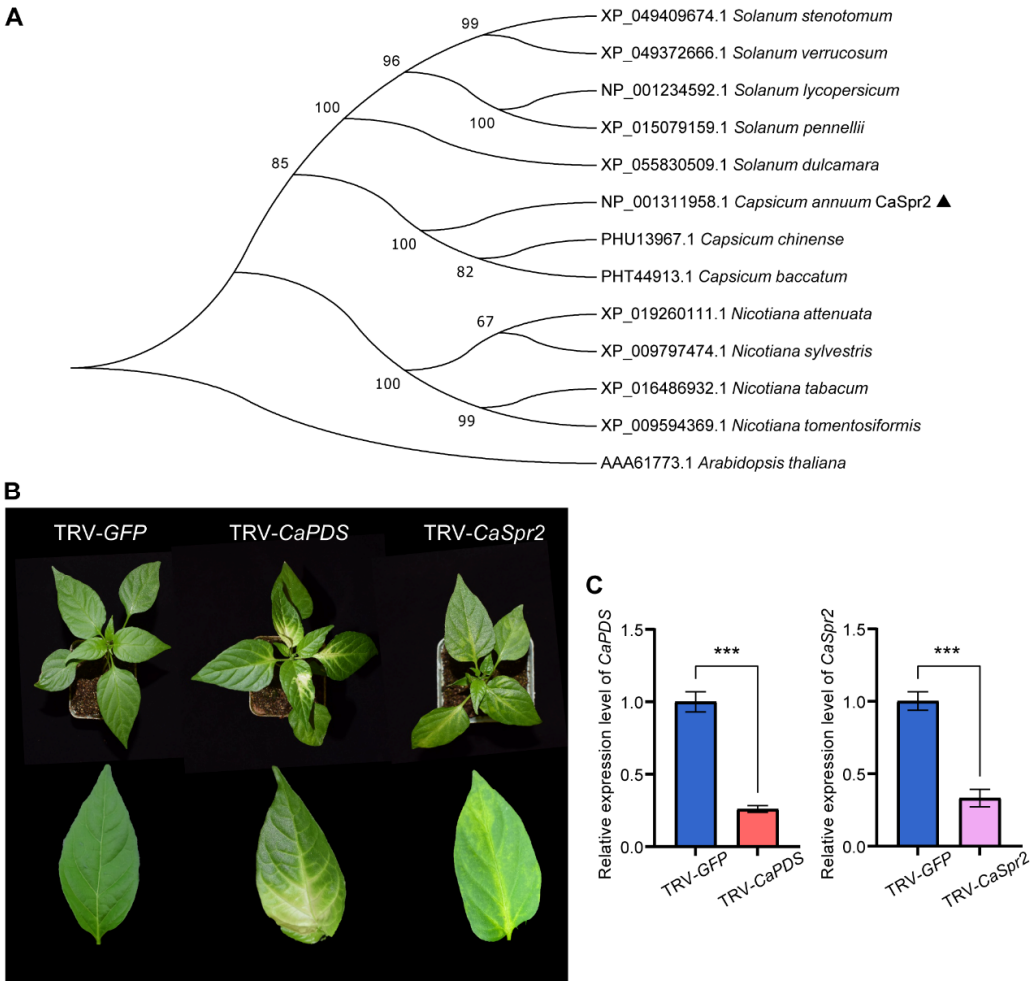

Figure 1. Phylogenetic characterization of Spr2 proteins and phenotypic profiling of *CaSpr2*-silenced pepper plants. (**A**) Phylogenetic relationship of partial Spr2 proteins in Solanaceae family. The tree was generated by MEGA 7.0.2 using the Neighbor-Joining method with 1000 bootstraps. The triangular symbol indicated the focal protein CaSpr2. (**B**) The phenotypes of pepper plants infected by TRV-*GFP* (negative control), TRV-*CaPDS* (positive control), and TRV-*CaSpr2*. (**C**) RT-qPCR analysis of the relative expression levels of *CaPDS* and *CaSpr2* in gene-silenced plants. Statistical differences (***: $p < 0.001$) were evaluated using Student's *t*-test.

Given the substantial challenges associated with acquiring transgenic pepper materials, we opted to employ a TRV-based VIGS to silence *CaSpr2* in pepper plants. Meanwhile, TRV-*GFP* served as the negative control and TRV-*CaPDS* was utilized as the positive control due to its characteristic photobleaching phenotype [41]. Following this, the full-length coding sequences (CDS) of *CaSpr2* and *CaPDS* were amplified (Figure S1A,B). Leveraging the VIGS online tool (https://vigs.solgenomics.net/, accessed on 6 July 2022), specific sequences of *CaSpr2* and *CaPDS* were elected and amplified (Figure S1C,D), which were then individually

cloned into the pTRV2-vector. Initially, *N. benthamiana* plants were infected with the TRV construct, and then the infected tissue was used to inoculate pepper plants. Compared with the control TRV-*GFP*-infected plants, a marked bleaching phenotype was evident on the *CaPDS*-silenced pepper leaves, and *CaSpr2*-silenced pepper leaves exhibited a mild chlorosis (Figure 1B). The transcript levels of *CaPDS* and *CaSpr2* were quantified by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) analysis. The results revealed that, relative to the *GFP* control, the expression level of *CaPDS* was reduced by approximately 80%, and the expression level of *CaSpr2* was diminished by roughly 70% (Figure 1C). These findings underscore the efficacy of the TRV-based VIGS system in effectively suppressing gene expression in pepper plants.

3.2. Silencing of CaSpr2 Exerts a Negative Impact on JA Accumulation and Attracts the Feeding of WFTs

To delve into the impact of *CaSpr2* silencing on the accumulation of JA and its bioactive conjugate JA-Ile [42], we evaluated the phytohormone levels in leaves of pepper plants infected with either TRV-*GFP* or TRV-*CaSpr2*. The results revealed that silencing of *CaSpr2* led to a reduction in the levels of JA and JA-Ile (Figure 2A,B). Subsequently, we conducted a further investigation into the effect of *CaSpr2* silencing on the accumulation of JA and JA-Ile in pepper plants in response to thrips infestation. Statistically significant differences were observed at both 12 h and 24 h post-infestation for JA and JA-Ile levels (Figure 2C,D). These findings indicated that *CaSpr2* silencing compromised the induction of JA and JA-Ile in pepper leaves subjected to thrips feeding. Collectively, they also underscore that *CaSpr2* is pivotal for the biosynthesis of JA and JA-Ile in response to herbivore attack.

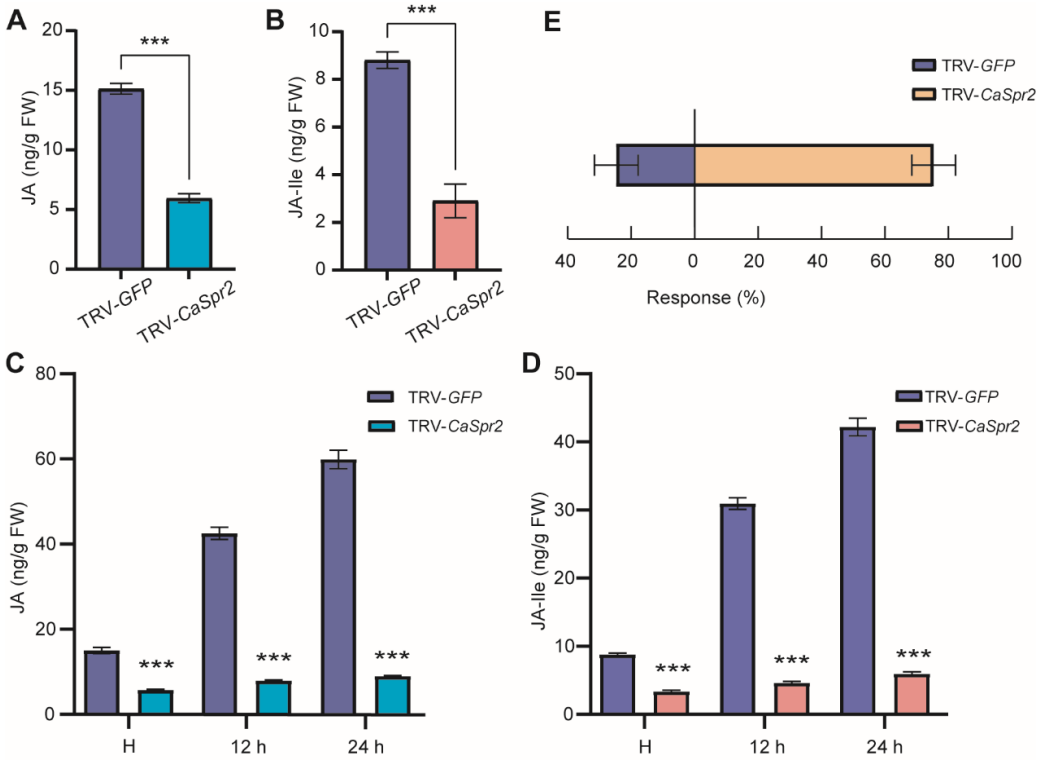

Figure 2. Silencing of *CaSpr2* reduces the JA accumulation and attracts the feeding of WFTs. (**A**,**B**) Quantification of JA and JA-Ile in TRV-*GFP* or TRV-*CaSpr2*-infected pepper leaves in the absence of thrips feeding. Data were mean ± SE of three biological replicates. Asterisks indicated significant differences (Student's *t*-test, ***: $p < 0.001$). (**C**,**D**) Quantification of JA and JA-Ile in TRV-*GFP* or TRV-*CaSpr2*-infected pepper leaves at 12 and 24 h post thrips infestation. Data were presented as mean ± SE with three biological replicates. Asterisks indicated statistically significant differences determined by

paired *t*-test (***: $p < 0.001$). (**E**) Thrips preference for TRV-*GFP* or TRV-*CaSpr2*-infected pepper leaves. Statistical comparisons were performed using χ^2 test. Data represented mean ± SE (n = 30 thrips per replicate; five independent replicates), expressed as percentage choice.

To investigate the behavioral response of WFTs to *CaSpr2*-silenced pepper plants, we evaluated the orientation preference patterns of thrips exposed solely to plant odors (odors from either TRV-*GFP* or TRV-*CaSpr2*-infected pepper leaves) under conditions devoid of any additional visual, gustatory, or tactile cues. The results revealed that 74.2% of WFTs exhibited a pronounced preference for TRV-*CaSpr2*-infected pepper leaves, demonstrating a statistically significant difference compared to those exposed to TRV-*GFP*-infected pepper leaves (Figure 2E). These results underscore the WFTs were attracted by the plant odors of *CaSpr2*-silenced pepper plants.

3.3. Silencing of CaSpr2 Is Beneficial for the Developmental Durations of WFTs

The duration of the immature stages of WFTs fed on TRV-*GFP* and TRV-*CaSpr2*-infected pepper leaves were stated in Table 1. With the exception of a marginal disparity in the developmental time during the second instar larval stage, there was virtually no difference in the other developmental time between the two treatment groups (Table 1). Similarly, no significant variations were detected for the survival rates from egg to adult stage (preadult survival rate). Notably, the longevity of both female and male thrips that fed on *CaSpr2*-silenced leaves was significantly prolonged compared to those that fed on control leaves (Table 1). Regarding the total preoviposition period (TPOP), no statistically significant difference was observed between two treatment groups. Furthermore, the mean fecundity of females exposed to TRV-*CaSpr2*-infected pepper leaves reached 79.58 eggs, a value that was markedly elevated compared to the mean fecundity (48.64 eggs) of females subjected to TRV-*GFP*-infected pepper leaves (Table 1). These findings collectively suggest that the dietary intake of *CaSpr2*-silenced pepper leaves by WFTs primarily exerts an influence on their longevity and fecundity.

Table 1. Developmental duration, survival, and reproduction of WFTs fed on TRV-*GFP* and TRV-*CaSpr2*-infected pepper leaves.

Parameter	TRV-*GFP*		TRV-*CaSpr2*		
	n	Mean ± SE	n	Mean ± SE	
First instar larval stage (d)	99	1.000 ± 0.000	93	0.973 ± 0.012	*
Second instar larval stage (d)	90	4.428 ± 0.081	88	4.620 ± 0.083	*
Prepupal stage (d)	88	1.006 ± 0.017	88	0.989 ± 0.018	ns
Pupal stage (d)	88	2.460 ± 0.048	88	2.403 ± 0.048	ns
Preadult duration (d)	88	12.869 ± 0.084	88	13.012 ± 0.079	ns
Preadult survival rate	99	0.889 ± 0.032	93	0.946 ± 0.023	ns
Female longevity (d)	40	12.296 ± 1.379	41	17.463 ± 0.889	*
Male longevity (d)	48	9.135 ± 0.536	47	12.138 ± 0.762	*
Total preoviposition period (d)	31	15.097 ± 0.249	41	14.976 ± 0.162	ns
Fecundity	40	48.643 ± 7.747	41	79.584 ± 5.922	*

n: The number of surviving individuals of WFTs of the specific period. Adult preoviposition period is defined as the duration from eclosion to the first day of oviposition. Total preoviposition period is defined as the duration from eggs to the first day of oviposition. Values are means ± standard error. Statistical analysis comparing TRV-*GFP* and TRV-*CaSpr2* is based on paired bootstrap test. The "ns" indicated not significant ($p > 0.05$), whereas the asterisk indicated significant difference ($p < 0.05$).

3.4. The Survival Rates of WFTs Reared on CaSpr2-Silenced Leaves Significantly Increased

The age-stage-specific survival rate (s_{xj}) of WFTs reared on *CaSpr2*-silenced and control leaves delineated the probability of a newly oviposited egg surviving to age x and stage j (Figure 3A,B). The survival curves for consecutive life stages overlapped substantially, reflecting the natural variation in developmental rates among individual thrips. Male

thrips fed on *CaSpr2*-silenced leaves exhibited greater longevity, surviving up to 40 days compared to 34 days for males on control leaves (Figure S2A). Adult survival rates were also significantly higher on *CaSpr2*-silenced leaves than on control leaves. The peak survival rate for females on TRV-*CaSpr2* leaves was 0.44, whereas it reached only 0.37 on TRV-*GFP* leaves (Figure S2B).

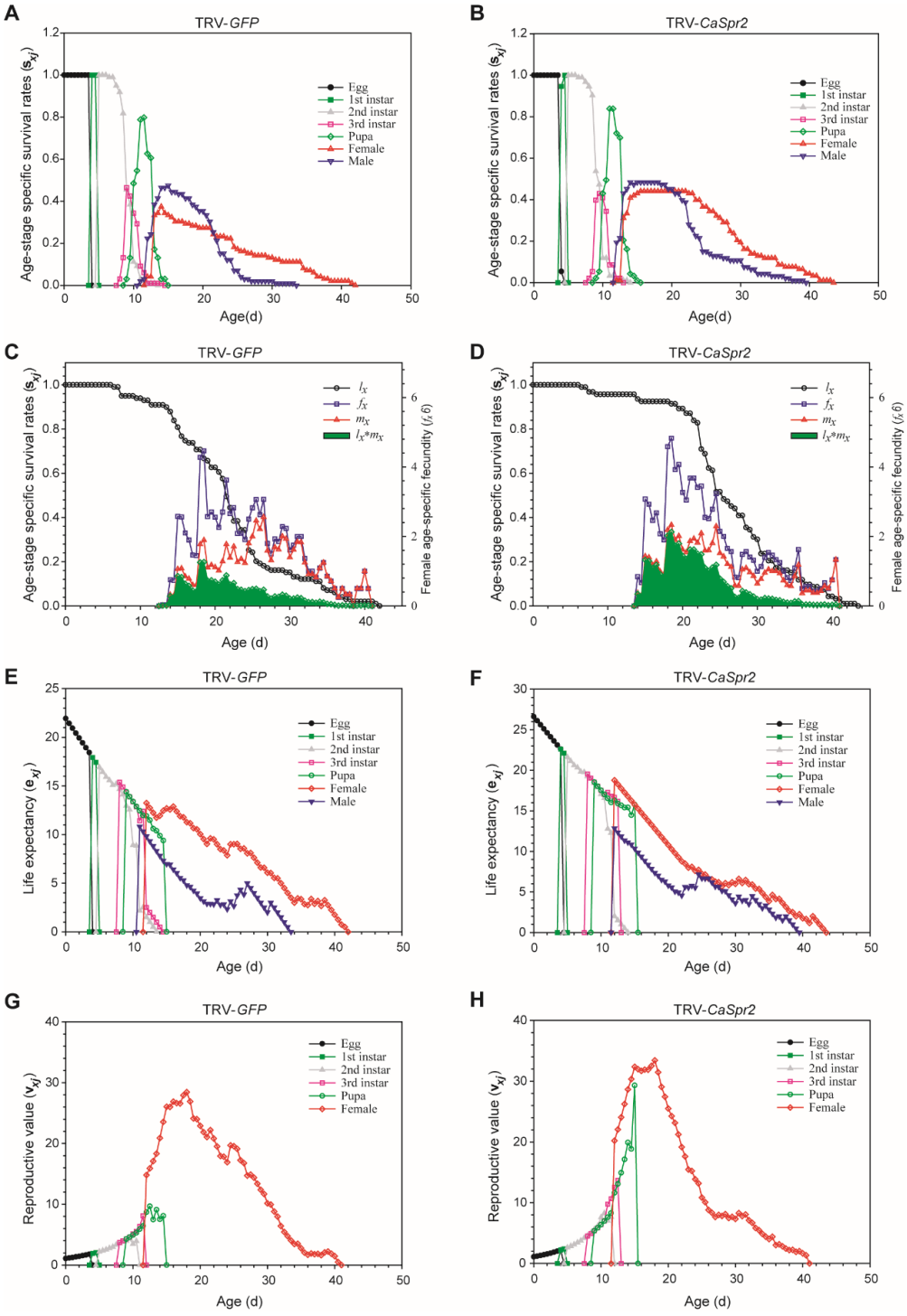

Figure 3. Life history traits of WFTs on TRV-*GFP* or TRV-*CaSpr2*-infected pepper leaves. (**A**,**B**) Age-stage-specific survival rate (s_{xj}) of WFTs on the two leaf groups. (**C**,**D**) Age-specific survival rate (l_x), age-stage-specific fecundity (f_x), age-specific fecundity (m_x), and age-specific net maternity (l_x*m_x) of WFTs on the two leaf groups. (**E**,**F**) Life expectancy (e_{xj}) of WFTs on the two leaf groups. (**G**,**H**) Age-stage reproductive value (v_{xj}) of WFTs on the two leaf groups.

The age-specific survival rate (l_x), age-stage-specific fecundity (f_x), age-specific fecundity (m_x), and age-specific net maternity (l_x*m_x) of WFTs fed on *CaSpr2*-silenced leaves and control leaves were presented in Figure 3C,D. The l_x curve revealed a decline in survival probability as the age of the thrips increased. In the control group, survivorship decreased rapidly beginning on 14th day, with all WFTs succumbing by the 42nd day. Also, for the *CaSpr2*-silenced group, survivorship declined rapidly from the 20th day onwards, and complete mortality was observed by the 44th day. The age-stage-specific fecundity curve (f_x) indicated that female adults in both groups commenced reproduction at 13 days of age. The peak of mean fecundity was recorded at 18.5 days, with 4.46 eggs laid for WFTs fed on control leaves and 4.83 eggs for those on *CaSpr2*-silenced leaves (Figure 3C,D). Notably, the net maternity of WFTs was higher when they were fed on *CaSpr2*-silenced leaves compared to the control leaves. The peak value of l_x*m_x for the TRV-*CaSpr2* group was 2.13 at 18.5 days, while the peak for the TRV-*GFP* group was 1.26 at the same age (Figure S2C).

3.5. The Life Expectancy and Reproductive Values of WFTs Fed on TRV-CaSpr2-Infected Pepper Leaves Are Prolonged

The life expectancy (e_{xj}) represents the probability that an individual of age x and stage j will survive to age and stage. The mean longevity of WFTs, defined as the life expectancy at age zero (e_{01}), was 21.92 days on TRV-*GFP*-infected plants, and 26.62 days on TRV-*CaSpr2*-infected plants (Figure 3E,F). The age-stage reproductive value (v_{xj}) quantifies the contribution of an individual at age x and stage j to the future population. The v_{xj} demonstrated an initial increase followed by a decrease with advancing age of WFTs (Figure 3G,H). Furthermore, the peak v_{xj} values for female adults were recorded at 18 days, with values of 33.44/d on TRV-*CaSpr2*-infected leaves and 28.45/d on TRV-*GFP*-infected leaves (Figure S2D).

3.6. WFTs Demonstrates Better Population Parameters When Feeding on CaSpr2-Silenced Leaves

The impacts of *CaSpr2*-silenced leaves and control leaves on the population parameters of WFTs are detailed in Table 2. When compared to WFTs fed on control leaves, those consuming *CaSpr2*-silenced leaves displayed a marked and statistically significant increase in their population parameters. Specifically, the intrinsic rate of increase (r = 0.174 d^{-1}), the finite rate of increase (λ = 1.190 d^{-1}), and net reproductive rate (R_0 = 35.105) were all substantially higher for WFTs on *CaSpr2*-silenced leaves than for those on control leaves (r = 0.143 d^{-1}, λ = 1.154 d^{-1}, R_0 = 19.661, respectively). There was no statistically significant difference in the mean generation time (T) between WFTs reared on *CaSpr2*-silenced leaves (T = 20.393d) and those on control leaves (T = 20.701 d). These findings suggest that WFTs demonstrate enhanced fecundity or accelerated developmental rates when feeding on *CaSpr2*-silenced leaves.

Table 2. Estimated population parameters of western flower thrips fed on TRV-*GFP* and TRV-*CaSpr2* pepper leaves.

Population Parameter	TRV-*GFP*	TRV-*CaSpr2*	
r (d^{-1})	0.143 ± 0.011	0.174 ± 0.008	*
λ (d^{-1})	1.154 ± 0.012	1.190 ± 0.009	*
R_0 (offspring/individual)	19.661 ± 3.932	35.105 ± 4.856	*
T (d)	20.701 ± 0.510	20.393 ± 0.323	ns

Values are means ± standard error. r: intrinsic rate of increase; λ: finite rate of increase; R_0: net reproductive rate; T: Mean generation time. Statistical analysis comparing TRV-*GFP* and TRV-*CaSpr2* is based on paired bootstrap test. The "ns" indicated not significant ($p > 0.05$), whereas the asterisk indicated significant difference ($p < 0.05$).

4. Discussion

The regulatory mechanisms of JA in non-model plants like pepper (*C. annuum*) remain incompletely understood due to limited genetic and genomic resources. Recent advances, including VIGS and pepper genomic data, now allow functional dissection of hormone-regulated defense networks. These methodological breakthroughs provide a valuable opportunity to bridge fundamental plant molecular biology with applied insect pest management research.

The *Spr* gene family was identified in tomato as suppressors of 35S::prosystemin-mediated phenotypes, regulating systemic induction of defense genes triggered by herbivory or wounding [43,44]. Extensive studies on *spr* mutants reveal that these genes not only modulate JA accumulation but also influence herbivore behavior, performance, and population dynamics. For example, the *spr1* mutant exhibits defects in prosystemin perception and the transmission of systemic wound signals [45], while the *spr2* mutants show impaired wound-induced and constitutive JA synthesis [28]. Interestingly, it exhibits increased susceptibility to infestation by tobacco hornworm and silverleaf whitefly [29], but negatively impacts the settling behavior, survival rate, and fecundity of the potato aphid [26]. Additionally, the *spr6* mutant displays deficiencies in wound- and prosystemin-induced defense gene expression, and its responsiveness to exogenous JA shows a dosage dependency [46]. Notably, *spr2* mutants display contrasting effects on different insect guilds, influencing phytophagous insects in distinct ways, highlighting the ecological complexity of JA-mediated defenses and their role in shaping plant–insect interactions. These findings from tomato mutants lay a foundation for investigating the effects of *Spr* homologs on insect resistance in solanaceous crops and provide a reference for validating the function of *Spr* genes in pepper.

Building upon these findings, our study extends the functional characterization of *Spr* homologs to pepper, a non-model but globally important crop that suffers severe losses from WFTs. By integrating VIGS, phytohormone quantification, insect behavioral assays, and life-history analyses, we demonstrate that *CaSpr2* functions as a positive regulator of JA and JA-Ile accumulation and restricts WFT growth, survival, and reproduction. The role of *CaSpr2* in regulating JA accumulation also raises questions regarding potential growth–defense trade-offs. JA signaling antagonizes growth-related pathways, and excessive or constitutive JA activation can impair photosynthesis, biomass accumulation, and reproductive output [47]. Thus, while loss of *CaSpr2* compromises JA-mediated defense and increases WFT susceptibility, our data do not suggest that constitutive *CaSpr2* overexpression would enhance resistance without fitness costs. Instead, *CaSpr2* appears to function as a regulatory node that contributes to balancing defense activation with plant physiological performance.

This study confirms the essential role of *CaSpr2* in JA biosynthesis and defense against western flower thrips (WFTs) in pepper, offering direct evidence for JA-mediated regulation of insect resistance in a non-model plant. From an applied perspective, *CaSpr2* should be considered a strategic target for the fine-tuned modulation of JA signaling rather than a single-gene solution. For instance, tissue-specific, inducible, or stress-responsive regulation of *CaSpr2* or its downstream components could enhance resistance during herbivory while mitigating potential growth penalties under non-stress conditions. Beyond direct genetic manipulation, these findings suggest alternative routes for translating *CaSpr2*-associated mechanisms into pest management. *CaSpr2* and related JA biosynthetic elements may serve as molecular markers in breeding programs to select pepper genotypes with optimized basal defense. Moreover, targeting specific downstream outputs of *CaSpr2*-mediated JA signaling, such as herbivore-induced volatiles or defensive metabolites, could provide a more selective means of deterring WFTs without broadly activating JA responses. This approach would be particularly valuable within an integrated pest management framework.

Current thrips management in pepper cultivation depends predominantly on chemical insecticides, a practice that has resulted in widespread pest resistance, environmental contamination, and the disruption of beneficial arthropod communities. Consequently, augmenting endogenous JA-mediated resistance via precisely regulated genetic or molecular breeding offers an environmentally compatible supplement to, rather than a substitute for, conventional control measures. Together, these results establish *CaSpr2* as a crucial regulatory element in pepper defense against WFTs and furnish a conceptual and experimental framework for future endeavors to harness hormone-mediated resistance within sustainable pest management strategies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/insects17020152/s1, Figure S1: Agarose gel electrophoresis of PCR products amplified from *C. annuum* genomic DNA with specific primers. (A) PCR amplification of the full-length sequences of *CaPDS* and *CaSpr2*. (B) PCR amplification of the partial sequences of *CaPDS* and *CaSpr2* for VIGS assays. Figure S2: Comparison of partial life history traits of WFTs on TRV-*GFP* or TRV-*CaSpr2*-infected pepper leaves. (A) Age-stage-specific survival rate (s_{xj}) of male thrips on the two leaf groups. (B) Age-stage-specific survival rate (s_{xj}) of female thrips on the two leaf groups. (C) Female age-specific net maternity (l_x*m_x) of WFTs on the two leaf groups. (D) Age-stage reproductive value (v_{xj}) of female thrips on the two leaf groups. Table S1: List of the primer sequences. Table S2: List of abbreviations.

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