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Tomato Mutants *SlIAA9* Exhibit Thermo-Morphophysiological Characters and Enhanced *SlDREBA4* Gene Expression

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Abstract

Rising temperatures associated with climate change threaten tomato productivity, yet the contribution of auxin signaling components to heat-stress adaptation remains incompletely understood. The *IAA9* gene, encoding an Aux/IAA transcriptional repressor, is well known for its role in auxin-regulated development, but its role in heat responses is still unclear. This study aims to elucidate the function of *IAA9* in modulating tomato responses under heat stress conditions. Researchers utilized tomato *iaa9-3* and *iaa9-5* mutants and exposed them to prolonged elevated temperatures of 40 to 45 °C for 6 weeks to assess morphophysiological traits, and to 38 to 40 °C for 6 days to evaluate molecular responses through *SlDREBA4* gene expression analysis. Under prolonged heat stress, all genotypes exhibited reduced leaf area, leaf number, and total chlorophyll content, accompanied by increased plant height compared to plants grown under normal conditions. Specifically, wild-type Micro-Tom (WT-MT) showed the lowest values in leaf area (165.89 cm^2), leaf number (23 leaves), and total chlorophyll content (115.7 $\mu g\ g^{-1}$). In contrast, the *iaa9-3* and *iaa9-5* mutants recorded the highest plant heights at 11.98 and 12.13 cm, respectively, indicating a differential growth response under stress. Gene expression analysis revealed that *SlDREBA4* expression was upregulated in both *iaa9-3* and *iaa9-5* mutants compared to normal temperature conditions, with increases of 0.45-fold and 1.78-fold, respectively. These results indicate that *IAA9* mutations confer enhanced thermotolerance in tomato, as reflected by altered morphology and increased heat-responsive gene expression. This study highlights *IAA9* as a potential genetic target for improving heat stress resilience in tomato breeding programs.

Keywords: auxin; heat stress; *IAA9*; *SlDREBA4* gene expression; thermo-morphogenesis

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a high-value horticultural commodity with strong agribusiness prospects, driven by increasing global demand associated with population growth and rising income levels (Djangsou et al., 2019). Despite this upward consumption trend, improving tomato productivity remains challenging for many conventional farmers, particularly under the accelerating impacts of climate change and global warming (Jadid et al.,

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2022). Heat stress disrupts critical developmental stages in tomato, including accelerated flowering, pollen sterility, impaired gamete and embryo formation, and reduced fruit set following pollination. Sustained exposure to temperatures above 38 °C for 5 to 10 days can inhibit fruit growth and compromise pollen development (Mubarok et al., 2024). In addition, high temperature has been widely reported to induce detrimental morphological changes such as reduced plant vigor, shorter shoots and roots, fewer branches, lower yield, smaller fruits, and reduced seed number per fruit (Alsamir et al., 2021).

High-temperature stress triggers significant physiological alterations in tomatoes, leading to reduced chlorophyll levels and stomatal numbers, along with increased proline and lycopene concentrations. Both proline and lycopene are recognized as essential constituents that play a pivotal role in determining the color of fully ripened tomato fruit (Suliman et al., 2024; Yuanhao et al., 2025). Furthermore, molecular biology tools can scrutinize these morphophysiological parameters involving thermo-related gene expression analysis. Graci and Barone (2024) highlighted several genes, including *SlDREBA4*, encoding a dehydration-responsive element-binding transcription factor. *SlDREBA4* exhibits upregulation in response to various environmental stresses, including heat, drought, cold, and high salinity.

Addressing the low productivity of tomato plants under heat stress necessitates strategic interventions to prevent yield reduction. Therefore, developing new tomato varieties with enhanced heat-stress tolerance capability could offer alternative strategies for tackling this challenge. Saito et al. (2011) highlighted the role of AUXIN RESPONSE FACTOR8 (*SlARF8*) and INDOLE-3-ACETIC ACID 9 (*SlIAA9*) in controlling tomato plant fertilization. *SlIAA9* functions as a transcriptional suppressor in the auxin signalling pathway. Previous studies have shown that downregulation of *SlIAA9* induces parthenocarpy (Mubarok et al., 2023). The tomato mutants, *iaa9-3* and *iaa9-5*, exhibited genetic mutations in the *IAA9* gene, with distinct mutation locations. The variations in mutation sites are projected to elicit distinct responses in the *iaa9-3* and *iaa9-5* mutants.

Although earlier studies have examined selected traits in *iaa9-3* and *iaa9-5* under normal and heat-stress conditions, such as pollen viability, stomatal anatomy, and changes in proline and chlorophyll content, comprehensive morphophysiological and molecular evaluations remain limited (Rahmat et al., 2023). Rahmat et al. (2023) also reported that *IAA9* mutants exhibited delayed flowering but increased flower number and fruit set, alongside higher photosynthetic rates and lower malondialdehyde levels, suggesting reduced leaf damage under heat stress. However, literature still lacks an integrated, allele-aware characterization that connects broader morphophysiological performance with thermo-related molecular responses under heat stress. In particular, the linkage between heat-induced leaf architectural adaptation (thermomorphogenesis), including hyponastic responses, and stress-related gene expression across *IAA9* alleles has not been explicitly and systematically addressed.

Therefore, this study aims to elucidate the role of *IAA9* in modulating tomato responses under heat stress by integrating morphophysiological characterization with thermo-related gene expression analysis. Specifically, researchers compare heat-induced morphological indicators, including leaf phenotypes associated with thermomorphogenesis, evaluate key morphophysiological traits under prolonged high-temperature exposure, and assess the molecular response to heat stress by quantifying *SlDREBA4* expression under elevated temperature conditions. Collectively, this approach seeks to determine whether *IAA9* mutations confer genotype-dependent thermotolerance and to establish *IAA9* mutants as valuable genetic resources for developing heat-resilient tomato cultivars, thereby supporting sustainable agriculture under increasingly frequent climate extremes.

MATERIALS AND METHOD

Growth conditions and plant material

This study utilized *iaa9-3* (TOMPJE2811) and *iaa9-5* (TOMJPG0114-1) mutant lines, previously developed and characterized by the University of Tsukuba, Japan (Saito et al., 2011). The wild-type Micro-Tom cultivar (WT-MT) served as the control. All tomato seeds were sown in polybags containing a 1:1 mixture of cocopeat and rice husk charcoal and grown under greenhouse conditions following the protocol described by Rahmat et al. (2023). After 2 weeks of growth, the plants were exposed to heat stress at 40 to 45 °C for 3 hours daily (from 11:00 to 14:00 WIB, GMT+7) over 6 weeks. Meanwhile, an acute heat stress at 38 to 40 °C was used for

gene expression analysis to capture the early transcriptional response of the *SlDREBA4* gene. The stability of the temperature was controlled using a thermostat. A control group was maintained under normal temperature conditions of 30 to 35 °C. These treatments were designed to assess the morphophysiological responses of the tomato plants to high-temperature stress. Hyponastic traits and *SlDREBA4* gene expression analyses were conducted on plants 48 days after the heat stress treatment.

Plant morphophysiological responses observation

Leaf area was quantified at the end of the vegetative stage using the gravimetric method. This involved tracing leaf outlines onto paper, weighing the cutouts using an analytical balance, and comparing them to a 10 cm × 10 cm reference paper square. The leaf area was then calculated using Equation 1.

$$\text{Leaf area} = \frac{\text{Leaf replica weight}}{\text{10 cm x 10 cm paper weight}} \times 100\ \text{cm}^2 \quad (1)$$

Leaf number and plant height were observed weekly from 1 to 6 weeks after transplanting under both temperature conditions. Leaf number was recorded by counting both primary leaves (emerging from the main stem) and secondary leaves (arising from branches or lateral shoots). Plant height was measured using a ruler, from the surface of the growing medium to the apex of the tallest shoot.

Chlorophyll content analysis

Fully expanded mature leaves from the central and basal regions of the plant were selected as samples. Leaf samples were homogenized and extracted using 3 ml of an acetone-hexane reagent. The resulting supernatant was collected in a microcentrifuge tube and centrifuged at 13,000 rpm for 5 minutes. Absorbance of the extract was measured using a spectrophotometer at wavelengths of 663, 645, 505, and 453 nm, following the method of Jadid et al. (2017). Chlorophyll concentrations were calculated using Equations 2 and 3.

$$\text{Chlorophyll a (mg 100 ml}^{-1}) = 0.999\ A_{663} - 0.0989\ A_{645} \quad (2)$$

$$\text{Chlorophyll b (mg 100 ml}^{-1}) = 1.77\ A_{645} - 0.328\ A_{663} \quad (3)$$

Total RNA extraction and gene expression analysis

Total RNA extraction was carried out using the Geneaid™ Total RNA Mini Kit and quantified according to Jadid et al. (2016). Gene expression analysis was conducted using SensiFAST™ SYBR® No-ROX One Step Kit (Meridian Bioscience). Primers used in this study include *SlDREBA4*-F (5’ TTG AGT CGG AAG AAT CGA AGA C 3’); *SlDREBA4*-R (5’ TAC CCA TCA AAG TCG CCA TC 3’); *SlActin*-F (5’ CTT GTC TGT GAC AAT GGA ACT G 3’); *SlActin*-R (5’ ATA CCC ACC ATC ACA CCA GTA T 3’). The tomato *Actin* (*SlActin*) gene was used as the internal reference gene, supported by prior studies reporting its suitability and stable performance for normalization in tomato qPCR analyses (Cui et al., 2024; Mitalo et al., 2024). The Real-Time qRT-PCR conditions include reverse transcription at 45 °C (10 minutes); polymerase activation at 95 °C (2 minutes); denaturation at 95 °C (5 seconds); annealing at 54 °C (10 seconds); extension at 72 °C (5 seconds). The cycle used was 40 cycles. The *SlDREBA4* expression was analyzed according to Livak and Schmittgen (2001).

Data analysis

Three genotypes of *Solanum lycopersicum* cv. Micro-Tom were used in this study, with 12 plants per genotype per treatment, resulting in 36 experimental units per treatment. Morphophysiological parameters were measured and subjected to Student’s *t*-test at a significance level of $p < 0.05$ to assess the effects of the *iaa9-3* and *iaa9-5* mutations relative to WT-MT under both temperature conditions. In addition, the molecular response to heat stress was evaluated by analyzing the expression of the heat-responsive gene *SlDREBA4* using Real-Time qRT-PCR with 3 biological replications, and relative expression levels were statistically analyzed using Student’s *t*-test at *p*-value < 0.05.

RESULTS AND DISCUSSION

Heat stress treatment results in hyponastic characteristics in tomato

High temperature is a major abiotic factor that constrains plant growth and development. In response to elevated ambient temperatures, plants undergo morphological and physiological modifications (thermomorphogenesis). This study revealed distinct phenotypic alterations in the leaves of *iaa9-3* and *iaa9-5* mutants under heat

stress, which were absent under normal conditions (Figure 1). Under heat stress, a hyponastic leaf posture was visually observed in *iaa9-3* and *iaa9-5* mutants. This indicates a typical thermomorphogenic adjustment that can reduce heat load and excess irradiance by modifying leaf angles. The *IAA9* encodes an Aux/IAA transcriptional repressor; therefore, a mutation in this gene might increase auxin responsiveness (Rahmat et al., 2023). The appearance of hyponasty under heat stress is consistent with an auxin-mediated differential growth response between adaxial and abaxial tissues (Jonsson et al., 2025). This architectural change is plausibly beneficial under heat stress by improving canopy cooling and limiting photoinhibition, thereby functioning as an early morphological acclimation trait. Importantly, the presence of hyponasty in both mutant alleles supports the premise that *IAA9* is involved in leaf-architecture plasticity during heat stress, beyond its established roles in reproductive traits.

Auxin homeostasis is central to controlling asymmetric growth between the adaxial and abaxial sides of leaves, thereby generating hyponastic or epinastic leaf movements (Lee et al., 2025). In this study, the hyponastic posture observed in *iaa9-3* and *iaa9-5* under heat stress is plausibly driven by an auxin-dependent thermomorphogenic program, involving phytochrome B inactivation and stabilization of *PIF4* signaling under warm conditions (Gommers, 2020). Such leaf-angle adjustments can reduce excess light interception and help alleviate photoinhibition during heat episodes (Jayawerdana et al., 2018). Although auxin is a major determinant, hyponasty/epinasty is also influenced by other phytohormones, including ethylene, abscisic acid, and brassinosteroids; nevertheless, evidence suggests auxin remains the dominant regulator, particularly when ethylene signaling is compromised (Gommers, 2020).

Morpho-physiological responses of tomato mutants to heat stress

Leaf area is one of the important parameters in analyzing plant growth because the processes of photosynthesis, respiration, and transpiration occur in the leaves, which determine the direction of plant growth and development (Yavas et al., 2024). The difference in leaf shape of *iaa9*-3 and *iaa9*-5 mutants due to mutations in the *IAA9* gene compared to WT-MT affects leaf area per plant. The observation results showed significant differences in leaf area parameters under normal and heat stress conditions (Figure 2).

Under normal conditions, the WT-MT exhibited the largest leaf area, measuring 603.88 cm², which was significantly greater than that of the *iaa9-3* and *iaa9-5* mutants, with leaf areas of 324.81 and 282.17 cm², respectively. In contrast, under heat stress conditions, the *iaa9-3* mutant displayed the largest leaf area at 217.83 cm², significantly exceeding that of WT-MT, which had the lowest value at 165.89 cm². The *iaa9-5* mutant recorded a leaf area of 181.78 cm², which was not significantly different from WT-MT. These results indicate that all genotypes

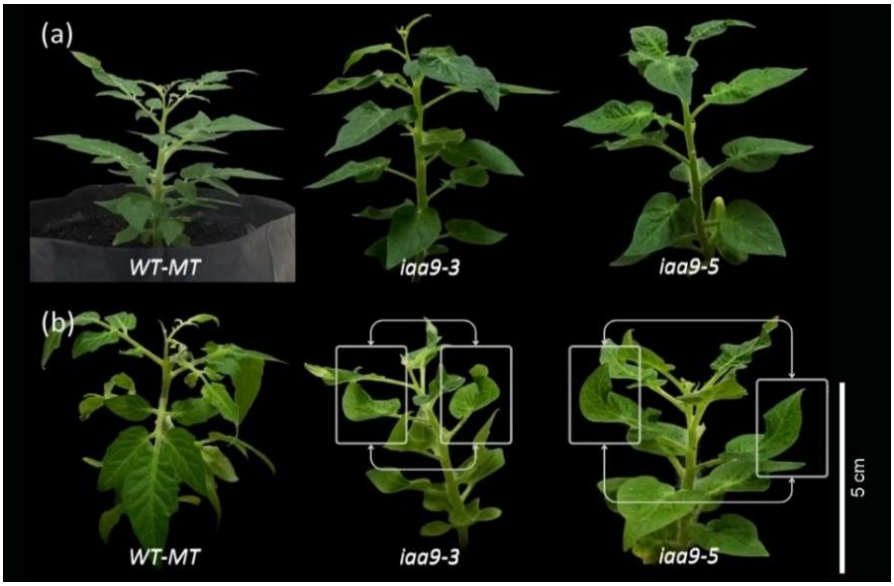


Figure 1. Hyponastic characters: (a) normal conditions, (b) heat stress conditions (38 to 40 °C)
Note: Rectangular symbols indicate hyponastic leaves on plants aged 48 days after transplanting, following a 6-day heat-stress treatment

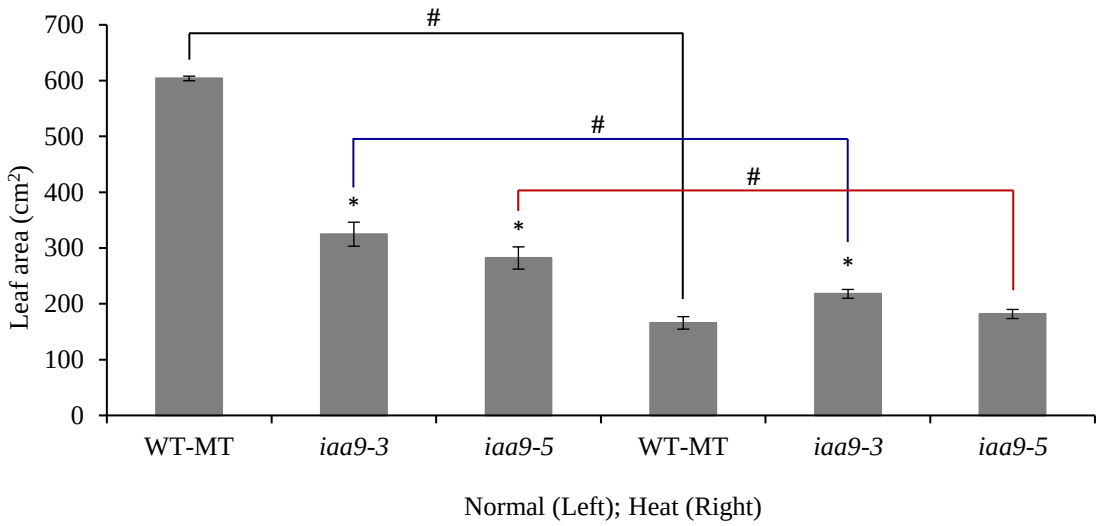


Figure 2. Leaf area under normal conditions (30 to 35 °C) and heat stress conditions (40 to 45 °C)
Note: The average value±standard error (SE) (n = 4), followed by an asterisk (*), indicates a significant difference compared to the control (WT-MT) under normal or heat stress conditions. The average value±standard error (SE) (n = 4), followed by a hashtag (#), indicates a significant difference in each genotype between the 2 temperature conditions according to Student's t-test at p-value < 0.05

experienced a significant reduction in leaf area under heat stress compared to normal conditions, with WT-MT showing the most pronounced decrease.

Leaves serve as vital indicators of plant growth and physiological status. In higher plants, leaves are broadly categorized as either simple or compound, with compound types further classified as pinnate or palmate. Cultivated tomato typically produces unipinnate compound leaves. Several tomato mutants–including *clausa* (*clau*), *Mouse ears* (*Me*), *Petroselinum* (*Pts*), and *tripinnate* (*tp*)–exhibit increased leaf complexity. Conversely, mutants such as *entire* (*e*), *potato leaf* (*c*), *trifoliate* (*tf*), and *wiry* series (*w*, *w3*, *w4*, *w6*), as well as *Lanceolate* (*La*), display reduced leaf complexity (Cao et al., 2025).

Under normal conditions, WT-MT plants exhibit broader leaves than the *iaa9-3* and *iaa9-5* mutants (Figure 2). This difference is likely attributable to mutations in the *SlIAA9* gene, which induce morphological alterations in Micro-Tom. Notably, *iaa9-3* and *iaa9-5* leaves are non-trifoliate, resulting in a more compact leaf architecture, and exhibit hyponastic character (Figure 1). This suggests that auxin signaling influences the expression of the *trifoliate* (*Tf*) gene, a critical regulator of trifoliate leaf formation in tomato. Previous study also reported that downregulation of *IAA9* alters leaf structure and vascular patterning, reinforcing the gene's central role in leaf morphogenesis (Wang and de Maagd, 2025).

Following heat stress treatment, the leaf area of the *iaa9-3* mutant surpassed that of WT-MT, potentially due to its greater leaf number (Figure 3). Nevertheless, heat stress reduced the leaf area across all genotypes (*iaa9-3*, *iaa9-5*, WT-MT), consistent with findings by Rahmat et al. (2023). Leaf area contraction under elevated temperatures is a known plant adaptation to reduce heat load and transpiration (Yuan et al., 2017). This response is often mediated by increased abscisic acid (ABA) synthesis in the roots, which, upon translocation to the shoots, triggers stomatal closure and limits leaf expansion (Rehaman et al., 2025).

Leaf number is an important determinant of photosynthetic capacity by influencing resource uptake and assimilation (Farjon et al., 2021). Under normal conditions, *iaa9-3* showed the highest leaf number (33.3), which was not significantly different from WT-MT (33.25), while *iaa9-5* had the lowest value (30.8) and did not differ significantly from WT-MT. Under heat stress, *iaa9-3* maintained the highest leaf number (29.9), significantly exceeding WT-MT, whereas *iaa9-5* averaged 24.4 leaves and did not differ from WT-MT (Figure 3). Overall, heat stress caused a significant reduction in leaf number across all genotypes compared with normal conditions.

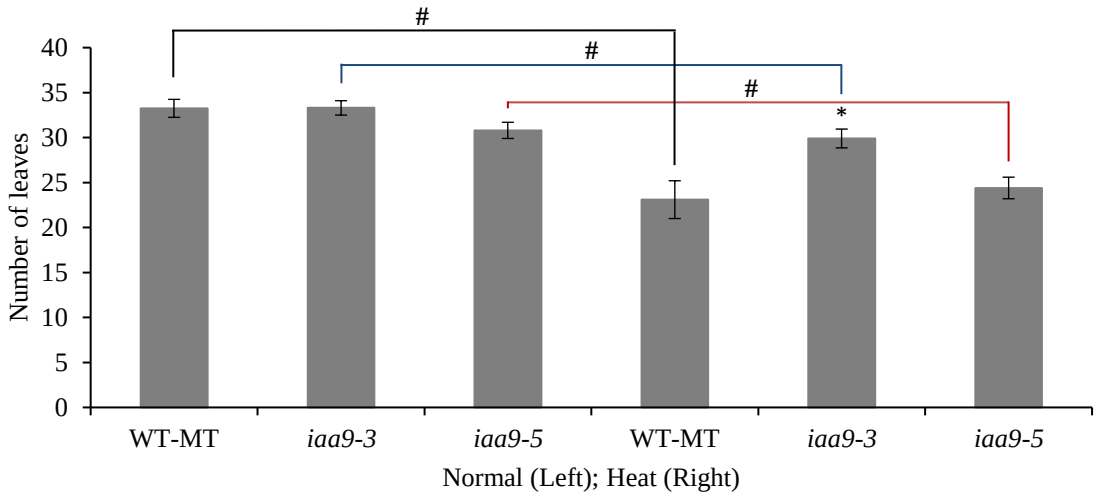


Figure 3. Number of leaves under normal conditions (30 to 35 °C) and heat stress conditions (40 to 45 °C)

Note: The average value±standard error (SE) (n = 12), followed by an asterisk (*), indicates a significant difference compared to the control (WT-MT) under normal or heat stress conditions. The average value±standard error (SE) (n = 12), followed by a hashtag (#), indicates a significant difference in each genotype between the 2 temperature conditions according to Student's t-test at p-value < 0.05

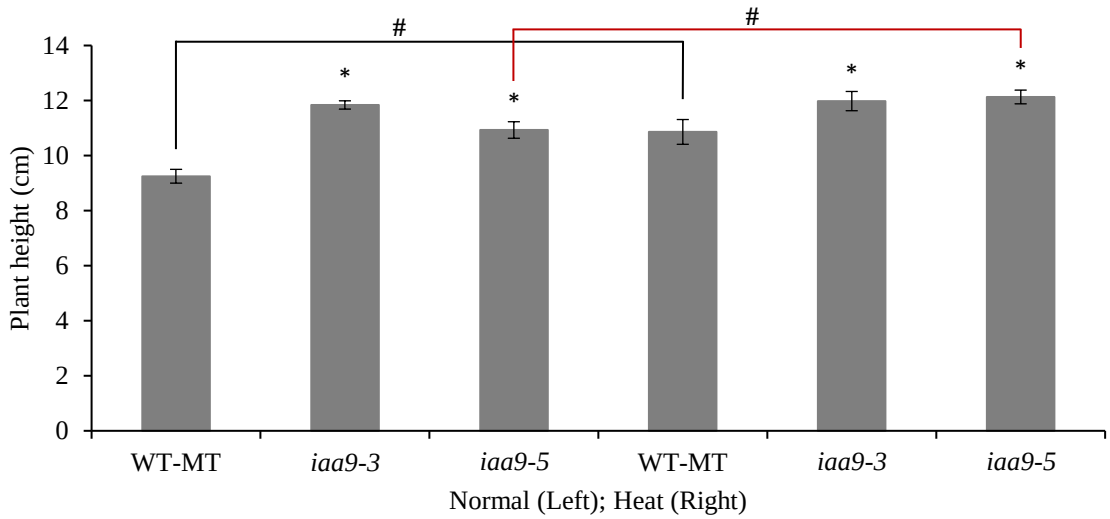


Figure 4. Plant height under normal conditions (30 to 35 °C) and heat stress conditions (40 to 45 °C)

Note: The average value±standard error (SE) (n = 12), followed by an asterisk (*), indicates a significant difference compared to the control (WT-MT) under normal or heat stress conditions. The average value±standard error (SE) (n = 12), followed by a hashtag (#), indicates a significant difference in each genotype between the 2 temperature conditions according to the Student's t-test at p-value < 0.05

WT-MT plants showed the highest number of branches under normal conditions, resulting in a higher leaf count. Although *iaa9-3* exhibited fewer branches, its leaf number was comparable to WT-MT under normal conditions and significantly higher under heat stress. This increase may reflect enhanced auxin responsiveness due to *IAA9* mutations. Auxin

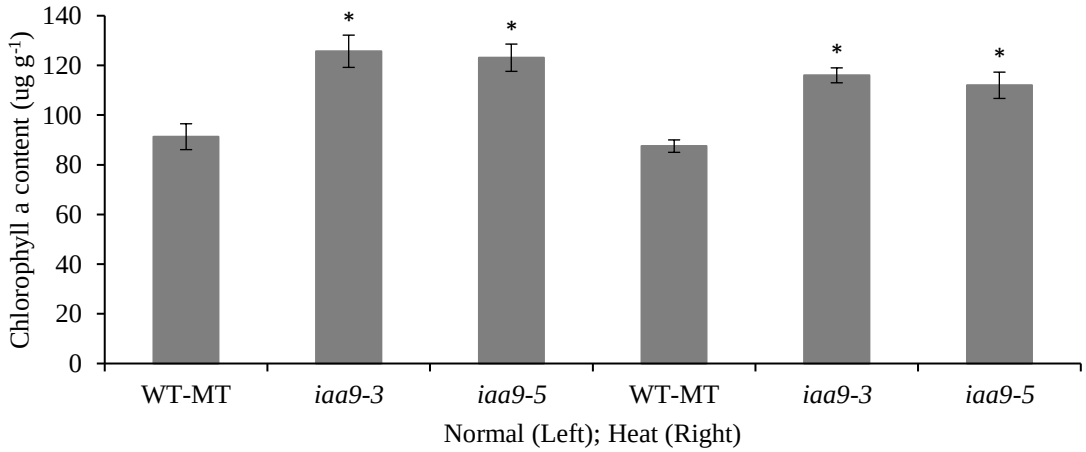
orchestrates key processes in leaf initiation and morphogenesis, influencing cell recruitment, polarity establishment, blade outgrowth, and final leaf shape (Xiong and Jiao, 2019). Under heat stress, leaf production declined in all genotypes, as heat disrupts water balance, cell division, and assimilation rate (Park et al., 2023).

In addition, statistical analysis revealed a significant difference in plant height between normal and high-temperature conditions (Figure 4). Mutations in the *IAA9* gene–specifically in the *iaa9-3* and *iaa9-5* mutants–resulted in significantly different plant height compared to the WT-MT under both normal and heat stress conditions. Under normal conditions, the *iaa9-3* mutant exhibited a plant height of 11.84 cm, which was significantly greater than the WT-MT, which had the lowest height at 9.25 cm. Similarly, the *iaa9-5* mutant, with a height of 10.93 cm, differed significantly from the WT-MT. Under heat stress, both *iaa9-3* and *iaa9-5* mutants reached heights of 11.98 and 12.13 cm, respectively–again showing significant increases compared to the WT-MT, which remained the shortest at 10.86 cm. These findings indicate that plant height tends to increase under heat stress relative to normal conditions, with statistically significant differences observed particularly in the WT-MT and *iaa9-5* genotypes. In contrast, the *iaa9-3* mutant displayed relatively stable plant height across both environmental conditions, with no significant variation.

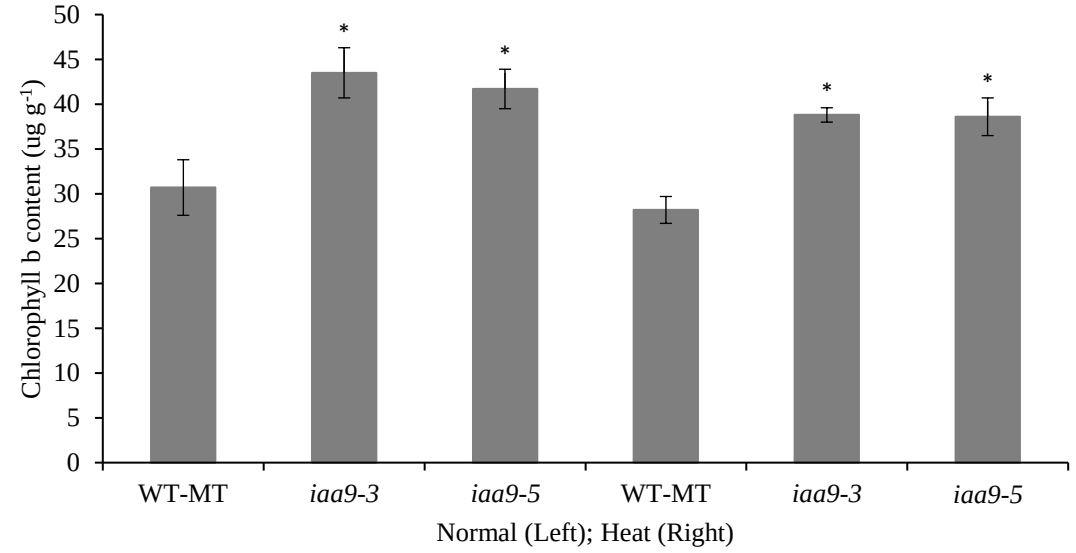
Both *iaa9-3* and *iaa9-5* were higher than WT-MT under both normal and heat-stress conditions, consistent with *IAA9* loss-of-function reducing repression in auxin signaling and thereby enhancing auxin-driven cell elongation in apical tissues (Ariizumi et al., 2013). Mechanistically, auxin promotes elongation by activating wall-loosening processes (expansins), increasing vacuolar turgor, and remodeling cell-wall polysaccharides (Kumar et al., 2025; Zhao et al., 2025). Heat stress can further amplify stem elongation as a thermomorphogenic response that elevates photosynthetic tissues above the warmer soil surface (Zhang et al., 2025), in part via warm-stabilized *PIF4*, which induces auxin biosynthesis genes such as *YUCCA* (Li et al., 2021). Collectively, all morphological patterns support a model where reduced *IAA9* repression enhances auxin-driven elongation and, in some contexts, mitigates the decline of vegetative growth traits under heat, although the extent of compensation appears allele-dependent.

The *iaa9-3* mutant exhibited the highest concentrations of chlorophyll a, chlorophyll b, and total chlorophyll–125.7, 43.5, and 174.2 µg g^{-1}, respectively–significantly higher than those of the WT-MT, which recorded the lowest values at 91.3, 30.7, and 122.1 µg g^{-1}, respectively, under normal conditions (Figure 5). Similarly, the *iaa9-5* mutant also showed significantly greater chlorophyll levels than WT-MT, with 123.1 µg g^{-1} (chlorophyll a), 41.7 µg g^{-1} (chlorophyll b), and 164.8 µg g^{-1} (total chlorophyll).

Under heat stress conditions, the trend persisted. The *iaa9-3* mutant maintained the highest chlorophyll a, b, and total chlorophyll contents at 116.0, 38.8, and 154.8 µg g^{-1}, respectively, while WT-MT recorded the lowest levels at 87.5, 28.2, and 115.7 µg g^{-1}, respectively (Figure 5). The *iaa9-5* mutant under heat stress also had significantly higher values than WT-MT, with 112.0, 38.6, and 150.6 µg g^{-1} for chlorophyll a, b, and total chlorophyll, respectively. These



a.



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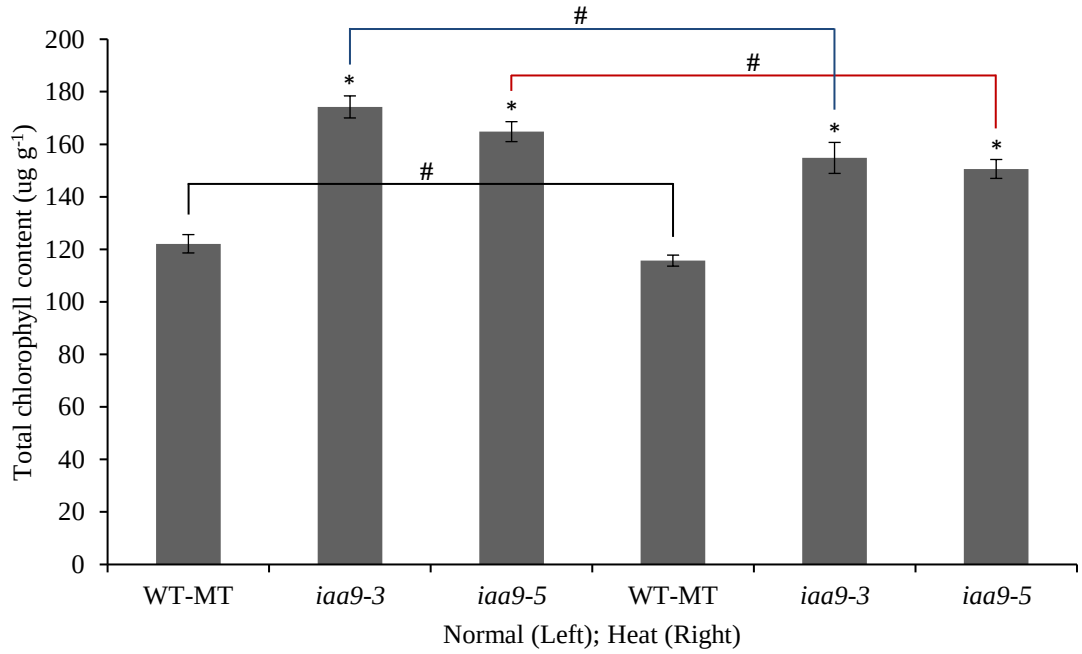

c.

Figure 5. Chlorophyll a (a), chlorophyll b (b), and total chlorophyll content (c) under normal conditions (30 to 35 °C) and heat stress conditions (40 to 45 °C)

Note: The average value±standard error (SE) (n = 4), followed by an asterisk (*), indicates a significant difference compared to the control (WT-MT) under normal or heat stress conditions. The average value±standard error (SE) (n = 4), followed by a hashtag (#), indicates a significant difference in each genotype between the 2 temperature conditions according to the Student's t-test at p-value < 0.05

findings indicate a consistent decrease in chlorophyll a, b, and total chlorophyll levels under heat stress across all genotypes, with a statistically significant reduction particularly evident in total chlorophyll content.

Heat stress commonly accelerates chlorophyll loss through impaired pigment biosynthesis and enhanced oxidative damage to the photosynthetic machinery. In this study, total chlorophyll decreased under heat in all genotypes, yet both

IAA9 mutants consistently retained higher chlorophyll levels than WT-MT. This pattern suggests that the mutants may experience relatively lower damage or better maintenance of chloroplast function under heat. Given that auxin signaling can influence leaf developmental programs and stress acclimation, the higher chlorophyll content in *IAA9* mutants may reflect improved photosynthetic apparatus protection, potentially through enhanced antioxidant capacity and/or delayed stress-induced senescence. From an agronomic perspective, preserving chlorophyll under heat is relevant because it supports continued light harvesting and carbon assimilation during stress episodes, which can contribute to better performance under high-temperature conditions (Tanaka and Ito, 2025).

The higher chlorophyll content observed in *iaa9-3* and *iaa9-5* is consistent with enhanced auxin signaling, which can promote chloroplast development, chlorophyll-associated protein expression, and RuBisCO activity (Yuan et al., 2018). Under heat stress, auxin may further delay chloroplast senescence and reduce oxidative injury by strengthening antioxidant defenses (Castro-Estrada et al., 2025). However, elevated temperatures still disrupt chlorophyll metabolism by impairing biosynthetic enzymes (POR and magnesium-chelatase) and damaging photosynthetic structures and proteins (thylakoids, PSII, and RuBisCO), while stress-induced ROS accelerates pigment loss and depresses photosynthetic efficiency (Fahad et al., 2017; Wang et al., 2017; Rahmat et al., 2023).

SlDREBA4 gene expression analysis

In this study, the analysis of *SlDREBA4* gene expression that is responsive to heat stress was carried out using Real-Time qRT-PCR with the actin reference gene. Under normal conditions, *SlDREBA4* expression was significantly downregulated in the *iaa9-3* and *iaa9-5* mutants, with expression levels reduced by 0.31-fold and 0.75-fold, respectively, relative to WT-MT (Figure 6). Under heat stress, the *iaa9-3* mutant continued to show reduced expression, with a 0.45-fold decrease compared to WT-MT, which remained statistically significant. In contrast, *SlDREBA4* expression in the *iaa9-5* mutant was significantly upregulated by 1.78-fold under heat stress, compared to WT-MT. Overall, *SlDREBA4* expression tended to increase in all genotypes under heat stress compared to normal conditions. However, only the *iaa9-5* mutant exhibited

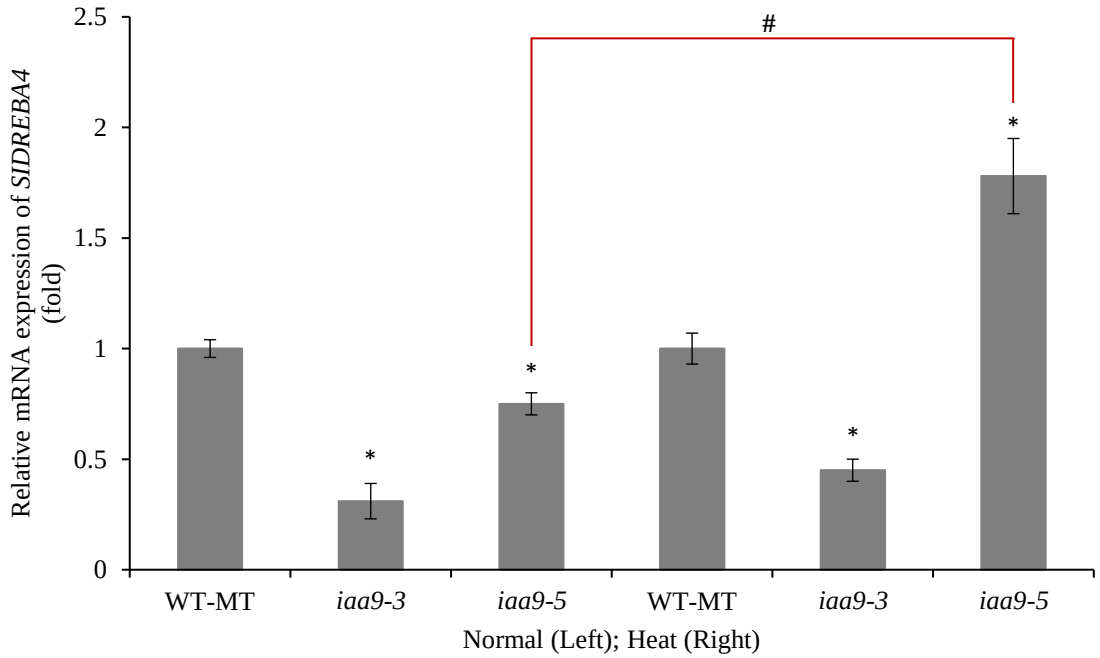

Figure 6. Relative mRNA expression of *SlDREBA4* gene

Note: The average value±standard error (SE) (n = 3), followed by an asterisk (*), indicates a significant difference compared to the control (WT-MT) under normal or heat stress conditions. The average value±standard error (SE) (n = 3), followed by a hashtag (#), indicates a significant difference in each genotype between the 2 temperature conditions according to the Student's t-test at p-value < 0.05

a statistically significant change, whereas the differences observed in WT-MT and *iaa9-3* were not significant. Notably, the highest relative mRNA expression of *SlDREBA4* was detected in the *iaa9-5* mutant under heat stress, while the lowest expression was observed in the *iaa9-3* mutant under normal conditions.

Hormonal signaling networks, particularly involving ABA and ethylene, are central to plant thermotolerance, with ABA more strongly linked to acquired stress responses (Vidya et al., 2018). *SlDREBA4*, a dehydration-responsive element-binding transcription factor, is the only DREB family member in tomato known to respond to heat stress (Deng et al., 2020). In this study, *SlDREBA4* expression was lower in *iaa9-3* and *iaa9-5* under normal conditions, likely because this gene is stress-inducible. Under heat stress, *iaa9-5* exhibited significantly higher *SlDREBA4* expression than WT-MT, reflecting a stronger auxin response linked to ABA accumulation (Mao et al., 2020). By contrast, *iaa9-3* showed only a moderate increase in *SlDREBA4* expression, potentially due to a premature stop codon in the mutated gene. These findings align with Rahmat et al. (2023), who reported a stronger auxin response in *iaa9-5* than in *iaa9-3*. *SlDREBA4* enhances heat tolerance by regulating osmolyte accumulation, stress hormone levels, and antioxidant enzyme activity. It also modulates HSP gene expression and interacts with calcium-binding proteins involved in ER protein processing and plant defense responses. Furthermore, *SlDREBA4* activates biosynthetic pathways for jasmonic acid, salicylic acid, and ethylene, binding to DRE motifs (A/GCCGAC) in target HSP promoters.

Interestingly, *iaa9-3* exhibited comparatively higher leaf area and chlorophyll content than WT-MT under prolonged heat exposure, yet showed a more modest *SlDREBA4* induction than *iaa9-5* under 38 to 40 °C heat stress. This suggests that *iaa9-3* allele may rely more strongly on morphophysiological responses and maintenance of photosynthetic integrity. Additionally, it might engage alternative protective pathways, which are not captured by *SlDREBA4* alone. In contrast, *iaa9-5* may exhibit a stronger early transcriptional activation of the DREB-related signaling axis, consistent with its higher *SlDREBA4* upregulation under acute heat conditions.

Taken together, the results of this study support the hypothesis that *IAA9* contributes to heat-stress adaptation through coordinated effects on thermomorphogenesis (leaf hyponasty and elongation), maintenance of vegetative growth, and regulation of stress-responsive gene expression, with clear allele-specific differences between *iaa9-3* and *iaa9-5* mutants. A limitation of this study is that hyponasty was documented qualitatively. Therefore, future work should quantify leaf angles to strengthen inferences regarding thermomorphogenic responses. In addition, integrating morpho-physiological analyses under a unified heat-stress regime with an expanded panel of molecular markers, including genes involved in ROS scavenging, would enable stronger inference about causal links between thermomorphogenic traits and molecular thermotolerance pathways.

CONCLUSIONS

The present study demonstrates that mutations in the *IAA9* gene significantly influence tomato responses to heat stress by modulating morphophysiological traits and stress-responsive gene expression. Under elevated temperature conditions, *iaa9-3* and *iaa9-5* mutants exhibited improved heat tolerance compared to WT-MT, as evidenced by higher plant height, greater leaf area and number, and elevated chlorophyll content. These mutants also showed upregulated expression of the heat-inducible *SlDREBA4* gene, particularly in *iaa9-5*, suggesting a genotype-dependent transcriptional response to thermal stress. Collectively, the findings highlight the regulatory role of *IAA9* in auxin-mediated thermomorphogenesis and stress adaptation in tomato. From a broader perspective, *IAA9* mutants hold potential as genetic resources for developing heat-resilient tomato cultivars, which is especially critical in the face of ongoing climate change and increasing global temperature extremes.

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