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The Roles of Different Types of Trichomes in Tomato Resistance to Cold, Drought, Whiteflies, and *Botrytis*

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Abstract: The tomato (*Solanum lycopersicum)* is one of the most popular vegetables in the world. In tomato production, due to the effects of diseases, insect pests, drought, and cold damage, large-scale production reduction is often caused. Plant trichomes are protruding attachments distributed on the surface of different plants, providing protection for plants. When the plant is under external stress, the trichomes can play an important role in protecting the plant from damage through its physical structure. The density and type of different trichomes are closely related to the stress resistance of tomatoes. The tomato *wo* mutant LA3186 (referred to herein as "3186M"), LA3186 (referred to herein as "3186L"), the *ln* mutant LA3-071 (referred to herein as "3-071"), and the tomato cultivar Jia Ren (referred to herein as "JR", used as the control), which possess different numbers of trichomes on the surface of the leaves, were used as materials; the glandular characteristics, types, and densities of the trichomes were observed under a scanning electron microscope (SEM); and transmission electron microscopy (TEM) was used to observe the subcellular structure in the leaves. The relationship between the different tomato trichomes and stress resistance was investigated with treatments of low temperature, drought, disease, and insects. This study provides a theoretical foundation and practical basis for the further utilization and regulation of the trichome-related characteristics of tomatoes.

Keywords: tomato; stress; trichomes; microscope

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1. Introduction

Trichomes originate from the outer epidermal cell tissue of the plant, and they are morphologically diverse appendages present on the surface of many plants [1]. Trichomes can play a buffering role in the interaction between plants and biotic or abiotic stresses. Plant trichomes not only increase the thickness of the epidermis but also act as a physical barrier against external invasion [2]. They are not necessary for the growth and development of the plant itself; however, they have many important biological functions, including reducing the loss of plant heat, increasing the resistance of plants to cold damage and drought, and protecting plant tissues from ultraviolet light and insects [3–5]. Additionally, trichomes provide a model system for studying the regulation of plant cell differentiation [6,7]. The trichomes of *Arabidopsis thaliana* have been widely investigated and have become the main model for the study of plant cell differentiation.

Trichomes can be divided into two major categories: glandular trichomes and nonglandular trichomes. Tomato trichomes can be further divided into eight different types (I–VIII); I, IV, VI, and

VII are glandular trichomes, and II, III, V and VIII are nonglandular trichomes [8]. Type I glandular trichomes consist of six to 10 cells of slender glandular trichomes, are 2–3 mm in length, and have spherical and multicellular bases with small, round glandular cells at the hairy tip. Type I trichomes mainly contain acyl glucose. Type II nonglandular trichomes resemble type I trichomes but are nonglandular and short (0.2–1.0 mm) and have spherical and multicellular bases. Both type I glandular trichomes and type II nonglandular trichomes are spherical and multicellular at their base. The glands of the type I, IV, and VI trichomes contain acyl flavonoids, terpenoids, and sugars. Of these compounds, acyl sugar has a very high viscosity and can thus stick to herbivores and prevent them from feeding freely on the surface of a plant [9]. In addition, an increase in the flavonoid content on the surface of plants can prevent the exposure of the plant to highly penetrating UV-A (longwave blackspot-effect ultraviolet rays) and excessive damage [5]. Tomatoes can also produce or release some highly toxic compounds to prevent pest damage [2]. Dong and Huang found that trichomes of wheat cultivars resistant to root rot were significantly longer than those of commonly susceptible cultivars. This finding suggested that trichomes on plant leaves play an important role in resistance to wheat root rot pathogens [10,11].

Trichomes can also protect plants from being damaged by excessive temperature and drought stress [12–14]. The density and types of the different trichomes are closely related to stress resistance in tomatoes. Previous investigations have shown that tomatoes rich in trichomes might have greater cold tolerance [15,16]. The published results indicated that under the same cold stress, wild tomatoes with dense trichomes have a higher survival rate than other common tomatoes without dense glandular trichomes. Speculations have been made that tomatoes with well-developed trichomes can better regulate their growth and response to the external environment during exposure to different adversity. Tomatoes with many trichomes can adjust their metabolism at the fastest rate, which can somewhat improve the cold tolerance of tomatoes [17]. Many reports exist on the study of plant trichomes and their resistance to stress, with previous studies focusing mainly the molecular, physiological and biochemical aspects. The microscopic or ultrastructure of plant leaves, particularly the relationship between trichome types and their response to adversity stress, the relationship between microscopic morphological characteristics and the internal structure of leaves, and the relationship between varieties and diseases have rarely been investigated.

To assess the relation between the type or density of trichomes and stress resistance, differences in the type, structure, glandular status, and gross density of the trichomes among two wo mutant LA3186 varieties (a hairy cultivar and a hairless cultivar), the natural ln mutant LA3-071, and a common tomato cultivar were compared. The Wo (Wolly) gene can promote the formation of multicellular trichome involved in modulating wax biosynthesis in tomato [18]. 3186M and 3186L are two mutants of wo, showing hairy and hairless traits, respectively. The Ln gene is one of the hairy genes, which dominate the hairy characters in tomato, and 3-071 is an ln mutant. JR is a common cultivated tomato; we use it as a control. The function of tomato trichomes in the process of stress resistance was then studied in combination with assessments of the internal structure and morphological observations by stressing the selected accessions with cold, drought, disease, and pests. The results of this study provide a theoretical and practical foundation for the further use of the physical characteristics of the trichomes.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The tomato *wo* mutant LA3186 hairy accession, the wo mutant LA3186 hairless accession, and the ln mutant LA3-071 hairy accession were gifts from the Tomato Genetics Resource Center, and these tomatoes, along with the tomato cultivar Jiaren, were conserved and propagated in our lab. Tomato seedlings were grown for 8 weeks in a greenhouse with 16-h light and 8 h dark cycles of natural light on soil under day and night temperatures of 26 °C and 16 °C, respectively. The seedlings at the four-leaf stage were sampled for transmission electron microscopy and scanning electron microscopy observations. When the seedlings grew to the five-leaf stage, they were treated with abiotic (cold and drought) and biotic (*Botrytis cinerea* and whitefly) stresses.

2.2. Observation of Trichomes Difference Using Scanning Electron Microscope

When the uniformly developed seedlings grew to the four-leaf stage, tomato leaves (cut to approximately 4 mm^2) and stems (cut to approximately 3–4 mm) were collected from the four different test materials. The leaves of the second morphological upper leaves of the plant were sampled, and the stem sections between the cotyledons and true leaves were gathered. The samples were placed in a 2% glutaraldehyde fixative, removed, and fixed for approximately 24 h, and the material was dehydrated in gradient alcohol (25%, 50%, 75%, 85%, 95%) and was finally treated with anhydrous alcohol twice, and the dehydration time of each step was about 2–3 h. Then, we place gradient-treated materials in a critical point dryer (HITACHIHCP-2). The adhesive table and the gold-sprayed and dried materials are adhered to the metal table with black double-sided adhesive under an ordinary magnifying glass, and the materials are gold sprayed with an ion sprayer (JFC-1600). The sample material was first observed and analyzed using a scanning electron microscope (JSM-6390/LV, Hitachi Limited, Japan) to determine the observation site, and then the samples were observed and photographed at different magnifications.

The leaf blades of 4 different types of tomato materials were cut into 3 mm × 5 mm with a blade, placed in a solution with a pH of 6.8, fixed with 2.5% glutaraldehyde solution, and were prefixed and stored at 4 °C for at least 2 h. The samples were then rinsed, postfixed, rinsed, dehydrated, replaced, soaked, polymerized, and trimmed. The pruned embedding block was placed on a microtome, the thickness of the slice was set to 0.75–1 μm, and a semi-thin section was obtained using an ULTRACUT-E ultrathin microtome. The semi-thin sections were placed on an ultramicrotome, and the section thickness was set to 50-70 nm. Finally, the ultrathin sections were stained and observed [19].

2.3. Abiotic Stress Treatment

For cold stress treatment, plasma membrane permeability was used as a reference. When the plants had grown to the five-leaf stage, 15 well-grown seedlings of each material were collected and placed in a growth chamber under a 16-h light/8-h dark cycle at a temperature of 4°C. After 0 h, 24 h, 48 h, 96 h, and 144 h of treatment, the leaf samples were obtained to determine the permeability of the plasma membrane at each of these time points and there were three biological replicates per treatment. Briefly, 0.5 g of fresh leaves was washed with deionized water and placed in a test tube. Distilled water (deionized water) was then added to obtain a final volume of 10 mL, and the samples were then shaken slowly for 30 min and incubated at room temperature for 30 min. The conductivity (E1) of the solution was measured with a conductivity meter (0.96 electrode). The samples were boiled for 10 min in a water bath and were then allowed to cool to room temperature; the total conductivity (E2) of the solution was then measured with a conductivity meter. The mean from the three measurements for each solution was obtained, and the permeability of the plasma membrane was determined [20]. Three biological replicates per group. For drought stress, when the plants reached the five-leaf stage, 12 tomato seedlings with different trichome characteristics were used as the experimental materials, and the water content of the potted soil was controlled by the weighing method to 75%–80% of the maximum water content. For the drought stress treatment, the plants were not given any water for 4 days, and then they were rewatered at 1, 2, 4, and 7 days after initiation of the drought stress treatment. On day 2 of rehydration, the relative water content (RWC) of the second leaf was determined. The measured values for Wf, Wd, and WT (averages from three replicates) were substituted into the formula to calculate the sample water content, relative water content, and the water saturation deficit, as previously described [21]. (Wf: fresh weight of tissue; Wd: dry weight of tissue; WT: weight of tissue fully saturated with water).

The formula is as follows:

$$\mathrm{RWC} = \frac{Wf - Wd}{Wt - Wd} \times 100$$

2.4. Botrytis Cinerea Inoculation

Botrytis cinerea was inoculated into liquid media and shaken at 25°C for approximately 1 week [22], after which *B. Cinerea* inoculum was diluted to obtain a suspension of 10^6cfu/mL. When seedlings reached the five-leaf stage, the suspension was sprayed onto both sides of the leaves. One week after inoculation, the disease index, superoxide dismutase (SOD) and peroxidase (POD) activities, and the chlorophyll content were measured. The chlorophyll concentration was determined according to Porra [23], and the absorbance of a volume of 250μL was read using a Sunrise Remote Microplate Reader (Tecan, Austria). The standard index used to grade the *B. cinerea* disease is based on the Guidelines for Pesticide Field Efficacy Tests edited by the Health Laboratory of the Ministry of Agriculture in China (0-9 grade, the proportion of the lesion area to the total leaf area is 0%, 0%–5%, 6%–10%, 11%–25%, 26%–50%, 50%–100%). The Peroxidase (POD)activity was measured as described by Fu and Huang [24], with relevant modifications. The Superoxide dismutase (SOD) activity was determined according to the methods described by Hao et al. and Li [4,25], with some improvements. The data analyses were performed using SAS v8.0 software, and an ANOVA method was used to assess the significance of the differences.

2.5. Whitefly Treatment

The whiteflies were collected from greenhouse tomato plants. When the plants grew to the five-leaf stage, 18 well-grown seedlings from each material were collected and placed in an insect box. Approximately 12 whiteflies were added to each seedling using a brush and magnifying glass. The density of the insect population was measured on 3 randomly selected leaves. The samples were collected every 2 days, specifically, after pest treatment for 1, 3, 5, 7, 9, and 11 days. The SOD and POD activities also were measured. Each experiment was repeated three times.

3. Results

3.1. Different Tomato Trichome Types

We obtained different types of tomato accessions related to trichome development on the TGRC (Tomato Genetics Resource Center), and planted plants with wo-regulated 3186M and 3186L, and ln-regulated 3-071, and observed their morphology by scanning electron microscopy. The trichomes in accession 3186M mainly included type II and III nonglandular trichomes and type I and IV glandular trichomes, although more type II and III nonglandular trichomes than type I glandular trichomes were observed (Table S1). Visual observation revealed that this accession showed high trichome coverage and density (Figure 1)

The trichomes of 3186L mainly included type III and V nonglandular trichomes and type VI glandular trichomes, which grew in an interphase manner, mostly with small glandular trichomes. The trichomes covering 3186L were small and short, with few glands and a relatively low density. In addition, the type VI trichomes comprised four glandular cells arranged into intermediate cells and a stem cell. The height of this type of trichome was lower than those of the other types, and this trichome type was not easily damaged by dehydration, either under experimental conditions or in nature (Figure 2).

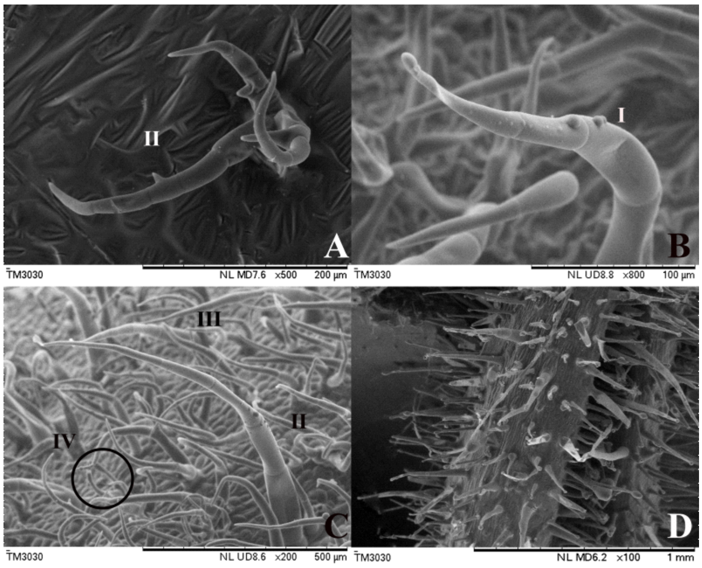


Figure 1. Morphological observation of different types of trichomes of tomato 3186M (tomato *wo* mutant, hairy) under a scanning electron microscope. The gross state of the trichomes of 3186M was observed by scanning electron microscopy, and different types of trichomes are marked in the picture. Scale bars (**A**) = 200 μm, (**B**) = 100 μm, (**C**) = 500 μm, (**D**) =1 mm.

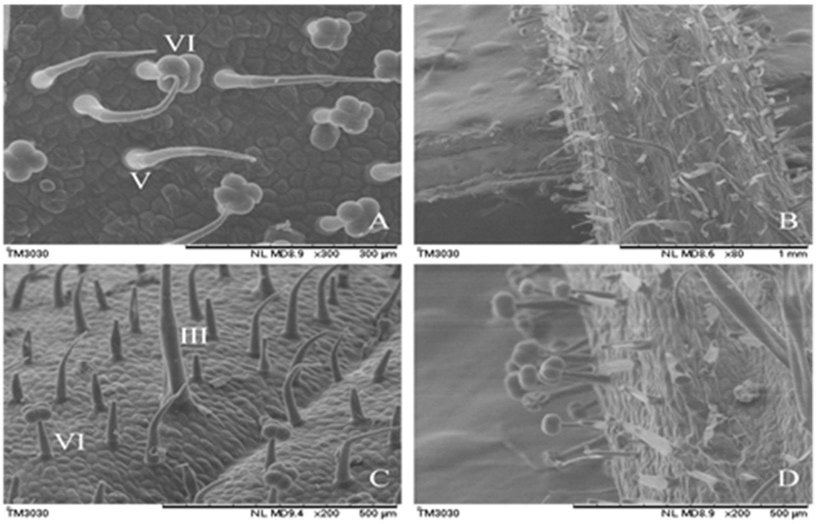


Figure 2. Morphological observation of different types of trichomes of tomato 3186 L (tomato *wo* mutant, hairless) under a scanning electron microscope. The gross state of the trichomes of 3186L was observed by scanning electron microscopy, and different types of trichomes are marked in the picture. Scale bars (**A**) = 300 μm, (**B**) = 1 mm, (**C**) = 500 μm, (**D**) = 500 μm.

The trichomes of accession 3-071, controlled mainly by *ln*, included more type III and V nonglandular trichomes and fewer type VI glandular trichomes. The trichomes in accession 3-071 exhibited small coverage and relatively low density and included long and short trichomes that grow together. In addition, accession 3-071 had the same type VI glandular trichomes as 3186L (Figure 3).

The trichomes of the control cv. JR mainly included type III and V nonglandular trichomes and type VI glandular trichomes. The type III trichomes were few in number, and the type V nonglandular trichomes and VI glandular trichomes grew in an interphase manner. The glands were less dense, consisting mainly of short glandular trichomes. In addition, the trichome coverage exhibited by the control cultivar JR was relatively low, with few species, a relatively low density, and highly uniform and short trichomes. (Figure 4).

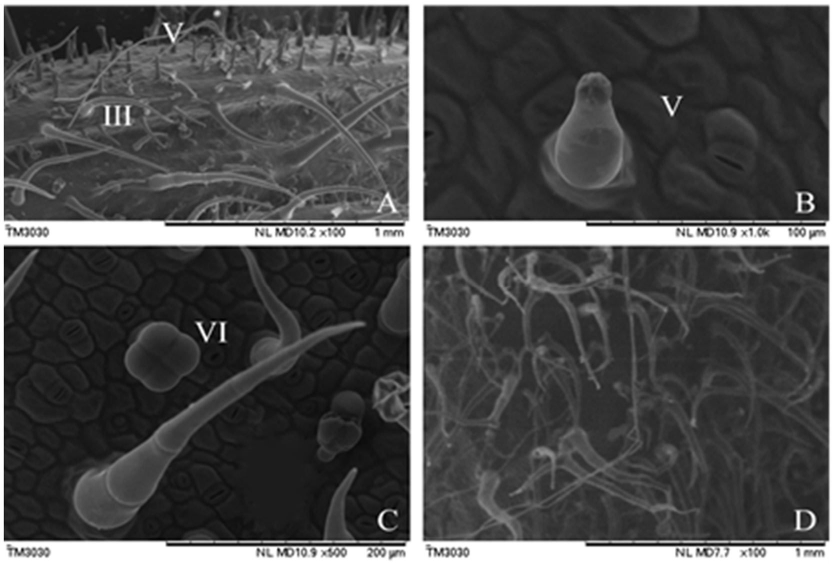


Figure 3. Morphological observation of different types of trichomes of tomato 3-071 (tomato *ln* mutant, hairless) under a scanning electron microscope. The gross state of the 3-071 accession was observed by scanning electron microscopy, and different types of trichomes are marked in the picture. Scale bars (**A**) =1 mm, (**B**) = 100 μm, (**C**) = 200 μm, (**D**) = 1 mm.

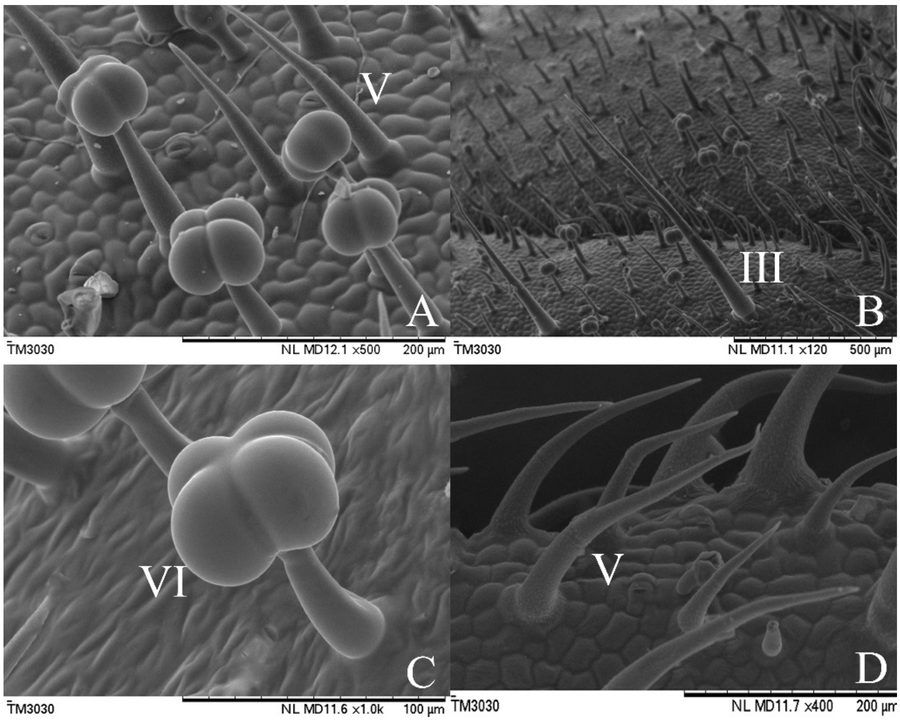


Figure 4. Morphological observation of different types of trichomes of tomato JR (tomato cultivar Jia Ren) under a scanning electron microscope. The gross state of the JR cultivar was observed by scanning electron microscopy, and different types of trichomes are marked in the picture. Scale bars (**A**) = 200 μm, (**B**) = 500 μm, (**C**) = 100 μm, (**D**) = 200 μm.

The densities of the tomato trichomes were observed and the results showed that the number of trichomes per unit area at 100 x magnification in 3186M, 3186L, 3-071, and JR were 118, 93, 84, and 103, respectively (Figure 5)

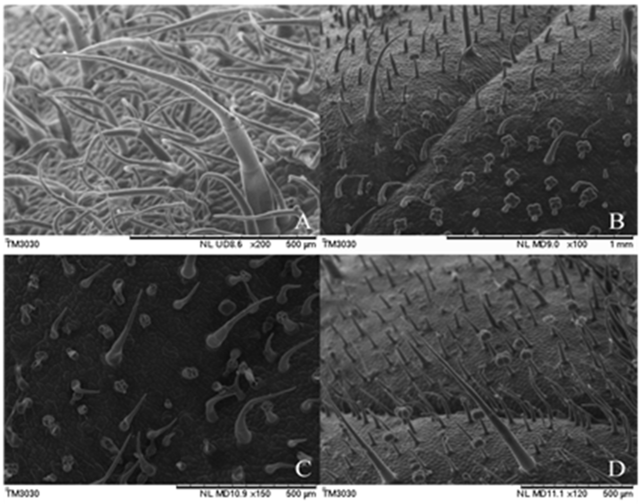


Figure 5. Scanning electron microscopy images of trichome density (per unit area) in 3186M, 3186L, 3-071, JR. 3186M (tomato *wo* mutant, hairy), 3186L (tomato *wo* mutant, hairless), 3-071 (tomato *ln* mutant, hairless), and JR (tomato cultivar Jia Ren). A scanning electron microscope observation of the state of trichome of each tomato accession per unit area. (**A**) The 3186M trichome numbers of the per unit area at 100x magnification. Bar = 500µm. (**B**) The 3186L trichome numbers of the per unit area at 100x magnification. Bar=1 mm. (**C**) The 3-071 trichome numbers of the per unit area at 100x magnification. Bar = 500 µm. (**D**) The JR trichome numbers of the per unit area at 100x magnification. Bar = 500 µm.

3.2. The Chloroplast Structure of Four Tomato Trichome Types

We observed through projection electron microscopy that the shape of the 3186M chloroplast was mostly round. This chloroplast had many basal laminates and starch granules, with an average of two to three starch granules per chloroplast (Supplemental Figure S1). The chloroplast of 3186Lwas a long circle with many basal laminae and matrix layers but few special plastids. (Supplemental Figure S2). The chloroplast of accession 3-071 was elliptic, with very few basal laminae but many stroma lamellae. The internal starch granules were abundant and bulky. Each of these chloroplasts had approximately three to five starch grains, and obvious folds were observed on the starch granules (Supplemental Figure S3). The chloroplast of the JR comprised a long circle with a unique circular plastid. This structure had many basal laminates and matrix films but few starch granules (Supplemental Figure S4).

3.3. Response to Abiotic Stress

Tomato plants at the 5-leaf stage were treated with low temperature (4 °C), and the degree of injury to the cell membrane was measured by the conductivity. As shown in Figure 6A, an increase in the duration of the low temperature treatment was associated with an increase in the plasma membrane permeability in all the accessions, and a significant increase was observed after 96 h of cold treatment. The membrane of 3186M exhibited the lowest permeability, followed by the membrane of the common tomato. A considerable increase in plasma membrane permeability was observed in the accessions with fewer trichomes, indicating that 3186M exhibits some resistance to low temperature.

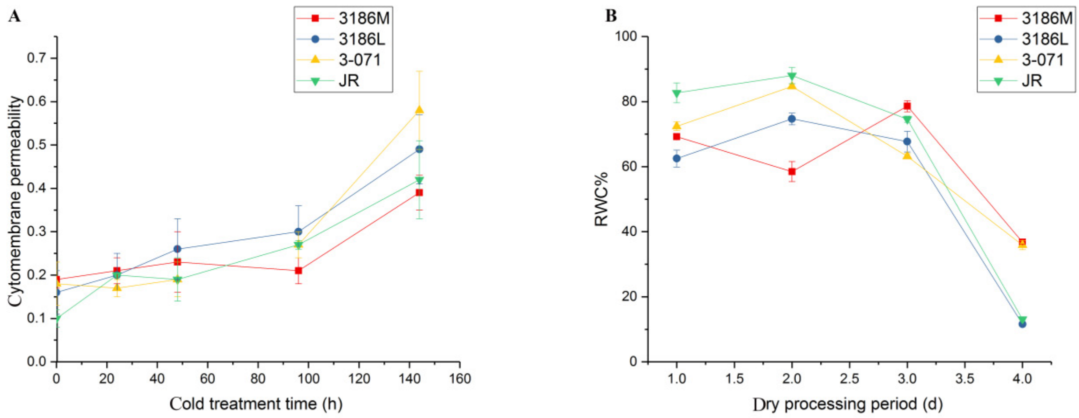


Figure 6. (**A**) Determination of the relative permeability of the plasma membrane by measuring the conductivity of several test tomato materials at different time points after the low temperature treatment. (**B**) During drought treatment, the relative water content (RWC) was measured at different times. The data represent the means of three replicates ± SD. All experiments were repeated three times with similar results.

The drought resistance of the plants was measured by the relative water content (RWC). As shown in Figure 6B, an extension in the duration of drought stress was associated with a gradual decrease in the RWC of the tomato leaves (fourth from last to last) and a gradual increase in the membrane permeability. After 2 days of drought treatment, the RWC began to show an obvious decrease, and on day 4, the RWC exhibited a sharp decrease. The RWC of 3186M and 3-071 showed a slower decrease than that of 3186L and JR after 4 days of drought treatment. The results indicated that the trichomes help plants to slow down water loss during water deficit and may contribute to drought tolerance of the plants.

Under mild and moderate drought stress, the chloroplast expanded and deformed, the number of starch granules was reduced, the basal laminates became more loosely arranged, and the thylakoid cavity was enlarged. Under severe drought stress, the chloroplasts expanded to achieve a nearly round shape; the chloroplast membranes ruptured, and severe vesicular degeneration was observed under severe drought conditions; and the chloroplasts of 3186M and 3-071 showed less ultrastructural damage than those of 3186L. The integrity of the cell structure was well maintained. The hairy tomato 3186 accession can grow better in arid environments (Supplemental Figure S5). The above results indicated that the varieties with abundant trichomes, particularly those with abundant nonglandular trichomes, had a strong water storage capacity and showed better drought resistance.

3.4. Resistance to B. Cinerea

The disease index in 3186M was not significant, but it was significant for JR and 3186L. These results showed that the accessions could be ranked in terms of disease index as JR > 3186L > 3-071 > 3186M (Figure 7A). As shown in Table 1, the accessions could be ranked in terms of increasing chlorophyll b content as 3-071 < JR < 3186 L < 3186 M. No significant difference in the chlorophyll content was found among 3186L, 3-071, and JR, but the content in these varieties was significantly different from that found in 3186M (Table 2). The analysis of the SOD activity revealed that it first increased and then decreased in all four accessions, and the accessions could be ranked based on the SOD activity after treatment as 3186M < JR < 3-071 < 3186L (Figure 7B). The changes in the POD activity after spraying with *B. cinerea* were also observed in the tomato varieties, and all accessions showed a similar trend consisting of an initial decrease followed by an increase. In addition, the varieties could be stably ranked in terms of POD activity 9 days after the application of this pathogen as 3186M > JR > 3186L > 3-071 (Figure 7C). These results showed that the accessions with more type III nonglandular trichomes were more resistant to *Botrytis cinerea.* Additionally, the accessions with the longer trichomes had a strong disease resistance.

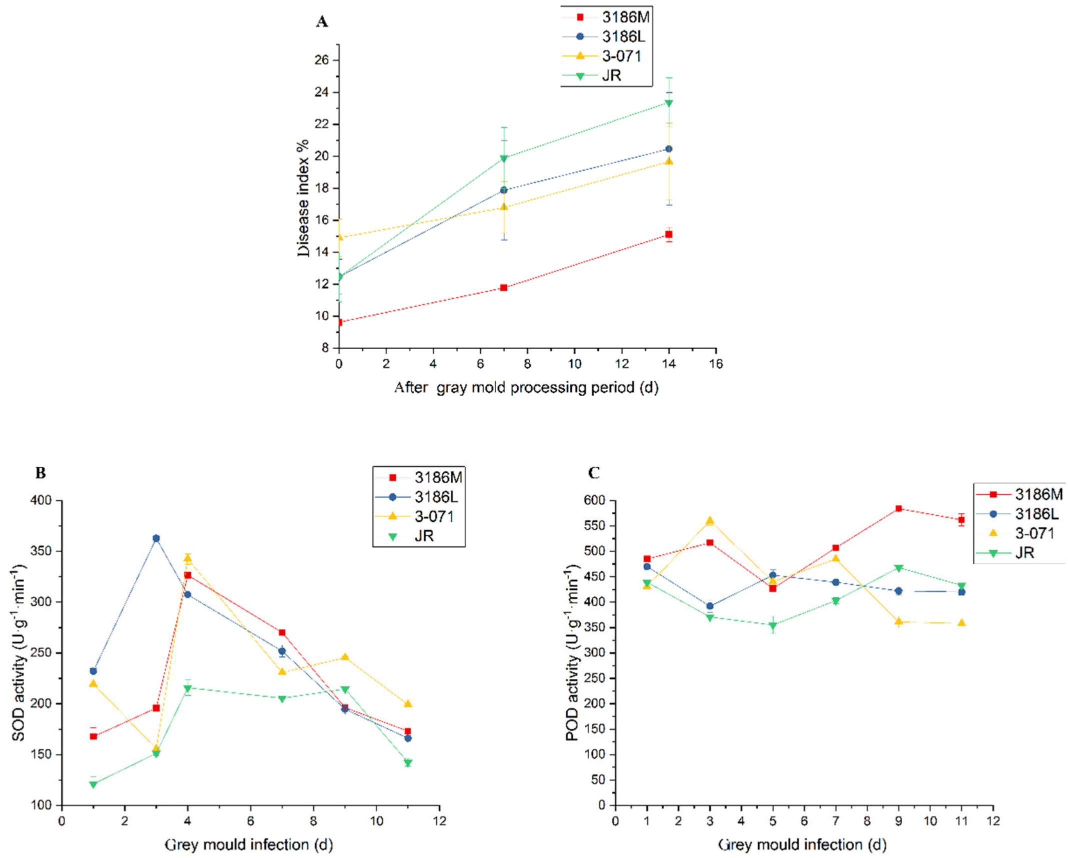


Figure 7. (**A**) Disease index of tomatoes with different trichome types at different time points after being inoculated with *Botrytis cinerea*. (**B**) Changes in the SOD activity of different tomato varieties 1 week after being inoculated with *Botrytis cinerea*. (**C**) Changes in the POD activity of the different tomato varieties 1 week after being inoculated with *Botrytis cinerea*. The content of SOD and POD were measured as previously reported [24]. The data represent the means of three replicates ± SD. All experiments were repeated three times with similar results.

Table 1. Peroxidase (POD) and Superoxide dismutase (SOD) activities in tomato seedlings that were sprayed with *Botrytis cinerea* at the five-leaf stage. The activities were measured one week after spraying. Among them, a, b, c, and d represent different degrees of POD and SOD activities. The values represent the average of three technical replicates ± SD. 3186M (tomato *wo* mutant, hairy), 3186L (tomato *wo* mutant, hairless), 3-071 (tomato *ln* mutant, hairless), JR (tomato cultivar Jia Ren).

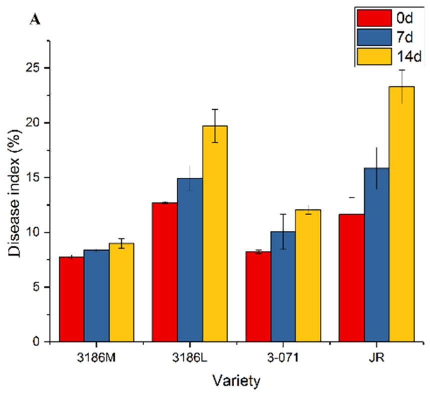
Accession	POD Activities /U·g^{-1}·min^{-1} (FW)	SOD Activities /U·g^{-1}·min^{-1} (FW)
3186M	503 ± 3.33d	270.11 ± 1.62d
3186L	439 ± 2.52b	251.82 ± 1.02c
3-071	485 ± 0.60c	230.72 ± 0.85b
JR	403 ± 6.03a	205.40 ± 1.08a

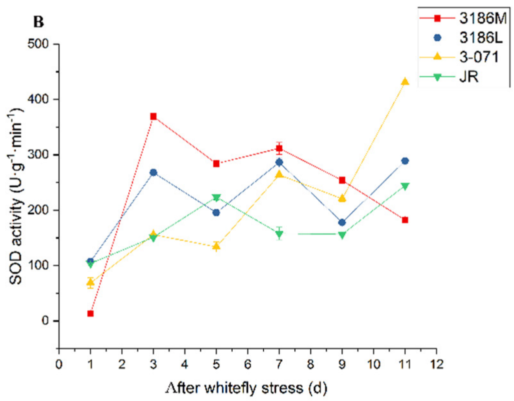
Table 2. Chlorophyll content of tomato accession seedlings in the six-leaf stage when treated with *Botrytis cinerea*. Among them, a and b represent different degrees of chlorophyll content. The values represent the average of three technical replicates ± SD. 3186M (tomato *wo* mutant, hairy), 3186L (tomato *wo* mutant, hairless), 3-071 (tomato *ln* mutant, hairless), JR (tomato cultivar Jia Ren).

Accession	Chlorophyll a Content /mg·g^{-1}	Chlorophyll b Content /mg·g^{-1}	Total Chlorophyll/mg·g^{-1}
3186M	5.47 ± 0.03b	11.13 ± 0.03b	16.60 ± 0.06b
3186L	3.74 ± 0.07a	7.82 ± 0.05a	11.56 ± 0.12a
3-071	3.79 ± 0.04a	7.81 ± 0.04a	11.60 ± 0.08a
JR	3.85 ± 0.07a	7.79 ± 0.04a	11.64 ± 0.11a

3.5. Resistance to Whiteflies

The accessions could be ranked in terms of population density (from high to low) as JR > 3186L > 3-071 > 3186M. Moreover, no significant difference was found between 3186L and 3-071, but a significant difference was detected between 3186L and JR (Table 3). Chlorophyll content was determined and resulted in the following ranking for the accessions: 3186M < 3-071 < JR < 3186L (Table 4). The SOD activity of the tomatoes with different trichome characteristics at the different time points during stress treatment was statistically analyzed, and the highest SOD activity was detected in 3186M. The general trend for SOD activity was an initial increase followed by a decrease. The varieties could be ranked based on their increasing SOD activity as 3186M < JR < 3186L < 3-071 (Figure 8A). The activities of the POD in all the species, except for 3186L, steadily decreased, and the descending trends found for these three varieties were similar. On day 9, these three varieties continued to show a stable trend (Figure 8B). The results of insect density, SOD and POD are basically the same. Using the results of electron microscopy in combination with these results, we were able to analyze that type I and IV glandular trichomes, particularly the long trichomes, and determined that they have a beneficial effect on insect resistance.





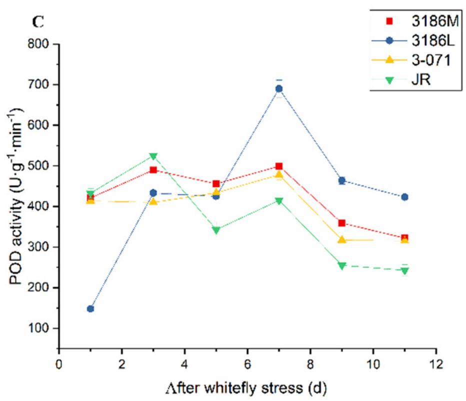


Figure 8. (**A**) Insect density on tomatoes with different trichome types; the density was measured at various times after treatment with whiteflies. (**B**) Changes in the SOD activity in the four tomato accessions at various times after treatment with whiteflies. (**C**) Changes in the POD activity in the four tomato accessions at various times after treatment with whiteflies. The content of SOD and POD were measured as previously reported [24]. The data represent the means of three replicates ± SD. All experiments were repeated three times with similar results.

Table 3. POD and SOD activities of whitefly-treated tomato seedlings at the six-leaf stage. The measurements were made 2 weeks after treatment with whiteflies. Among them, a, b, c, and d represent different degrees of POD and SOD activities. The values represent the average of three technical replicates ± SD. 3186M (tomato *wo* mutant, hairy), 3186L (tomato *wo* mutant, hairless), 3-071 (tomato *ln* mutant, hairless), JR (tomato cultivar Jia Ren)

Accession	POD Activity /U·g^{-1}·min^{-1} (FW)	SOD Activity /U·g^{-1}·min^{-1} (FW)
3186M	499 ± 3.33c	311.73 ± 0.71c
3186L	690 ± 1.32d	286.34 ± 0.52bc
3-071	478 ± 2.56b	263.77 ± 1.66b
JR	415 ± 1.05a	157.98 ± 0.36a

Table 4. Chlorophyll content of whitefly-treated tomato seedlings at the six-leaf stage. The measurements were made 2 weeks after treatment with whiteflies. Among them, a, b, c, and d represent different degrees of chlorophyll content. The values represent the average of three technical replicates ± SD. 3186M (tomato *wo* mutant, hairy), 3186L (tomato *wo* mutant, hairless), 3-071 (tomato *ln* mutant, hairless), JR (tomato cultivar Jia Ren).

Accession	Chlorophyll a Content /mg·g^{-1}	Chlorophyll b Content /mg·g^{-1}	Total Chlorophyll /mg·g^{-1}
3186M	3.66 ± 0.03a	7.95 ± 0.03a	11.61 ± 0.06a
3186L	5.98 ± 0.07c	12.19 ± 0.05c	18.17 ± 0.12c
3-071	4.49 ± 0.04b	9.21 ± 0.04b	13.7 ± 0.08b
JR	4.85 ± 0.07b	9.95 ± 0.04b	14.80 ± 0.11b

4. Discussion

As the first important barrier between plants and their environment, plant trichomes constitute an important component for reducing external pressures and resisting external threats to facilitate normal plant growth. At present, studies on the response of tomato trichomes to stress are mostly focused on molecular, physiological, and biochemical research. Studies of the leaf microstructure or ultrastructure, particularly studies focusing on the relation between microscopic morphological characteristics and internal differences in the leaf structure, and whether accessions are susceptible to disease, are relatively rare. This study is the first to observe the trichome characteristics of tomatoes using electron microscopy with an auxiliary physiological index measurement. In addition, the relation between tomato trichomes and resistance was studied, and the results provide a theoretical and practical foundation for further use of the physical characteristics of trichomes.

4.1. Tomatoes with a High Density of Type I Glandular and Type II Nonglandular Trichomes were Tolerant to Cold Stress

A low temperature can cause plant damage and even death. Compared to other plant species, the leaves, stems, and fruits of *Solanum habrochaites* all have obvious hairy body coverage. With increases in cold treatment duration, the accessions with more trichomes showed obvious advantages, laying a solid foundation for studying the cold resistance mechanism of tomatoes. In this study, tomato accessions with several different types of trichomes were treated with low temperature. The plasma membrane permeability of 3186M increased slightly and that of 3186L increased substantially compared to that of the other accessions. Tomato membrane lipids were postulated to have been damaged under low temperature conditions. We observed the structure type of trichomes with an electron microscope, and obtained dense trichomes, especially the unique type I gland and type II non-glandular trichomes of 3186M, which are more resistant to cold. This is consistent with the results reported by Foolad and Lin [26]. The dense trichomes in 3186M contain unique type I glandular hairs and type II nonglandular hairs. Presumably, spherical and multicellular bases can increase the trichome coverage, which would increase the physical cover protection and cold tolerance of the tomato. Moreover, a tomato accession that has more Trichome type I is more tolerant to cold stress compared to accessions that have less of this trichome type. Based on these results, we speculated that, at low temperatures, when the tomato membrane system is destroyed, trichome-rich tomatoes can increase their metabolic rate to minimize membrane lipids and thus exhibit better survival.

Previous studies have shown that, under low-temperature stress, plant cell membranes are damaged first [20]. One of the important signs of frozen injury in plants is the enhanced permeability of the plasma membrane; thus, a determination of current conductivity can be used to reflect the degree of damage to the cell membrane [27]. The magnitude of changes in the permeability of the plasma membranes of 3186M and JR were relatively small. A comparison of the density and type of trichomes within a unit area showed that the trichome density in 3186M and JR was relatively high. However, the former cultivar mostly contains long trichomes, and the latter is usually short haired and exhibits good cold resistance. The other two breeds have short and long trichomes and a have weak cold tolerance ability. Therefore, increasing the trichome density of the same altimeter can improve the cold tolerance of tomato accessions to some extent. A higher density of trichomes at the same height has been hypothesized to be associated with a larger coverage area and fewer gaps, which would be beneficial for preventing the direct contact of cold air with the leaf epidermis. This characteristic physically protects tomato leaves and improves their cold resistance. Therefore, the accessions containing type I glandular trichomes and type II nonglandular trichomes have strong cold resistance.

4.2. Abundant Nonglandular Trichomes in Tomatoes can Promote Drought Tolerance

After drought treatment, the RWC of a plant will decrease [22,23]. Compared with those of 3186L, the chloroplast ultrastructure of 3186M and 3-071 showed decreased injury under severe drought conditions and reasonable maintenance of the integrity of their cell structure. Therefore, 3186M, which has abundant trichomes, can grow better in an arid environment, which is consistent with our observations. Both 3186M and 3-071 have many nonglandular trichomes and fewer glandular trichomes compared with 3186L and JR. The 3186M accession showed better drought resistance.

In addition, related studies have shown that nonglandular trichomes can increase the drought resistance of plants by increasing the absorption of solar radiation and increasing the absorption of water vapor in the leaves [5]. The vitrification of nonglandular trichomes after differentiation and maturation, surrounded by a ring of epidermal supporting cells, has been hypothesized to result in a certain reflection of irradiated light. These trichomes reduce the penetration of solar radiation and increase the reflection of solar radiation to reduce the harmful effects of solar radiation. We speculated that because of the reduction of UV damage and thus the avoidance of high temperatures, water evaporation is reduced, and water storage performance is better. For drought-tolerant breeding, accessions with many nonglandular trichomes should be bred.

4.3. Abundant Type III Nonglandular Trichomes and a Higher Average Height of Tomato Trichomes can Result in Pathogen Resistance

Gray mold can cause extensive harm and is difficult to prevent [28]. When Botrytis cinerea comes in contact with a plant, this fungus usually kills the plant cells first and then absorbs nutrients from the dead tissue for rapid propagation. The disease index of all accessions increased at different degrees after the application of Botrytis cinerea.

The increase found in 3186M was lower than that found for 3-071, but, in 3186L and JR, the disease index rose significantly. The accessions could be ranked in order of disease severity after treatment with Botrytis cinerea as follows: 3186M < 3-071 < 3186L < JR. The results show that the highest trichome height found in 3186M yielded the strongest disease resistance, which is consistent with the results of Patil [29].

The determination of chlorophyll content reflects the strength of plant photosynthesis to a certain extent. Some studies have shown that chlorophyll content is positively correlated with plant resistance. Previous results have shown that disease induces an increase in the SOD activity, which effectively inhibits the production of reactive oxygen species and improves plant resistance to stress [30,31]. The POD plays an important role in disease resistance in plants. This enzyme can eliminate excess free radicals, catalyze the synthesis of bactericidal substances, increase the synthesis of lignin and corkwood, form a physical barrier, and then enhance the resistance of plants. Most studies believed that the disease resistance-related enzyme activity is positively correlated with the disease resistance index, and it is a protective measure against plant pathogens. The enzyme activity of the diseased plants was lower than that of the disease-resistant plants [32–34], which is consistent with the results of this study. Therefore, in this article, we analyzed their ability to resist external stress by measuring the chlorophyll content and POD and SOD activity in 3186M, 3186L, 3-071, and JR. The results show that 3186M exhibited the strongest disease resistance and the highest chlorophyll content.

An analysis of the trichome characteristics of the tomatoes using an SEM suggested that 3186M and 3-071, which show resistance to Botrytis cinerea, are rich in type III nonglandular trichomes. The other two accessions, which have a lower average height, were less resistant to Botrytis cinerea. A greater number of trichomes can block pathogens from contacting the epidermis and improve the disease resistance of tomatoes. Type III nonglandular trichomes consist of four to eight cells and are 0.4–1.0 mm in length, with a unicellular and flat base. Their external walls lack intercellular sections. Lipophilic substances in type III nonglandular trichomes can cross the borders of the trichomes and pass through the body cells. These trichomes can play an important role in the interaction between plants and the environment and protect tomatoes from pathogen infections.

This study reveals that accessions with a higher average length of trichomes and type III nonglandular trichomes exhibit strong disease resistance. This finding provides insights for the future cultivation of disease-resistant accessions.

4.4. With Longer Trichomes and a Higher Trichome Density, Particularly Type I and IV Glandular Trichomes, Tomatoes can be Resistant to Whiteflies

Insect resistance refers to a type of insect resistance mechanism produced by the crops themselves after being subjected to insect pests [35]. Plants with insect resistance will produce substances that influence the normal growth and development of plant-eating insects. Some of these might make insects small and weak, whereas others can make the insects display continued restlessness, which increases their risk of discovery by insect-eating animals and thus their death rate. Glandular trichomes secrete mucous, resins, volatile oils, and other substances, which can interfere with damage from herbivores and pathogens [8,36,37]. Studies have shown that the glands on the surface of trichomes can secrete toxic chemicals, such as resins and nicotine, to ward off external hazards [3,5]. The glandular trichomes of alfalfa can secrete chemicals to kill pests that attack its leaves and stems [38].In addition, the trichomes of the plants themselves block the free movement of insects and can also secrete volatile or nonvolatile toxic substances, making it difficult for pests to feed on the plant surface and thereby affecting the feeding position of insects. Several annual or perennial plants of the genus alfalfa also exhibit this characteristic. Anemones with long glandular trichomes also secrete flavonoids and terpenes to prevent leafhoppers from feeding on the plants, and the hairy body of the cowpea also plays a similar role in biological control [39]. Recent studies provide evidence that trichomes may function as sensors for detecting insect movement on the leaf surface [40].

According to the analysis of enzyme activity and population density, the accessions could be ranked in terms of severe infestation with insect pests as 3186M < 3-071< JR < 3186L. The literature indicates that the trichome density of plants is significantly correlated with their insect resistance. In addition, the high density of trichomes in *Solanum habrochaites* prevents insects from moving freely among the trichomes, reducing the destructive power of the insect.

The type I and IV trichomes of 3186M can secrete acyl sweets, and the type VI trichomes of 3-071 can secrete methyl ketone and other substances, such as mucus, which prevents insects from eating freely on the plant, providing resistance to pests. This finding is consistent with the results obtained in this study, which show that 3186M is more resistant to insects. However, although 3186L also contains type VI glandular trichomes, its resistance to insects is poor; thus, whether type VI trichomes are associated with insect resistance and needs be further investigated. The following conclusions can be obtained from the following SEM observation: accessions with type I and IV glandular trichomes, particularly long trichome-rich accessions, show greater insect resistance.

The adaptive changes of different accessions of tomato to adversity are not only related to its trichome density and type, but also to the molecular mechanism of resistance induced by stress, such as whether the expression of some resistance genes in plants plays a key role. Therefore, the analysis of the relationship between trichome characteristics and the resistance to stress from the aspects of genetic or micro-ecological regulation needs to be further explored. The results show that increased trichome density can enhance plant resistance to adversity. However, it is interesting that it has not happened during the evolution process, and there is no related research report. We hypothesize that an increase in trichome density will lead to associated yield losses.

In this article, we observe the status, type difference, and density of different types of trichomes of four different tomato materials and compare and analyze the above accessions with stress treatments, such as a low temperature, drought, disease and insect pests. Thus, the relationship between trichomes and stress resistance was determined. Finally, it was found that under cold stress, accessions with the same height glandular hair density had a certain cold tolerance. Under drought stress, accessions with abundant trichomes, especially those with more non-glandular trichomes, have stronger drought resistance. Under gray mold stress, type III non-glandular trichomes are more abundant, and breeds with higher average height of trichomes have better antibacterial properties. Under whitefly stress, accessions with longer trichomes and higher densities or with glandular trichomes of type I and IV have stronger insect resistance.

The adaptation of leaves of different tomato plants is related to not only the degree of adversity but also to the internal physiological mechanisms induced under stress, and further research is needed. Therefore, the relationship between trichome characteristics and stress resistance needs to be further tested in terms of gene or microecological regulation. In future studies of insect resistance, we can attempt to breed tomato cultivars with longer trichomes and a higher trichome density. In addition, the presence of type I and IV glandular trichomes is beneficial to insect resistance.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/3/411/s1, Figure S1: Structure of the chloroplast of 3186M, as viewed under a transmission electron microscope; Figure S2: Structure of the chloroplast of 3186L, as viewed under a transmission electron microscope; Figure S3: Structure of the chloroplast of 3-071, as viewed under s transmission electron microscope; Figure S4: Structure of the chloroplast of JR, as viewed under a transmission electron microscope; Figure S5: Chloroplast structures of the various experimental accessions before and after drought treatment, Table S1: The surface coat types and density distribution of four tomato materials.

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