Review

Presenting the Secrets: Exploring Endogenous Defense Mechanisms in Chrysanthemums against Aphids

Changchen Xia¹,²,†, Wanjie Xue¹,²,†, Zhuozheng Li¹,², Jiaxu Shi¹,², Guofu Yu² and Yang Zhang¹,²,*

¹ Key Laboratory of Saline-Alkali Vegetation Ecology Restoration, Ministry of Education, College of Life Science, Northeast Forestry University, Harbin 150040, China; changmx1111@163.com (C.X.); xuewanjie0209@163.com (W.X.); 2018212989@nefu.edu.cn (Z.L.); jiaxu011006@163.com (J.S.)
² College of Life Science, Northeast Forestry University, Harbin 150040, China; yuguofu@nefu.edu.cn
* Correspondence: summerzhang@126.com
† These authors contributed equally to this work.

Abstract: As the second-largest cut flower plant globally and one of the top ten traditional flowers in China, chrysanthemums hold significant economic value, encompassing both ornamental and medicinal applications. However, aphids pose a considerable threat as one of the most critical pests affecting chrysanthemums. These pests not only diminish the ornamental value of chrysanthemums through feeding and reproduction but also transmit numerous plant viruses, causing irreversible damage. This review examines aphids’ feeding and damage patterns as a starting point, highlighting the unique endogenous defense mechanisms that have evolved in chrysanthemums during their continuous struggle against aphids. These mechanisms include constitutive defense and induced defense. In addition, we enumerate aphid-resistance genes that have been reported in chrysanthemums. Furthermore, this paper compares and predicts the aphid-resistance genes of other species based on the published Chrysanthemum nankingense genome, aiming to provide a valuable reference for future research on aphid-resistance genes in chrysanthemums.

Keywords: constitutive defense; induced defense; aphid-resistance genes; chrysanthemum; aphid; reference genes

1. Introduction

As the global economy expands, the ornamental plant industry has experienced rapid growth in recent years. Statistics indicate that the worldwide value of ornamental cut flower plants was estimated to reach USD17.6 million in 2021, accounting for one-third of the total global market share for ornamental plants [1]. Among these, chrysanthemums hold a significant position in the global ornamental plant market, being one of the ten most famous traditional flowers in China and the second-largest cut flower plant globally, following roses [2]. However, chrysanthemum cultivation faces challenges from various pests, with aphids posing a significant threat to their growth [3]. As hemipteran insects, aphids are widespread, encompassing around 4000 species [4]. Three primary species affect chrysanthemums: Macrosiphoniella sanborni, Aphis gossypii, and Myzus persicae [5,6]. These aphids uniquely select chrysanthemums as host plants and intensify the damage through their specific life history.

Research has shown that aphids are attracted to the odor of host plant extracts, while non-host plants repel them [7]. Under natural conditions, aphids employ a set of criteria to identify suitable host plants (Figure 1). It is the special color and volatile compounds in chrysanthemums that make aphids choose chrysanthemums as one of their host plants [8]. Additionally, aphids have developed a unique life history to adapt to the environment (Figure 2), which can allow better survival on the chrysanthemum. The mechanisms of parthenogenesis and sexual reproduction enable aphids to rapidly increase their population density and accelerate their encroachment on the host plant, the chrysanthemum.
In contrast, sexual reproductive generations of aphids enhance their adaptability to host plant defenses through genetic recombination, which not only maintains the species’ characteristics but also produces mutations that ensure genetic diversity [9]. Furthermore, they can utilize a specific feeding strategy to minimize the plant defense response during infestations [10–12]. Therefore, aphids become a major pest of chrysanthemums during their growth and cultivation.

Figure 1. Aphid selection of host plants [13,14]: (a) The migrating aphid’s choice of landing on a particular plant depends on receiving the plant-reflected wavelengths (between about 500 nm and 600 nm) [15,16]; upon landing, antennal receptors detect the plant surface volatiles for initial assessment. (b) After making contact with the plant surface, the aphid briefly and tentatively pierces the epidermis using its stylet (<1 min) and ingests a small quantity of plant sap for further evaluation by a gustatory organ in the epipharyngeal area. (c) If the initial assessment is favorable, the aphid penetrates the epidermis to pierce the mesophyll and parenchyma tissues and briefly ingests more sap from vacuoles for additional evaluation and to determine the appropriateness of further ingestion (<1 min). (d) Upon identifying the host plant, the aphid pierces the epidermis of the leaf and passes through the intercellular air spaces of the mesophyll cells using its stylet to reach the sieve tube element in plant phloem, releasing salivary enzymes to protect the mouthparts and prevent plant tissue repair, enabling continuous sap consumption. If ingestion in the sieve tube exceeds 10 min, the host plant is deemed suitable for aphid reproduction and growth, allowing for extended feeding periods (>1 h).
Agricultural control, such as regulating the growing environment and changing growing wingless. In the case of heteroecious aphids, winged female and winged male aphids migrate to wingless female aphids produce both male and female larvae. In general, antoecious aphids are commonly managed through physical, chemical, and agricultural methods. However, consequently inhibiting their further reproduction [20–22].

Under lengthy exposure to aphid attacks, chrysanthemums have developed a multi-tiered, three-dimensional defensive strategy consisting of two primary aspects: constitutive defense and induced defense [19]. Constitutive defense, the first line of defense, can interfere with aphids’ choice of chrysanthemums as host plants and then directly or indirectly slow down aphid infestation through physical barriers and chemicals produced by their metabolism. On the other hand, induced defense comes into play when chrysanthemums experience feeding damage caused by aphids. This type of defense diminishes the fitness of aphids to consume chrysanthemums by impacting food digestion and nutrient absorption, consequently inhibiting their further reproduction [20–22].

To preserve the ornamental and economic value of chrysanthemums, aphids are commonly managed through physical, chemical, and agricultural methods. However, physical control is labor-intensive, time-consuming, and can be costly [23]. Although chemical pesticides are progressively becoming more specific and less toxic, their misuse can heighten aphid resistance in plants, resulting in a vicious cycle of increased pesticide usage and resistance, as well as environmental pollution and ecological imbalance [24]. Agricultural control, such as regulating the growing environment and changing growing patterns, is more limited, subject to certain spatial and seasonal constraints, and is slower to show the effects of control. Therefore, this paper offers the possibility of controlling aphids by breeding transgenic crops by describing the endogenous defense mechanisms of chrysanthemums and tapping into the genes that can produce aphid-resistant characters in chrysanthemums.
2. Damage Caused by Aphids to Chrysanthemums

Aphids can cause damage to chrysanthemum growth, development, and ornamental value in the following three ways.

2.1. Aphid Feeding

The distribution of aphid damage on chrysanthemum plants is closely related to the plant’s physiological age. This may be due to varying nutrient levels and plant secondary metabolite requirements for aphid growth [7]. To obtain the necessary nutrients, aphids alter the chrysanthemum’s normal physiological resource allocation, such as transferring nitrogen from the apical growth zone to the feeding site and mobilizing amino acids from other plant organs to the sieve tube. This disruption can cause issues like leaf curling, slow growth, abnormal flower bud development, and even total death in chrysanthemums [11]. Aphids also release their saliva during the feeding process. Aphid saliva contains various enzymes, such as cellulases and pectinases, which facilitate the penetration of the aphids’ stylets into plant cell walls [25,26]. Additionally, polyphenol oxidase and peroxidases reduce the toxicity of phenolic compounds secreted by the plant, ensuring continuous feeding by aphids [27]. In legumes, the saliva contains calcium-binding proteins, preventing phloem blockage and allowing aphids to feed on the same part for several hours, which can cause more serious damage to plants [28,29]. However, the presence of this phenomenon in chrysanthemums needs to be further verified. Furthermore, aphid saliva interferes with the plant’s photosynthesis [30].

2.2. Aphid Secretions

Honeydew secreted by aphids can block chrysanthemum leaf pores and negatively impact the plant’s photosynthesis [5]. As honeydew dries, its sugars produce a high osmotic pressure concentration, leading to the separation of plant leaf cell mass walls, making them thinner and more susceptible to pathogens. Additionally, honeydew can create a suitable environment for black molds, causing spots on chrysanthemum leaves. The aphid cuticle can also remain on the plant’s surface, affecting the plant’s ornamental value (Figure 3) [7].

Figure 3. Aphid secretions and aphid-borne viruses’ damage to chrysanthemums.
2.3. Aphid-Borne Viruses

Aphids can transmit Chrysanthemum virus B (CVB), Tomato Aspermy virus V (TAV), and Tobacco Cucumber mosaic virus (CMV). CVB infection can cause brown scabs on chrysanthemum leaves and even necrotic streaks on petals [31]. TAV can lead to dwarfing and deformation of chrysanthemum plants, significantly reducing their ornamental value. The presence of aphid-borne plant viruses increases the attractiveness and suitability that aphids have for the host plant, promoting the production of winged aphids that can migrate with the virus [5,32]. Due to the mechanism of aphid selection of host plants [8], even non-host plants can become infected with plant viruses. In short, the virus carried by aphids is far more harmful to chrysanthemums than the aphids themselves are to chrysanthemums.

3. Constitutive Defense

Constitutive defenses refer to the physical and chemical factors that plants inherently possess to deter external attacks. These defenses can be categorized into physical and chemical defenses. Physical defenses involve creating mechanical barriers that impede aphids from feeding through the plant’s physical composition. In contrast, chemical defenses prevent aphids from feeding or poisons them using naturally occurring volatile or non-volatile substances.

3.1. Physical Defense

3.1.1. Leaf Structure

_Chrysanthemum morifolium_ Ramat. (chrysanthemum) ‘Hangbaiju’ was grafted as scions and _Artemisia annua_ (mugwort), which is highly resistant to aphids, was used as rootstocks. The aphid resistance of the grafted seedlings was significantly improved [33]. The leaves of grafted seedlings exhibit a tighter cell structure than the cuttings of ‘Hangbaiju’, such as thicker cell layers of fenestrated tissue and thinner cell layers of spongy tissue. Alterations in the leaf structure of grafted seedlings and cuttings can inform the characterization of highly resistant chrysanthemum varieties.

3.1.2. Leaf Color

As mentioned earlier, aphids initially select their hosts by detecting light waves reflected from the plant surface [34,35], meaning that the plant’s color is selected first. Aphids exhibit varying sensitivity to plant color, being more attracted to land when chrysanthemum leaves are yellow-green, brighter, and more colorful [16]. Consequently, aphid-resistant chrysanthemum leaves are dark green.

3.1.3. Leaf Surface Barriers

In several groups of experiments involving aphid-resistant and non-aphid-resistant chrysanthemums, it was discovered that aphid-resistant chrysanthemums tend to develop greater mechanical barriers to aphid feeding. These barriers manifest as increased height, length, and density of leaf epidermal hairs; thicker and more abundant leaf epicuticular waxes; and deeper location of vascular bundles within the plant [36,37]. When selecting a host plant (Figure 1), aphids must pierce the cells using their stylets to access the sap [13]. In summary, the chrysanthemum structure mentioned above prevents aphids’ mouthparts from penetrating the cells to reach the sieve tube, thereby exerting an anti-aphid effect.

Among the many structures of chrysanthemum leaf surface barriers, trichomes play a crucial role in physical defense and are classified into two types: glandular and non-glandular. The primary trichomes on the chrysanthemum surface are multicellular peltate glandular trichomes, T-trichomes, and others [38]. Glandular trichomes poison or trap aphids by secreting toxins and mucus on the plant surface [39], while non-glandular trichomes reduce chrysanthemum damage by mechanically preventing aphids from moving across the plant surface. _Dendranthema morifolium_ (chrysanthemum) variety ‘Zhongshan-jingui’ hybridizes with _Artemisia vulgaris_ (mugwort) ‘Variegata’, which exhibits a higher...
abundance of glands and trichomes than their parent plants, making them more resistant to aphids \[40\].

3.2. Chemical Defense
3.2.1. Volatile Compounds

Volatile compounds, such as terpenoids and their oxygenated derivatives, play a crucial role in the interactions among chrysanthemums, aphids, and their natural enemies, forming a line of defense in the chemical defense of chrysanthemums. One significant factor contributing to the hybrid Chrysanthemum’s greater resistance to aphids compared to its parent is its increased capacity for terpenoid synthesis.

Among these compounds, germacrene D significantly inhibits arthropod feeding (Figure 4a) \[41,42\]. Furthermore, \((E)\)-\(\beta\)-farnesene, an aphid alarm pheromone (Figure 4a), causes aphids to cease feeding and rapidly flee their hosts while strongly attracting the aphid’s natural enemy, the ladybird \[43,44\]. This compound can also be combined with pesticides for more effective aphid control \[42,45\]. Pyrethrins, unique aphid-resistant substances found in \(\text{Tanacetum cinerariifolium}\) (pyrethrum), consist of six compounds (Figure 4b): Pyrethrins I, Pyrethrins II, Cinerin I, Cinerin II, Jasmolin I, and Jasmolin II. These compounds exhibit repellent and touch-killing action against aphids and act as neurotoxins that inhibit excitation transmission \[46\].

![Chemical structure formulae of germacrene D and (E)-\(\beta\)-farnesene](image)

![Chemical structure formulae of pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin I, and jasmolin II](image)

![Chemical structure formulae of chrysindins A-D](image)

Figure 4. (a) Chemical structure formulae of germacrene D and (E)-\(\beta\)-farnesene, (b) Chemical structure formulae of pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin I, and jasmolin II; (c) Chemical structure formulae of chrysindins A-D.

3.2.2. Non-Volatile Compounds

Non-volatile secondary metabolites often protect chrysanthemums by allowing aphids to feed on them and subsequently producing toxic or repellent effects. The primary non-volatile secondary metabolites in chrysanthemums are flavonoid compounds, including flavonols and isoflavonoids. Due to their oxidation, these compounds are toxic to phytophagous insects, and their content is positively correlated with plant resistance to aphids \[47\]. Flavonoids can also cause aphids to reject food and limit their growth and development. The primary alkaloids in chrysanthemum are pyrrolizidine alkaloids, which
are entomotoxic and exhibit toxic effects on aphids and other pests. These alkaloids interfere with insects’ nervous systems, causing nerve paralysis and ultimately death [48,49]. Chrysanthemum also contains various polyacetylenes (Figure 4c), such as Chrysindins A–D, which are cytotoxic [50]. However, their precise effect on aphids remains to be investigated.

4. Induced Defense

When chrysanthemums experience biotic stress from aphids, they initiate changes in their nutrient levels, phytohormones, and defense proteins to counter the aphid attack. These defense mechanisms involve repairing damaged cells, disrupting aphid nutrient uptake and digestion, and releasing toxins. This type of defense is referred to as induced defense. In contrast to constitutive defense, induced defense is only activated in response to biotic stresses.

4.1. Nutrients and Reactive Oxygen Species

Soluble sugars and soluble proteins are nutrients closely associated with induced defenses. When aphids attack chrysanthemums, soluble sugar metabolism will increase to synthesize other defensive substances. Thus, aphids have reduced sugar uptake due to the consumption of soluble sugars [51]. Aphid infestation also elevates soluble protein levels, likely because the plant requires numerous enzymes to initiate its defense system [52].

Upon mechanical damage to chrysanthemum cells, large clusters of reactive oxygen species (ROS) are produced, including hydroxyl free radical (OH•), alkyl free radical (RO•), nitrosyl free radical (NO•), hypochlorous acid (HOCl), peroxyl free radical (ROO•), hydrogen peroxide (H2O2), and superoxide anions (O2−) [53]. These ROS play a crucial role in defending chrysanthemums against aphids by directly preventing aphid feeding, such as causing oxidative damage to the aphid’s intestines and oxidizing plant nutrients required by the aphid, thereby preventing nutrient acquisition [54]. Moreover, ROS can act as secondary messengers, activating the plant’s internal defense system [55].

4.2. Defense Proteins

Chrysanthemum plants contain defense proteins, which are generally classified into two categories: antioxidant enzymes and defense enzymes. Antioxidant enzymes, including superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase (POD), primarily function to counterbalance the elevated oxidation levels caused by aphid-inflicted damage to chrysanthemum cells. In contrast, defense enzymes generate toxic secondary metabolites. Other substances that alleviate cell damage can be generated too. Upon exposure to aphid stress, defense protein content increases, with higher resistance in chrysanthemum plants correlating to a faster rate of defense protein augmentation [37].

When aphids penetrate the chrysanthemum’s epidermis, they disrupt the plant’s redox balance, leading to elevated ROS content and the accumulation of oxidants such as hydrogen peroxide. This accumulation can damage plant membrane structures [13,56]. However, the plant’s secondary metabolites and antioxidant enzymes can significantly reduce ROS levels, restoring the oxygen-reduction balance and preventing irreversible damage to chrysanthemums caused by excessive oxidant buildup [57].

Polyphenol oxidase (PPO) is a defense enzyme in chrysanthemums, serving four primary functions: (1) PPO catalyzes the synthesis of phenolic compounds into o-benzoquinone, which is toxic to aphids (Figure 5) [58]. (2) Due to quinones’ reactive nature, they can undergo complex chemical reactions with proteins containing nucleophilic groups, such as amino and sulfhydryl groups, resulting in phenolic complexes. The growth of aphids requires proteins containing lysine, histidine, cysteine, and methionine. However, treating these proteins with PPO leads to significant losses in these amino acids, which reduces the nutritional value of proteins for aphid feeding and provides further resistance (Figure 5) [59]. (3) When plant cells experience physical damage, PPO is released, initiating an enzymatic reaction in the presence of oxygen that produces a melanin-like brown pigment, which inhibits the spread of pathogenic bacteria [60]. (4) PPOs secondary metabolism
promotes plant cell lignification, enhancing lignin synthesis, repairing damaged cell walls, and thickening undamaged cell walls. This process increases mechanical barriers to aphid feeding and deprives nutrients of pathogenic bacteria, ultimately leading to their death [61].

The phenylpropanoid pathway is a crucial defense signaling pathway in plants, with phenylalanine ammonia-lyase (PAL) serving as a key catalyst. In the presence of PAL, phenylalanine can be deaminated to form cinnamate, a precursor of numerous physiologically important compounds. This process enables the synthesis of essential plant compounds, such as lignans, lignin, flavonoids, and coumarins, catalyzed by p-coumaroyl CoA (Figure 6) [62]. Among these compounds, lignans and lignin strengthen the toughness of cell walls and promote lignification, creating mechanical barriers that hinder aphid feeding. Flavonoids can exhibit toxic effects on aphids [63]. Furthermore, peroxidase (POD) also functions as a defense enzyme, catalyzing the biosynthesis of lignin and suberin. This process reinforces the chemical connections between cell wall components, increasing stability and further impeding aphid feeding [64].

![Figure 5](image1.png)

**Figure 5.** PPO catalyzes the synthesis of o-benzoquinone, toxic to aphids, and forms phenolic complexes which reduce the nutritional value of proteins ingested by aphids.

![Figure 6](image2.png)

**Figure 6.** Phenylalanine ammonia lyase (PAL) synthesizes numerous compound precursors, cinnamic acid, and p-coumaroyl CoA catalyzes synthesis of physiologically important compounds.
4.3. Cell Wall Modifications

The chrysanthemum, a type of vascular plant, illustrates an intriguing cellular composition. Its cell wall is composed of approximately 30% cellulose, 30% hemicellulose, and 35% pectin; the residual 1–5% consists of structural proteins [65]. Aphid resistance is primarily exhibited through two mechanisms involving plant cell wall modification. The first implicates key genes that govern the structural modification and composition of the cell wall, thereby enhancing resistance to aphids via mechanical fortification of the regulated cell wall structure. Secondly, the hydrolysis of cell wall polysaccharides by aphid saliva generates oligomers and small molecules. These compounds can be identified by resistance receptors, triggering a defensive response. And the two often work together because of their close association. Notably, the area of cell wall modification in chrysanthemums remains relatively underexplored; therefore, the references provided below should serve as potential subjects for future substantiation.

Pectin, a major component of plant cell walls, plays a significant role in both mechanisms of cell wall modifications, causing aphid resistance. Structurally, pectin can be classified into homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II) [66]. HG, which constitutes about 60% of cell wall pectin, is the most extensively investigated variety [67]. While feeding on plants, aphids secrete cell wall-modifying enzymes (CWMEs) within their saliva, such as pectin methyltransferases (PMEs), polygalacturonases (PGs), and pectate lyase enzymes (PLs) [68]; these degrade cell wall polysaccharides, thereby extracting nutrients from the cells’ contents. One reaction involves HG undergoing unmethyl-esterification catalyzed by PMEs with increased activity. This reaction, along with depolymerization facilitated by PGs or PLs, results in the production of oligogalacturonides (OGs). Plants perceive OGs as damage-associated molecular patterns (DAMPs) [69,70], due to the binding of these OGs to the extracellular pectin-binding domain of wall-associated kinase receptors (WAK). And then, an innate defense response is triggered via the mitogen-activated protein kinase (MAPK) signaling pathway [71,72].

Beyond OGs, the process of unmethyl-esterification also produces methanol [73,74], a volatile compound—another DAMPs capable of initiating both personal and communal defenses across neighboring plants against aphids [73,75]. However, the timing and concentration of methanol emissions are inconsistent and can thus exhibit varying effects on aphid resistance, which may differ between species [76]. As such, further experiments are required in chrysanthemums.

Plants can produce modifying enzymes via their gene regulation. For example, pectin methylesterase inhibitors (PMEIs) inhibit the unmethyl-esterification of HGs to enhance the antimicrobial property of plants [77], while polygalacturonase inhibitors (PGIPs) specifically counteract pathogens’ efforts to hydrolyze HGs [78]. Extraordinarily, these alterations not only enhance the mechanical strength of the cell wall but also indirectly affect methanol release, amplifying the defensive capabilities of the cell wall against both pathogens and pests [75,79]. Concurrently, the plant reinforces its protective system by activating other defense pathways, such as those involving jasmonic acid and ethylene, releasing signaling molecules that further strengthen its defenses [11].

4.4. Plant Hormones

Constructing phytohormone overexpression vectors in chrysanthemum poses a challenge due to the complex metabolic pathways of phytohormones. Consequently, researchers have examined the impact of phytohormones on endogenous defense in chrysanthemums through spraying exogenous plant hormones.

It has been observed that abscisic acid (ABA) in chrysanthemums enhances the expression of CmTPS1 and CmTPS2 genes [80], increases resistance toward aphids, and elevates ROS content, thereby activating antioxidant enzyme activity. In conclusion, the regulation of aphid populations by ABA may be associated with the equilibrium between ROS concentrations and the activity of antioxidant enzymes in chrysanthemums [81].
In contrast, the salicylic acid (SA) pathway is more intricate [82]: (1) SA can induce a reduction in chlorophyll content, initiating the plant’s defenses for improved resistance against aphids. (2) SA can promote the conversion of soluble sugar metabolism toward the synthesis of other aphid-resistant materials. (3) SA can increase the total protein content, including soluble and insoluble in chrysanthemums, likely due to the replenishment of numerous enzymes required to initiate the plant’s defense system under stress. (4) After SA spraying, ROS and malondialdehyde (MDA) content in chrysanthemum increased in advance, but the increase was smaller than that in aphid stress alone, potentially protecting plant cells from high ROS concentration and acting as an advanced defense. (5) SA also promoted the early and efficient expression of flavonoid synthesis genes and significantly increased the flavonoid content in plants when exposed to aphid stress after spraying.

Methyl jasmonate (MeJA) is a well-studied phytohormone that plays a crucial role in aphid resistance in chrysanthemums. Although MeJA is not directly toxic to insects when ingested, it can be employed to control pests when spraying externally. This implies that MeJA achieves control not through inherent toxicity but by modulating the plant’s defense system [83]. The specific effects are as follows (Figure 7) [51,84,85]: (1) MeJA induces lignin accumulation, reinforcing the cell wall and acting as a defense against aphids by maintaining the activity of PAL and POD enzymes and enabling the responsive expression of lignin-synthesizing genes. (2) MeJA reduces the amount of MDA accumulation in cells, thereby decreasing cellular damage. (3) MeJA increases the activity of defense or antioxidant enzymes, such as SOD, POD, PAL, and PPO, which can prevent extensive cellular damage by ROS and synthesize other essential substances, including phenols and flavonoids that constitute the plant defense system. (4) MeJA rapidly enhances proline content to stabilize damaged plant structures, reduce cell acidity, and stabilize biomacromolecules. (5) MeJA increases the soluble protein content, thereby enhancing the enzyme content of the plant defense system and the content of defense proteins involved in aphid resistance. (6) MeJA decreases the soluble saccharides content of the plant body, which can be used by the metabolism to produce other defense tissues and reduce nutrient uptake by aphids. (7) MeJA increases the activity of trypsin proteinase inhibitor (TI) and chymotrypsin proteinase inhibitor (CI) to prevent important proteins in chrysanthemums from being hydrolyzed by their high protease content. Upon entering the aphid’s body, these substances can bind to proteases present within the insect, consequently inhibiting or impairing the aphid’s ability to absorb essential nutritional proteins. (8) MeJA increases the content of endogenous jasmonic acid by enhancing the activity of lipoxygenase (LOX), which activates the defense system in chrysanthemums.
Moreover, TcCHS can generate pyrethrin derivatives as well as volatile chrysanthemol. The overexpression of the TcCHS gene in Chrysanthemum morifolium (chrysanthemum) results in constitutive defense mechanisms, such as synthesizing defensive substances and regulating phytohormone signaling pathways.

5. Aphid-Resistance Genes

Through the observation of aphid resistance in various chrysanthemum varieties, it was discovered that certain strains stably inherit the aphid resistance trait, indicating the presence of specific aphid-resistance genes in the plant to maintain this characteristic. The identified aphid-resistance genes primarily contribute to the chrysanthemum plants’ constitutive defense mechanisms, such as synthesizing defensive substances and regulating phytohormone signaling pathways.

WRKY is a family of transcription factors that are unique to plants. It is associated with plant growth and development, responses to abiotic stresses such as salt damage, low temperature, high temperature, drought, and plant defense against pathogenic bacteria and aphid stresses [86]. As a defense response, SA can induce WRKY family gene expression [87]. Currently, CmWRKY33 (GenBank: AJF11718.1), CmWRKY48 (GenBank: AJF11724.1), and CmWRKY53 (GenBank: AJF11719.1) have been found to play crucial roles in aphid resistance in chrysanthemum, regulating secondary metabolites involved in plant defense and acting through the SA or MeJA pathway [88].

The MYB family of transcription factors has numerous roles in plants. MYB proteins serve as key elements in the regulatory network controlling plant hormone synthesis and regulation, primary and secondary metabolism, and responses to biotic or abiotic stresses [89]. Among them, CmMYB15 (GenBank: ALF46700.1) and CmMYB19 (GenBank: ALF46701.1) can promote lignin synthesis for defense against aphids [90,91]. Additionally, CgCOMT (GenBank: BAK42963.1) is identified to be expressed in roots, stems, and leaves for lignin synthesis, exhibiting the highest expression in the stem.

The initial step in pyrethrin synthesis depends on the chrysanthemol synthase (CHS), which is regulated by TcCHS (GenBank: AFZ61535.1) in Tanacetum cinerariifolium (pyrethrum). Moreover, TcCHS can generate pyrethrin derivatives as well as volatile chrysanthemol. The overexpression of the TcCHS gene in Chrysanthemum morifolium (chrysanthemum) results

---

**Figure 7.** The pathways of methyl jasmonate for aphid resistance. MeJA, methyl jasmonate; MDA, malondialdehyde; LOX, lipoxygenase; TI, trypsin proteinase inhibitor; CI, chymotrypsin proteinase inhibitor; JA, jasmonic acid; PAL, phenylalanine ammonia lyase; POD, peroxidase; PPO, Polyphenol oxidase; SOD, superoxide dismutase; ROS, reactive oxygen species.
in the production of a chrysanthemol glycoside derivative, as well as volatile chrysanthemol. Volatile chrysanthemol functions to repel aphids, while non-volatile glycosides, stored in vacuoles, deter aphids by affecting them upon ingestion and discouraging further feeding [92].

Plants contain 200 times more carotenoids, the precursors of ABA, than ABA. They accumulate in large quantities, forming a “carotenoid pool” [93]. The 9-cis-epoxycarotenoid dioxygenase (NCED) has been demonstrated to be a crucial enzyme in the ABA synthesis pathway [94]. CmNCED3-1 (GenBank: BAF36655.1) is a key gene in Chrysanthemum that influences the ABA synthesis pathway, which can enhance the expression level of NCED, thereby utilizing ABA to regulate defense against aphids.

Aphid feeding-induced expression of CmHSFB1 (GenBank: AUG69001.1) enhances the expression of genes involved in the synthesis or positive regulation of ROS and stimulates the expression of downstream heat stress proteins. Both mechanisms contribute to aphid stress resistance [95].

In addition to the previously reported genes, we also selected genes from other species with aphid resistance functions for prediction in Chrysanthemum nankingense (Table 1). We compared the protein data obtained from NCBI: https://www.ncbi.nlm.nih.gov/ (accessed on 24 March 2023) with protein sequences from Chrysanthemum nankingense using local blast and selected those protein sequences with potential aphid resistance as our prediction in chrysanthemum. The protein sequence of Chrysanthemum nankingense was derived from www.amwayabrc.com, accessed on 24 March 2023.

**Table 1. Predicted aphid-resistance genes in Chrysanthemum nankingense.**

<table>
<thead>
<tr>
<th>Gene Abbreviation</th>
<th>Accession Number</th>
<th>Source</th>
<th>Function</th>
<th>Reference</th>
<th>Predictive Protein</th>
<th>% Identity</th>
<th>Alignment Length</th>
<th>E-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rm</td>
<td>XP_007225245.1</td>
<td>Prunus persica</td>
<td>beta-xyluloylase/alpha-L-arabinofuranosidase 2</td>
<td>[96]</td>
<td>CHR00089971-RA</td>
<td>77.242</td>
<td>747</td>
<td>0.0</td>
</tr>
<tr>
<td>CHS</td>
<td>AAA32771.1</td>
<td>Arabidopsis thaliana</td>
<td>Chalcone synthase</td>
<td>[97]</td>
<td>CHR00028844-RA</td>
<td>85.751</td>
<td>386</td>
<td>0.0</td>
</tr>
<tr>
<td>Cu/ZnSOD</td>
<td>NP_001077494.1</td>
<td>Arabidopsis thaliana</td>
<td>copper/zinc superoxide dismutase</td>
<td>[98]</td>
<td>CHR00045542-RA</td>
<td>82.590</td>
<td>659</td>
<td>0.0</td>
</tr>
<tr>
<td>PRX7</td>
<td>NP_199033.1</td>
<td>Arabidopsis thaliana</td>
<td>Peroxidase superfamily protein</td>
<td>[99]</td>
<td>CHR00037301-RA</td>
<td>73.810</td>
<td>294</td>
<td>3.63 × 10^{-166}</td>
</tr>
<tr>
<td>TCH4</td>
<td>KAJ4877013.1</td>
<td>Raphanus sativus</td>
<td>Tyrosine decarboxylase 1</td>
<td>[100]</td>
<td>CHR00084602-RA</td>
<td>73.222</td>
<td>478</td>
<td>0.0</td>
</tr>
<tr>
<td>GbRac</td>
<td>NP_001314362.1</td>
<td>Gossypium hirsutum</td>
<td>ROP/Rac protein</td>
<td>[101]</td>
<td>CHR00004359-RA</td>
<td>93.333</td>
<td>180</td>
<td>8.85 × 10^{-127}</td>
</tr>
<tr>
<td>Vat</td>
<td>XP_008465871</td>
<td>Cucumis melo</td>
<td>Heavy metal-associated isoprenylated plant protein 39</td>
<td>[102]</td>
<td>CHR00066820-RA</td>
<td>93.056</td>
<td>72</td>
<td>4.58 × 10^{-30}</td>
</tr>
</tbody>
</table>

Furthermore, apart from directly predicting based on the reported aphid-resistance genes, we also explored other potential aphid-resistance genes indirectly. For example, if epidermal wax thickness plays a crucial role in aphid defense, then genes in the synthetic epidermal wax pathway can also be predicted as aphid-resistance genes in chrysanthemums (Table 2) [103]. The predictions are made using the same method as the direct predictions.
### Table 2. Predicted epidermal wax synthesis genes in *Chrysanthemum nankingense*.

<table>
<thead>
<tr>
<th>Gene Abbreviation</th>
<th>Accession Number</th>
<th>Source</th>
<th>Recname</th>
<th>Reference</th>
<th>Predictive Protein</th>
<th>% Identity</th>
<th>Alignment Length</th>
<th>E-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTP2</td>
<td>Q9S7I3.1</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Non-specific lipid-transfer protein 2</td>
<td>[104]</td>
<td>CHR00049734-RA, CHR00038208-RA</td>
<td>50.847</td>
<td>118</td>
<td>5.15 × 10⁻³⁵</td>
</tr>
<tr>
<td>FAR3</td>
<td>Q93ZB9.1</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Fatty acyl-CoA reductase 3</td>
<td>[104]</td>
<td>CHR00025791-RA, CHR00092239-RA</td>
<td>57.407</td>
<td>486</td>
<td>2.16 × 10⁻¹⁷⁰</td>
</tr>
<tr>
<td>WSD1</td>
<td>Q9S2ZR6.1</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Wax ester synthase / diacetylcerol acyltransferase 1</td>
<td>[105]</td>
<td>CHR00049351-RA, CHR00071533-RA</td>
<td>39.738</td>
<td>458</td>
<td>3.81 × 10⁻¹¹⁹</td>
</tr>
<tr>
<td>ABCG11</td>
<td>Q8RXN0.1</td>
<td><em>Arabidopsis thaliana</em></td>
<td>ABC transporter G family member 11</td>
<td>[106]</td>
<td>CHR00028885-RA, CHR00053055-RA</td>
<td>77.874</td>
<td>696</td>
<td>7.34 × 10⁻⁴⁵</td>
</tr>
<tr>
<td>LACS1</td>
<td>O22898.1</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Long chain acyl-CoA synthetase 1</td>
<td>[104]</td>
<td>CHR00023806-RA, CHR00075380-RA</td>
<td>62.367</td>
<td>659</td>
<td>0.0</td>
</tr>
<tr>
<td>KCS1</td>
<td>Q9MMAM3.1</td>
<td><em>Arabidopsis thaliana</em></td>
<td>3-ketoyl-CoA synthase 1</td>
<td>[104]</td>
<td>CHR00017132-RA, CHR00049310-RA, CHR00035436-RA</td>
<td>71.734</td>
<td>467</td>
<td>0.0</td>
</tr>
<tr>
<td>KCR1</td>
<td>Q8L9C4.1</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Very-long-chain 3-oxoacyl-CoA reductase 1</td>
<td>[104]</td>
<td>CHR00038450-RA, CHR00022927-RA, CHR00032783-RA</td>
<td>64.762</td>
<td>315</td>
<td>9.70 × 10⁻¹⁰⁹</td>
</tr>
<tr>
<td>ECR</td>
<td>Q9M2U2.1</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Very-long-chain enoyl-CoA reductase</td>
<td>[104]</td>
<td>CHR00007949-RA, CHR00032181-RA</td>
<td>78.333</td>
<td>300</td>
<td>1.42 × 10⁻¹⁴⁰</td>
</tr>
<tr>
<td>CER3</td>
<td>Q8H1Z0.1</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Very-long-chain aldehyde decarboxylase CER3</td>
<td>[104]</td>
<td>CHR00091218-RA, CHR00066836-RA</td>
<td>66.341</td>
<td>615</td>
<td>0.0</td>
</tr>
<tr>
<td>MAH1</td>
<td>Q9FVS9.1</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Alkane hydroxylase MAH1</td>
<td>[104]</td>
<td>CHR00033702-RA, CHR00073663-RA</td>
<td>45.669</td>
<td>508</td>
<td>4.13 × 10⁻¹⁶⁷</td>
</tr>
</tbody>
</table>

### 6. Concluding Remarks

In recent years, the effectiveness of conventional physical methods and chemical pesticides for insect control has shown limited progress and significant drawbacks, including adverse side effects. As a result, biological control of aphids has become a prominent research topic. Studies on the mechanisms of aphid resistance in chrysanthemums and aphid-resistant transgenic research have attracted increasing attention. This paper presents an overview of the defense mechanisms and unique aphid-resistance genes that have evolved in the ongoing battle between chrysanthemums and aphids.

By analyzing the inherent defense mechanisms of chrysanthemums, several aspects of aphid resistance strategies beyond poisoning aphids have been identified, such as modifying the tissue structure of the chrysanthemum epidermis, increasing defense protein content, regulating phytohormone levels, and attracting aphids’ natural enemies. These factors are all closely related to the regulation of chrysanthemum genes. By using the reported and predicted aphid-resistance genes discussed in this paper, it is possible to further develop aphid-resistant plants at the genetic level. It is now crucial to examine the impact of chrysanthemum genes on their physical structure, secondary metabolites, and phytohormonal pathways. A comprehensive understanding of chrysanthemum genes may hold the key to addressing future pest challenges.

**Author Contributions:** Conceptualization, C.X. and Y.Z.; methodology, W.X.; software, Z.L., C.X. and G.Y.; validation, C.X., W.X. and Y.Z.; formal analysis, J.S.; investigation, C.X.; data curation, C.X.; writing—original draft preparation, C.X.; writing—review and editing, Y.Z. and W.X.; visualization, C.X.; supervision, W.X. and Y.Z.; project administration, Y.Z.; funding acquisition, Y.Z. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by a grant from the Natural Science Foundation of Heilongjiang Province of China (grant number: LH2021C006).

**Data Availability Statement:** The sequence data of reference genes that were used and analyzed during this study are available in the NCBI (https://www.ncbi.nlm.nih.gov, accessed on 24 March 2023) and Chrysanthemum Genome Database (www.amwayabrc.com, accessed on 24 March 2023).
Conflicts of Interest: The authors declare no conflict of interest.

References


84. Liu, Q.; Luo, L.; Zheng, L. Lignins: Biosynthesis and Biological Functions in Plants. *Int. J. Mol. Sci.* 2018, 19, 335. [CrossRef]
93. Parry, A.D.; Horgan, R. Carotenoids and abscisic acid (ABA) biosynthesis in higher plants. *Physiol. Plant.* 1991, 82, 320–326. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.