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# Herbivore defense responses and associated herbivore defense mechanism as revealed by comparing a resistant wild soybean with a susceptible cultivar



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## ABSTRACT

Plants have evolved sophisticated defense mechanisms against herbivores to help them adapt to the environment. Understanding the defense mechanisms in plants can help us control insects in a more effective manner. In this study, we found that compared with Tianlong 2 (a cultivated soybean with insect susceptibility), ED059 (a wild soybean line with insect resistance) contains sharper pubescence tips, as well as lower transcript levels of wound-induced protein kinase (WIPK) and salicylic acid-induced protein kinase (SIPK), which are important mitogen-activated protein kinases involved in early defense response to herbivores. The observed lower transcript levels of WIPK and SIPK induced higher levels of jasmonic acid (JA), JA biosynthesis enzymes (AOC3) and some secondary metabolites in ED059. Functional analysis of the *KTI1* gene via *Agrobacterium*-mediated transformation in *Arabidopsis thaliana* indicated that it plays an important role in herbivore defense in ED059. We further investigated the molecular response of third-instar *Helicoverpa armigera* (Hübner) larvae to Tianlong 2 and ED059. We found apoptotic cells only in the midguts of larvae that fed on ED059. Compared with larvae reared on the susceptible cultivar Tianlong 2, transcript levels of catalase (CAT) and glutathione S-transferase (GST) were up-regulated, whereas those of CAR, CHSB, and TRY were down-regulated in larvae that fed on the highly resistant variety ED059. We propose that these differences underlie the different herbivore defense responses of ED059 and Tianlong 2.

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## 1. Introduction

Soybean, the main oil crop of China, has a long history of providing the country with plant protein and oil. However,

various diseases and damage due to insect pests have led to huge yield losses in recent years. Pest control in China has relied mainly on chemical pesticides. However, use of such pesticides causes serious environmental pollution and

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increases pest resistance to pesticides. Breeding and deployment of resistant varieties is a new way of pest management that is crucial for agricultural sustainable development and environmental protection.

Plants have evolved a diverse set of defense responses to herbivores. They use both constitutive and induced defenses to combat challenges posed by pests [1]. Constitutive defenses include morphological and biochemical features such as sharp and thick trichomes [2,3], heavy epicuticular waxes [4], and secondary compounds, which are constitutively present in the plant. Induced defense is a complex physiological and biochemical process that can be enhanced upon recognition of herbivore attack and includes several signal transduction pathways (the mitogen-activated protein kinase (MAPK) signaling pathway, jasmonic acid (JA) pathway, salicylic acid (SA) pathway, and ethylene (ET) pathway, among others) [1]. These signaling pathways produce many anti-herbivore compounds [5], including secondary metabolites such as proteinase inhibitors (PIs) and nicotine, which exert direct effects on herbivores by interfering with their physiology [6,7]. Other compounds, such as volatile organic compounds, act by interfering with the behavior of herbivores [8]. However, very little is known about the herbivore defense mechanisms in soybean.

Plants can also distinguish damages from herbivores and mechanical wounding. Some genes and proteins are activated both by mechanical wounding and by herbivores [9]. However, other studies indicate that very different transcript profiles are induced by mechanical wounding and insect attack [10–12]. Studies of the mechanism by which insects induce plant response have led to the discovery of fatty acid–amino acid conjugates (FACs) in herbivore oral secretions that elicit herbivory-specific responses, such as JA and ET bursts, that are greater than those elicited by mechanical wounding; high levels of trypsin proteinase inhibitor (TPI); and the release of volatile organic compounds (VOCs) [13]. However, whether there is any difference in the early steps and the final defense metabolites between soybean response to herbivore attack and that to wounding is unknown.

When a plant is attacked by herbivores, the induced defense reaction not only is activated in the local leaf being attacked, but also is transferred to other leaves through a series of signal transduction events [14]. Ultimately, the whole plant shows enhanced resistance against herbivores. In tomato, PIs induced by herbivores provide an ideal model for elucidating the molecular basis of systemic resistance signaling in plants [15]. A recent study showed that a signaling molecule that undergoes long-distance transport is JA, which induces expression of PIs in leaves throughout the plant [16]. Systemin, a polypeptide signaling molecule, regulates the biosynthesis of JA [17]. Systemin and JA collaborate to induce the plant to produce defense compounds after attack by herbivores. However, whether there is systemic resistance in soybean following local insult by herbivores remains unknown.

Differences in phenotypic characteristics can be ultimately attributed to genetic differences. In recent years, along with the growing number of completed genome sequences and transcript profiles of organisms, large-scale gene expression analyses have become more easily. A large number of studies have focused on gene expression-level polymorphisms (ELPs) in different individuals to analyze diverse phenotypic traits

[18], such as rust resistance [19], herbivore-induced early signaling events, and secondary metabolite production in insect resistance [20]. ELPs are of great help for understanding the complete metabolic, regulatory, and developmental pathways that underlie different phenotypic traits.

In a previous study, we found that the wild soybean line ED059 (*Glycine soja* Sieb. et Zucc.) was highly resistant to the cotton bollworm *Helicoverpa armigera* (Hübner), and that a cultivar Tianlong 2 [*Glycine max* (L.) Merr.] was highly susceptible to *H. armigera* [3]. In the present study, we investigated differences in morphology, early response to stresses, and anti-herbivore metabolites between ED059 and Tianlong 2 after attack by *H. armigera*. Our results showed that ED059 not only had physical barriers against herbivores, but also exhibited an induced herbivore defense reaction. Our results reveal large differences in phenotype and molecular response to herbivores in ED059 and Tianlong 2 and provide important new insights into the metabolic, regulatory and developmental pathways that underlie the resistance of wild soybean variety ED059 to herbivores.

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## 2. Materials and methods

### 2.1. Plant materials

The cultivated soybean (Tianlong 2) and the wild soybean (ED059) used in this study were obtained from the Institute of Oil Crops Research, Chinese Academy of Agricultural Sciences, Wuhan, China. Tianlong 2 is susceptible and ED059 resistant to *H. armigera*.

Seeds were pre-germinated on moistened filter paper in a plant growth chamber at 27 °C, 85% ambient humidity, and 16:8 light:dark photoperiod for 3 to 4 days. The seedlings were transferred into 18 cm × 18 cm individual plastic pots containing 2:3 Pinnstrup Substrate (Pinnstrup Substrate, Denmark):vermiculite at 27 °C under 16 h of light. All plants used in the experiments had three fully expanded trifoliates.

### 2.2. Trichome density and the shape of pubescence tip on ED059 and Tianlong 2

Tianlong 2 and ED059 were observed for pubescence tip morphology and density using 10 randomly selected 4-week-old plants (plants in the V3 stage) from each variety. First, soybean leaves were cut into small pieces and fixed in phosphate buffer with 4% glutaraldehyde for 24 h. The leaves were then cleaned with phosphate buffer and dehydrated in an ethanol concentration series. Lastly, the leaves were sprayed with metal film and their pubescence tips were photographed under a scanning electron microscope (Hitachi SU8010) to classify the trichome tips as either sharp or blunt [21].

Using a punch with a diameter of 5 mm, 5 samples from each leaf were obtained for examination. Then, the trichome density was quantified for both Tianlong 2 and ED059 by measurement of the number of trichomes in 1 mm<sup>2</sup> of the upper and under leaf surfaces of 10 plants of each variety using a fluorescence microscope (OLYMPUS IX71) at 20 × magnification. The trichome density was represented as the number of trichomes per square millimeter (mm<sup>2</sup>).

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