Front. Agr. Sci. Eng. 2021, 8(2): 292–301 https://doi.org/10.15302/J-FASE-2021395

RESEARCH ARTICLE

CHARACTERISTICS OF HERBIVORY/WOUND-ELICITED ELECTRICAL SIGNAL TRANSDUCTION IN TOMATO

Chaoyi HU[1], Siqi DUAN[1], Jie ZHOU[1], Jingquan YU (✉)[1,2]

1 Department of Horticulture, Zijingang Campus, Zhejiang University, Hangzhou 310058, China.
2 Key Laboratory of Horticultural Plant Growth and Development, Ministry of Agriculture and Rural Affairs of China, Hangzhou 310058, China.

KEYWORDS

electrical signal, glutamate receptor-like, herbivory, jasmonic acid, tomato

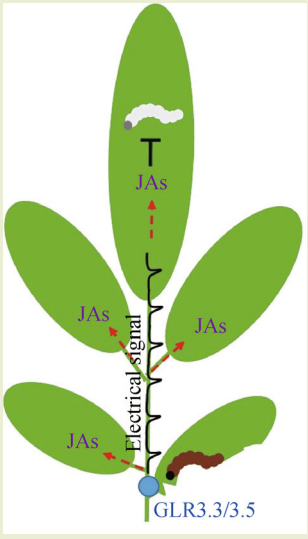
HIGHLIGHTS

- Herbivory and mechanical wounding elicited electrical signals.
- Petiole wounding elicited stronger electrical signals than did leaflet wounding.
- Leaflet wounding elicited electrical signals and JA signaling within a compound leaf.
- GLR3.3 and GLR3.5 mediated leaflet-to-leaflet electrical signal transduction.
- JA synthesis and *Helicoverpa armigera* resistance were reduced in *glr3.3/3.5* plants.

Received December 9, 2020;
Accepted April 13, 2021.

Correspondence: jqyu@zju.edu.cn

GRAPHICAL ABSTRACT



ABSTRACT

Electrical signals commonly occur in plants in response to various environmental changes and have a dominant function in plant acclimation. The transduction of wound-elicited electrical signals in the model plant species *Arabidopsis* has been characterized but the characteristics of electrical signal transduction in response to herbivory or wounding in crop species remain unknown. Here, the features of electrical signals elicited by insect herbivory and wounding in tomato were investigated. Unlike those in *Arabidopsis*, wounding tomato leaves did not cause leaf-to-leaf electrical signal transduction. In contrast, electrical signals elicited in response to petiole wounding were stronger and more strongly transduced. Leaflet wounding also activated electrical signal transduction and jasmonic acid (JA) signaling within the whole compound leaf. It was also demonstrated that tomato glutamate receptor-like 3.3 (GLR3.3) and GLR3.5 mediated leaflet-to-leaflet electrical signal transduction. Herbivory-induced JA accumulation and *Helicoverpa armigera* resistance were reduced in *glr3.3/3.5* plants. This work reveals the nature of electrical signal transduction in tomato and emphasizes the key roles of GLR3.3 and GLR3.5 in electrical signal transduction and JA signaling activation.

1 INTRODUCTION

Environmental changes such as fluctuations in temperature and light, mechanical damage, drought, salinity, insect herbivory and pathogens, directly impact plant growth. Unlike animals, plants cannot escape. Plants must endure these changes and make appropriate acclimation or defense responses in a fixed location to survive. Signal transduction events generally occur during the early growth of plants and in response to abiotic or biotic environment interactions. Previous studies show a range of signals in plants including reactive oxygen species signals, Ca^{2+} fluxes, and electrical signals, all of which have essential functions in plant responses to abiotic and biotic stresses[1–7].

The existence of electrical signals in plants was first demonstrated by Burden-Sanderson[8] and Charles Darwin[9]. Since then, electrical signals have generally been reported in plants in response to various environmental changes and have been shown to have biological function. For example, electrical signals are induced by mechanical and flame-induced damage and are involved in activating protease inhibitor genes in tomato[10]. Mousavi et al. reported that electrical signals could be detected after insect herbivory, cold water stimuli and mechanical wounding of *Arabidopsis* and after exogenous electric shock, activating jasmonic acid (JA) signaling[6]. During the process of systemic acquired resistance, electrical signals are integrated together with reactive oxygen species and Ca^{2+} flux to participate in the rapid propagation of systemic signals[3]. Rapid electrical signals generated in response to local heat stimuli regulate systemic changes in nonphotochemical quenching and photosystem II quantum efficiency in *Arabidopsis* and dandelion[11]. In addition, previous work shows that electrical signals are generated in response to nematode attack in tomato roots, after which the signals are then propagated to the shoots to activate JA accumulation[12].

Recent work has linked glutamate receptor-like (*GLR*) genes to electrical signal transduction and JA signaling in *Arabidopsis* in which GLR3.3 and GLR3.6 were shown to mediate leaf-to-leaf wound signaling[6]. GLR proteins belong to a family of cation-permeable ion channels that function in various plant processes ranging from growth and development to stress response and defense. *GLR* genes facilitate Ca^{2+} influx across the plasma membrane to modulate the apical $[Ca^{2+}]_{cyt}$ gradient and consequently affect pollen tube growth and morphogenesis[13]. In addition, *GLR* genes regulate lateral root initiation and root development[14,15], and *GLR* genes have been reported to participate in both the salt stress response in *Arabidopsis* and cold tolerance in *Arabidopsis* and tomato[16–19]. In terms of biotic stress responses, *GLR* genes are dominant in defense against pathogens and herbivores. AtGLR3.3 is a key component of resistance against *Hyaloperonospora arabidopsidis*, *Pseudomonas syringae* pv. tomato DC3000 and the herbivore *Spodoptera littoralis*[20–22]. Also, earlier studies have characterized the role of GLR3.5 in the long-distance transduction of electrical signals elicited by nematodes, leading to nematode resistance[12]. The functions of *GLR* genes have mostly been studied in the model plant species *Arabidopsis* but the possible functions of *GLR* genes in crop plant species remain largely unknown.

Tomato (*Solanum lycopersicum*) is an important crop species worldwide and has been used as a model species to study interactions between plants and herbivores. Here, the characteristics of electrical signals induced by herbivory and mechanical wounds in tomato were investigated. In addition, *glr3.3* and *glr3.5* mutants and the *glr3.3/3.5* double mutant were used to examine the functions of *GLR* genes in electrical signal transduction and JA signaling.

2 MATERIALS AND METHODS

2.1 Plant materials and growth conditions

Tomato (*S. lycopersicum* cv. Ailsa Craig) was used in all experiments. The target sequence (TGAGTAGGAATGGCACTTCA) for *GLR3.3* was designed using the web tool CRISPR-P[23]. A *GLR3.3* CRISPR/Cas9 vector was constructed as described by Pan *et al.*[24] and then transformed into *Agrobacterium tumefaciens* strain EHA105. The transformed *A. tumefaciens* was then introduced into tomato Ailsa Craig (AC) and *glr3.5* mutants for the generation of *glr3.3* mutants and *glr3.3/3.5* double mutants, respectively[25]. Mutations induced by CRISPR/Cas9 were genotyped by DNA sequencing and homozygous lines in the F_2 generation were selected and used. Seedlings were cultivated in a plant nursery and treated with Hoagland's nutrient solution. Plants were grown under a 12:12 h L:D photoperiod at 25 and 20°C with a photosynthetic photon flux density of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

2.2 Plant treatments

Tomato seedlings with three fully expanded leaves were used. Cotton bollworm (*Helicoverpa armigera*) larvae were obtained from Jiyuan Baiyun Industry Co., Ltd. (Jiyuan, Henan, China). *H. armigera* larvae at the fourth-instar stage were starved for 1 d and then used for herbivory treatments. Mechanical wounding was conducted by crushing one-third of the terminal leaflet of the second true compound leaf with plastic hemostatic forceps.

Herbivory was simulated by crushing both sides of the leaflets of plants with plastic hemostatic forceps followed by the immediate application of 10 μL of 20% oral secretions (OS) from *H. armigera* at each wound side (W + OS). In herbivory trials, uniform *H. armigera* third-instar larvae (~ 5 mg) reared on an artificial diet were starved for 1 d. After starvation three larvae were placed on one tomato plant at the six-leaf stage, and there were eight plants of each treatment. After the larvae fed on the plants for 3 d the larval masses were determined.

2.3 Electrical signal recording

Electrical signals were detected as previously reported[6,12] with minor modifications. Briefly, silver electrodes (0.5 mm in diameter, World Precision Instruments, Sarasota, FL) were connected to a drop (10 μL) of 10 mmol·L^{-1} KCl in 0.5% (w/v) agar placed on the tomato leaf or petiole, and the ground electrode was placed in the soil. Two dual-channel amplifiers (FD 223 and Duo 773, World Precision Instruments) were used to detect the signals. The amplitude was the potential difference relative to the baseline before the changes, '*n*' was the total number of plants detected, and '*x*' was the number of plants in which changes in potential were detected. Each experiment comprised at least five separate plants.

2.4 qRT-PCR analysis

Total RNA was extracted from leaf tissues using an RNA Prep Pure Plant Kit (Tiangen, Beijing, China) and then reverse transcribed to cDNA using a ReverTra Ace qPCR RT Kit (Vazyme Biotech Co., Ltd., Nanjing, China) according to the manufacturer's instructions. The qRT-PCR experiments were conducted on a Light Cycler 480 II Real-Time PCR detection system (Roche, Mannheim, Germany). Each 20 μL reaction system consisted of 10 μL of SYBR Green PCR Master Mix (AceQ qPCR SYBR Green Master Mix Kit, Vazyme Biotech Co., Ltd., Nanjing, China), 1 μL of cDNA, and forward and reverse primers (0.1 μmol·L^{-1} each), according to the manufacturer's instructions. The housekeeping genes *ACTIN2* and *UBI3* were used as internal references to calculate the relative expression of target genes[26]. The sequences of the primer pairs are listed in supplemental materials (Table S1).

2.5 Determination of jasmonic acid, jasmonoyl-isoleucine, salicylic acid and indole-3-acetic acid concentrations

The concentrations of the phytohormones indole-3-acetic acid (IAA), JA, salicylic acid (SA) and jasmonoyl-isoleucine (JA-Ile) were determined by HPLC-MS/MS (Agilent 6460; Agilent Technologies, Santa Clara, CA) with D5-IAA, D5-JA, D4-SA (OlChemlm, Olomouc, Czechia) and D6-JA-Ile (Quality Control Chemicals, Newark, DE) used as internal standards, as described previously[27].

2.6 Statistical analysis

At least three independent biological replicates were included in each experiment. The data were statistically analyzed by analysis of variance using SAS software (version 8, SAS Institute, Cary, NC). The significance of treatment differences was determined using Student's *t*-test or Tukey's test as indicated in the Figure legends.

3 RESULTS

3.1 Herbivory activated electrical signals in tomato leaves

Three electrodes were placed, one on the terminal leaflet at the midrib (P1), one on the petiole of the second true compound foliage (P2) and the third on the petiole of the first leaf (P3) to investigate whether insect chewing is associated with the induction of electrical signals in tomato. After 1 d of starvation, cotton bollworm (*H. armigera*) larvae at the fourth-instar stage were placed on the second leaf at a position 1 cm away from P1 (Fig. 1(a)). No electrical activity was induced at P1, P2 or P3 but the larvae were crawling or resting on the leaves. However, larval herbivory on the lamina elicited significant changes in the surface potential, with a reduction in amplitude of about −20 mV at P1. In contrast, no change was observed at P2 or P3 (Fig. 1(b)). However, *H. armigera* larvae feeding on the base of the petiole elicited a surface potential with an amplitude of about −40 mV and both the frequency and duration were greater in response to feeding on the base of the petiole than on the lamina (Fig. 1(c,d)).

3.2 Characteristics of leaf/petiole wound-activated surface potential changes

Insect chewing usually induces mechanical wounding with the release of chemical elicitors. The effects of mechanical wounding on electrical activity were investigated to gain insight into the characteristics and propagation of the electrical signals. One-third of the terminal leaflet of the second true compound leaf was mechanically wounded by crushing the leaflet with plastic forceps. Electrical signals were measured at 1 and 3 cm from the wounding position, respectively (P1 and P2) and the junction of

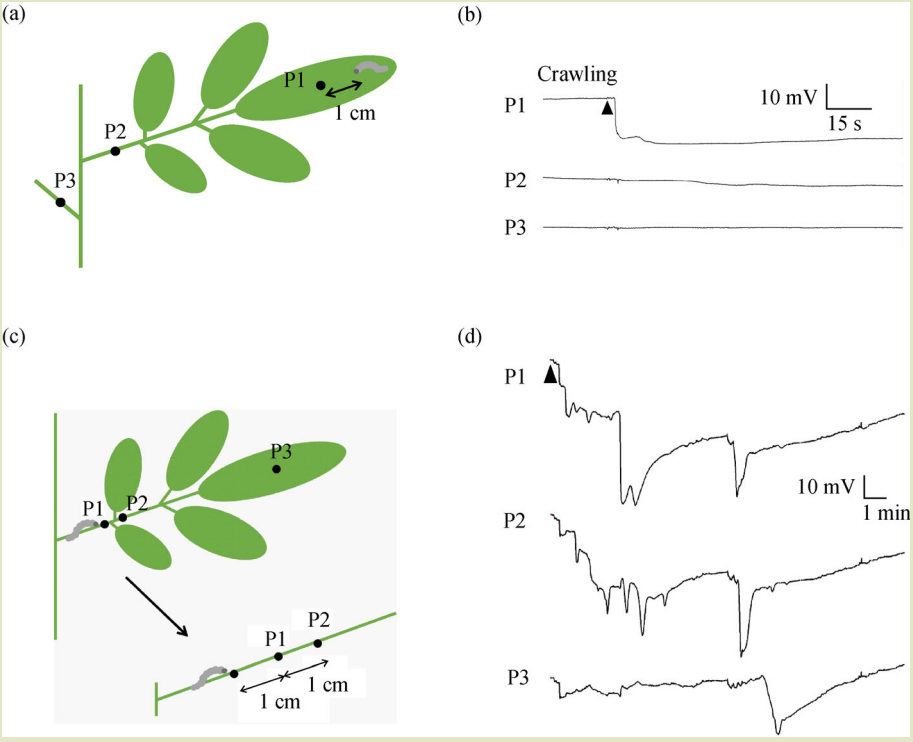


Fig. 1 Insect herbivory induced changes in the surface potential of tomato leaves. (a) Experimental design for measuring electrical signals elicited by herbivory on a leaf. Measuring electrodes: P1, midrib; P2, petiole; P3, petiole of the first leaf. *Helicoverpa armigera* larvae were allowed to feed at a position about 1 cm from electrode P1. (b) Electrical signals measured at electrodes P1, P2 and P3 of (a). (c) Experimental design for measuring electrical signals elicited by herbivory of the petiole. Measuring electrodes: P1, petiole (1 cm from the larval herbivory site); P2, petiole (1 cm from P2); P3, midpoint of the midrib of the terminal leaflet. (d) Electrical signals measured at electrodes P1, P2 and P3 of (c). The starting time of larval herbivory is indicated with a filled triangle. For (b) and (d), typical surface potential changes are shown (n = 6).

the terminal leaflet and petiole (P3) (Fig. 2(a)). After wounding, changes in surface potential were soon detected thereafter at P1 in a total of 9 of 12 plants, with an amplitude of –26.3±10.8 mV (Fig. 2(b) and Table 1). In addition, the surface potential varied for several seconds to several minutes and failed to return to normal levels in some leaves. Several seconds later a surface potential with an amplitude of –14.8±7.48 and a frequency of 5 of 12 plants was observed at P2. Wounding elicited a potential amplitude of about –15 mV at P3 in 3 of 12 observations (Fig. 2(b) and Table 1). By determining the delay time between P1 and P2 it was estimated that the rate of electrical signal transduction within the leaflet was 6.91±2.52 cm·min^{-1} (Table 1).

We then determined the characteristics of petiole wound-elicited surface potential changes. Petiole wound-elicited surface potential was first detected at P1, which was 1 cm from the wounding site, and these electrical signals were transduced to the junction of the petiole and terminal leaflet (P2) or the midrib of the terminal leaflet (P3, Fig. 2(c,d)). The amplitude of the surface potential decreased from –39.3 mV at P1 to –16.4 mV at P2 and to –14.2 mV at P3. Also, the percentage of leaves with detectable changes in surface potential (x/n) decreased from 100% at P1 to 25% at P3. The speed of signal propagation in the petiole from P1 to P2 was estimated to be about 10 cm·min^{-1}, greater than in the leaves (Table 1).

We then examined whether the electrical signals could propagate between leaflets. To this end, three electrodes (P1, P2 and P3) were placed on the junction of the petiole and leaflet (three leaflets: L1, L2 and L3). The middle of leaflet L1 was wounded (Fig. 3(a)). After wounding, changes in the surface potentials were detectable at P1 and P2 but very small changes were detected at P3. Also, wound-induced changes in the surface potential decreased from P1 to P3 (Fig. 3(b) and Table 2). When L2 was wounded, substantial changes in the surface potential were detected at P2 and P3, but few changes were detected at P1 (Fig. S1(a,b)).

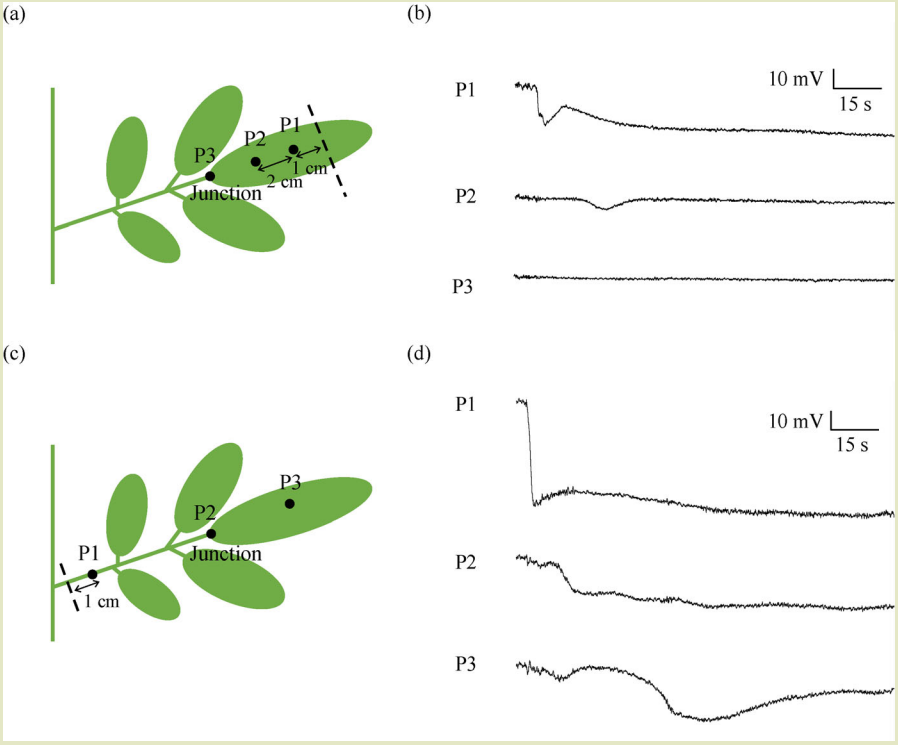


Fig. 2 Electrical signals caused by leaf wounding and petiole wounding. (a) Experimental design for measuring electrical signals elicited by mechanical wounding of a leaf. Measuring electrodes: P1, midrib 1 cm from the wounding position; P2, midrib 2 cm from electrode P1; P3, junction of the terminal leaflet and petiole. Dashed line, position of the mechanical wounding of one-third of the terminal leaflet. (b) Electrical signals measured at electrodes P1, P2 and P3 of (a). (c) Experimental design for measuring electrical signals activated by mechanical wounding of the petiole. Measuring electrodes: P1 (petiole 1 cm from the wound position; P2, the junction of the terminal leaflet and petiole; P3, the midpoint of the midrib of the terminal leaflet. Dashed line, position of the mechanical wound of the petiole. (d) Electrical signals measured at electrodes P1, P2 and P3 of (c). For (b) and (d), typical surface potential changes are shown (n = 12).

Table 1 Amplitude and speed of leaf and petiole wound-elicited electrical signals

Position	P1		P2		P3		Speed (cm·min^{-1})
	Amplitude (mV)	x/n	Amplitude (mV)	x/n	Amplitude (mV)	x/n	
Leaf wound	−26.32±10.76 a	9/12	−14.78±7.48 a	5/12	−14.74±2.53 a	3/12	6.91±2.52
Petiole wound	−39.33±9.31 a	12/12	−16.43±13.10 b	8/12	−14.17±5.22 b	3/12	10.05±4.14

Note: x, the number of plants in which changes in potential were detected; n, the total number of plants tested. The means denoted by the same letter do not significantly differ at $P < 0.05$ according to Tukey's test among P1, P2 and P3.

3.3 GLR3.3 and GLR3.5 mediated electrical signal transduction within a compound leaf

The role of tomato GLR3.3 and GLR3.5, which are homologs of *Arabidopsis* GLR3.3 and GLR3.6[6], in electrical signal transduction was examined by generating *glr3.3*, *glr3.5*[12] and *glr3.3/3.5* double mutants via CRISPR/Cas9 technology (Fig. S2). In comparison with untransformed plants, the wound-elicited surface potential at P1 was significantly reduced in the *glr3.5* mutants and *glr3.3/3.5* double mutants but not in the *glr3.3* mutants. The propagation of the electrical signals was also attenuated in all three mutants, with lower amplitude and decreased frequency at P2 and P3. Notably, the transduction of the electrical signal from P1 to P3 was totally abolished in all *glr3.3*, *glr3.5* and *glr3.3/3.5* plants (Fig. 3 and Table 2).

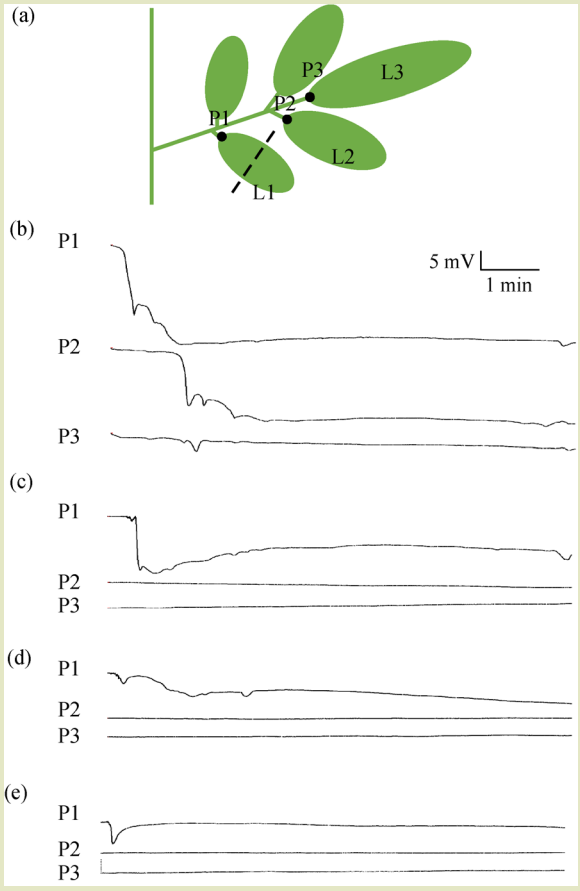


Fig. 3 *GLR* mutants present reduced amplitude and transduction of electrical signals. (a) Experimental design for measuring electrical signals within a compound leaf. Measuring electrodes: P1, junction of the petiole and leaflet 1; P2, junction of the petiole and leaflet 2; P3, junction of the petiole and leaflet 3. Dashed line, position of the mechanical wound at the center of leaflet 1. L1, leaflet 1; L2, leaflet 2; L3, leaflet 3. (b) Electrical signals measured at P1, P2 and P3 in untransformed plants. (c) Electrical signals measured at P1, P2 and P3 in *glr3.3* mutants. (d) Electrical signals measured at P1, P2 and P3 in *glr3.5* mutants. (e) Electrical signals measured at P1, P2 and P3 in *glr3.3/3.5* double mutants. For (b–e), typical surface potential changes are shown (n = 10).

3.4 Herbivory-induced JA accumulation and *Helicoverpa armigera* resistance were reduced in *glr3.3/3.5* plants

We next investigated how the transcription of JA biosynthesis- and JA signaling-related genes was altered by wounding. The transcript levels of the JA synthesis-related genes *LOXD*, *AOC* and *OPR3* and the JA signaling-related gene *JAZ10* were determined in L1, L2 and L3 45 min after L1 wounding (Fig. 3(a)). Wounding caused 18, 15, 17 and 54 times increases in transcript levels of *LOXD*, *AOC*, *OPR3*, and *JAZ10*, respectively, in L1 leaves compared with undamaged L1 leaves (control). The transcript levels of *LOXD*, *OPR3* and *JAZ10* also increased significantly in L2 and L3 after L1 wounding (Fig. 4). Similarly, L2 wounding greatly increased the transcription of these four genes in L2. However, this wounding induced fewer but significant increases in the transcript numbers of these genes in L1 and L3 (Fig. S3). Using UPLC-MS/MS we compared the accumulation of JA, JA-Ile, SA and IAA in the untransformed plants and *glr3.3/3.5* mutants in response to herbivory. Herbivory was simulated by crushing both sides of the leaflets followed by the immediate application of OS from *H. armigera* at the wound sites (W + OS). There were no significant differences in the accumulation of JA, JA-Ile, SA or IAA in the control leaves between the untransformed plants and *glr3.3/3.5* plants (Fig. 5(a) and Fig. S4). W + OS induced 20- and 88-times increases in the accumulation of JA and JA-Ile, respectively, in the leaves of the untransformed plants. However, these increases were greatly attenuated in the *glr3.3/3.5* mutants (Fig. 5(a)). W + OS induced little change in the accumulation of SA but a significant increase in the accumulation of IAA in the leaves. However, no significant differences were found between the *glr3.3/3.5* mutants and untransformed plants (Fig. S4). Herbivory trials show that, compared with untransformed plants, *glr3.5* single mutant and *glr3.3/3.5* double mutant plants were less resistant to *H. armigera* as indicated by the greater larval mass in response to feeding on the mutant plants (Fig. 5(b)).

Table 2 Amplitude of electrical signals in *GLR* mutants

Position	P1		P2		P3	
	Amplitude (mV)	x/n	Amplitude (mV)	x/n	Amplitude (mV)	x/n
WT	−43.21±15.25 a	10/10	−36.94±18.67	5/10	−6.12±1.89	4/10
*glr*3.3	−38.37±18.13 a	10/10	−20.78±14.60	2/10	/	0/10
*glr*3.5	−17.10±3.64 b	10/10	/	0/10	/	0/10
*glr*3.3/3.5	−13.11±6.71 b	10/10	−8.26±2.08	2/10	/	0/10

Note: x, the number of plants in which changes in potential were detected; n, the total number of plants tested. The means denoted by the same letter do not significantly differ at $P < 0.05$ according to Tukey's test.

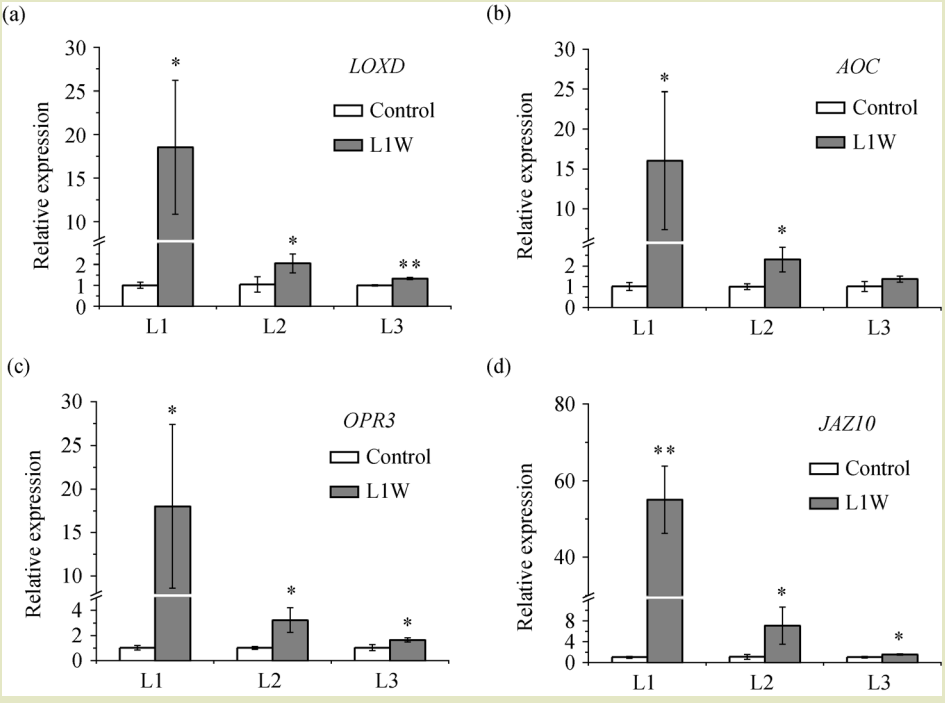


Fig. 4 L1 Wounding induced JA biosynthesis and signaling-related gene expression within compound leaves. (a) Transcript level of *LOXD* after L1 wounding. (b) Transcript level of *AOC* after L1 wounding. (c) Transcript level of *OPR3* after L1 wounding. (d) Transcript level of *JAZ10* after L1 wounding. Samples were collected 45 min after L1 wounding. Three biological samples were used for qRT-PCR determination. *ACTIN2* and *UBI3* were used as internal references to calculate the relative expression of the target genes, and the gene expression in L1/L2/L3 under control conditions was defined as 1. The data represent the mean±SD (n = 3). L1, leaflet 1; L2, leaflet 2; L3, leaflet 3. Statistically significant differences are indicated with asterisks (*, $P<0.05$; **, $P<0.01$) according to Student's t-test.

4 DISCUSSION

Electrical signals are universal signals in response to various environmental changes in plants. A previous study in *Arabidopsis* showed that wounding causes changes in surface potential, with an amplitude close to –70 mV, in local rosette leaves and unwounded systemic leaves. The speed of electrical signal transduction in the lamina is approximately 2.6 cm·min^{-1} but reaches 9 cm·min^{-1} along the midrib[6]. Here, we show that (1) herbivory and mechanical wounding induced electrical signals; (2) the amplitude of herbivory- and mechanical wounding-induced electrical signals was about –20 to –40 mV and the rates of electrical signal transduction in the midrib and petiole were about 7 cm·min^{-1} and 10 cm·min^{-1}, respectively; (3) electrical signals elicited by petiole wounds were stronger than those caused by leaf wounds, with larger amplitude and greater propagation; and (4) electrical signals were not transduced from leaf to leaf in tomato but could propagate from leaflet to leaflet within a compound leaf. The characteristics of electrical signals shown in this study display some similarities to and differences from those in *Arabidopsis*. First, the rates of electrical signal transduction were similar to the signal propagation speed in *Arabidopsis*. Second, the amplitude of electrical signals in tomato was much lower than in *Arabidopsis*. In addition, electrical signals caused by root knot nematode infection were even weaker with an amplitude of about –5 mV and a duration of about 30 s[12]. Third, electrical signal transduction from leaf to leaf was not detected in the tomato seedlings. Consistently, Wildon et al. reported that mechanical damage of one cotyledon of tomato seedlings led to the propagation of electrical activity to the petiole of the same leaf and stem and systemic accumulation of protease inhibitor activity at the one-expanded-leaf stage, but such induction was not as reproducible at two-expanded-leaf stage. Much greater reproducibility was observed when a heat stimulus was applied to the leaf[10]. Thus, electrical signals in plants vary widely and the strength and transduction of electrical signals depend on the type of damage, wound position, plant species and plant growth stage.

JA is crucial in plant defense against herbivores and pathogens[28–30]. JA accumulates within minutes in both wounded and intact tissues of *Arabidopsis*[31–33]. This may be due to the fast systemic transduction of electrical signals which

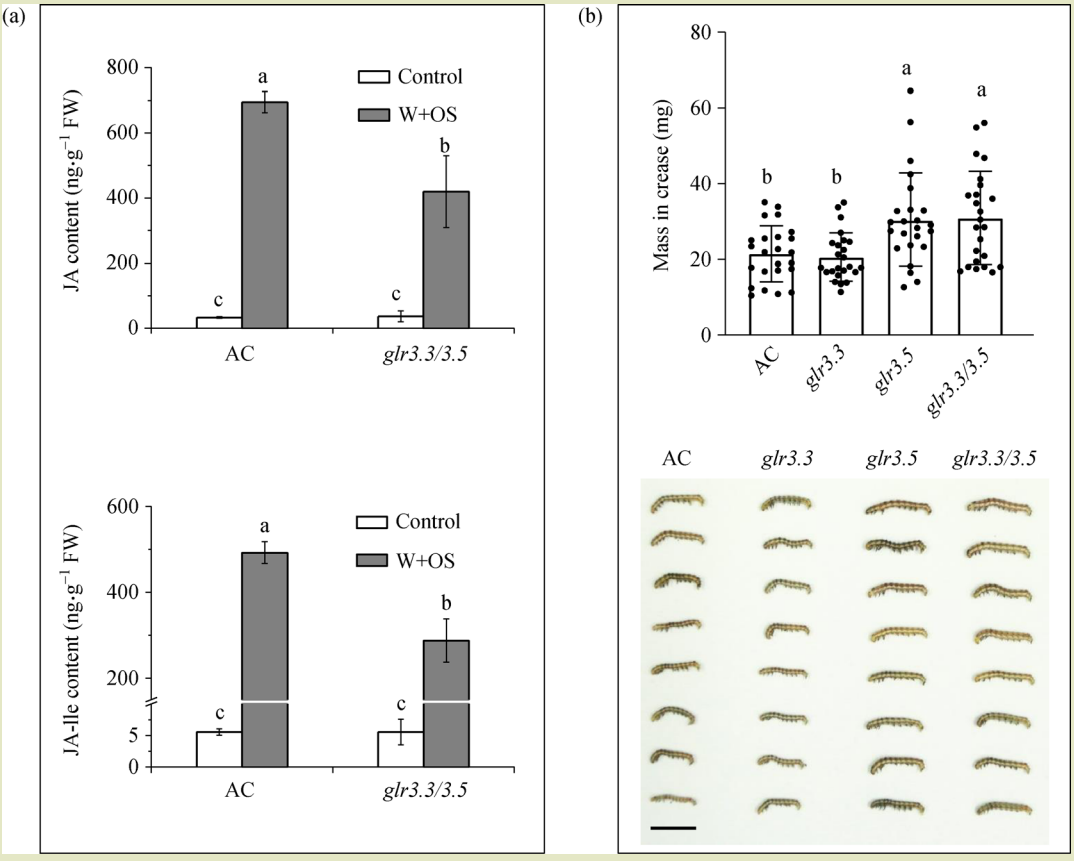


Fig. 5 *GLR* mutants present reduced herbivory-induced JA and JA-Ile accumulation and *Helicoverpa armigera* resistance. (a) JA and JA-Ile contents in WT plants and *glr3.3/3.5* mutants upon W + OS. Samples were collected 1 h after W + OS. Three biological samples were used. The data represent the mean±SD (n = 3). The means denoted by the same letter do not significantly differ at $P<0.05$ according to Tukey's test. (b) Mean weight increase (upper panel) and representative images of larvae after 3 d of herbivory on *glr3.3* and *glr3.5* single mutants and on *glr3.3/3.5* double mutants (lower panel). The data represent the mean±SD (n = 24). Bar = 1 cm.

occurs upstream of JA signaling[6]. Here, we provide evidence that JA signaling was activated within compound leaves, and the intensity was consistent with the transduction of electrical signals (Fig. 3, 4, S1, and S3). Leaf-to-leaf systemic propagation of herbivore-induced electrical signals was not observed in tomato, as it was in *Arabidopsis*. These findings are strongly supported by evidence from Li et al.[34] who suggested that JA, or a related compound derived from the octadecanoid pathway, may act as a transmissible wound signal in tomato. Importantly, we found that wounding of different organs caused different levels of response. Petiole damage from wounding or herbivory resulted in stronger electrical signals than leaf damage (Fig. 2 and Table 1). Previous studies show that electrical signals can inhibit photosynthesis[11,35], suggesting that electrical signals may function as a potential regulator in balancing defense and growth tradeoffs in plants in response to herbivory. However, further research is needed to confirm this.

GLR genes have been reported to mediate electrical signals, Ca^{2+} signals and JA signaling in *Arabidopsis*[5,6,22]. The roles of tomato GLR3.3 or/and GLR3.5 in regulating electrical signals and JA accumulation are demonstrated in the present study. First, *glr3.5* mutants and *glr3.3/3.5* double mutants showed reduced electrical signal intensity in response to mechanical wounding, displaying lower amplitudes within the wounded leaflets compared with those displayed by untransformed plants (Fig. 3 and Table 2). Second, the propagation of electrical signals from wounded leaflet 1 to undamaged leaflet 3 was abolished in the *glr3.3*, *glr3.5* and *glr3.3/3.5* mutants (Fig. 3 and Table 2). Third, the *glr3.3/3.5* mutants accumulated reduced amounts of JA and JA-Ile upon W + OS (Fig. 5(a)). Fourth, the *glr3.5* and *glr3.3/3.5* mutants presented significantly decreased resistance to *H. armigera* (Fig. 5(b)). These results are consistent with those of an earlier study that showed that GLR3.5-mediated nematodes induced both systemic transduction of electrical signals and JA

accumulation[12]. Given that there were no differences in electrical signal amplitude detected in the wounded leaflets between the *glr3.3* mutants and untransformed plants, we suggest that GLR3.3 may function mainly in the propagation of electrical signals rather than in the generation of these signals.

5 CONCLUSIONS

This study shows the characteristics of electrical signals in tomato elicited by herbivory and wounding. Both herbivory and mechanical wounding induced electrical signals. However, petiole wounding produced stronger electrical signals than did leaflet wounding. Wounding of a leaflet caused electrical signal transduction and JA signaling within a whole compound leaf. Further investigation revealed that GLR3.3- and GLR3.5-mediated herbivory/wound-elicited the propagation of electrical signals within a compound leaf, further activating JA signaling.

Supplementary materials

The online version of this article at https://doi.org/10.15302/J-FASE-2021395 contains supplementary materials (Table S1; Figs. S1–S4).

Acknowledgements

This work was funded by the National Natural Science Foundation of China Projects of International Cooperation and Exchange (32020103013) and the Modern Agro-industry Technology Research System of China (CARS-25-02A).

Compliance with ethics guidelines

Chaoyi Hu, Siqi Duan, Jie Zhou, and Jingquan Yu declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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