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SHORT COMMUNICATION

Intraplant communication in maize contributes to defense against insects

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ABSTRACT

The vasculature of plants act as a channel for transport of signal(s) that facilitate long-distance intraplant communication. In maize, Maize insect resistance1-Cysteine Protease (Mir1-CP), which has homology to papain-like proteases, provides defense to different feeding guilds of insect pests. Furthermore, accumulation of Mir1-CP in the vasculature suggests that Mir1-CP can potentially function as a phloem-mobile protein. In a recent study, we provided evidence that Mir1-CP can curtail the growth of phloem-sap sucking insect, corn leaf aphid (CLA; *Rhopalosiphum maidis*). Our current study further examined whether aboveground feeding by CLA can induce resistance to subsequent herbivory by belowground feeding western corn rootworm (WCR; *Diabrotica virgifera virgifera*). Aboveground feeding by CLA systemically induced the accumulation of Mir1-CP in the roots. Furthermore, foliage feeding by CLA provided enhanced resistance to subsequent herbivory by belowground feeding of WCR. Taken together, our previous findings and results presented here indicate that long-distance transport of Mir1-CP is critical for providing enhanced resistance to insect attack in maize.

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The vascular system of higher plants is a probable conduit for transport of signal(s) that enables above and belowground plant structures to translocate signal(s) throughout the plant.^{1,2} When plants are being challenged by different stresses, including abiotic and biotic stresses, the plants systemically translocate defense signal(s) to induce defense responses.^{3,4} Biotic stresses caused by herbivore attack induce plant defenses both locally and systemically. It has been shown that aboveground feeding by insect herbivores activates root-based defenses, which subsequently influences the physiology and behavior of aboveground feeding herbivores, suggesting that roots play a vital role in inducing defenses.⁵⁻¹⁰ Previous studies have shown that aboveground feeding by leaf attacking caterpillars can have negative effects on the root-feeding herbivores.¹⁰⁻¹² Similarly, belowground feeding by root-feeders increases foliar resistance against leaf attacking caterpillars.^{10,11} These studies suggest that shoot-to-root communication and vice versa is critical to fine-tune defense against attacking herbivores.

Maize (*Zea mays*) is attacked by a plethora of insect pests that feed both above and belowground. Aboveground feeding insects include both chewing (e.g., Fall armyworm [FAW; *Spodoptera frugiperda*]) and piercing/sucking (e.g., Corn leaf aphid [CLA; *Rhopalosiphum maidis*]) groups of insects. Belowground feeding insects include the root feeders such as western corn rootworm (WCR; *Diabrotica virgifera virgifera*). Chewing insects' strong mandibles enables them to feed externally on the plant and results in considerable mechanical damage to the leaves.^{13,14} By contrast, insects with piercing/sucking mouthparts seemingly cause

less physical damage to tissues; however, they feed on phloem sap by piercing plant tissue and extracting plant fluids resulting in considerable crop yield loss.^{15,16} In addition, several piercing/sucking insects vector plant viruses.¹⁷ Root feeders are major insect pests that attack belowground plant tissues and cause considerable damage to crop plants. Belowground feeding is debilitating to crops because it weakens the roots system, diminishes nutrient uptake, and creates entry points for fungal and bacterial pathogens and increases the chances of plant lodging.¹⁸

The maize genotype, Mp708, developed by classical plant breeding is remarkably resistant to different feeding styles of herbivore pests.^{12,19-21} Previous studies have shown that Mp708 provides resistance to caterpillars by rapidly accumulating Maize insect resistance1-Cysteine Protease (Mir1-CP), a papain-like protease.¹⁹ Furthermore, it has been shown that Mir1-CP is localized in maize vascular tissues.²² Interestingly, Mir1-CP, a phloem-mobile protein, accumulates in roots in response to foliar feeding by FAW. Recently, we have also identified that feeding by CLA triggers the accumulation of *mir1* transcripts in Mp708.²¹ Feeding-trial bioassays with recombinant rMir1-CP indicated that Mir1-CP provides toxicity to CLA.²¹ Interestingly, root removal prior to CLA infestation dramatically reduced the expression of *mir1* in whorls, suggesting that roots act as an important site for synthesis of defense-related proteins/compounds.²¹ The current study was aimed at addressing the role of Mir1-CP in aboveground-belowground communication and how aboveground feeding by CLA impact subsequent herbivory by belowground feeding of WCR.

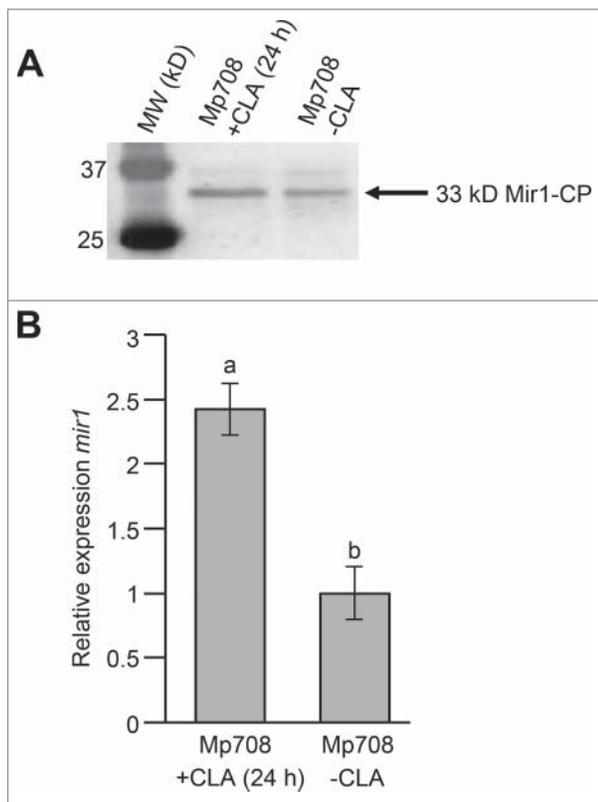


Figure 1. Aboveground feeding by corn leaf aphids (CLA) promotes accumulation of Mir1-CP protein and expression of *mir1* transcripts in the roots of Mp708 genotype. (A) Immunoblot analysis of Mir1-CP protein: Mir1-CP accumulation in the roots of V3 Mp708 plants after 24 h of foliar feeding of CLA (Mp708 +CLA 24 h). Mp708 plants that did not receive CLA (Mp708 -CLA) were used as the control. MW, Molecular weight markers in kD. (B) Relative expression of *mir1* transcripts: qRT-PCR analysis of *mir1* transcripts in V3 Mp708 plants with (+) and without (–) CLA infestation ($N = 4$). Different letters above the bars indicate values that are significantly different from each other ($P < 0.05$). Error bars represent \pm SE. The experiments in (A) and (B) were conducted twice independently with similar results.

To determine if Mir1-CP accumulates in the roots after foliar feeding by CLA, we monitored the Mir1-CP levels in the roots using immunoblot analysis. Our results suggest an increased accumulation of Mir1-CP levels in the roots after CLA feeding in the whorl for 24 h (Fig. 1A). Similarly, our results indicate that aboveground feeding by CLA for 24 h trigger belowground expression of *mir1* transcripts (Fig. 1B). These results are supportive of our previous finding that CLA feeding rapidly induces the expression of *mir1* in Mp708 genotype.²¹ To test the importance of aboveground feeding induced resistance to subsequent herbivory by belowground root-feeding insect, insect bioassays were performed using naïve WCR larvae. Aboveground feeding by CLA for 24 h provides enhanced resistance to subsequent herbivory by belowground feeding of WCR (Fig. 2), suggesting that roots may also act as a site for toxin synthesis in response to aboveground herbivory. These results further support our hypothesis that intraplant communication in maize can act as a critical component in modulating defenses against insect attack.

A recent study by Erb et al. (2015)¹⁰ suggested that aboveground feeding by Egyptian cotton leafworm (*S. littoralis*) resulted in the reprogramming of soluble root

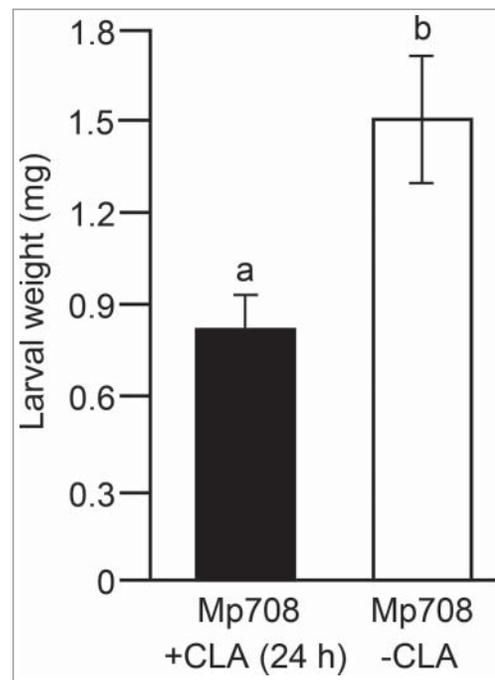


Figure 2. Aboveground feeding by corn leaf aphids (CLA) negatively effects the growth of belowground feeding western corn rootworm (WCR). Mp708 plants (V3 stage) were initially infested with 25 adult apterous corn leaf aphids (+CLA) on the whorl region. Two newly hatched neonates of WCR were used to infest each plant after removing the CLA that were feeding on Mp708 plants for 24 h. After 12 d the fresh weight of WCR was measured. Mp708 plants that were not exposed to CLA (-CLA) were used as the control ($N = 10$). Means were separated using LSD procedure. Different letters above the bars indicate values that are significantly different from each other ($P < 0.05$). Error bars represent \pm SE. The bioassay was repeated 3 times with similar results. Data represent one of 3 independent experiments achieving similar results.

components that potentially affected the avoidance behavior of rooting feeding WCR. However, it remains unclear how foliar feeding by CLA can manipulate the systemic changes in the root metabolites. It is highly plausible that CLA feeding, in addition to triggering the accumulation of Mir1-CP in roots, can also modulate the composition of root defense compounds, thereby reducing the fitness of root feeders on maize plants. Additional experiments are required to determine the signaling partner(s) of Mir1-CP and other possible mechanisms that are involved in long-distance defense signaling mechanisms against insect attack in maize. Furthermore, plants respond differently to different feeding styles of insect pests. Elucidating the differences and similarities of how signaling networks in maize respond to different feeding guilds of insect pests will help us to better understand the key regulatory mechanisms underlying plant resistance to insect pests.

Disclosure of potential conflict of interest

No potential conflicts of interest were disclosed.

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