Short Communication

Maize defense elicitor, 12-oxo-phytodienoic acid, prolongs aphid salivation

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Running Title: 12-OPDA extends aphid salivation

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Abstract

12-oxo-phytodienoic acid (OPDA), an intermediate in the jasmonic acid (JA) biosynthesis pathway, regulates diverse signaling functions in plants, including enhanced resistance to insect pests. We previously demonstrated that OPDA promoted enhanced callose accumulation and heightened resistance to corn leaf aphid (CLA; *Rhopalosiphum maidis*), a phloem sap-sucking insect pest of maize (*Zea mays*). In this study, we used the electrical penetration graph (EPG) technique to monitor and quantify the different CLA feeding patterns on the maize JA-deficient *12-oxo-phytodienoic acid reductase* (*opr7opr8*) plants. CLA feeding behavior was unaffected on B73, *opr7opr8* control plants (- OPDA), and *opr7opr8* plants that were pretreated with OPDA (+ OPDA). However, exogenous application of OPDA on *opr7opr8* plants prolonged aphid salivation, a hallmark of aphids' ability to suppress the plant defense responses. Collectively, our results indicate that CLA utilize its salivary secretions to suppress or unplug the OPDA-mediated sieve element occlusions in maize.

TEXT

The corn leaf aphid (CLA; *Rhopalosiphum maidis*), a piercing-sucking insect pest, is one of most damaging pests of many cereal crops, including maize (*Zea mays*).[1–6] Unlike chewing herbivores, CLA feed by inserting their slender stylets into phloem sieve elements to consume the nutrients required for their growth and development. CLA feeding also transmits various plant viral diseases.[2,7,8] In addition, the aphid honeydew, the digestive waste produced by aphids, which are deposited on the leaves promotes sooty mold growth, thereby reducing the photosynthetic efficiency of plants.[9]

We have previously shown that 12-oxo-phytodienoic acid (OPDA), an intermediate in the jasmonic acid (JA) biosynthesis pathway, promotes heightened maize resistance against aphids.[6] In addition, exogenous application of OPDA enhanced callose accumulation, one of the defense mechanisms utilized by plants against insect attack, and also enhanced the expression of ethylene biosynthesis and receptor genes that act as an important modulator in regulating *maize insect resistance1* (*mir1*)-dependent maize defense to CLA.[5,6] However, artificial diet aphid bioassays confirmed that OPDA does not have a direct negative impact on CLA population, rather the OPDA-induced activation of downstream defenses contributed to enhanced maize resistance to CLA.[6]

Exogenous application of OPDA does not affect feeding of CLA on maize plants

In maize, two *12-Oxo-Phytodienoic acid Reductase* (*OPR7* and *OPR8*) genes are involved in the conversion of OPDA to JA.[10] Basal and wound-induced OPDA levels in *opr7 opr8* double mutants were reduced as compared to wild-type B73 plants, whereas JA induction was undetectable in *opr7opr8* plants.[11] Previously, we showed that there was comparable CLA numbers on B73 and *opr7opr8* plants, however, exogenous application of OPDA showed significantly lesser aphid numbers on *opr7opr8* plants.[6] Similarly, exogenous application of OPDA and feeding by CLA on *opr7opr8* plants increased the callose accumulation compared to *opr7opr8* control plants and wild-type plants.[6] These findings suggested that the OPDA-mediated resistance to CLA in maize can occur independently of the JA pathway and signaling mechanisms. Strong antibiosis, which curtails insect fecundity and population growth, can also influence insect's feeding behavior.[9] To determine if exogenous OPDA application can affect CLA feeding behavior, we utilized the electrical penetration graph (EPG) technique[6,12-14] to

monitor and quantify the different CLA feeding activities on *opr7opr8* plants. Using EPG, the various parameters measured included the time taken to first probe (FP), time taken to reach first sieve element phase (f-SEP), time spent in the pathway phase that represent both the inter- and/or intracellular aphid stylet routes during feeding (PP), total time spent in the SEP, total time spent in the xylem phase (XP), and total time spent in nonprobing phase (NP). As shown in **Figure 1**, there were no significant differences in any of these parameters measured for the CLA feeding behavior on the wild-type (B73), *opr7opr8* control plants (- OPDA) and OPDA pretreated *opr7opr8* (+ OPDA) plants. The EPG result suggests that OPDA pretreatment does not have an effect on aphid feeding behavior.

OPDA pretreatment extends aphid salivation on maize plants

The SEP consists of E1 (salivation) and E2 (sap ingestion) phases.[15] E1 phase, the initial phase in the SEP, represents aphid salivation and in general, could remain approximately for one minute. E2 waveform represents subsequent ingestion of phloem sap with continuous salivation and it could range from several minutes to hours.[15] Aphids secrete watery saliva during E1 SEP, which contains salivary effectors that alter host physiology for their own benefit and to assist continued feeding from the sieve elements, before start ingesting phloem sap (E2).[9,15-17] Our results indicate that CLA spent significantly longer time in the E1 phase of OPDA pretreated *opr7opr8* (+ OPDA) plants compared to the wild-type (B73) and *opr7opr8* control plants (- OPDA) (**Figure 2A**). In contrast, there was no significant difference in the E2 phase of CLA feeding on the wild-type (B73), *opr7opr8* control plants (- OPDA) and OPDA pretreated *opr7opr8* (+ OPDA) plants (**Figure 2B**). **Figure 2C** shows the representative E1 and E2 waveform patterns produced by CLA feeding on maize plants.

Upon aphid feeding, as a counter-defense mechanism, plants induce the phloem wound responses, such as aggregation of phloem proteins and callose deposition.[15,18] It should also be noted that the wound responses in sieve elements by aphid stylets compared to severing the sieve elements by a glass needle, which mimics aphid feeding, are distinct and does not lead to the activation of similar set of phloem proteins.[15] Furthermore, studies have shown that an extended E1 phase is indicative of the ability of the aphids to suppress the wound defense responses induced by insect feeding.[15,20] It was previously shown that OPDA pretreatment enhanced callose accumulation on maize plants.[6] It is highly likely that CLA may inject more watery saliva into the sieve elements to suppress the defense responses, for example, suppression of sieve element occlusion by dissolving callose accumulation. However, it remains unclear how aphid salivation suppresses OPDA-mediated defenses. Ca^{2+} is reported to have a major role in phloem occlusion through its effect on callose deposition and coagulating phloem proteins.[19,21,22] Ca^{2+} binding proteins are identified in the salivary glands of aphids[23], suggesting that aphids may inject these proteins during E1 phase to suppress the wound responses. Whether similar Ca^{2+} binding proteins and/or other salivary gland proteins are required for E1 salivation in CLA are yet to be determined.

Disclosure of potential conflict of interest

No potential conflicts of interest were disclosed.

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Figure Legends

Figure 1. Electrical penetration graph (EPG) comparison of time spent by CLA in various feeding activities on maize B73, *opr7opr8*, and *opr7opr8* plants pretreated with OPDA in 8h of recording time. FP, time taken to first probe; f-SEP, time taken to reach first sieve element phase; PP, time spent in pathway phase; XP, total time spent in the xylem phase; SEP, total time spent in the sieve element phase or phloem phase; NP, total time spent in nonprobing phase during the 8 h recording time. Boxplots represent median and range for each treatment (n = 5-7). EPG was analyzed by the non-parametric Kruskal–Wallis test. Statistically significant differences were not observed among any of the aphid feeding parameters on B73, *opr7opr8*, and *opr7opr8* plants pretreated with OPDA.

Figure 2. Electrical penetration graph (EPG) comparison of time spent by CLA in the E1 (salivation) (A) and E2 (ingestion) (B) phases during the sieve element phase (SEP) on maize B73, *opr7opr8*, and *opr7opr8* plants pretreated with OPDA in 8h of recording time. Boxplots represent median and range for each treatment (n= 5-7). Asterisks indicate significant difference ($P < 0.05$; Kruskal–Wallis test) among individual CLA feeding parameter on different maize plants. C) Representative EPG waveform patterns of E1 and E2 during the CLA feeding on maize plants for 10 seconds.

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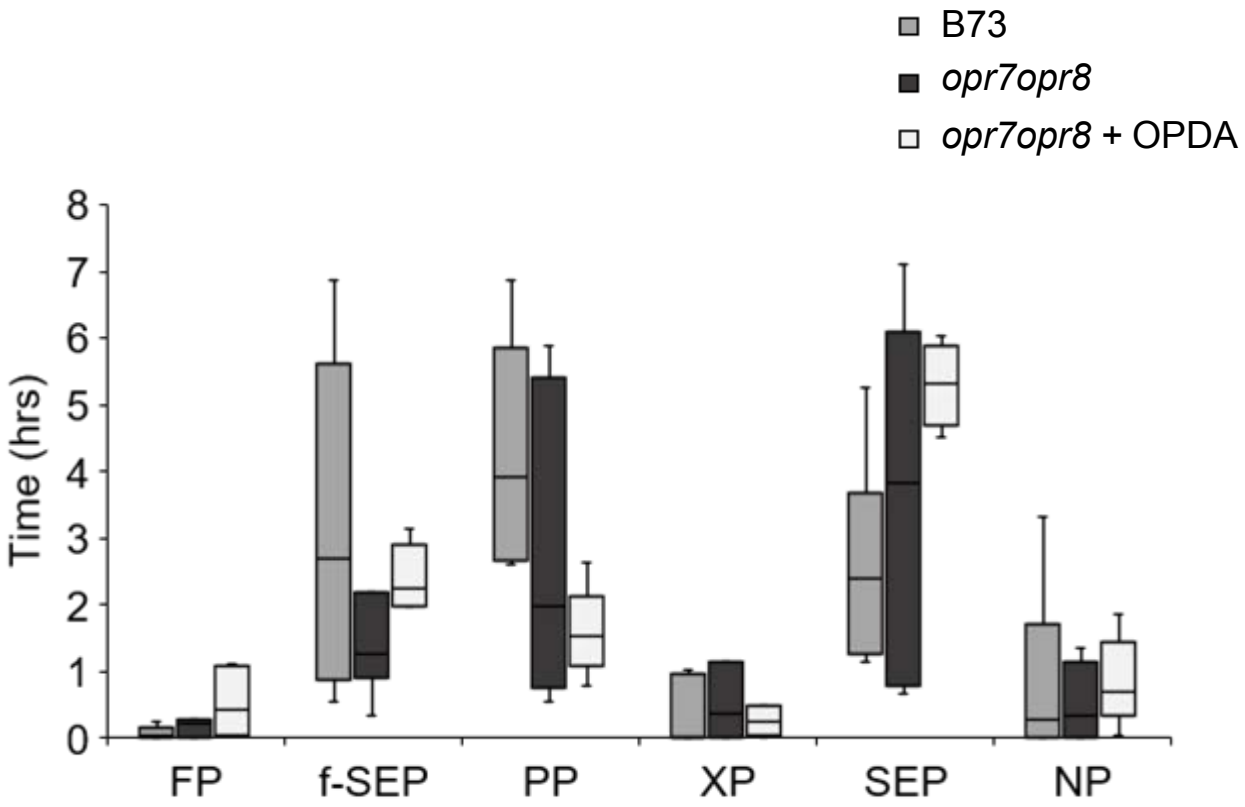

Figure 1. Electrical penetration graph (EPG) comparison of time spent by CLA in various feeding activities on maize B73, *opr7opr8*, and *opr7opr8* plants pretreated with OPDA in 8h of recording time. FP, time taken to first probe; f-SEP, time taken to reach first sieve element phase; PP, time spent in pathway phase; XP, total time spent in the xylem phase; SEP, total time spent in the sieve element phase or phloem phase; NP, total time spent in nonprobing phase during the 8 h recording time. Boxplots represent median and range for each treatment (n = 5-7). EPG was analyzed by the non-parametric Kruskal–Wallis test. Statistically significant differences were not observed among any of the aphid feeding parameters on B73, *opr7opr8*, and *opr7opr8* plants pretreated with OPDA .

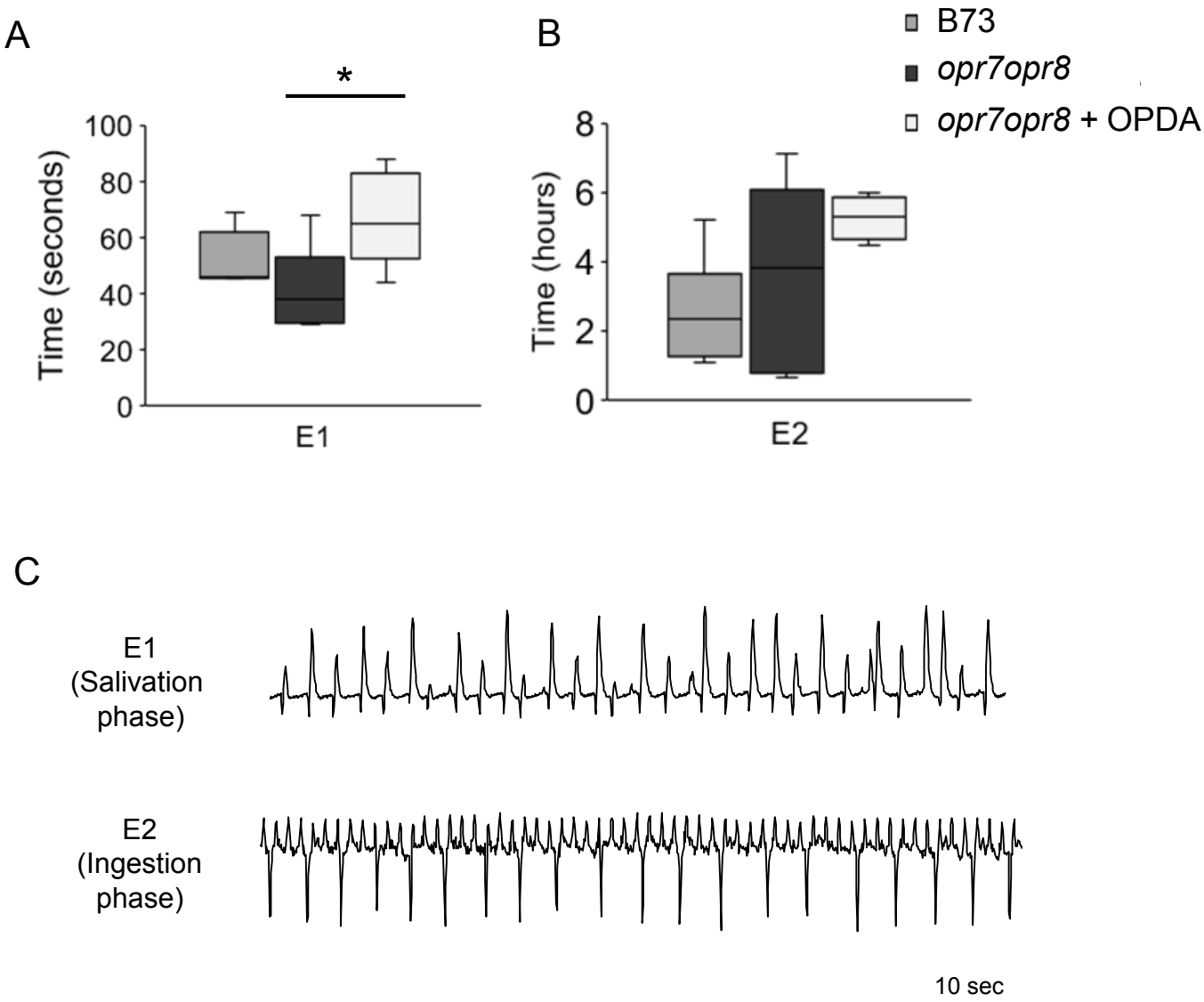

Figure 2. Electrical penetration graph (EPG) comparison of time spent by CLA in the E1 (salivation) (A) and E2 (ingestion) (B) phases during the sieve element phase (SEP) on maize B73, *opr7opr8*, and *opr7opr8* plants pretreated with OPDA in 8h of recording time. Boxplots represent median and range for each treatment (n= 5-7). Asterisks indicate significant difference ($P < 0.05$; Kruskal–Wallis test) among individual CLA feeding parameter on different maize plants. (C) Representative EPG waveform patterns of E1 and E2 during the CLA feeding on maize plants for 10 seconds.