

Maize defense against insect herbivory: A novel role for 9-LOX-derived oxylipins

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Up to 40% of global crops are destroyed by arthropod pests, either prior to harvest or during storage (Gullino et al., 2021). This problem is exacerbated by current and anticipated climate changes that will allow the long-term survival of pest populations in new habitats, as well as by the introduction of new pest species into areas where they were not previously present. For instance, the recent introduction of *Spodoptera frugiperda* (fall armyworm), which previously was a major pest of *Zea mays* (maize) only in the Americas, led to the expansion of this species throughout the maize growing regions of Africa and Asia in less than five years (Tay et al., 2022). Given current yield losses and the high probability of future range expansions by pest species, meeting the anticipated demands for food and bioenergy provides significant challenges and requires innovative research to bolster plant resistance to insect herbivory. Basic plant research, such as that conducted by Yuan *et al.* (2023), provides new insight into the mechanisms of herbivory-induced defenses and holds promise for devising novel strategies that can be implemented to promote crop plant resilience through breeding or transgenic approaches.

In response to herbivore attack, plants initiate a signaling cascade that leads to the production of toxic and/or deterrent small molecules and proteins. Research conducted initially with *Arabidopsis thaliana* (Arabidopsis) and *Solanum lycopersicum* (tomato) showed that oxylipins and, in particular, jasmonic acid (JA) and its derivatives play a central role in the initiation of plant defenses against insect herbivory (Howe and Jander, 2008). A defense signaling cascade is initiated when α -linolenic acid (ALA) is converted to *cis*-(+)-12-oxo-phytodienoic acid (12-OPDA) by the sequential action of 13-lipoxygenase (13-LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC) (Figure 1). Further reduction and β -oxidation reactions lead to the production of JA, which is conjugated to L-isoleucine (Ile) to form JA-Ile (Wasternack and Feussner, 2018). Defense-related gene expression is activated when JA-Ile binds to the F-box protein coronatine insensitive 1 (COI1) and promotes the interaction between COI1 and the jasmonate-ZIM-domain (JAZ) transcriptional repressor proteins, which triggers

ubiquitination (via the E3 ubiquitin ligase SCF^{COI1} complex) and degradation (via the 26S proteasome) of the JAZ repressors. Mutations in both the synthesis and perception of JA-Ile lead to increased herbivore susceptibility in several plant species. For instance, maize *opr7 opr8* (12-oxo-phytodienoic acid reductase 7 and 8) double mutants have reduced JA levels and increased susceptibility to *Spodoptera exigua* (beet armyworm) herbivory (Yan et al., 2012). Moreover, JA-Ile precursors such as 12-OPDA seem to have a more direct role activating plant defenses against piercing-sucking insects (Guo et al., 2014).

In addition to 13-LOX enzymes, which initiate the production of JA and related metabolites, 9-lipoxygenase (9-LOX) enzymes can also use ALA as a substrate. By oxidizing ALA at carbon 9 instead of carbon 13, 9-LOX enzymes produce 9-hydroperoxy-octadecatrienoic acid (9-HPOT), which serves as a precursor for 9-hydroxy-10-oxo-12(Z),15(Z)-octadecadienoic acid (9,10-KODA) and other oxylipins (Figure 1). Transcriptomic studies of maize responses to caterpillar herbivory show that expression of some 9-LOX genes, namely *LOX4* and *LOX5*, is highly induced (Park et al., 2010; Tzin et al., 2017). Two *Mutator* (*Mu*) transposon insertions that knocked out expression of the *LOX4* gene increased maize susceptibility to *S. exigua* feeding. However, until recently published research (Yuan et al., 2023), there was no evidence for the mechanism by which 9-LOX activity leads to the induction of maize defense responses.

To investigate the in vivo function of *LOX5* in maize defense against insect herbivory, Yuan et al. (2023) used *Mu* transposon insertions to generate three independent *lox5* mutants and back-crossed these into the maize inbred line B73 genetic background to generate near-isogenic lines. Over several field seasons, insect damage was greater on the *lox5* mutant plants than on wildtype B73. These results were recapitulated in a laboratory setting, where fall armyworm larvae caused more damage on the *lox5* mutant plants and, after nine days of feeding, were about six-fold heavier than on wildtype B73 maize. This dramatic result showed that *LOX5* plays a major role in maize defense against fall armyworm herbivory.

Analysis of the *LOX5* amino acid sequence identified an N-terminal signal sequence that suggested localization to the vacuolar membrane (tonoplast) of plant cells. GFP fusions were used to confirm the subcellular localization of *LOX5*, showing that the protein localized to the tonoplast and the cytosol when expressed in both maize and *Arabidopsis* protoplasts. This finding is somewhat unexpected, as previously investigated 13-LOXs were found to be localized in the chloroplasts, the site of 12-OPDA biosynthesis, and suggested that *LOX5* may have a different function in plant defense.

Biochemical analysis of oxylipins and other plant signaling molecules in *lox5* mutant and wildtype maize showed only a small decrease in the accumulation of the presumed product, 9-HPOT (Figure 1). There was also no significant change in the accumulation of death acids, jasmonate-like 9-LOX-derived compounds that were previously shown to affect herbivore resistance (Christensen et al., 2015). However, 9,10-KODA, as well as the 13-LOX-derived 12-OPDA, JA and JA-Ile were significantly reduced in *lox5* mutants. This indicates that: (i) in addition to the previously identified regulation of 9,10-KODA accumulation by JA, there is also reciprocal regulation of the 13-LOX branch of the oxylipin pathway by 9-LOX products, and (ii) other 9-LOX enzymes, e.g., LOX1/2/3/4/12, may play a more direct role in death acid biosynthesis. Interestingly, the induction of plant defenses by the 9-LOX pathway appears to occur independently of JA signaling. Whereas exogenous addition of 9,10-KODA or 12-OPDA restored herbivore resistance in *lox5* plants, JA-Ile did not. Nevertheless, there were additive effects on herbivore resistance in a *lox5 lox10* double mutant, suggesting two independent pathways to the induction of plant defenses, including green leaf volatile production, dependent on 9,10-KODA and 12-OPDA biosynthesis, respectively.

Other assays conducted by Yuan *et al.* (2023) point to the proximal causes of increased herbivore sensitivity in *lox5* mutant maize. Compared to wildtype, *lox5* mutants exhibited a significantly lower accumulation of 6-methoxy-benzoxazolin-2-one (MBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside (DIMBOA-Glc), two metabolites of the maize hydroxamic acid pathway which contributes to insect resistance (Zhou et al., 2018). Furthermore, artificial diet assays suggested that 9,10-KODA itself has insecticidal properties and contributes to maize defense.

Together, the results presented by Yuan *et al.* (2023) provide evidence for a previously unknown 9-LOX role in maize defense against insect herbivory. These findings also highlight the complexity of oxylipin signaling, showing that it is not a simple linear pathway but rather a network of interacting signals involving both positive and negative regulation of plant defense responses. Because both ALA and 9-LOX enzymes are present in most plants, it would be worth testing whether this anti-herbivore mechanism is conserved in other species. To investigate the involvement of JA-Ile in this crosstalk, it will be interesting to further explore the crosstalk between 9,10-KODA and JA-Ile in a plant model system where *coi1* mutants are available. It also needs to be determined whether the observed crosstalk is driven in part by a dual 9-/13-regiospecificity of ZmLOX5, an enzymatic property that has been described for other LOXs in different plant species. Nevertheless, irrespective of the exact mechanism, the study by Yuan *et al.* (2023) unveils a previously unknown plant defense signaling pathway that provides new opportunities for enhancing crop plant resistance to insect herbivory, particularly if this pathway is broadly conserved in other plant species.

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Figure 1: Model of ZmLOX5-mediated fatty acid-derived metabolism and signaling. Tonoplast-localized production of ALA-derived 9,10-KODA is positively regulated by the canonical jasmonate pathway. In turn, core JA-Ile-dependent jasmonate signaling is negatively regulated by 9,10-KODA via feedback loop inhibition, while upstream 12-OPDA production is induced. The potential of 9,10-KODA as an insecticide is also depicted. Abbreviations for compounds: ALA, α -linolenic acid; OPC-8:0, 3-oxo-2-(2-pentenyl)-cyclopentane-1-octanoic acid; HPOT, hydroperoxy-octadecatrienoic acid; 12-OPDA, *cis*-(+)-12-oxo-phytodienoic acid; JA, (+)-7-*iso*-jasmonic acid; JA-Ile, JA isoleucine conjugate. Abbreviations for enzymes: LOX, lipoxygenase; OPR, 12-OPDA reductase; AOS, allene oxide synthase; AOC, allene oxide cyclase; HPL, hydroperoxide lyase; JAR1, jasmonate resistant 1 (jasmonate-amidosynthetase). GLVs, green leaf volatiles. Hollow arrows denote translocation events. A dashed arrow indicates a hypothetical event. Arrows in bold indicate a positive (full arrow head) or negative (blunt arrow head) regulation postulated. Core signaling components described by Yuan et al. (2023) are highlighted in magenta color. This figure is adapted from (Wasternack and Feussner, 2018).

