Natural variability in jasmonic acid signaling among maize parental NAM lines

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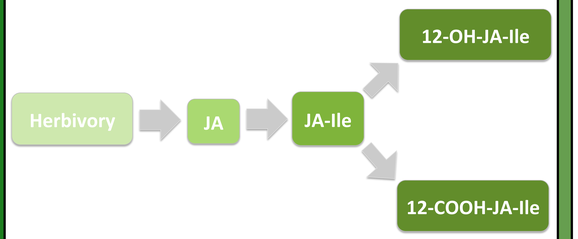
Introduction

The phytohormone jasmonic acid (JA) and its receptor-active derivative, jasmonoyl-L-isoleucine (JA-Ile), are key components in induced immunity against a variety of environmental stresses such as attack by insect herbivores, pathogens, and other forms of tissue damage. Induced immunity may be preferential to constitutive immunity due to its lower resource costs and potential for specialized response [1]. In a matter of hours after herbivory, the spike in JA-Ile levels is nearly completely metabolized into less active derivatives 12-OH-JA-Ile and 12-COOH-JA-Ile [2]. This targeted response allows plants to better balance growth-defense antagonism in order to optimize both growth and defense. Because JA is produced upon insect-induced damage, it is a key determinant in plant-insect interactions [3]. Our purpose was to quantify the natural variation in constitutive and herbivore-induced levels of JA and its derivatives among the founding maize Nested Associated Mapping (NAM) population lines [4].



Materials and methods

Herbivore induction
4 control and 4 herbivore-induced plants for each of the 26 parental NAM lines

Figure 1 experimental setup

Phytohormone extraction
Powdered leaves suspended in extraction buffer (ethyl acetate)

Creation of JA standards
Pure standard diluted to 100, 10, and 1 ng/µL

GC-MS Analysis
Integration of relevant peaks to determine peak area

Graphic analysis
See Figures 8 and 9

GC-MS analysis of experimental and control samples after phytohormone extraction

Peaks having the proper molecular ion and retention time for JA were found in many samples (see Figure 6). In addition to JA peaks, we looked for OH-JA-Ile peaks by filtering the chromatograms for molecular ion 339, as some lines may have rapidly metabolized the JA spike into OH-JA-Ile prior to the collection of tissue 3 hours after initial herbivore induction. As seen in Figure 6, the OH-JA-Ile had a consistent retention time of 23.5 minutes, and was ultimately visible in more samples than JA.

overall chromatogram
filtered for molecular ion 282
filtered for molecular ion 339

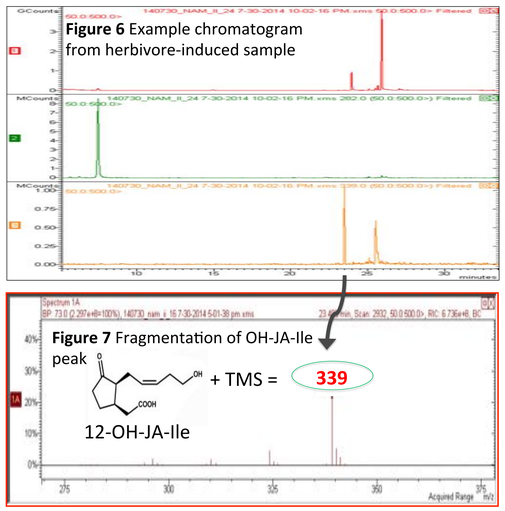


Figure 6 Example chromatogram from herbivore-induced sample

Figure 7 Fragmentation of OH-JA-Ile peak

+ TMS = 339

12-OH-JA-Ile

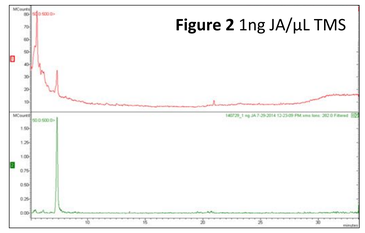


Figure 2 1ng JA/µL TMS

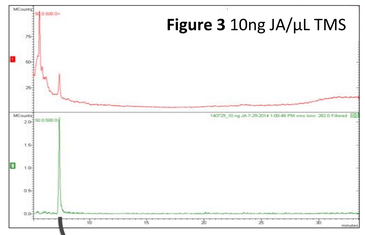


Figure 3 10ng JA/µL TMS

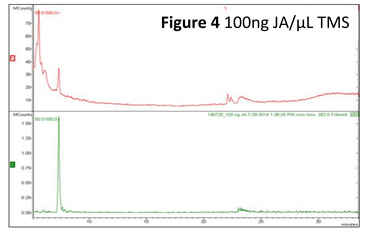


Figure 4 100ng JA/µL TMS

overall chromatogram
filtered for molecular ion 282

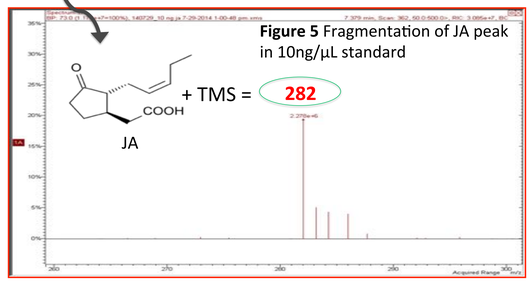


Figure 5 Fragmentation of JA peak in 10ng/µL standard

Gas chromatography-mass spectrometry (GC-MS) analysis of JA standards

Before experimentation began, we ran jasmonic acid standards with concentrations of 1, 10, and 100 ng/µL to test the machine's sensitivity to and retention time for the compound (Figures 2, 3, and 4, respectively). After trimethylsilyl (TMS) derivatization of the standards, the GC-MS was able to detect all three concentrations.

The chromatogram was filtered for molecular ion 282, TMS-derivatized JA. The samples were found to have a retention time of about 7.5 minutes, as shown by the consistency of the peaks in Figures 2, 3, and 4. In the fragmentation of the filtered chromatogram (Figure 5), the peak was shown to come from molecular ion 282, confirming the presence of JA.

Results

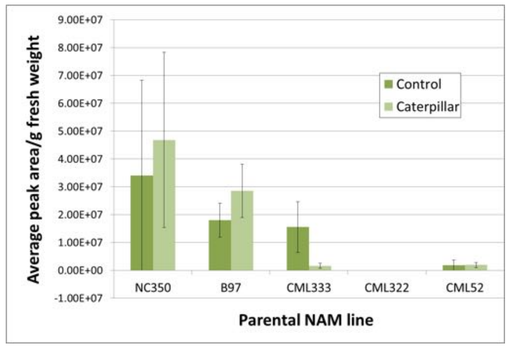


Figure 8 JA levels among 5 selected parental NAM lines

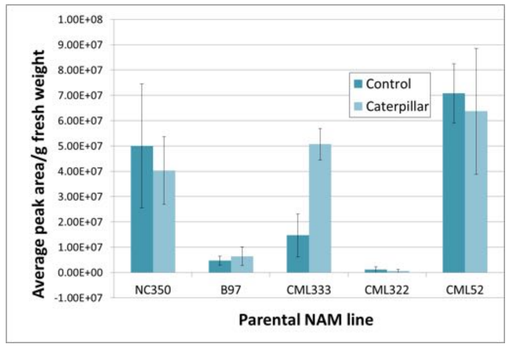


Figure 9 OH-JA-Ile levels among 5 selected parental NAM lines

Conclusions and perspectives

- We observed variability among the 5 selected parental NAM lines analyzed with respect to both JA levels and rate of metabolism to 12-OH-JA-Ile.
- The variability quantified here can be used as a starting point for pinpointing genes responsible for magnitude of JA signaling.
- The high presence of JA and OH-JA-Ile in some control lines, sometimes even surpassing that of the experimental, remains to be explored.

Literature

1. **Campos, M. *et al.* (2014)** Jasmonate-Triggered Plant Immunity: Review.
2. **Koo, A. *et al.* (2011)** Ctyochrome P450 CYP94B3 mediates catabolism and inactivation of the plant hormone jasmonoyl-L-isoleucine. *PNAS* 108(22): 9298-9303.
3. **Wasternack, C. and Hause, B. (2013)** Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. *Annals of Botany* 111: 1021-1058.
4. **Jianming, Y. *et al.* (2008)** Genetic Design and Statistical Power of Nested Association Mapping in Maize. *Genetics* 178: 539-551

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