Short Communication

Oil body-mediated defense against fungi: From tissues to ecology

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Abbreviations: α-DOX1, α-dioxygenase1; CLO3, caleosin3; 2-HOT, 2-hydroxy-octadecatrienoic acid; HPOT, hydroperoxyoctadecatrienoic acid; LTP3, lipid transfer protein 3; GPAT2, glycerol-3-phosphate acyltransferase 2, LOX, lipoxygenase

Abstract

Oil bodies are localized in the seed cells and leaf cells of many land plants. They have a passive function as storage organelles for lipids. We recently reported that the leaf oil body has an active function as a subcellular factory that produces an antifungal oxylipin during fungal infection in *Arabidopsis thaliana*. Here, we propose a model for oil body-mediated plant defense. Remarkably, senescent leaves develop oil bodies and accumulate α-dioxygenase1 (α-DOX1) and caleosin3 (CLO3) on the oil-body membrane, which catalyze the conversion of α-linolenic acid to the phytoalexin 2-hydroxy-octadecatrienoic acid (2-HOT). The model proposes that senescent leaves actively produce antifungal oxylipins and phytoalexins, and abscised leaves contain a mixture of antifungal compounds. In natural settings, the abscised leaves with antifungal compounds accumulate in leaf litter and function to protect healthy tissues and young plants from fungal infection. Plants might have evolved this ecological function for dead leaves.

Introduction

We recently reported evidence that leaf oil bodies produce the phytoalexin 2-hydroxy-octadecatrienoic acid (2-HOT) when challenged with fungal infection.[1] Proteomic analysis of leaf oil bodies from *Arabidopsis thaliana* identified α-dioxygenase1 (α-DOX1) and caleosin3 (CLO3) as leaf oil-body enzymes. These two enzyme activities were induced in the perilesional area of the infection site in response to infection with the pathogenic fungus *Colletotrichum higginsianum*. Therefore, oil bodies could have an active role in phytoalexin production in addition to a passive role in lipid storage. We found that α-DOX1 and CLO3 were strongly induced in senescent leaves. The 2-HOT content in senescent leaves was approximately 200 times greater than that in green leaves. These data indicate that senescent leaves actively produce 2-HOT, although cell death or apoptotic pathways have already been activated. Therefore, senescent leaves might have a functional role in plant defense. Here, we focus on a possible functional role for senescent leaves in plant defense mechanisms.

Leaf Oil Bodies Emerge during Senescence: Model for an Ecological Function of Dead Leaves

Senescence is a phenomenon in which tissues begin to die off. Senescent leaf tissues have yellow or red coloration due to nutrient recycling.[2] Cells in senescent *A. thaliana* leaves have higher expression of α-DOX1 and CLO3.[1] Leaf oil bodies were observed in senescent *A. thaliana* leaves, while they were hardly observed in young leaves (Fig. 1A). The number of oil bodies in the senescent leaves was approximately seven times more than that in the young leaves (Fig. 1B). These results indicate that the formation of leaf oil bodies is induced in senescent leaves. The induction of leaf oil bodies is correlated to increase of the 2-HOT contents in senescent leaves. Senescence also induces up-regulation of several genes and pathways involved in lipid metabolism, such as glycerolipid synthesis and β-oxidation.[3] Microarray data show that genes involved in lipid metabolism, such as *lipid transfer protein 3* (*LTP3*), *LTP4*, and *glycerol-3-phosphate acyltransferase 2* (*GPAT2*), are induced by senescence (Fig. 1C). These results suggest that lipid-related pathways have higher activity during senescence.

Why do plants accumulate high levels of the phytoalexin 2-HOT in senescent leaves? Senescent leaves actively produce the phytoalexin 2-HOT, undergo abscission, fall down to the surrounding environment, and ultimately accumulate on lower branches, shorter plants, or the

ground (Fig. 2A). The 2-HOT in abscised leaves has antifungal activity against fungi in the surrounding environment, and protects healthy tissues and susceptible young plants from fungal infection (Fig. 2A). We propose that dead leaves have a functional role in oil body-mediated plant defense. The two oil-body enzymes α-DOX1 and CLO3 cooperatively catalyze the conversion of α-linolenic acid to the phytoalexin 2-HOT (Fig. 2B).[1] Within the oil body, the substrate (α-linolenic acid) and the two localized enzymes (α-DOX1 and CLO3) could be in close enough proximity to drive catalysis of phytoalexin 2-HOT. Genes with homology to *CLO3* and *α-DOX1* were identified in numerous land plants (SALAD Database, http://salad.dna.affrc.go.jp/salad/),[4] suggesting that oil body-mediated defense is conserved in numerous land plants. Land plants might have evolved this defense-related ecological function for dead leaves.

Senescence leads to up-regulation of lipoxygenase genes (*LOX1*, *LOX3*, and *LOX4*) in *A. thaliana*.[5] Previous work shows that 9-lipoxygenses convert α-linolenic acid to 9-hydroperoxyoctadecatrienoic acid (9-HPOT),[6] whereas 13-lipoxygenses convert α-linolenic acid to 13-hydroperoxyoctadecatrienoic acid (13-HPOT).[6] These oxylipins (9-HPOT and 13-HPOT) have antifungal activities against *Phytophthora parasitica* and *Cladosporium herbarum*.[6] These results suggest that the antifungal oxylipins 9-HPOT and 13-HPOT could have functional roles in plant defense mediated by senescent leaves. We propose that these antifungal oxylipins in senescent leaves contribute to the defense-related ecological function against antifungal-oxylipin-susceptible fungi. In contrast, some species of fungi such as *Alternaria brassicicola* are resistant to the oxylipins,[6] suggesting that pathogenic fungi might have evolved a countermove against the antifungal oxylipins.

Abscised leaves might be utilized for applied biosciences and/or applied agronomy. Deciduous trees shed abundant quantities of abscised leaves, particularly before the onset of winter. These abscised leaves might contain phytoalexins and antifungal oxylipins. Large quantities of these antifungal compounds could potentially be isolated on an industrial scale from abscised leaves. Because the mixture of antifungal compounds could contain 2-HOT, 9-HPOT, 13-HPOT, and other oxylipins, it could have broad-spectrum antifungal activity against numerous agronomically important fungi.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Figure Legends

Figure 1. Lipid-related pathways in senescent leaves. (A) Micrographs of leaf oil bodies in young and senescent leaves. Young green leaves were from 2-week-old plants. Senescent leaves were from 5-week-old plants; aerial parts were removed and transferred to dark conditions for one week to generate senescent leaves. Both young and senescent leaves were vacuum-infiltrated for 5 min with 20 μg/ml of Nile-red stain in water. Nile-red fluorescence was observed using confocal laser scanning microscopy. Nile-red images (red) were merged with differential interference contrast (DIC) images. A leaf oil body is marked with the arrowhead. (B) Quantitative analysis of numbers of leaf oil bodies in cross-sections of confocal images of young and senescent leaves. The number of leaf oil bodies was counted in each micrograph (147.5 μm × 128.3 μm); five micrographs of young and senescent leaves were used each other (n = 5). Error bars show standard deviation. (C) Senescence-induced gene-expression patterns. Organ-specific gene-expression patterns were obtained from developmental data at AtGenExpress. The y-axis represents the absolute signal intensity value for gene expression. Average values from three independent experimental datasets are shown. AtGenExpress datasets used in this study were ATGE_17 for green leaf (green) and ATGE_25 for senescent leaf (yellow). Data analysis was performed using DART (http://dandelion.liveholonics.com/dart/index.php). *LTP3*, At5g59320; *LTP4*, At5g59310; and *GPAT2*, At1g02390.

Figure 2. Model of oil body-mediated plant defense. (A) Proposed ecological function of dead leaves in plant defense. Abscised leaves contain antifungal compounds and have an important role to protect healthy tissues and susceptible young plants from fungal infection. (B) Oil body-mediated defense protects healthy tissues, young plants, and seedlings from fungal infection. Oil bodies in senescing cells contain two enzymes (α-DOX1 and CLO3) and a substrate (α-linolenic acid); enzyme catalysis converts the substrate to the phytoalexin 2-HOT, which disrupts pathogenic fungal invasion and proliferation.

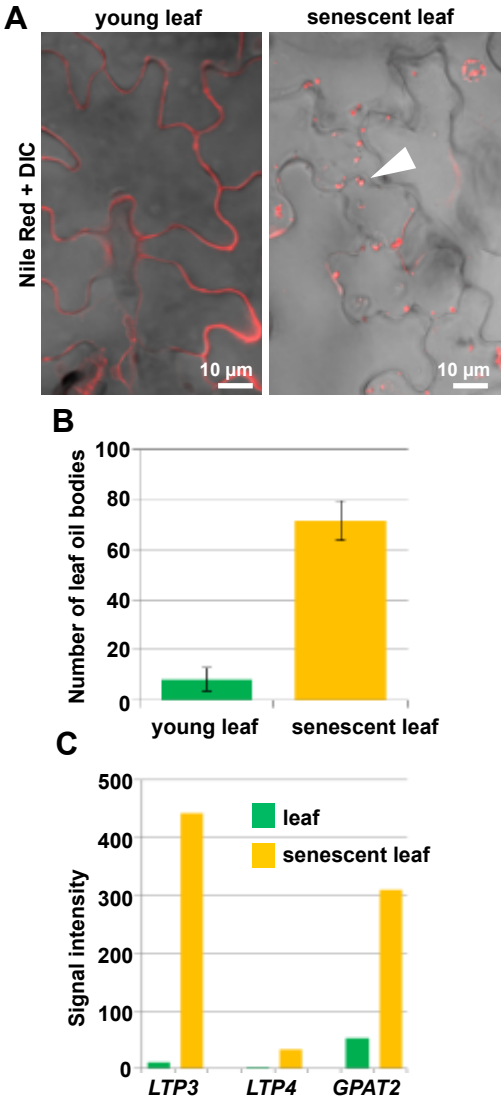
A
young leaf
senescent leaf
Nile Red + DIC
10 µm
10 µm
B
Number of leaf oil bodies
100
80
60
40
20
0
young leaf
senescent leaf
C
Signal intensity
500
400
300
200
100
0
leaf
senescent leaf
LTP3
LTP4
GPAT2

Figure 1

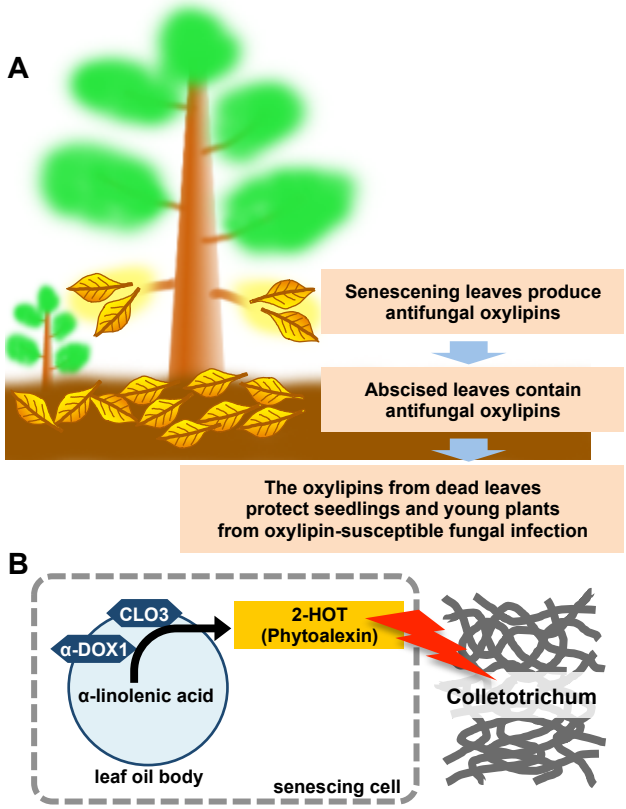
A
Senescening leaves produce antifungal oxylipins
Abscised leaves contain antifungal oxylipins
The oxylipins from dead leaves protect seedlings and young plants from oxylipin-susceptible fungal infection
B
CLO3
α-DOX1
α-linolenic acid
leaf oil body
2-HOT (Phytoalexin)
senescing cell
Colletotrichum

Figure 2