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Role of OsPLDα4 and α5 in rice defense responses

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The Chloroplast-Localized Phospholipases D α4 and α5 Regulate Herbivore-Induced Direct and Indirect Defenses in Rice

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ABSTRACT

The oxylipin-pathway is of central importance for plant defensive responses. Yet, the first step of the pathway, the liberation of linolenic acid (LeA) following induction, is poorly understood. Phospholipases D (PLDs) have been hypothesized to mediate this process, but data from Arabidopsis regarding the role of PLDs in plant resistance has remained controversial. Here we cloned two chloroplast-localized PLD genes from rice, OsPLDα4 and OsPLDα5, both of which were up-regulated in response to feeding by rice striped stem borer (SSB) Chilo suppressalis, mechanical wounding and treatment with jasmonic acid (JA). Antisense expression of OsPLDα4 and α5 (as- pld), which resulted in a 50% reduction of the expression of the two genes, reduced elicited levels of LeA, JA, green leaf volatiles (GLVs) and ethylene, and attenuated the SSB-induced expression of a mitogen-activated protein kinase (MAPK; OsMPK3), a lipoxygenase (OsHI-LOX), a hydroperoxide lyase (OsHPL3) as well as an ACC synthase (OsACS2). The impaired oxylipin and ethylene signaling in as- pld plants decreased the levels of herbivore-induced trypsin protease inhibitors (TrypPIs) and volatiles, improved the performance of SSB and rice brown planthopper (BPH) Nilaparvata lugens, and reduced the attractiveness of plants to a larval parasitoid of SSB, Apanteles chilonis. The production of TrypPIs in as- pld plants could be partially restored by JA, while the resistance to BPH and SSB was restored by GLV application. Our results show that phospholipases function as important components of herbivore-induced direct and indirect defenses in rice.
INTRODUCTION

Induced plant defenses play an important role in protecting plants from herbivore attack (Browse and Howe, 2008). Their activation depends on a complex signaling network with the oxylipin pathway at its center (Bostock, 2005; Browse and Howe, 2008; Howe and Jander, 2008). The biosynthesis of oxylipins starts with the release of linolenic acid (LeA) from chloroplast membranes. LeA is then oxygenated via 9- or 13-lipoxygenase (LOX) into hydroperoxy polyunsaturated fatty acids, which are the common substrates for at least seven different enzymes including allene oxide synthase (AOS) and hydroperoxide lyase (HPL, Feussner and Wasternack, 2002). The metabolites of the AOS and HPL branch of the pathway are jasmonates and green leaf volatiles (GLVs), both of which are important signals in plant defenses (Kessler and Baldwin, 2001; Shiojiri et al., 2006; Browse and Howe, 2008). Until today, the first crucial step in oxylipin biosynthesis and induction, the release of LeA from chloroplast membranes, is not well understood. Plant phospholipases D (PLDs) have received special attention as possible mediators of this reaction (Wang et al., 2000; Bargmann et al., 2009). PLDs cleave phospholipids into phosphatidic acid (PA) and free-head groups such as choline (Li et al., 2009; Hong et al., 2010). PA may then be converted to LeA (Ryu et al., 1998; Sang et al., 2001), thereby enabling an increase in jasmonate production. PLDs may also stimulate acylhydrolases or phospholipases A (PLA) activity, which may induce jasmonate signaling (Wang et al., 2000). In accordance with these two hypotheses, antisense expression of AtPLDα1 in Arabidopsis reduced PA levels, LOX activity, wound-induced JA levels and wound-induced gene expression (Wang et al., 2000). However, Bargmann et al. (2009) recently demonstrated that pldα1 and pldα1/δ double knockouts of Arabidopsis do not show any changes in wound-induced protein kinase activity, AtLOX2 gene expression, JA biosynthesis and resistance against the herbivore Pieris rapae. The authors attributed this surprising difference to the possibility that the antisense constructs used by Wang et al. (2000) may have suppressed other PLD isoforms in Arabidopsis.
PLDs are a large family of heterologous enzymes with a variety of functions. It has been reported that PLDs and their main product PA, because of their involvement in membrane degradation, vesicular transport, membrane tethering, signal transduction and hormone production or action, play important roles in plant growth, development and response to various abiotic and biotic stresses, including wounding and pathogen infection (Choi et al., 2005; Wang et al., 2006; Hong, 2008; Hong et al., 2009; Wan et al., 2009; Yamaguchi et al., 2009; Hong et al., 2010). Different PLDs, have distinct, but sometimes overlapping functions (Li et al., 2009). In Arabidopsis for example, both PLDα1 and α3 enhance plants osmotic stress responses, but they act through different mechanisms; PLDα1 mediates the ABA effect on stomatal movement to reduce water loss whereas PLDα3 promotes root growth (Hong et al., 2010). Several PLDs have been reported to be involved in plant defense responses. In rice, silencing OsPLDβ1 caused hypersensitive response-like cell death, phytoalexin production and increased plant resistance to pathogens (Yamaguchi, et al., 2009). Krinke et al (2009) found that PLD activation is an early component of the salicylic acid signaling pathway in Arabidopsis. PLDs in soybean activated a wound-activated MAPK (Lee et al., 2001), a homologue of which is responsible for the production of wound-induced JA, SA and ethylene in Nicotiana attenuata (Wu et al., 2007). AtPLDα1 finally positively regulated reactive oxygen species (ROS) generation, and was involved in ethylene signaling in Arabidopsis (Fan et al., 1997; Li et al., 2009; Zhang et al., 2009). In summary, while PLDs seem to be central to many plant regulatory processes, their role in the wound-response remains unclear. This is especially evident for PLDs in monocots, where nothing is known about their involvement in oxylipin mediated defenses against herbivores.

We therefore isolated two herbivore-induced rice PLD genes, OsPLDα4 and OsPLDα5, both of which localize in chloroplasts (McGee et al., 2003) and share 90% sequence identity. Using a reverse genetics approach, we obtained rice lines with reduced expression of OsPLDα4 and OsPLDα5 (as-PLD). We determined the role of
these two genes in herbivore induced defense by measuring the expression of several
oxylipin and ethylene biosynthesis-related genes, concentrations of phytohormones as
well as the direct and indirect resistance against a chewing herbivore, the striped stem
borer (SSB, *Chilo suppressalis*) and a phloem-feeder, the rice brown planthopper (BPH,
*Niaparvata lugens*). Our results provide evidence for the involvement of *OsPLDa4* and
*a5* in rice herbivore defense.
RESULTS

Isolation and characterization of rice OsPLDα4 and α5

By RT-PCR, we cloned the full-length cDNAs of two SSB-induced OsPLD genes. The two genes included an open reading frame of 2499 and 2529bp, both of which encode 832 amino acids with a predicted molecular mass of 92.39kDa and a pI of 6.183, and 842 amino acids with a calculated Mr of 93.19kDa and a theoretical pI of 6.148, respectively (Supplemental Fig. S1 and S2). Sequence alignment revealed high similarities (99% and 99%) of the two PLD genes to those of previously identified rice PLD genes (AK100278 and AK119861, Supplemental Fig. S1), suggesting that the cloned two OsPLD genes are OsPLDα4 (AK100278) and α5 (AK119861, Yamaguchi et al., 2009). Moreover, both of the two PLD genes share 90% identity in nucleotide sequence and 84% identity in amino acid sequence (Supplemental Fig. S2).

Quantitative RT-PCR (qRT-PCR) analysis of rice stems revealed low constitutive expression of OsPLDα4 and α5, while SSB caterpillar attack, mechanical wounding and JA treatment resulted in a rapid increase in transcript levels in infested or treated stems (Fig. 1). In contrast, five other tested rice PLDs, OsPLDα1, OsPLDα2, OsPLDα3, OsPLDη1 and OsPLDη2, did not change their expression profile after SSB infestation (Supplemental Fig. S6, A-E). BPH infestation or SA treatment did not induce transcript accumulation of OsPLDα4 and α5, except for the 24h harvest of BPH infestation, when transcript levels of OsPLDα5 in BPH treatment were higher than in non-infested treatment (Supplemental Fig. S3). Hence, OsPLDα4 and α5 share similar induced expression profiles and may be involved in JA-related insect responses in rice.

Antisense expression of OsPLDα4 and α5 reduces elicited levels of LeA as well as OsMPK3 mRNA, but not H2O2

As OsPLDα4 and α5 share high sequence identity, similar induced expression profiling and chloroplast localization, it is likely that they are functional homologues. Therefore, we co-silenced the two genes by Agrobacterium-based transformation (Supplemental Fig. S4) and obtained three independently transformed lines (L10-2, L10-6 and L10-22).
with a single insertion (Supplemental Fig. S5). qRT-PCR showed that expression of OsPLDα4 and α5 in SSB infested stems decreased markedly in two of the as- pld lines compared to identically treated WT plants; the levels of OsPLDα4 and OsPLDα5 mRNA in the two lines, L10-2 and L10-6, were 38.92-59.25% and 47.99%-61.46%, respectively, of those in WT plants 1 and 2 h after SSB infestation (Fig. 2A). The OsPLDα4 and α5 antisense construct did not co-silence the transcript accumulation of OsPLDα1, OsPLDα2, OsPLDα3, OsPLDη1 and OsPLDη2 (Supplemental Fig. S6, A-E), all of which share 66%-77% sequence similarity with OsPLDα4 (Supplemental Fig. S6, A-E). There was no obvious difference in growth phenotype between WT plants and as- pld lines during their whole development (Supplemental Fig. S5). For most of the following experiments, we compared two as- pld lines (L10-2 and L10-6) with untransformed plants.

The maximal accumulation of LeA occured 15 min after SSB infestation (Fig. 2B). Constitutive and SSB-induced LeA levels (15 min after infestation) in two as- pld lines were lower than those in WT plants (Fig. 2B). Antisense expression of OsPLDα4 and α5 had no influence on the SSB-induced expression levels of OsMPK6 (Supplemental Fig. S6F), but significantly reduced the expression of OsMPK3 less than 1h after SSB infestation (Fig. 2C). Basal H2O2 levels were significantly higher in WT plants than in as- pld lines. However, after BPH infestation for 3, 8 and 24 h, there was no difference in H2O2 levels between as- pld lines and WT plants any more (Supplemental Fig. S7).

OsPLDα4 and α5 influence JA, SA, ethylene and GLV biosynthesis

Phytohormone analysis showed that basal JA levels in the as- pld lines did not differ significantly from those of WT plants (Fig. 3A). However, JA levels increased more strongly in WT plants than in as- pld after infestation with SSB (Fig. 3A). Equally, there was no difference in constitutive SA levels between the as- pld lines and WT plants (Fig. 3E), but 3 h after SSB infestation, SA levels in as- pld lines were significantly higher than those in WT plants (Fig. 3E). Ethylene accumulation in as- pld plants was less than in WT plants 6-48 h after SSB infestation (Fig. 3C). Consistent with the results of JA
and ethylene, antisense expression of OsPLDα4 and α5 significantly decreased the
transcript levels of OsHI-LOX (starting <0.5h after infestation, Fig. 3B), a
13-lipoxygenase gene involved in herbivore-induced JA biosynthesis in rice (Zhou et al.,
2009) and OsACS2 (starting <1h after infestation, Fig. 3D), an ACC synthase gene that
is involved in herbivore-induced rice ethylene production (Lu et al., 2011).

We also analyzed the emission of GLVs in as- pld and WT plants. The levels of
constitutive (Z)-3-hexenal and (Z)-3-hexen-1-ol from as- pld lines (L10-2 and L10-6)
did not differ from those from WT plants (Fig. 3, F and G), but the wound-induced
emission of (Z)-3-hexenal and (Z)-3-hexen-1-ol from as- pld lines was significantly
lower than those from WT plants (Fig. 3, F and G). The reduction in expression levels
of OsHPL3, a wound induced hydroperoxide lyase that exclusively catalyzes the
cleavage of 13-hydroperoxy linolenic acid into GLVs (Chehab et al., 2006), was also
reduced in as- pld lines, albeit somewhat later, at 1 h and 2 h after herbivore infestation
(Fig. 3H).

**Antisense expression of OsPLDα4 and α5 reduces elicited TrypPI levels**
Constitutive TrypPI levels in as- pld lines did not differ from those in WT plants (Fig. 4,
A and B). However, SSB- or BPH-elicited TrypPI activity in as- pld lines L10-2 and
L10-6, measured 3 days after the start of herbivore infestation, was reduced compared
to WT plants (Fig. 4, A and B). Simultaneous JA treatment and SSB infestation induced
higher TrypPI levels in both WT and as- pld plants compared to BUF+SSB treatment.
However, the TrypPI levels in as- pld plants treated by JA+SSB were still lower than
those in identically treated WT plants (Fig. 4C), suggesting an important but not
exclusive role of the oxylipin pathway in the herbivore-induced production of TrypPI in
rice. Treatment by GLVs, (Z)-3-hexenal or (Z)-3-hexen-1-ol, had no effect on SSB
induced TrypPI levels (Fig. 4D), indicating that the reduced TrypPI levels in as- pld
lines were related to the reduced elicited JA levels rather than the reduced GLV levels.

**Antisense expression of OsPLDα4 and α5 reduces resistance against SSB and BPH**
SSB caterpillars gained more mass on as- pld lines than on WT plants (Fig. 5A).
Consistent with this finding, as-\textit{pld} plants were more severely damaged by SSB and survived less than WT plants did (Fig. 5D). When the different rice genotypes were exposed to a BPH colony on the other hand, BPH female adults were more often found on as-\textit{pld} lines than on WT plants (Fig. 6, A and B). Similarly, BPH female adults laid significantly more eggs on as-\textit{pld} lines than on WT plants (Fig. 6C). BPH nymphs also preferred to feed on as-\textit{pld} lines over WT plants (Fig. 6D and E) and BPH nymphs that fed on the as-\textit{pld} lines had higher survival rates than those that fed on WT plants (Fig. 6F). There were differences between the as-\textit{pld} lines (L10-2 and L10-6) and WT plants in terms of how they tolerated BPH infestation: as-\textit{pld} lines died more rapidly than WT plants when they were infested by BPH female adults (Fig. 6G). Fourteen days after infestation by 12 BPH female adults, as-\textit{pld} plants had completely wilted, whereas only the outer leaf sheaths showed necrosis in WT plants (Fig. 6G).

To determine if the improved performance of SSB could be due to the reduced GLV production and if the improved performance of BPH resulted from the decrease in GLVs or JA, we conducted a series of complementation experiments. Exogenous application of GLVs (containing (Z)-3-hexenal and (Z)-3-hexen-1-ol, each with 250 nmol per plant) on WT plants reduced the increases in SSB caterpillar mass (Fig. 5B). For BPH preference, application of JA on as-\textit{pld} plants did not alter the feeding and oviposition preference of BPH female adults for the as-\textit{pld} line L10-6 (Figs 6B and C, 7C and Supplemental Fig. S8J), whereas the preference of BPH female adults changed when GLVs were applied on as-\textit{pld} plants: BPH females gradually shifted their preference from as-\textit{pld} plants to WT plants when GLVs were applied in increasing doses (from 0 to 500 nmol GLVs) (Fig. 6, 7 and Supplemental Fig. S8), suggesting a repellent role of GLVs to BPH. When the as-\textit{pld} plants were individually supplemented with 125 or 250 nmol of (Z)-3-hexen-1-ol, BPH female adults preferred to settle and oviposit on WT plants (Fig. 7B and Supplemental Fig. S8D and E). Exogenous application of (Z)-3-hexenal on the as-\textit{pld} or WT plants did not have this repellent effect (Fig. 7B and C and Supplemental Fig. S8F-I). Hence, (Z)-3-hexen-1-ol supplementation
fully restored the feeding and oviposition preference of BPH female adults for the
as-\textit{pld} plants. These and our previous findings suggest that GLVs are involved in rice
resistance to SSB and BPH, and that \textit{OsPLDa4} and \textit{a5} increases the plant’s defense
capacity by stimulating their release.

\textbf{Antisense expression of \textit{OsPLDa4} and \textit{a5} decreases herbivore-induced volatiles
and their attraction of a larval parasitoid of SSB}

We collected and analyzed the volatiles emitted from WT and as-\textit{pld} plants that were
infested by SSB. The results show that SSB infestation significantly enhanced the
release of volatiles in both WT and as-\textit{pld} plants (Fig. 8, Table 1). While there was no
difference in constitutive volatile-release between WT plants and as-\textit{pld} plants, SSB
infested plants of as-\textit{pld} lines L10-2 or L10-6 emitted significantly lower amounts of
induced volatiles than WT plants. The emission of 14 compounds was significantly
reduced in as-\textit{pld} lines (Fig. 8, Table 1).

Consistent with the decrease in SSB-induced volatiles in as-\textit{pld} lines, the volatiles
emitted from infested as-\textit{pld} lines were less attractive to \textit{Apanteles chilonis}, a larval
parasitoid of SSB, than those from SSB-infested WT plants (Fig. 5C). This suggests that
\textit{OsPLDa4} and \textit{a5} participates in the indirect defense response in rice.
DISCUSSION

Here, we present evidence for the involvement of two PLDs in the wound- and insect-mediated induction of the oxylipin and ethylene pathways, and direct and indirect defenses of rice. Several lines of evidence support the hypothesis that OsPLDα4 and α5 play an important, specific role in the wound response. First, transcripts of OsPLDα4 and α5 increase after infestation with SSB caterpillars, wounding and JA, but not after treatment with SA and BPH (Fig. 1 and Supplemental Fig. S3). Second, antisense expression of OsPLDα4 and α5 decreases elicited levels of JA, ethylene and GLVs (Fig. 3), but not H2O2 (Supplemental Fig. S7). Third, OsPLDα4 and α5 are localized in chloroplasts (McGee et al., 2003) in which most steps of JA biosynthesis take place (Feussner and Wasternack, 2002). Although the role of AtPLDα1 in the wound-induced JA production in Arabidopsis has recently been debated (Bargmann et al., 2009), the authors could not exclude that other PLDs play important roles in this process. Since the antisense sequence that we used to inhibit expression levels of PLDα4 and α5 had no high similarity to other rice genes except for the ones tested here, we estimate the probability of co-silencing as very low. Our study thus clearly supports the notion that PLDs can have a central function in the wound response. Further studies will be required to elucidate if PLDs have a different role in monocotyledons or if they are universal triggering points of oxylipin biosynthesis in plants. Furthermore, the identification of insertion mutants of OsPLDα4 and OsPLDα5 will make it possible to assess if the two PLDs are indeed functionally redundant.

PLDs have been reported to be implicated in ethylene signaling (Pinhero et al., 2003; Testerink et al., 2007) and also have long been hypothesized to be involved in JA production by regulating LeA levels (Ryu and Wang, 1998), LOX activity (Wang et al., 2000) and MAPK signaling (Lee et al., 2001). Here we also found that antisense expression of OsPLDα4 and α5 decreases elicited levels of GLVs (Fig. 3). We therefore determined the changes in LeA concentrations and the expression levels of OsHI-LOX, OsHPL3, OsACS2 and two MAPK genes, OsMPK3 and OsMPK6, the homologues
(NaMAPK3 and NaMAPK6) of which are responsible for the production of
wound-induced JA, SA and ethylene in Nicotiana attenuata (Wu et al., 2007). Our
results show that antisense expression of OsPLDα4 and α5 did not influence the
expression levels of OsMPK6 (Supplemental Fig. S6F), but decreased the levels of
induced LeA and the expression levels of OsHI-LOX, OsMPK3, OsHPL3 and OsACS2
(Fig. 2, 3). The release of GLVs after wounding is transient, thus the decreases in levels
of GLVs in as- pld lines were not the result of the reduction of OsHPL transcript levels
that were significantly lower than those in WT plants 1 and 2h after SSB infestation
(Fig. 3). Therefore, the influence of OsPLDα4 and α5 on the oxylipin pathway may
occur via two routes: First, via influencing LeA, the common substrate for JA and GLV
biosynthesis, and second, via modulation of LOX activity and MAPK signaling, which
subsequently regulate the biosynthesis of JA and GLVs. The effect of OsPLDα4 and α5
on ethylene biosynthesis may occur mainly via regulating MAPK signaling, which in
turn affects ACS activity (Lee et al., 2001; Wu et al., 2007). In the future, it will be
important to measure actual MAPK activity in addition to gene expression to
substantiate this hypothesis.

Antisense expression of OsPLDα4 and α5 in rice decreased plant resistance against
SSB (Fig. 5, A and D). This coincided with impaired induced levels of TrypPIs (Fig. 4B)
and GLVs (Fig. 3), suggesting that the OsPLDα4 and α5 -mediated signal cascade plays
an important role in rice defenses, including TrypPI and GLV production. Both TrypPIs
(Zavala et al., 2004) and GLVs (Vancanneyt et al., 2001; Shiojiri et al., 2006; Chehab et
al., 2008) have been implicated in plant defense against herbivores. In rice, TrypPIs
have been reported to influence the larval performance of SSB (Zhou et al., 2009), and
here we found that GLVs ((Z)-3-hexenal and (Z)-3-hexen-1-ol) negatively affected the
growth of SSB caterpillars (Fig. 5B). Thus, the reduction of herbivore resistance in
as- pld rice plants can at least partially be explained by a decrease in induced TrypPI
activity and GLV levels. Similar to our previous results found on as-lox rice mutants
(Zhou et al., 2009), exogenous application of JA on as- pld plants also partially restored
the induced TrypPI accumulation, while GLVs did not (Fig. 4, C and D). This suggests that other herbivore-induced signals, in addition to OsPLDα4 and α5-derived JA, are involved in OsPLDα4 and α5-dependent TrypPI accumulation. Ethylene, for example, has been reported to be involved in the production of herbivore-induced rice TrypPIs (Wang et al., 2011). Given the obvious decrease in ethylene levels in as-pld lines (Fig. 3), ethylene may be one of these additional signals.

Similar to the larval performance of SSB, female adults or nymphs of BPH, a homopteran phloem feeder of rice, also showed a preference for settling, ovipositing and feeding on as-pld plants rather than on WT plants, and survived better on the former (Fig. 6). Moreover, as-pld plants died more rapidly than WT plants when infested with an equal number of BPH female adults (Fig. 6G). This finding contrasts with our previous results showing that as-lox plants, which had lower elicited JA levels than WT plants, were more resistant to BPH (Zhou et al., 2009). This difference may be related to the levels of H₂O₂ and GLVs. Compared to WT plants, as-lox plants had higher levels of BPH-induced H₂O₂ and equal GLV levels (Zhou et al., 2009), whereas as-pld plants had equal H₂O₂ levels (Supplemental Fig. S7) and lower elicited GLV levels (Fig. 3). The GLV (Z)-3-hexen-1-ol repelled BPH (Fig. 7B and Supplemental Fig. S8D and E), while JA had no obvious effect on BPH preference (Fig. 7C and Supplemental Fig. S8J). Therefore, the increased resistance to BPH in as-lox plants might be related to its higher BPH-induced H₂O₂ levels, which, together with elicited SA, induce a HR-like cell death that inhibits BPH feeding (Zhou et al., 2009), whereas the reduced resistance in as-pld lines may be the result of reduced (Z)-3-hexen-1-ol levels.

Apart from their direct effects, herbivore-induced plant volatiles (HIPVs) also attract natural enemies of the herbivores, thereby indirectly protecting plants (Kessler, 2004; Frost et al., 2008; Dicke, 2009). For the production of HIPVs, JA signaling plays a central role (Kessler, 2004; Dicke, 2009). Here we also found that as-pld plants, which had reduced elicited JA and ethylene levels compared to WT plants (Fig. 3A and C), released lower levels of induced volatiles than WT plants did (Fig. 8, Table 1), followed
by a reduced attractiveness to the larval parasitoid of SSB, *A. chilonis* (Fig. 5C).

Previous experiments have shown that exogenous application of JA on rice plants
induced volatile emission (Lou et al., 2005). Moreover, volatiles emitted from
SSB-infested plants were attractive to the parasitoid *A. chilonis* (Chen et al., 2002).
Together with these findings, our results demonstrate that *OsPLDα4* and *α5* are
involved in the SSB-induced, volatile-mediated rice indirect defense via increases of the
biosynthesis of JA.

In summary, *OsPLDα4* and *α5* can regulate the rice oxylipin and ethylene signaling
cascade, including JA, GLVs and ethylene, possibly by influencing the levels of LeA, as
well as the activity of LOX and ACS, and MAPK signaling, which, taken together, results
in an effective herbivore-induced direct and indirect defense response. Our study
provides a compelling example of how genes that act at the very beginning of the
wound- and herbivore-induced oxylipin and ethylene pathways can influence
plant-insect interactions up to the third trophic level.
MATERIALS AND METHODS

Plant growth

The rice genotypes used in this study were Xiushui 11 wild type (WT) and as-pled transgenic lines (see below). Pre-germinated seeds were cultured in plastic bottles (diameter 8 cm, height 10 cm) in a greenhouse (28±2°C, 14L: 10D). Ten-day-old seedlings were transferred to 50-L communal hydroponic boxes with a rice nutrient solution (Yoshida., et al 1976). After 30-35 days, seedlings were transferred to individual 500-ml hydroponic plastic pots, each pot with one or two plants (for two plants, one was WT plant and the other was as-pled transgenic line plant). Plants were used for experiments 4-5 days after transplanting.

Insects

Colonies of SSB and BPH were maintained on Shanyou 63 (a susceptible variety to SSB and BPH) rice seedlings using the same method as described in Zhou et al. (2009). Apanteles chilonis (Munakata) colonies were obtained from rice fields in Hangzhou, China, and maintained on SSB larva.

Generation and characterization of as-pled transgenic lines

A 1060 bp portion (Supplemental Fig. S1) of the OsPLDa4 cDNA present on plasmid pPLDa4 (see below) was PCR-amplified using the primers 5’-CAGTCGCTCGGCATCAAG-3’ and 5’-TTCCCAAGCAGAAGAAGGTG -3’, which has 91% sequence similarity with OsPLDα5, and then was cloned into pCAMBIA1301, yielding an antisense transformation vector (Supplemental Fig. S4). The vector was inserted into the rice variety Xiushui 11 using Agrobacterium tumefaciens-mediated transformation. The procedure of rice transformation, screening of the homozygous T2 plants and identification of the number of insertions followed the same method as described in Zhou et al. (2009). For most experiments, two T2 homozygous lines, L10-2 and L10-6, each harboring a single insertion (Supplemental Fig. S5), and WT plants were used.

Plant treatments
Pots with one plant were used for this experiment. For SSB treatment, plants were individually infested using a third-instar larva of SSB that had been starved for 2 h. Control plants (Con) were left herbivore-free. For BPH treatment, plants were individually infested with 15 gravid BPH females that were confined in a glass cylinder (diameter 4 cm, height 8 cm, with 48 small holes (diameter 0.8 mm)). One empty cylinder was attached to control plants (non-infested). For mechanical wounding, plants were individually damaged using a needle on the lower part of rice stems (about 2 cm long), with 200 holes (W). Control plants (Con) were not pierced. The method for JA and SA treatment was the same as described in Zhou et al. (2009). Plants were individually sprayed with 2 ml of JA (100μg ml⁻¹) or SA (70μg ml⁻¹) in 50 mM sodium phosphate buffer. Control plants were sprayed with 2 ml of buffer (BUF). For GLV treatment, plants from each genotype were individually treated with 125-500 nmol of (Z)-3-hexenal or (Z)-3-hexenol in lanolin paste (see details for each experiment) on stems. Control plants received the same volume of pure lanoline paste (Lanolin).

Isolation of the full-length cDNA of OsPLDα4 and OsPLDα5

Full-length cDNA of a SSB-induced OsPLDα4 and α5 were obtained by RT-PCR from total RNA isolated from WT plants infested by an SSB larva for 24 h. The primers PLDα4-F (5' - GTGCTTCTTTCTCCCGTTCTT -3'), PLDα4-R (5' - GCAGGTGCATACTCATCATTAC -3'), PLDα5-F (5' - ATGTGTTTCTGAGCTTCTTCC -3'), PLDα5-R (5' - CGATGAACATATCATTAGA -3') were designed based on the sequence of the rice phospholipase D genes (accession number AK100278 and AK119861, belong to OsPLDα4 and α5 (Yamaguchi et al., 2009)), which have high homology with the partial sequences of the two OsPLD that were cloned by suppression subtractive hybridization (SSH) (G. Z. and Y.L., unpublished data). The PCR products were cloned into pMD19-T vector (Takara, http://www.takara.com.cn/) (pPLD) and sequenced.

qRT-PCR

For QRT-PCR analysis, five independent biological samples were used. Total RNA was
isolated using the SV Total RNA Isolation System (Promega). Fifty ng of each total RNA sample was reverse transcribed using the PrimeScript™ RT-PCR Kit (TaKaRa). qRT-PCR was performed on an ABI PRISM 7500 sequence detection system (Applied Biosystems) with OsACT RNA for normalization. The primers, Taqman probe sequences used for Taqman qRT-PCR (Premix Ex Taq™ Kit, TaKaRa), and primer sequences for SYBR green-based qRT-PCR (SYBR premix ExTaq, Takara) are shown in Table S1. The relative expression levels of the target genes were determined using standard curves (Livak, 1997).

**JA, SA, ethylene and GLV analysis**

Plants (one plant per pot) were randomly assigned to SSB and control treatment. Two or three as-ald lines (L10-2, L10-6, L10-22) and one WT line were used. The stems were harvested at 0, 1.5 and 3 h after SSB treatment, and JA and SA levels were analyzed by GC-MS using labeled internal standards as described by Lou and Baldwin (2003). Plants were individually covered with a sealed glass cylinder (diameter 4 cm, height 50 cm) and ethylene production was determined using the same method as described by Lu et al. (2006). Each treatment at each time interval was replicated 5-6 times.

GLV emissions [(Z)-3-hexenal and (Z)-3-hexen-1-ol] were analyzed with a portable gas analyzer (zNose™4200, Electronic Sensor Technology, [http://www.estcal.com](http://www.estcal.com)) using the same method as described by Zhou (2009). Five (controls) and thirteen (wounding) replicates were carried out for each genotype (L10-2, L10-6 and one WT line).

**Quantification of hydrogen peroxide**

WT plants and plants of as-ald lines L10-2 and L10-6 were randomly assigned to BPH and control treatment. Leaf sheaths were harvested at 0, 3, 8 and 24 h after treatment. Each treatment at each time interval was replicated 5 times. H₂O₂ concentrations were determined as described by Lou and Baldwin (2006).

**LeA Analysis**

We compared LeA levels in two as-ald lines and one WT line at 0, 15, 30, 60 and 120
min after SSB treatment. For each treatment and each time interval, stems (0.15g) of five plants were sampled. LeA was extracted for GC-MS analysis, which was similar to the method as described by Qu et al. (2006) with a small modification. Samples were individually added with 10μL of ethyl decanoate (2000ppm) as internal standards before LeA extraction, and the extracts were derivatized with diazomethane. Quantification of LeA in samples was achieved according to a stand curve that was obtained by authentic standard LeA.

TrypPI analysis
Plants (one plant per pot) from each line (L10-2, L10-6 and WT line) were randomly assigned to nine groups (see Fig. 4). For JA+ SSB, and BUF+ SSB treatment, the plants were treated with either JA or the buffer for 1 day, followed by infestation of SSB third-instar larvae (one larva per plant); for (Z)-3-hexenal+SSB, (Z)-3-hexen-1-ol+ SSB and lanolin+ SSB treatment, the plants were treated with 250 nmol of either (Z)-3-hexenal, (Z)-3-hexen-1-ol or lanolin, immediately followed by infestation of SSB third-instar larvae (one larva per plant). Stems (0.12-0.15 g per sample) were harvested 3 days after the start of herbivore infestation. The TrypPI concentrations were measured using a radial diffusion assay as described by van Dam et al. (2001). Each treatment at each time interval was replicated five times.

Collection, isolation and identification of rice volatiles
The collection, isolation and identification of rice volatile was used the same method as described in Lou et al (2005). Volatiles emitted from individual plants (one plant per pot) of each line (L10-2, L10-6 and WT line) that were infested with SSB for 24 h or non-manipulated were collected. Collections were replicated five times for each treatment. The compounds were expressed as percentage of peak areas relative to the internal standard (IS) per 8 h of trapping one plant.

Olfactometer bioassays
Responses of A. chilonis (Munakata) females to rice volatiles were measured in a Y-tube olfactometer using the same method as described by Lou et al. (2005). The
behavioral response of the parasitoid exposed to the following pairs of odor sources was observed: SSB-infested plants of each as-\textit{pld} line (L10-2, L10-6) vs. SSB-infested WT plants (infestation for 24 h); control plants of each line vs. control WT plants. For each treatment, 10 plants were used, and the odor sources were replaced by a new set of 10 plants after testing 8 wasps. For each odor source combination, a total of 48 females were tested.

**Herbivory experiments**

Seven-day-old larvae of SSB, which had been weighed and starved for 2 h, were placed individually on each plant of the two as-\textit{pld} lines (L10-2, L10-6) and the WT line. To test the effect of GLVs on the larval performance of SSB, the larvae were placed individually on each WT plant that were treated with either GLVs (10 $\mu$L lanolin containing (Z)-3-hexenal and (Z)-3-hexen-1-ol, with 250 nmol each) or Lanolin only (10 $\mu$L lanolin). Twenty-four to 30 replicate plants from each line and treatment were used. Larval mass (to an accuracy of 0.1 mg) was measured 7 days after the start of the experiment and the increased percentages of larval mass on each line or treatment were calculated.

To determine the colonization and oviposition preference of BPH, pots with two plants (an as-\textit{pld} line plant versus a WT plant) were confined with glass cylinders into which 15 gravid adult BPH females or nymphs were introduced. In complementation experiments, transgenic line L10-6 and WT plants were used; the colonization and oviposition preferences of BPH female adults were determined for 11 pairs of plants (see details in Fig. 7). The number of BPH at different time and BPH eggs at 48h on each plant were counted followed the method as described in Zhou et al. (2009). The experiment was repeated 5-6 times.

The survival rates of BPH nymphs on WT and as-\textit{pld} plants were recorded 6 days after the introduction of the herbivore using the method as described in Zhou et al. (2009). The experiment was repeated 8 times.

The differences in plant tolerance to herbivore attack between WT and as-\textit{pld}
plants were determined following the method as described in Zhou et al. (2009).

**Data analysis**

Differences in SSB-induced JA, SA, ethylene, LeA and volatiles, TrypPIs, and GLVs as well as expression levels of *OsHI-LOX, OsACS2* and *OsHPL3* were analyzed by one-way ANOVA, if the ANOVA analysis was significant (*P*<0.05), Duncan’s multiple range tests was used to detect significant differences between groups. Differences in attractiveness of HIPVs to the parasitoid between lines were tested by $\chi^2$. Differences in experiments using two treatments were determined by Student’s *t*-tests. Data were analyzed with Statistica (Statistica, SAS Institute Inc., Cary, NC, USA).

**ACKNOWLEDGMENTS**

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LITERATURE CITED


allopolyplploid *Nicotiana* host plants. Proc. Natl. Acad. Sci. USA **100**: 14581-14586


Supplemental Data

The following materials are available in the online version of this article.

Supplemental Table S1. Primers and probes used for QRT-PCR of target genes.

Supplemental Figure S1 cDNA sequence alignment of the cloned two PLD genes (OsPLD1 and OsPLD2), AK100278 (OsPLDα4) and AK119861 (OsPLDα5).

Supplemental Figure S2 Alignment of deduced amino acid sequences of the cloned two PLD genes, OsPLD1 and OsPLD2.

Supplemental Figure S3. Mean expression levels (relative to expression levels of OsACT)(+SE, n=5) of OsPLDα4 and α5 in rice stems after different treatments.

Supplemental Figure S4. Rice transformation vector pCAMBIA-PLD (13.7 kb) with hgh and gus as plant selectable marker genes.

Supplemental Figure S5. DNA gel-blot analysis and growth phenotypes of as-PLD lines and WT plants.

Supplemental Figure S6. Mean expression levels (relative to expression levels of OsACT)(+SE, n=5) of OsPLDα1, OsPLDα2, OsPLDα3, OsPLDη1, OsPLDη2 and OsMPK6 in stems of WT plants and two as-PLD lines at different time after SSB infestation.

Supplemental Figure S7. Mean H₂O₂ concentrations (+SE, n=5) in as-PLD lines L10-2 and L10-6 and WT plants at various times after plants were individually infested with 15 brown planthopper female adults (BPH) or not infested.

Supplemental Figure S8. Mean number of BPH female adults per plant (+SE, n=5) on pairs of plants with different treatments, 48 h after 5 replicated plant pairs were exposed to 15 female adults.
**Figure legends**

**Figure 1.** Mean expression levels (relative to expression levels of OsACT, +SE, n=5) of OsPLDα4 and α5 in rice stems after different treatments. (A, D) SSB, rice stripped stem borer; (B, E) W, mechanical wounding; (C, F) JA, jasmonic acid; BUF, sodium phosphate buffer. Asterisks indicate significant differences between treatments and controls (0h time point for A, B, D and E; BUF for C and F; *, P < 0.05; **, P < 0.01; Student’s t-test).

**Figure 2.** Mean expression levels (relative to expression levels of OsACT, +SE, n=5) of OsPLDα4 and OsPLDα5 (A) and OSMPK3 (C) and LeA levels (+SE, n=5) (B) in WT plants and as-plit lines at different time points after SSB infestation. Letters indicate significant differences between lines within one time point (P < 0.05, Duncan’s multiple-range test). Asterisks indicate significant differences between treatments and controls (0 h time point) within one line (*, P < 0.05; **, P < 0.01; Student’s t-test).

**Figure 3.** Levels of JA, ethylene, SA, (Z)-3-hexenal, (Z)-3-hexen-1-ol and expression levels of OsHI-LOX, OsACS2 and OSHPL3 in as-plit lines and WT plants elicited by various treatments. (A, E) Mean levels (+SE, n=5) of JA and SA levels in stems of three as-plit lines and WT plants, 0, 1.5 and 3 h after infestation by SSB. (C) Mean levels (+SE, n=6) of ethylene in two as-plit lines and WT plants that were individually infested by a third-instar SSB larva. (F, G) Mean levels (+SE) of (Z)-3-hexenal and (Z)-3-hexan-1-ol (peak area/mg fresh mass) in leaves of two as-plit lines and WT plants before (Con, n=5) and immediately after leaves were cut into small pieces (W, n=13). (B, D, H) Mean expression levels (relative to expression levels of OsACT, +SE, n=5) of OsHI-LOX (B), OsACS2 (D) and OSHPL3 (H) in stems of three as-plit lines and WT plants, 0, 0.5, 1 and 2 h after infestation by SSB. Letters in (G) and (F) indicate significant differences between treatments. In the other graphs, letters indicate significant differences between lines within time points (P < 0.05, Duncan’s multiple-range test). Asterisks indicate significant differences between treatments and controls (0 h time point) within one line (*, P < 0.05; **, P < 0.01; Student’s t-test).

**Figure 4.** Mean TrypPI levels (+SE, n=5) in as-plit lines and WT plants elicited by various treatments. (A) Stems of two as-plit lines and WT plants 3 days after plants were individually infested by 15 female BPH adults (BPH) or not infested. (B) Stems of two as-plit lines and WT plants 3 days after plants were individually infested by a
third-instar SSB larva (SSB) or kept untreated (Con). (C) Stems of two as-<i>pld</i> lines and
WT plants, which were first individually sprayed with 2 ml of either JA (100μg ml<sup>-1</sup>)
(SSB+JA) or the sodium phosphate buffer (SSB+BUF) for 1 day, followed by
third-instar SSB larva infestation for 3 days. (D) Stems of two as-<i>pld</i> lines and WT
plants, which were first individually treated with 250 nmol of either (Z)-3-hexenal
(SSB+HAL), (Z)-3-hexenol (SSB+HOL) or lanolin (SSB+Lan), followed by third-instar
SSB larva infestation for 3 days. Letters indicate significant differences among
treatments (A-C) or among lines within the same treatment (D, <i>P</i> < 0.05, Duncan’s
multiple range test).

**Figure 5.** Direct and indirect resistance of WT plants and as-<i>pld</i> Lines to SSB. (A, B)
Mean increased mass (% +SE) of individual seven-day-old SSB larvae fed on WT
plants and as-<i>pld</i> lines L10-2 and L10-6 (A, n=24), or WT plants that were treated with
lanolin or GLVs (B, n=30), 7 days after the larvae were placed on plants. (C) Number of
<i>A. chilonis</i> attracted by volatiles released from either non-manipulated plants (Con) or
plants infested by SSB for 24 h (SSB)(n=48): WT vs. L10-2, and WT vs. L10-6. (D)
Damaged phenotypes of as-<i>pld</i> lines L10-2, L10-6 and WT plants that were individually
infested with a SSB third-<i>pld</i> larva for 9 days. Letters indicate significant differences
among lines (A) or treatments (B, <i>P</i> < 0.05, Duncan’s multiple range test). Asterisks
indicate significant differences between odor sources (*, <i>P</i> < 0.05; **, <i>P</i> < 0.01; χ<sup>2</sup>
-test).

**Figure 6.** BPH performance on as-<i>pld</i> lines L10-2 and L10-6 and WT plants, and
tolerance to BPH infestation. (A, B, D, E) Mean number (+SE, n=5-6) of BPH female
adults (A, B) or nymphs (D, E) per plant on pairs of plants, L10-2 versus WT and L10-6
versus WT at different time points. (C) Mean percentage (+SE, n=5-6) of BPH eggs per
plant on pairs of plants as stated above, 48 h after the release of the insects. (F) Mean
survival rate (+SE, n=8) of BPH nymphs on as-<i>pld</i> lines L10-2 and L10-6 and WT
plants, 6 day after the release of the insects. (G) Damage phenotypes of as-<i>pld</i> lines
L10-2, L10-6 and WT plants after they were infested by 12 BPH female adults for 14
days. Asterisks indicate significant differences between pairs of lines (A-E, *, <i>P</i> < 0.05;
**, <i>P</i> < 0.01; Student’s t-test). Letters indicate significant differences between lines (F, <i>P</i>
< 0.05, Duncan’s multiple range test).

**Figure 7.** Mean percentage (+SE, n=5) of BPH eggs per plant on pairs of plants 48 h
after exposure to different treatments: (A) WT lanolin treatment vs. L10-6 GLV
treatment (containing (Z)-3-hexenal and (Z)-3-hexenol, each with 0, 125, 250 or 500
nmol); (B) WT lanolin treatment vs. L10-6 GLV treatment (125 or 250 nmol of
(Z)-3-hexenal or (Z)-3-hexenol); (C) WT Lanolin treatment vs. WT GLV treatment (125
or 250 nmol of (Z)-3-hexenal) and WT BUF (sodium phosphate buffer) treatment vs.
L10-6 JA treatment. Asterisks indicate significant differences between pairs of lines (*,
$P < 0.05$; **, $P < 0.01$; Student’s t-test).

**Figure 8.** Typical chromatograms obtained by headspace collections from
non-manipulated Plants (Con) and SSB-infested plants (for 24 h) (SSB) of WT plants
and as-tpld Lines L10-2 and L10-6. Numbers represent chemicals that are the same as in
Table 1.
Table 1. Volatile compounds emitted by WT plants and as-Pla lines L10-2 and L10-6 by heath plants (Con) and plants infested by striped stem borer for 24 h (SSB). Data represent the mean amount (% of internal standard peak area) of five replications. Letters in a same row indicate significant differences among treatments (P < 0.05, Duncan’s multiple range test).

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</tbody>
</table>
Figure 1. Mean expression levels (relative to expression levels of OsACT, +SE, n=5) of OsPLDα4 and α5 in rice stems after different treatments. (A, D) SSB, rice stripped stem borer; (B, E) W, mechanical wounding; (C, F) JA, jasmonic acid; BUF, sodium phosphate buffer. Asterisks indicate significant differences between treatments and controls (0h time point for A, B, D and E; BUF for C and F; *, P < 0.05; **, P < 0.01; Student’s t-test).
Figure 2. Mean expression levels (relative to expression levels of OsACT, +SE, n=5) of OsPLDα4 and OsPLDα5 (A) and OsMPK3 (C) and LeA levels (+SE, n=5) (B) in WT plants and as-plt lines at different time points after SSB infestation. Letters indicate significant differences between lines within one time point ($P < 0.05$, Duncan’s multiple-range test). Asterisks indicate significant differences between treatments and controls (0 h time point) within one line (*, $P < 0.05$; **, $P < 0.01$; Student’s t-test).
Figure 3. Levels of JA, ethylene, SA, (Z)-3-hexenal, (Z)-3-hexen-1-ol and expression levels of OsHI-LOX, OsACS2 and OSHPL3 in as-pld lines and WT plants elicited by various treatments. (A, E) Mean levels (+SE, n=5) of JA and SA levels in stems of three as-pld lines and WT plants, 0, 1.5 and 3 h after infestation by SSB. (C) Mean levels (+SE, n=6) of ethylene in two as-pld lines and WT plants that were individually
infested by a third-instar SSB larva. (F, G) Mean levels (+SE) of (Z)-3-hexenal and (Z)-3-hexan-1-ol (peak area/mg fresh mass) in leaves of two as-pld lines and WT plants before (Con, n=5) and immediately after leaves were cut into small pieces (W, n=13). (B, D, H) Mean expression levels (relative to expression levels of OsACT, +SE, n=5) of OsHI-LOX (B), OsACS2 (D) and OSHPL3 (H) in stems of three as-pld lines and WT plants, 0, 0.5, 1 and 2 h after infestation by SSB. Letters in (G) and (F) indicate significant differences between treatments. In the other graphs, letters indicate significant differences between lines within time points (P < 0.05, Duncan’s multiple-range test). Asterisks indicate significant differences between treatments and controls (0 h time point) within one line (*, P < 0.05; **, P < 0.01; Student’s t-test).
Figure 4. Mean TrypPI levels (+SE, n=5) in as-ple lines and WT plants elicited by various treatments. (A) Stems of two as-ple lines and WT plants 3 days after plants were individually infested by 15 female BPH adults (BPH) or not infested. (B) Stems of two as-ple lines and WT plants 3 days after plants were individually infested by a third-instar SSB larva (SSB) or kept untreated (Con). (C) Stems of two as-ple lines and WT plants, which were first individually sprayed with 2 ml of either JA (100μg ml⁻¹) (SSB+JA) or the sodium phosphate buffer (SSB+BUF) for 1 day, followed by third-instar SSB larva infestation for 3 days. (D) Stems of two as-ple lines and WT plants, which were first individually treated with 250 nmol of either (Z)-3-hexenal (SSB+HAL), (Z)-3-hexenol (SSB+HOL) or lanolin (SSB+Lan), followed by third-instar SSB larva infestation for 3 days. Letters indicate significant differences among treatments (A-C) or among lines within the same treatment (D, P < 0.05, Duncan’s multiple range test).
Figure 5. Direct and indirect resistance of WT plants and as-�د Lines to SSB. (A, B) Mean increased mass (%, +SE) of individual seven-day-old SSB larvae fed on WT plants and as-�د lines L10-2 and L10-6 (A, n=24), or WT plants that were treated with lanolin or GLVs (B, n=30), 7 days after the larvae were placed on plants. (C) Number of A. chilonis attracted by volatiles released from either non-manipulated plants (Con) or plants infested by SSB for 24 h (SSB)(n=48): WT vs. L10-2, and WT vs. L10-6. (D) Damaged phenotypes of as-�د lines L10-2, L10-6 and WT plants that were individually infested with a SSB third-instar larva for 9 days. Letters indicate significant differences among lines (A) or treatments (B, $P < 0.05$, Duncan’s multiple range test). Asterisks indicate significant differences between odor sources (*, $P < 0.05$; **, $P < 0.01$; $\chi^2$-test).
Figure 6. BPH performance on as- pld lines L10-2 and L10-6 and WT plants, and tolerance to BPH infestation. (A, B, D, E) Mean number (+SE, n=5-6) of BPH female adults (A, B) or nymphs (D, E) per plant on pairs of plants, L10-2 versus WT and L10-6 versus WT at different time points. (C) Mean percentage (+SE, n=5-6) of BPH eggs per plant on pairs of plants as stated above, 48 h after the release of the insects. (F) Mean survival rate (+SE, n=8) of BPH nymphs on as- pld lines L10-2 and L10-6 and WT plants, 6 day after the release of the insects. (G) Damage phenotypes of
as-pld lines L10-2, L10-6 and WT plants after they were infested by 12 BPH female adults for 14 days. Asterisks indicate significant differences between pairs of lines (A-E, *, $P < 0.05$; **, $P < 0.01$; Student’s t-test). Letters indicate significant differences between lines (F, $P < 0.05$, Duncan’s multiple range test).
Figure 7. Mean percentage (+SE, n=5) of BPH eggs per plant on pairs of plants 48 h after exposure to different treatments: (A) WT lanolin treatment vs. L10-6 GLV treatment (containing (Z)-3-hexenal and (Z)-3-hexenol, each with 0, 125, 250 or 500 nmol); (B) WT lanolin treatment vs. L10-6 GLV treatment (125 or 250 nmol of (Z)-3-hexenal or (Z)-3-hexenol); (C) WT Lanolin treatment vs. WT GLV treatment (125 or 250 nmol of (Z)-3-hexenal) and WT BUF (sodium phosphate buffer) treatment vs. L10-6 JA treatment. Asterisks indicate significant differences between pairs of lines (*, $P < 0.05$; **, $P < 0.01$; Student’s t-test).
Figure 8. Typical chromatograms obtained by headspace collections from non-manipulated Plants (Con) and SSB-infested plants (for 24 h) (SSB) of WT plants and as-pld Lines L10-2 and L10-6. Numbers represent chemicals that are the same as in Table 1.