A cell wall degrading enzyme and its putative receptor/co-receptor activate rice and Arabidopsis immune responses via different signalling pathways

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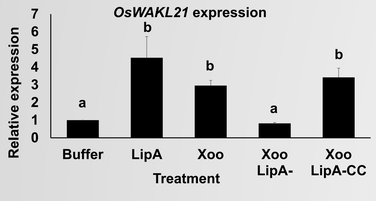
Abstract

Plant pathogens secrete various cell wall degrading enzymes (CWDE) to degrade different components of the plant cell wall. Plants can perceive damage caused by these CWDE and mount immune responses (IRs). But how do plants perceive it and mount immunity is not well explored. Lipase/esterase (LipA) is a CWDE that is secreted by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the bacterial blight pathogen of rice. Treatment of rice or Arabidopsis tissue with LipA or LipA treated cell wall extract induces IRs (1, 2). Using transcriptome analysis, we identified a rice receptor kinase *OsWAKL21* which is upregulated following treatment of rice leaves with LipA or *Xoo* but not after treatment of LipA mutant of *Xoo*. Downregulation of *OsWAKL21* in rice attenuated LipA induced IRs. Overexpression of *OsWAKL21* in rice mimics LipA treatment in terms of IRs. Ectopic expression of OsWAKL21 in Arabidopsis also induces similar IRs. OsWAKL21 is a moonlighting kinase that has kinase and guanylate cyclase (GC) activities. Interestingly, kinase activity of OsWAKL21 is required for induction of rice IRs while in Arabidopsis it needs its GC activity. The expression profile of defence genes and experiments with mutant Arabidopsis lines indicates LipA treatment or overexpression of OsWAKL21 induces JA pathway in rice while it acts via SA pathway in Arabidopsis. Thus, our results indicate that LipA and its putative receptor/co-receptor OsWAKL21 activate rice and Arabidopsis immunity via two different signaling pathways (3).

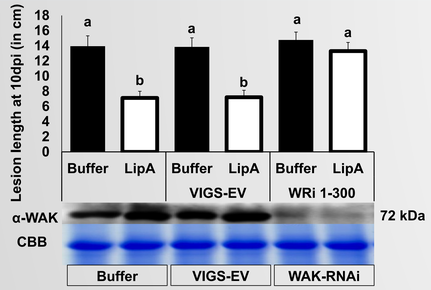
Key findings

1. Damage caused by *Xoo* secreted LipA induces expression of rice Wall Associated Receptor Kinase (OsWAKL21)



- Previously a microarray analysis indicated OsWAKL21 is upregulated after 2hr and 12hr of treatment of rice leaves with LipA.
- qRT-PCR analysis indicates *OsWAKL21* is upregulated following treatment of rice leaves with LipA or *Xoo* but not after treatment of LipA mutant of *Xoo*.
- *OsWAKL21* is also upregulated following treatment of rice tissue with cell wall degradation products released after LipA treatment.

2. OsWAKL21 is a key signaling intermediate for perception of LipA induced cell wall damage in rice



- Virus induced gene silencing mediated transient downregulation of *OsWAKL21* in rice leaves attenuates LipA induced immune responses such as
  - Enhanced tolerance against subsequent *Xoo* infection (as shown in figure)
  - Enhanced callose deposition

3. OsWAKL21 is a moonlighting kinase exhibiting two biochemical activities

Purified kinase domain of OsWAKL21 shows *in-vitro*

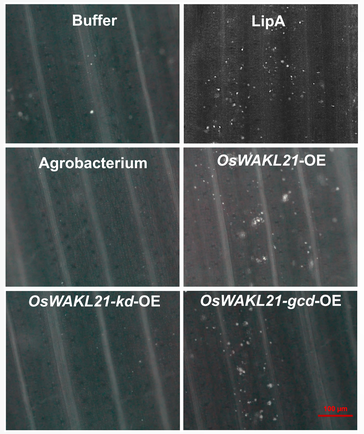
- Kinase activity as observed by autoradiography (Fig A)
- Guanylate cyclase (GC) activity which is observed by detecting produced cGMP using anti-cGMP antibody in dot-blot (Fig B)

A. Kinase assay

B. Guanylate cyclase (GC) assay

We also generated kinase deficient (OsWAKL21-kd) and GC deficient (OsWAKL21-gcd) versions of OsWAKL21 by site directed mutagenesis and observed loss of respective biochemical activities while retaining the other activity.

4. Overexpression of *OsWAKL21* in rice mimics LipA treatment and activates immunity by kinase activity

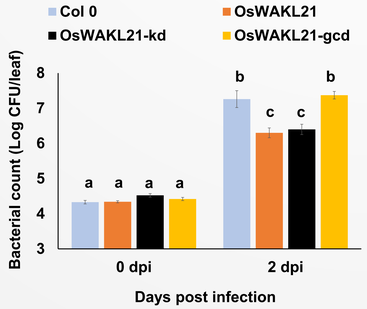


Agrobacterium mediated transient overexpression of *OsWAKL21* in rice mimics LipA treatment in

- Induction of callose deposition (as shown in image)
- Expression of key defense related genes
- Enhanced tolerance against subsequent *Xoo* infection.

Additionally, experiments with mutant versions of kinase activity (OsWAKL21-kd) or GC activity (OsWAKL21-gcd) indicates kinase activity but not the GC activity of OsWAKL21 is required for induction of Arabidopsis immune responses.

5. Ectopic expression of *OsWAKL21* in Arabidopsis induces immunity by GC activity



Ectopic expression of *OsWAKL21* in Arabidopsis transgenic lines enhances

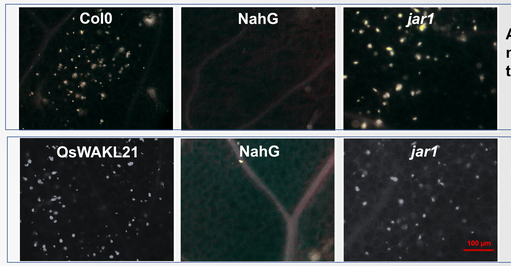
- Callose deposition.
- Expression of key defense related genes
- In-vivo cGMP level
- Tolerance against subsequent *Pseudomonas syringae* pv. *tomato* infection (as shown in figure)

Additionally, experiments with mutant versions of kinase activity (OsWAKL21-kd) or GC activity (OsWAKL21-gcd) indicates GC activity but not the kinase activity of OsWAKL21 is required for induction of Arabidopsis immune responses.

6. LipA treatment or OsWAKL21.2 overexpression induces JA pathway in rice and SA pathway in Arabidopsis

Expression profile of key JA and SA pathway genes indicated treatment of LipA or overexpression of OsWAKL21 in rice induces expression of JA pathway related genes but not SA pathway related genes. Similar treatment in Arabidopsis shows enhanced expression of SA pathway related genes but not JA pathway related genes.

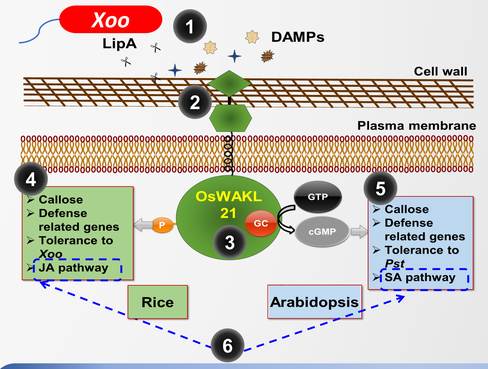
To further validate these findings in Arabidopsis we utilized NahG expressing lines that are SA pathway deficient and *jar1* mutant lines that are JA pathway deficient. We either treated these lines with LipA or crossed them with OsWAKL21 expression lines.



Arabidopsis NahG expressing lines do not induce immunity after LipA treatment

Arabidopsis NahG expressing lines do not induce immunity after ectopic expression of OsWAKL21

Conclusion



1. Treatment of rice tissue with LipA, *Xoo* or LipA activity derived cell wall degradation products enhance expression of *OsWAKL21*.
2. OsWAKL21 is a key intermediate of LipA induced immune responses.
3. Kinase domain of OsWAKL21 shows kinase and guanylate cyclase biochemical activity.
4. OsWAKL21 induces rice immune responses via its kinase activity.
5. OsWAKL21 induces Arabidopsis immune responses via its GC activity.
6. OsWAKL21 activates JA pathway in rice while it activates SA pathway in Arabidopsis.

Future directions

- Identification of LipA derived cell wall degradation products that induce immunity in rice and Arabidopsis.
- Identification of downstream signaling intermediate of OsWAKL21 induced immune responses in rice.
- Confirmation of results in rice in stable transgenic lines and gene edited lines.

References

1. Jha et al., MPMI, 2007
2. Aparna et al., Plant Cell, 2009
3. Malukani et al., Plant Physiology 2020.

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