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Short communication

Suppression of bacterial infection in rice by treatment with a sulfated peptide

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SUMMARY

The rice XA21 receptor kinase confers robust resistance to bacterial blight disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). A tyrosine-sulfated peptide from *Xoo*, called RaxX, triggers XA21-mediated immune responses, including the production of ethylene and reactive oxygen species and the induction of defence gene expression. It has not been tested previously whether these responses confer effective resistance to *Xoo*. Here, we describe a newly established post-inoculation treatment assay that facilitates investigations into the effect of the sulfated RaxX peptide *in planta*. In this assay, rice plants were inoculated with a virulent strain of *Xoo* and then treated with the RaxX peptide 2 days after inoculation. We found that post-inoculation treatment of XA21 plants with the sulfated RaxX peptide suppresses the development of *Xoo* infection in XA21 rice plants. The treated plants display restricted lesion development and reduced bacterial growth. Our findings demonstrate that exogenous application of sulfated RaxX activates XA21-mediated immunity *in planta*, and provides a potential strategy for the control of bacterial disease in the field.

Keywords: bacterial blight disease of rice, post-inoculation treatment, sulfated RaxX, XA21-mediated immunity, *Xanthomonas oryzae* pv. *oryzae*.

INTRODUCTION

Pattern recognition receptors (PRRs), which detect microbial molecules, are critical components of the innate immune system in both animals and plants (Jones and Dangl, 2006; Ronald and Beutler, 2010; Schwessinger and Ronald, 2012). In animals, Toll-like receptors (TLRs) recognize conserved microbial molecules (Kawai and Akira, 2010). In plants, plasma membrane-localized receptor kinases (RKs) and receptor-like proteins serve as PRRs to perceive microbial molecules. Well-studied PRRs include *Arabidopsis* FLAGELLIN-SENSITIVE2 (FLS2), which detects bacterial flagellin, elongation factor Tu receptor (EFR), which recognizes bacterial elongation factor Tu (EF-Tu), and rice *Xanthomonas* resistance 21 (XA21), which is activated by a tyrosine-sulfated peptide, called RaxX (*r*equired for *a*ctivation of *X*A21-mediated immunity *X*), derived from *Xanthomonas oryzae* pv. *oryzae* (*Xoo*; Cao *et al.*, 2014; Chen *et al.*, 2006; Gomez-Gomez and Boller, 2000; Hayafune *et al.*, 2014; Pruitt *et al.*, 2015; Song *et al.*, 1995; Sun *et al.*, 2004; Zipfel *et al.*, 2006).

The recognition of microbial molecules by plant RKs triggers a broad spectrum of immune responses in plants, including Ca^{2+} uptake, activation of mitogen-activated protein kinases (MAPKs), reactive oxygen species (ROS) burst, production of ethylene and large-scale transcriptional reprogramming (Ausubel, 2005; Boller and Felix, 2009; Pruitt *et al.*, 2015; Schwessinger and Ronald, 2012). Researchers have also shown that pretreatment of *Arabidopsis* plants with flg22, an active epitope of bacterial flagellin, prevents subsequent infection of *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000). In contrast, an *Arabidopsis* mutant lacking FLS2 is not protected from infection by pretreatment with flg22 (Zipfel *et al.*, 2004). Similarly, pretreatment of *Arabidopsis* with chitooligosaccharides enhances resistance to subsequent inoculation with the necrotrophic fungus *Alternaria brassicicola*. An *Arabidopsis* mutant carrying a T-DNA insertion in the cognate PRR, *LysM-RLK1*, is not protected (Wan *et al.*, 2008).

The rice PRR XA21 confers robust resistance to most *Xoo* strains, the causal agent of bacterial blight disease (Khush *et al.*, 1990; Song *et al.*, 1995). Recently, we have identified the sulfated RaxX peptide (RaxX-sY) derived from *Xoo* strain PXO99 as the activator of XA21-mediated immunity (Pruitt *et al.*, 2015). *Xoo* strains that lack RaxX (PXO99Δ*raxX*) or RaxST (PXO99Δ*raxST*), which is required for sulfation of RaxX, develop long water-soaked lesions on XA21 plants (Pruitt *et al.*, 2015; da Silva *et al.*, 2004). Sulfated, but not non-sulfated, RaxX peptides trigger hallmarks of the immune response in detached rice leaves from XA21 plants, including a ROS burst, ethylene production and the induction of defence-responsive genes (Pruitt *et al.*, 2015). However, it has not yet been demonstrated that RaxX-sY activates XA21-mediated immunity *in planta*, and whether the immune response triggered by RaxX-sY is sufficient to suppress *Xoo* infection.

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Here, we describe a novel post-inoculation treatment method to test the *in planta* activity of RaxX-sY. We treated *Xoo*-inoculated plants with RaxX-sY, 2 days after inoculation, and found that this post-inoculation treatment enhances resistance to virulent strains of *Xoo* in an XA21-dependent manner. This study demonstrates that exogenous application of sulfated RaxX triggers XA21-mediated immunity *in planta*, and provides a potential method for the control of bacterial disease caused by *Xanthomonas* species.

RESULTS

Clipping and water pretreatment delays lesion development on *Xoo* infection

We have reported previously that clipping and treatment with water before *Xoo* inoculation affects lesion development (Lee *et al.*, 2013). In this study, we further investigated this observation. For this purpose, fully expanded leaves from 6-week-old TP309 and transgenic plants carrying XA21 (XA21-TP309) were clipped with sterile scissors and treated with water for 6 h before inoculation with PXO99Δ*raxST*. The lesion lengths on the pretreated plants were significantly shorter than those on untreated plants regardless of the presence or absence of *Xa21* (Fig. 1). These results demonstrate that pretreatment of clipped leaves with water leads to enhanced resistance to PXO99Δ*raxST*, which probably reflects a non-specific immune response in rice. Partly based on these results, we retracted the 2009 report (Lee *et al.*, 2009, 2013; Ronald, 2013).

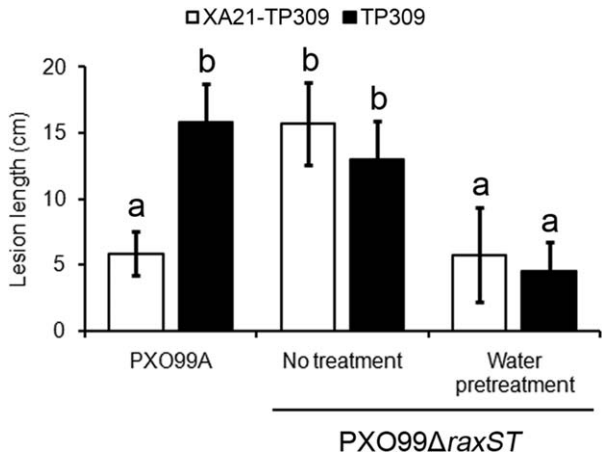

Fig. 1 Water pretreatment of clipped leaves leads to enhanced resistance to a virulent PXO99Δ*raxST Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain. Leaves from 6-week-old XA21-TP309 (open bar) and TP309 (filled bar) were clipped 2 cm from the tip using sterile scissors, dipped into water for 6 h and inoculated with *Xoo* inoculum immediately after pretreatment. Bars represent the mean lesion length ± standard deviation (SD) measured at 14 days after inoculation ($n \geq 6$). Different letters indicate significant differences between the samples [Tukey's honestly significant difference (HSD) test, $\alpha < 0.05$].

Pretreatment or co-treatment with RaxX39-sY does not enhance resistance to PXO99Δ*raxX*

To assess the ability of RaxX-sY to trigger XA21-mediated immunity *in planta*, we established a new assay. For this purpose, we used three treatments: pretreatment, co-treatment or post-treatment of Kitaake and transgenic plants carrying XA21 (Ubi::XA21) with sulfated or non-sulfated RaxX39 peptides (RaxX39-sY and RaxX39-Y, respectively). For the pretreatment assay, we clipped the leaves and then soaked them in the peptide solution for 6 h before inoculation with PXO99Δ*raxX*. For co-treatment, we dipped clipped leaves in PXO99Δ*raxX* inoculum supplemented with the peptides for 6 h. For post-inoculation treatment, we treated inoculated leaves with the peptides for 6 h, 2 days after inoculation with PXO99Δ*raxX*.

As shown in Fig. 2A, Kitaake and Ubi::XA21 plants pretreated with RaxX39-sY both developed short lesions similar to those on plants treated with RaxX39-Y or water. These results indicate that the resistance induced by pretreatment is not specific to RaxX39-sY. Plants co-treated with water, RaxX39-sY or RaxX39-Y developed long lesions, which are characteristic of bacterial blight disease, similar to the untreated plants, indicating that co-treatment had no significant effect on resistance. In contrast, plants treated with RaxX39-sY, 2 days after inoculation, developed significantly shorter lesions than the untreated plants, whereas plants treated with RaxX39-Y developed long lesions (Fig. 2A). These results demonstrate that treatment with RaxX39-sY, 2 days after *Xoo* inoculation, triggers an effective immune response, and that this response requires both treatment with sulfated RaxX and the presence of *Xa21*.

Post-inoculation treatment with RaxX21-sY or RaxX39-sY, 2 and 3 days after inoculation, suppresses infection of a virulent *Xoo* strain

We next tested the effects of post-inoculation treatment with RaxX39-sY at different time points after inoculation. As shown in Fig. 2B, XA21 plants treated with RaxX39-sY, 2 and 3 days after PXO99Δ*raxX* inoculation, had significantly shorter lesions than those on plants treated with RaxX39-Y or water. Treatment with RaxX39-sY, 4 and 5 days after inoculation, conferred no effects on lesion development compared with controls. Based on these experiments, we carried out further post-inoculation treatment experiments 2 days after inoculation.

We have identified previously a 21-amino-acid sulfated RaxX peptide as the minimal epitope required for activation of XA21-mediated immunity (Pruitt *et al.*, 2015). We therefore tested whether post-inoculation treatment with RaxX21-sY could also suppress infection of PXO99Δ*raxX*. We found that XA21 plants treated with RaxX21-sY developed significantly shorter lesions than those on plants treated with RaxX21-Y (Fig. S1, see Supporting Information). These results demonstrate that RaxX21-sY, like

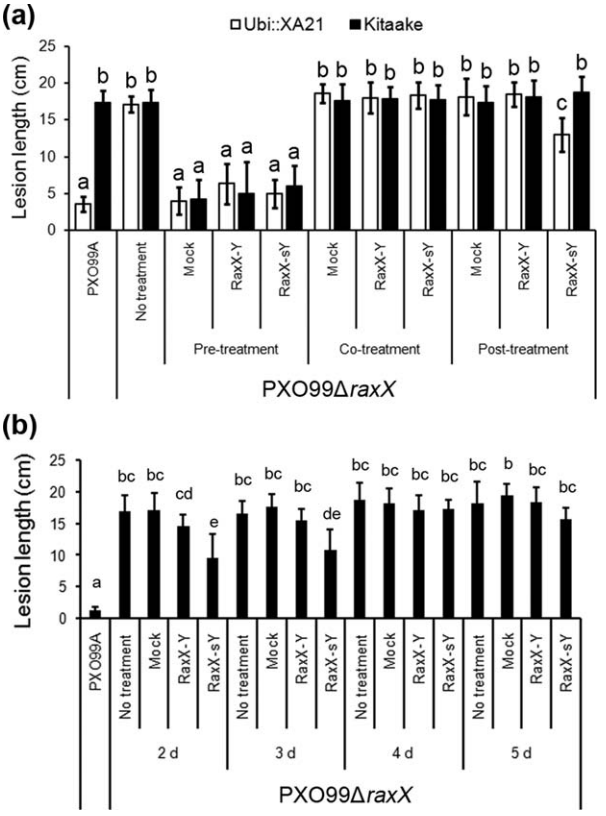

Fig. 2 Post-inoculation treatment with sulfated RaxX enhances resistance to PXO99Δ*raxX* strain in an XA21-dependent manner. (A) Six-week-old Ubi::XA21 (open bars) and Kitaake (filled bars) plants were used for pretreatment, co-treatment and post-treatment with 39-amino-acid RaxX. For pretreatment, rice leaves were clipped using sterile scissors and dipped into 1 μM RaxX-Y or RaxX-sY for 6 h before inoculation with PXO99Δ*raxX*. For co-treatment, leaves were clipped and dipped into PXO99Δ*raxX* inoculum supplemented with 1 μM RaxX-Y or RaxX-sY for 6 h. For post-treatment, leaves inoculated with PXO99Δ*raxX* were dipped into 1 μM RaxX-Y or RaxX-sY for 6 h, 2 days after inoculation (dai). Bars represent the mean lesion length ± standard deviation (SD) measured at 14 dai ($n \geq 6$). (B) Six-week-old XA21-TP309 plants were inoculated with PXO99Δ*raxX*, and treated with water (Mock), 1 μM RaxX-Y or RaxX-sY for 6 h at the indicated time points (d, day) after inoculation. Untreated plants and those inoculated with PXO99 were used as controls. Bars represent mean lesion length ± SD measured at 13 dai ($n \geq 6$). Different letters indicate significant differences between the samples [Tukey's honestly significant difference (HSD) test, $\alpha < 0.05$].

RaxX39-sY, can suppress infection of the virulent *Xoo* strain in XA21 plants.

Treatment of hydroponically grown XA21 plants with RaxX21-sY suppresses infection of the virulent *Xoo* strain PXO99Δ*raxX*

Soil-grown rice plants require space and glasshouse facilities, a large volume of water with nutrients and effective pest control. Another challenge is that the growing conditions in the glasshouse vary in quality of sunlight and cloud cover. For this reason, we have established a hydroponic system in light- and temperature-controlled growth chambers that is space and cost efficient (Pruitt *et al.*, 2015). We tested whether our newly developed post-inoculation treatment assay could also be applied on plants grown under these well-controlled growth conditions. We used Kitaake rice in this hydroponic system because it has a much shorter generation time and adapts better to growth chamber conditions than TP309.

Four-week-old hydroponically grown Kitaake and Ubi::XA21 plants were inoculated with PXO99Δ*raxX* and treated with RaxX21-sY 2 days later. As shown in Fig. 3A, Kitaake and Ubi::XA21 leaves inoculated with PXO99Δ*raxX* developed long, water-soaked lesions. Only the XA21 plants treated with RaxX21-sY, 2 days after inoculation, showed reduced lesion lengths, as short as the XA21 plants inoculated with PXO99. The lesion lengths on XA21 plants treated with RaxX21-sY were significantly shorter than those on plants treated with RaxX21-Y or water, and untreated plants (Fig. 3B). Consistent with the lesion measurements, the bacterial population in RaxX21-sY-treated XA21 leaves was significantly (>10-fold) lower than those on plants treated with RaxX21-Y or water, and untreated controls (Fig. 3C). The lesion lengths and bacterial populations in XA21 plants inoculated with PXO99Δ*raxX* followed by RaxX21-sY treatment were similar to those in the resistant XA21 plants inoculated with PXO99. These results demonstrate that post-inoculation treatment with RaxX21-sY suppresses *Xoo* infection in hydroponically grown XA21 plants.

DISCUSSION

We have identified previously the tyrosine-sulfated RaxX peptide as the activator of XA21-mediated immunity. Sulfated RaxX triggers XA21-dependent immune responses in detached rice leaves, including ROS burst, ethylene production and the induction of defence gene expression (Pruitt *et al.*, 2015). These results suggest, but do not demonstrate, that the peptide induces effective resistance *in planta*. Indeed, it has been demonstrated that the induction of defence markers by treatment with microbial molecules does not necessarily correlate with effective resistance *in planta*. For example, exogenous application of elf18, a functional epitope of EF-Tu, on transgenic rice expressing *Arabidopsis* EFR results in ROS production and the induction of defence gene expression, but does not lead to effective resistance to PXO99 (Schwessinger *et al.*, 2015). For these reasons, we tested whether the immune responses activated in XA21 leaf clippings in response to RaxX-sY treatment correlated with enhanced resistance *in planta*.

It has been demonstrated previously that pretreatment of *Arabidopsis* plants with microbial molecules can enhance resistance to bacterial and fungal pathogens (Wan *et al.*, 2008; Zipfel *et al.*,

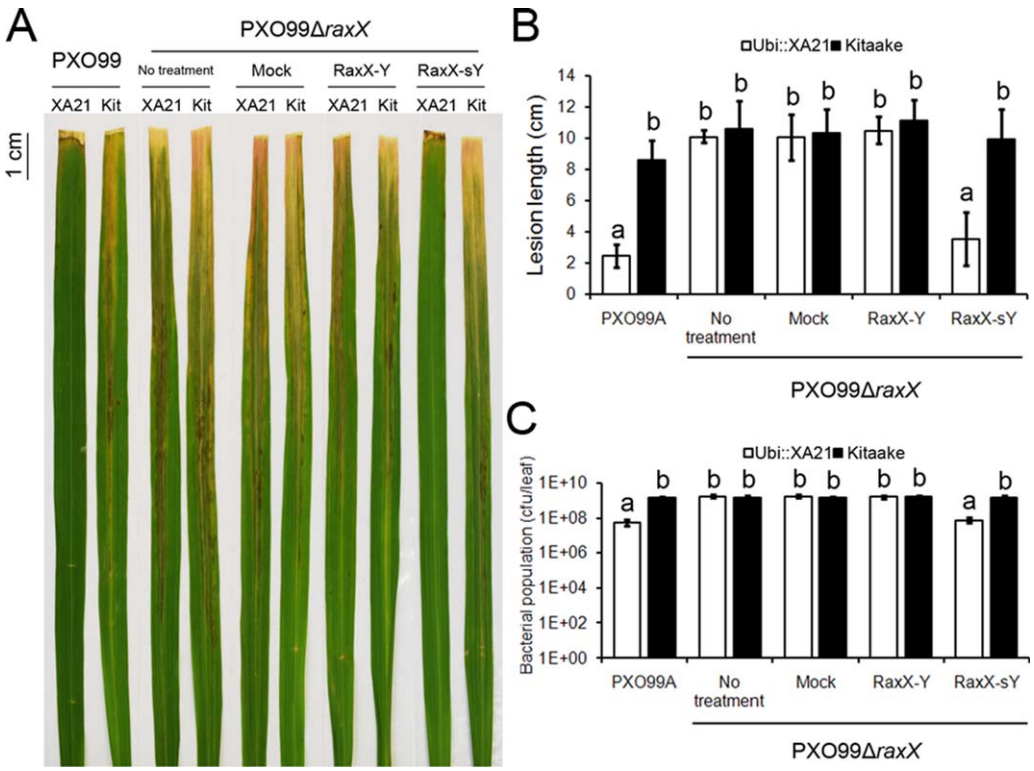

Fig. 3 Post-inoculation treatment with sulfated RaxX enhances resistance to PXO99Δ*raxX* strain in hydroponically grown XA21 plants. (A) Rice leaves from Ubi::XA21 (XA21) and Kitaake (Kit) plants, 13 days after inoculation (dai). (B) Lesion lengths of Ubi::XA21 (open bars) and Kitaake (filled bars) at 13 dai. Five-week-old hydroponic Ubi::XA21 and Kitaake plants were inoculated with PXO99Δ*raxX*, and treated with water (Mock), 1 μM 21-amino-acid RaxX-Y or RaxX-sY for 6 h at 2 dai. XA21 plants with no treatment and those inoculated with PXO99 were used as controls. Bars represent mean lesion length ± standard deviation (SD) measured at 13 dai ($n \geq 6$). (C) Bacterial population was quantified as the number of colony-forming units (cfu) per inoculated leaf at 13 dai. Different letters in (B, C) indicate significant differences between the samples [Tukey's honestly significant difference (HSD) test, $\alpha < 0.05$]. The experiment was repeated three times with similar results.

2004, 2006). In contrast, we found that pretreatment with water alone enhances resistance to the virulent mutant *Xoo* strains, PXO99Δ*raxST* and PXO99Δ*raxX* (Figs 1 and 2). The observed resistance may be caused by a non-specific stress response triggered by clipping and water pretreatment. As an alternative approach, we established a post-inoculation treatment assay to avoid this non-specific response. We demonstrated that *in planta* treatment with RaxX39-sY and RaxX21-sY can suppress the infection of virulent strains of the bacterial pathogen in an XA21-dependent manner. We also showed that this assay can be extended to hydroponically grown XA21 plants. We demonstrated that post-inoculation treatment with sulfated RaxX triggers an effective immune response against a virulent *Xoo* strain, as reflected in restricted lesion lengths and reduced bacterial growth (Fig. 3). The robust resistance triggered by the treatment is comparable with the resistance observed in XA21 plants inoculated with the wild-type *Xoo* PXO99 strain, which expresses RaxX.

XA21 has been introgressed into diverse rice varieties and planting of these Xa21 varieties has served as an effective strategy to control outbreaks of bacterial blight disease (Chen *et al.*, 2000; Sundaram *et al.*, 2008; Win *et al.*, 2012). We have discovered recently that *Xoo* isolates carrying mutations in *raxX* are able to evade XA21-mediated immunity (Mishra *et al.*, 2013; Pruitt *et al.*, 2015). The discovery that plants treated with the sulfated RaxX peptides can suppress the infection of virulent *Xoo* strains provides a new strategy for the suppression of infection of these virulent *Xoo* field isolates. Furthermore, this strategy can be applied to other crop plants that are susceptible to *Xanthomonas* infection. For example, bacterial blight of cassava (caused by *X. axonopodis* pv. *manihotis*), bacterial spot of citrus (caused by *X. axonopodis* pv. *citrumelo*) and bacterial spot of tomato (caused by *X. campestris* pv. *vasculorum*) are all serious diseases for which there is currently no effective control. Each of these *Xanthomonas* species carries a *raxX* allele that does not activate XA21-mediated immunity (Pruitt *et al.*, 2015). The expression of XA21 in these crop plants and treatment with sulfated RaxX provides an opportunity to control these devastating diseases. Another approach is to engineer XA21 to recognize these RaxX variants and to introduce the novel XA21 alleles into these crops.

EXPERIMENTAL PROCEDURES

Plant growth conditions

Oryza sativa ssp. *japonica* rice variety Taipei 309 (TP309), a TP309 transgenic line carrying XA21 driven by its own promoter (XA21-TP309 106-17-derived progeny; Song *et al.*, 1995), Kitaake and a Kitaake transgenic line expressing XA21 tagged with Myc under the maize ubiquitin promoter (Ubi::XA21 7A-8-derived progeny; Park *et al.*, 2010) were used in this study. Rice seeds were surface sterilized using 15% bleach, rinsed with water and germinated in distilled water at 28°C for 1 week. Plants were transplanted into a soil mixture (80% sand, 20% peat from Redi-Gro, Sacramento, CA, USA) and grown in a glasshouse, as described previously (Pruitt *et al.*, 2015). Before bacterial inoculation, 5-week-old rice plants were transferred to a walk-in growth chamber (14-h light/10-h dark photoperiod, 28°C/24°C, 80%/85% humidity). For hydroponic growth, 1-week-old rice seedlings were transplanted into a tray filled with A-OK Starter Plugs (Grodan, The Netherlands) and grown in the growth chamber. The seedlings were watered twice a week with Hoagland's solution.

Bacterial culture

Xoo Philippines race 6 (referred to as PXO99) and two marker-free mutants generated from PXO99, PXO99Δ*raxX* and PXO99Δ*raxST* (Pruitt *et al.*, 2015), were used in this work. *Xoo* strains were cultured on peptone sucrose agar (PSA) plates supplemented with 20 mg/L cephalexin for 3 days, and then scraped off and resuspended in sterilized water. The concentration was adjusted to an optical density at 600 nm (OD_{600}) of 0.5 for inoculation [approximately 1×10^8 colony-forming units (cfu)/mL].

Bacterial inoculation and peptide treatments

Two RaxX variants previously shown to activate the XA21-dependent ROS burst, ethylene production and induction of defence gene expression were used in the post-inoculation treatment assays: a 39-amino-acid peptide, called RaxX39 (KGRPEPLDQRLWKHVGGGDYPPPGANPKHDPPPRNPGHH), and a 21-amino-acid peptide, called RaxX21 (HVGGGDYPPP-GANPKHDPPPR) (Pruitt *et al.*, 2015). The sulfated and non-sulfated peptides (RaxX-sY and RaxX-Y, respectively) were synthesized at Pacific Immunology, Ramona, CA, USA. Plants were inoculated with *Xoo* using the scissors clipping method (Song *et al.*, 1995). For post-inoculation treatment assays, the *Xoo*-inoculated leaves were dipped into a 1 μM peptide solution containing 0.02% Tween-20 for 6 h. The lesions were measured 10–14 days after inoculation. Measurement of bacterial population growth *in planta* was performed as described previously (Bahar *et al.*, 2014).

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REFERENCES

Ausubel, F.M. (2005) Are innate immune signaling pathways in plants and animals conserved? *Nat. Immunol.* **6**, 973–979.

Bahar, O., Pruitt, R., Luu, D.D., Schwessinger, B., Daudi, A., Liu, F., Ruan, R., Fontaine-Bodin, L., Koebnik, R. and Ronald, P. (2014) The Xanthomonas Ax21 protein is processed by the general secretory system and is secreted in association with outer membrane vesicles. *Peer J.* **2, e242**.

Boller, T. and Felix, G. (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol. 60*, 379–406.

Cao, Y.R., Liang, Y., Tanaka, K., Nguyen, C.T., Jedrzejczak, R.P., Joachimiak, A. and Stacey, G. (2014) The kinase LYK5 is a major chitin receptor in *Arabidopsis* and forms a chitin-induced complex with related kinase CERK1. *Elife*, **3**, e03766.

Chen, S., Lin, X.H., Xu, C.G. and Zhang, Q.F. (2000) Improvement of bacterial blight resistance of 'Minghui 63', an elite restorer line of hybrid rice, by molecular marker-assisted selection. *Crop Sci.* **40**, 239–244.

Chen, X.W., Shang, J.J., Chen, D.X., Lei, C.L., Zou, Y., Zhai, W.X., Liu, G., Xu, J., Ling, Z., Cao, G., Ma, B., Wang, Y., Zhao, X., Li, S. and Zhu, L. (2006) A B-lectin receptor kinase gene conferring rice blast resistance. *Plant J.* **46**, 794–804.

Gomez-Gomez, L. and Boller, T. (2000) FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. *Mol. Cell*, **5**, 1003–1011.

Hayafune, M., Berisio, R., Marchetti, R., Silipo, A., Kayama, M., Desaki, Y., Arima, S., Squeglia, F., Ruggiero, A., Tokuyasu, K., Molinaro, A., Kaku, H. and Shibuya, N. (2014) Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization. *Proc. Natl. Acad. Sci. USA*, **111**, E404–E413.

Jones, J.D.G. and Dangl, J.L. (2006) The plant immune system. *Nature*, **444**, 323–329.

Kawai, T. and Akira, S. (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* **11**, 373–384.

Khush, G.S., Bacalangco, E. and Ogawa, T. (1990) A new gene for resistance to bacterial blight from *O. longistaminata*. *Rice Genet. Newsl.* **7**, 121–122.

Lee, S.W., Han, S.W., Sririyanum, M., Park, C.J., Seo, Y.S. and Ronald, P.C. (2009) A type I-secreted, sulfated peptide triggers XA21-mediated innate immunity (Retracted article. See vol. 342, pg. 191, 2013). *Science*, **326**, 850–853.

Lee, S.W., Han, S.W., Sririyanum, M., Park, C.J., Seo, Y.S. and Ronald, P.C. (2013) A type I-secreted, sulfated peptide triggers XA21-mediated innate immunity (Retraction of vol. 326, pg. 850, 2009). *Science*, **342**, 191.

Mishra, D., Vishnupriya, M.R., Anil, M.G., Konda, K., Raj, Y. and Sonti, R.V. (2013) Pathotype and genetic diversity amongst Indian isolates of *Xanthomonas oryzae* pv. *oryzae*. *PLoS One*, **8, e81996**.

Park, C.J., Lee, S.W., Chern, M., Sharma, R., Canlas, P.E., Song, M.Y., Jeon, J.S. and Ronald, P.C. (2010) Ectopic expression of rice *Xa21* overcomes developmentally controlled resistance to *Xanthomonas oryzae* pv. *oryzae*. *Plant Sci.* **179**, 466–471.

Pruitt, R.N., Schwessinger, B., Joe, A., Thomas, N., Liu, F., Albert, M., Robinson, M.R., Chan, L.J., Luu, D.D., Chen, H., Bahar, O., Daudi, A., De Vleesschauwer, D., Caddell, D., Zhang, W., Zhao, X., Li, X., Heazlewood, J.L., Ruan, D., Majumder, D., Chern, M., Kalbacher, H., Midha, S., Patil, P.B., Sonti, R.V., Petzold, C.J., Liu, C.C., Brodbelt, J.S., Felix, G. and Ronald, P.C. (2015) The rice immune receptor XA21 recognizes a tyrosine-sulfated protein from a Gram-negative bacterium. *Sci. Adv.* **1**, e1500245.

Ronald, P. (2013) Lab Life: the anatomy of a retraction. *Sci. Am.* Available at http://blogs.scientificamerican.com/food-matters/lab-life-the-anatomy-of-a-retraction/

Ronald, P.C. and Beutler, B. (2010) Plant and animal sensors of conserved microbial signatures. *Science*, **330**, 1061–1064.

Schwessinger, B. and Ronald, P.C. (2012) Plant innate immunity: perception of conserved microbial signatures. *Annu. Rev. Plant Biol.* **63**, 451–482.

Schwessinger, B., Bahar, O., Thomas, N., Holton, N., Nekrasov, V., Ruan, D.L., Canlas, P.E., Daudi, A., Petzold, C.J., Singan, V.R., Kuo, R., Chovatia, M., Daum, C., Heazlewood, J.L., Zipfel, C. and Ronald, P.C. (2015) Transgenic expression of the dicotyledonous pattern recognition receptor EFR in rice leads to ligand-dependent activation of defense responses. *PLoS Pathog.* **11**, e1004809.

da Silva, F.G., Shen, Y., Dardick, C., Burdman, S., Yadav, R.C., de Leon, A.L. and Ronald P.C. (2004) Bacterial genes involved in type I secretion and sulfation are required to elicit the rice Xa21-mediated innate immune response. *Mol. Plant–Microbe. Interact.* **17**, 593–601.

Song, W.Y., Wang, G.L., Chen, L.L., Kim, H.S., Pi, L.Y., Holsten, T., Gardner, J., Wang, B., Zhai, W.X., Zhu, L.H., Fauquet, C. and Ronald, P. (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *XA21*. *Science*, **270**, 1804–1806.

Sun, X.L., Cao, Y.L., Yang, Z.F., Xu, C.G., Li, X.H., Wang, S.P. and Zhang, Q. (2004) *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. *Plant J.* **37**, 517–527.

Sundaram, R.M., Vishnupriya, M.R., Biradar, S.K., Laha, G.S., Reddy, G.A., Rani, N.S., Sarma, N.P. and Sonti, R.V. (2008) Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica*, **160**, 411–422.

Wan, J., Zhang, X.-C., Neece, D., Ramonell, K.M., Clough, S., Kim, S.-Y., Stacey, M.G. and Stacey, G. (2008) A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in *Arabidopsis*. *Plant Cell*, **20**, 471–481.

Win, K.M., Korinsak, S., Jantaboon, J., Siangliw, M., Lanceras-Siangliw, J., Sirithunya, P., Vanavichitc, A., Pantuwand, G., Jongdeee, B., Sidhiwongf, N. and Toojinda, T. (2012) Breeding the Thai jasmine rice variety KDML105 for non-age-related broad-spectrum resistance to bacterial blight disease based on combined marker-assisted and phenotypic selection. *Field Crops Res.* **137**, 186–194.

Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E.J., Jones, J.D.G., Felix, G. and Boller, T. (2004) Bacterial disease resistance in Arabidopsis through flagellin perception. *Nature*, **428**, 764–767.

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J.D.G., Boller, T. and Felix, G. (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell*, **125**, 749–760.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1 Post-inoculation treatment with 21-amino-acid sulfated RaxX enhanced resistance to PXO99Δ*raxX* in XA21 plants. Six-week-old Ubi::XA21 (open bars) and Kitaake (filled bars) were inoculated with PXO99Δ*raxX*, and treated with water (Mock), 1 μM 21-amino-acid RaxX-Y or RaxX-sY for 6 h, 2 days after inoculation (dai). Untreated plants and those inoculated with PXO99 were used as controls. Bars represent mean lesion length ± standard deviation (SD) measured at 11 dai ($n \geq 6$). Different letters indicate significant differences between the samples [Tukey's honestly significant difference (HSD) test, $\alpha < 0.05$].