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Commentary

Drought treatment: A new tool for dissecting XA21 signaling in rice

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Microbial infection can cause cell damage in both plants and animals, as well as triggering stress responses commonly induced by environmental (abiotic) cues. While significant progress has been made in understanding how host immunity restricts pathogen growth, little is known about the role of the immune system in controlling abiotic stress responses associated with pathogen attack. The immune receptor chitin elicitor receptor kinase 1 (CERK1), which recognizes the fungal cell wall component chitin [1], also plays a role in salt stress signaling. This novel function is thought to be mediated by the detection of elevated Na^+ ion levels caused by fungal infection [2].

The rice (*Oryza sativa*) gene *Xa21* provides immunity against a broad range of strains of the Gram-negative bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), which causes the vascular disease bacterial leaf blight [3,4]. *Xa21* encodes a receptor-like kinase (RLK) consisting of an extracellular leucine-rich repeat (LRR) domain, a single transmembrane helix, and an intracellular region composed of a charged domain, a juxtamembrane (JM) motif, and a serine/threonine kinase domain (**Figure 1)** [3]. XA21 acts as an immune receptor that recognizes a sulfated peptide from the *Xoo* protein RaxX (Required for activation of XA21-mediated immunity X) [5-8]. Notably, XA21 shares structural similarity with the immune receptors discovered in flies, mice, and humans [8].

The intracellular portion of XA21 can undergo autophosphorylation on multiple serines and threonines *in vitro*, including the Ser-686, Thr-688, and Ser-689 residues in the JM domain, which also possesses a putative proteolytic cleavage motif (PCM) (**Figure 1)** [9,10]. Immunoblot analysis of wild-type XA21 and its autophosphorylation variants in transgenic rice plants revealed that the immune receptor can be cleaved at a site(s) near the transmembrane domain by an unidentified protease, although the precise location of the cleavage site(s) remains to be determined [10-12]. Mutations of the three autophosphorylated amino acids in the JM domain or Lys-736, a well-conserved

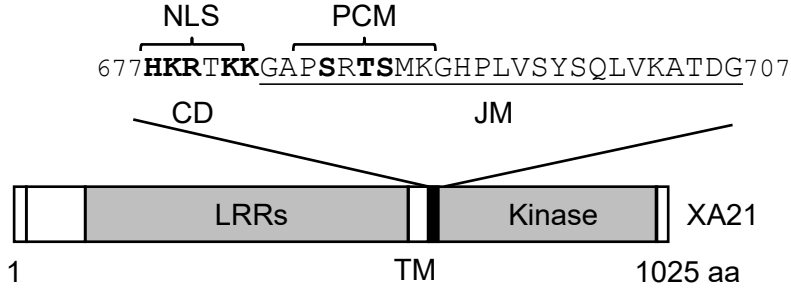

Figure 1. Schematic representation of XA21. The domains of XA21 were described previously [3]. The positively charged residues in the NLS and the autophosphorylated residues in the JM domain are shown in bold. LRRs: Leucine-Rich Repeats; TM: Transmembrane domain; CD: Charged Domain; JM: Juxtamembrane domain; NLS: Nuclear Localization Signal; PCM: Putative proteolytic Cleavage Motif.

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residue located inside the XA21 kinase domain that is essential for its kinase activity, led to markedly reduced steady-state accumulation of XA21 in rice and increased sensitivity to cleavage. These findings suggest that XA21 is stabilized by autophosphorylation. Given that the over-accumulation of immune receptors might lead to heightened immune responses, which could have detrimental effects on plants [13,14], we hypothesized that proteolytic cleavage might play a role in regulating XA21 levels to maintain homeostasis in rice [10]. Accumulating evidence indicates that, besides XA21 in rice, three well-characterized *Arabidopsis thaliana* receptor kinases/RLKs (SYMBIOSIS RECEPTOR-LIKE KINASE [SYMRK], CERK1, and BRASSINOSTEROID-INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1 [BAK1]) can be proteolytically cleaved [15-18]. Notably, the corresponding proteases and the physiological significance of the cleavages remain to be identified.

XA21 is constitutively expressed in rice leaves [10,11]. The protein localizes to the plasma membrane and endoplasmic reticulum of plant cells [11,19] and forms complexes with six partners comprising XA21 binding protein 3 (XB3), XB24, Luminal-binding protein 3, XB25, OsSERK2, and Paladin, as revealed by co-immunoprecipitation experiments using rice protein extracts [11,12,20-23]. These findings support the idea that, once activated, XA21 might be capable of initiating multiple signaling pathways.

After infection, *Xoo* primarily accumulates in the xylem vessels of rice leaves causing disease symptoms that resemble those of drought-stressed plants [24]. We previously demonstrated that, in addition to immunity against *Xoo*, XA21 confers resistance to drought stress [25]. A question raised by this study was how drought conditions are perceived by the immune receptor to activate downstream signaling. We cannot exclude the possibility that water-deficit stress induces the production of a yet-to-be-discovered rice protein/peptide that is recognized by XA21, possibly through its LRR domain. This hypothesis would imply that XA21 can recognize two distinct ligands: one for pathogen defense and one for the water-deficit stress response. Indeed, in Arabidopsis, the damage-associated elicitor PEPTIDE 1 (PEP1) binds to the LRR-receptor kinase PEP1 RECEPTOR 1 (AtPEPR1) and activates immune responses [26,27]. However, XA21 was shown to be a highly selective receptor that

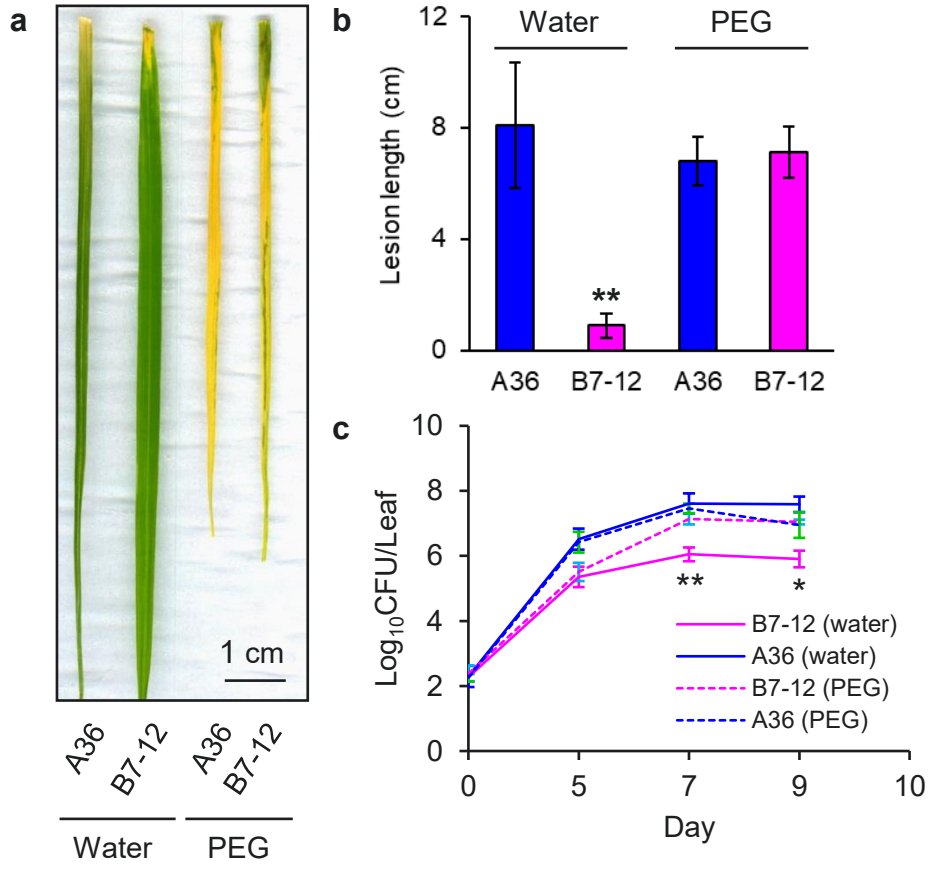

Figure 2. PEG treatment suppresses XA21-mediated resistance to incompatible *Xoo* strain PXO99A. Two-week-old seedlings of the indicated lines were inoculated with incompatible *Xoo* strain PXO99A using the leaf-clipping method. After inoculation, the seedlings were cultured in water or 13% (w/v) PEG in a growth chamber (27ºC, under fluorescent light with a 16-h-light/8-h-dark photoperiod) for the indicated time. Lesion length in diseased leaves (n = 15 per line) at 10-days post inoculation (dpi) (**a, b**) and the growth of PXO99A in inoculated seedlings (**c**) were measured. Asterisks indicate statistically significant differences from the controls (**, $p<0.01$; *, $p<0.05$).

specifically recognizes the bacterial ligand RaxX, but not plant PSY (plant peptide containing sulfated tyrosine) peptides, which shares similarities with RaxX [28].

An alternative possibility is that water-deficit stress induces the production of a protease that catalyzes the proteolysis of XA21. Given that the charged domain (CD) in the intracellular region of XA21 harbors a functional nuclear localization signal (NLS) (**Figure 1**) [12], the proteolytic cleavage of XA21 might trigger the nuclear translocation of the intracellular part of this protein together with its binding proteins. In mammals, membrane-localized receptors (e.g., Notch) can be processed by multiple proteases for maturation as well as activation [29]. The activation of immune receptors in the absence of pathogens would represent a non- canonical, ligand-independent avenue to stimulate the immune system. Such a mechanism might provide the flexibility required for the immune system to function in complex environmental and possibly developmental contexts.

Here, we examined the potential interplay between XA21-mediated drought resistance and *Xoo* immunity. To this end, we inoculated 2-week-old seedlings of the previously characterized Myc-XA21-FLAG rice line B7-12 and the empty vector control line A36 with the incompatible *Xoo* strain PXO99A. Following inoculation, we cultured the seedlings in water or 13% (w/v) polyethylene glycol (PEG), a nonionic water-soluble polymer used to simulate drought in plants [30]. Ten days later, B7- 12 seedlings cultured in water displayed normal resistance to *Xoo*, as evidenced by the reduction in lesion lengths and bacterial growth (**Figure 2**). However, PEG treatment suppressed XA21-mediated resistance to *Xoo*. Therefore, distinct defense signaling pathways, i.e., drought signaling and disease resistance signaling, are likely initiated by XA21 after *Xoo* infection and water-deficit treatments, respectively; and there might be a trade-off between the PEG-induced dehydration response and bacterial immunity.

A major technical barrier that has long hindered the reliable detection of molecular events associated with XA21 signaling under physiological conditions is the molecular events associated with XA21 signaling under physiological conditions is the difficulty in synchronizing signaling due to the slow spread of *Xoo* in the xylem following inoculation using the leaf-clipping method [31]. We overcame this obstacle in our newly developed XA21-mediated drought resistance system. Drought treatment exerts intense stress throughout the plant to generate signals for XA21 activation. Our method should facilitate the identification of additional components involved in XA21 signaling, such as the hypothesized proteases that can cleave the immune receptor, using currently available molecular tools.

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