Evaluation and Licensing Opportunities

For further information on this technology and evaluation / licensing opportunities please contact:

Dr Lars von Borcke
lars@pbltechnology.com
Tel: +44 (0)1603 456500

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Patent Literature

Publications: EP3318638, US 2019-0292559-A1

Pathogen Resistant Plants

The modification of stomata dynamics by genome editing of the JAZ2 co-receptor protects plants against bacterial infection

Genome edited pathogen resistant tomato germplasm is available for breeding

Targets for genome editing identified in other crops

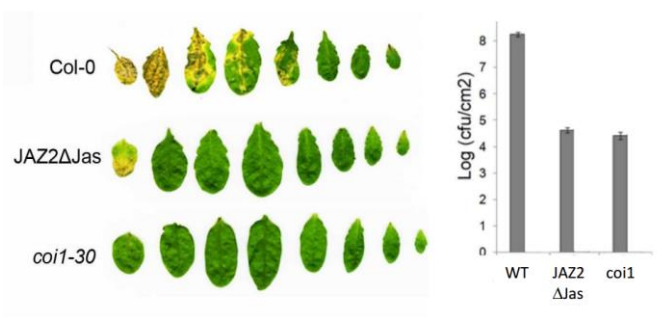
In plants, defence against pathogens is partly reliant on perceiving the pathogen and mounting an effective immune response involving highly conserved microbe-associated molecular patterns (MAMPs) by host cell transmembrane proteins that function as pattern recognition receptors, which in turn, activate MAMP-triggered immunity. Plants rapidly close stomata upon perception of MAMPs during pathogen infection to inhibit the entry of pathogens. The bacterial-triggered stomatal closure response is an integral part of the plant immune system, as phytopathogenic bacteria must enter by natural surface openings such as stomata or wounds.

Plant hormones are important regulators of responses to biotic stress, salicylic acid (SA) signalling mediates resistance against biotrophic and hemi-biotrophic microbes (e.g. *Pseudomonas syringae*), whereas a combination of JA and ethylene (ET) pathways activates resistance against necrotrophs (e.g. fungal pathogen *Botrytis cinerea*). SA and JA/ET defence pathways generally antagonize each other and thus, elevated resistance against biotrophs is often correlated with increased susceptibility to necrotrophs. Therefore, strategies to obtain broad-spectrum disease resistance in crops by manipulating these pathways have not been effective so far.

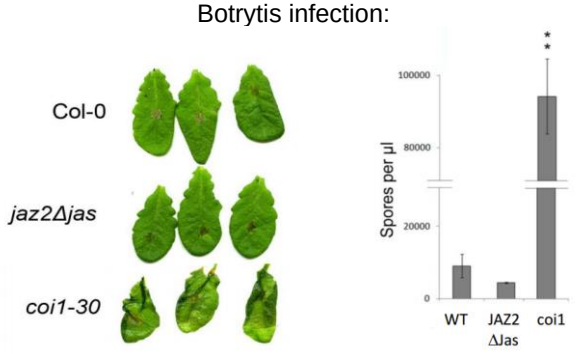
Coronatine (COR) is a virulence factor produced by different strains of the hemibiotrophic plant pathogens and is a mimic of the bioactive hormone JA-isoleucine (JA-Ile) and can bind to the co-receptor COI1-JAZ. COR suppresses host defences by activating the JA pathway, which has an inhibitory effect on SA-mediated defences against the bacteria. As a result, production of COR allows *P. syringae* to manipulate the interactions between these hormonal pathways to open stomata, grow in the apoplast and induce disease symptoms in plants.

Roberto Solano and co-inventors at the CSIC Centro Nacional de Biotecnologia, Madrid, have studied and manipulated the JAZ2 co-receptor (a family member of the jasmonate zim-domain (JAZ) proteins) which is constitutively expressed in stomata guard cells and is required for stomatal closure after bacterial recognition. Mutation of JAZ2 (JAZ2ΔJas) confers resistance to bacterial pathogens by decoupling it from COR manipulation without affecting susceptibility to necrotrophs.

Pseudomonas infection:

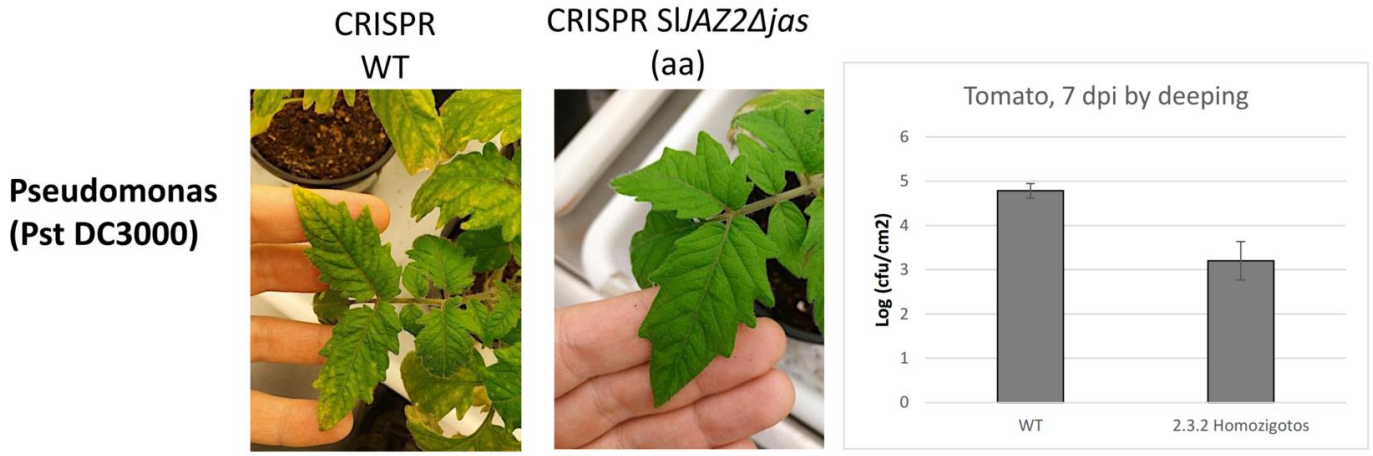


Botrytis infection:



In the experiement above leaves of wild-type Arabidopsis (Col-0), the JAZ2 mutant (JAZ2ΔJas) and the Col1 mutant (coi1-30) were infected with either Pseudomonas (left) or Botrytis (right). The results clearly demonstrate that the JAZ2 mutations confers resistance to biotrophs while not impacting necrotroph resistance.

In further work the inventors identified the JAZ2 homolog in tomatoes and generated SlJAZ2Δjas tomato plants by genome editing. These plants have been shown to be resistant to *Pseudomonas* infection:



In the above experiment non-modified (wt) and JAZ2 deactivated genome-edited plants (SlJAZ2Δjas) were infected with *P. syringae*. The JAZ2 edited plants showed much better resistance to this biotrophic pathogen. Furthermore, in experiments testing the resistance to necrotrophs these plants were not compromised in their defence mechanism. Below the same plants were infected with Botyrtis and the JAZ2 mutant plants show the same level of resistance as the wild-type plants.

Pseudomonas is the agent causing bacterial speck disease in tomatoes, which affects 18 million tonnes annually. The disease is an important economic factor for tomato cultivation. PBL can now make bacterial spot **resistant** genome edited **tomato germplasm** available for breeding into commercial cultivars.

In addition other strains of Pseudomonas affect a wide range of crops causing diseases such as bacterial leaf spot, halo blight, blister spot, grease spot apical chlorosis, bacterial canker, bacterial brown spot and others and as the **target (JAZ2 receptor) for genome editing** is known the technique can easily be transferred to **any crop species** that suffer from these type of diseases and that are amenable to genome editing.

References:

Ortigosa A *et al* (2018). Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of *SlJAZ2*. *Plant Bio Journal*; doi: 10.1111/pbi.13006.

Gimenez-Ibanez S *et al* (2017). JAZ2 controls stomata dynamics during bacterial invasion. *New Phytologist*; 213: 1378-1392.