AMSH2 – Insect Resistance

Modification of AMSH2 increases plant insect defence response

Genome editing approach to insect resistance

Evaluation and Licensing Opportunities

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

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Patent Literature

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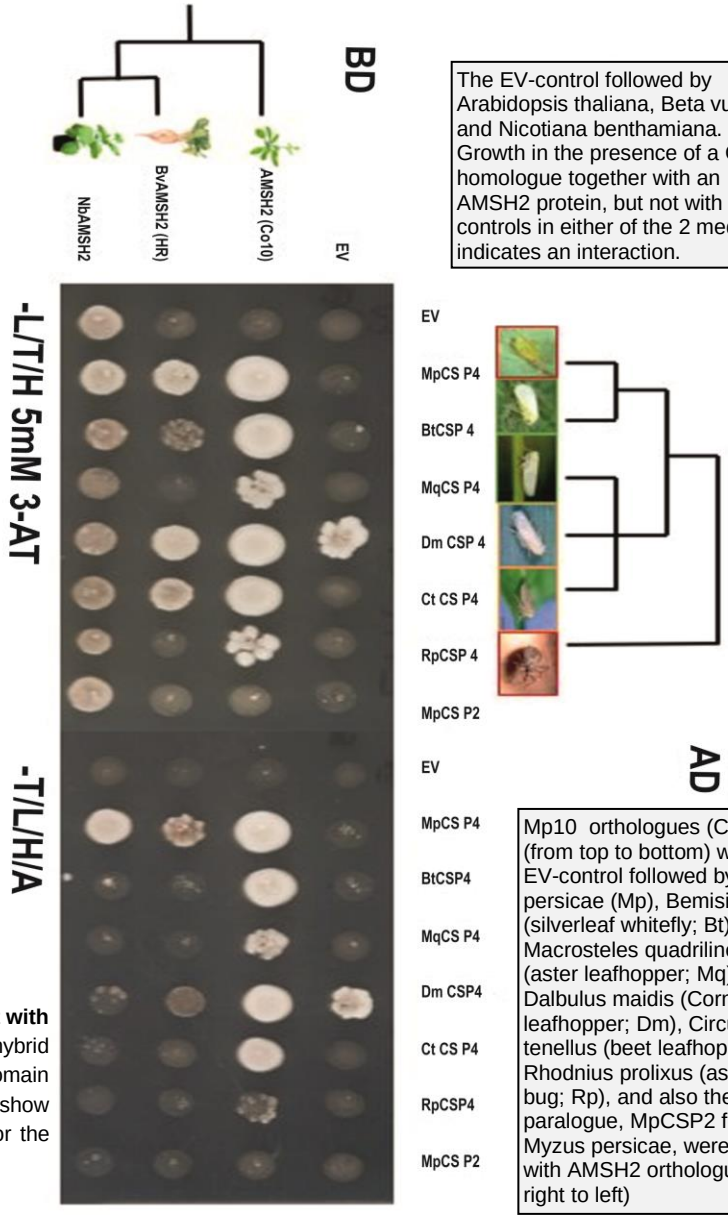
In addition to weakening crop plants by sucking sap, sap-feeding insects like aphids, whiteflies, leafhoppers and planthoppers cause major crop yield losses by transmitting plant pathogens including viruses, bacteria and plasmodium-like organisms, and naked RNA molecules known as viroids. Furthermore, some aphid species are resistant to common insecticide classes including carbamates, organophosphates, and pyrethroids. An integrated pest management strategy using biological pest control can work, but is difficult to achieve except in enclosed environments such as glasshouses.

Professor Saskia Hogenhout and Sam Mugford at the John Innes Centre studied the Mp10 effector protein from the green peach aphid. Mp10 supresses plant defences and is required for successful colonisation of host plants by the aphid. During the early stages of aphid feeding, Mp10 is delivered into the plant mesophyll cells where it supresses the early stages of plant immune signalling, by binding to the plant protein AMSH2 (Associated Molecule with the SH3 domain of STAM 2). Both proteins are **conserved across plant-feeding hemipteran insects and their hosts**. The study revealed that modifying certain well-defined amino acids in AMSH2 disrupts the binding of Mp10 to AMSH2 resulting in the **reduction of plant defence suppression and ultimately lowering virus infection,** as confirmed in field trials.

As Mp10 is well conserved across insect species and it interacts with AMSH2 which is easily identified in plant species, the team has been able to identify **target mutation sites in many crop species including wheat, maize, soybean, cotton and rice**, so that the technology can be easily applied in many important crop species, a full list of these mutations is available and included the patent application.



The EV-control followed by Arabidopsis thaliana, Beta vulgaris, and Nicotiana benthamiana. Growth in the presence of a CSP homologue together with an AMSH2 protein, but not with the EV controls in either of the 2 media indicates an interaction.

Mp10 orthologues (CSP4) (from top to bottom) with the EV-control followed by Myzus persicae (Mp), Bemisia tabaci (silverleaf whitefly; Bt), Macrosteles quadrilineatus (aster leafhopper; Mq), Dalbulus maidis (Corn leafhopper; Dm), Circulifer tenellus (beet leafhopper; Ct), Rhodnius prolixus (assassin bug; Rp), and also the Mp10 paralogue, MpCSP2 from Myzus persicae, were tested with AMSH2 orthologues (from right to left)

Fig 1: AMSH2 homologues from diverse plant species interact with Mp10 homologues from diverse hemipteran insects: Yeast 2-hybrid assays using Mp10 or its homologues fused to the Activation domain (AD) and AMSH2 proteins fused to the DNA-binding domain (BD) show growth on selective media (SD -L, -T, - H, + 5 mM 3-AT (topt) or the more stringent SD -L, -T, - H, -A (bottom)).

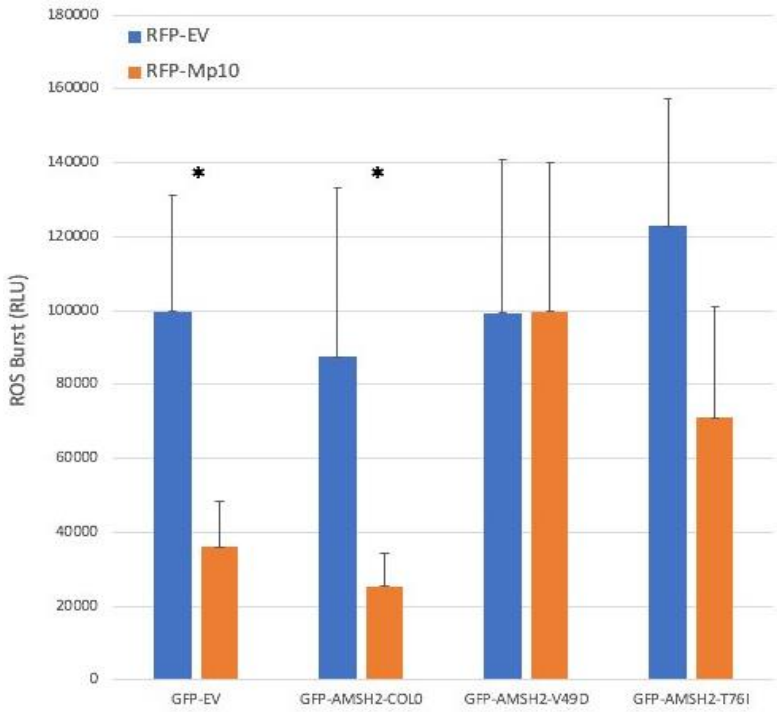


Fig 2: AMSH2 proteins that do not interact with Mp10 block the immune-suppressive activity of Mp10 in plants. *Nicotiana benthamiana* leaf discs transiently expressing Mp10 (fused to RFP) show a supressed reactive oxygen species (ROS) burst in response to treatment with the bacterial elicitor-flg22, compared to control leaf discs (RFP-EV). In leaf discs co-expressing non-interacting alleles of AMSH2 (fused to GFP; with V49D or T76I mutations) together with Mp10, Mp10 is no longer able to supress the flg-induced ROS burst (right), but in leaf discs co-expressing the Mp10-interacting AMSH2 (Col0) or the control (GFP-EV) Mp10 does suppress the ROS burst (left).

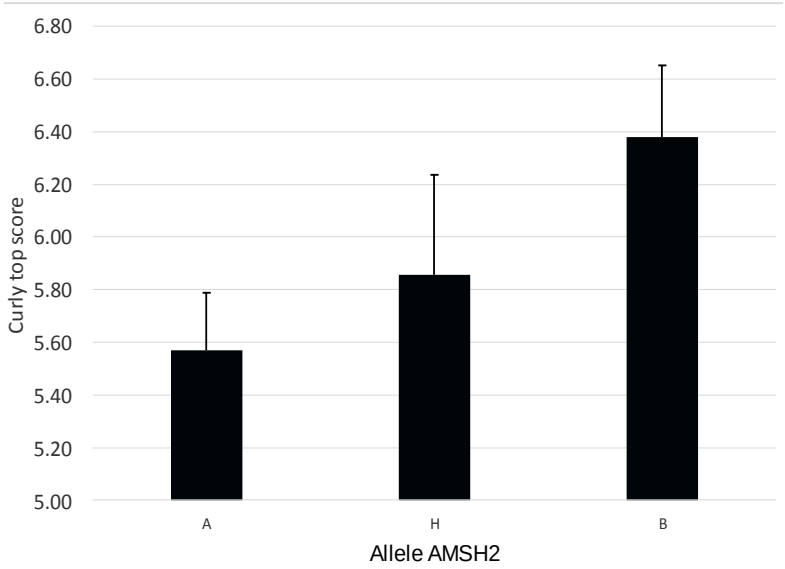


Fig 3: Sugar beet field trial resistance to Curly top virus in sugar beet plants expressing the N109I mutation in AMSH2. Scoring scale of 1 (not damaged plants) to 9 (plants completely destroyed by Curly top virus).
A are plants that are homozygous, having two mutated alleles of the AMSH2 gene.
H are the hemizygous plants and **B** are the wild-type plants with the wild-type AMSH2 allele.
A clear dose effect of virus resistance can be seen.

In summary:

- AMSH2 has been identified as the target for insect Mp10 effector protein
- Binding of Mp10 to AMSH2 suppresses plant defence responses
- Mp10 is conserved across sap-feeding insects
- AMSH2 is conserved across plant species
- Certain mutations at clearly defined amino acid positions results in Mp10 not being able to bind to AMSH2 and therefore enabling the plant to activate defence responses
- Field trials demonstrate the effectiveness of the AMSH2 mutation to reduce presence of virus
- Target sites for mutating AMSH2 have been identified in many crop species

For more information or licensing interest, please contact PBL

References:

Not yet published in the scientific literature