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MOS2 has redundant function with its homolog MOS2H and is required for proper splicing of *SNC1*

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Plant immunity is essential for plant survival and resistance (R) proteins serve essential roles in pathogen detection and defense signal initiation. A gain-of-function mutation in *SNC1*, a TIR-type *R* gene, results in a characteristic autoimmune phenotype in *Arabidopsis*. From a forward genetic suppressor screen using *snc1*, *MOS2* (*MODIFIER of snc1*), which encodes an RNA-binding protein, was identified. When *MOS2* function is lost, the autoimmunity caused by *snc1* is abolished and basal resistance against virulent pathogens is attenuated. Recently, it was shown that *mos2* mutants also have defects in miRNA processing. However, it is not known how the role of MOS2 in miRNA production is related to the suppression of *snc1*-mediated autoimmunity. Here, we show that MOS2 contributes to proper splicing of *SNC1* transcript, agreeing with its potential association with the MOS4-associated complex (MAC). In addition, although mutant plants carrying a mutation in the *MOS2* homolog *MOS2H* are wild-type like, the double mutant *mos2 mos2h* is lethal. These data suggest that *MOS2* and *MOS2H* have unequally redundant functions. Overall, MOS2 and MOS2H probably have diverse functions in both alternative splicing and miRNA processing.

Like all organisms, plants face constant threats from pathogens in their environment. The plasma membrane layer of plant defense involves the recognition of molecules often conserved among large groups of microbial organisms, such as bacterial flagellin.[1] However, successful pathogens are able to secrete or inject molecules termed effectors into plant cells to subvert this layer of defense. Through evolution, plants have evolved many resistance (*R*) genes, which encode proteins that are able to recognize the effectors directly or indirectly.[2] Following recognition, a very strong immune response is usually triggered, which often includes accumulation of salicylic acid (SA), production of reactive oxygen species and hypersensitive response (HR), which is a localized cell death around the site of infection. R gene mediated defense is usually very effective in preventing establishment of infection and thus has been widely used by breeders for crop protection.

Despite the importance of R proteins in plant immunity, little is known about their activation mechanisms. A Modifier of *snc1* (MOS) forward genetic screen was conducted to identify components required for R protein activation, using an autoimmune *snc1* mutant.[3] This allele encodes an altered form of a TIR-NB-LRR-type of R protein which is less vulnerable to protein degradation and therefore accumulates to higher levels in the plant.[4] The over-accumulation of snc1 confers a dwarf, autoimmune phenotype. Mutants in several genes were identified that suppressed the autoimmunity of *snc1*, including *MOS2*.[5,6] As *mos2* suppresses the small stature, high constitutive SA accumulation and enhanced disease resistance characteristics of *snc1*, *MOS2* is necessary for the function of *snc1*. Basal resistance and resistance conferred by other R proteins are also reduced in *mos2* single mutants, suggesting a more general role for *MOS2* in defense. Recently it was further shown that MOS2 is involved in micro RNA (miRNA) maturation by binding to long primary miRNA (pri-miRNA) transcripts and facilitating their recruitment to HYL1, a protein that assists in the production of miRNAs through the cleavage of pri-miRNA.[7] However, how miRNA maturation affects *snc1*-mediated autoimmunty is unclear.

The *Arabidopsis* genome also contains a close homolog of *MOS2*, which was named *MOS2H*.[5] The deduced amino acid sequences of these two proteins share 52% identity. While the null *mos2-2* plants exhibit a slightly stunted morphology and round leaves, the *mos2h-1* knockout mutant (SAIL_223_H01), which carries a T-DNA insertion in the exon of *MOS2H*, does not show any obvious morphological phenotypes (**Fig. 1A**). When we crossed *mos2-2* with *mos2h-1* plants, no homozygous *mos2-2 mos2h* plants were recovered in the F2 generation. Furthermore, several *mos2-2/mos2-2 MOS2h/mos2h-1* plants were allowed to self-fertilize and their offspring were examined for the presence of a double mutant. However, no double mutant plants were recovered. This suggests that *MOS2* and *MOS2H* are partially redundant and required for plant survival, with *MOS2* playing a larger or broader role.

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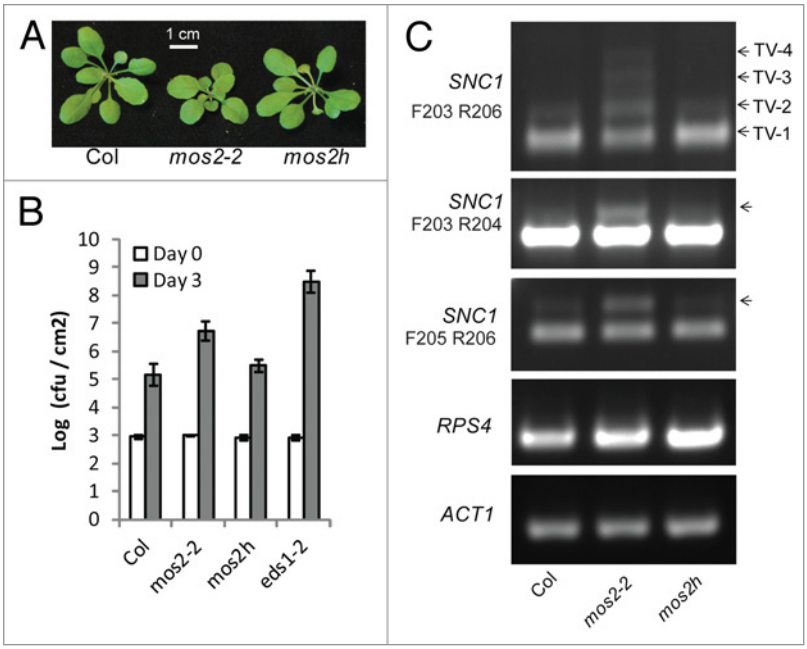

Figure 1. *MOS2* has a role in basal immunity and *SNC1* splicing. (**A**) Morphology of 3-wk-old soil-grown wild-type (Col), *mos2-2* and *mos2h* plants. (**B**) Susceptibility of wild-type, *mos2-2* and *mos2h-1* to *P.s.m.* ES4326. Leaves of 4-wk-old soil-grown plants were infiltrated with a suspension of bacteria at OD_{600} = 0.001 and leaf discs from the infiltrated area were taken at 0 or 3 d to quantify the colony forming units (cfu). Bars represent the means of five replicates and the error bars represent the standard deviations. (**C**) Splicing pattern of *SNC1* and *RPS4*, as determined through RT-PCR. cDNA was amplified using a combination of primers F203, F205, R204 and R206 for *SNC1* and *RPS4-F* and *RPS4-R* for RPS4.[10] Bands representing intron-retained splice variants are indicated by arrowheads. These bands were directly sequenced and confirmed to be intron-retained forms of *SNC1*.

In addition to the morphological phenotypes, *mos2-2* plants also exhibit enhanced disease susceptibility to the virulent pathogen *Pseudomonas syringae* pv *maculicola* (*P.s.m.*) ES4326 (**Fig. 1B**), demonstrating a role for *MOS2* in basal resistance. The growth of *P.s.m* ES4326 in *mos2-2* was not as high as observed in *eds1-2*, which was used as a positive control for defects in basal immunity. However, the resistance of *mos2h-1* plants is not different from wild-type, in agreement with their wild-type-like morphology.

MOS2 binds pri-miRNA and facilitates production of miRNA.[7] Several miRNAs have been shown to be involved in antibacterial resistance.[8,9] The function of MOS2 in defense may be partly explained by its role in miRNA maturation. However, it remains elusive whether MOS2 also can regulate resistance though additional mechanisms. *MOS12,* another gene recovered from the MOS screen, has previously been characterized as a positive regulator of immunity, with a role in the proper splicing of the *R* genes *SNC1* and *RPS4*.[10] MOS12 seems to function together with the spliceosome-interacting MOS4-associated complex (MAC).[10-12] Interestingly, the yeast MOS2 homolog Spp2 and human MOS2 homolog GPKOW/T54 were identified as putative Nineteen complex (NTC) components, with NTC as the equivalent MAC in those organisms.[13,14] Therefore, we examined the contribution of *MOS2* and *MOS2H* to *R* gene splicing. An increase in intron-retained splice forms of *SNC1* was seen in *mos2-2* mutants but not *mos2h* (**Fig. 1C**). Sequencing of the upper bands further confirmed that they are indeed intron-retained splice forms of *SNC1*. However, no difference was observed in the splicing pattern of *RPS4*. This suggests that like MOS12 and the MAC components, MOS2 is also required for the proper splicing of *SNC1*. The autoimmune phenotype of *snc1* mutants is due to increased accumulation of snc1 protein, which activates the immune signaling pathway.[4] A positive feedback loop then increases the transcriptional level of *snc1*, ultimately resulting in further elevated snc1 protein levels. Because of the reduced efficiency of *snc1* mRNA splicing in *mos2* mutant plants, snc1 protein probably cannot accumulate to levels high enough to cause autoimmunity. This partly explains the *snc1*-suppressing phenotype of *mos2*.

It is not known what role MOS2 performs in mRNA splicing, nor how this is related to its other known roles in miRNA biogenesis. It has been suggested that the RNA-binding activity of MOS2 may be involved in the recruitment of pri-miRNA molecules to HYL1 and other proteins responsible for processing into miRNAs.[7] It is possible that MOS2 also binds mRNA and recruits it to the MAC, which further facilitates proper splicing of *SNC1* transcript.[10] Members of the MAC and other pre-mRNA splicing factors have also been implicated in control of RNA-directed DNA methylation, suggesting that splicing-related proteins may play roles in diverse cellular processes.[15] Future in-depth transcriptome analysis of these mutants and refined biochemical dissection of the protein-protein interactions may help us reveal how MOS2 mechanistically regulates plant immunity and plant development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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