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Auxin Binding Protein 1 Reinforces Resistance to Sugarcane Mosaic Virus in Maize

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Abstract
Dear Editor,

Sugarcane mosaic virus (SCMV) causes severe viral diseases in maize worldwide (Fuchs and Gruntzig, 1995), resulting in significant losses in grain and forage yield in susceptible cultivars of maize and related crops. The most promising solution is to cultivate resistant varieties, which contribute to sustainable crop production. Two epistatically interacting major SCMV resistance loci (Scmv1 and Scmv2) are required to confer complete resistance against SCMV in the resistant nearisogenic line F7RR/RR (the letters left of the slash refer to the genotype at Scmv2 on chromosome 3 and those on the right refer to the genotype at Scmv1 on chromosome 6, with R indicating a resistance allele and S a susceptibility allele) (Xing et al., 2006). Scmv2 adds a second layer of resistance to the immediate response mediated by Scmv1. Scmv1 has recently been identified to encode ZmTrxh, which acts as a molecular chaperone to suppress viral RNA accumulation in cytoplasm without eliciting a salicylic acid- or jasmonic acidmediated defense response (Liu et al., 2017).

Disciplines
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Comments
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Auxin Binding Protein 1 Reinforces Resistance to *Sugarcane Mosaic Virus* in Maize

Dear Editor,

Sugarcane mosaic virus (SCMV) causes severe viral diseases in maize worldwide (Fuchs and Gruntzig, 1995), resulting in significant losses in grain and forage yield in susceptible cultivars of maize and related crops. The most promising solution is to cultivate resistant varieties, which contribute to sustainable crop production. Two epistatically interacting major SCMV resistance loci (*Scmv1* and *Scmv2*) are required to confer complete resistance against SCMV in the resistant near-isogenic line $F7^{RR/RR}$ (the letters left of the slash refer to the genotype at *Scmv2* on chromosome 3 and those on the right refer to the genotype at *Scmv1* on chromosome 6, with R indicating a resistance allele and S a susceptibility allele) (Xing et al., 2006). *Scmv2* adds a second layer of resistance to the immediate response mediated by *Scmv1*. *Scmv1* has recently been identified to encode ZmTrxh, which acts as a molecular chaperone to suppress viral RNA accumulation in cytoplasm without eliciting a salicylic acid- or jasmonic acid-mediated defense response (Liu et al., 2017).

The quantitative nature of SCMV resistance, causing escapes and incomplete penetrance (Xing et al., 2006), impedes the functional validation of *Scmv2*. By genotyping all recombinants from our previous study (Ingvardsen et al., 2010), we narrowed *Scmv2* down to a 250 kb region on chromosome 3 between markers 184B1 and 212FG08, covered by overlapped B73 (c0023O09, B0654c18, and c0281K07) and FAP1360A (46H09, 223J01, and 009E08) BAC clones (Figure 1A). Five candidate genes were predicted, encoding auxin binding protein 1 (ABP1), glutathione synthase (GSS), and three unknown/hypothetical proteins (Figure 1A). All five candidate genes were sequenced for both resistant FAP1360A and susceptible F7 parental lines (Figure 1B). Non-synonymous variants between FAP1360A and F7 alleles were exclusively found for *ZmABP1* and *ZmGSS* genes.

We used a transgenic approach to evaluate the contribution of candidate genes to SCMV resistance. T_0 RNAi transgenic plants, either carrying pMCG1005-*ABP1* or pMCG1005-*GSS* constructs (Supplemental Figure 1A), were first crossed with $F7^{RR/RR}$ and then backcrossed to $F7^{RR/RR}$ (Xing et al., 2006), and BC_1 plants homozygous for the *Scmv2* resistance allele were selected for comparison. No plants carrying pMCG1005-*GSS* construct were symptomatic during 4 weeks of observation. From four transgenic events, a total of 10 BC_1 plants harboring the pMCG1005-*ABP1* construct showed obvious SCMV symptoms within 4 weeks post inoculation (wpi) (Figure 1C) with a significantly (P = 0.05) lower *ZmABP1* expression level (Supplemental Figure 1B) and a larger amount of SCMV accumulated in symptomatic transgenic plants compared with non-transgenic siblings determined by ELISAenzyme-linked immunosorbent assay (ELISA) (Supplemental Figure 1C). Thus, we focused on *ZmABP1* in subsequent experiments. The *ZmABP1* alleles of FAP1360A and F7 were designated $ABP1^{FAP}$ and $ABP1^{F7}$, respectively. The complementation construct (pTF-$ABP1^{FAP}$) including native full-length gene$ABP1^{FAP}$ (Figure 1D) was introduced into the maize transformable genotype Hill, and T_0 progeny were crossed with near-isogenic line $F7^{SS/RR}$ and backcrossed to $F7^{SS/RR}$ (Xing et al., 2006). For transgenic event of Aux7-1, 96% BC_1 plants lacking the pTF-$ABP1^{FAP}$ construct were susceptible, in contrast to 45% symptomatic transgenic plants carrying pTF-$ABP1^{FAP}$ (Figure 1E). For Aux 29-1, 92% of plants lacking pTF-$ABP1^{FAP}$ were susceptible, in contrast to 55% carrying pTF-$ABP1^{FAP}$ (Figure 1E). Exogenous $ABP1^{FAP}$ significantly (P = 0.01) increased SCMV resistance, which was associated with higher *ZmABP1* expression levels (Figure 1F).

To identify causal variants responsible for resistance, we characterized *ZmABP1* genomic sequences from resistant inbreds FAP1360A, Pa405, D32, D21, 1145, and susceptible lines F7, D145, and Mo17. No consistent differences were found between resistance and susceptibility alleles. Major differences between FAP1360A and F7 are five SNPs and a 524 bp insertion in FAP1360A (Supplemental Figure 2). FAP1360A and F7 alleles share the same cDNA sequence (603 bp) from RACE (rapid amplification of cDNA ends), except for two non-synonymous SNPs (A18C and A85G) in the first exon, and three synonymous SNPs (G333C, T483A, and A492G) in the third and fourth exons. The 524 bp insertion was also found in Pa405, which is distantly related to FAP1360A. A deletion construct (pTF-$dABP1^{FAP}$) was created by deleting the 524 bp region from the pTF-$ABP1^{FAP}$ construct (Figure 1D), and validated in transgenic plants as complementation construct. Of 80% plants lacking pTF-$dABP1^{FAP}$ were symptomatic, in contrast to 18% of plants carrying pTF-$dABP1^{FAP}$ from transgenic event Mid9-1, as well as for transgenic event Mid15-4 (Figure 1G). Increased resistance of plants harboring pTF-$dABP1^{FAP}$ suggest that the 524 bp insertion/deletion has no or negligible impact on SCMV resistance. *ZmABP1* expression level again was significantly (P = 0.01) associated with the disease resistance (Figure 1H).

Increased *ZmABP1* expression was observed at 14 days post inoculation (dpi) for both F7 and $F7^{RR/RR}$ plants compared with mock treatment, and it was 1.7-2.2 fold higher for inoculated $F7^{RR/RR}$ compared with inoculated F7 at later time points (Figure 1I). In contrast, no late response was found in the expression of other positional candidate genes (Supplemental Figure 3). The higher and late SCMV-induced *ZmABP1* expression level is consistent with the previous finding that *Scmv2* is

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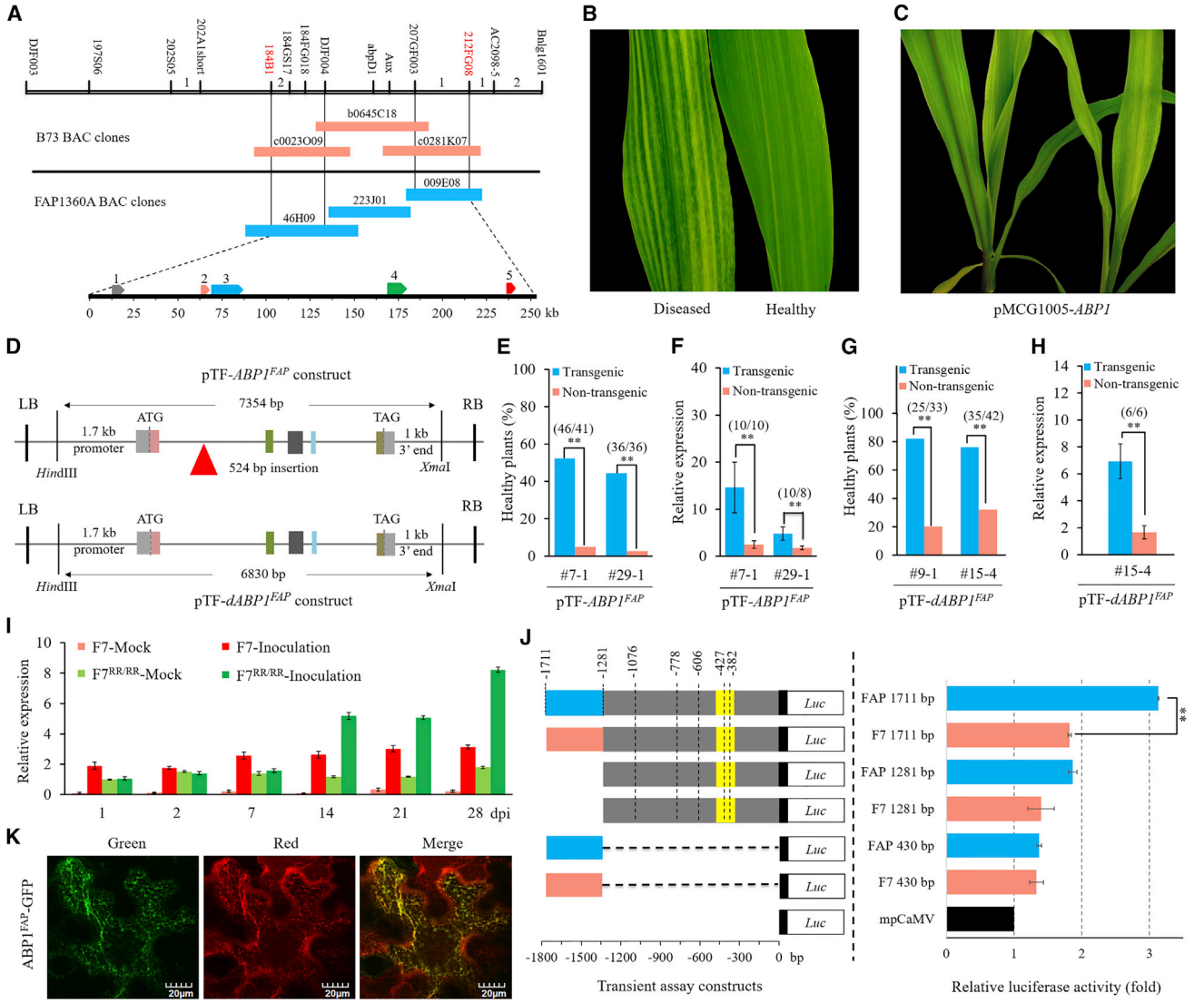

Figure 1. *ZmABP1* Underlies *Scmv2* and Its Promoter Variation Matters Its SCMV Resistance.

(A) Map-based cloning of *Scmv2*. 12 polymorphic markers were used for mapping *Scmv2*. The numbers above the bar indicate the recombinants found between adjacent markers. The *Scmv2* gene was fine mapped to the interval between the boundary markers 184B1 and 212FG08 (in red) on chromosome 3. The three blue clones from FAP1360A composed the minimal tiling path for *Scmv2*. Five genes were predicted for the *Scmv2* region (AGPv3). 1, hypothetical protein; 2, putative uncharacterized protein; 3, glutathione synthetase; 4, auxin binding protein 1; 5, unknown.

(B) SCMV infection causing typical chlorosis in susceptible parental line F7 (left) and resistant parental line FAP1360A (right).

(C) RNAi of the *ZmABP1* allele resulted in symptomatic lines (right) with typical chlorosis in emerging leaves. Resistant control (left).

(D) The complementation construct of *ZmABP1* (pTF-$ABP1^{FAP}$) included a ~1.7 kb native promoter, 4.5 kb coding, and 1 kb terminator sequence. It was excised from the FAP1360A BAC clone and inserted into pTF101.1. The deletion construct (pTF-$dABP1^{FAP}$) includes a 1.7 kb native promoter, 4.0 kb coding, and 1 kb terminator sequence modified from the pTF-$ABP1^{FAP}$ construct by deleting the 524 bp insertion and inserted into pTF101.1.

(E) Transgenic lines carrying the pTF-$ABP1^{FAP}$ construct (from transgenic events 7-1 and 29-1) showed increased SCMV resistance. Numbers on the top of each column refer to the individual lines used for comparison, the same as below; $^{**}P = 0.01$.

(F) Higher *ZmABP1* RNA expression level exists in resistant transgenic plants carrying pTF-$ABP1^{FAP}$ construct. Data are shown as means ± SE. Asterisks on the top of each column indicate significant differences ($^{**}P = 0.01$).

(G) Increased resistance of plants containing pTF-$dABP1^{FAP}$ suggesting that the 524 bp insertion/deletion has no impact on SCMV resistance; $^{**}P = 0.01$.

(H) Transgenic lines carrying pTF-$dABP1^{FAP}$ constructs showed an increased *ZmABP1* RNA expression level for events 15-4 in comparison with F7. Data are shown as means ± SE. Asterisks on the top of each column indicate significant differences ($^{**}P = 0.01$).

(I) Expression of the *ZmABP1* alleles in resistant $F7^{RR/RR}$ and susceptible F7 under virus and mock inoculation. The mean ± SE was obtained from three biological and three technical replications for each. Inoculation, plants were inoculated with SCMV. Mock, plants were inoculated with SCMV-free PBS buffer. dpi, days post inoculation.

(J) Promoter variation between *ZmABP1* alleles determines its expression level. Left, the construct backbone consists of different *ZmABP1* promoter fragments controlling expression of the firefly luciferase activity (*Luc*, white box). 430 bp (red/blue box), 1281 bp (gray box), and 1711 kb (red/blue + gray box) promoter components from F7 and FAP1360A were cloned into restriction sites upstream of the minimal promoter (black box). Five SNPs (dashed

(legend continued on next page)

active at later stages post SCMV infection (Xia et al., 1999; Xing et al., 2006). We characterized allelic *ZmABP1* promoter activity for FAP1360A and F7 using maize protoplast transient expression assays. Compared with the 1711 bp $ABP1^{F7}$ promoter, the corresponding $ABP1^{FAP}$ promoter segment led to 2-fold and thus substantial and significant increase ($P = 0.01$) of LUC activity (Figure 1J). We observed a slightly but not significantly increased LUC activity for the 1281 bp promoter of $ABP1^{FAP}$ compared with $ABP1^{F7}$. However, no significant difference was found between the respective 430 bp promoters (Figure 1J). These results indicate that the 430 bp $ABP1^{FAP}$ promoter segment between −1282 bp and −1711 bp contains a functional *cis*-regulatory element, promoting luciferase activity and by inference, *ZmABP1* expression *in vivo*, thereby promoting SCMV resistance. We identified two *cis* elements in the FAP1360A 430 bp promoter region that are absent in the corresponding region of F7: a BoxII motif, and three Sp1 motifs arranged in tandem. Both are light responsive, which might be required for increased *ZmABP1* expression levels in response to light, resulting in light-dependent virus resistance (Chandra-Shekara et al., 2006).

The endoplasmic reticulum (ER) is the site for potyvirus 6K2 protein-induced vesicle assembly and viral replication (Restrepohartwig and Carrington, 1994). ZmABP1 encoded by either $ABP1^{FAP}$ or $ABP1^{F7}$ colocalized with ER- (CD3-959) and plasma membrane (PM) (CD3-1007)-specific markers, revealing the presence of ZmABP1 at both ER and PM (Figure 1K; Supplemental Figures 4–6). These findings suggest no detectable effect of two polymorphic amino acids of ZmABP1 on its subcellular localization in either resistant or susceptible genotypes. Viral RNA colocalized with ER, where the virus replication complex (VRC) is initiated and viral replication takes place. We evaluated the effect of *ZmABP1* alleles on SCMV replication using a transient assay based on maize protoplasts *in vivo*. SCMV replication was not impaired by increasing *ZmABP1* expression (Supplemental Figure 7). This suggests that ER-localized ZmABP1 has no effect on SCMV replication and is not the cause of SCMV resistance.

We next screened for ZmABP1-interacting proteins by yeast two-hybrid assays using $F7^{RR/RR}$ derived ABP1 as bait, and obtained ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (RbCS), which showed the strongest interaction with ZmABP1 (Supplemental Figure 8). Recently, NtRbCS was reported to be the host target of potyvirus P3 protein and Tobamovirus multifunctional movement protein for virion replication (Danci et al., 2009) and systemic movement (Zhao et al., 2013). Transiently expressed fluorescence was detected in *Nicotiana benthamiana* lines co-infiltrated with pABP1-YFP^C and pRbCS-YFP^N through a bimolecular fluorescence complementation (BiFC) assay (Supplemental Figure 9). Co-immunoprecipitation (Co-IP) assays further demonstrated an interaction between ZmABP1 and RbCS (Supplemental Figure 10). ZmRbCS also interacted with $ABP1^{F7}$ (Supplemental Figure 11). Whether and how the interaction between ZmABP1 and ZmRbCS is associated with SCMV resistance remains unknown; further studies are needed to reveal the *ZmABP1*-mediated SCMV resistance mechanism.

Scmv1 most probably suppresses SCMV replication and slows local spread as the "frontline of defense" probably around 2 wpi (like $F7^{SS/RR}$) (Xing et al., 2006). When SCMV overcomes this "frontline of defense", rapid systemic movement follows via sieve elements. RbCS might be recruited for VRC formation by viral proteins and for systemic movement. *ZmABP1* expression is greatly stimulated by SCMV infection in resistant genotypes, reinforcing resistance to viruses that escaped from the *Scmv1* barrier at later stages. As a consequence, ZmRbCS would neither be available for SCMV genome amplification nor utilized for potyvirus long-distance movements (Danci et al., 2009; Zhao et al., 2013). *Scmv2* also confers resistance to other potyviruses, such as *Maize dwarf mosaic virus* and *Wheat streak mosaic virus* (Lübberstedt et al., 2006). Our study provides evidence for a crucial role of *ZmABP1* in potyvirus resistance and will be beneficial for breeding of potyvirus multiresistant cultivars, adding an unexpected and exciting aspect to its biological functions. Further studies toward understanding the molecular and genetic mechanisms of *ZmABP1*-mediated SCMV resistance are still needed.

ACCESSION NUMBERS

Sequence data are deposited with NCBI GenBank under accession numbers: Genbank: KY364860–KY364868.

SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

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AUTHOR CONTRIBUTIONS

The project was conceived and designed by T.L, P.L., and Q.J. P.L. and Q.J. performed experiments, data analysis, and generated data figures. T.A., C.R.I., and B.S. screened the BAC clone library, U.K.F. and Y.X. helped with sequence analysis, M.R. provided SCMV antibody, M.R. and M.J. assisted with virus inoculation. P.G., S.L., and F.L. performed part of the RT–PCR experiments, P.L. and Q.J. wrote the manuscript, G.P., T.L. and M.X. edited and revised the manuscript, and all authors read and approved the final manuscript.

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lines) are the major difference in the 1281 bp region, two in the *Tourist-Zm11* (yellow box) region, three in the remaining promoter region. Right, horizontal bars show the corresponding expression levels of firefly Luciferase relative to Renillaluciferase. The data are shown as means ± SE, obtained from three biological replicates. Asterisks on the right of the column indicate significant differences (**$P = 0.01$).

(K) $ABP1^{FAP}$ co-localized with the endoplasmic reticulum (ER) marker ER-rk CD3-959 in transiently transformed *N. benthamiana* as indicated by the overlap of green and red fluorescence under a confocal laser scanning microscope. Image was taken 72 hours post transformation.

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