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Commentary

Frontline Warrior microRNA167: A Battle of Survival

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Abstract: Plant pathogens such as viruses are detrimental to the survivorship of plant species. Coinfection of maize chlorotic mottle virus (MCMV) and the sugarcane mosaic virus (SCMV) causes a deadly disease in maize. An investigation by Liu et al. (2022) showed the role of *Zma-miR167* in positively imparting resistance against the MCMV and SCMV. The authors identified *ZmARF3* and *ZmARF30* as the targets of *Zma-miR167*. *ZmARF3* and *ZmARF30* were identified as transcription factors that bind the cis-element in *ZmPAO1* promoters to activate its expression. The authors showed how the *Zma-miR167-ZmARF3/30-ZmPAO1* module functions differently in resistant and susceptible lines with high expression of *Zma-miR167* in resistant lines correlated with the resistant phenotype. Finally, the authors concluded that MCMV-encoded p31 protein enhances ZmPAO1 enzyme activity for its survival in the host.

Keywords: ARF; maize chlorotic mottle virus; maize lethal necrosis; microRNA; polyamine oxidase 1; sugarcane mosaic virus

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Maize is considered a major staple food crop vulnerable to disease outbreaks. Maize Lethal Necrosis (MLN) is one of the severe diseases caused by coinfection of the maize chlorotic mottle virus (MCMV) and the sugarcane mosaic virus (SCMV) [1]. Potyviruses, including SCMV, are diverse and ubiquitous in nature, unlike MCMV; hence, MLN disease outbreaks depend on the emergence of MCMV [1,2]. Until now, no maize varieties have complete resistance to MCMV [3,4] and the molecular mechanism of MCMV infection remains unexplored. Recent MLN outbreaks have become a significant concern for maize production and global food security [5].

How do the host and pathogen help each other to evolve? Pathogen infection is critical for any host to increase fitness and sustain itself in an unfavorable environment. Similarly, pathogens exploit the host machinery to thrive and escape the host defense response. Liu et al. (2022) investigated the role of maize *Zma-miR167-ARF3/30*-mediated resistance against MCMV and the use of *ZmPAO1* as a counter-defense response employed by MCMV [6]. MicroRNAs are post-transcriptional regulators of gene silencing [7]. They are reported to regulate plant growth and development processes as well as biotic and abiotic stress responses [8,9]. Authors discovered that *Zma-miR167* was substantially upregulated upon MCMV infection. Overexpression of *pre-MIR167b* (*Zma-miR167OE*) decreased MCMV genomic RNA and coat protein (CP) levels and showed mild mosaic symptoms in plants compared to chlorotic and mottle symptoms in wild-type plants upon viral infection. At the same time, *Zma-miR167* silencing resulted in severe leaf chlorosis and an increased MCMV genomic RNA and CP accumulation. Moreover, the basal level of *Zma-miR167* was examined in resistant and susceptible lines upon MCMV infection, and higher expression of *Zma-miR167* in resistant lines suggested that *Zma-miR167* may impart resistance against MCMV. Further, the author observed lower coinfection efficiency of MCMV and SCMV in resistant lines than in susceptible lines. These results, in conjunction, indicated that increased expression of *Zma-miR167* plays an essential role in providing resistance to MLN.

What is the underlying mechanism of *Zma-miR167*-mediated resistance to MCMV? To address this question, the authors used an array of experiments for identifying *Zma-miR167* target candidates, *ZmARF3* and *ZmARF30*. Increased expression of *ZmARF3* and *ZmARF30* was observed in *Zma-miR167* knockdown, while *ZmARF3* and *ZmARF30* expression was decreased in *Zma-miR167* overexpression lines. Further, *Nicotiana benthamiana* leaves expressing the GFP fusion of these two target candidates showed reduced green fluorescence and corresponding protein levels upon an increase in *Zma-miR167* levels. The virus-induced gene silencing of *ZmARF3* and *ZmARF30* led to a similar phenotype to *Zma-miR167* overexpression, i.e., mild symptoms and reduced genomic RNA and CP levels upon MCMV infection. The findings indicated that *Zma-miR167* targets *ZmARF3* and *ZmARF30,* and *ZmARF3/30* negatively regulates plant resistance against MCMV.

To understand further the *Zma-miR167-ZmARF*-mediated signaling cascade, the authors performed transcriptomic analysis of *Zma-miR167* overexpression and knockdown lines and their respective controls. The antagonistically expressing common DEGs were evaluated for downstream analysis, including GO enrichment, which revealed that these DEGs primarily regulate the oxidation–reduction biological process. The authors have further performed a promoter scan on DEGs to determine whether these DEGs are also directly regulated by ARF. They narrowed it down to seven DEGs, particularly *polyamine oxidase 1* (*ZmPAO1*), the hydrogen peroxide (H_2O_2) production gene that has binding sites for ZmARFs and participates in the oxidation–reduction process.

To understand the role of *ZmPAO1* in the *Zma-miR167-ZmARF* signaling cascade during MCMV infection, the authors performed various promoter binding and transcriptional activity assays to validate the strong affinity between ZmARF3/ZmARF30 and *ZmPAO1* promoter. *ZmPAO1* followed the same expression pattern trend as *ZmARF3/ZmARF30* in overexpression and knockdown lines of *Zma-miR167,* and silenced plants of *ZmPAO1* are also less sensitive to MCMV infection. Moreover, to explore the correlation between ZmPAO activity and MCMV infection, the authors treated maize plants with the ZmPAO activity inhibitor, Guazatine. The treated plants showed fewer MCMV-CP than the control plants. These findings suggest that the *Zma-miR167–ZmARF3/30* module minimizes MCMV infection, whereas *ZmPAO1* helps in viral infection.

Intriguingly, although the authors found less MCMV infection in *Zma-miR167OE* compared to the control, MCMV can still survive in *Zma-miR167OE*, indicating that MCMV employs a counter-defense mechanism. To understand the MCMV-mediated defense mechanism, the authors performed the yeast two-hybrid interaction between the ZmPAO1 and each of seven MCMV-encoded proteins. The screening revealed an interaction between ZmPAO1 and MCMV-encoded p31 protein. Additional in vivo and in vitro interaction techniques validated the physical interaction between ZmPAO1 and MCMV p31. Moreover, co-expression of p31 with ZmPAO1 enhanced ZmPAO1 activity and hence increased H_2O_2 production compared to the control, indicating p31 is positively maintaining the stability of ZmPAO1. Overall findings suggested *Zma-miR167* plays a vital role in resisting the MCMV infection, and MCMV reiterates back involving a counter-defensive approach by enhancing PAO activity (Figure 1). This study provided a promising candidate, *Zma-miR167*, that can be used to screen the MLN/MCMV resistant varieties. Also, the *Zma-miR167-ZmARF3/30-ZmPAO1* module can be utilized in genetic engineering and breeding programs to generate virus-resistant maize plants. Future research may seek to understand the molecular mechanism behind *Zma-miR167–ZmARF3/30*-mediated regulation of the jasmonic acid, salicylic acid and brassinosteroid signaling pathway to generate MCMV-resistant lines.

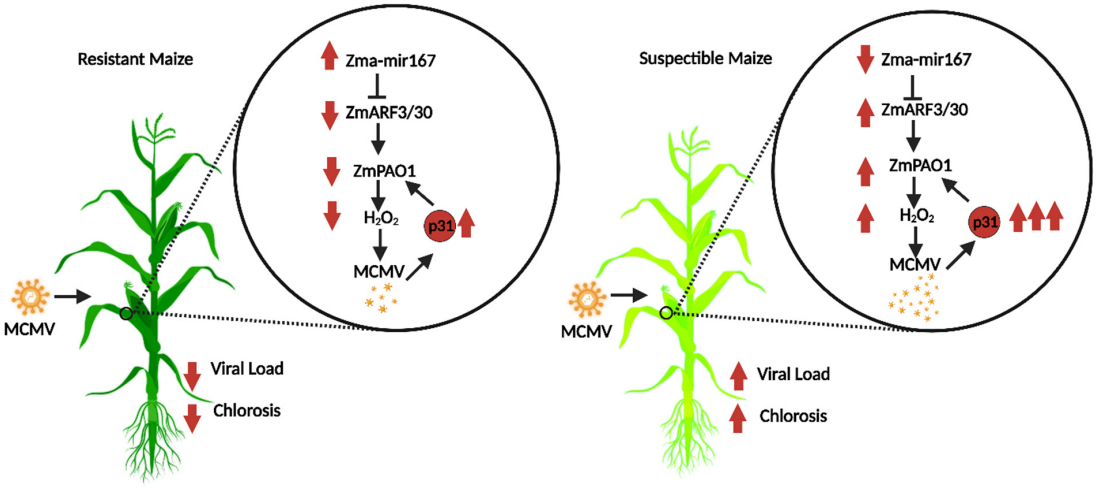

Figure 1. A schematic representation showing *Zma-miR167*-mediated resistance against maize chlorotic mottle virus (MCMV). *Zma-miR167* targets maize auxin response factor genes *ZmaARF3* and *ZmaARF30.* ZmaARF3/30 protein acts as a transcription factor to activate the expression of *ZmaPAO1*, resulting in H_2O_2 production and helping MCMV infection. The resistant maize lines have higher expression of *Zma-miR167*; hence, expression of target genes *ZmaARF3/30* and *ZmaPAO1* decreases. Lower levels of ZmaPAO1 protein results in less H_2O_2 production and MCMV infection. MCMV tries to use the viral p31 protein to stabilize ZmaPAO1 for increased H_2O_2 production and viral load. Contrastingly, susceptible maize lines show lower *Zma-miR167* expression and correspondingly higher expression of *ZmaARF3/30* and *ZmaPAO1.* Higher ZmaPAO1 protein levels result in H_2O_2 production and increased MCMV infection. Downward and upward-facing arrows indicate lower and higher levels of respective genes/proteins/ and viral load or chlorosis. The figure is adapted and modified from Liu et al., 2022 and created on Biorender.com.

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