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RESEARCH ARTICLE

Silencing *ZmPP2C-A10* with a foxtail mosaic virus (FoMV) derived vector benefits maize growth and development following water limitation

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ABSTRACT

- Global climate change is causing more frequent and severe droughts, which can have negative impacts on plant growth and crop productivity. Under drought conditions, plants produce the hormone ABA (abscisic acid), which regulates adaptive responses, such as stomatal closure and root elongation. Plant viruses have been used in the lab to convey new traits to plants and could also be used to increase production of ABA or to enhance downstream plant drought resistance responses.
- In this study, foxtail mosaic virus (FoMV) was used to silence *ZmPP2C-A10*, a negative regulator of ABA signalling, in maize (*Zea mays* L.). Both silenced and control plants were exposed to an 8-day drought treatment, followed by a 30-day period of rewatering, after which indicators of drought resistance were measured.
- After drought treatment, we observed a nearly twofold increase in expression of a stress-mitigation gene, *ZmRAB17*, reduced chlorophyll fluorescence changes (indicator of stress), and increased plant biomass and development in the *ZmPP2C-A10*-silenced maize compared to controls.
- These results demonstrate that the FoMV system can be used to silence endogenous expression of *ZmPP2C-A10* and increase maize tolerance to drought. This could offer a useful tool to improve crop traits and reduce yield loss during the growing season.

INTRODUCTION

Drought is a natural phenomenon that can have disastrous effects on natural and agricultural ecosystems (Madadgar *et al.* 2017; Berg & Sheffield 2018). Drought stress can reduce crop photosynthetic efficiency, productivity, and yield (Bartels & Sunkar 2005; Farooq *et al.* 2009; Madadgar *et al.* 2017). Furthermore, over 39% of global maize (*Zea mays* L.) yields are lost to drought each year (Daryanto *et al.* 2016). As climate change worsens, we will experience more frequent and severe droughts (Pokhrel *et al.* 2021), which will make it increasingly difficult to sustain the global population and ensure food security (Gupta *et al.* 2020). Developing novel methods to enhance crop drought resistance and tolerance, such as with bioengineering tools, will be crucial for sustaining our growing global population amid the challenges posed by climate change.

Plants respond to drought stress by regulating the biosynthesis, accumulation, and processing of the phytohormone abscisic acid (ABA; Vishwakarma *et al.* 2017; Chen *et al.* 2020). ABA can help plants tolerate drought stress by regulating the osmotic potential of stomatal guard cells, which reduces water loss through transpiration (Daszkowska-Golec & Szarejko 2013; Gupta *et al.* 2020). Additionally, accumulation of ABA activates various downstream drought-responsive genes that have a range of functions in osmotic stress responses. These genes encode late embryogenesis abundant (LEA) proteins, aquaporins, enzymes involved in osmoprotectant synthesis, and transcription factors involved in osmotic stress protection (Chandler & Robertson 1994; Zhang *et al.* 2006; Fujita *et al.* 2011). Previous research has shown that increasing ABA concentrations through chemical application (Zhang *et al.* 2016; He *et al.* 2019a; Li *et al.* 2020), overexpression of ABA-biosynthetic genes (Zhao *et al.* 2016; Hong *et al.* 2022; Zhang *et al.* 2022), and knocking out ABA catabolic genes (Umezawa *et al.* 2006; Zhang *et al.* 2020) positively influence plant responses to drought and osmotic stress.

Recent studies have shown that virus-infected plants are more drought resistant compared to uninfected plants (Xu *et al.* 2008; Aguilar *et al.* 2017; Shteinberg *et al.* 2021; Mishra *et al.* 2022; Prakash *et al.* 2023), and the expression of certain viral proteins can increase drought tolerance in host plants (Westwood *et al.* 2013; Corrales-Gutierrez *et al.* 2020; Prakash *et al.* 2023). Viruses have also been used as tools to silence different components of the ABA signalling pathway using virus-induced gene silencing (VIGS) (Manmathan *et al.* 2013; Ramegowda *et al.* 2014; Ogata *et al.* 2017; Shinwari *et al.* 2020), which can either decrease or increase plant tolerance to drought stress. For example, targeting positive regulators like SNF1-related kinase2 (SnRK2) in cotton (*Gossypium hirsutum* L.) using tobacco rattle virus (TRV) reduces plant survival under drought conditions (Bello *et al.* 2014), while targeting negative regulators like ERA1 (ENHANCED RESPONSE TO ABSCISIC ACID) and SAL1 (INOSITOL POLYPHOSPHATE 1-PHOSPHATASE) using barley stripe mosaic virus (BSMV) in wheat (*Triticum aestivum* L.) increases plant relative water content and reduces leaf transpiration rates (Xiong *et al.* 2001;

Manmathan *et al.* 2013; Jalakas *et al.* 2017). Similarly, silencing *GmERA1A* in soybean (*Glycine max* L.) using apple latent spherical virus (ALSV) reduces stomatal aperture under drought stress, resulting in reduced plant water loss and wilting (Ogata *et al.* 2017). These findings suggest that virus-based methods can be used to dynamically regulate plant tolerance to drought stress, however other targets for VIGS still need to be identified.

One potential VIGS target for enhancing drought tolerance is clade A 2C protein phosphatases (PP2C-A). PP2C-A proteins act as negative regulators of ABA signalling and drought responses by suppressing SnRK2, an important regulator of ABA-responsive transcription factors (Fig. 1; Wei & Pan 2014; Hirayama & Umezawa 2010; Chen *et al.* 2020). Under water-limited conditions, ABA binds to receptors (PYR/PYL/RCAR family proteins), which then suppress the activity of PP2C-A and increases the induction of drought responses through SnRK2. Previous research has shown that overexpression of certain *ZmPP2C-A*s (*ZmPP2C-A2*, *-A6*, and *-A10*) in maize and *Arabidopsis* decreases drought tolerance and delays recovery after drought (Liu *et al.* 2009; Xiang *et al.* 2017; He *et al.* 2019b). However, to our knowledge, the potential to use viruses to silence *ZmPP2C-A*s and enhance drought tolerance has not been evaluated.

Foxtail mosaic virus (FoMV) is a positive sense single stranded (ss) RNA member of the Potexvirus family (Bancroft *et al.* 1991; Robertson *et al.* 2000; Bruun-Rasmussen *et al.* 2008). Recently, a DNA-based VIGS vector was developed from FoMV which can establish whole-plant systemic infections (Mei *et al.* 2016). The FoMV vector has been successfully utilized in maize, barley, wheat, and foxtail millet for RNA silencing (Liu *et al.* 2016; Mei *et al.* 2019), as well as in virus-mediated overexpression (VOX) experiments in maize and wheat (Bouton *et al.* 2018; Prakash *et al.* 2023). In this study, FoMV was used to silence *ZmPP2C-A10* in maize, and indicators of drought tolerance were measured in silenced plants. Our results demonstrate that even mild silencing of *ZmPP2C-A10* (~25%) increased expression of *ZmRab17*, an osmotic stress mitigating gene, and reduced changes in chlorophyll fluorescence, an indicator of stress. Furthermore, silenced plants exhibited increased growth and development after rewatering compared to control plants, suggesting they were better able to recover from the drought event. In summary, our study identifies another target for enhancing drought tolerance and indicates virus-based tools could be a promising mid-season strategy to overcome ongoing climatic changes.

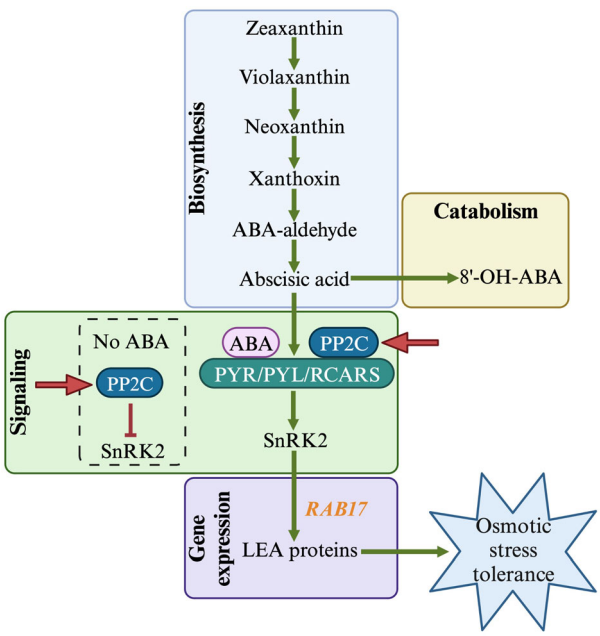

Fig. 1. ABA biosynthesis, catabolism, signalling, and downstream gene expression. *ZmPP2C-A10* (denoted by red arrows) was silenced in maize plants using an FoMV-derived vector. Gene expression was measured for *ZmPP2C-A10* and a downstream dehydrin-encoding gene (*RAB17*: RESPONSIVE TO ABA 17) in this experiment. This figure was modified from Leng *et al.* (2014) and Zhang *et al.* (2019).

MATERIAL AND METHODS

FoMV constructs and infection

A 192-bp fragment of *ZmPP2C-A10* (GRMZM2G177386) was cloned into multiple cloning site II (MCSII) of the pFoMV plasmid vector using XbaI and XhoI to generate the construct pFoMV-*ZmPP2Ci*. *Agrobacterium tumefaciens* (GV3101 strain) containing the pFoMV or pFoMV-*ZmPP2Ci* construct was grown on LB plates supplemented with 50 μM kanamycin and 10 μM rifampicin. Single colonies were then inoculated in LB broth containing the same antibiotics and grown overnight. *Agrobacterium* liquid cultures were pelleted using a centrifuge and resuspended in an infiltration buffer containing 0.5 M $MgSO_4$, 0.5 M MES, and 200 μM acetosyringone. After a 2-h dark period, 9-day-old *Z. mays* (var. Golden × Bantam) were agroinfiltrated with one of the *Agrobacterium* solutions (OD_{600} = 1.0). Plants were infiltrated by injecting 1 ml agroinfiltration solution into the coleoptile node at the base of the stem using a needle syringe (0.4 × 13.0 mm). After infiltration, plants were placed in the dark at 25 °C for 24 h, then moved to a growth chamber (16-h light: 8-h dark, 25–27 °C).

RNA extraction and RT-PCR validation of infection

Two weeks after infiltration, systemic leaves were harvested, and total RNA isolated using the Quick-RNA™ MiniPrep kit (Zymo Research, Irvine, CA, USA). First-strand cDNA was synthesized from 1000 ng RNA using oligo dT according to the manufacturer's instructions (SMART® MMLV; Takara Bio, San Jose, CA, USA). To determine infection status and confirm there was no loss of the *ZmPP2C-A10 silencing* insert, RT-PCR was performed using FoMV-specific primers flanking the MSCII (Table S1) using the following parameters: 95 °C for 2 min, then 95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s for 31 cycles, followed by 72 °C for 5 min and 4 °C for ∞.

Drought and rewatering treatment

One week after RT-PCR confirmation of infection, potted maize plants were watered until run-off. Twenty-four hours later, the potted maize plants were weighed to obtain baseline mass. Plants were then subjected to an 8-day drought, where soil water content was reduced and maintained at 40% of baseline pot mass to simulate drought conditions (following Kansman

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et al. 2022). The 40% baseline mass was maintained by weighing potted plants each day during the drought period and adding water if the pot mass fell below the baseline. At the end of the 8-day drought period, the soil of each pot was water-saturated, and plants were then watered normally for 30 days. For transcript analysis, leaf tissue samples were collected from developmentally matched leaves before drought and on the final day of drought treatment (day 8). For ABA analysis, leaf tissue samples were collected from developmentally matched leaves on the final day of drought treatment (day 8) and after the 30-day rewatering period. Each experiment contained 5–22 plants per treatment and was repeated three times, with individual traits measured in at least two of the three experiments.

Concentration of ABA

To examine the effect of silencing *ZmPP2C-A10* on drought-induced ABA accumulation in maize, we quantified ABA levels in FoMV- and FoMV-*ZmPP2Ci*-infected plants under water-limited conditions and after a rewatering period, using LC/MS as previously described (Blundell *et al*. 2020; Prakash *et al*. 2023). In brief, plant samples were lyophilized, weighed, then ground to a fine powder. A total of 1 ml phytohormone extraction buffer (2:1:0.005 of iso-propanol: HPLC grade H_2O: hydrochloric acid; v:v:v) spiked with 1000 ng·µl^{-1} deuterated standard of ABA ((+)-ABA-d6; Cayman Chemical, Ann Arbor, MI, USA) was added to each sample. Next, samples were extracted and injected into a Dionex UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) with a Kinetix C18 column of particle size 1.7 µm, length 150 × 2.1 mm, 100 Å (Phenomenex, Torrance, CA, USA). The gradient used was 1% (v/v) isocratic acetonitrile for 3 min, then linear increase of acetonitrile 98% (v/v) for 17 min, followed by isocratic acetonitrile 98% (v/v) for 5 min, and a linear decrease to 1% (v/v) for 3 min. Phytohormones were detected in an Orbitrap-Q Exactive mass spectrometer (Thermo Fisher Scientific) with a mass-to-charge scan range of 75–300 and polarity of the run set as negative. Individual hormones were identified by the signature ions and retention time of ABA and the deuterated ABA-d6 standard, and peak areas determined using the Xcalibur 3.0 program (Thermo Fisher Scientific, Waltham, MA, USA). ABA concentrations were quantified by comparing the peak area of the endogenous ABA with 1000 ng spiked deuterated ABA-d6, and standardized to sample dry mass.

Drought-responsive gene expression

We performed quantitative real-time PCR (qRT-PCR) to evaluate silencing efficiency of *ZmPP2C-A10* and to determine the effects of silencing *ZmPP2C-A10* on the expression of a *Z. mays* dehydrin-encoding gene (*RESPONSIVE TO ABA 17* [*ZmRAB17*]). RNA was isolated and cDNA synthesized as described above. cDNA was diluted 1:10 with molecular grade water and used as template for qRT-PCR. Gene-specific primers were designed for genes of interest and actin was used as the maize reference gene (Table S1). All qRT-PCR reactions used SsoAdvance™ Universal SYBR® Green Supermix (BioRad Laboratories, Hercules, CA, USA) and were run on a CFX384™ Optics Module Real-Time System (BioRad Laboratories). The program had an initial denaturation for 2 min at 94 °C, followed by 40 cycles of 94 °C for 15 s and 55 °C for 1 min. Relative expression of genes was calculated using the delta-delta Ct method ($2^{-\Delta\Delta Ct}$) (Livak & Schmittgen 2001).

Chlorophyll fluorescence

To determine the effect of silencing *ZmPP2C-A10* on drought-induced plant stress, we assessed changes in chlorophyll fluorescence (F_v/F_m) using a handheld FluorPen FP 110/D (Photon Systems Instruments, Drásov, Czech Republic). Chlorophyll fluorescence is widely used to monitor photosynthetic performance and as an indicator of plant stress (Baker 2008; Murchie & Lawson 2013; Pérez-Bueno *et al*. 2019). The F_v/F_m ratios were measured on plants prior to the start of the drought treatment (*i.e*., under normal, well-watered conditions) and on the final day of the drought treatment. Three measurements were taken on a single leaf blade of each plant and averaged. Change in chlorophyll fluorescence was calculated as the percentage difference between F_v/F_m ratios of individual plants before and during drought treatment.

Tassel production and plant biomass

To assess the effect of silencing *ZmPP2C-A10* on the growth and development of maize plants following drought, we allowed plants to develop for another 30 days under well-watered conditions. Next, we recorded the proportion of FoMV- and FoMV-*ZmPP2Ci*-infected plants producing tassels. Plants were then cut at the base and aboveground shoots dried in an oven at 40 °C for 7 days. Roots were washed and cleaned of all soil before drying in an oven at 40 °C for 7 days. Once dry, shoots and roots were weighed separately. Root:shoot ratio was calculated by dividing the dry mass of plant roots by the dry mass of the plant shoots.

Statistical analysis

All statistical analyses were performed using the R statistical software (R Core Team 2021). Data transformations were performed when needed to meet the assumptions of each statistical test. Linear mixed-effects model ANOVAs ('*lmer*') were used to examine silencing efficiency of *ZmPP2C-A10*, impacts of silencing on transcript abundance of *ZmRAB17*, ABA concentration, and root and shoot biomass. Experimental replicate was included as a random factor in each model. Data transformations did not correct for the violations of the assumptions of normality for comparison of chlorophyll fluorescence (F_v/F_m), total biomass, and root:shoot ratio, so non-parametric Wilcoxon rank sum tests ('*wilcox.test*') were performed separately for these datasets. Log-likelihood ratio test of independence ('*GTest*') was performed to assess the effect of silencing *ZmPP2C-A10* on maize tassel production. All figures were created with the '*ggplot2*' package in R (Wickham 2016).

RESULTS

Silencing *ZmPP2C-A10* increased *RAB17* expression in maize under drought conditions

The expression of *ZmPP2C-A10* significantly decreased by 24.6% in maize infected with the FoMV-*ZmPP2Ci* silencing

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construct compared to plants infected with the FoMV control construct (Fig. 2A; $F_{1,73} = 6.804$, $P = 0.011$). Expression of the dehydrin-encoding gene, *ZmRAB17*, which is downstream from *PP2C* (Fig. 1), was also significantly reduced in *ZmPP2C-A10*-silenced plants compared to controls before drought treatment (Figure S1; $F_{1,30} = 5.885$, $P = 0.022$). During these experiments we noticed the FoMV-*ZmPP2Ci*-infected plants appeared slightly larger than the FoMV controls 14 days after infiltration (Fig. 2B), however there were no other noticeable phenotypic differences before drought treatment.

During the drought treatment, no differences in ABA concentrations were observed between *ZmPP2C-A10*-silenced and control maize (Fig. 3A; $F_{1,7} = 2.586$, $P = 0.152$), which is expected as *ZmPP2C-A10* is downstream from ABA (Fig. 1). However, expression of *ZmRAB17* was significantly increased by nearly twofold in *ZmPP2C-A10*-silenced plants compared to controls during drought (Fig. 3B; $F_{1,27} = 5.472$, $P = 0.027$).

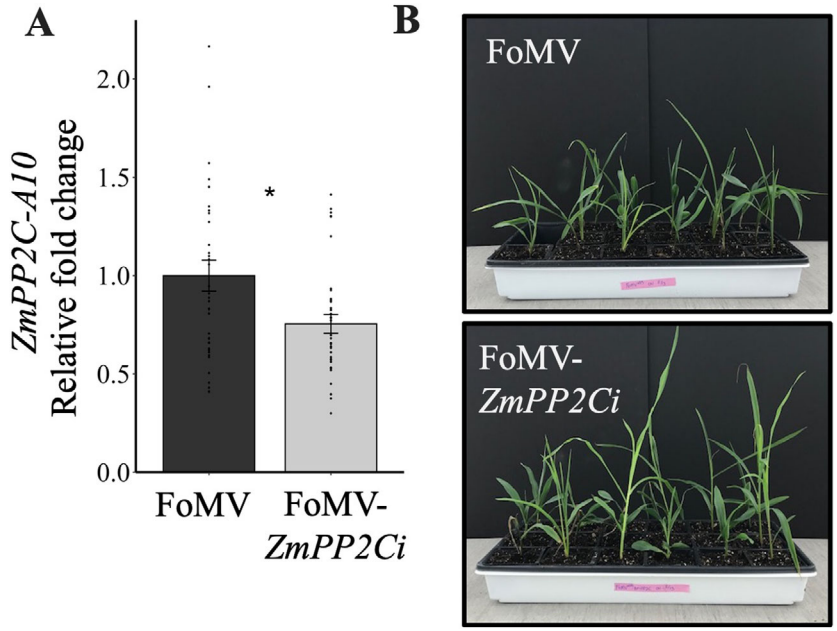

Fig. 2. *ZmPP2C-A10* gene expression is silenced in FoMV-*ZmPP2Ci*-infected plants without drought. *Zea mays* seedlings were agroinfiltrated with FoMV or FoMV-*ZmPP2Ci*. A: Expression of *ZmPP2C-A10* was significantly lower in FoMV-*ZmPP2Ci*-infected plants 14 days post-infiltration ($N = 38$–39). *ZmPP2C-A10* expression is relative to *ZmActin*. B: Visual phenotypic differences between FoMV- and FoMV-*ZmPP2Ci*-infected *Z. mays* before drought. Bars indicate ±SE of mean. Asterisks (*) indicate significant differences between treatments as determined by an ANOVA ($P < 0.05$).

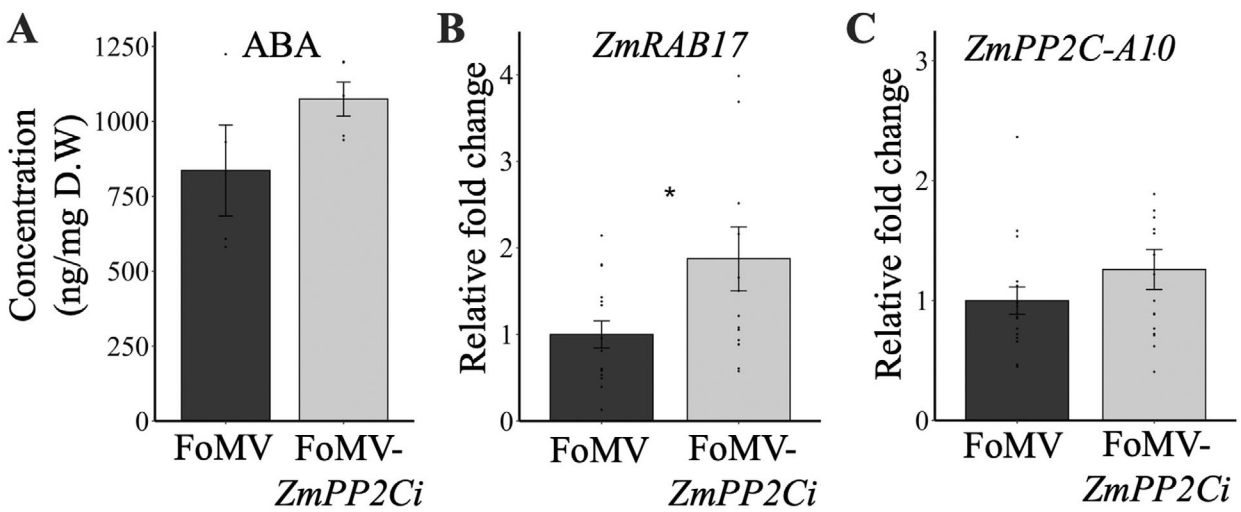

Fig. 3. *ZmPP2C-A10* silencing enhances downstream expression of the dehydrin gene, *ZmRAB17*, under drought conditions. A: ABA concentration. B: Relative fold change in transcript abundance of the dehydrin gene *ZmRAB17*. C: Relative fold change in transcript abundance of *ZmPP2C-A10* in FoMV- or FoMV-*ZmPP2Ci*-infected *Zea mays* on the 8th day of drought. *ZmRAB17 and ZmPP2C-A10* expression is relative to *ZmActin*. Bars indicate ±SE of mean. Asterisks (*) indicate significant differences between treatments as determined by an ANOVA ($P < 0.05$). For (A) $N = 4$–5, (B) $N = 15$–17, (C) $N = 17$.

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On the final day of drought, we did not observe a difference in *ZmPP2C-A10* transcript levels between FoMV- and FoMV-*ZmPP2Ci*-infected plants (Fig. 3C; $F_{1,30} = 1.419$, $P = 0.234$), suggesting that *ZmPP2C-A10* expression was reduced in the controls in response to drought treatment. These results suggest that even mild silencing of *ZmPP2C-A10* (~25%) early during a drought period is effective in mitigating the negative regulation on *ZmRAB17* gene expression.

Drought-induced changes in chlorophyll fluorescence were reduced in *ZmPP2C-A10*-silenced maize

Chlorophyll fluorescence is widely used as an indicator of plant stress (Baker 2008; Murchie & Lawson 2013; Pérez-Bueno *et al.* 2019). Before drought, F_v/F_m values were higher in control plants compared to *ZmPP2C-A10*-silenced plants (Fig. 4A; W = 1093, $P < 0.001$). However, at the end of the 8-day drought, *ZmPP2C-A10*-silenced plants had higher F_v/F_m values than control plants (Fig. 4A; W = 564.5, $P = 0.073$). Overall, relative F_v/F_m decreased 25.3% and 18.7% in control and *ZmPP2C-A10*-silenced maize plants, respectively (Fig. 4B; W = 522.5, $P = 0.026$). On the final day of drought treatment, both FoMV- and FoMV-*ZmPP2Ci*-infected maize displayed severe symptoms of drought stress (Fig. 4C). Four days after rewatering, most plants from both treatments displayed phenotypes similar to plants grown under well-watered conditions. However, the FoMV-*ZmPP2C-A10i*-infected plants appeared to recover more photosynthetic area than FoMV-infected plants according to their greener appearance (Fig. 4D). Taken together, these results suggest *ZmPP2C-A10*-silenced plants were less stressed under drought and better able to recover following rewatering compared to control plants.

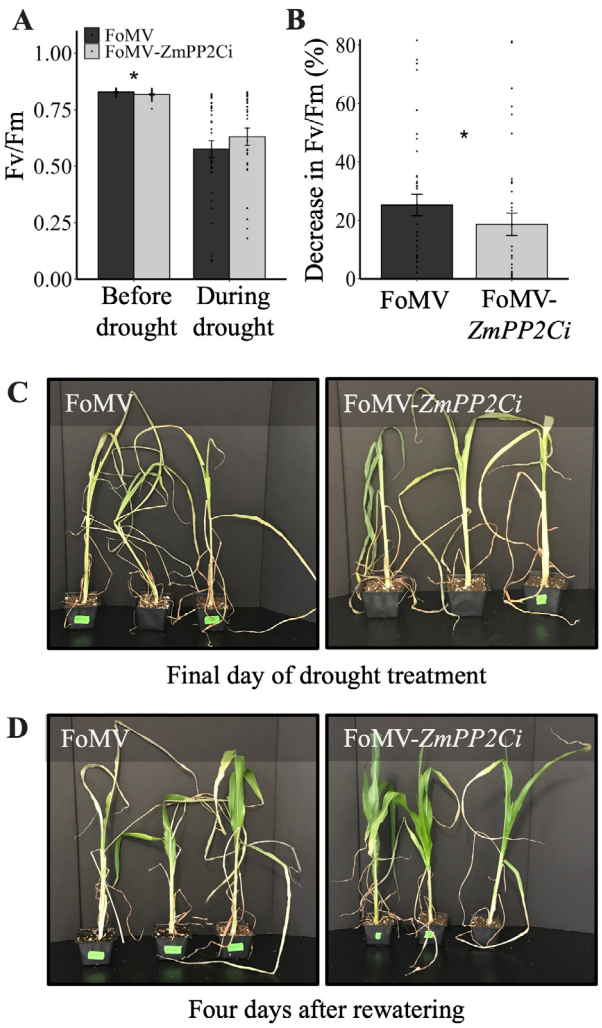

Fig. 4. Changes in chlorophyll fluorescence were reduced in *ZmPP2C-A10*-silenced plants under drought conditions. A: Chlorophyll fluorescence (F_v/F_m) of FoMV- and FoMV-*ZmPP2Ci*-infected maize under well-watered conditions (before drought) and on the 8th day of drought stress (during drought). B: Percentage decrease in chlorophyll fluorescence after 8 days of drought stress in FoMV- and FoMV-*ZmPP2Ci*-infected *Z. mays*. C,D: Photos of FoMV- and FoMV-*ZmPP2Ci*-infected plants (C) on the 8th day of drought and (D) following a 4-day rewatering period. Bars indicate ±SE of mean. Asterisks (*) indicate significant differences between treatments as determined by Wilcoxon rank sum test ($P < 0.05$, $N = 38–39$).

Silencing *ZmPP2C-A10* reduced biomass loss and development delays following drought

Plants were allowed to recover for 30 days with regular watering then ABA content and biomass were measured. Both FoMV- and FoMV-*ZmPP2Ci*-infected plants had significantly lower levels of ABA after rewatering compared to during drought (Fig. S2; FoMV: W = 20, $P = 0.016$; FoMV-*ZmPP2Ci*: W = 25, $P = 0.008$), and there were no significant differences in ABA concentration between the two treatments after the rewatering period ($F_{1,8} = 4.227$, $P = 0.074$). After the 30-day rewatering period, *ZmPP2C-A10*-silenced plants had greater roots ($F_{1,51} = 12.654$, $P < 0.001$), shoots ($F_{1,51} = 13.016$, $P < 0.001$), and total dry biomass (W = 155, $P < 0.001$) compared to control plants (Fig. 5A). There was no difference in the root:shoot ratio between maize infected with the FoMV constructs (Fig. S3; W = 371, $P = 0.918$). At the end of the 30-day rewatering period, 62.9% of *ZmPP2C-A10*-silenced maize produced tassels, compared to only 14.8% of plants infected with the FoMV control construct (Fig. 5B; G = 13.924, df = 1, $P < 0.001$). These results suggest that silencing *ZmPP2C-A10*

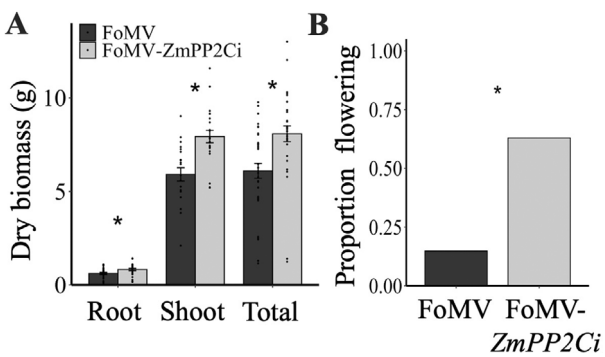

Fig. 5. Silencing *ZmPP2C-A10* decreased biomass loss and development delays following drought. A: *ZmPP2C-A10*-silenced maize (FoMV-*ZmPP2Ci*) had larger root, shoot, and total dry biomass, and B: more tassels compared to FoMV-infected control plants after a 30-day rewatering period. Bars indicate ±SE of mean. Asterisks (*) indicate significant differences between treatments (root and shoot biomass: ANOVA; total biomass: Wilcoxon rank sum test; flowering: log-likelihood ratio test of independence, $P < 0.05$, $N = 27$).

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reduced the negative impacts of drought on plant growth and development.

DISCUSSION

In this study, we show that silencing *ZmPP2C-A10* in maize using a FoMV-derived VIGS system reduced plant stress, plant biomass loss, and developmental delays that are caused by osmotic stress (Figs. 4 and 5). *ZmPP2C-A10* acts downstream of ABA as a negative regulator of signalling and drought mitigation responses. Consistent with this, we found that there was no change in ABA concentration between silenced and control plants (Fig. 3A). However, transcript abundance of the downstream dehydrin gene, *ZmRAB17*, was significantly increased in *ZmPP2C-A10*-silenced plants compared to controls during drought stress (Fig. 3B). The FoMV vector has been previously used in both VOX and VIGS experiments (Liu & Kearney 2010; Liu *et al.* 2016; Mei *et al.* 2016; Bouton *et al.* 2018; Prakash *et al.* 2023), and more recently, a FoMV virus-enabled gene editing (VEdGE) vector was also developed for Cas9-mediated gene editing (Mei *et al.* 2019). In these previous studies, the FoMV-derived systems were used to engineer visual phenotypic markers into plants. In contrast, our study goes further, and uses the FoMV VIGS vector system to target genes that convey ecologically relevant phenotypes (Figs. 4 and 5). While previous studies have shown that overexpressing *ZmPP2C-A*s in maize increases drought susceptibility (He *et al.* 2019b), and that natural variation in *ZmPP2C-A10* mediates drought resistance among maize varieties (Xiang *et al.* 2017), this is the first study showing *ZmPP2C-A10* transcript levels can be genetically engineered to enhance ABA drought mitigation responses and drought resistance. As the FoMV vector system does not require stable plant transformation, this suggests that it can be used midseason to enhance plant stress tolerance in the field.

As mentioned above, previous studies have shown that overexpressing clade A *ZmPP2C* in plants reduces drought tolerance (Liu *et al.* 2009; Xiang *et al.* 2017; He *et al.* 2019b). For example, *Arabidopsis* plants overexpressing *ZmPP2C* had reduced photosynthesis rate, reduced proline content, and lost more water under drought treatment compared to controls (Liu *et al.* 2009). Similarly, overexpression of *ZmPP2C-A2* and *ZmPP2C-A6* in *Arabidopsis* increased leaf water loss, reduced accumulation of proline, and ultimately reduced plant survival during drought (He *et al.* 2019b). Clade A ZmPP2Cs are considered the primary protein phosphatases that negatively regulate ABA signalling and drought tolerance; however, recently it was shown that overexpression of a clade F PP2C phosphatase, *ZmPP84*, reduced relative water content and plant survival under drought stress compared to controls. CRISPR/CAS9-mediated knockout of *ZmPP84* increased drought tolerance, with edited plants having higher relative water content and survival following drought (Guo *et al.* 2022). These results suggest that additional PP2C targets may exist and can potentially be silenced to improve drought tolerance.

RAB17 encodes hydrophilic dehydrin-type late-embryogenesis abundant (LEA) proteins (Vilardell *et al.* 1990; Kizis & Page 2002), which have been shown to play a critical role in maintaining plant tolerance to osmotic stress (Fig. 1; Sun *et al.* 2021; Chakraborty & Roychoudhury 2022). Dehydrin-type LEA proteins act as chaperones protecting proteins, DNA, and membranes during stress (Sun *et al.* 2021), and are strongly induced in plants by exogenous ABA application, as well as by water deficit (Welin *et al.* 1994; Matsukura *et al.* 2010; Sun *et al.* 2021). In wheat, overexpressing another dehydrin type LEA protein, RAB7, increased plant survival, relative water content, chlorophyll content, proline content, antioxidant enzyme activity, and expression of genes conferring abiotic stress tolerance under drought conditions, compared to controls (El-Esawi & Alayafi 2019). Similarly, transgenic tobacco (*Nicotiana tabacum* cv. Xanthi) overexpressing *PgRab7* from *Pennisetum glaucum* had higher germination and survival rates, longer and larger roots, and larger leaf area and seedling fresh weight when treated with mannitol to induce osmatic stress compared to control plants (Agarwal *et al.* 2008). *Arabidopsis* plants overexpressing *ZmRAB17* had higher proline and carbohydrate content and recovered faster from mannitol treatment compared to control plants, indicating *ZmRAB17* overexpression improved plant tolerance under water-limited conditions (Figueras *et al.* 2004). While expression of only one ABA-responsive gene (*i.e.*, *ZmRAB17*) was measured in our study, plants have evolved multiple protective mechanisms downstream of ABA that may also contribute to improved drought tolerance and should be the focus of further studies.

Plant metabolic and physiological processes rely heavily on water, and thus water limitation in the soil can adversely affect plant homeostasis, growth, and development. For example, plants experiencing water-limited conditions generally have reduced photosynthetic efficiency (Lawlor & Cornic 2002; Reddy *et al.* 2004). A reduction in plant chlorophyll fluorescence indicates a decline in optimal quantum yield of photosynthesis (Krause & Weis 1991). Under well-watered conditions, both the FoMV- and FoMV-*ZmPP2Ci*-infected plants had chlorophyll fluorescence levels similar to unstressed leaves (Fig. 4A; Murchie & Lawson 2013). While drought decreased chlorophyll fluorescence in both FoMV- and FoMV-*ZmPP2Ci*-infected maize (Fig. 4A), the reduction was less drastic for maize infected with the FoMV-*ZmPP2Ci* silencing construct (Fig. 4B). This suggests FoMV-*ZmPP2Ci*-infected maize have more efficient photosynthetic activity compared to control plants and experience less physiological stress under water-limited conditions.

In this study, silencing *ZmPP2C-A10* increased total plant biomass and tassel production in maize following drought treatment (Fig. 5A, B). Wei & Pan (2014) showed that mutation of *Arabidopsis* FLOWERING ASSOCIATE PTPase1 (*FPTP1*), a homologue to *ZmPP67*, promoted expression of FLOWERING LOCUS T, while overexpression delayed flowering time. Similarly, transgenic *Arabidopsis* and lines overexpressing clade F PP2C (*AtPP2C-F1*) displayed later flowering phenotypes compared to wild-type plants (Sugimoto *et al.* 2014). The link between *ZmPPC2-A10* silencing and increases in flowering deserves additional attention in future studies.

Drought is a major factor limiting maize productivity worldwide (Daryanto *et al.* 2016; Fahad *et al.* 2017). Engineered drought resistance has been successfully used to counter productivity losses in the past. For example, maize overexpressing nuclear transcription factor Y subunit beta (*ZmNF-YB*) have improved grain yield when grown in drought conditions, compared to control plants (Nelson *et al.* 2007; Wang *et al.* 2018). Additionally, overexpression of the downstream ABA-responsive gene *OsRAB7* in rice not only increased plant

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survival under drought conditions, but also increased grain yield compared to control plants (El-Esawi & Alayafi 2019). Our work demonstrates that foxtail mosaic virus-derived vectors can be used to transiently engineer maize drought resistance *via* virus-induced gene silencing, potentially offering a more dynamic solution that can be implemented when drought is predicted. This approach has the potential to not only target genes related to drought tolerance, but could also be used by growers to target other biotic and abiotic challenges as a mid-season intervention.

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AUTHOR CONTRIBUTIONS

CLC conceived the project. CTN and CLC designed the research. CTN and IAG performed the research. CTN and CLC analyzed and interpreted the data. CTN and CLC wrote the article with contributions from IAG.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. *ZmRAB17* expression was reduced in *ZmPP2C-A10* silenced plants before drought. Relative fold change of downstream dehydrin (*ZmRAB17*) gene expression was measured under well-watered conditions (before drought) in FoMV- or FoMV-*ZmPP2Ci*-infected *Zea mays* plants. *ZmRAB17* expression is relative to *ZmActin*. Bars indicate standard errors of the mean. Asterisks (*) indicate significant differences between treatments as determined by an ANOVA ($P < 0.05$, $N = 30$).

Figure S2. ABA concentration was elevated in *Z. mays* during drought compared to after rewatering. ABA concentrations were measured in FoMV- and FoMV-*ZmPP2Ci*-infected maize plants on the final day of drought and after a 30-day rewatering period. Bars indicate standard errors of the mean. Asterisks (*) indicate significant differences in ABA concentrations during drought and after rewatering as determined by Wilcoxon rank sum test ($P < 0.05$, $N = 4–5$).

Figure S3. Root to shoot ratio was not different between *ZmPP2C-A10* silenced and control plants following drought. Roots and shoots were harvested after a 30-day rewatering period and dried at 30 °C for 7 days. Root to shoot ratio was calculated by dividing the root dry mass by shoot dry mass. Bars indicate standard errors of the mean. There were no significant differences between treatments as determined by Wilcoxon rank sum test ($P > 0.05$, $N = 27$).

Table S1. PCR and qRT-PCR primers used in this study.

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