Research article

**Ectopic expression of the *Aechmea fasciata* APETALA2 gene AfAP2-2 reduces seed size and delays flowering in *Arabidopsis***

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**Abstract**

The Bromeliaceae family, which is distributed pantropically, is one of the most morphologically diverse families. Except for the edible pineapple (*Ananas comosus*), the vast majority of bromeliads cultivated worldwide are appreciated mainly for their ornamental value. As subtropical and tropical flowering plants, these bromeliads, among with *Aechmea fasciata*, have significant economic importance. However, the molecular mechanism of flowering in bromeliads remains unrevealed. In this study, an APETALA2 (AP2) homologue, AfAP2-2, which belongs to the AP2/ethylene response element binding protein (AP2/EREBP) transcription factor superfamily, was identified in *A. fasciata*. AfAP2-2 contains two conserved AP2 domains and is a nuclear-localized transactivator. The expression level of AfAP2-2 was predominantly higher in vegetative organs of the reproductive phase than in those of the vegetative phase. Ectopic expression of AfAP2-2 in *Arabidopsis* specifically delayed flowering in short-day (SD) conditions. Furthermore, the size and weight of seeds of AfAP2-2-overexpressing *Arabidopsis* plants were significantly reduced compared to those of the wild type (WT). Our findings suggest that AfAP2-2 might be a negative regulator of flowering and seed size and weight. These results may help facilitate the molecular breeding of bromeliads.

**ARTICLE INFO**

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**1. Introduction**

As components of signal transduction pathways, transcription factors (TFs) modulate the expression of numerous genes (Hernandez-Garcia and Finer, 2014). The APETALA2/ethylene response element binding protein (AP2/EREBP) transcription factor superfamily, which is one of the most well-known TF families, regulates various developmental and stress-responsive pathways (Licauisi et al., 2013). Based on their different numbers or structures of AP2 and other conserved domains, AP2/EREBPs could be classified into AP2, Related to Abscisic acid insensitive3/Viviparous1 (RAV), Dehydration Responsive Element Binding Proteins (DREB), Ethylene Responsive Factors (ERF), and other categories (Sakuma et al., 2002; Nakano et al., 2006). The members of the AP2 subfamily are characterized primarily by a tandem repetition of two AP2 domains, each of which comprises approximately 60–70 amino acid residues (Nakano et al., 2006). Previous studies have demonstrated that members of the AP2 subfamily play important roles in floral development. For example, there are six members of the AP2 subfamily in *Arabidopsis*, including AP2, TARGET OF ARABIDOPSIS 1 (TOE1), TOE2, TOE3, SCHLAFMUTZE (SMZ), and SCHNARCHZAPFEN (SNZ) (Aukerman and Sakai, 2003), and all are regulated by microRNA172 (miR172) (Chen, 2004). AP2 has been shown to affect the floral meristem (FM) and to determine floral organ identity and development (Jofuku et al., 1994; Chen, 2004; Wollmann et al., 2010; Dinhardt et al., 2012; Krogan et al., 2012; Liu et al., 2014). TOEs could delay flowering as repressors of variable flowering enhancers or integrators (Jung et al., 2007, 2014; Tao et al., 2012; Zhai et al., 2015; Zhang et al., 2015). Additionally, SMZ and SNZ act redundantly with TOEs to repress flowering (Aukerman and Sakai, 2003; Chen, 2004; Mathieu et al., 2009; Yant et al., 2010). Further investigations showed that at least TOE1 and SMZ were able to bind directly to the promoter sites of the putative florigen *Flowering Locus T* (*FT*) to prevent flowering (Zhang et al., 2015; Mathieu et al., 2009). Furthermore, among these six AP2 members, AP2 was also required for ovule and seed coat development.

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and subsequently regulated seed size and seed weight (Jofuku et al., 1994, 2005; Léon-Kloosterziel et al., 1994; Western et al., 2001; Ohto et al., 2005, 2009), indicating its potential impact on agronomic traits such as seed yield.

The Bromeliaceae, which comprises 3248 species in 58 genera, is one of the largest vascular plant families with a Neotropical distribution, outstanding ecological range, and rich diversity of life forms (Crayn et al., 2004; Versieux et al., 2012). Among them, numerous bromeliads cultivated worldwide are appreciated for the astonishing flavour and fragrance of their fruits (for example, pineapple, *Ananas comosus*) or for their ornamental value of their vegetative forms and flowers. To date, some efforts have been made to reveal the molecular mechanism of flowering in bromeliads with significant economic importance. Previous studies identified several crucial factors participating in flowering pathways (Lv et al., 2012a, b; Lei et al., 2016, 2018). In particular, genes related to ethylene biosynthesis and ethylene-inducing flowering pathways in bromeliads have been characterized (Trusov and Botella, 2006; Cong et al., 2012; Li et al., 2016a, b). With the release of pineapple genome data (Zhang et al., 2014; Ming et al., 2015), several Tfs that might be involved in flowering regulation were identified (Ali et al., 2017; Xie et al., 2018). All these results are valuable for better understanding the various molecular mechanisms of flowering in bromeliads.

In this study, *AfAP2-2*, a gene that encodes an AP2 homologue, was identified in the genome of *Aechmea fasciata*, a popular ornamental flowering bromeliad. The expression level of *AfAP2-2* was predominantly higher in vegetative organs during the reproductive phase than during the vegetative phase. Transactivation assays in yeast demonstrated that *AfAP2-2* was a transactivator. In *AfAP2-2*-overexpressing (*AfAP2-2-OX*) *Arabidopsis*, the bolting time exhibited no significant difference from that of the wild type (WT) under long-day (LD) conditions, but flowering was delayed in *AfAP2-2-OX Arabidopsis* in short-day (SD) conditions. Furthermore, the size and weight of seeds of *AfAP2-2-OX Arabidopsis* plants were significantly reduced compared to those of the WT. These results suggested that *AfAP2-2* might be a flowering regulator in *A. fasciata*.

### 2. Materials and methods

#### 2.1. Plant materials

The *A. fasciata* specimens used in this study were planted in a greenhouse (ambient temperature of 30–32°C) located in the experimental area of the Institute of Tropical Crop Genetic Resources, Chinese Academy of Tropical Agricultural Sciences. Juvenile (6 months), adult (12 months) and 39-day-after-flowering (DAF) (18–20 months) *A. fasciata* plants grown in pots were selected for investigation. Different tissue samples, including mature leaves, central leaves, stems, roots and various flower organs, were collected and immediately frozen in liquid nitrogen for further research.

The seeds of both WT and transgenic *Arabidopsis* plants were surface-sterilized in 0.1% HgCl₂ for 10 min and then washed with sterilized distilled water five times before being plated on Murashige and Skoog (MS) medium containing sugar (2%) and agar (0.8%). After being incubated in the dark at 4°C for 2 days, the plates were moved to a chamber at 23°C under LD (16 h light) or SD (8 h light) conditions with a photon flux density of 120 μmol m⁻² s⁻¹. Ten days later, the seedlings were transplanted into turf soil for continuous growth.

#### 2.2. Sequence identification and bioinformatics analysis

Total RNA was extracted from the central leaves of *A. fasciata* using the hexadecl trimethyl ammonium bromide (CTAB) method (Lei et al., 2016). The cDNA of *AfAP2-2* was characterized based on transcriptome data, and the 5’ and 3’ ends were identified using the SMARTer™ RACE cDNA Amplification Kit (Clontech, Tokyo, Japan). The primers used are listed in Table S1 online.

The online Open Reading Frame (ORF) Finder (https://www.ncbi.nlm.nih.gov/orffinder/) was used to predict the ORF of *AfAP2-2*. The physicochemical properties of the *AfAP2-2* protein were predicted and calculated using Expasy ProtParam (http://web.expasy.org/protparam/). MEGA version 6.0 was used to construct the phylogenetic tree (Tamura et al., 2013). The sequence logo was generated by the online WebLogo 3 platform (http://weblogo.threeplusone.com/). Gene Structure Display Server 2.0 (http://gsds.cbi.pku.edu.cn/index.php) was used to generate the scheme of exon-intron structures. The Multiple EM for Motif Elicitation (MEME) online software (http://meme-suite.org/tools/meme) was used with the default settings to identify putative motifs in the variable AP2s.

#### 2.3. Reverse transcription followed by quantitative real-time PCR (RT-qPCR)

First-strand cDNA was synthesized using the TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgen, Beijing, China), and qPCR was performed using the TransStart Tip Green qPCR...
2.6. Exogenous ethephon treatment of A. fasciata line.

The primers used are listed in Table S1 online. Y2HGold using the Yeastmaker Yeast Transformation System 2 amplified and cloned into the destination vector pGBKT7 (pBD). AfAP2-2(1–305AA) and the C terminus of AfAP2-2(296–468AA) were respectively.

2.5. Transactivation analysis of AfAP2-2 in yeast cells

The coding sequence of full-length AfAP2-2, the N terminus of AfAP2-2 (1–305 AA) and the C terminus of AfAP2-2 (296–468 AA) were amplified and cloned into the destination vector pGBK7 (pBD). Transactivation analysis assays were carried out in the yeast strain Y2HGold using the Yeastmaker Yeast Transformation System 2 (Clontech, Tokyo, Japan). The primers used are listed in Table S1 online.

2.4. Subcellular localization

The 1480 bp coding sequence (CDS) region of AfAP2-2 was amplified and subcloned into the pEASY-blunt vector (Transgene, Beijing, China) and confirmed by sequencing. Then, the inserts were transferred to the destination vector pBI221. The Agrobacterium-mediated transformation of onion epidermal cells was conducted as previously reported (Sun et al., 2007). GFP fluorescence was observed by confocal scanning microscopy (Olympus FV1000, Olympus, Tokyo, Japan). For GFP, 488 and 505–530 nm were used for excitation and emission, respectively.

2.3. Plant transformation

To test ethylene response, adult (12-month-old) A. fasciata plants grown in pots in our greenhouse were treated with 10 mL of ethephon at 0.3 g L⁻¹, 0.6 g L⁻¹, 1.2 g L⁻¹, 2.4 g L⁻¹, 4.8 g L⁻¹ for 1, 2, 4, 8, 24 or 48 h or with the same quantity of water as a control. The central leaves were then physically isolated and immediately frozen in liquid nitrogen for further research.

2.7. Plant transformation

To generate the transgenic constructs, the CDS of AfAP2-2 was cloned into the binary vector Cam35S-gfp under the control of the cauliflower mosaic virus (CaMV) 35S promoter. Transgenic Arabidopsis plants were generated by Agrobacterium-mediated transformation using the floral dipping method (Clough and Bent, 1998). The selection of transgenic plants was carried out on MS agar medium supplemented with hygromycin (25 mg/L). Transgenic plants were verified by genomic PCR, RT-PCR and GUS staining. The primers used (AfAP2-2-OX F and AfAP2-2-OX R) are listed in Table S1 online. T3 transgenic plants were used for the subsequent experiments.

3. Results and discussion

3.1. AfAP2-2 is a transcriptional activator that belongs to the AP2 subfamily

The members of the AP2 family, which have two AP2 domains, can be further divided into two clades, AP2 and AINTEGUMENTA (ANT) (Mizukami and Fischer, 1999). In addition to Arabidopsis, the AP2 subfamily has also been found in many other plant species (Zumajo-Cardona and Pabón-Mora, 2016), such as petunia (Maes et al., 2001), tomato (Chung et al., 2010), maize (Stephen and Moose, 1994; Chuck et al., 1998), Picea abies (Vahala et al., 2001; Nilsson et al., 2007), Pinus thunbergii (Shigyo and Ito, 2004), larch (Guillaumot et al., 2008), Capsicum annuum (Borovsky et al., 2015), peanut (Arachis hypogaea) (Park and Grabau, 2016), and Brassica (Zhang et al., 2018).

In the present study, we identified the AP2-like gene AfAP2-2 from A. fasciata, an economically valuable, SD ornamental plant. The CDS of AfAP2-2 was 2160 bp long and contained a 606-bp 5′ untranslated region (UTR), a 150-bp 3′ UTR and a 1404-bp ORF. An exon-intron organization analysis showed that the structure of AfAP2-2 was composed of nine exons and eight introns (Fig. 1); this structure was similar to that of TOE1, indicating putative evolutionary conservation between these genes.

A phylogenetic tree was constructed with AfAP2-2, AfAP2-1 and the AP2 proteins of several other species using the neighbour-joining method with 1000 bootstrap replicates, confirming that AfAP2-2 is a member of the AP2 subfamily (Fig. S1). Furthermore, the unrooted phylogenetic tree showed that AfAP2-2 was mostly closely related to AfAP2-1, implying that they have similar origins. In addition, AfAP2-1 and AfAP2-2 were both clustered with six other AP2-like proteins of monocots (Aeglops tausschi, Dendrobium crumenatum, Hordeum vulgare ssp. vulgare, Oryza sativa Japonica Group and Zea mays) (Fig. S1), indicating that these proteins diverged prior to the divergence of dicots and monocots.

A sequence comparison of AfAP2-2 with AfAP2-1, and six AP2 family members in Arabidopsis showed that the putative nuclear localization signal (NLS) was completely conserved among them (Fig. S2). In addition, both the AP2 domains and the linker between them (called the joint peptide, Jp) were highly similar (Fig. S2).

Further conserved sequence examination between AfAP2-2, AfAP2-1 and six AP2 members in Arabidopsis using the online MEME tool showed that all members shared the eight conserved motifs except SMZ and SNZ, and these motifs had similar distributions. Motif 1 belonged to AP1R1, motif 2 belonged to AP1R1 and Jp, motifs 3 and 5 belonged to AP2R2, and motif 4 belonged to NLS (Figs. S3A and B). In addition, motifs 6, 7 and 8 were all conserved in these AP2 members (Figs. S3A and B).
and B), indicating putative functional conservation among them.

A large number of genes can be regulated by TFs by being bound at specific sites or motifs (Krishnaswamy et al., 2011; Krogan et al., 2012). APA2-2 was shown to be localized predominantly to the nucleus, indicating that it might be an active TF (Fig. 2A). A previous study demonstrated that two AP2 domains separated by a spacer region were crucial for AP2 subfamily members to bind the promoter regions of target genes (Okamura et al., 1997). Here, a sequence analysis showed that two AP2 domains and Jp were highly conserved in APA2-2 (Figs. S2, S3A, B), indicating that it might have a conserved ability to bind target genes. Further assays in yeast cells showed that APA2-2 was a transactivator, and the activation domain was located in the C terminus, which does not contain two AP2 domains (Fig. 2B). This result was consistent with that obtained for APA2-1, another AP2 member isolated from A. fasciata (Lei et al., 2016).

3.2. Ectopic expression of APA2-2 in Arabidopsis delayed flowering under SD conditions

Flowering, which is regulated by numerous genes, is a complex developmental process (Nilsson et al., 2007). Among these genes, numerous AP2 members have been demonstrated to repress floral induction. In Arabidopsis, all six AP2 family members act redundantly to repress flowering (Aukerman and Sakai, 2003; Jung et al., 2007; Mathieu et al., 2009; Yant et al., 2010; Zhang et al., 2015), and overexpression of TOE1, SMZ or SNZ can directly downregulate the expression of FT under LD conditions (Jung et al., 2007; Mathieu et al., 2009). In Cardamine flexuosa, CITOE1 negatively regulates the expression of SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 of C. flexuosa (CfSOC1), which encodes a floral induction factor, to repress flowering (Zhou et al., 2013). In maize, the expression of GLOSSY15 (GL15), an AP2-related gene, is correlated with the juvenile-to-adult phase transition and inhibits flowering (Lauter et al., 2005). The constitutive expression of the conifer Picea abies (Norway spruce) APETALA2 LIKE2 (PaAP2L2) gene in Arabidopsis conferred a late-flowering phenotype compared with that of WT Arabidopsis (Nilsson et al., 2007). The APETALA2 transcription factor homologue CaAP2 in Capsicum annuum was demonstrated to be a candidate flowering repressor (Borovsky et al., 2015). In Ipomoea nil, InAP2-like was involved in photoperiodic flower induction (Glazinska et al., 2009).

To gain insights into the role of APA2-2 in A. fasciata, RT-qPCR was performed to analyse its expression patterns. APA2-2 transcripts could be detected in all tested vegetative and reproductive tissues (Fig. 3A and B). Notably, the expression level of APA2-2 was relatively lower in a variety of reproductive tissues, especially in most flowering organs, than in the vegetative tissues (Fig. 3B). In addition, the quantity of

Fig. 2. Subcellular localization and transactivation activity assay of APA2-2. (A) Subcellular localization of APA2-2-GFP fusion protein. Plasmids harbouring a C-terminal GFP driven by the CaMV 35S promoter (35S:GFP) were used as a control. Recombinant plasmids harboured a C-terminal GFP fusion with APA2-2 driven by the CaMV 35S promoter (35S:APA2-2-GFP). Green fluorescence indicates GFP, and blue fluorescence indicates nuclear staining by 4′,6-diamidino-2-phenylindole (DAPI). Bars = 50 μm. (B) Transactivation activity assay of APA2-2 in yeast cells. The constructs of pBD vectors fused with the full-length APA2-2 (pBD-APA2-2), the N terminus of APA2-2 (pBD-APA2-2-N) and the C terminus of APA2-2 (pBD-APA2-2-C) were transformed into Y2HGold cells, which contain the reporter genes AUR1-C and MEL1. pBD and pGAL4 plasmids were transformed into Y2HGold cells and used as negative and positive controls, respectively. Yeast clones containing the confirmed constructs grew on SD/-Trp medium at dilutions of 1, 1/10, 1100 and 1/1000 for three to five days and then were transferred onto SD/-Trp/+AbA/+X-α-Gal medium for continuous growth for three days to test their transactivation activities. SD: synthetic dropout; AbA: aureobasidin A; SD/-Trp: SD medium without Trp; SD/-Trp/+AbA/+X-α-gal: SD medium without Trp but with 40 mg/L X-α-gal and 200 μg/L AbA. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
AfAP2-2 transcripts were higher in the central leaves of juvenile plants than in those of adult plants, indicating that AfAP2-2 might be involved in the juvenile-to-adult phase transition (Fig. 3C).

To assess the function of AfAP2-2 in flowering, we induced the ectopic expression of AfAP2-2 under the CaMV 35S promoter (Pro35S::AfAP2-2) in Arabidopsis ecotype Columbia (Col-0) (WT) (Fig. S4). Pro35S::AfAP2-2 transgenic plants showed no significant difference from WT in flowering time under LD conditions (Figs. S5A and B), and no defects in floral morphology were observed in the transgenic plants (Figs. S5C, D, E, F, G). Interestingly, the flowering time of Pro35S::AfAP2-2 transgenic plants was significantly later than that of WT under SD conditions (Fig. 4A and B). Compared to that of WT, the expression level of the genes SOC1 and LEAFY (LFY), which encode floral induction factors, was substantially reduced at the shoot apex of Pro35S::AfAP2-2 transgenic plants (Fig. 5A, C). In addition, the expression of another gene, FRUITFULL (FUL), which also encodes a floral induction factor, was significantly downregulated at the shoot apex of lines 77 and 157 of Pro35S::AfAP2-2 transgenic plants but upregulated in line 74 (Fig. 5B) compared to that of WT. The expression of FT, an integrator of flowering pathways, and floral organ identity genes, such as AP1, AP2 and AP3, was also considerably downregulated (Fig. 5D, E, F, G). These results indicated that AfAP2-2 was a putative flowering repressor involved in flowering induction and might be a potential target of genetically engineered breeding, as a large number of bromeliads, including A. fasciata, are SD plants.

The gas hormone ethylene is widely used to induce flowering in bromeliads. Previous studies have demonstrated that exogenous ethylene mediates the flowering of bromeliads by stimulating the synthesis of endogenous ethylene (Wang et al., 2007; Cong et al., 2012). To date, several efforts have been made to identify the functional factors...
participating in ethylene transduction pathways and ethylene-induced-flowering signalling pathways in bromeliads. Li et al. identified four ethylene receptor genes in pineapple and suggested that AcERS1b, AcETR2a, and AcETR2b were involved in pineapple flowering induced by exogenous ethephon (Li et al., 2016b). Our previous studies also identified two ethylene-responsive genes, \textit{AfAP2-1} and \textit{AfSPL14}, which were significantly repressed or induced, respectively, by exogenous ethylene treatment, indicating their important roles in the ethylene-induced flowering of \textit{A. fasciata} (Lei et al., 2016, 2018). Here, the possible response of \textit{AfAP2-2} in adult plants prior to flower bud differentiation in \textit{A. fasciata} after exogenous ethephon treatment was investigated at different concentrations. As shown in Fig. 3D, the expression of \textit{AfAP2-2} was significantly induced by exogenous ethephon treatment, independent of concentration. Interestingly, the expression level reached a maximum after treatment with exogenous ethephon at relatively high concentrations (2.4 g/L and 4.8 g/L) at 24 h, while the expression of \textit{AfAP2-2} rose gradually when treated with exogenous ethephon at lower concentrations (0.3 g/L, 0.6 g/L and 1.2 g/L), even after 24 h (Fig. 3D). These results were in contrast to those of \textit{AfAP2-1}, for which transcript levels were repressed but not induced by exogenous ethylene treatment (Fig. 3D). Furthermore, the expression of \textit{AfAP2-2} induced by exogenous ethylene treatment was dose dependent (Fig. 3D). Interestingly, the expression level of \textit{AfAP2-2} generally appeared to gradually increase in various vegetative organs with age (Fig. 3A). All these results indicated that \textit{AfAP2-2} might be involved in the development of vegetative organs and might not be involved in the ethylene-induced flowering of \textit{A. fasciata}.

3.3. Ectopic expression of \textit{AfAP2-2} in \textit{Arabidopsis} altered seed size and weight

The \textit{Arabidopsis} seed consists of the seed coat, embryo, and endosperm. These three components are derived from the ovule integuments, the fusion of the egg cell and one sperm cell, and the fusion of the central cell of the embryo sac with the second pollen sperm cell, respectively (Becker et al., 2014; Figueiredo and Kohler, 2014; Khan et al., 2014; Lafon-Placette and Kohler, 2014). Seed development was influenced by both environmental factors and endogenous genetic signals (Orozco-Arroyo et al., 2015). Among these agronomic traits, seed size and mass are considered important determinants of seedling survival and vigour upon germination (Jofuku et al., 2005). In \textit{Arabidopsis}, seed size is mainly influenced by the rapid proliferation of the endosperm and the seed coat cells and to date, several pathways regulating seed size are known (Orozco-Arroyo et al., 2015). For example, genome imprinting and parent-of-origin effects could influence the developmental process of the endosperm and thereby regulate seed size. In addition, factors controlling integument cell proliferation and/or elongation could influence the developmental process of the seed coat, altering seed size. Furthermore, phytohormones such as brassinosteroids, cytokinins, auxins and abscisic acid have also been proposed to coordinate seed development (Sun et al., 2010). Among these pathways, several key TFs have been characterized, and AP2 was demonstrated to play a crucial role in controlling endosperm cellularization and seed coat development (Jofuku et al., 2005; Ohto et al., 2005, 2009). The endosperm proliferation stage of \textit{ap2} mutant seeds was extended, and cellularization was then delayed (Ohto et al., 2009). In addition, the cell number and cell size of \textit{ap2} mutants were increased in association with enlarged embryo size (Jofuku et al., 2005; Ohto et al., 2005). Our studies showed that floral morphology did not show observable defects in \textit{Pro3SS::AfAP2-2} transgenic \textit{Arabidopsis} plants (Figs. S5C, D, E, F, G), in contrast to that in \textit{TOE3}-overexpressed (\textit{TOE3-OX}) \textit{Arabidopsis} plants (Jung et al., 2014). Interestingly, the seeds of \textit{Pro3SS::AfAP2-2} transgenic \textit{Arabidopsis} plants were smaller than those of WT (Fig. 4C, D, E, F, G, H), and the average seed weight of \textit{Pro3SS::AfAP2-2} transgenic \textit{Arabidopsis} plants was decreased by 24.55%–27.23% compared to that of WT (Fig. 4I), indicating that \textit{AfAP2-2} might play crucial roles in the regulation of seed size and weight. \textit{A. fasciata} is an ornamental plant that has only flowers but not fruits or seeds, the fact of which may be the reason that the transcript level of \textit{AfAP2-2} was lower in the reproductive organs than in the
vegetative organs of *A. fasciata* (Fig. 3B). Except for ornamental *A. fasciata*, a variety of bromeliads cultivated worldwide are appreciated for the astonishing flavour and fragrance of their fruits (for example, pineapple, *A. comosus*). Interestingly, an AP2 homologue, which was identified in *A. comosus* and named as AcAP2-2, shares very similar amino acid sequences with AfAP2-2. Here, we speculate that AcAP2-2 might have similar functions with AfAP2-2 in the regulation of flowering time and the size and weight of fruits. Further studies should focus on the characterization of floral morphology in *AfAP2-2-OX A. fasciata* plants and/or the size and weight of fruits of *AcAP2-2-OX A. comosus* plants.

**Author contributions**

All authors read and commented on the manuscript. LX, ZYL and ML designed the study and guided the research. ML performed the experiments, prepared all the figures and wrote the draft manuscript text. JBW and YLF performed some of the experiments. LX modified this manuscript.

**Conflicts of interest**

The authors declare no conflict of interest.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.03.034.

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