Article

Over-Expression of ZmAIA29, an AUX/IAA Transcription Factor, Improved Maize Flowering Time

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Abstract: Maize is one of the most important food and feed crops in China due to the high demand for its use by both humans and animals. The Huang-Huai-Hai region is a major maize cultivation area in China, and the special cropping rotation system of wheat and maize requires a suitable reproductive period for maize. The AUX/IAA and ARF transcription factors are two key genes that regulate auxin signals in plants, which play an important role during the process of auxin regulation in plant growth and development. In this study, we obtained two overexpressed lines of ZmAIA29 belonging to the AUX/IAA transcription factor family. Compared with the Wide type, the overexpressed strains of ZmAIA29 were significantly regulated in maize during the flowering time and had increased ear weight. Moreover, ZmAIA29 was assayed via the yeast two-hybrid and BiLUC systems for interaction, and the results showed that ZmAIA29 interacted with several ZmARF transcription factors. Furthermore, RNA-seq and DAP-seq analysis revealed that ZmAIA29 regulates maize growth and development by influencing pathways such as those for plant signal transduction and the key genes related to maize flowering. Taken together, our results suggest that ZmAIA29 plays a key role in the development of maize flowering. This study provides new insight for better understanding the combined regulation of the AUX/IAA and ARF transcription factors during flowering time in maize.

Keywords: AUX/IAA TF; ARF TF; florescence

1. Introduction

Maize is one of the most important cereal crops for food and feed, and it is also used for biofuel production and industrial raw material. China is the second-largest maize production country, and its maize is classified based on the planting period, i.e., summer, autumn, and spring maize [1,2]. The Huang-Huai-Hai region is among the important maize-producing areas in China; it has a unique crop rotation system for wheat and maize. This cultivation pattern means that after the summer maize harvest period, around October and during winter, wheat continues to be sown. After winter, wheat matures in June of the following year, and summer maize continues to be sown. The growth period of maize not only affects maize yield but also the sowing time of wheat [3]. With global climate change, high-temperature weather is occurring more frequently, and maize varieties with short growth periods can be planted later to avoid the adverse effects of weather. Therefore, the cultivation of maize varieties with suitable growth time periods is important for improving total grain yield. The growth period is generally positively correlated with crop yield, meaning that varieties with longer growth periods have higher yields per plant. However, some genes that have been reported recently also exhibit the effect of simultaneously increasing yield and shortening the growth period. For example, OsDREB1C can simultaneously improve photosynthesis efficiency and nitrogen utilization.
efficiency and can also lead to the early heading of rice, shorten the growth period, and increase crop yield by more than 30% [4]. Continuously looking for similar genes can lead to the discovery of further genetic resources for both aims.

Auxins are phytohormones and are involved in many important developmental processes of plants, such as flowering, organ development and maturation, the maintenance of apical dominance, and phototropism as well as the division of the downward growing somatic cells of roots, the generation of germ cells, and delays in leaf senescence [5,6]. Three gene families have been identified in the response to early auxin signaling, named the Aux/IAA, SAUR, and GH3 gene family [7]. Among them, AUX/IAA TFs usually regulate plant growth and development by regulating the expression of the downstream genes of auxin and crosstalk with other hormones [8,9]. For example, AtIAA14 regulates lateral root development in Arabidopsis [10]. The silencing of the tomato gene SLIAA9 results in monosexual fruiting and the phenotype of a single leaf replacing that of a compound leaf [11]. Aux/IAA proteins have a conserved structure and contain four highly and typically conserved domains, I, II, III, and IV, which play distinct functions [9]. Domain I is located at the N-terminus, where it can bind to the promoter to inhibit the initiation of transcription. Domain II is also located at the N-terminus, where it functions to maintain Aux/IAA protein stability, and the 13 amino acids that make up Domain II comprise a degron that is degraded by proteases following ubiquitination by SCFTR1 when auxin levels are high in the plant. Domains III and IV are located at the C-terminal of Aux/IAA proteins and can interact with ARF proteins to co-regulate the expression of genes under different auxin levels [12,13].

ARFs (auxin response factors) are key factors that specifically regulate the auxin signaling pathway by binding the promoter region of the auxin downstream response gene [14]. Similar to AUX/IAA, ARFs participate in a variety of growth and development processes, such as the creation of the vas deferens, lateral root production, floral bud differentiation, and the embryonic axis [15,16]. A number of auxin-responsive transcription factors, totally 23, 25, and 31 ARFs, have been identified in arabidopsis, rice, and maize, respectively [17,18]. The regulation of ARFs on auxin genes largely depends on whether or not dimers of ARFs and AUX/IAA are formed under different concentrations of auxin [19]. For example, AtIAA3, AtIAA14, and AtIAA18 control lateral root formation by interacting with two different ARF7 and ARF19 ARFs [20], whereas AtIAA7/AXR2 controls light-induced morphological responses such as the inhibition of hypocotyl elongation, promoting leaf development [21]. The aberrant binding of AtARF6 and AtARF8 with AtIAA8 mutated by Domain II leads to reduced levels of jasmonic acid and abnormal floral organ development [22,23].

The ZmIAA29 transcription factor was previously identified via florescence in a genome-wide association study on maize [24]. In this study, we analyzed the overexpression of the ZmIAA29 transcription factor and generated two lines against the background of KN5585 background. Our results showed that the ZmIAA29 could be helpful during the flowering growth time in maize and increase ear weight. Moreover, yeast two-hybrid, BiLUC, RNA-seq, and DAP-seq analyses revealed that ZmIAA29 may interact with multiple ZmARFs to collectively regulate maize flowering time and growth via pathways such as those for hormone signaling, amino acid metabolism, and transcriptional regulation. Overall, the findings of this study provide new insight into the regulation of Aux/IAA and ARFs during the flowering development time in maize.

2. Materials and Methods
2.1. Plant Material and Transgenic Identification

The CDs of ZmIAA29 (Zm00001d011588, Zm-B73-REFERENCE-GRAMENE-4.0) were obtained from Gramene (http://ensembl.gramene.org/index.html, accessed on 26 July 2023). A CUB-modified vector with a UBI promoter and bar resistance gene was used as an overexpression carrier. ZmIAA29 transgenic material with a KN5585 background provided by WeiMi Biotech (Jiangsu, China) was constructed. T1 transgenic plants were planted
in Sanya (Hainan, China) in 2022. The T2 transgenic plants were planted in Zhengzhou (Henan, China), and T3 transgenic plants were planted in Sanya (Hainan, China) during 2023. The background materials of KN5585 were planted at the same time. Plants were grown to 3 m in length, with 0.2 m between plants and 0.6 m from row to row. Field management techniques such as water manure use were implemented according to local routine management. Upon T3 generation, SAMs (apical meristems) from the OE and KN5585 littermates grown to V6 were used for RNA extraction, RNA-seq, and qRT-PCR.

Transgenic maize material was tested using herbicides and a PCR. The specific operation was as follows: when the transgenic materials had grown to the V10 stage, daubed herbicide basta with a concentration of 0.2% was sprayed on the leaves; after one week, DNA was extracted from the young leaves that had not turned to yellow to perform the PCR test. The forward and reverse primer sequences are detailed in Supplementary Table S1. The PCR program was set as follows: pre-denaturation at 95 °C for 3 min; amplification through 35 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 40 s; and final extension at 72 °C for 5 min. Plants were identified as positive selfing and numbering. Furthermore, genotypes were judged to be homozygous by observing the progeny of selfing for the segregation of resistance genes. Moreover, the expression pattern was examined between the OEs and KN5585 using qRT-PCR. RNA extraction, reverse transcription, and qRT-PCR procedures were performed strictly in accordance with the instructions of the Fastpure universal plant total RNA Isolation Kit, Hiscript II 1st strand cDNA synthesis Kit (+gDNA Piper), and Chamq universal SYBR qPCR Master Mix (RC411, R212, Q711, Vazyme Biotech Co., Ltd., Nanjing, China).

2.2. Phenotypic Identification

The cultivation and flowering time traits of the OEs were investigated during T3 generation in Sanya in 2023. The pumping stage was described as beginning on the day of sowing and ending on the day that half of a plant’s male panicle was fully exposed from the bract. The time in which pollen was scattered by the main axis of the male panicle from the sowing date was defined as the pollination period. The number of days during which we noticed that a flower had appeared 1 cm from the bract was defined as the silking period, with counting beginning from the sowing date. These three florescence traits were investigated and recorded daily during the fixation times (9:00–10:00 a.m.). The statistical analysis of relevant yield traits including kernel number per row (KNR), kernel row number (KRN), kernel number (KN), and kernel weight (KW) was recorded, and different tests were performed using SPSS 19.0 software.

2.3. Vector Construction, Subcellular Localization, Yeast Two-Hybrid, and BiLUC

The full-length CDS sequence of *ZmIAA29* was amplified using the Phanta Max super fidelity DNA polymerase (p505, Vazyme Biotech Co., Ltd., Nanjing, China), and the PCR reaction was performed with pre-denaturation at 95 °C for 3 min; amplification through 35 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 40 s; and final extension at 72 °C for 5 min. All of the linearized vectors required for vector construction in this assay were obtained via enzymatic digestion, and all of the vectors were stored in our laboratory. Vectors and primer sequences are detailed in Supplementary Table S1. The ligation of vectors and fragments was performed using the Gibson homologous recombination method. The enzyme digest vector and PCR products with corresponding adaptors connected were used in the reaction at 50 °C for 15 min with a ratio of 2:1 moles, and the specific procedure was described using the Clonexpress ultra one-step cloning kit, (C115, Vazyme Biotech Co., Ltd., Nanjing, China).

The subcellular localization of *ZmIAA29* was assayed in maize, and the constructed vectors 35S:*ZmIAA29-GFP* and 35S:*NF-YA4-RFP* (nuclear marker gene) were transformed into maize protoplasts using a polyethylene glycol-mediated transient transformation system in accordance with the previous method of Yoo et al. [25]. Fluorescence signals were observed with a confocal laser scanning microscope (LSM710, Zeiss, Germany).
The constructed AD-ZmIAA29 and corresponding BD-ZmARFs were co-transformed into yeast strains of Y2H gold. The negative and positive controls were pGADT7-T + pGBK7-lam and pGADT7-T + pGBK7-53. Only the combination growing on a defective medium, SD/-Leu-Trp-His-Ade, was interactive [26]. The constructed interactive vector mixture of BiLUC was inoculated into tobacco leaves, and after 12 h of dark treatment and 48 h of light recovery growth, the tobacco leaves were treated with luciferin substrate in accordance with the manufacturer’s instructions (L9504, Sigma-Aldrich, St. Louis, MO, USA) under dark conditions for 10 min; the fluorescence signal was detected using a fully automated chemiluminescence image analysis system (Tanon 5200, Shanghai, China) [27].

2.4. Combined Analysis of RNA-seq and DAP-seq

Total RNA was extracted from OEs and KN5585 in the SAM V6 stage in accordance with the manufacturer’s instructions for TransZol reagent (Transgene, China). Total RNA was used for RNA-seq (Berry Genomics, Beijing, China). The raw reads were produced after the exclusion of low-quality reads and adaptor contaminants. The unique RNAs were aligned to maize genome Zm-B73-REFERENCE-GRAMENE-4.0. Only perfectly matching sequences were selected for further analysis. The count information was used to determine normalized gene expression levels as reads per kilobase per million mapped reads [28].

Multiple testing carried out with the Benjamini–Hochberg procedure for the false discovery rate (FDR) was taken into account using an adjusted \( p \)-value. A \( p \)-adj value of \( \leq 0.05 \) and \( |\log_{2}\text{fc}| \) value of \( \geq 1 \) were used as thresholds for DEG (differential expression gene) screening, and DEseq2 edgeR was also used. DEGs were aligned to the KEGG (Kyoto Encyclopedia of Genes and Genomes) database and GO (gene ontology) database entries for enrichment and annotation, with a \( p \)-value of <0.05 [29].

DAP-seq raw data of ZmRAF7 and ZmARF25 were downloaded from the NCBI-SRA database [9]. The raw fastaq files were filter-adapted using Trimmomatic. BWA was used to align the fastaq files to the genome reference Zm-B73-REFERENCE-GRAMENE-4.0. Samtools was used to convert the sam files into bam files. Macs2 was used for peak finding, and the \( p \)-value was set as 10. IDR software was used to merge the peaks of biological duplicates with \( p < 0.05 \) and to score the reliability of these repeated peaks, and then chip seeker was used for peak annotation. We defined target genes that contained DAP-seq peaks located within 2 kb upstream of ATG. The target genes were also aligned to the KEGG database and GO database entries for enrichment and annotation, with a \( p \)-value of <0.05, using TBtools software.

The DEGs of RNA-seq and commonly significant genes in ZmARF7 and ZmARF25 of DAP-seq overlapped. Furthermore, the overlapping genes were aligned to the KEGG and GO databases [30].

3. Results
3.1. Identification of ZmIAA29 Overexpression in Maize

During T3 generation, all eight and six maize lines in both tested strains were tested and found to be positive via herbicide application. Additionally, the bar gene was also successfully amplified at 615 bp (Supplementary Figure S1A). Next, driven by maize-specific strong promoter UBI, the expression level of these lines was 25- and 12-fold higher than that of KN5585 in SAM tissue (Supplementary Figure S1B). Thus, these two strains were confirmed as ZmIAA29 transgenic plants, named OE1 and OE2.

3.2. Phenotype Characterization of ZmIAA29 Overexpression Maize

During T3 generation, OE1 and OE2 flowered significantly earlier than KN5585 did. Tassels were extended from the bract almost 3 days earlier than in KN5585 (at 57.66 ± 2.08 days for OE1 and 56.09 ± 1.75 for OE2, both earlier than the control at 59.66 ± 1.58 days). Meanwhile, the time for pollination and silking was also three days earlier (Figure 1A, Supplementary Figure S2A), with times of 62.44 ± 0.72 and 63.88 ± 0.78 days.
for the WT, 60.33 ± 0.57 and 61.66 ± 0.57 for OE1, and 58.72 ± 2.45 and 59.45 ± 2.02 for OE2.

Figure 1. Phenotype analysis of OE and KN5585: (A) maize pollination and silking occurs earlier in OE than in KN5585; (B) the smaller kernel size of OE compared with that of KN5585.

The yield traits of OE1 and OE2 were analyzed. Although the kernel sizes of transgenic maize were significantly smaller than those of the control KN5585 (Figure 1B), the KNR and KRN were increased conversely (Supplementary Figure S2B). The KN increased, with the average grain number increasing by 68.45 ± 2.47 grains, from 126.25 ± 11.44 for WT to 193 ± 43.47 for OE1 and 196.5 ± 20.09 for OE2 (Supplementary Figure S2C). Therefore, due to the increase in the total number of kernels, the kernel weight from one cob of the transgenic lines was significantly higher than that of the control, from 36.63 ± 5.03 g for the WT to 53.40 ± 8.36 g for OE1 and 53.20 ± 6.68 g for OE2.

3.3. The Subcellular Localization of ZmIAA29

ZmIAA29 is a member of the AUX/IAA transcription factor family and is always located in the nucleus. To verify the location of ZmIAA29, we constructed pRTL-35S::ZmIAA29-GFP and nuclear localization marker pRTL-35S::NF-YA4-RFP. After treatment, the GFP fluorescent signal of the ZmIAA29-GFP protein and the RFP fluorescent signal of the nuclear marker NF-YA4 could be co-observed in the nucleus (Figure 2) of the maize protoplast, with the results showing that ZmIAA29 could also be located in the nucleus.

Figure 2. The subcellular localization of ZmIAA29. From left to right, these are fluorescence images of GFP, RFP, bright field images, and images merging the first three images. The first row of images is of ZmIAA29 and the nuclear marker NF-YA4 for the co-transfection of maize protoplasts. The second row of images is the negative control and its NF-YA4.

3.4. ZmIAA29 Interacts with ZmARF2, ZmARF7, and ZmARF25

Generally, AUX/IAA TFs can interact with ARF TFs and form heterodimers, responding to auxin signals. Next, we constructed multiple yeast two-hybrid systems of ZmIAA29...
with mounts of ZmARFs. However, only a few pairs of reactions were positive. ZmIAA29 with ZmARF2, ZmARF7, ZmARF15, ZmARF19, and ZmARF25 can grow normally on SD-/Leu-Trp-His-Ade medium (Figure 3A). The BiLUC system in tobacco finally confirmed that only ZmARF2, ZmARF7, and ZmARF25 can interact with ZmIAA29 (Figure 3B).

![Image of experimental setup](image_url)

**Figure 3.** Interaction of ZmIAA29 and ARFs: (A) yeast two-hybrid system verifying that ZmIAA29 interacts with ZmARFs; $10^{-2}$, $10^{-1}$, $10^0$ are three concentrations of yeast. The left figure of A is the SD defect culture medium without Leu and Trp. The right figure of A is the SD defect culture medium without Leu, Trp, His, and Ade. From top to bottom, the seven groups represent the negative and positive control and the interaction between ZmIAA29 and ZmARF2, ZmARF7, ZmARF15, ZmARF19, and ZmARF25; (B) one tobacco leaf divided into four parts and infected with bacterial solution separately. The upper left part shows ZmIAA29 and ZmARFs. The lower left part shows the negative control of n-LUC and c-LUC. ZmIAA29 + c-LUC and N-luc + ZmARFs are in the upper right and lower right, respectively.

### 3.5. ZmIAA29 and ZmARFs Regulate Maize Growth through Plant Signal Transduction and Other Metabolic Pathways

The above results indicate that the overexpression of ZmIAA29 can influence maize florescence and that ZmIAA29 interacts with ZmARF2, ZmARF7, and ZmARF25. According to reports, when ZmARFs are combined with AUX/IAA, it usually affects maize reproductive growth [31,32]. Therefore, we combined the RNA-seq of OE ZmIAA29 and the DAP-seq of ZmARF7 and ZmARF25 to explore the regulatory network of maize flowers.

The 2146 DEGs were obtained via the RNA-seq of OE1 and OE2 overlap, with 891 being up-regulated in overexpressed materials and 1255 being down-regulated (Figure 4A). All of the DEGs can enrich certain pathways, such as those for the generation of mature mRNA, the development of the number and structure of floral organs, and the process of cell development. Additionally, 3820 genes were found to be overlapped via the DAP-seq of ZmARF7 and ZmARF25, which also involves a pathway-like signal; see Supplementary Table S2. Briefly, 296 and 378 overlapped genes were revealed from ZmIAA29 RNA-seq with ZmARF7 DAP-seq and ZmARF25 DAP-seq, respectively (Figure 4B). The most reliable metabolic pathways are annotated as those for the metabolic processes of hormones, transcription regulatory region nucleic acid binding, transcription cis-regulatory region binding, plant hormone signal transduction, alpha-Linoleic acid metabolism, and signal transduction in the GO and KEGG database (Figure 4C).
The domestication of modern maize led to the selection of certain earlier-mature alleles [35]. During flowering time [36–39], in this study, we analyzed the overexpression of ZCN8 delaying the growing time for wheat could cause a decline in wheat harvest rates [33,34]. After maize is harvested, winter wheat should be grown immediately. Otherwise, this planting mode in the Huang-Huai-Hai region in northern China [3].

4. Discussion

The rotation cropping system of summer maize and winter wheat is a main double-cropping system in the Huang-Huai-Hai region in northern China [3]. In this planting mode, after maize is harvested, winter wheat should be grown immediately. Otherwise, delaying the growing time for wheat could cause a decline in wheat harvest rates [33,34]. Some genes are directly related to growth, maize flowering, and plant development, and these genes were also identified in a combined analysis. However, ZmHB53 and ZmMADS69 are the only downstream genes of ZmARF7, while ZmMYB43, ZmMYB93, and ZmCCD7 are common downstream genes of ZmARF7 and ZmARF25. In addition, the expression pattern of the key genes ZmHB53, ZmMADS69, ZmMYB43, ZmMYB93, and ZmCCD7 in the SAMs of ZmIAA29 was examined in overexpressed material at the V6 stage using qRT-PCR (Supplementary Figure S3); the results show that the expression levels of ZmHB53 and ZmCCD7 in overexpression materials were significantly increased by almost 8- and 40-fold, while the expression levels of ZmMADS69, ZmMYB43, and ZmMYB93 were significantly decreased, which was consistent with the transcriptome data.

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of ZmIAA29 identified from GWAS analysis conducted during flowering time. ZmIAA29 exhibits a positive regulatory effect on maize flowering. Therefore, ZmIAA29 might have a similar function to that of other candidate genes, and haplotypes of this gene could be investigated to achieve pyramiding among early-flowering alleles via marker-assisted selection. The early flowering time usually leads to a decrease in fruit size and yield [40]. The grain size of the OE maize line is also reduced, but ear weight can be increased by increasing the number of KNR, KRN, and kernel number per ear. Different from other flowering genes, such as ZmKNR6, two alleles can balance the flowering time and ear length [41]. ZmIAA29 can improve the flowering time and maize yield at the same time. Therefore, ZmIAA29 plays a potential role in earlier mature flowering but also increases yield and seed production via a greater number of kernels.

ZmIAA29 is a member of an AUX/IAA class transcription factor that is mainly involved in plant auxin signal transduction [9]. Mutations of these proteins can lead to a variety of typical auxin pathway defect phenotypes, such as those related to embryonic development, lateral root growth and elongation, hypocotyl growth, phototropism, flower organ development, and other processes that are inhibited [42,43]. ZmIAA10 can inhibit the expression of the LATERAL ROOT PRIMORDIA1 (LRP1) gene by directly binding to the promoter in maize [44]. FaARF4 promotes flowering by activating AP1 and FUL [45], floral meristem identity genes, in strawberry. ARF3 plays a key role in strawberry flowering development [46]. In our study, the overexpression of ZmIAA29 significantly advanced the tassel, pollen, and silking time of maize, which means that ZmIAA29 plays a key role in reproductive transformation and flower morphogenesis, which is a typical plant growth stage regulated by auxin.

AUX/IAA proteins usually bind to ARFs under low auxin levels, inhibiting their regulation of downstream genes. Under high auxin levels, AUX/IAA was degraded via ubiquitination, and the ARF was released [9]. The interaction between AtIAA8 and TIR1 regulates the formation of lateral roots, and the abnormal expression of this complex can cause geotropism abnormalities [47]. AtIAA12 regulates the initiation of the early root meristem by interacting with the AtARF5 protein [48]. TaIAA15-1A controls flowering time by interacting with BdARF16 in wheat [49]. Our research demonstrates that ZmIAA29 can interact with ZmARF2, ZmARF7, and ZmARF25 proteins, regulating pathways such as hormone signaling and transcriptional activity. These results might explain the influence of ZmIAA29 on flowering time, causing it to be earlier.

ARF can both positively and negatively regulate the downstream genes of auxin response [50]. ZmHB53, a type II HD-zip transcription factor, is negatively regulated by ZmARF7 [9]. Overexpressed ZmHB53 in Arabidopsis exhibits a phenotype with narrower leaves, early flowering, and enhanced expression of shading response genes. These results suggest that ZmHB53 may be involved in the regulation of the maize shading response, leading to an earlier flowering time [51]. The high expression of ZmIAA29 may enhance the inhibitory effect on ZmARF7, leading to the initiation of the shade response via the inhibitory relieving effects of ZmHB53. ZmMADS69 belongs to the MADS-box gene family, and the MADS-box gene family is one of the biggest plant transcription factor families. It is an essential regulatory gene in plants and plays a key role in flowering, flower development, nutrient absorption in plant roots, and starch biosynthesis [52,53]. It has been confirmed that ZmMADS69 can act on the promoter region of the ZCN8 gene, regulating the flowering period of maize [54]. IAA9/ARF interaction with MADS in tomato [55] and the overexpression of ZmIAA29 might cause a decrease in ZmMADS69 expression levels through ZmARFs. ZmMADS69, in turn, affects flowering through downstream genes such as ZCN8. Overall, the complex regulatory system of ZmARFs and ZmIAA29 is likely to co-regulate the expression of downstream flowering-related genes.

5. Conclusions

In this study, we investigated the overexpression of ZmIAA29 in maize via analysis. The ZmIAA29 encoding protein was located in the nucleus. Moreover, the overexpression
of ZmIAA29 was positively regulated during the flowering time and increased ear weight. In addition, ZmIAA29 interacted with several ZmARFs such as ZmARF2, ZmARF7, and ZmARF25, and these genes are possibly involved in regulating maize growth through hormone signaling and cell development. Taken together, our results suggest that the ZmIAA29 transcription factor is positively involved in the regulation of growth and development during flowering time in maize.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13082028/s1, Supplementary Figure S1: Identification of ZmIAA29 OE; Supplementary Figure S2: Flowering time, kernel weight, and kernel number; Supplementary Figure S3: Relative expression of key genes from RNA-seq and DAP-seq; Supplementary Table S1: Primer and vector information; Supplementary Table S2: RNA-seq and DAP-seq information.

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**Data Availability Statement:** The RNA-seq data can be downloaded from NCBI-SRA, PRJNA974863.

**Conflicts of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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