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The promoters of two CpMYB106-like paralog genes drive differential expression in contrasting cultivars of Cucurbita pepo and respond to abiotic stresses and phytohormones

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Abstract:	Zucchini fruit (Cucurbita pepo L.) is susceptible to chilling injury (CI) during its postharvest life, with this response being cultivar-dependent. A previous transcriptomic analysis comparing cold-tolerant fruit from the cultivar 'Natura' with cold-sensitive fruit from the cultivar 'Sinatra', revealed the transcription factor (TF) CpMYB106-like as a relevant candidate gene for the acquisition of postharvest low-temperature tolerance. C. pepo has two CpMYB106-like paralogs, which are differentially present in the 'Natura' (CpMYB106-likeA) and 'Sinatra' (CpMYB106-likeB) cultivars due to gene and promoter deletions. The aim of this study was to investigate the transcriptional regulation of the CpMYB106-like paralogs to unravel their role in the postharvest life of zucchini fruit. For this, gene expression was analyzed and promoter sequences and their activities were studied through reporter β-glucuronidase (GUS) gene analysis in heterologous systems. An expression analysis showed that CpMYB106-like mRNA levels were induced only in the cold-stored fruit of 'Natura'. The analysis of the promoter sequences showed numerous cis-regulatory elements (CREs), many of which have a differential distribution or frequency between the two paralogs. Promoter basal activity was determined by transient transformation of Nicotiana benthamiana leaves, which showed a higher expression driven by the promoter from the cold-tolerant cultivar 'Natura', which could be explained by the presence of three additional copies of the enhancer element EECCRCAH1 in proMYB106A. The tissue-specific expression pattern, and the response to abiotic stresses and phytohormones controlled by CpMYB106-like promoters was analyzed in transgenic Arabidopsis thaliana plants. GUS activity was mainly detected in the vascular system, leaves, and inflorescences, especially in siliques. With respect to abiotic stresses, low temperature decreased GUS expression in Arabidopsis, but increased it in plants carrying the 'Natura' promoter after a 24-h acclimation period at 22 ºC. Salt treatment induced GUS activity driven by both promoters in Arabidopsis seedlings, but it was higher with the 'Natura' promoter. Furthermore, methyl jasmonate (MeJA) also induced a sharp increase of GUS controlled by proMYB106A. These results suggest that the regulation of CpMYB106-like TFs in zucchini is promoter-driven, and reveal the involvement of these genes in the acquisition of cold tolerance in fruit during postharvest stress conditions.
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Dear Dr. G. Manganaris

Please find enclosed our paper entitled “The promoters of two *CpMYB106-like* paralog genes drive differential expression in contrasting cultivars of *Cucurbita pepo* and respond to abiotic stresses and phytohormones”, to be published in Scientia Horticulturae.

In this work we present the sequencing and heterologous expression of two promoters of a *CpMYB106-like* transcription factor that are differentially expressed in two zucchini cultivars during cold storage. The cultivars analysed show a contrasting behaviour to chilling injury. The promoter structure and the response to different stresses will be explained in the MS. We hope it can be considered for publication in this journal.

Thank you very much for your time and consideration.

Sincerely,

Dolores Garrido
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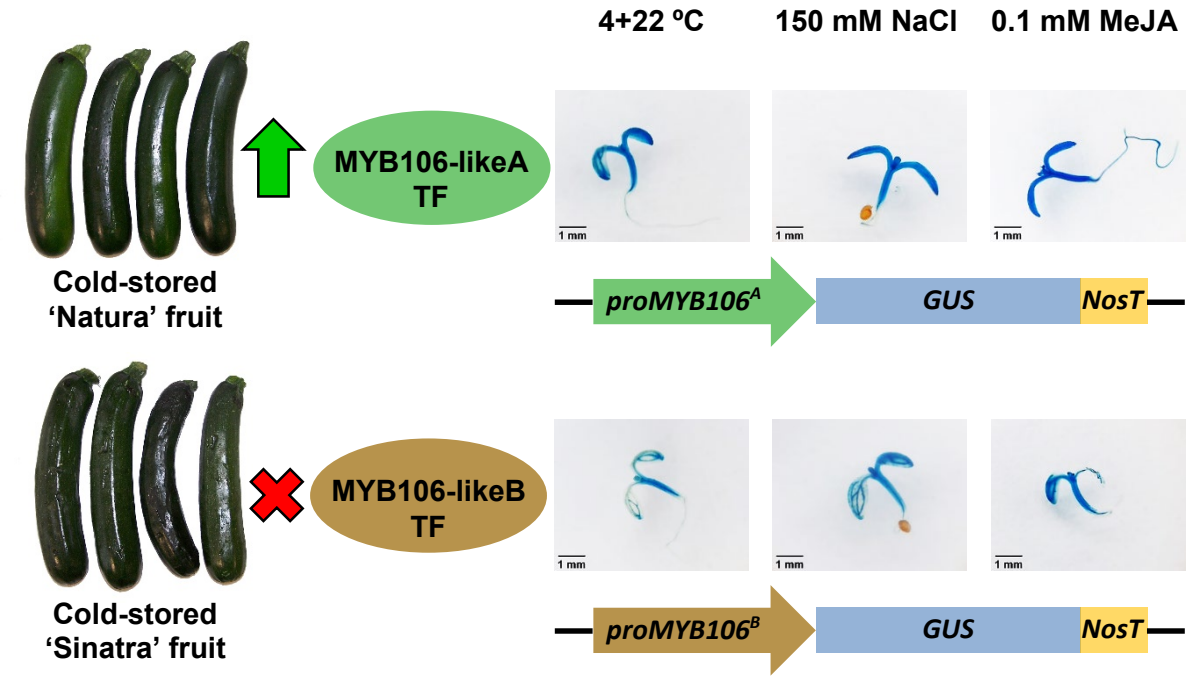
Graphical Abstract



Highlights

- Low temperature induces *CpMYB106-like* in the cold-tolerant ‘Natura’ fruit
- ‘Natura’ promoter, *proMYB106*^{A}, shows higher basal expression
- Acclimation after cold stress increases GUS expression driven by *proMYB106*^{A}
- Salt and MeJA upregulate both promoters, but most prominently *proMYB106*^{A}
- CREs occurrence and distribution in ‘Natura’ promoter could lead to higher expression

The promoters of two *CpMYB106-like* paralog genes drive differential expression in contrasting cultivars of *Cucurbita pepo* and respond to abiotic stresses and phytohormones

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Abstract

Zucchini fruit (*Cucurbita pepo* L.) is susceptible to chilling injury (CI) during its postharvest life, with this response being cultivar-dependent. A previous transcriptomic analysis comparing cold-tolerant fruit from the cultivar 'Natura' with cold-sensitive fruit from the cultivar 'Sinatra', revealed the transcription factor (TF) *CpMYB106-like* as a relevant candidate gene for the acquisition of postharvest low-temperature tolerance. *C. pepo* has two *CpMYB106-like* paralogs, which are differentially present in the 'Natura' (*CpMYB106-likeA*) and 'Sinatra' (*CpMYB106-likeB*) cultivars due to gene and promoter deletions. The aim of this study was to investigate the transcriptional regulation of the *CpMYB106-like* paralogs to unravel their role in the postharvest life of zucchini fruit. For this, gene expression was analyzed and promoter sequences and their activities were studied through reporter β-glucuronidase (GUS) gene analysis in heterologous systems. An expression analysis showed that *CpMYB106-like* mRNA levels were induced only in the cold-stored fruit of 'Natura'. The analysis of the promoter sequences showed numerous *cis*-regulatory elements (CREs), many of which have a differential distribution or frequency between the two paralogs. Promoter basal activity was determined by transient transformation of *Nicotiana benthamiana* leaves, which showed a higher expression driven by the promoter from the cold-tolerant cultivar 'Natura', which could be explained by the presence of three additional copies of the enhancer element EECCRCAH1 in $proMYB106^{A}$. The tissue-specific expression pattern, and the response to abiotic stresses and phytohormones controlled by *CpMYB106-like* promoters was analyzed in transgenic *Arabidopsis thaliana* plants. GUS activity was mainly detected in the vascular system, leaves, and inflorescences, especially in siliques. With respect to abiotic stresses, low temperature decreased GUS expression in Arabidopsis, but increased it in plants carrying the 'Natura' promoter after a 24-h acclimation period at 22 ºC. Salt treatment induced GUS activity driven by both promoters in Arabidopsis seedlings, but it was higher with the 'Natura' promoter. Furthermore, methyl jasmonate (MeJA) also induced a sharp increase of GUS controlled by $proMYB106^{A}$. These results suggest that the regulation of CpMYB106-like TFs in zucchini is promoter-driven, and reveal the involvement of these genes in the acquisition of cold tolerance in fruit during postharvest stress conditions.

Keywords:

Zucchini; Fruit; Postharvest, MYB106; Promoter; Arabidopsis

1. Introduction

The maintenance of fruit quality after harvest, throughout storage, and transportation, is a major concern for producers, especially when dealing with tropical or subtropical fruits. In the case of zucchini (*Cucurbita pepo* L.), the fruit is stored at low temperatures to prevent decay and water loss. However, for many zucchini cultivars, this can lead to chilling injury (CI), consisting in peel pitting, weight loss, and softening, and resulting in a decrease in quality and economic losses. Many studies on CI in zucchini have been published, which have described effective physical and chemical treatments for decreasing damage during cold storage, such as a preconditioning at 15 ºC before the cold storage, or the application of nitric oxide, polyamines or γ-amminobutiric acid (Carvajal et al., 2015a, 2015b; Jiménez-Muñoz et al., 2021; Palma et al., 2014a, 2019). Additionally, it has been demonstrated that different cultivars respond differently to low temperatures during postharvest (Carvajal et al., 2011; Megías et al., 2016). In this sense, our group has characterized the contrasting cultivars 'Natura', whose fruit is tolerant to cold conservation, and 'Sinatra', with cold-sensitive fruit. One differential trait between these cultivars, which has a high impact on postharvest performance, is the development of the fruit cuticle (Carvajal et al., 2021). ‘Natura’ fruit accumulate cuticular wax during storage at low temperature, mainly through the induction of the alkane biosynthetic pathway, whereas ‘Sinatra’ fruit do not increase their cuticular wax load during cold conservation.

To select genes involved in cold tolerance, a transcriptome analysis was performed to compare fruits from these two cultivars, ‘Natura’ and ‘Sinatra’ (Carvajal et al., 2018). Several transcription factors (TFs) that were differentially expressed were identified, such as an important candidate named *CpMYB106-like*, belonging to the MYB family. This gene is a member of the R2R3-MYB subfamily, which includes proteins involved in

many developmental and stress-related processes in plants (He et al., 2023, Wang et al., 2021a). Homologs of *CpMYB106-like* from different species have been described to be involved in cuticle development, suggesting its potential role in postharvest cold resistance (Oshima et al., 2013).

The *de novo* assembly of the *Cucurbita pepo* genome has revealed a whole-genome duplication event (Montero-Pau et al., 2018). Accordingly, two *MYB106-like* paralog genes have been identified, one located in chromosome 3 (LOC111791574, *CpMYB106-likeB*), while the second gene (LOC111785532, *CpMYB106-likeA*) has not been mapped yet. A differential deletion affecting the two paralog genes has been found through a whole-genome sequencing analysis of the cultivars 'Natura' and 'Sinatra'. In the cold-tolerant cultivar 'Natura', the deletion affected the paralog located in chromosome 3 (*CpMYB106-likeB*); whereas in the cold-sensitive cultivar 'Sinatra' the deletion affected the unplaced paralog (*CpMYB106-likeA*). These findings make these cultivars very suitable for the analysis of CpMYB106-like TFs as potential markers for tolerance to cold and other stresses during fruit postharvest storage.

To gain further insights on the involvement of *CpMYB106-like* TFs in the acquisition of cold tolerance of zucchini fruit after harvest, an expression analysis and functional characterization of the promoter sequences of both paralogs, *CpMYB106-likeA* and *CpMYB106-likeB,* is presented here. To achieve this, a β-glucuronidase (GUS) reporter gene analysis was conducted in transiently transformed *Nicotiana benthamiana* and transgenic *Arabidopsis thaliana* plants, as there are currently no efficient genetic transformation protocols available for *Cucurbita pepo*.

2. Material and Methods

2.1. Plant material and postharvest treatment

Cucurbita pepo L. Morphotype Zucchini fruit from the commercial hybrids 'Natura' (EnzaZaden) and 'Sinatra' (Clause-Tezier) were provided by "Hortofrutícola La Ñeca S.L.". Freshly-harvested fruit with uniform length and free from mechanical damage or disease symptoms were randomly divided into three biological replicates of 6 fruits for each storage time, and stored in a temperature-controlled chamber in darkness at 4 ºC and 85–90% relative humidity (RH) for 14 days. Exocarp samples were taken after 0, 1, 5, 10, and 14 days, frozen in liquid N_2, milled to a powder, and stored at −80 ºC.

Cucurbita pepo 'Natura' and 'Sinatra' seeds were surface-sterilized and germinated in culture dishes containing two layers of wet filter paper at 26 ºC. Seeds of *Nicotiana benthamiana* and *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) were surface-sterilized and sown on 1/2 Murashige and Skoog (MS) medium supplemented with 0.8 % (w/v) agar (MSA). The plants were grown in a growth chamber under a 16-h light/8-h dark photoperiod at 22/18 ºC (day/night), 60–80% RH, and 350 μmol $m^{-2}s^{-1}$ photosynthetically active radiation (PAR). Seedlings were either cultured on MSA plates or transferred to a mixture of organic substrate:perlite:vermiculite (3:2:1, w/w/w).

2.2. Semi-quantitative and quantitative RT-PCR

Total RNA was extracted from roots, cotyledons, leaves, female and male flowers, pollen, and exocarp of cold-stored fruit of the zucchini cultivars 'Natura' and 'Sinatra'. The RNA was then treated with DNase, and purified using TRIsure™ reagent (Bioline) and the Direct-zol™ RNAMiniprep kit (Zymo Research). The quality and quantity of RNA was determined by agarose gel electrophoresis and a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific). Reverse transcription was performed using PrimeScript™ RT Master Mix (Takara). The primer pairs used are included in Table S1. For semi-quantitative RT-PCR, the reactions were performed in an XT thermal cycler (Bioer Technology). For qRT-PCR, the reactions were performed in an iCycler iQ thermal cycler

(Bio-Rad) and quantification was performed with the iCycler iQTM associated software (Real Time Detection System Software, version 2.0). Relative gene expression was calculated using non-stored 'Natura' fruit as the calibration sample, and EF-1α as the internal reference gene for normalizing the transcript profiles following the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.3. Isolation and characterization of the full-length promoter sequences

Genomic DNA from the cultivars 'Natura' and 'Sinatra' was isolated using a HigherPurity™ Plant DNA Purification Kit (Canvax) and used as a template. On the basis of the *Cucurbita pepo* MYB106-like (LOC111785532 and LOC111791574) promoter sequences obtained from the NCBI database, different primer sets were designed (Table S1) and employed to amplify the full-length promoter region using FastPANGEA™ High Fidelity DNA Polymerase (Canvax). The amplified fragments were purified using a CleanEasy™ Agarose Purification Kit (Canvax), and inserted via TA-cloning into the pSpark® vector (Canvax). Plasmids containing inserts of the expected size, identified by PCR, were sequenced. The analysis of the *cis*-regulatory elements (CREs) and the transcription factor binding sites of the promoter sequences was performed using the New PLACE (Higo et al., 1999) and PlantPAN 3.0 (Chow et al., 2019) databases.

2.4. Construction of the GUS fusion vector and Agrobacterium transformation

To obtain the *proMYB106::GUS* constructs, the full-length promoter sequences were amplified with forward and reverse primers, CAMHindIIIf and CAMBglIIr, containing *HindIII* and *BglII* restriction sites, respectively (Table S1). The amplified PCR products were purified with a CleanEasy™ Agarose Purification Kit (Canvax), digested with *HindIII* and *BglII* restriction enzymes, and then ligated into the digested pGFPGUS*Plus* plant transformation vector (Vickers et al., 2007), replacing the cauliflower mosaic virus (CaMV) 35S promoter. The recombinant plasmids *proMYB106*^{A}*::GUS* and

143 *proMYB106^{B}::GUS*, or the vector pGFPGUS*Plus*, were employed to transform
144 *Agrobacterium tumefaciens* strain GV3101 using a MicroPulser Electroporator (Bio-
145 Rad).

146 *2.5. Agrobacterium-mediated transient expression assays*

147 *Agrobacterium*-mediated transient expression assays were performed in 6-week old
148 *Nicotiana benthamiana* plants as described previously (Yang et al., 2000). The agro-
149 infiltration was carried out on the abaxial surface of leaves using needleless syringes.
150 After the infiltration, plants were kept under normal growing conditions, and the agro-
151 infiltrated leaves were collected after 3, 4, and 5 days, sampled for histochemical GUS
152 staining, and then frozen in liquid N_2, milled to a powder, and stored at −80 ºC. Two
153 biological replicates of agro-infiltrated leaves from 3 different plants were used per
154 condition in each experiment.

155 *2.6. Arabidopsis thaliana transformation and treatments*

156 Arabidopsis plants were transformed with *A. tumefaciens* GV3101 harboring the
157 recombinant plasmids or pGFPGUS*Plus* vector using the floral-dip method (Clough and
158 Bent, 1998). Transformants were selected for hygromycin resistance according to
159 Harrison et al. (2006). Hygromycin-resistant T_1 transgenic seedlings were transplanted
160 into soil and confirmed by PCR. The transgene copy number and heterozygosis were
161 determined through the segregation ratio of the T_2 and T_3 plants and by qRT-PCR
162 (Traewachiwiphak et al., 2018)

163 Abiotic stress treatments were conducted in 5-day-old T_3 homozygotic Arabidopsis
164 seedlings. For the cold treatment, seedlings in MSA medium were placed in a growth
165 chamber at 4 ºC for 7 days with or without an additional day of acclimation at 22 ºC. For
166 osmotic and salt stress analyses, seedlings were transferred to MSA medium
167 supplemented with 150 mM mannitol or NaCl for 7 days. For phytohormone treatments,

seedlings were transferred to MSA medium supplemented with 0.1 mM abscisic acid (ABA) or methyl jasmonate (MeJA) for 3 days. Treated and control seedlings were used for GUS histochemical staining or frozen in N_2 liquid and stored at -80 ºC for GUS fluorometric assay. Two biological replicates of 25-30 plants were used per condition for each experiment.

2.7. Histochemical staining and fluorometric GUS assay

GUS staining and fluorometric activity assays were performed in the transiently-transformed *N. benthamiana* leaves and T_3 homozygotic Arabidopsis plants, as described by Jefferson et al. (1987) with some modifications. For histochemical assays, discs excised from agro-infiltrated leaves, different Arabidopsis organs or seedlings, were vacuum infiltrated in 100 mM sodium phosphate buffer at pH 7.0, supplemented with 0.5 mM potassium ferricyanide and potassium ferrocyanide, 10 mM EDTA, 0.05% (v/v) Triton X-100, and 1 mM 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (X-Gluc) for 10 min and incubated overnight at 37 ºC. After washing the samples with 100 mM sodium phosphate buffer at pH 7.0, chlorophyll was removed in a solution of increasing ethanol concentration, being rehydrated afterwards.

For the fluorometric assays, samples were homogenized in 50 mM sodium phosphate buffer at pH 7.0, supplemented with 10 mM 2-mercaptoethanol, 10 mM EDTA, 0.1% (w/v) sodium lauryl sarcosine, and 0.1% (v/v) Triton X-100. After centrifugation at 10,000 x *g* and 4 ºC for 10 min, the supernatants were collected for the GUS assay using 10 mM 4-methylumbelliferyl β-d-glucuronide (MUG) as a substrate. Reactions were performed in extraction buffer for 45 min at 37 ºC, and were stopped by adding 200 mM Na_2CO_3. Fluorescence was measured with excitation at 365 nm and emission at 455 nm using a Synergy HTX plate reader (BIO-TEK). The GUS activity was calculated based on a standard curve of 4-methyl umbelliferone (MU) and expressed in nmol of MU

formed per min and mg protein. The protein content was quantified using the method described by Lowry et al. (1951), with bovine serum albumin (BSA) as the standard.

2.8. Bioinformatic and statistical analysis

Alignments were performed using CLUSTAL OMEGA at EMBL-EBI (https://www.ebi.ac.uk/Tools/msa/clustalo/). The phylogenetic relations between MYB106 TFs from different species were analyzed using MEGA11 software (Tamura et al., 2021) through the construction of phylogenetic trees using the Maximum Likelihood method based on the Poisson correction model (Zuckerkandl and Pauling, 1965), with 2000 bootstrap replicates. Protein sequences were obtained using The Arabidopsis Information Resource (https://www.arabidopsis.org/), NCBI, and the Cucurbit Genomics Database (http://cucurbitgenomics.org/).

The experimental designs were completely randomized. The average of three independent experiments was used for statistical analysis. The data were subjected to an ANOVA using the SPSS 26.0 software (SPSS Inc.). Pairwise comparisons of means were assessed with a t-test, and multiple comparisons of means were assessed by Duncan's test, and differences at $p < 0.05$ were considered significant.

3. Results

3.1. Expression profile and sequence analysis of the transcription factor MYB106-like in Cucurbita pepo

Gene expression of CpMYB106-like was analysed in fruit of cultivars 'Natura' and 'Sinatra' during their postharvest storage at 4 ºC (Fig. 1A). At harvest, mRNA levels of *CpMYB106-like* were barely detected in the fruit. However, during postharvest cold storage, the expression of *CpMYB106-like* was induced in the cold-tolerant cultivar 'Natura' fruit after 5 days, and reached its maximum level after 10 days of cold exposure. On the other hand, in the cold-sensitive cultivar 'Sinatra', there was no significant change

in *CpMYB106-like* expression under the same postharvest stress conditions. Fig. 1B depicts the external appearance of 'Natura' and 'Sinatra' fruit after 14 days of cold storage, showing the differential response of the two cultivars to low temperature stress.

Organ-specific expression patterns of *CpMYB106-like* were investigated (Fig. S1). *CpMYB106-like* mRNA was not detected in roots or cotyledons, but a strong accumulation was present in leaves of both cultivars. The expression in reproductive organs was differential between them: *CpMYB106-like* transcripts were detected in female flowers of 'Natura', whereas in 'Sinatra', the expression appeared in male flowers, and a faint signal was found in mature pollen grains.

The *C. pepo* genome has two duplicate copies of MYB106-like TF (*CpMYB106-likeA* and *CpMYB106-likeB*), with a high degree of similarity between them (98.6%). The protein sequences of the *CpMYB106-like* paralogs were aligned with homologous sequences from other cucurbits and with the gene from the model species *Arabidopsis thaliana* (Fig. 2A). The deduced MYB106-likeA and MYB106-likeB proteins presented the structure of the R2R3-MYB TF class, with an N-terminal DNA-binding domain (the MYB domain) shaped by two repeats of three α–helices R2 and R3. Two single nucleotide polymorphisms (SNPs) located in the coding region of *CpMYB106-likeA* were found in the cultivar 'Natura' at positions 210 and 212, respectively, resulting in an amino acid substitution of serine by threonine at residue 71 (S71T) in the CpMYB106-likeA protein.

To assess the genetic relationships between homologs, a phylogenetic tree was inferred using MYB106 TFs from different cucurbit species and *Arabidopsis thaliana* (Fig. 2B). In the *Cucurbita* genus, the proteins clustered together, with CpMYB06-likeA being closer to the MYB106-like TF from *C. maxima* and CpMYB106-likeB closer to the MYB106-like TF from *C. moschata*.

3.2. Sequence analysis of the promoters from the CpMYB106-like paralog genes and basal expression level

In total, 1530 and 1527 base pairs upstream of the ATG start codon were sequenced from promoters *proMYB106*^{A} and *proMYB106*^{B}, respectively, which showed only a 4.08% of divergence between them. The analysis of putative *cis*-regulatory elements (CREs) revealed 392 and 396 CREs for *proMYB106*^{A} and *proMYB106*^{B}, respectively (Table S2). Among these elements, 223 and 226 had a differential occurrence or frequency (Table 1). The most prevalent was CAATBOX1, followed by CACTFTPPCA1, and DOFCOREZM. Several TATA box elements were located at different positions, with the nearest to the ATG start codon located at -54 and -52 in *proMYB106*^{A} and *proMYB106*^{B}, respectively. The presence of 6 copies of the enhancer element EECCRCAH1 in *proMYB106*^{A} was noteworthy, while *proMYB106*^{B} only had 3 copies (Fig. S2). Additionally, *proMYB106*^{B} also contained two negative regulatory elements: S1FBOXSORPS1L21 and S1FSORPL21.

Both promoters presented a high number of CREs associated with both abiotic and biotic stress responses, including ACGTTBOX, BIHD1OS, DRE1COREZMRAB17, GT1GMSCAM4, LTRECOREATCOR15, MYBST1, and WBOXNTERF3. Additionally, various phytohormone-responsive elements were found with differential occurrence or frequency, including -300CORE, ACGTATERD1, ARFAT, ARR1AT, ASF1MOTIFCAMV, DPBFCOREDCDC3, PYRIMIDINEBOXHVEPB1, and SEF4MOTIFGM7S, which are associated with responses to abscisic (ABA), jasmonic (JA), and salicylic (SA) acids, auxins, cytokinins, and gibberellins.

The analysis of the putative *trans*-elements revealed a number of TF families with binding sites (TFBSs) showing a differential distribution and frequency between *proMYB106*^{A} and *proMYB106*^{B} (Table S3). Among them, the families with the higher number of TFBSs

were APETALA 2/ethylene-responsive (AP2/ERF) factors, NF-Y TF complex, and basic leucine zipper (bZIP) TFs.

The basal promoter activities of *CpMYB106-likeA* and *CpMYB106-likeB* were analysed through transient expression assays in *Nicotiana benthamiana* leaves using *Agrobacterium* carrying the recombinant vectors *proMYB106^{A}::GUS* and *proMYB106^{B}::GUS* (Fig. 3). The promoter activities displayed significant differences, with the highest GUS activity associated to promoter *proMYB106^{A}*, which belongs to the cold-tolerant cultivar 'Natura'.

3.3. Tissue-specific expression pattern in transgenic Arabidopsis thaliana

Functional characterization was performed by analyzing the reporter GUS activity under the transcriptional control of *CpMYB106-likeA* and *CpMYB106-likeB* promoters. To accomplish this, different T_3 homozygous Arabidopsis lines harboring only a single transgenic insert were obtained. Transgene copy number was estimated by segregation ratio and qRT-PCR in T_2, and heterozygosity was determined in T_3 using the same methods (Fig. S3). Different transgenic lines for the same recombinant vector varied in GUS activity intensity (Fig. S4). This variation could be attributed to differences in transgene integration. In this study, we presented two lines harboring the binary vectors *proMYB106^{A}::GUS* (L7) and *proMYB106^{B}::GUS* (L8) with a similar range and pattern of GUS expression. Transcriptional activity was analyzed throughout the plant's development (Fig. 4A), as well as in reproductive organs (Fig. 4B). In seedlings, GUS activity was observed in the root and cotyledon vascular tissues, hypocotyl, and hydathodes, being more intense in plants transformed with the *CpMYB106-likeA* promoter. The GUS gene was strongly expressed in leaves of adult plants, particularly in vascular tissues. Inflorescences from both transgenic lines showed GUS activity in pedicels, sepals, petals, stamen filaments, upper part of the carpels, and stigmatic tissue.

The most intense staining was observed in mature siliques, especially in the junction region. Additionally, GUS activity was also detected in the radicles of imbibed seeds.

3.4. Transcriptional regulation in Arabidopsis seedlings under abiotic stresses and phytohormone application

To investigate the potential role of *CpMYB106-like* in the response to low temperature stress, cold was applied to 5-day-old transgenic Arabidopsis seedlings carrying the GUS gene under control of either *proCpMYB106*^{A} or *proCpMYB106*^{B} for a period of 7 days (Fig. 5). A low temperature decreased GUS expression, as compared to control plants maintained at 22 ºC, leading to reduced histochemical staining and activity. Following the cold treatment, a 24 hour-acclimation period at 22 ºC was applied. The *proMYB106*^{A}*::GUS* line exhibited a significant increase in GUS activity after the acclimation. The GUS activity did not change significantly in seedlings carrying the *35S* promoter in these treatments.

The response of the transgenic Arabidopsis seedlings to osmotic stress (150 mM mannitol) or salt stress (150 mM NaCl) was also studied (Fig. 6). The application of mannitol did not affect GUS activity, and no differences were detected between promoters. However, salt stress triggered GUS expression in transgenic lines harboring *proCpMYB106*^{A} and *proCpMYB106*^{B}, although a higher activity was measured in the promoter from ‘Natura’. In seedlings carrying the *35S* promoter, no significant induction of GUS activity was observed.

The effect of stress phytohormones on promoter activity was studied through the exogenous application of 0.1 mM of ABA (Fig. S5) or MeJA (Fig. 7). The ABA treatment led to reduced GUS histochemical staining in all transgenic lines. In contrast, MeJA enhanced GUS activity in transgenic seedlings, with the most notable effect observed with the *CpMYB106-likeA* promoter, which increased the activity 5-fold with respect to

its control, much higher than in *CpMYB106-likeB* transformed plants. Seedlings carrying the *35S* promoter did not change GUS activity after MeJA treatment.

4. Discussion

Postharvest management of tropical and subtropical fruit, such as zucchini fruit, remains a challenge due to the limitations imposed by their own physiology. Since one of the main limitations for farmers and marketers is the maintenance of postharvest quality and overcoming chilling injury after the fruits are collected, several studies have been conducted in order to undermine this problem with treatments and procedures that preserve quality (Carvajal et al., 2015a; Castro-Cegrí et al., 2023a; Jiménez-Muñoz et al., 2021). Several metabolites involved in the resistance to cold storage have been identified in the exocarp of zucchini fruit (Palma et al. 2016, 2014a, 2014b). However, the molecular mechanism underlying the responses to adverse environmental conditions are still unclear. Finding candidate genes to regulate the defense against postharvest stress, and unravelling their transcriptional regulation could help us obtain new zucchini cultivars, which use could reduce economic losses due to fruit decay. After the transcriptome comparison between cold-resistant and cold-sensitive cultivars, TF MYB106-like was selected as a candidate gene involved in cold stress resistance (Carvajal et al., 2018). Its highest homology was found with the MIXTA-like TFs, which are known to coordinate the development of trichomes and cuticle (Jakoby et al., 2008; Oshima et al., 2013; Shi et al., 2018). According to this homology, it is possible that *CpMYB106-like* could also be involved in cuticle development, the first line of defense of the fruit against a negative environment. When comparing the fruit cuticle of the cold-tolerant cultivar ‘Natura’ with the cold sensitive ‘Sinatra’, an accumulation of cuticular waxes and an induction of the alkane biosynthesis pathway were detected in the cold-tolerant cultivar, whose fruit also lost less water during low temperature postharvest storage (Carvajal et al., 2021). In

tomato, mutants of a MIXTA-like TF also showed fruit with altered postharvest water loss and resistance to pathogens (Lashbrooke et al., 2015).

To decipher the transcriptional regulation underlying the *CpMYB106-like* expression pattern, promoter sequences from the paralog genes were isolated and sequenced. The *in silico* analysis revealed the presence of multiple CREs, many of them with differential distribution and frequency in *proMYB10*^{A} and *proMYB106*^{B}. The most abundant CREs identified were CAATBOX1, which are core promoter elements that contribute to tissue-specific activity (Shirsat et al., 1989), CACTFTPPCA1, which drives mesophyll-specific gene expression (Gowik et al., 2004), and DOFCOREZM, a core site required for binding of DOF (DNA-binding One Zinc Finger) proteins, a TF family involved in many biological processes in higher plants (Noguero et al., 2013). The analysis of the TF families binding to *CpMYB106-like* promoters revealed that the APETALA2/ETHYLENE-RESPONSIVE ELEMENT BINDING PROTEINS (AP2/EREBP) family, which includes the WIN/SHINE (SHN) clade, were identified as regulators of *CpMYB106-like* expression. The member of that clade with the highest expression levels in zucchini fruit, *CpWIN1-like*, increased its mRNA levels significantly in 'Natura' fruits during the first 24 h of exposure to cold (Carvajal et al., 2021). In tomato fruit, SlSHINE3 has been proven to act upstream of *Sl*MIXTA-like, thus regulating cuticle development (Lashbrooke et al., 2015).

The transient expression in *Nicotiana benthamiana* leaves enabled the comparison of the basal expression level of the GUS reporter gene controlled by both *CpMYB106-like* promoters, with *proMYB106*^{A} showing the highest level of expression. The CAATBOX1 element, which was found in a higher copy number in the *CpMYB106-likeA* promoter, has been described as a positive regulator that potentially enhances gene expression (Bhalothia et al., 2016; Jiang et al., 2018). The enhancer element EECCRCAH1,

associated with a high rate of transcription, was detected in both promoters. In cassava, the deletion of two of these enhancer elements sharply decreased expression of a starch synthase promoter (Guan et al., 2016). In the case of zucchini, *proMYB106*^{A} from the cold-tolerant cultivar 'Natura' had six copies of EECCRCAH1, whereas only three copies were detected in the *proMYB106*^{B} of 'Sinatra'. This difference could contribute to the higher levels of transcription detected in 'Natura'. Furthermore, *proMYB106*^{B} contained two negative regulatory elements, S1FBOXSORPS1L21 and S1FSORPL21, which could contribute to the down-regulation of *CpMYB106-likeB* and to lower its expression levels. These former elements are responsible for a decrease in the promoter activity of several photosynthetic-related genes in spinach (Villain et al., 1994; Zhou et al., 1992).

In young Arabidopsis plantlets, GUS staining was detected mainly in the vascular system; whereas in adult plants, GUS activity was mainly located in leaves and inflorescences, especially in siliques. These results are consistent with the expression pattern found in *Cucurbita pepo*; where *CpMYB106-like* mRNA was accumulated in leaves, male and female flowers. In 'Natura', the expression was found in leaves and female flowers, whereas in 'Sinatra', it was prominent in leaves and male flowers, including mature pollen grains. This difference in expression could be a result of variations in the promoter region, as described for Wuschel-related Homeobox (WOX) TFs in the cultivars 'Chardonnay' and 'Cabernet Sauvignon' of *Vitis vinifera*, where differences between promoter sequences were found to drive the differential expression of these TFs in flowers and seedlings (Boccacci et al., 2017). In spite of the differential expression found in the sexual tissues of zucchini 'Natura' and 'Sinatra' plants, no differences were detected between the *CpMYB106-like* promoters, in regard to male or female reproductive tissues and fruit in Arabidopsis. This divergence could be related to the fact that *Arabidopsis thaliana* is a bisexual species which produces hermaphroditic flowers, whereas zucchini is unisexual

monoecious, with both male and female flowers on the same plant (Martínez and Jamilena, 2021). When searching for TF families that could bind to *CpMYB106-like* promoters, a binding site for the AP2-like ethylene-responsive TF AINTEGUMENTA, a gene is required for the development of the female gametophyte (Klucher et al., 1996), was found only in the 'Natura' promoter *proMYB106*^{A}. The expression of *CpMYB106-like* in the female organs in 'Natura' could indicate an involvement of the gene in sex determination.

The transcriptional activation of *CpMYB106-like* genes under cold stress conditions was studied in transgenic Arabidopsis. The GUS activity was down-regulated in plants exposed to cold. However, after an acclimation period of 24 h at 22 ºC, a significantly higher induction in plants carrying the 'Natura' promoter *proMYB106*^{A} was observed. This response could be related to the presence of one more copy of LTRECOREATCOR15 in its sequence closer to the transcription start site (TSS). In *Brassica napus*, two copies of LTRECOREATCOR15 in the promoter region of the cold-inducible gene *BN115* were critical for inducing a high gene expression, with the one closer to the TSS being the factor that most contributed to this effect (Jiang et al., 1996). MYB proteins are TFs involved in the adaptation to salt stress conditions, with some of them related with the metabolism of the cuticle, as is the case of MYB49 in Arabidopsis. The overexpression of MYB49 produced plants more tolerant to salt, with a thicker leaf cuticle due to an induction of cutin biosynthesis and the intracuticular wax deposited within the cutin matrix (Zhang et al., 2020). In this work, a NaCl treatment triggered the up-regulation of GUS in both promoters, with a higher activity observed in plants transformed with the 'Natura' promoter *proMYB106*^{A}. The *cis* element GT1GMSCAM4, which is necessary for pathogen- and salt-induced gene expression (Park et al., 2004), was present in both promoters, with one more binding site in the *proMYB106*^{A}.

The role of ABA in the postharvest life of zucchini fruit is crucial, and an increase in its concentration has been associated with enhanced tolerance to chilling stress (Benítez et al., 2022; Carvajal et al., 2017; Castro-Cegrí et al., 2023b). Both promoter sequences from 'Natura' and 'Sinatra' contained a significant number of CREs associated with responses to ABA. However, the application of this phytohormone down-regulated GUS expression in Arabidopsis seedlings carrying either promoter. On the contrary, MeJA induced the promoter activity of *CpMYB106-like*, with GUS activity being significantly higher for the 'Natura' *proMYB106*^{A} promoter. It has been proposed that EECCRCAH1 and GT1GMSCAM4 play a significant role in biotic defense responses in maize, potentially via *trans*-acting factors that are dependent on plant hormones such as MeJA (Shi et al., 2013). Both CREs were present in a higher copy number in the *CpMYB106-likeA* promoter sequence. The effect of exogenous MeJA on fruit production and quality during their postharvest life has been widely studied (García-Pastor et al., 2019, Kondo, 2022, Wang et al., 2021b). As *C. pepo CpMYB106-like* paralogs are MeJA inducible, similar to another R2R3-MYB TF such as *GsMYB15* (Shen et al., 2018), new strategies to preserve the quality of this fruit during the postharvest period could be designed with this phytohormone.

Taken together, the final conclusion is that *CpMYB106-like* paralogs are stress-inducible genes regulated by stress-responsive promoters. A set of *cis*-regulatory elements is responsible for the induction of *CpMYB106-like* promoters during acclimation after the exposure to low temperature and by application of salt stress and MeJA. The higher number of copies of the enhancer element EECCRCAH1 found in the *CpMYB106-likeA* promoter sequence could explain the higher expression levels in the cold-tolerant cultivar. Overall, the outcomes of this study suggest that the TFs CpMYB106-like are involved in

the regulation of the response of zucchini fruit during postharvest stress conditions, with these genes being excellent candidates for future breeding programs.

CRediT authorship contribution statement

Fátima Carvajal: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Raquel Jiménez-Muñoz:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Alejandro Castro-Cegrí:** Investigation, Writing – review & editing. **Francisco Palma:** Funding acquisition. **Dolores Garrido:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Data Availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version.

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Figure Captions

Fig. 1. (A) Relative expression of *CpMYB106-like* transcription factor in exocarp of 'Natura' and 'Sinatra' fruit during the postharvest cold-storage at 4ºC. Different letters indicate significant differences according to Duncan's test ($p < 0.05$). Data presented are means ± SE of triplicate samples of six fruit each. (B) External appearance of fruit from the cultivars 'Natura' and 'Sinatra' after 14 days of cold storage.

Fig. 2. (A) Multiple sequence alignment of the conserved domains in the deduced amino acid sequence of CpMYB106-likeA (LOC111785532) and B (LOC111791574), other cucurbits, and *Arabidopsis thaliana* MYB106 transcription factors (TFs). The shading represents different degrees of conservation among sequences; black indicates identical residues, dark grey identical residues in cucurbits, and light grey identical residues in *Cucurbita* genus. Red residue indicates amino acid substitution. The positions of the three α-helices that form each repeat are marked as Helix 1 to Helix 3. (B) Phylogenetic relationships between MYB106-like TFs of the cucurbit species *Cucurbita pepo*, *Cucurbita maxima* (LOC111482355), *Cucurbita moschata* (LOC111441815), *Cucurbita argyrosperma* (KAG6580950), *Benincasa hispida* (LOC120091763), *Cucumis melo* (LOC103486978) and *Cucumis sativus* (LOC101212616), and the homologous protein from *Arabidopsis thaliana* (AT3G01140). Node numbers represent the percentage of bootstrap replicates containing each clade. The length of the branch lines indicates the extent of divergence.

Fig. 3. Basal GUS expression driven by *CpMYB106-like* promoters isolated from *C. pepo* cultivars 'Natura' (*proMYB106*^{A}) and 'Sinatra' (*proMYB106*^{B}) in transiently transformed *Nicotiana benthamiana* leaves. (A) Fluorometric GUS quantification. Statistical

significance is shown at $**p < 0.01$. Error bars represent standard error (SE) from mean of three independent experiments. (B) Histochemical GUS staining.

Fig. 4. GUS reporter activity driven by *CpMYB106-like* promoters isolated from *C. pepo* cultivars 'Natura' (*proMYB106*^{A}) and 'Sinatra' (*proMYB106*^{B}) or *35S* in T_3 Arabidopsis transgenic lines at different stages of development (A) and in reproductive organs (B). Scale bar correspond to 1 or 0.5 mm.

Fig. 5. Transcriptional regulation of *CpMYB106-like* promoters isolated from *C. pepo* cultivars 'Natura' (*proMYB106*^{A}) and 'Sinatra' (*proMYB106*^{B}) or *35S* in 5-day-old Arabidopsis seedlings after 7 days at 4 °C and with an additional day of acclimation at 22 ºC. (A) Fluorometric GUS quantification. Different letters indicate significant differences according to Duncan's test ($p < 0.05$), ns indicates non-significant differences. Error bars represent standard error (SE) from mean of three independent experiments. (B) Histochemical GUS staining. Scale bar correspond to 1 mm.

Fig. 6. Transcriptional regulation of *CpMYB106-like* promoters isolated from *C. pepo* cultivars 'Natura' (*proMYB106*^{A}) and 'Sinatra' (*proMYB106*^{B}) or *35S* in 5-day-old Arabidopsis seedlings treated with 150 mM mannitol or NaCl during 7 days. (A) Fluorometric GUS quantification. Different letters indicate significant differences according to Duncan's test ($p < 0.05$), ns indicates non-significant differences. Error bars represent standard error (SE) from mean of three independent experiments. (B) Histochemical GUS staining. Scale bar correspond to 1 mm.

Fig. 7. Transcriptional regulation of *CpMYB106-like* promoters isolated from *C. pepo* cultivars 'Natura' (*proMYB106*^{A}) and 'Sinatra' (*proMYB106*^{B}) or *35S* in 5-day-old Arabidopsis seedlings treated with 0.1 mM methyl jasmonate (MeJA) during 3 days. (A)

720 Fluorometric GUS quantification. Different letters indicate significant differences
721 according to Duncan's test ($p < 0.05$), ns indicates non-significant differences. Error bars
722 represent standard error (SE) from mean of three independent experiments. (B)
723 Histochemical GUS staining. Scale bar correspond to 1 mm.

724

725

Table 1. Putative *cis*-regulatory elements present with different copy number in the *CpMYB106-likeA* and *CpMYB106-likeB* promoter sequences identified from New Place.

***Cis*-regulatory element (CRE)**	**Sequence**	***proMYB106*^{A}**		***proMYB106*^{B}**		**Description**
		Copies	Position	Copies	Position	
-300CORE	TGTAAAG	0	-	1	-1355 (-)	Endosperm; ABA and auxin-responsive element
5659BOXLELAT5659	GAAWTTGTGA	0	-	1	-650 (+)	Pollen-specific expression
ACGTATERD1	ACGT	6	-1409,-993,-558 (+/-)	8	-1406,-996,-559,-453 (+/-)	Seed; ABA and auxin-responsive element
ACGTTBOX	AACGTT	0	-	2	-454 (+/-)	Drought, ABA, and auxin-responsive element
ARFAT	TGTCTC	1	-402 (-)	0	-	Auxin-responsive element
ARR1AT	NGATT	16	-1493,-1192,-1131,-1019,-910,-694,-544,-108 (+) -1426,-1345,-754,-491,-446,-329,-210,-61 (-)	15	-1490,-1022,-912,-695,-191,-106 (+) -1423, -1365,-1349,-1097,-756,-492,-327,-210,-59 (-)	Cellular metabolism and environmental responsiveness, cytokinin-regulated transcription
ASF1MOTIFCAMV	TGACG	1	-1148 (-)	2	-1189 (+) -1152 (-)	Root-specific; ABA, SA, JA, and auxin-responsive element
B2GMAUX28	CTTGTCGTCA	0	-	1	-1157 (+)	Auxin-responsive element
BIHD1OS	TGTCA	9	-1009,-323 (+) -1406,-1186,-1159,-1042,-523, -302,-290 (-)	7	-1012,-321 (+) -1403,-1043,-524,-300,-290 (-)	Disease resistance response
BS1EGCCR	AGCGGG	1	-1001 (-)	0	-	Secondary metabolism; glucose-regulated genes
CAATBOX1	CAAT	28	-1346,-1324,-1296,-904,-755,-689,-684,-638, -587,-551,-520,-509,-370,-299,-191,-32 (+) -1438,-1226,-1211,-1017,-896,-830,-765,-760, -718,-585,-542,-513 (-)	26	-1350,-1328,-1300,-1048,-906,-757,-690,-639, -685,-588,-552,-521,-510,-297,-282,-30 (+) -1435,-1229,-1214,-1020,-898,-832,-767-762, -719,-586, -514 (-)	Tissue specific gene expression, seed; enhancer, CAAT box
CACTFTPPCA1	YACT	23	-1329,-1283,-1249,-1156,-984,-914,-725,-652, -471,-375,-261,-203 (+) -1026,-769,-598,-504, -464,-436,-182,-150,-70 (-)	25	-1338,-1333,-1287,-1253,-1159, -1007,-987, -916,-727,-653,-472,-373 (+) -1057,-771,-599, -505,-465,-456,-437,-385,-182,-150,-68 (-)	Mesophyll-specific gene expression
CGACGOSAMY3	CGACG	1	-1101 (+)	0	-	Sugar signal response
DPBFCOREDCDC3	ACACNNG	2	-1411 (+) -1028 (-)	3	-1408 (+) -1059,-1031 (-)	Embryo and ABA-specific response element
DRE1COREZMRAB17	ACCGAGA	1	-405 (+)	0	-	Drought and ABA-responsive element
EECCRCAH1	GANTTNC	6	-1287,-1076,-1063,-762,-720 (+) -1096 (-)	3	-1291,-1080,-764 (+)	Enhancer element
GATABOX	GATA	10	-1331,-1273,-1221,-883,-879,-125 (+) -1293, -1117,-743,-7 (-)	11	-1263,-1224,-1164,-885,-881,-602,-123 (+) -1197,-1121,-745,-7 (-)	Response to light and core element
GT1GMSCAM4	GAAAAA	8	-1468,-1234,-198 (+) -1142,-750,-678,-606, -133 (-)	7	-1465,-1238,-198 (+) -1146,-752,-679,-131 (-)	Pathogen and salt-induced element
HEXAMERATH4	CCGTCG	1	-1101 (-)	0	-	Meristematic specific expression
IBOXCORE	GATAA	1	-1118 (-)	2	-1164,-1122 (-)	Light regulation, involved in sugar repression
INRNTPSADB	YTCANTYY	3	-964,-691 (+) -544 (-)	2	-967,-692 (+)	Light responsive; initiator motif
LTRECOREATCOR15	CCGAC	3	-733,-429,-362 (-)	2	-735,-430 (-)	Low temperature-responsive element (LTRE), drought, ABA
MARARS	WTTTATRTTTW	0	-	1	-956 (+)	Play a role in scaffold attachment region

MARTBOX	TTWTWTTWTT	2	-951 (+) -935 (-)	7	-954,-138,-137,-136 (+) -943,-940,-937 (-)	Play a role in scaffold attachment region
MYBST1	GGATA	2	-743,-7 (-)	3	-1165 (+) -745,-7 (-)	Stress and ABA-responsive element, transcriptional activator, sugar repression
NODCON1GM	AAAGAT	3	-546 (+) -753,-328 (-)	2	-755, -326 (-)	Nodule-specific expression
NODCON2GM	CTCTT	5	-1082,-259 (+) -1431,-1134,-393 (-)	6	-1086,-259 (+) -1428,-1138,-575,-391 (-)	Nodule-specific expression
NONAMERMOTIFTAH3H4	CATCCAACG	1	-1052 (-)	0	-	Meristematic specific expression
OSE1ROOTNODULE	AAAGAT	3	-546 (+) -753,-328 (-)	2	-755,-326 (-)	Root-specific expression element
OSE2ROOTNODULE	CTCTT	5	-1082,-259 (+)- 1431,-1134,-393 (-)	6	-1086,-259 (+) -1428,-1138,-575,-391 (-)	Root-specific expression element
POLASIG1	AATAAA	10	-1112,-903,-853,-837,-550,-538 (+) -1392, -1127,-950,-620 (-)	11	-1116,-940,-905-855,-839,-551,-539,-146 (+) -1389,-1131,-621(-)	Polyadenylation signal
POLASIG2	AATTAAA	3	-813,-519 (+) -943 (-)	2	-815,-520 (+)	Polyadenylation signal
POLASIG3	AATAAT	14	-1276,-868,-865,-862,-859,-856,-840,-683,-646 (+) -1441,-970,-660,-532 (-)	15	-1280,-943,-870,-867,-864,-861,-858,-842, -684,-647 (+) -1438,-973,-661,-625,-533 (-)	Polyadenylation signal
POLLEN1LELAT52	AGAAA	6	-789,-145 (+) -1321,-1123,-676,-274 (-)	5	-723 (+) -1325,-1127,-677,-274 (-)	Pollen-specific transcription
PREATPRODH	ACTCAT	3	-1248,-1178 (+) -1311 (-)	2	-1252,-1181 (+)	Proline response
PYRIMIDINEBOXHVEPB1	TTTTTTCC	1	-199 (-)	0	-	Element required for GA induction
QELEMENTZMZM13	AGGTCA	0	-	1	-1090 (-)	Pollen-specific enhancing factor, Q(quantitative)-element
RHERPATEXPA7	KCACGW	6	-1501,-1067,-471,-117 (+) -1409,-1291 (-)	5	-1498,-481,-115 (+) -1406,-1295 (-)	Root hair-specific element
ROOTMOTIFTAPOX1	ATATT	15	-1518,-1418,-1228,-898,-878,-832,-626,-310, -124 (+) -1295,-1229,-899,-833,-311,-83 (-)	16	-1515,-1415,-1231,-952,-900,-880 -834,-627, -308,-122 (+) -1299,-1232,-901,-835,-309,-81 (-)	Root-specific expression
S1FBOXSORPS1L21	ATGGTA	0	-	1	-279 (-)	Plastid-related genes repressor
S1FSORPL21	ATGGTATT	0	-	1	-281 (-)	Plastid-related genes repressor
SEF3MOTIFGM	AACCCA	1	-218 (-)	0	-	SEF3 binding site
SEF4MOTIFGM7S	RTTTTTR	11	-821,-670,-178 (+) -1356,-1280,-1183,-892, -665,-565,-158,-77 (-)	10	-1165,-823,-671,-178 (+) -1284,-894,-666,-566, -158,-75 (-)	SEF4 binding site; ABA-responsive element
SREATMSD	TTATCC	0	-	1	-1165 (-)	Tissue-specific element and sugar repression
SURECOREATSULTR11	GAGAC	1	-402 (+)	0	-	Sulfur and auxin responsive factor
TATABOX5	TTATTT	10	-1391,-1126,-949,-623,-619 (+) -1277,-1113, -869, -841,-647 (-)	11	-1388,-1130,-624,-620 (+) -1281,-1117,-871, -843, -648,-458,-147 (-)	TATA box
WBOXNTERF3	TGACY	3	-579 (+) -206,-119 (-)	4	-1090,-580 (+) -206,-117 (-)	Wounding

Base abbreviations (IUPAC notation) are as follows: K=[G,T], N=[any nucleotide], R=[A,G], W=[A,T], Y=[C,T]. CREs were found either in sense (+) or antisense (−) DNA strands. The numbers indicate the nucleotide position from the translational initiate site, ATG (A as +1).

Figure 1

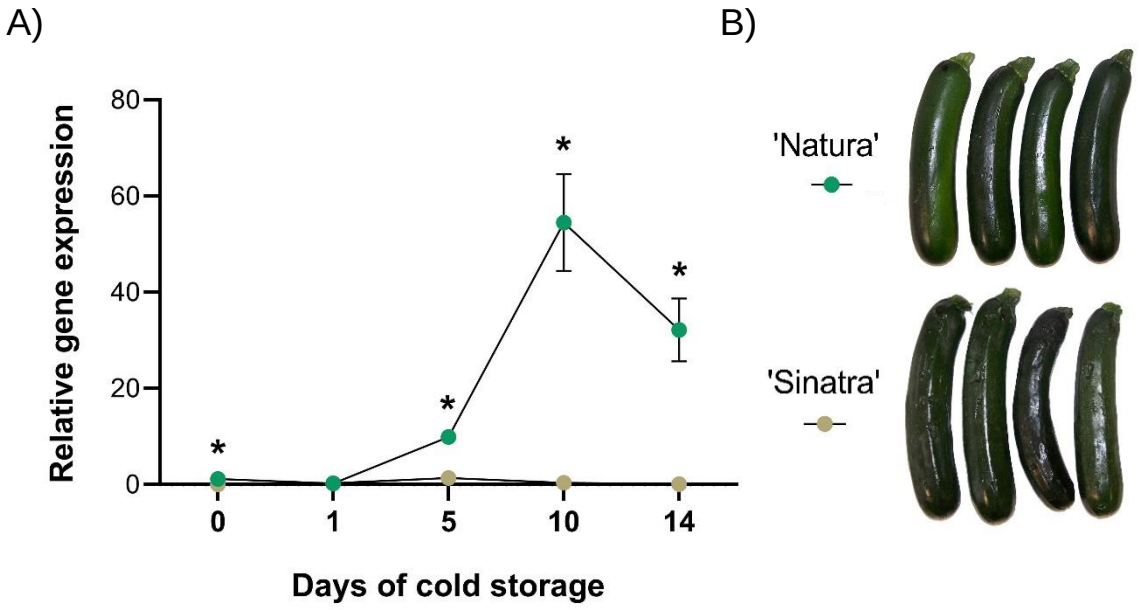
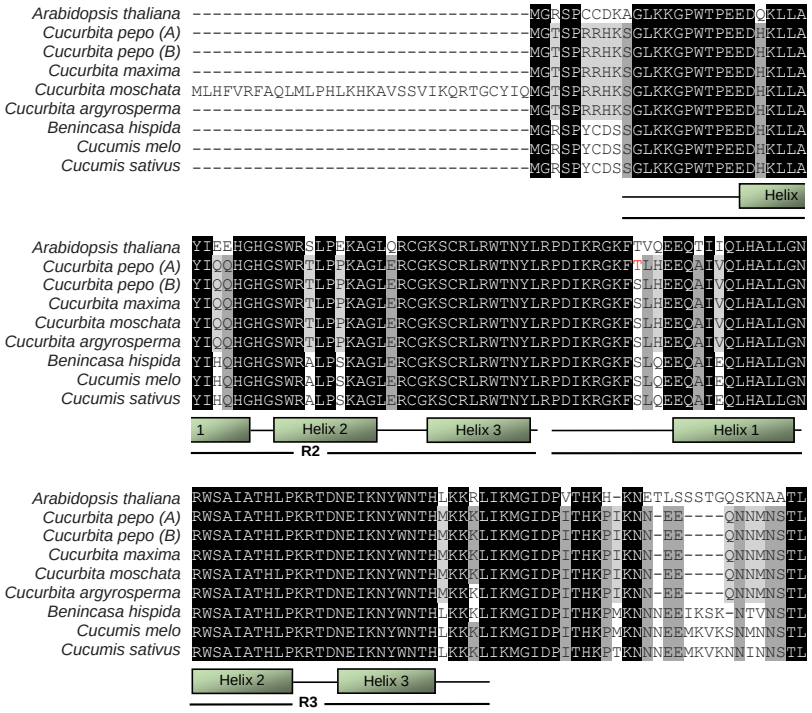


Figure 2

A)


Arabidopsis thaliana
Cucurbita pepo (A)
Cucurbita pepo (B)
Cucurbita maxima
Cucurbita moschata
Cucurbita argyrosperma
Benincasa hispida
Cucumis melo
Cucumis sativus
Helix
1
Helix 2
Helix 3
Helix 1
R2
R3

B)

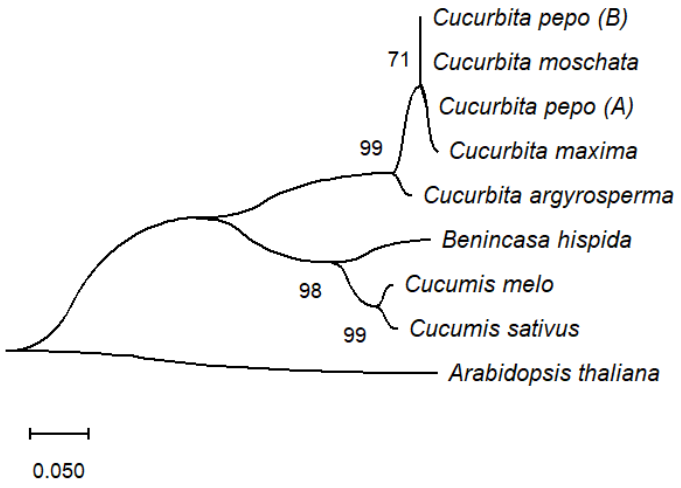

Cucurbita pepo (B)
71
Cucurbita moschata
Cucurbita pepo (A)
99
Cucurbita maxima
Cucurbita argyrosperma
Benincasa hispida
98
Cucumis melo
99
Cucumis sativus
Arabidopsis thaliana
0.050

Figure 3

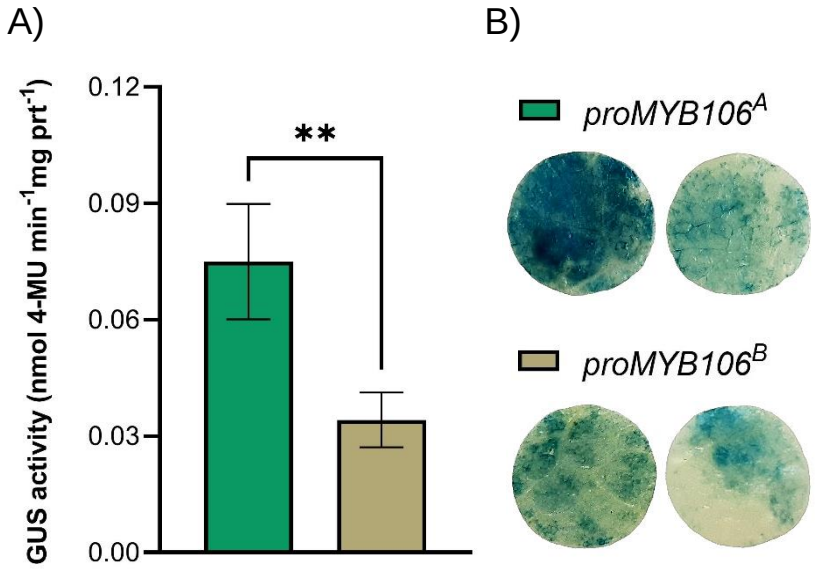

A)
B)
0.12
0.09
0.06
0.03
0.00
GUS activity (nmol 4-MU min-1mg prt-1)
**
proMYB106A
proMYB106B

Figure 4

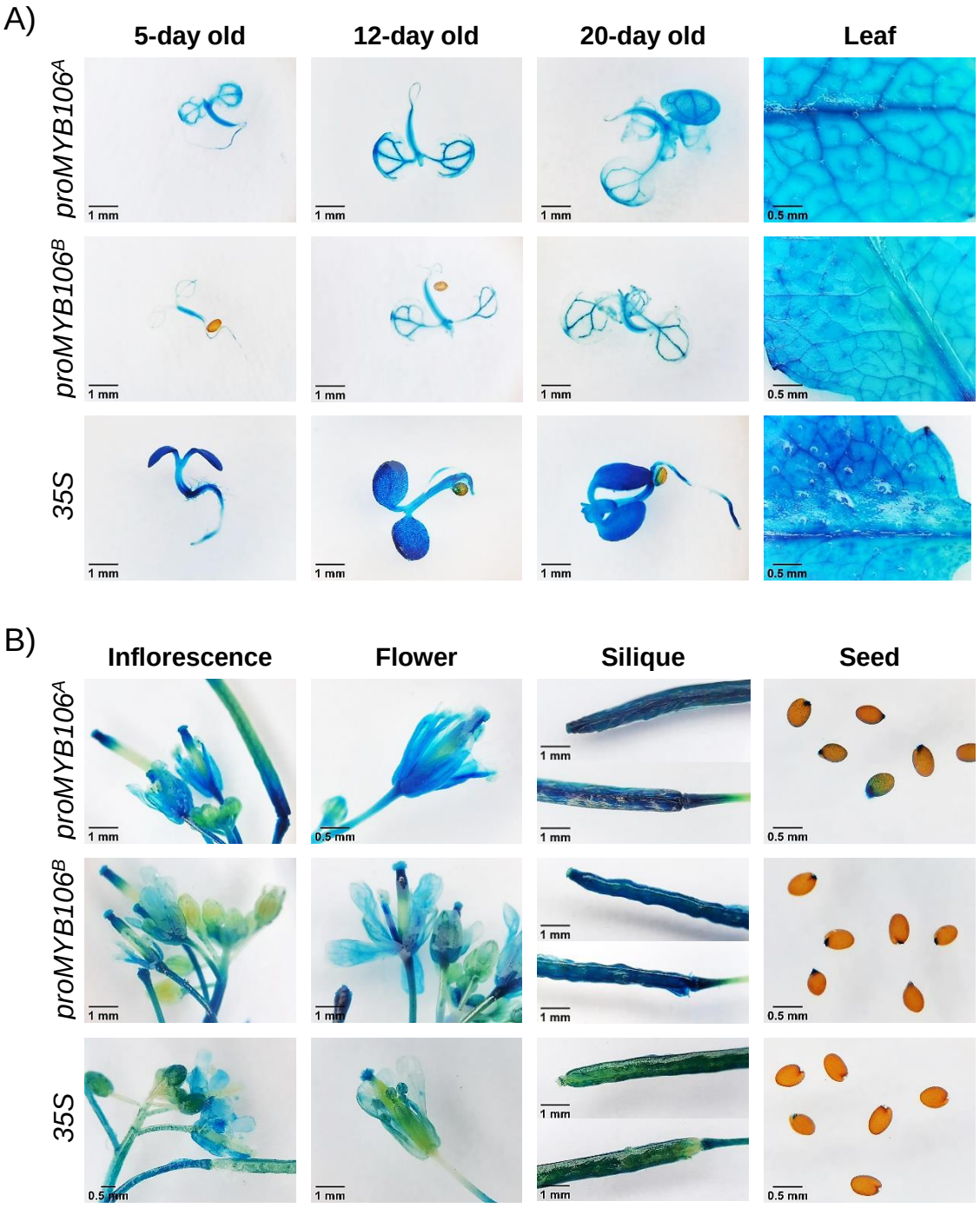

A)
5-day old
12-day old
20-day old
Leaf
proMYB106A
proMYB106B
35S
1 mm
0.5 mm
B)
Inflorescence
Flower
Silique
Seed
proMYB106A
proMYB106B
35S
1 mm
0.5 mm

Figure 5

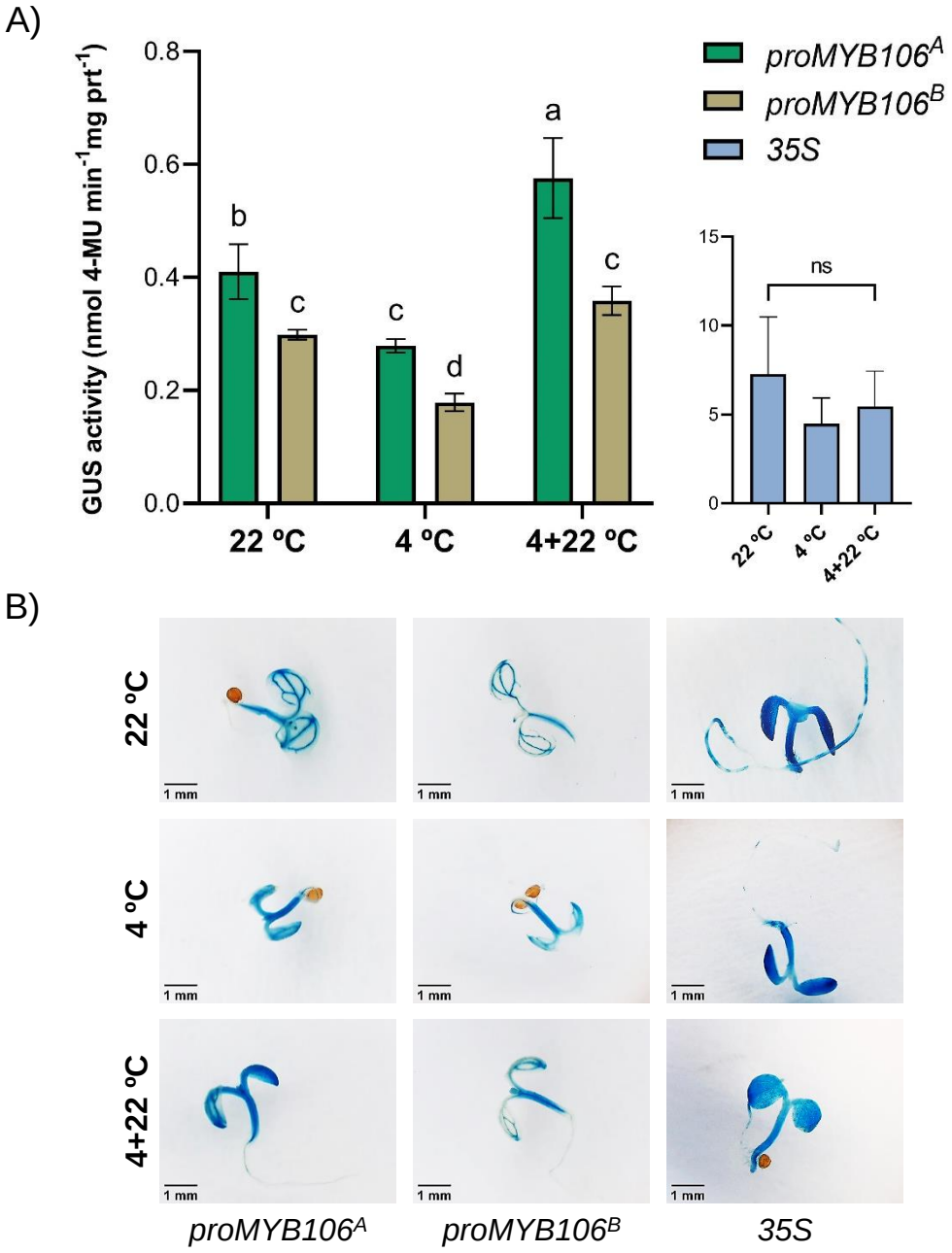

A)
GUS activity (nmol 4-MU min-1mg prt-1)
0.8
0.6
0.4
0.2
0.0
b
c
c
d
a
c
22 °C
4 °C
4+22 °C
proMYB106A
proMYB106B
35S
15
10
5
0
ns
22 °C
4 °C
4+22 °C
B)
22 °C
4 °C
4+22 °C
1 mm
proMYB106A
proMYB106B
35S

Figure 6

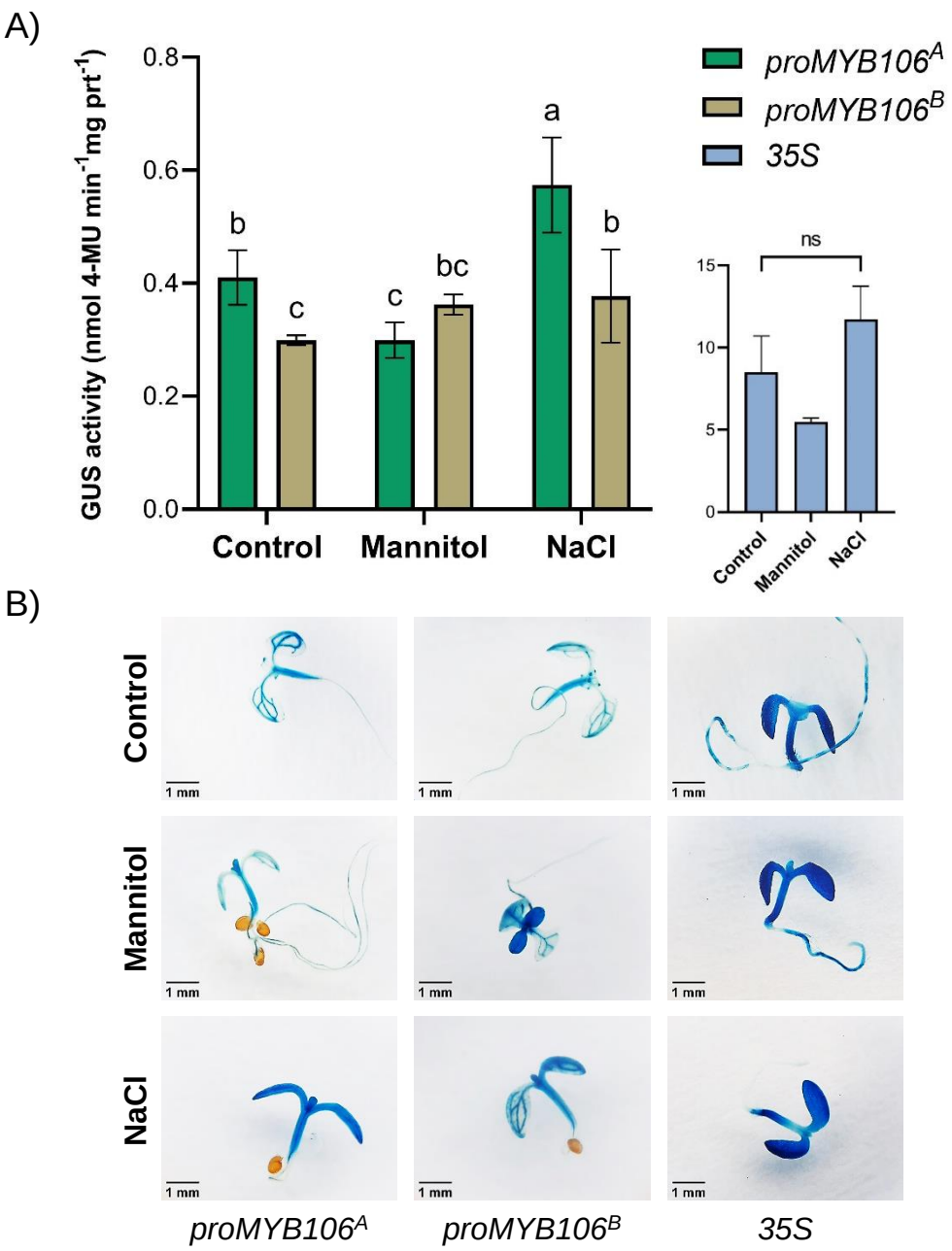
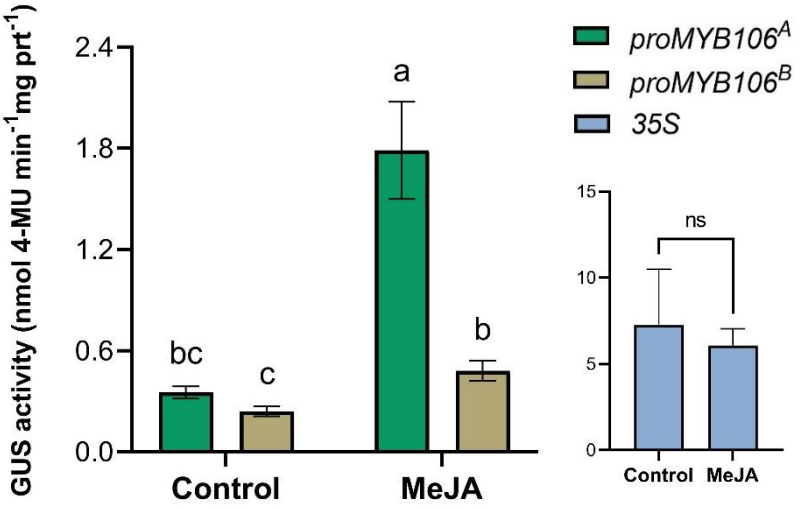
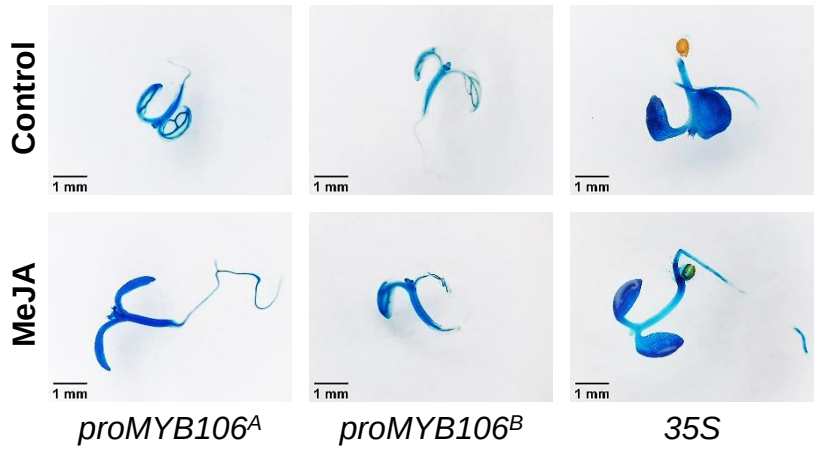

A)
GUS activity (nmol 4-MU min-1mg prt-1)
0.8
0.6
0.4
0.2
0.0
b
c
c
bc
a
b
Control
Mannitol
NaCl
proMYB106A
proMYB106B
35S
ns
15
10
5
0
Control
Mannitol
NaCl
B)
Control
Mannitol
NaCl
1 mm
proMYB106A
proMYB106B
35S

Figure 7

A)


GUS activity (nmol 4-MU min-1mg prt-1)
2.4
1.8
1.2
0.6
0.0
bc
c
a
b
Control
MeJA
proMYB106A
proMYB106B
35S
15
10
5
0
ns
Control
MeJA

B)


Control
MeJA
1 mm
proMYB106A
proMYB106B
35S

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: