Expression of MYB transcription factor gene *ZmMYB59* affects seed germination in *Nicotiana tabacum* and *Oryza sativa*

Kaihui Zhai
  Zhejiang A and F University

Guangwu Zhao (✉ gwuzhao@126.com)
  Zhejiang A and F University   https://orcid.org/0000-0001-5646-9922

Hongye Jiang
  Zhejiang A and F University

Caixia Sun
  Zhejiang A and F University

Jingyu Ren
  Zhejiang A and F University

Abstract

Background: MYB transcription factors are involved in many biological processes, including metabolism, development and responses to biotic and abiotic stresses. In our previous work, a new MYB transcription factor gene, *ZmMYB59* was induced by deep sowing and down-regulated during maize seed germination via Real-Time PCR. However, there are few reports on seed germination regulated by MYB proteins and the functions of *ZmMYB59* remain unknown.

Results: In this study, to examine its functions, *Agrobacterium*-mediated transformation was exploited to generate *ZmMYB59* transgenic tobacco and rice. In T_2 generation transgenic tobacco, germination rate, germination index, vigor index and hypocotyl length were significantly decreased by 25.0~50.9%, 34.5~54.4%, 57.5~88.3% and 21.9~31.3% compared to wild-type (WT) lines. In T_2 generation transgenic rice, germination rate, germination index, vigor index and mesocotyl length were notably reduced by 39.1~53.8%, 51.4~71.4%, 52.5~74.0% and 28.3~41.5%, respectively. On this basis, relative physiological indicators were determined. The activities of catalase, peroxidase, superoxide dismutase, ascorbate peroxidase and proline content of transgenic lines were significantly lower than those of WT, suggesting that *ZmMYB59* reduced their antioxidant capacity. As well, *ZmMYB59* expression extremely inhibited the synthesis of gibberellin A1 (GA_1) and cytokinin (CTK), and promoted the synthesis of abscisic acid (ABA) concurrently, which implied that seed germination was repressed by *ZmMYB59* in hormone levels. Furthermore, cell length and cell number of hypocotyl/mesocotyl in transgenic tobacco and rice were notably decreased.

Conclusions: Taken together, it proposed that *ZmMYB59* plays a negative regulatory role during seed germination in tobacco and rice, which also contributes to illuminate the molecular mechanisms regulated by MYB transcription factors.

Background

Seed germination is a remarkably pivotal stage in the biological clock of plants [1], involving the fast absorbing water by the dry seeds until all of the seed organs are hydrated thoroughly; a dynamic equilibrium in which the water potential of the seed remains unchanged; and a phase of rapid combination water for radical elongation [2-4]. For most plants, the germination stages are the most delicate to biotic and abiotic stresses. On that account, the investigation into seed germination is recommended to assess and breed the ecological adaptation of the crops. Recently, some researches have documented that MYB transcription factors have noteworthy influences on this developing phases [5-8].

MYB, the largest transcription factor family in plants, is widely distributed in both monocotyledons and dicotyledons [9, 10]. MYB proteins are characterized by a highly conserved DNA-binding domain: the MYB domain about 52 amino acids, the C-terminal region alters strikingly from one MYB protein to another, thereby allowing MYB superfamily to perform a considerable assortments of structures and functions [6,

11]. Depending on the number of conserved motifs, the superfamily is divided into four classes: R1-MYB, R2R3-MYB, R1R2R3-MYB and 4R-MYB [12]. The functions of MYB proteins have been probed in plentiful plant species such as *Arabidopsis*, maize, rice, petunia, snapdragon, grapevine, poplar and apple [9], involving the regulation of cell differentiation, plant development, organ morphogenesis, phytohormone response, stress tolerance, secondary metabolism [13, 14].

In view of the regulation of seed germination by MYB transcription factors, AtMyb7 mutants produced a lessened lateral root length, playing a key role in establishment of homeostasis during seed germination in *Arabidopsis* [6]. RSM1, an *Arabidopsis* MYB protein could modulate seed germination in response to ABA and salinity [7]. LcMYB2 involved the potential to increase root growth to enhance drought tolerance during sheepgrass seed germination [8]. However, there are relatively few reports on seed germination regulated by MYB proteins and the explicit mechanisms remain unidentified.

In our previous study, a new MYB transcription factor gene named ZmMYB59, was cloned from the B73 inbred line. Affymetrix GeneChip was employed to analyze the gene expression patterns of mesocotyl tissue during maize seed germination at 20 cm sowing depth, indicating that *MYB* genes were significantly expressed [15]. Thereafter, Real-Time PCR showed the expression of *ZmMYB59* in maize mesocotyl was fairly down-regulated during seed germination [16]. In this study, *ZmMYB59* transgenic tobacco and rice were produced by genetic transformation. Afterwards, germination experiment, antioxidant capacity, cellular morphology and phytohormone content were measured. The objective of this study was to further investigate the functions of *ZmMYB59* during seed germination in exogenous expressed tobacco and rice, which will also contribute to elucidate the regulatory mechanisms by MYB transcription factors affecting seed germination.

Results

Verification of the integration of the *ZmMYB59* gene into the tobacco and rice genomes

The expression vector pCAMBIA3301-Bar-ZmMYB59 construct was transferred into immature embryos to gain transgenic tobacco and rice. The regeneration of somatic embryos and their conversion into plants was attempted to each transgenic line. The different stages of plant regeneration of stable transgenic lines were described in Fig 1. T_0 generation transgenic plants were continually self-pollinated until T_2 generation. Then, to validate the presence of the *ZmMYB59* gene, T_2 generation transgenic tobacco and rice were tested by PCR amplification, wild-type was as negative control, *ZmMYB59* gene was as positive control. PCR products were tested by agarose gel electrophoresis. In tobacco, the result of electrophoresis showed that 18 of 22 transgenic lines were consistent with the positive control (Additional file 1: Figure S1). In rice, the results suggested that 16 of 19 lines were successfully transformed (Additional file 1: Figure S2). During all electrophoretic bands, three over-expression lines were selected to be employed in this study (OE1, OE2, OE3 in tobacco, OE2, OE4, OE6 in rice), which was showed in Fig 2a, 2b.

Effect of *ZmMYB59* expression on seed germination

The results showed that *ZmMYB59* significantly improved seed germination among three independent homozygous transgenic lines. This improvement was reflected on higher germination rate, germination index, vigor index, as well as longer hypocotyl/mesocotyl length. In T_2 generation transgenic tobacco, germination rate, germination index, vigor index and hypocotyl length were decreased by 25.0~50.9%, 34.5~54.4%, 57.5~88.3% and 21.9~31.3% compared to WT lines (Table 2, Fig 3a). In T_2 generation transgenic rice, the corresponding indexes were reduced by 39.1~53.8%, 51.4~71.4%, 52.5~74.0% and 28.3~41.5%, respectively (Table 2, Fig 3b). The above results suggested that *ZmMYB59* played a negative regulatory role in the process of seed germination in both transgenic tobacco and rice.

Effect of *ZmMYB59* expression on antioxidant capacity

To investigate whether *ZmMYB59* expression decreases antioxidant capacity, the contents of malondialdehyde (MDA), proline content and the activities of CAT, POD, SOD and APX were measured (Table 3). In T_2 generation transgenic tobacco, MDA content was enhanced by 5.7~21.5% and proline content was reduced by 32.9~60.6% compared to WT lines. In T_2 generation transgenic rice, MDA content was increased by 4.3~8.0% and proline content was decreased by 6.6~10.9%. Moreover, the activities of CAT, POD, SOD and APX of transgenic tobacco were significantly decreased by 32.3~46.2%, 18.0~25.3%, 9.8~18.9%, 19.8~29.0%, respectively. In transgenic rice, the above enzymatic activities were decreased by 8.3~12.8%, 9.0~19.4%, 24.8~43.5%, 36.5~59.6%, respectively. It could be safely documented that *ZmMYB59* could decrease antioxidant capacity of transgenic tobacco and rice, which was generally consistent with the results of germination experiment.

Effect of *ZmMYB59* expression on cellular morphology

Considering that *ZmMYB59* reduced hypocotyl/mesocotyl length, cellular morphology of hypocotyl/mesocotyl in tobacco and rice was observed in this experiment to determine whether and how MYB affects cell proliferation and elongation. This is indeed the case (Fig 4, Table 4). In T_2 generation transgenic tobacco, cell number and cell length of hypocotyl were significantly decreased by 12.8~22.2% and 21.7~42.7% compared to WT lines (Fig 4a, 4b). In T_2 generation transgenic rice, cell number and cell length of mesocotyl were significantly reduced by 20.0~28.2% and 10.8~17.6% (Fig 4c, 4d). The results suggested the decrease of hypocotyl/mesocotyl length caused by *ZmMYB59* was attributed to the inhibition of cell growth including cell number and cell length.

Effect of *ZmMYB59* expression on content of phytohormone

To investigate the influence of *ZmMYB59* expression on endogenous phytohormones during seed germination, the contents of ABA, IAA, GA_1, GA_3, GA_4, CTK in hypocotyl/mesocotyl were determined. In T_2 generation transgenic tobacco, compared to WT lines, the contents of endogenous GA_1, GA_3, GA_4, IAA, CTK were reduced by 21.1~39.2%, 18.7~29.9%, 3.3~15.4%, 3.4~7.5% and 27.9~44.8%, whereas the content of ABA was increased by 23.8~43.9% (Table 5). In T_2 generation transgenic rice, the contents of endogenous GA_1, GA_3, GA_4, IAA and CTK were decreased by 29.4~47.6%, 14.9~22.3%, 15.4~24.3%,

5.7~10.0% and 15.7~37.8%, whereas the content of ABA was increased by 17.9~26.9% (Table 5). Among them, the changes of endogenous GA_1, CTK and ABA reached significant level while there were no significant changes in those of endogenous GA_3, GA_4 and IAA. These results indicated that the inhibiting effect of ZmMYB59 might be ascribed to the promotion of endogenous GA_1 and CTK synthesis and the inhibition of endogenous ABA synthesis.

Discussion

Seed germination is directly related to field establishment and crop yield. There are some documents that some transcription factors normally defined as proteins are capable of mobilizing or suppressing plant growth [17]. Nonetheless, the contributions of MYB transcription factors during seed germination have not yet been functionally characterized. In our previous study, to elucidate the molecular mechanisms of maize seed germination at 20 cm sowing depth, gene expression of mesocotyl was analyzed by Affymetrix GeneChip [15]. The results demonstrated that there was the highest expression level of MYB genes, accounting for 8.15% of total number of transcription factor family [15].

However, its functions of MYB protein on seed germination have not been investigated thoroughly. The limited research for MYB transcription factors in plant primarily concentrated on *Arabidopsis thaliana*. MYB family was positively involved in plant-specific processes including physiological and biochemical processes and various biotic and abiotic stresses [18, 19], such as AtMYB15, AtMYB30, AtMYB60 and AtMYB96 [20]. On the other hand, AtMYB59, was reported to negatively regulate cell cycle progression of root tips, and inhibited root growth by extending the metaphase of mitotic cells [21]. Recently, AtMYB59 was found to be involved in plant growth and stress responses by acting as a negative regulator of Ca^{2+} signaling and homeostasis [10]. These reports suggested that MYB59 transcription factor might play important roles in plant growth, however, its functions on seed germination remained unclear.

The sequence of *ZmMYB59* gene was first issued in 2009. Through BLASTp of NCBI, comparing the amino acid sequence of ZmMYB59 protein [GenBank: ACG37097.1] with AtMYB59 protein [GenBank: NP_200786.1], sequence identity between them attained 53.65%, suggesting that *ZmMYB59* is indeed the homologous gene of *AtMYB59*. We speculated that *ZmMYB59* might also play negative regulation and made some research to prove our conjectures. The expression of *ZmMYB59* in maize mesocotyl was remarkably down-regulated after incubation for 6~8 d [16]. It was well known that seed germination was attributable primarily to the elongation of the mesocotyl and the first internode [22, 23]. In like manner, AtMYB30 was highly expressed in brassinosteroid pathway to manipulate hypocotyl cell prolongation during *Arabidopsis thaliana* seed germination [24]. Consequently, mesocotyl/hypocotyl length was an essential index to inflect germination. In this study, germination experiments revealed that hypocotyl/mesocotyl length of transgenic tobacco and rice was significantly lower than wild-type lines. To further detect the effect of *ZmMYB59* expression on cell growth, cell morphology of hypocotyl/mesocotyl was observed. Our results suggested that the reduction of hypocotyl/mesocotyl length caused by *ZmMYB59* was due to reducing both cell length and cell number among three transgenic lines.

Seed germination involved high metabolic activity and generation of reactive oxygen species (ROS) [25]. ROS, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH·), and singlet oxygen ($^1O_2$), was generated in both stressed and unstressed cells [26]. Abiotic stress could cause the accumulation of ROS, which might initiate destructive oxidative processes, such as lipid peroxidation (inflected by MDA content), chlorophyll and protein oxidation [27]. Besides, increased production of ROS might lead to cellular damage resulting in seed deterioration [28]. To minimize the negative effects of ROS, aerobic organisms had evolved both non-enzymatic and enzymatic antioxidant defence [29]. CAT, POD, SOD, APX, as major antioxidant enzymes of plants provided cells with highly efficient machinery to scavenge the ROS. SOD catalyzes the dismutation of O_2^-, CAT, POD and APX mainly scavenge H_2O_2 [30]. Meanwhile, proline, under extreme adversity, will protect plant protein from osmotic stress [31]. In this study, compared to the wild-type lines, MDA content was increased and proline content and the activities of CAT, POD, SOD, APX were decreased in *ZmMYB59* transgenic tobacco and rice. These results implied that *ZmMYB59* expression could inhibit seed germination by reducing antioxidant capacity.

The phytohormones GA, CTK, ABA were reported to play antagonistic roles in the control of seed germination [30]. GA released dormancy and stimulates seed germination by enhancing the proteasome-mediated destruction of RGL2 (RGA-LIKE2), a key DELLA factor repressing germination [32, 33]. ABA biosynthesis was associated with the maintenance of seed dormancy, leaf senescence and inhibited germination [34, 35]. Cytokinin (CTK) regulated diverse processes from embryonic development to adult plant growth [36]. It can be safely concluded that some MYB transcription factors played important roles in phytohormone regulation. For example, *AtMYB60* and *AtMYB96* could synergistically control stomatal aperture, drought and disease resistance by ABA signal pathway [9]. *GAMYB* expression in the first internode was substantially increased by GA_3 application in a wheat variety, named Hong Mang Mai [37]. *AtMYB7* negatively regulated ABA-induced inhibition of seed germination by blocking the expression of a bZIP transcription factor ABI5 [6]. Overexpression of *OsMYBR1* conferred improved drought tolerance and decreased ABA sensitivity in rice [38]. CLAU was a MYB transcription factor that modulated leaf morphogenesis by constraining the morphogenetic potential, in part due to attenuation of CTK signaling [39].

Gibberellin (GA) was essential intermediate in the stimulation of seed germination, including GA_1, GA_3, GA_4 [40, 41]. In our previous study, discovering that the expression level of *ZmMYB59* in maize mesocotyl was inhibited when seed soaked with 10^{-5} M GA_3, and in plumules and roots were strongly increased by ABA [13]. Furthermore, after GA_3 soaking, endogenous GA_3, GA_4 and ABA were controlled at a relatively low level, but endogenous GA_1 was controlled at a relatively high level in seed embryos of Masson pine [41]. The results showed that exogenous GA_3 improves seed germination through lowering the ABA level and stimulating GA_1 biosynthesis, which indicated that endogenous GA_1 might play more important roles during seed germination rather than endogenous GA_3, GA_4 [41]. In this study, our results showed that *ZmMYB59* expression decreased the total levels of endogenous GA_1, GA_3, GA_4, IAA, CTK and increased the level of endogenous ABA, but only endogenous GA_1, CTK and ABA had significant changes.

Therefore, it could be concluded that the inhibitory effect of *ZmMYB59* was attributed to endogenous GA_1, but not GA_3 and GA_4. Taken together, the results suggested that endogenous GA_1 could play more important roles during seed germination in *ZmMYB59* transgenic tobacco and rice, which was generally consistent with the research results of embryos of Masson pine according to the study of Zhao et al. (2014) [41]

In summary, the molecular mechanisms regulated by *ZmMYB59* gene during seed germination of tobacco and rice can be elucidated in Fig 5. Expressing *ZmMYB59* inhibited the synthesis of endogenous GA_1 and CTK, and promoted the synthesis of endogenous ABA. This effect reduced the antioxidant capacity, which directly affected cell growth. All above effects together inhibited hypocotyl/mesocotyl elongation, which suggested that *ZmMYB59* gene was a negative regulatory factor during seed germination in tobacco and rice. In our future work, genetic transformation of *ZmMYB59* gene in maize will be performed to further validate its functions. Gene knockout is advised as an effective strategy to breeding new maize varieties, which improve seed germination.

Conclusions

The results reported here demonstrated that *ZmMYB59* heterogenous expression in tobacco and rice had a negative effect on seed germination by inhibiting the synthesis of GA, CTK and IAA and promoting the synthesis of ABA, then reducing the contents of proline and the activities of CAT, POD, SOD, APX, as a result, repressing cell proliferation and elongation. The result also confirms that endogenous GA_1 may play more important roles during seed germination rather than GA_3, GA_4. Our findings suggest that *ZmMYB59* plays a negatively regulatory role in tobacco and rice, which will contribute to elucidate the mechanisms of seed germination regulated by MYB transcription factors and also provides a key gene affecting seed germination.

Methods

Plant materials

T_2 generation transgenic seeds of tobacco (*Nicotiana tabacum* L.), rice (*Oryza sativa* L. ssp. *Japonica*) were provided by Hangzhou Biogle Co., LTD by *Agrobacterium*-mediated transformation. The embryogenic callus from wild-type plants was inoculated with *agrobacterium tumefaciens* strain EHA105 and expression vector plasmid pCAMBIA3301-Bar-ZmMYB59 to generate *ZmMYB59* transgenic tobacco, rice. In each crop, three independent *ZmMYB59* transgenic lines were used in this study.

RNA isolation and PCR amplification

Total RNA was extracted using All-in-one DNA/RNA Mini-preps kit (B618203, Sangon Biotech, shanghai, China) with a genomic DNA purification according to the manufacturer's instructions. RNA concentration and purity were measured with a NanoDrop ONE (Thermo Fisher Scientific, Madison, USA), and RNA

integrity was estimated by 1% agarose gel electrophoresis. cDNA (20 μL) was synthesized from 1000 ng of total RNA using the One-step gDNA Removal and cDNA Synthesis Kit using PrimerScript TM RT regent Kit with gDNA Eraser (RR047, Takara, Beijing, China). Primers were designed by Primer Premier 5.0 software and showed in Table 1. The amplification reaction system was as follows (20 μL): Master mix 10 μL, ddH$_2$O 7 μL, 10 μmolL^{-1} up-stream and down-stream primers 1 μL, cDNA 1 μL. PCR reaction procedure: 95 °C for 5 min; followed by 30 s at 95 °C, 30 s at 55 °C; 40 s at 72 °C for 35 cycles; finally maintained at 72 °C for 10 min.

Germination experiment

Germination experiment was conducted on three replicates. Twenty seeds were randomly selected for each replicate. Wild-type and *ZmMYB59* transgenic seeds were evenly floored in the germination boxes. In tobacco, culture conditions were set up in 16 h at 20 °C dark and 8 h at 30 °C light. In rice, culture conditions were set up in 8 h at 25 °C dark and 16 h at 30 °C light. Considering the fact that sowing covered with soil was generally required in crop production, seeds of tobacco and rice were sowed at the depth of 1 cm and 4 cm, respectively. After incubation for 2 d, the number of germinated seeds was recorded each day until 14 d. Germination rate, germination index, vigor index were determined according to following formulas:

Germination rate = n_1 within 14 days / n_2 × 100%. Here n_1 is the number of germinated seeds; n_2 is the number of tested seeds.

Germination index = $\sum$ Gt / Dt.

Vigor index = $\sum$ (Gt / Dt) × S. Here Gt is corresponding number of seeds germinated in the t day; Dt is time corresponding to Gt in days; S is the average length of 10 seedlings.

Determination of antioxidant capacity

Malondialdehyde (MDA) content was measured by using 2-thiobarbituric acid [27]. Fresh leaves 0.5 g were ground with 5 ml 0.6% TBA in 10% trichloroacetic acid. The mixture was heated at 100 °C for 15 min and then cooled in ice bath. Finally, read the absorbance at 450, 532 and 600 nm. computational formula was as follows: μmol MDA g^{-1} fresh weight (FW) = 6.45 (OD$_{532}$ − OD$_{600}$) − 0.56 OD$_{450}$. The proline content was assayed from freeze dried leave material, using a 3% sulfosalicylic and ninhydrin extraction buffers [42]. Dry leaves 0.04 g was homogenized with 3% sulfosalicylic acid and centrifuged at 3000 g/min for 10 min. 200 μl supernatant was mixed with 400 μl reagent mixture and heated in sealed test tubes at 100 °C for 1 h. After cooling down, 4 ml toluene was added to each sample. Proline content was measured by spectrophotometer at 520 nm.

Until the seedlings grew out of 4~5 cm, the activities of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX) were measured by employing 0.5 g samples in 5 mL extraction buffer containing 0.05 M phosphate buffer [30, 43]. CAT was determined

spectrophotometrically based on the decrease in absorbance of H_2O_2 at 240 nm. POD was measured as the absorbance at 470 nm. SOD was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). APX was assayed from the decrease in absorbance at 290 nm as ascorbate was oxidized [44].

2.4 Observation of cell morphology

Hypocotyl/mesocotyl of wild-type and transgenic seedlings was cut longitudinally and the cell sections were made to determine the changes of cell length and cell number. Firstly, the middle position of hypocotyl/mesocotyl was cut and fixed with 25% glutaraldehyde stationary solution initially. Next five visual fields were randomly selected and cell length of hypocotyl/mesocotyl was measured by calibrated eyepiece, and cell number was counted by pictures.

2.5 Determination of phytohormone content

The concentration of the endogenous phytohormones involving gibberellin A1 (GA_1), gibberellin A3 (GA_3), gibberellins A4 (GA_4), cytokinin (CTK), abscisic acid (ABA), indole-3-acetic acid (IAA) during seed germination was determined by LC-MS/MS system [45, 46]. Each sample was transferred into a 2 mL LC/MS glass vial for LC-MS/MS analysis. MS system: ion spray voltage -4500 V, temperature 550 °C, Ion source Gas 1:50/Gas 2:50. Chromatographic system: HSS T3 liquid chromatography column (100 ≤ 2.1 mm, 1.8 μm), mobile phase A (0.1% formic acid-aqueous solution), mobile phase B (0.1% formic acid-acetonitrile). Multiple reaction monitoring detection method was used for the quantification of all analytes. Each sample was extracted three times.

2.6 Statistical analysis

Analysis of variance (ANOVA) was carried out with SPSS 19.0 (IBM SPSS Statistics, Chicago, USA). Duncan's multiple range test or Student's t test was employed to determine if there were significant differences between the determination indicators of transgenic and wild-type lines at $p < 0.05$.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All the necessary data generated or analyzed during this study have been included in this article.

Abbreviations

WT: Wild-type; OE: Over-expression; GA: Gibberellin; CTK: Cytokinin; ABA: Abscisic acid; PCR: Polymerase chain reaction; GA_1: gibberellin A1; GA_3: gibberellin A3; GA_4: gibberellin A4; CAT: Catalase; POD: Peroxidase; SOD: Superoxide dismutase; APX: Ascorbate peroxidase; MDA: Malondialdehyde; ROS: Reactive oxygen species; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; ANOVA: Analysis of variance

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Tables

Table 1 The nucleotide sequences of the primer pairs used to identify the PCR products of expressed *ZmMYB59* gene

primer	sequence（5'- 3'）	T_m (°C)	Product size (bp)
ZmMYB59-F	ATTGAGCTCCATGCTCGGTG	60	580
ZmMYB59-R	TAGCTGAGTGGCCTGACCAA	60	

Table 2 Measurement of phenotypic indexes of wild-type and *ZmMYB59* transgenic plants

Species	Lines	Germination rate (%)	Germination index	Vigor index	Hypocotyl/Mesocotyl length (cm)
Tobacco	WT	91.7±8.1 a	2.61±0.30 a	105.94±23.20 a	0.32±0.02 a
	OE1	51.0±6.0 bc	1.37±0.18 b	26.08±5.38 bc	0.23±0.02 b
	OE2	45.0±5.3 c	1.19±0.11 b	12.42±2.40 c	0.22±0.01 b
	OE3	68.8±7.5 b	1.71±0.44 b	45.05±8.61 b	0.25±0.03 b
Rice	WT	58.0±3.4 a	1.40±0.03 a	40.04±6.10 a	0.53±0.07 a
	OE2	26.8±4.2 b	0.40±0.01 c	10.43±3.20 b	0.31±0.01 b
	OE4	35.3±8.6 b	0.68±0.08 b	19.01±8.25 b	0.38±0.10 b
	OE6	28.7±6.3 b	0.49±0.04 c	13.85±5.37 b	0.33±0.08 b

Note: WT and OE represent wild-type and *ZmMYB59* transgenic plants, respectively. Means with standard deviations that do not followed by the same lower case letter between OE and WT lines significantly differ by

ANOVA analysis at 5% level of significance.

Table 3 Measurement of antioxidant capacity of wild-type and *ZmMYB59* transgenic plants

Species	Lines	MDA (µmol/g)	Proline (µg/g)	CAT (U/g·min)	POD (U/g·min)	SOD (U/g.min)	APX (U/g·min)
Tobacco	WT	22.8±2.0 b	97.1±14.0 a	318.0±39.0 a	125.6±8.0 a	88.2±10.0 a	50.4±9.0 a
	OE1	24.1±1.8 ab	48.7±8.1 bc	184.7±18.0 b	98.0±7.1 b	75.2±5.1 b	37.7±1.5 b
	OE2	27.7±3.0 a	38.3±10.0 c	171.1±9.0 b	93.8±6.0 b	71.5±3.0 b	35.8±4.0 b
	OE3	25.3±1.6 ab	65.2±5.0 b	215.3±15.2 b	103.0±11.2 b	79.6±4.8 ab	40.4±4.1 ab
Rice	WT	116.9±7.2 b	18.3±0.4 a	10.9±0.2 a	48.9±3.6 a	53.3±1.4 a	10.4±0.3 a
	OE2	126.3±1.6 a	16.3±0.3 b	9.5±0.1 b	39.4±2.2 b	30.1±2.5 c	4.2±0.1 c
	OE4	124.0±4.0 ab	16.5±0.7 b	10.0±0.5 b	44.5±6.1 ab	40.1±8.0 b	6.6±0.6 b
	OE6	121.9±2.3 ab	17.1±1.0 ab	9.7±0.4 b	40.7±5.5 ab	32.3±4.0 bc	4.9±0.7 c

Note: WT and OE represent wild-type and *ZmMYB59* transgenic plants, respectively. MDA, CAT, POD, SOD, APX represent malondialdehyde, catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, respectively. Means with standard deviations that do not followed by the same lower case letter between OE and WT lines significantly differ by ANOVA analysis at 5% level of significance.

Table 4 Measurement of cell length and cell number of wild-type and *ZmMYB59* transgenic plants

Species	Lines	Cell number	Cell length (µm)
Tobacco	WT	18.0±0.1 a	103.8±14.0 a
	OE1	14.5±0.5 c	70.2±8.3 bc
	OE2	14.0±0.1 c	59.5±5.0 c
	OE3	15.7±0.6 b	81.3±10.3 b
Rice	WT	22.0±0.6 a	242.1±7.1 a
	OE2	15.8±1.0 b	199.6±30.7 b
	OE4	17.6±4.6 ab	215.9±14.1 ab
	OE6	16.5±1.5 b	204.3±23.2 ab

Table 5 Measurement of phytohormone contents in wild-type and *ZmMYB59* transgenic lines

Species	Lines	GA_1 (ng/g)	GA_3 (ng/g)	GA_4 (ng/g)	CTK (ng/g)	IAA (ng/g)	ABA (ng/g)
Tobacco	WT	0.166±0.022 a	0.187±0.035 a	0.123±0.016 a	18.544±2.152 a	1.657±0.528 a	4.832±0.486 c
	OE1	0.127±0.019 b	0.150±0.010 ab	0.119±0.003 a	11.661±1.016 bc	1.601±0.019 a	6.082±0.242 b
	OE2	0.101±0.013 b	0.131±0.028 b	0.104±0.010 a	10.234±0.989 c	1.532±0.472 a	6.951±0.349 a
	OE3	0.131±0.016 b	0.152±0.011 ab	0.114±0.012 a	13.362±0.667 b	1.534±0.092 a	5.982±0.327 b
Rice	WT	0.187±0.029 a	0.202±0.030 a	0.169±0.046 a	45.142±5.317 a	1.914.±0.355 a	7.235±0.561 b
	OE2	0.098±0.021 b	0.157±0.042 a	0.128±0.057 a	28.085±3.391 c	1.722±0.863 a	9.179±0.380 a
	OE4	0.132±0.038 ab	0.172±0.011 a	0.143±0.008 a	38.074±4.284 ab	1.804±0.303 a	8.533±0.503 a
	OE6	0.124±0.027 b	0.163±0.015 a	0.133±0.007 a	32.362±5.552 bc	1.754±0.186 a	8.802±0.459 a

Note: WT and OE represent wild-type and *ZmMYB59* transgenic lines, respectively. GA, CTK, IAA, ABA represent gibberellin, cytokinin, indole-3-acetic acid, abscisic acid, respectively. Means with standard deviations that do not followed by the same lower case letter between OE and WT lines significantly differ by ANOVA analysis at 5% level of significance.

Figures
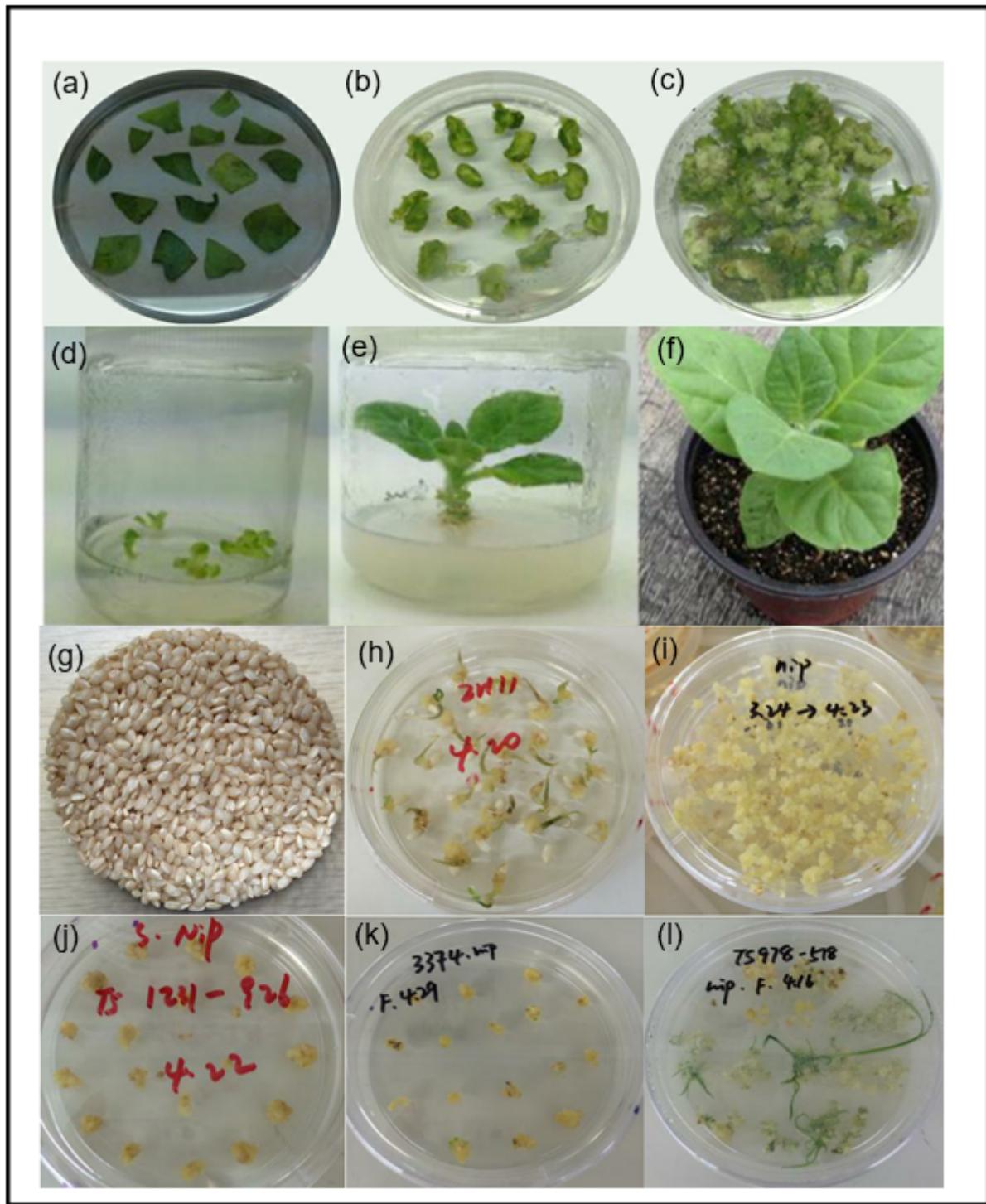
Figure 1

Description of regeneration from callus of ZmMYB59 transgenic plants. (a)~(f) Tobacco transformation: (a) EHA105 infected leaves, (b) Differentiation of callus, (c) Formation of resistant seedlings, (d) Rooting culture, (e) Transgenic seedlings, (f) Transplantation. (g)~(l) Rice transformation: (g) Screening seeds, (h) Co-culture with Agrobacterium tumefaciens containing target gene vector at EHA105, (i) Subculture of callus, (j) Screening resistant seedlings, (k) Differentiation of callus, (l) Rooting culture.
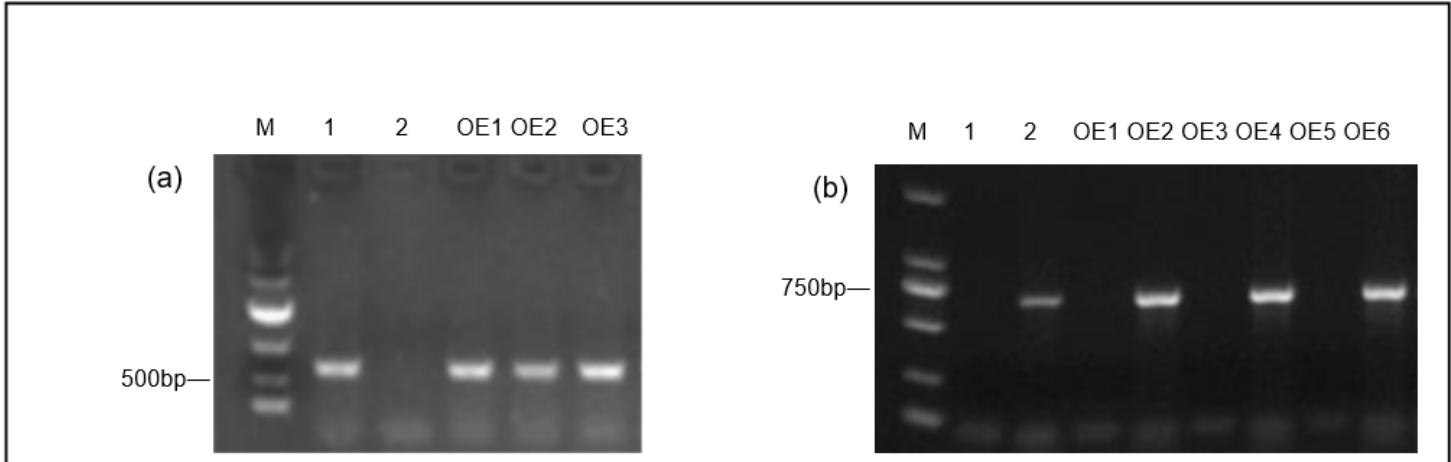
Figure 2

Electrophoretic results to confirm the presence of the ZmMYB59 gene in T2 generation transgenic tobacco and rice. (a) Detection of PCR in T2 generation transgenic tobacco. M: DL10000 DNA Marker, 1: positive control, 2: negative control, OE1~OE3: ZmMYB59 transgenic tobacco lines. (b) Detection of PCR in T2 generation transgenic rice. M: DL2000 DNA Marker, 1: negative control, 2: positive control, OE1~OE6: ZmMYB59 transgenic rice lines.
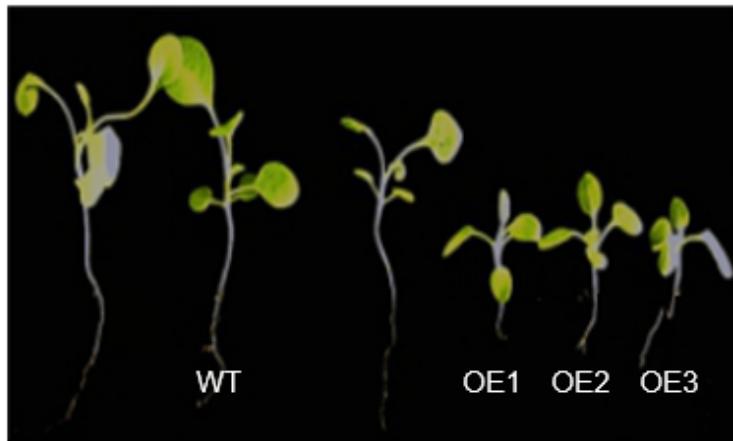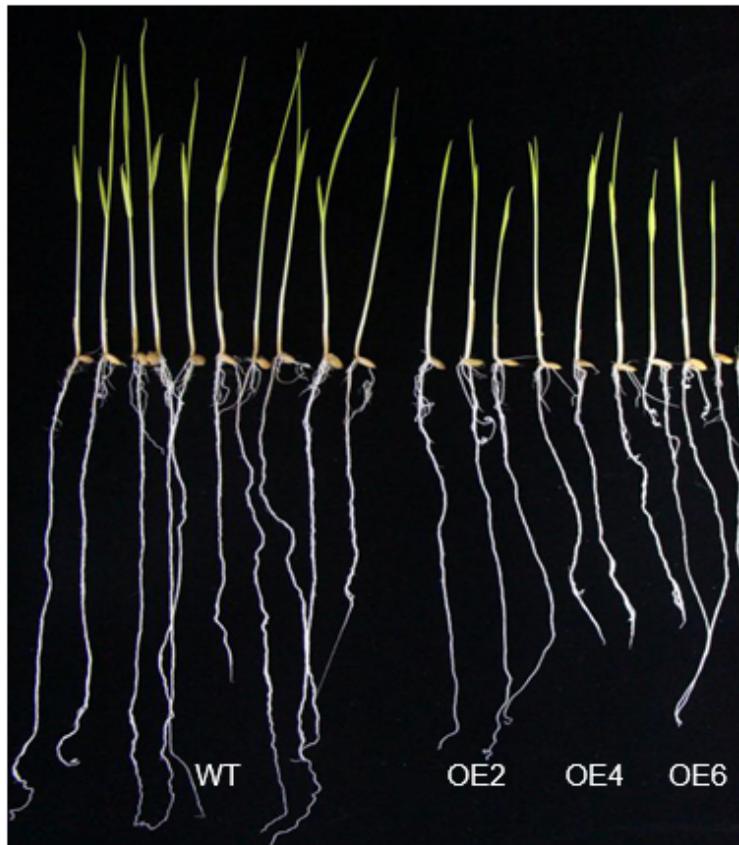
Figure 3

Phenotypic observation of wild-type and ZmMYB59 transgenic seedlings. (a) T2 generation transgenic tobacco with wild-type tobacco. (b) T2 generation transgenic rice with wild-type rice.
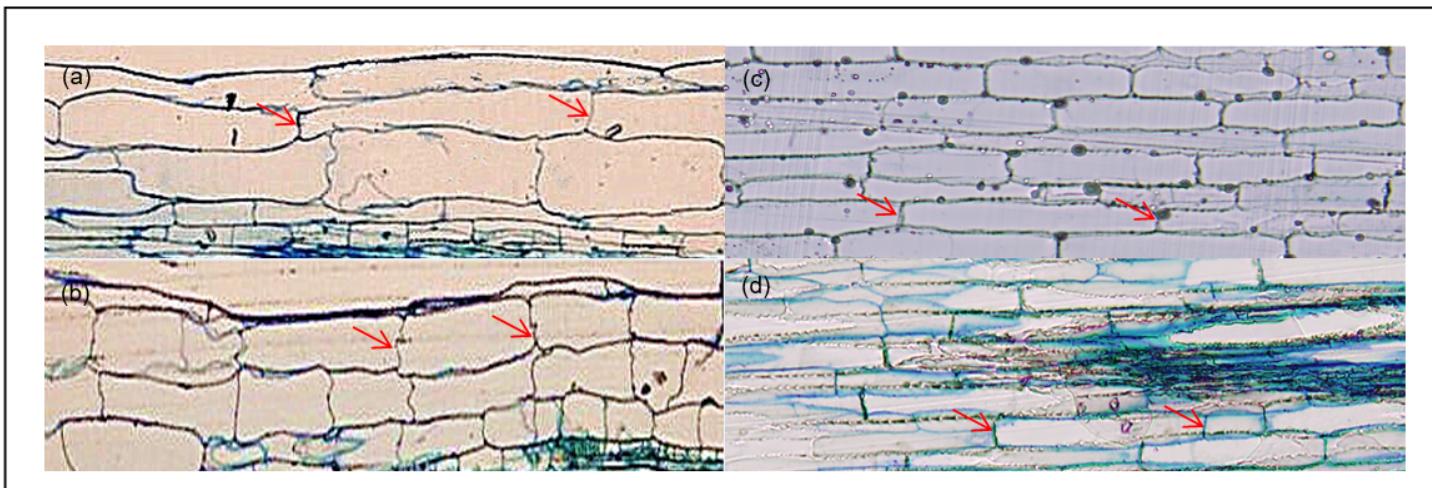
Figure 4

Microstructure of mesocotyl cells of wild-type and ZmMYB59 transgenic plants. (a) Wild-type tobacco. (b) T2 generation transgenic tobacco. (c) Wild-type rice. (d) T2 generation transgenic rice. The image is a 4 × fluorescence microscope, and the arrow refers to the longitudinal boundary position of the cell.
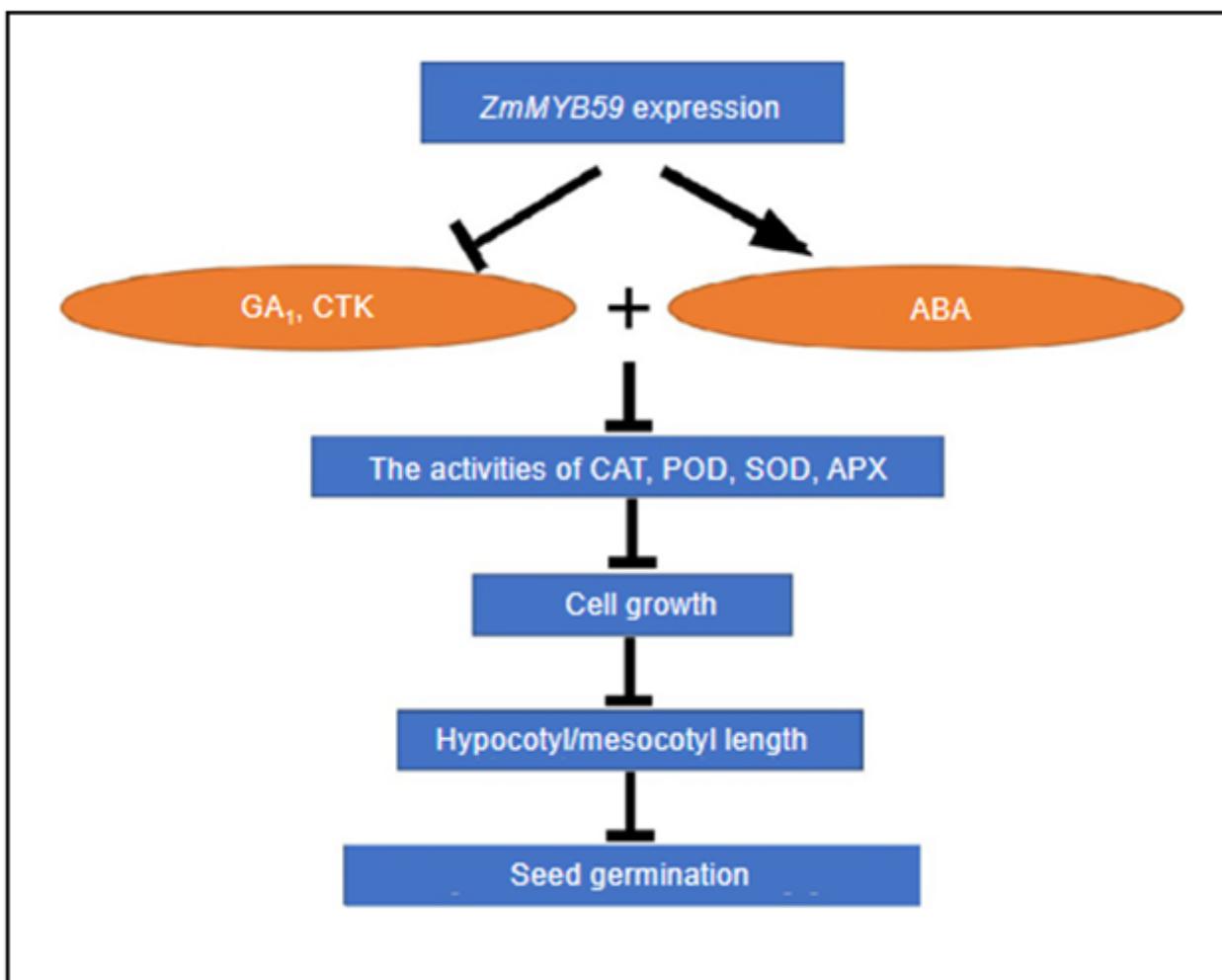
Figure 5

The regulatory mechanisms of seed germination regulated by ZmMYB59 in tobacco and rice. GA1, CTK, ABA represent gibberellin A1, cytokinin, abscisic acid, respectively. CAT, POD, SOD, APX represent catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, respectively.

Supplementary Files

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