Transcription factor myc2-like, binding to ABRE element of OsCYP2 promoter, enhance salt tolerance in Oryza sativa

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Abstract

Cyclophilins, a type of peptidyl-prolyl *cis-trans* isomerase, function as an important molecular chaperone in series of biological processes. However, the expression pattern and signal transduction pathway of cyclophilins is still unclear. Hereby, it was clarified that the promoter of *OsCYP2* is a constitutive expression pattern which by GUS staining in transformants with *pOsCYP2:GUS* genetic transformation. Simultaneously, it is found that the sequence of promoter had not only core elements but also contained inducible elements. Then, the ABA responsive element was used to cDNA library screening, a transcription factor myc2-like was identified by yeast one hybrid and confirmed though electrophoretic mobility shift assay. Furthermore, the relative expression showed that *myc2-like* was induced by abscisic acid. In addition, *myc2-like* overexpression could enhance salt tolerance in transformants and partial restore *cyp2*-RNAi line. Together, we explored a novel transcriptional signal mediated by myc2-like, a potential regulator of salt stress physiological processes in rice.

Introduction

Salt, drought, cold and toxic metals in the soil are major environmental factors that affect the geographical distribution of plants in nature, limit plant productivity in agriculture. Abiotic stresses are often unfavorable or stressful for plant growth and development (Gong et al., 2020). Conversely, crops lead to a series of morphological, physiological, biochemical and molecular changes in order to adapt to these stress conditions during their lifetime (Amaral et al., 2016; Verma et al., 2016). The development of crops with elevated levels of salt tolerance is therefore highly desirable. How plants sense stress signals and adapt to adverse environments are fundamental biological questions. Until now, there is still a lot of constraints in the complexity and low genetic variance of quantitative traits during previous genetic modification (Tester et al., 2005; Varshney et al., 2011). With the genomics, transcriptomics and proteomics resources development, a large array of stress responsive transcript factor has been identified with functional research in crops (Li et al., 2018; Yang et al., 2020; Zhanget al., 2021). Most important, promoter structure analysis and yeast one-hybrid have been applied as an efficient technology in transcription factor study (Ding et al., 2021; Li et al., 2021; Wani et al., 2021).

As well known, the basic/helix-loop-helix (bHLH) proteins are a superfamily of transcription factors that bind as homodimers and/or heterodimers to a consensus hexanucleotide sequence called the E-box (5'-CANNTG-3'). Among the different types of E-boxes, the most common palindromic G-box (5'-CACGTG-3') provide core consensus site for recognition of bHLH transcription factors (Toledo-Ortiz et al., 2003). In addition, flanking nucleotides outside of the hexanucleotide core sequence and loop residue in bHLH transcription factors could both affect the binding specificity (Massari et l., 2000; Nair et al., 2000). So far, a serial of myc2-like transcription factors contained bHLH domain have been well characterized in crop as crucial regulatory components in diverse abiotic and biotic stress response (Wamg et al., 2014; Li et al., 2021). More importantly, there is some evidence that bHLH transcription factor mediated hormone signaling to regulate crop biological processes (Wang et al., 2020; Wu et al., 2021).

Therefore, keeping in view the novel stress-inducible genes/proteins would help us to reveal the unknown molecular mechanisms of complex trait in abiotic stress tolerance. In previously study, *cyclophilin 2* (*CYP2*) as a molecular chaperone was characterized that confer salt tolerance in rice by modulating activities of antioxidant enzymes at translation level (Ruan et al., 2011). Moreover, it is hope to elucidate that the precise regulatory mechanism with underlying the key transcript factor involved in this signal pathway. Here, we are presenting the expression pattern of *OsCYP2* and charactered its transcriptional signal mediated by myc2-like, a potential regulator of salt stress physiological processes via abscisic acid (ABA) signal in rice.

Results

Promoter cloning and *cis* elements analysis

To better understand the expression pattern of *OsCYP2*, an upstream 1156 bp fragment of *OsCYP2* was cloned into pCAMBIA1301 for substituting 35S promoter to construct the *pOsCYP2:GUS* vector with *Bam*HI and *Bgl*II (Supplementary Fig. S2). Furthermore, data regarding predicted *cis*-acting regulatory elements have been listed in Supplementary Table S2. This promoter had not only core elements (TATA, CAAT and GATA box) but also contained three main types known as inducible elements (ABRE and MYBR) which were response to abiotic stresses.

Gus staining and transcript level analysis of *OsCYP2*

To investigate the type of *pOsCYP2* in plant cells, we further tested the GUS gene staining in rice. The results of histochemical assay showed that four tissues (panicle, leaf, stem and root) of transformants were stained blue which were induced by the promoter of OsCYP2 (Fig. 1E-1H), but not in wild type (Fig. 1A-1D). It means that the *pOsCYP2* is a constitutive promoter.

Determine minimal inhibitory concentration of Aureobasidin A (AbA) for bait report strain

The ABRE (p62) and its mutant (p63) elements with *Hin*dIII and *Xho*I overhanging sticky ends were as followed in Supplementary Table S1. Bait and mutant strains were selected on SD/-Ura media plates by small scale transformation into Y1H strain. Four types of Y1H strains (p62-AbAi, p63-AbAi, p53-AbAi, pAbAi) were conducted to determine the minimal inhibitory concentration of AbA. The results showed that neither positive control Y1H (p53-AbAi) nor negative control Y1H (pAbAi) can grow on SD/-Ura with plus 150-750 μg/L AbA. The effective inhibitory were validated at 750 μg/L to Y1H (p62-AbAi) and Y1H (p63-AbAi) for cDNA library screening (Supplementary Fig. S3).

Screening cDNA library and rescuing prey plasmid

Sixteen colonies were obtained on SD/-Leu/AbA plates by library scale transformation of Y1H [p62-AbAi] strain. Among of them, twelve clones could generate single colonies and grow healthily by streaking three times on SD/-Leu/AbA media plates (Supplementary Figure S4). The results of yeast colony PCR showed that 10 colonies (No.3, 5-8, 9, 11, 12, 14-16) had one band except for No.12 (two bands) (Fig. 2).

Rescue plasmids which had single band in yeast colony PCR were used to distinguish genuine positive from false positive interactions. Each rescued plasmid was transformed into Y1H [p62-AbAi] and Y1H [p63-AbAi], respectively. The transformation reactions were spread onto SD/-Leu and SD/-Leu/AbA media plates for culturing 3-5 days. The result showed that candidate preys (No. 11) could grow on selective media plates (Fig. 3) which was genuine interactions compared to other false interactions (Supplementary Figure S5). Furthermore, a myc2-like protein (GeneID: 4349484) was implied by sequencing and blast.

Myc2-like protein binds to ABRE element in *in vitro*

To investigate *in vitro* interaction between myc2-like protein and the ABRE element, a His-tagged fusion myc2-like protein was identified though SDS-PAGE and western blot (Supplementary Figure S6). Then purified protein was used to assess binding ability with EMSA. The results showed that Myc2-like protein could directly bind to biotin labeled probes with a shift band by comparison to no protein incubated and biotin mutated probes (Fig. 4). In addition, signal shift observed can be prevented by competition from 200-fold molar excess unlabeled probes.

*Myc2-like*was induced by salt and ABA

To better understand the expression pattern of *myc2-like* to response to salt and ABA, the transcript level was detected in series of treatments (Fig. 5). The expression of *myc2-like* could be induced both by salt and ABA in 2h, 12h and 24h. Furthermore, it demonstrated a different trend. For NaCl treatment, transcript level gradually decreases as the time goes with 2-24 hr, however, the maximal expression occurred at 12 hr under ABA treatment.

Phenotypic characterization and physiological responses to salt stress of transformants

Based on the results of qPCR (Supplementary Figure S7), three independent lines with relative higher expression level of *myc2-like* overexpression in wild type and *myc2-like* overexpression in *cyp2*-RNAi were selected for salt tolerance analysis. After treated 24 hrs, leaves of each line showed wilting, especially wild type and *cyp2*-RNAi (L3-61) exhibited even more sensitive to salt stress (Fig. 6). Obviously, *myc2-like* overexpression (L1-4) could exhibit better growth condition, also partial restore the *cyp2*-RNAi (L2-4) phenotype under salt stress (Fig. 6). The results of SOD, POD, and CAT activities in *myc2-like* overexpression transformants were significantly increased 44.6%, 73.2% and 68.4%, respectively, compared to wild type (Fig. 7A, 7B and 7C). The similar results were exhibited in *cyp2*-RNAi and *myc2-like* overexpression with *cyp2*-RNAi background. In addition, the content of MDA and proline suggested that the *myc2-like* overexpression may have a better resistant to salt stress by reducing lipid peroxidation (Fig. 7D and 7E). However, there were no significant differences in Na^+/K^+ ratio among these lines under control condition and NaCl treatment, respectively, suggested that of *myc2-like* cannot adapt to the salt stress through Na^+ and K^+ uptake selectively (Fig. 7F).

Discussion

An important feature of salt stress is that the hyperosmotic signal causes the accumulation of the phytohormone abscisic acid (ABA), which in turn elicits many adaptive responses in plants. Therefore, crops have developed a series of alterations in physiology and biochemistry at transcriptional and translational levels so as to offset the adverse effects which will allow them to avoid stress conditions (Singhal et al., 2015). There are two types of stress-responsive genes including functional and regulatory genes (Schwarzer et al., 2014). In previous study, a cyclophilin chaperone, named OsCYP2, was characterized to resistant to abiotic stresses, especially to salt stress when overexpressed (Ruan et al., 2011). However, the transcriptional mechanism was unclear in this signal pathway. In present study, we aimed to analyze the expression pattern and promoter sequence of *OsCYP2* for providing more pivotal information to elucidate the mechanism of transcriptional regulation in response to salt stresses. A 1156 bp fragment upstream of *OsCYP2* was cloned and identified that as a constitutive promoter, especially high expression in panicle (Supplementary Fig. S2 and Fig. 1). It was implied that the expression of *OsCYP2* might be related to fertility development in rice which was supported by Kurek et al., 2002.

As we know that, *cis* elements of promoter play an important role in activation and suppression of gene expression in transcriptional regulation process which depends on specific binding sequence for proteins (Hernandez-Garcia et al., Liu et al., 2014). ABA responsive element (ABRE, PyACGTGGC), including various type of DNA core sequences (G-box, CE3, hex3 and motif III), is the major binding site for ABA regulated transcription factors target (Fujita et al., 2005). The interaction between ABRE binding factor and ABRE elements have been demonstrated to play a critical role in ABA mediated response (Toledo-Ortiz et al., 2003; Fujita et al., 2005). In previous study, the expression of *OsCYP2* could be induced by several abiotic stresses and ABA (Ruan et al., 2011). It is worth pointing out that 5 of ABRE elements were found in *OsCYP2* promoter sequence. Hereafter, a myc2-like transcript factor was identified to bind the ABRE element by yeast one hybrid and EMSA assays (Fig. 3 and Fig. 4).

There were two types of plant growth response to salinity, osmotic phase and ionic phase (Munns et al., 2008). Firstly, organic solutes were accumulated in the cytosol and organelles at high salt concentration to balance the osmotic pressure of the ions in the vacuole. Secondly, plant salt tolerance is mainly associated with the low maintenance of cytosolic Na^+/K^+ ratio (Gupta et al., 2020; 2021). According to our results, *myc2-like* overexpression could elevate the content of proline in transformants that compared to wild type in the presence of 150 mM NaCl, but no significant differences in Na^+/K^+ ratio among these lines (Fig. 7). On the other hand, *myc2-like* overexpression transformants showed that higher activity of antioxidative enzyme in reactive oxygen species scavenging. So, we speculated that *myc2-like* gene increases the resistance to salt stress by increasing activity of antioxidative enzyme and post translational regulation in transformed plants, rather than through Na^+ and K^+ uptake selectively.

Conclusions

Until now, a clear understanding of the perception of salt and the signaling pathway that help us to develop crop cultivars resistant to salinity and bring the salt affected lands into productive cultivation. Through the identified and characterized interactive transcription factor expressed under salt responsive

promoter element could provide a promising approach for genetic breeding. In summary, our results indicated that OsCYP2 was involved in salt stress resistance through a novel transcriptional signal mediated by myc2-like transcript factor regulated in rice. More importantly, it is a potential regulator that would provide a foundation in these sophisticated regulation mechanisms to salt stress response and genetic improvement in rice.

Materials And Methods

Cloning and analysis of *OsCYP2* promoter

Seeds of wild type (*O. sativa* L. cv. Aichi-ashahi, kindly provided by Institute of Biotechnology, Hangzhou Academy of Agricultural Sciences) were germinated with distill water in dark incubator at 30 °C and cultured at 32 °C/28 °C with the photoperiod of 16 h/8 h (light/dark). The total DNA of seeding was extracted from wild type according to modified CTAB method. An upstream 1156 bp fragment of the *OsCYP2* was amplified by specific primers (Supplementary Table S1) and then used to substitute the promoter of *GUS* in pCAMBIA1301 to construct a *pOsCYP2:GUS* plasmid (Supplementary Fig. S1). The *cis* elements of the promoter were predicted by PLACE (Higo et al., 1999).

Histochemical GUS assay of *OsCYP2*

The *pOsCYP2:GUS* vector was transformed into wild type by *Agrobacterium tumefaciens* strain EHA105 according to previously reference described with minor modification (Hiei et al., 1994). GUS activity was conducted with various tissues (panicle, leaves, stem and root) of wild type and transformants via histochemical staining by 0.5 mg/mL X-gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronide), which contained 80 mM phosphate buffer, 0.5 mM $K_3Fe(CN)_6$, 0.5 mM $K_4Fe(CN)_6$, 0.1% Triton X-100, and one drop N,N-dimethyl formamide. All the samples were incubated at 37 °C for 24 hr, and rinsed 30 min with a series of 50%, 75%, 100% ethanol, respectively. Then, these samples were kept in 75% ethanol for analysis and photograph.

Construction of bait yeast strain and testing for AbA expression

Based on *cis* elements of the promoter, ABA responsive element (ABRE) target sequence (p62) was used as a bait and mutant sequence (p63) as a negative control simultaneously. Two antiparallel oligonucleotides sequence (Supplementary Table S1) of ABRE *cis* element with *Hind*III and *Xho*I overhanging sticky ends was synthesis, then anneal the oligonucleotides and ligation into the linearized pAbAi vector. Recombinant plasmids were linearized through *Bst*BI and transformed into Y1H for generating bait reporter yeast strains. The minimal inhibitory concentration of AbA was conducted to bait reporter yeast strains.

Yeast one-hybrid assay

The pre-constructed cDNA library was transformed into Y1H bait strain (pBait-AbAi) using the Yeastmaker Transformation System 2 (Clontech, Cat. No. 630439) (Cui et al., 2016). The transformation reaction was spread on SD/-Leu plates with plus minimal inhibitory concentraion of AbA. The candidate clones were restreaked onto SD/-Leu/AbA at least three times to generate single colonies. For eliminate duplicate clones, we conducted yeast colony PCR by Matchmaker Insert Check PCR Mix 2 (Clontech, Cat. No. 630497). The prey vectors that showed single band in yeast colony PCR was isolated by Easy Yeast Plasmid Isolation Kit (Clontech, Cat. No. 630467) and sequenced using T7 primer. To verify positive interactions, each prey should be transformed into bait and mutant bait strain on selective media with side by side positive and negative controls.

Electrophoretic mobility shift assay

The transcription factor myc2-like was cloned into pET28a for electrophoretic mobility shift assays (EMSA). The recombinant vector was transformed in *E. coli* BL21 competent cells and fusion protein was purified by Magnehis Protein Purification Kit (Promega, Cat. No. V8500). Analyze the samples by 12% SDS-PAGE and western blot with His tag antibody conjugated horseradish peroxidase. The oligonucleotides containing three tandem copies of the elements were listed in Table S1 and labelled by Biotin 3' End DNA Labelling Kit (Thermo, Cat. No. 89818). Electrophoretic mobility shift assays were conducted using Light Shift Chemiluminescent EMSA Kit (Thermo, Cat. No. 20148) according to the manufacturer's instructions.

Transcript level of *myc2-like* under salt and ABA induction

Seedlings of wild type were cultivated as previously conditions. Samples were treated by 150 mM NaCl and 50 μM ABA. Transcript level of *myc2-like* was conducted by qRT-PCR after treated 2 hr, 12 hr and 24 hr. Three replications and statistical analysis of experiments were calculated according to previous described. The primers of *myc2-like* was listed in Supplementary Table S1. Each sample was performed three replicates and calculated according to the method of Livak and Schmittgen 2001.

Construction of *myc2-like* overexpression transformants in wild type and *cyp2*-RNAi line

The recombinant vector of *p1300-Ubiquitin-myc2-like* was constructed, then transformed into wild type and *cyp2*-RNAi line L3-61 (Cui et al., 2017) through *A. tumefaciens* strain EHA105 to create overexpression transformants. Plant transformation was performed according to previously reference described with minor modification (Higo et al., 1999). Independent transformants of *myc2-like* overexpression in wild type and *cyp2*-RNAi line were identified by quantitative RT-PCR.

Physiological and biochemical assays of transformants

To evaluate the salt response of transformants, seedlings of wild type, *myc2-like* overexpression in wild type, *myc2-like* overexpression in *cyp2*-RNAi and *cyp2*-RNAi were treated by 150 mM NaCl for 24 hr. Various physiology and biochemistry index were measured according to Cui et al., 2016, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), malondialdehyde (MDA) and proline.

Sodium and potassium ions contents were analyzed through atomic absorption spectrophotometry (Munns et al., 2010).

Statistical analysis

Statistical analysis of the results presented here are the means of at least three replicates. Treatment means were compared by the analysis of variance (ANOVA) and using the least significant difference (LSD) test at the $P \leq 0.05$ level of significance.

Declarations

Ethics approval and consent to participate

All plant experiments were performed in compliance with relevant institutional, national, and international guidelines and legislation.

Availability of data and materials

The data that support this study are available in the article and accompanying online supplementary material.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

HL and PC conceived the research plan and wrote the manuscript. BZ and JZ did the Y1H assay and genetic analysis. CL and QL performed qPCR and antioxidant enzymes measurements. HL, PC and BZ analyzed the data. All authors read and approved this final version of manuscript.

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References

1. Amaral MN, Arge LWP, Benitez LC, Danielowski R, Silveira SFS, Farias DR, Deuner S, Oliveira AC, Braga EJB, Maia LC (2016) Differential expression of photosynthesis-related genes and quantification of gas exchange in rice plants under abiotic stress. Acta Physiol Plant 38: 1–11.

2. Cui P, Liu HB, Islam F, Li L, Farooq MA, Ruan SL, Zhou WJ (2016) OsPEX11, a Peroxisomal Biogenesis Factor 11, contributes to salt stress tolerance in *Oryza sativa*. Front Plant Sci 7: 1357.
3. Cui P, Liu HB, Ruan SL, Ali B, Gill RA, Ma HS, Zheng ZF, Zhou WJ (2017) A zinc finger protein, interacted with cyclophilin, affects root development via IAA pathway in rice. J Integr Plant Biol 59: 496–505.
4. Ding Y, Zhu J, Zhao D, Liu Q, Yang Q, Zhang T (2021) Targeting *cis*-regulatory elements for rice grain quality improvement. Front Plant Sci 12: 705834.
5. Fujita Y, Fujita M, Satoh R, Maruyama K, Parvez MM, Seki M, Hiratsu K, Ohme-Takagi M, Shinozaki K, Yamaguchi-Shinozaki K (2005) AREB1 is a transcription activator of novel AREB dependent ABA signaling that enhances drought stress tolerance in *Arabidopsis*. Plant Cell 17: 3470–3488.
6. Gong Z, Xiong L, Shi H, Ynag S, Herrera-Estrella LR, Xu G, Chao DY, Li J, Wang PY, Qin F, Li J, Ding Y, Shi Y, Wang Y, Yang Y, Guo Y, Zhu JK (2020). Plant abiotic stress response and nutrient use efficiency. Sci China-Life Sci 63: 635–674.
7. Gupta A, Shaw BP (2020) Biochemical and molecular characterisations of salt tolerance components in rice varieties tolerant and sensitive to NaCl: the relevance of Na^+ exclusion in salt tolerance in the species. Funct Plant Biol 48: 72–87.
8. Gupta A, Shaw BP, Sahu BB (2021) Post-translational regulation of the membrane transporters contributing to salt tolerance in plants. Funct Plant Biol 48: 1199–1212.
9. Higo K, Ugawa Y, Iwamoto M, Korenaga T (1999) Plant *cis*-acting regulatory DNA elements (PLACE) database. Nucleic Acids Res 27: 297–300.
10. Hiei Y, Ohta S, Komari T, Kumashiro T (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant J 6: 271–282.
11. Hernandez-Garcia CM, Finer JJ (2014) Identification and validation of promoters and *cis*-acting regulatory elements. Plant Sci 217–218: 109–119.
12. Kurek I, Dulberger R, Azem A, Tzvi BB, Sudhakar D, Christou P, Breiman A (2002) Deletion of the C-terminal 138 amino acids of the wheat FKBP73 abrogates calmodulin binding, dimerization and male fertility in transgenic rice. Plant Mol Biol 48: 369–381.
13. Li C, Yan C, Sun Q, Wang J, Yuan C, Mou Y, Shan S, Zhao X (2021) The bHLH transcription factor *AhbHLH112* improves the drought tolerance of peanut. BMC Plant Biol 21: 540.
14. Li W, Yu D, Yu J, Zhu D, Zhao Q (2018) Functional analysis of maize silk-specific *ZmbZIP25* promoter. Int J Mol Sci 19: 822.
15. Li X, Yu B, Wu Q, Min Q, Zeng R, Xie Z, Huang J (2021) OsMADS23 phosphorylated by SAPK9 confers drought and salt tolerance by regulating ABA biosynthesis in rice. Plos Genet 17: e1009699.
16. Liu JH, Peng T, Dai W (2014) Critical *cis*-acting elements and interacting transcription factors: key players associated with abiotic stress responses in plants. Plant Mol Biol Rep 32: 303–317.

17. Livak KJ, Schmittgen TD, (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}_{T}$ method. Methods 25: 402–408.
18. Massari ME, Murre C (2000) Helix-loop-helix proteins: regulators of transcription in eukaryotic organisms. Mol Cell Biol 20: 429–440.
19. Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59: 651–681.
20. Munns R, Wallace PA, Teakle NL, Colmer TD (2010) Measuring Soluble Ion Concentrations (Na^+, K^+, Cl^-) in Salt-Treated Plants. In: Sunkar R. (eds) Plant Stress Tolerance. Methods in Molecular Biology (Methods and Protocols), Humana Press 639: 371–382.
21. Nair SK, Burley SK (2000) Recognizing DNA in the library. Nature 404: 715–717.
22. Ruan SL, Ma HS, Wang SH, Fu YP, Xin Y, Liu WZ, Wang F, Tong JX, Wang SZ, Chen HZ, (2011) Proteomic identification of OsCYP2, a rice cyclophilin that confers salt tolerance in rice (*Oryza sativa* L.) seedlings when overexpressed. BMC Plant Biol 11: 34.
23. Schwarzer W, Spitz F (2014) The architecture of gene expression: integrating dispersed *cis*-regulatory modules into coherent regulatory domains. Curr Opin Genet Dev 27: 74–82.
24. Singhal P, Jan AT, Azam M, Haq QMR, (2015) Plant abiotic stress: a prospective strategy of exploiting promoters as alternative to overcome the escalating burden. Front Life Sci 9: 52–63.
25. Tester M, Bacic A (2005) Abiotic stress tolerance in grasses. From model plants to crop plants. Plant Physiol 137: 791–793.
26. Toledo-Ortiz G, Huq E, Quail PH (2003) The Arabidopsis basic/helix-loop-helix transcription factor family. Plant Cell 15: 1749–1770.
27. Varshney RV, Bansal KC, Aggarwal PK, Datta SK, Craufurd PQ (2011) Agricultural biotechnology for crop improvement in a variable climate: hope or hype? Trends Plant Sci 16: 363–371.
28. Verma V, Ravindran P, Kumar PP (2016) Plant hormone-mediated regulation of stress responses. BMC Plant Biol 16: 86.
29. Wang H, Ding C, Du H, Liu H, Wang Y, Yu D (2014) Identification of soybean MYC2-like transcription factors and overexpression of *GmMYC1* could stimulate defense mechanism against common cutworm in transgenic tobacco. Biotechnol Lett 36: 1881–1892.
30. Wang T, Wang S, Wang Y, Li J, Yan F, Liu Y, Zhao L, Wang Q (2020) Jasmonic acid-induced inhibition of root growth and leaf senescence is reduced by *GmbHLH3*, a soybean bHLH transcription factor. Can J Plant Sci 100: 477–487.
31. Wani SH, Anand S, Singh B, Bohra A, Joshi R (2021) WRKY transcription factors and plant defense responses: latest discoveries and future prospects. Plant Cell Rep 40: 1071–1085.
32. Wu H, Ren Z, Zheng L, Guo M, Yang J, Hou L, Qanmber G, Li F, Yang Z (2021) The bHLH transcription factor GhPAS1 mediates BR signaling to regulate plant development and architecture in cotton. Crop J 9: 1049–1059.
33. Yang W, Ju Y, Zou L, Shang L, Li X, Li X, Feng S, Ding X, Chu Z (2020) OsHsfB4d binds the promoter and regulates the expression of *OsHsp18.0-CI* to resistant against *Xanthomonas Oryzae*. Rice 13: 28.

34. Zhang H, Zhang Z, Zhao Y, Guo D, Zhao X, Gao W, Zhang J, Song B (2021) StWRKY13 promotes anthocyanin biosynthesis in potato (*Solanum tuberosum*) tubers. Funct Plant Biol 49: 102–114.

Figures

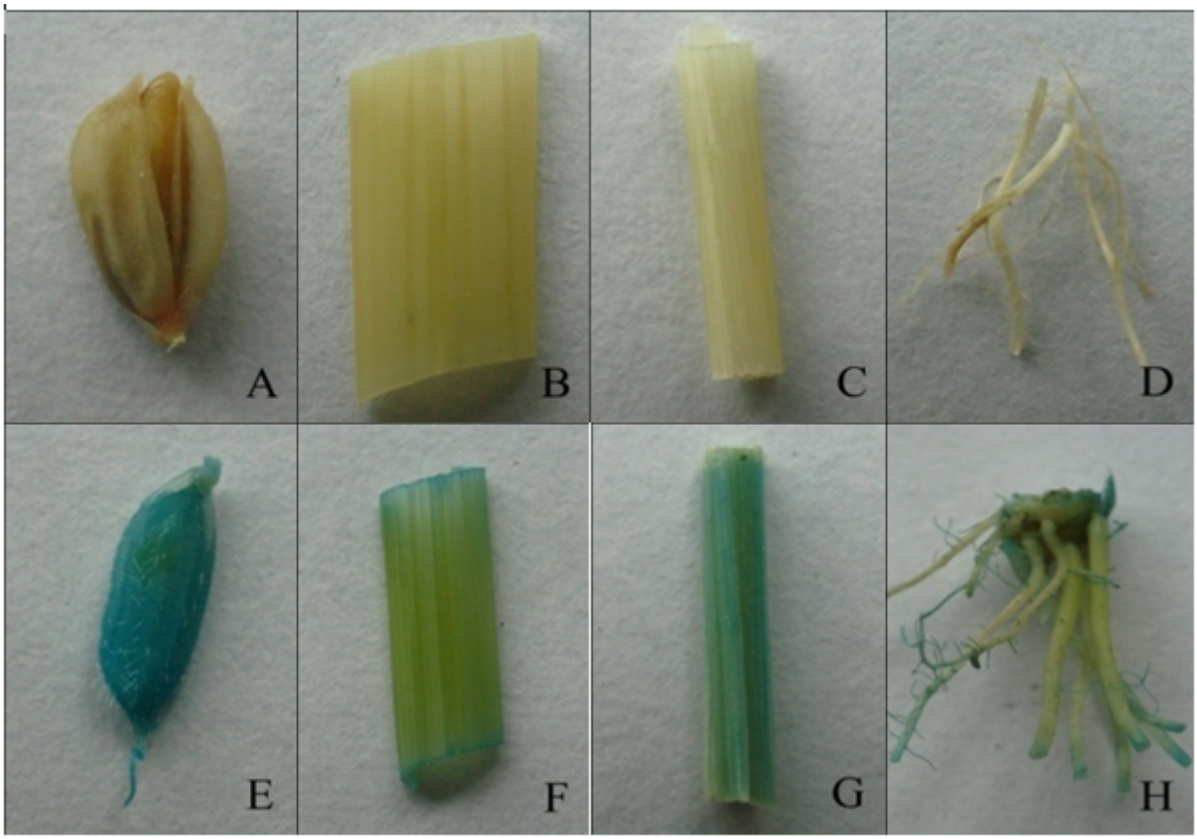

Figure 1

GUS staining showed expression patterns of OsCYP2 indicated by GUS reporter gene which was driven by promoter of OsCYP2. A-D: Panicle, leaf, stem and root of wild type; E-H: Panicle, leaf, stem and root of *pOsCYP2:GUS* transgenic plants.

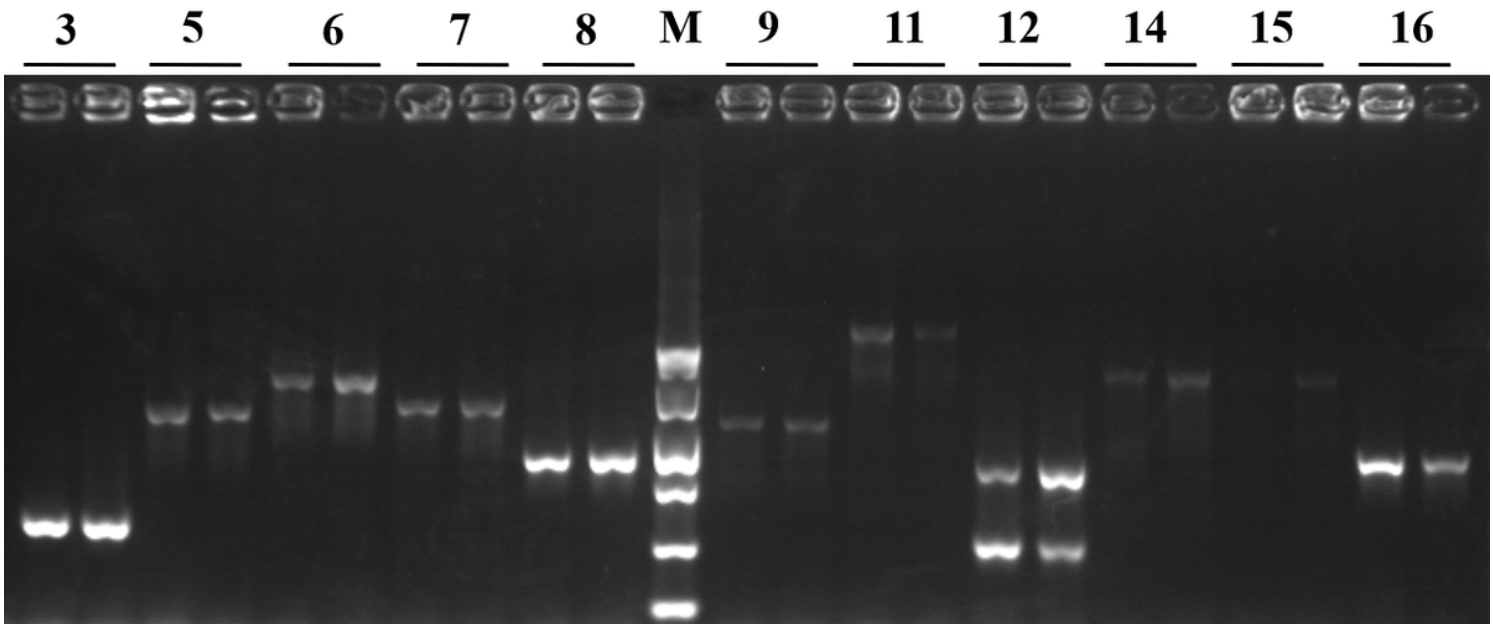

Figure 2

Detection preys by yeast colony PCR. Each preys using two independent colony, M: DNA Marker F (200-2000)

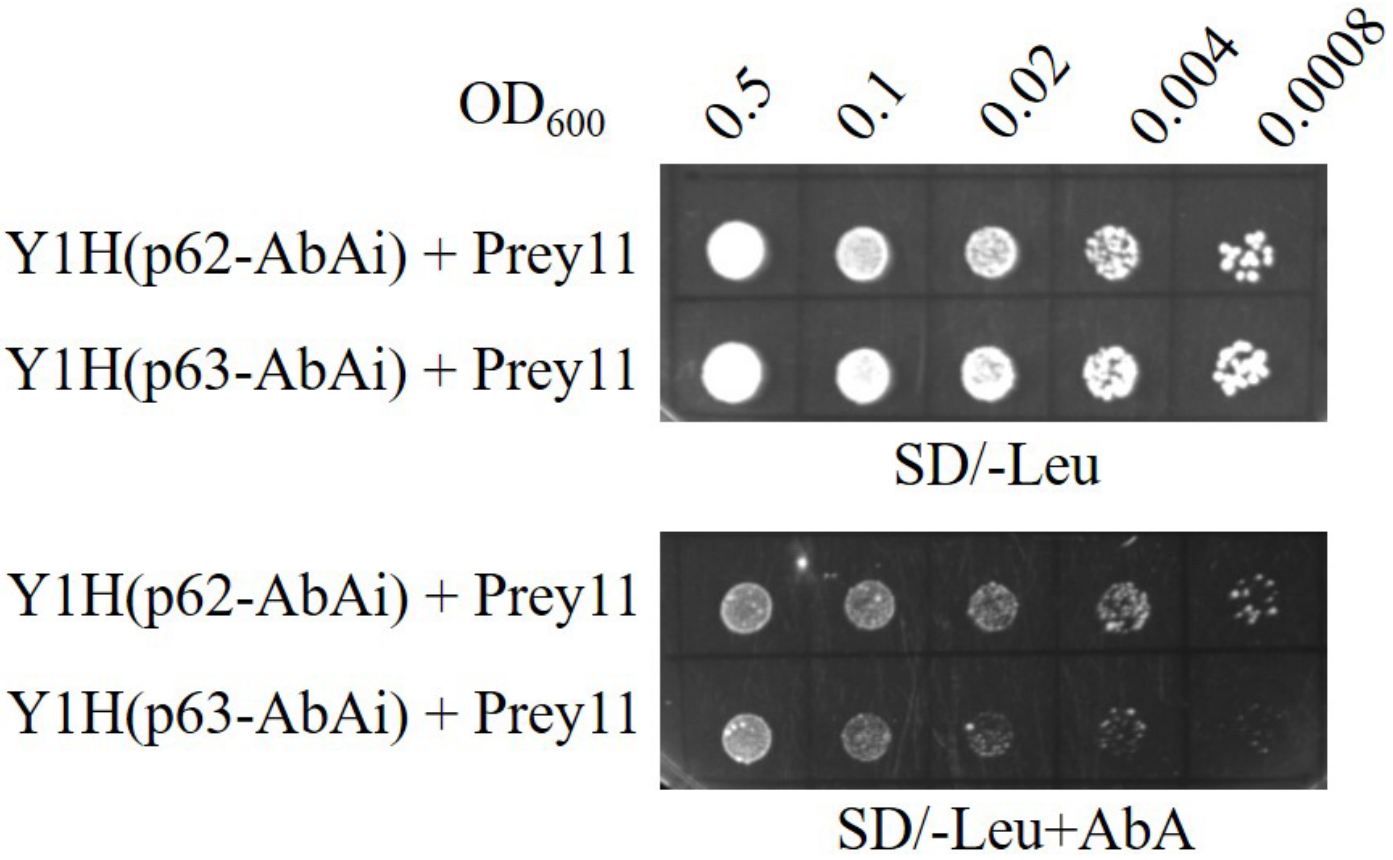

Figure 3

Cotransformation and serial diluted (1:5) with the initial optical density OD_{600}) of 0.5 on selective media to candidate interaction transcription factors (No. 11). SD/-Leu: synthetically defined medium with dropout leucine, SD/-Leu+AbA: synthetically defined medium with dropout leucine and plus 750 μg/L Aureobasidin A; Y1H (p62-AbAi): bait strain, Y1H (p63-AbAi): mutant bait strain.

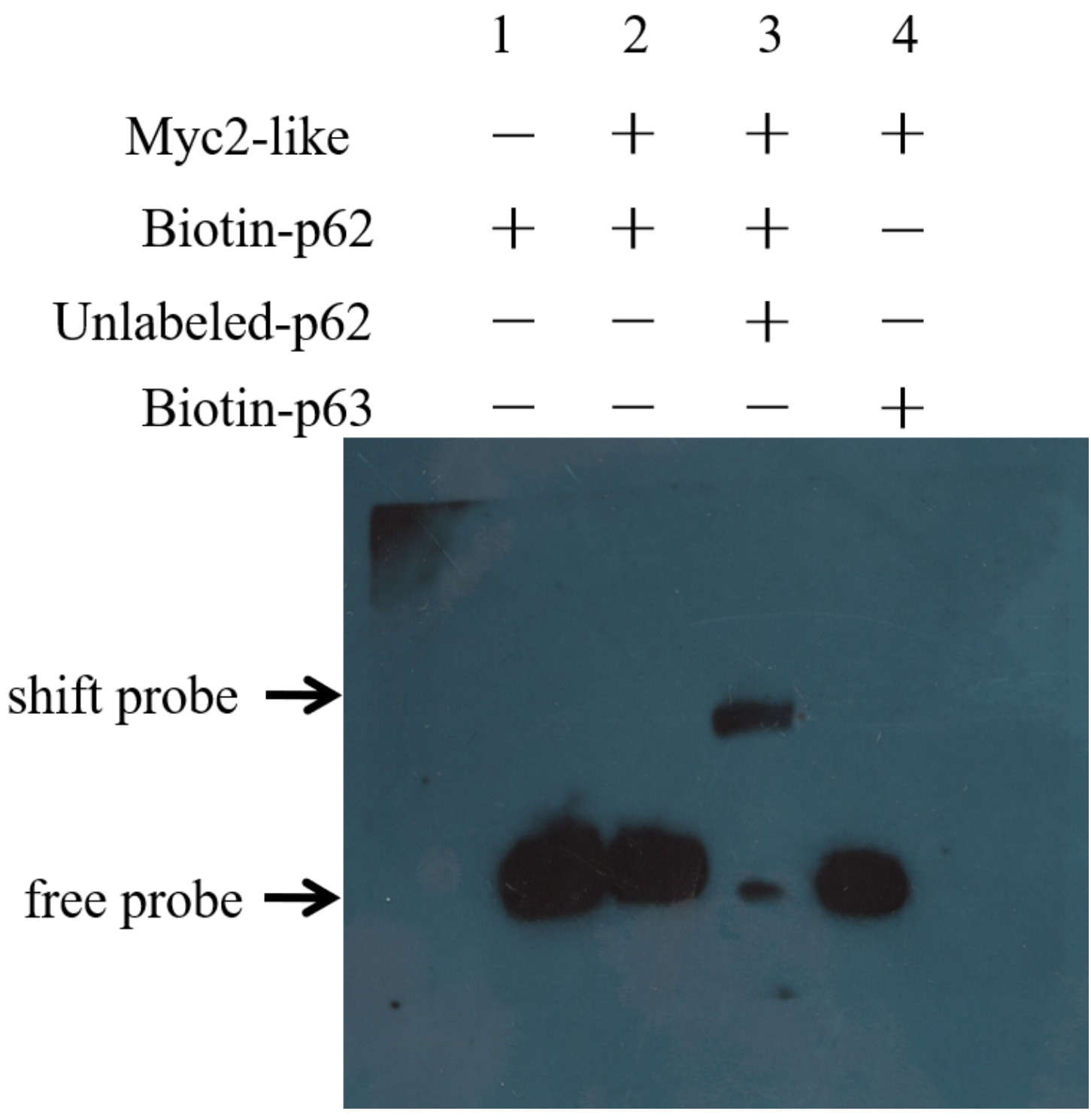

Figure 4

Myc2-like protein bind to the ABRE element by EMSA assays. 1: No myc2-like protein for biotin labeled probe (p62) to bind, establishes the position of an unshifted probe band; 2: The myc2-like protein was incubated with the biotin labeled probe (p62); 3: 200-fold excesses of unlabeled probes was used for competition, 4: Biotin mutated probes (p63) were used as a negative control. Mutated probe in which the ABRE element 5’-CACGTG-3’ was replaced with 5’-CACGCG-3’.

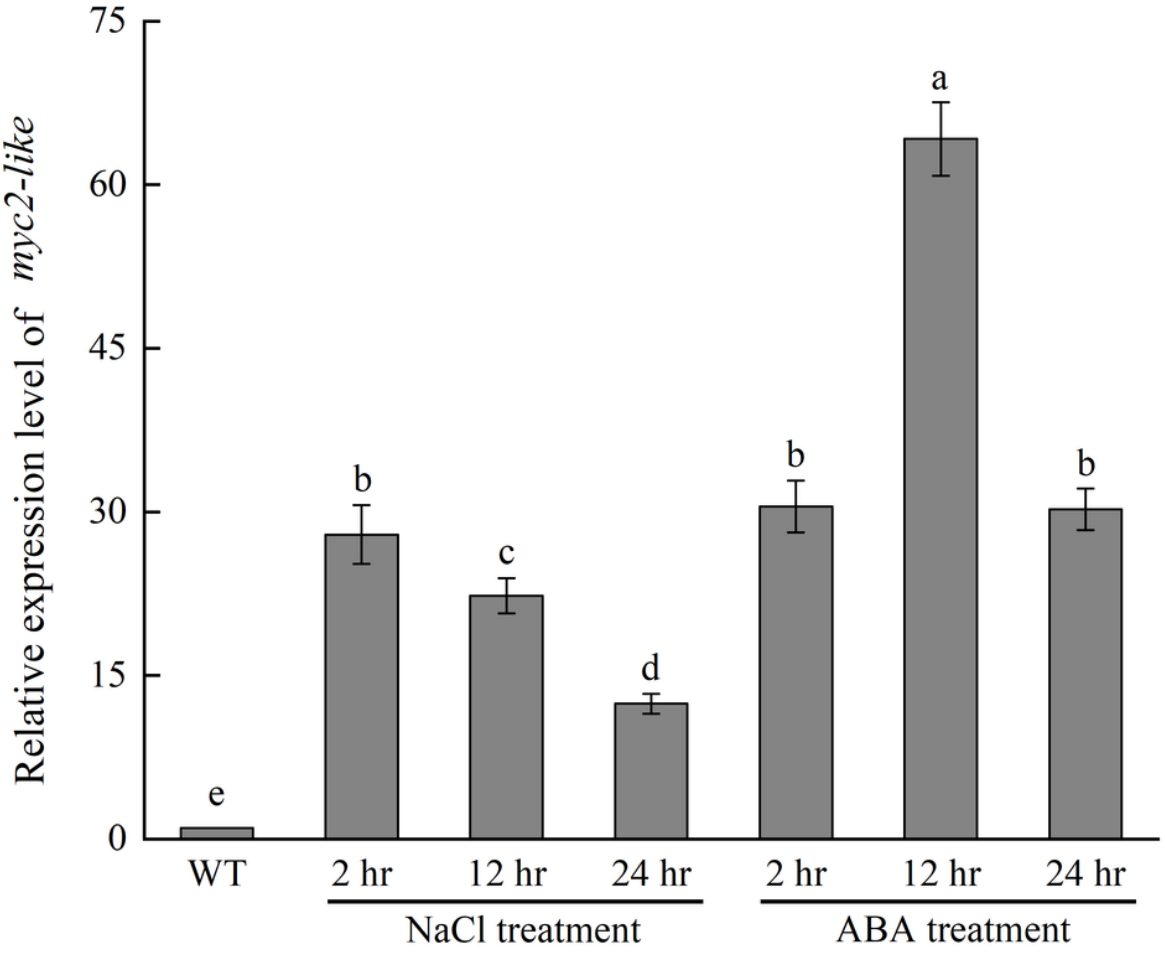

Figure 5

Relative transcript level of *myc2-like* induced by salt and ABA. Error bars indicate SD.

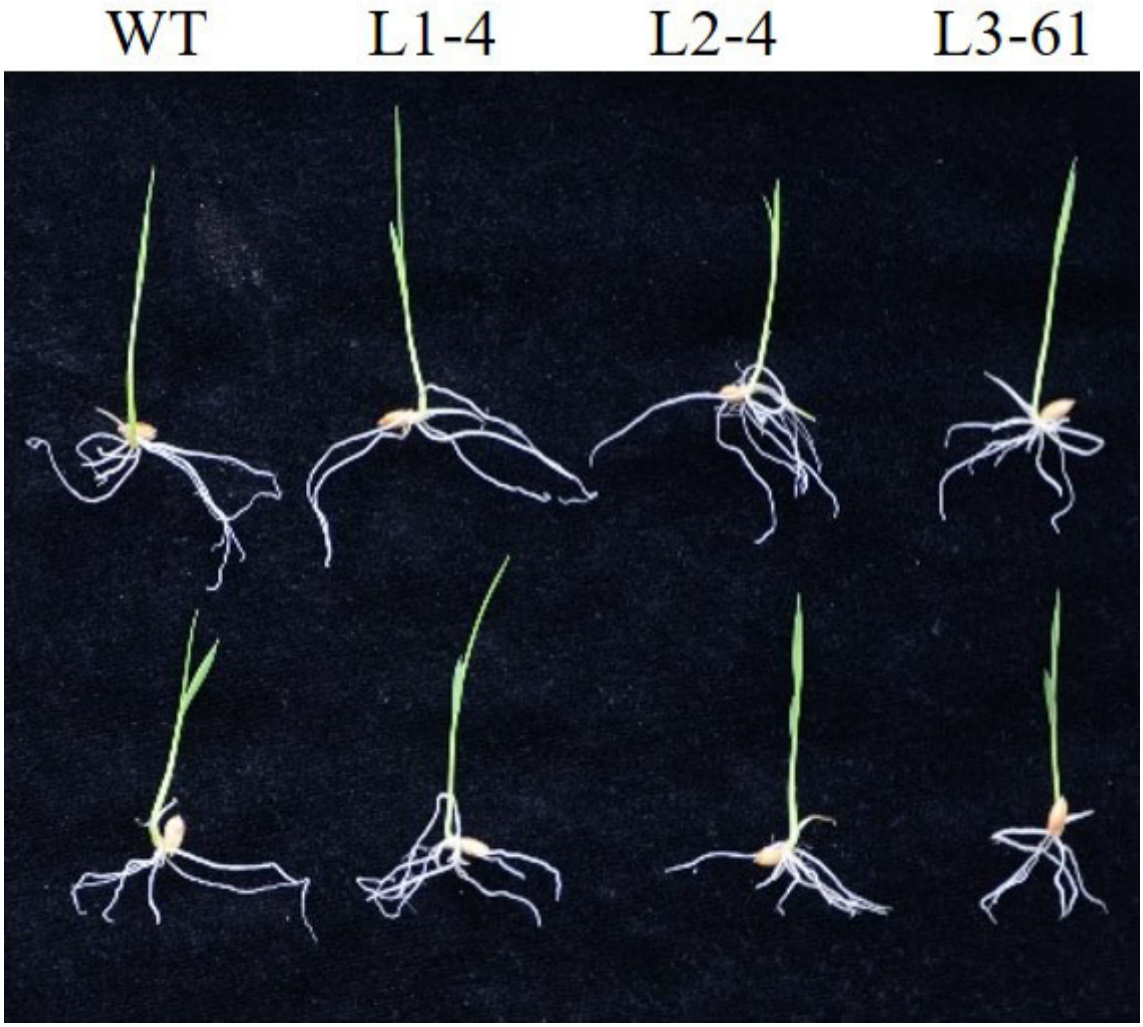

Figure 6

Phenotype of wild type, *myc2-like* overexpression (L1-4), *myc2-like* overexpression in *cyp2*-RNAi (L2-4) and *cyp2*-RNAi (L3-61) under salt stress (150 mM NaCl).

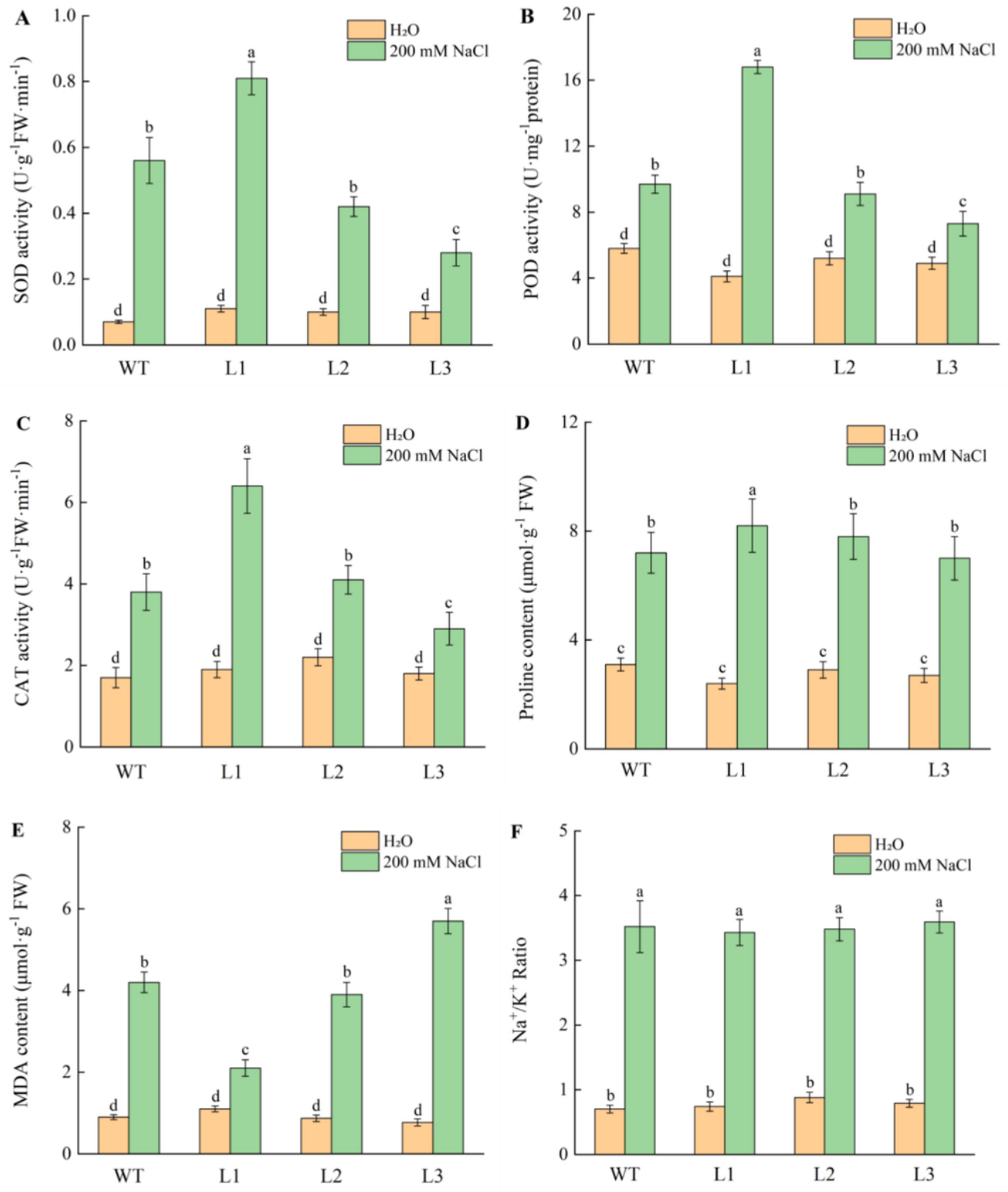

Figure 7

Comparison of antioxidative enzymes (SOD, POD, CAT), membrane lipid peroxidation index (MDA), Osmolyte (proline), and Na^+/K^+ ratio among wild type (WT), *myc2-like* overexpression (L1), *myc2-like* overexpression in cyp2-RNAi (L2) and *cyp2*-RNAi (L3) under salt stress. Values are mean ± S.D. (n=3) followed by the same letter did not significantly differences at $P \leq 0.05$ according to LSD test.

Supplementary Files

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- Supplementalfile.docx