FUNCTIONAL CHARACTERIZATION OF MYB GENE IN RICE

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Although MYB is an important transcription factor family gene for plant development and defense against drought stress, understanding the participation of MYB genes in rice growth and adaptation to drought stress is still largely unknown. Functional analysis of MYB98 showed that OsMYB98 is highly expressed during seed development and maturation and induced under drought stress. Here, OsMYB98 was amplified from rice Nipponbare variety and functionally over-expressed in Zhonghua11 (ZH11) as a wild type to verify the role of OsMYB98 in drought stress. Based on the results of transcription level and the relative expression analysis, three independent lines were selected for further analysis. Two week's seedlings of OsMYB98, and wild type were subjected to 20% PEG6000 to conduct drought stress. The results revealed that OsMYB98 was differentially expressed and regulated during drought pressure in roots of OsMYB98-3, OsMYB98-6, and OsMYB98-8 compared with the wild type. Over-express of the OsMYB98 gene in rice increases the rice roots' resistance to drought pressure, where the expression of OsMYB98 in roots was rapidly increased and consequently elevated during the tested time point and reached the highest after 24 hours. OsMYB98 plants enhance the resistance to drought compared with wild type, and resulted in lower MDA content, lower water loss, and higher proline content in OsMYB98 plants under drought. The results revealed that OsMYB98 is a stress-responsive gene, and it might be a future basic to develop the resistance of rice and other crops to drought.

Keywords: transcription factor, rice MYB98, functionally overexpressed, abiotic stress, drought

INTRODUCTION

Transcription factors are important candidates for targeting the complex traits in crops; they are the key regulators of cellular processes in plants. TFs have a wide range of genes contains DNA binding elements such

as; MYB, Zinc fingers, WRKY, Bzip, MYC, and ERF/AP2, which have been reported as an inducible signal (Peng et al., 2016 and Roy, 2016). MYB is a massive family of genes, which regulates and divers in plants with the major domains R2R3-MYB serving as DNA binding (Huang et al., 2022). MYB genes involved in a vast array of biochemical and physiological process (He et al., 2019; Cao et al., 2020 and Huang et al., 2022). In plants, MYB proteins are well documented as the key elements involved in abiotic and biotic stresses response and regulatory controlling, hormone signal transduction, metabolism, and cell development. Releasing the genome sequence in several plants lead to functionally characterized several MYB proteins in frequent plants species; petunia, *Arabidopsis*, snapdragon, maize, grapevine, rice, poplar, and apple and provided the basis for predicting the role of MYB proteins in plants (Dubos et al., 2010). Simultaneously, several research studies were released to provide a comprehensive insight to the dynamic mechanisms of MYB gene regulation and numbers of target genes were identified. Increasing the plant genome variability among the plant species have allowed the comparative and better understanding the association analysis and the evaluation of large MYB family (Dubos et al., 2010). R2R3-MYB genes have a key role in regulating rejoinders to abiotic tensions; cold, salt, and drought (Tang et al., 2019 and Wang et al., 2021).

Several MYB genes enhance drought stress response by controlling abscisic acid (ABA) signal (Ding et al., 2009; Seo et al., 2009 and Cao et al., 2022). AtMYB15 displayed higher tolerance to salinity through enhanced sensitivity to abscisic acid (Ding et al., 2009). Several GmMYB genes enhance *Arabidopsis* resistance to stress through regulating the stress related genes (Liao et al., 2008). MYB96 expressed in the roots of *Arabidopsis*, and altered the level of endogenous auxins during lateral root development and mediates ABA-auxin during drought stress through inducing GH3 genes providing tolerance strategy under drought stress (Seo et al., 2009). Physiologically and biochemically, OsMyb4 confer resistance to drought and cold stress, and altered metabolite accumulation in apple (Pasquali et al., 2008). Moreover, Osmyb4 cause changes in proline and sugars accumulations and enhance the freezing and cold tolerance in *Osteospermum ecklonis* (Laura et al., 2010). OsMYB6 functionally reported as a drought stress responsive gene (Tang et al., 2019), BpMYB123 was recently reported to regulate BpLEA14 to develop drought tolerance in *Betula platyphylla* (Lv et al., 2021). Globally, rice is an exemplary and essential grain crop for human population. Nevertheless, rice is a sensitive plant to salt and drought pressure than other crops (Huang et al., 2014). Therefore, functional verification of MYB genes in response to the drought pressure in rice crop will enhance and improve our vision for the network of stress signal in rich to improve rice resistance to drought.

MATERIALS AND METHODS

1. Plant Materials

For gene cloning, young leaf from Japonica rice Nipponbare was used to extract RNA and cDNA was immediately synthesized. Pdonr207 was used as an entry vector to deliver the gene into the destination over-expression vector PJC034 using the Gateway recombination reaction (Invitrogen).

2. Phylogenetic Tree Analysis

Rice Genome Annotation Project was used to download the full length of MYB98 CDS and amino acid. Based on protein BLAST search against NCBI data base, rice MYB98 consist of 1317 base pair encodes 438 amino acids, displayed similarity with hundred protein sequence from diverse of plant species. To reveal the correlation between the rice MYB98 protein and the resulted blasted proteins based on the amino acid sequence, MEGA-X software was used (Kumar et al., 2018).

3. Physiochemical Properties and Putative Tissue Expression Profile of Rice MYB98

PROTPARAM tool was used to conduct the physiochemical properties of the MYB98 gene. In addition, InterPro tools were used to predict the protein domain. RNA-Seq Atlas of rice (Rice eFP Browser) was used to extract the putative tissue expression profile. The *Arabidopsis* Information Resource (TAIR) is used for putative subcellular localization of MYB98 gene; it was inferred from their sequence similarity with characterized protein. Subcellular localization profile image was built using Cell eFP tool.

4. Rice MYB98 Gene Amplification, Vector Construction and Rice Plant Transformation

Full length sequence of OsMYB98 gene was amplified from cDNA of Japonica rice Nipponbare leaves using the specific primers of MYB98 (Table 1). The isolated fragment was immediately first cloned into the pDONR207 entry vector in *E. coli* DH5α competent cell using heat shock methods (Peng et al., 2017). The positive colony with the right sequence of pDONR207-MYB98 plasmid was delivered into the destination over-expression vector pJC034 using the Gateway recombination reaction (Invitrogen) (Peng et al., 2017). The PJC034-MYB98 construct was inserted into EHA105 *Agrobacterium tumefaciens* strain, and transformation was conducted in Japonica ZH11 as described by Lin and Zhang (2005). Among twenty-four transgenic lines, three independent overexpression lines were selected for further analysis.

Table (1). Primers used in this study.

Primer	Sequence	Usage
Forward	AAAAAGCAGGCTTAATGGCGGCTGCAGGTAAC	Gene amplification
Reverse	AGAAAGCTGGGTATCAGTTGATCCACCTGGCC	Gene amplification
Forward	GCACATGCTTCCCAGTGAAA	Relative expression
Reverse	CTGCACAACGGGTAGAGTTG	Relative expression
Forward	GATGGACCAACCATCTGCAC	Transcription level
Reverse	CTGCACAACGGGTAGAGTTG	Transcription level

5. RNA Extraction and Quantitative Real-time PCR (RT-PCR)

Young leaves were used to extract total RNA from Nipponbare variety for gene amplification, while roots of the OsMYB98 lines and ZH11 were used to extract RNA for expression level analysis. TRIZOL reagent was used to extract the RNA according to the methods described by Liu et al. (2009). By following its manufacturer's instructions, RevertAid First StrandcDNA synthesis kit (Fermentas, Lithuania) was used to reverse transcribed the total RNA to get the first strand cDNA. SYBR GREEN PCR Master Mix (Applied Biosystems, USA) was used with the MYB98 qRT-PCR specific primers to perform the quantitative real-time PCR, Ubi-actin primer was used as the reference endogenous gene (Table 1). The qRT-PCR was conducted with the following incubation condition: 50°C for 2 min, 50°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Data were collected and the expression data were analyzed by ΔCt method (fold change $= 2^{-\Delta\Delta C}$t) (Livak and Schmittgen, 2001).

6. Drought Stress for the MYB98 Transgenic Lines and Japonica Rice Variety Zhonghua11 (ZH11).

The experiment was implemented in controlled greenhouse. The seeds of MYB98-3, MYB98-6, and MYB98-8 transgenic lines and the Japonica rice variety Zhonghua11 (ZH11) were washed with sterilized water. 1% NaOCl solution was used to sterilize and eliminate the contaminants, then disinfected seeds were soaked in water at 37°C for two days, and immediately germinated at 30°C with a relative humidity set at 70% and 16 h (light)/8h (dark) photoperiod. Two weeks seedlings grown in 2000 ml pots containing a ½ strength Hoagland nutrient solution were subjected to drought stress by adding 20% PEG6000 to the nutrient solution. In three biological replicates for each point time, the roots were sampled after 0, 1, 3, 6, 12, and 24 hours.

7. Physiological Measurements

14-day old of the three MYB98 lines (MYB98-3, MYB98-6, and MYB98-8) compared with wild type seedlings were subjected for air-drying to determine the water loss, which calculated as percentage as follows: (lost weight/initial weight) × 100 as described by Bundo and Coca (2017). The rice

seedlings were put on the laboratory bench at room temperature for different time courses. For drought assay, the seeds of three OsMYB98 lines and wild type were germinated and grown in the green house as stated by Li et al. (2021). Four-week-old of the three OsMYB98 lines and wild type had their watering withheld for 21 days. Proline content and malondialdehyde (MDA) were detected after 21 days of drought in the leaves as stated by Xia et al. (2018).

RESULTS AND DISCUSSION

1. Phylogenetic Tree of Rice MYB98

The full-length nucleotide sequence of rice MYB98 consists of 1317 base pair, encodes 438 amino acids, displayed a homologue with one-hundred protein sequences in diverse plant species using protein BLAST search against NCBI database. To elucidate the association between the homologue proteins and the rice MYB98 protein, a diverse of 100 proteins and the MYB98 proteins were applied for cluster analysis using MEGA-X software (Fig. 1 a). The results showed that rice MYB98 has the highest similarity with MYB119 found in *Oryza Sativa* Japonica group, OsI_25392 (*Oryza sativa* Indica Group) and the MYB like transcription factor (*Oryza sativa* Japonica Group).

2. Physicochemical Properties and Putative Tissue Expression Pattern Rice *MYB98* Gene

The molecular weight of rice MYB98 proteins is 47467.99 Da. The theoretical pI is 5.7 (Table S1). The grand average of hydropathy (GRAVY) values of rice MYB98 proteins is -0.544 (moderately soluble) which is similar to MYBs in peach and brassica napus (Hajiebrahimi et al., 2017 and Zhang et al., 2018). The instability index of rice MYB98 protein is 54.42 which is very similar to MYB proteins in *Brassica napus* and peach (Hajiebrahimi et al., 2017 and Zhang et al., 2018). The instability index of MYB proteins in peach fluctuated between 30.27 to 91.89 with an average value of 53.99, which was very close to that (54.0) of MYB proteins in *Brassica napus*. The instability index (II) is computed to be 54.42, which means that the protein is unstable. These results agreed with that reported by Hajiebrahimi et al. (2017) and Zhang et al. (2018). To predict the domain analysis for the rice Os07g12130 (MYB98) gene, the amino acids sequence was shot against 13-member database using InterPro tools. The results showed that the rice MYB98 shares in SANT/Myb domain (IPR001005), Myb-like DNA binding domain (PF00249), Myb-like domain profile (PS50090), DNA-binding domains (SM00717), DNA-binding domains (cd00167), Myb domain (IPR017930), Myb-type HTH DNA-binding domain profile (PS51294) (Fig. 1 b).

a

b
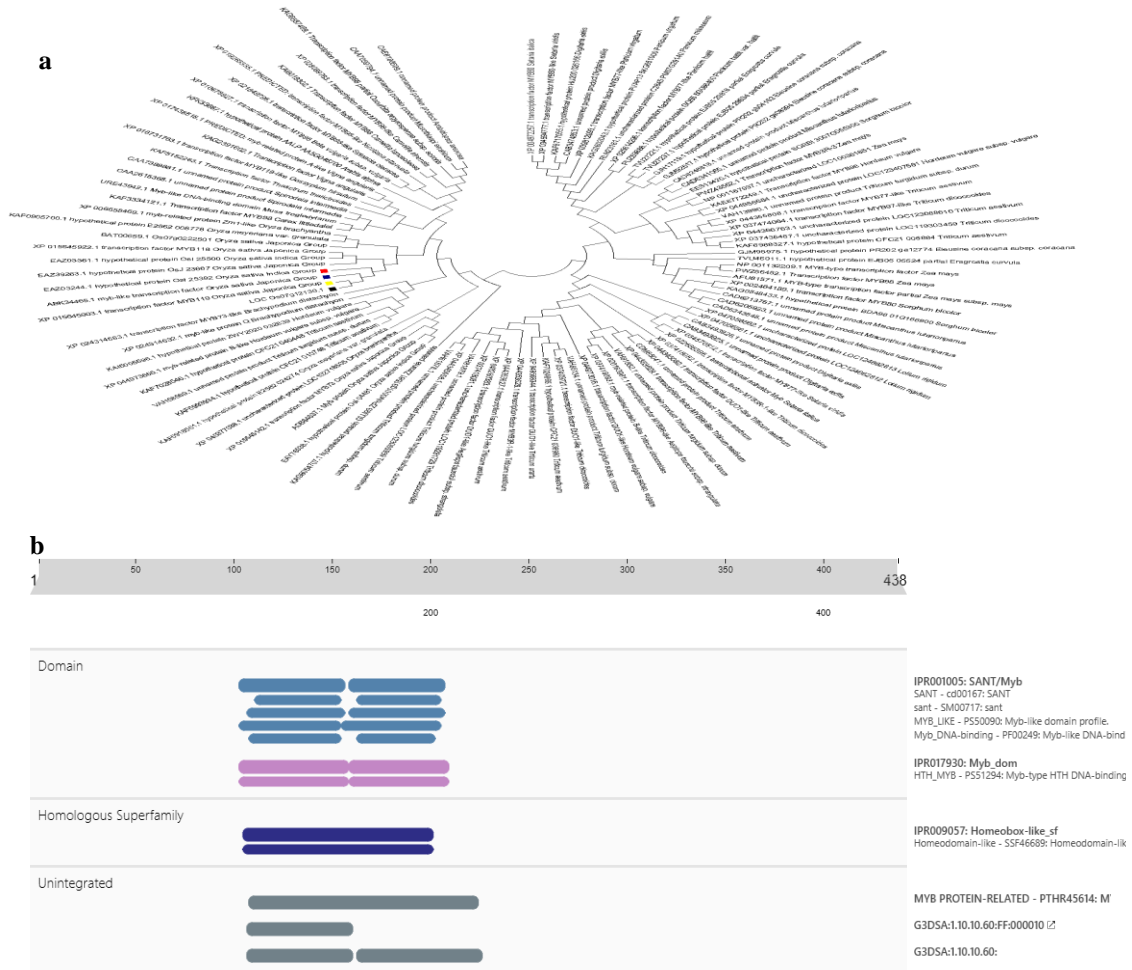
Fig. (1). a. The phylogenetic tree of rice MYB protein with 100 homologous proteins in different plant species. The phylogenetic tree was generated by ClustalW2 using standard parameters of the Likelihood method in MEGA-X. The 100 homologous MYB protein sequences were downloaded from NCBI database. Rice MYB is indicated by a black box, and the highest three similar genes indicated by yellow, blue and red box. **b.** Putative domain analysis for MYB using the InterPro protein sequence analysis & classification database.

Identification of the transcript abundance patterns of rice MYB98 genes that are similar to MYB proteins with known functions from rice or other plant species offers candidates for future studies aimed at comprehensively dissecting MYB function. Several studies use Electronic Fluorescent Pictograph (eFP) browser database for rapid comparisons with

putative orthologs from several plant species (Wilkins et al., 2009; Hawkins et al., 2017 and Ali et al., 2022). Based on the rice transcript, the putative MYB98 gene expression profile maps were investigated to recognize the role of the MYB98 gene at diverse rice tissue (Fig. 2). The rice data display that the MYB98 is highly expressed in seeds S4 (stage 4) followed by seed S3 (Stage 3), SAM, seeds S2 (stage 2), young leaf and roots (Fig. 2 a and d). The results coincide with the function of FveMYB10 in strawberry fruits where it was expressed highly at the receptacle fruit starts changing color (Hawkins et al., 2017). In context, Osmyb2, Osmyb3 and Osmyb5 genes were highly regulated in rice seed development, while Osmyb1 and Osmyb4 revealed a role in rice seed maturation (Suzuki et al., 1997), with the evidence that MYB genes induced by drought stress are increasing with the time (Yang et al., 2012 and Wei et al., 2017). MYB98 was highly expressed under drought stress in rice (Fig. 2 c). These results are in line with Tang et al. (2019), who stated that OsMYB6, was induced by drought. Compared with wild type; OsMYB6 plants showed a dramatic increased tolerance to drought. It is evident from the Rice Electronic Fluorescent Pictograph Expression Profile Browser that the MYB98 gene was expressed in most rice tissues. The results revealed that MYB98 gene was highly expressed in the section top of the maturated leaf (Fig. 2 b). The deep analysis of MYB genes in *Arabidopsis* and rice revealed that MYB genes are regulated in all tissues due to the distribution and position of MYB genes in the whole genome (Katiyar et al., 2012). Transcription factors are generally consist of two domains, a transcription activation and DNA-binding (Kim et al., 2017), which works together to regulate various developmental, biochemical, and physiological processes by triggering the genes correlated with the stress responses and development process (Riechmann et al., 2000). It is well known that rice seeds can germinate under anoxia and can show coleoptile elongation, where the anoxic coleoptile is usually longer than aerobic coleoptiles (Magneschi et al., 2009). Here, the results revealed that MYB98 was induced in both aerobic coleoptile and anoxic coleoptile during rice germination (Fig. 2 e). It is clear from the Cell Electronic Fluorescent Pictograph subcellular localizations profiles that the MYB98 gene was highly expressed and presented in nuclus (Fig. 2 f). These results are in line with Agarwal et al. (2006), who reported that MYB15 is present in the nucleus.
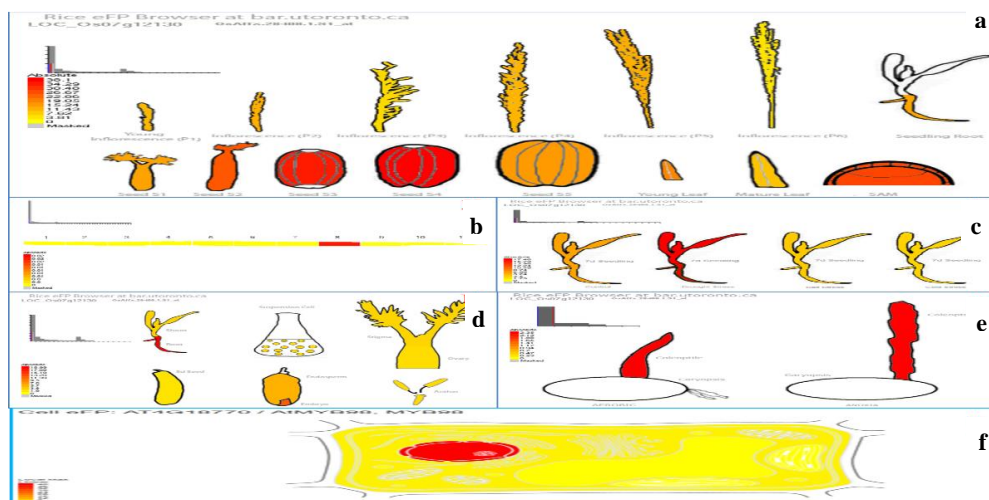
Fig. (2). Visualization of the putative "electronic fluorescent pictograph" browsers for exploring the putative tissue expression and cell localization of rice MYB98 (OsMYB98) and *Arabidopsis* (AtMYB98) gene, based on rice and *Arabidopsis* gene expression and protein localization at different tissues and cell organs. **a.** Expression data at different tissues from seedling to seeds. **b.** Expression data of tissue specific stem epidermis at top and bottom of rice leaf gradient. **c.** Expression data under different stresses (drought, salt, and cold) at 7-day old seedling. **d.** Expression data at root, shoot, suspension cell, stigma, ovary, 5-day seed, endosperm, embryo, and anthers. **e.** Expression data in aerobic coleoptile and anoxic coleoptile. The blue arrow points the expression scale (the more intense red color, the more gene expression). f. The localization of MYB98 gene in nucleus of rice cells.

3. Characterizations and Over-expression of Rice MYB98 Gene

Drought destructively influences the growth of plant and restricts plant yields through regulation of drought related genes (Chen et al., 2015). Constantly, transcription factors play crucial roles in the plant adaptation to drought, and the regulation of MYB genes in stress responses have been reviewed recently (Chen et al., 2015). Several MYB genes were stated to develop drought resistance in plants (Jung et al., 2008; Ding et al., 2009 and Chen et al., 2015). The destructive consequence of drought on the plant yields, lead the researcher to release a new drought responsive gene to break the harmful effect of drought on agriculture yield under drought stresses. Here, to understand the role of rice MYB98, CDS sequence with 1317 bp encodes 438 amino acids was amplified from the cDNA of Japonica rice Nipponbare leaves (Fig. 3 a and b). For over-expression vector construction, the isolated fragment was immediately first cloned into the entry pDONR207 plasmid (Fig. 3 c), and then into the destination vector pJC034 with govern of maize ubiquitin

promoter (Fig. 3 d and Fig. 4). The construct was inserted into *Agrobacterium tumefaciens* strain EHA105 (Fig. 3 e), and then transferred into Japonica ZH11. A total of 24 independent rice MYB transgenic lines were gained. The presence of MYB98 in the OsMYB98 rice lines genome was detected by PCR test from DNA. The transcription level of the 24 rice MYB transgenic lines was analyzed by RT-PCR (Fig. 3 f.a and f.b), based on the results of the transcription level, ten independent over-expression lines were selected for relative expression analysis (Fig. 5). MYB-3, MYB-6, and MYB-8 lines display the highest relative expression level among the ten transgenic lines (Fig. 5). Based on the quantitative RT-PCR analysis, MYB-3, MYB-8 and MYB-6 transgenic lines were selected for further experiments.
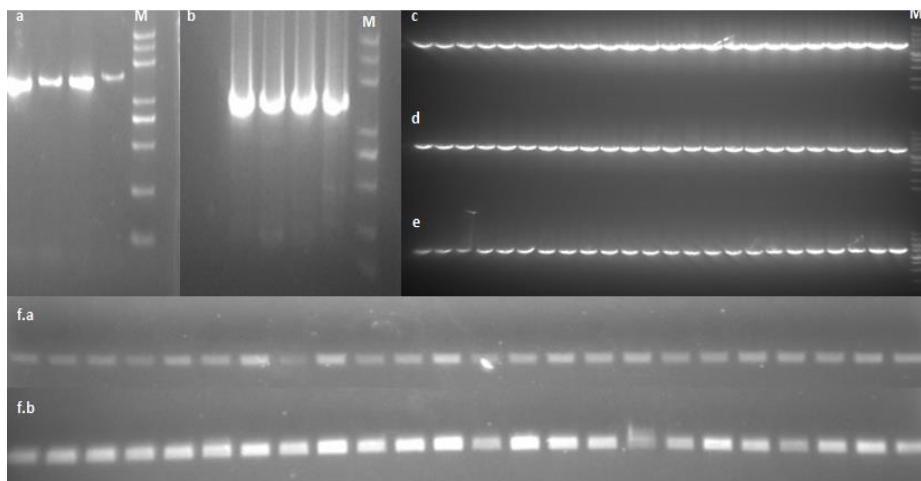

Fig. (3). a. First PCR amplification of MYB gene. **b.** Second PCR amplification. **c.** BP cloning. **d.** LR cloning. **e.** Transformation in *Agrobacterium tumefaciens* EHA105. **f.a.** The transcription level of actin in the rice transgenic plants. **f.b.** The transcription level of MYB-gene in the rice transgenic plants.

Fig. (4). Schematic representation of PJC034-Os07g12130 vector constructs. ORF of the MYB Os07g12130 fused with vector PJC034 to construct MYB overexpression vector under the control of maize ubiquitin promoter.

Fig. (5). The relative expression of osMYB98 gene among selected ten transgenic lines comparing with the wild type. Real time PCR data were standardized to the ubiquitin actinreference gene by using $2^{-\Delta\Delta CT}$ methods. Values are the means and the error bar indicates ±SD over three biological replicates. The expression analysis showed that MYB-3, MYB-8 and MYB-6 transgenic lines were the highest expression lines.

4. Over-expression of *OsMYB98* Increases Drought Tolerance

Drought is a complex environmental stress triggered by mixtures of climatic fluctuations that influence agricultural traits and results in a decrease in crop yields (Kim et al., 2020). Drought harshness is of gigantic anxiety because it has immense leverage worldwide. The Mediterranean basin countries suffer from the increasing severity of drought due to the sharp climate change, affecting crop growth, development, crop yields, and consequently, food security (Masih et al., 2014; Wang et al., 2017 and Naumann et al., 2018). The improvement of drought-resistant crops is of key significance strategy to avoid the loss of yield in crops from drought stress. Roots are the main organ for absorbing nutrients and water in plants and are the first organs affected by drought stress (Kim et al., 2020). Hence, several transcription factors were differentially expressed in a drought tolerant in several plant species (Ergen et al., 2009; Joo et al., 2013; Joshi et al., 2016). MYB family is among these family genes that govern the response of plants against drought (Dubos et al., 2010 and Baldoni et al., 2015). Here, a total of twenty-four over-expression rice OsMYB98 regulated by maize ubiquitin promoter were generated. Based on the transcriptional level results we select 10 OsMYB98 lines for expression pattern analysis. The OsMYB98-3, OsMYB98-6, and OsMYB98-8 were the highest expression lines and were used to further verified the expression level of OsMYB98 transgenic lines in roots under drought stress using PEG 6000 treatments compared with the wild type (Fig. 6). The results reveal that the three transgenic lines show a wide range of regulations in roots in drought pressure compared with the wild type during the six-time points. OsMYB98-3, OsMYB98-6, and OsMYB98-8 are extremely induced in roots by drought stress at 24-hour point time, whereas the OsMYB89-8 are extensively indued at 24 hours. The relative expression of the OsMYB98 gene in the three transgenic lines compared with the wild type revealed that the inducing of the OsMYB98 gene by drought in the roots was rapidly increased and consequently elevated during the tested time point and reached the highest at 24 hours. These data recommend that OsMYB98 is a drought-responsive gene, and when over-expressed in rice, it develops the resistance of transgenic rice to drought. These results coincide with (Tang et al., 2019). Expanding evidence has proved that over-expression of several MYB genes might improve the resistance of transgenic plants to drought (Wei et al., 2017 and Tang et al., 2019). Similarly, the results demonstrated that OsMYB98 was induced and activated by drought (Fig. 6), suggesting OsMYB98 may have a key influence on drought tolerance. It is concluded that over-expression of OsMYB98 in rice increased tolerance to drought. These results agreed with previous report for several drought responsive MYB genes in plants (Baldoni et al., 2015). Recently, wheat R2R3 MYB-Type (a TaODORANT1), overexpressed in tobacco and enhance the tolerance to drought (Wei et al., 2017). PbrMYB21 was stated to have an affirmative role

in drought tolerance in *Pyrus betulaefolia* (Li et al., 2017). The results also agreed with AtMYB44, TaMYB3R1, and AtMYB60, which have been showed tolerance to drought (Jung et al., 2008). In summary, these results conclude that OsMYB98 plays a regulatory role for developing the tolerance of rice to drought.
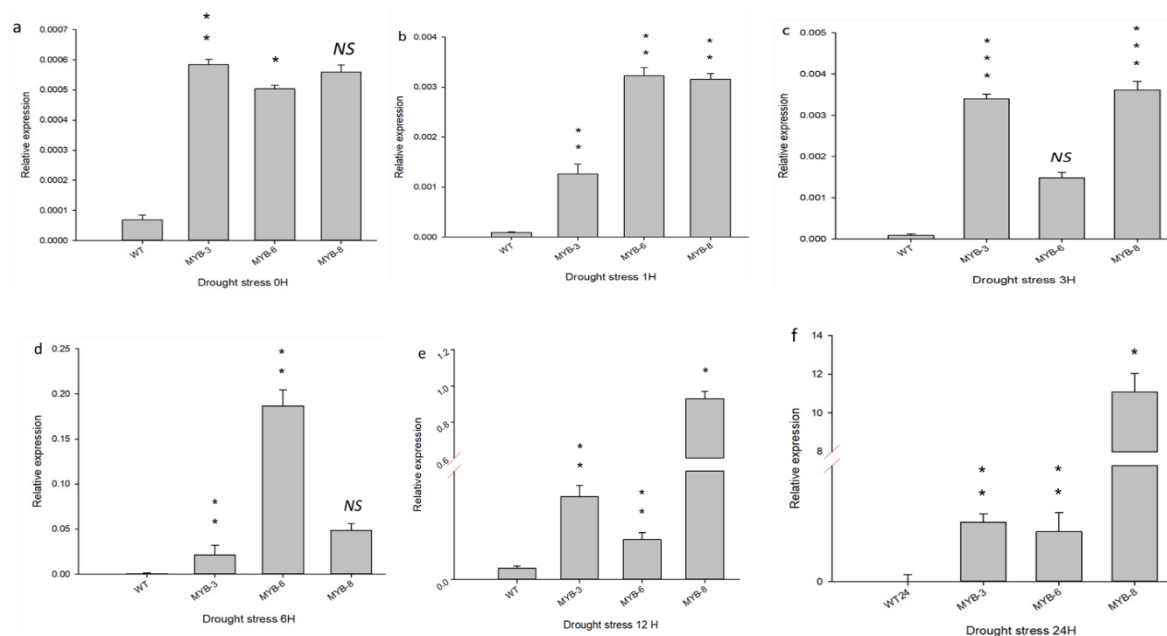

Fig. (6). The Relative expression of MYB98-3, MYB98-6, and MYB98-8 transgenic lines compared to the wild type under drought stress at different time point. **a.** the relative expression at 0 hours (0H), **b.** the relative expression at 1 hours (1H), **c.** the relative expression at 3 hours (3H), **d.** the relative expression at 6 hours (6H), **e.** the relative expression at 12 hours (12H), **f.** the relative expression at 24 hours (24H). The data are presented as mean ± SD, n = 3. $P < 0.05$, $**P < 0.01$, $***P < 0.001$, Student's t tests.

5. Physiological Measurements

The physiological indicator, water loss, MDA, and proline content is the main parametres that indicate the plant respond drought stress (Ben Rejeb et al., 2012; Wei et al., 2017; James et al., 2018; Zhao et al., 2018 and Tang et al., 2019). Water loss rates are mainly used extensively to reveal drought resistance in plants. The affirmative regulations of OsMYB98 under drought stress were confirmed by water loss assay through air drying of 14-day old seedlings for the three OsMYB98 lines and wild type. Fresh weight of OsMYB98 seedlings displayed lower rates of water loss at each time point

rather than wild type seedlings (Fig. 7). The MYB transcription factor TaMYB31 reduces the water loss and enhances drought tolerance in wheat (Zhao et al., 2018). At the same directon, The functional characterization of wheat R2R3-MYB, TaMYB30-B in *Arabidopsis* results in water loss and enhances the drought tolerance in the transgenic lines (Zhang et al., 2012).
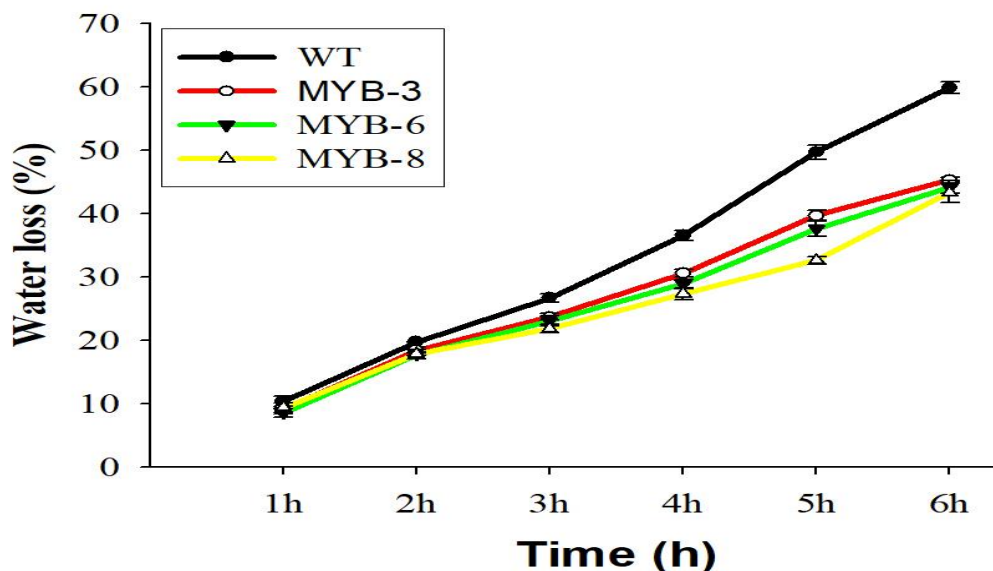
Fig. (7). Water loss rate of air-dried 14 day-old of MYB98-3, MYB98-6, and MYB98-8 transgenic and wild type seedlings. The seedlings were collected in three biological replicates.

The studies reported that proline is a key indicatore for drought tolerance (Ben Rejeb et al., 2012 and Tang et al., 2019). The content of proline in the leaves of OsMYB98 lines and wild type under drought stress and normal growth conditions is verified. The results showed that no difference in the proline content between the OsMYB98 lines and the wild type was observed under normal growth. Nevertheless, compared with the wild type, OsMYB98 plants revealed higher proline content after drought stress (Fig. 8), indicating that proline is a key evidence for the strong tolerance to drought as displayed by OsMYB98 transgenic lines. These results are in line with Tang et al. (2019) studies, which showed that OsMYB6 displays higher proline content than the wild type.
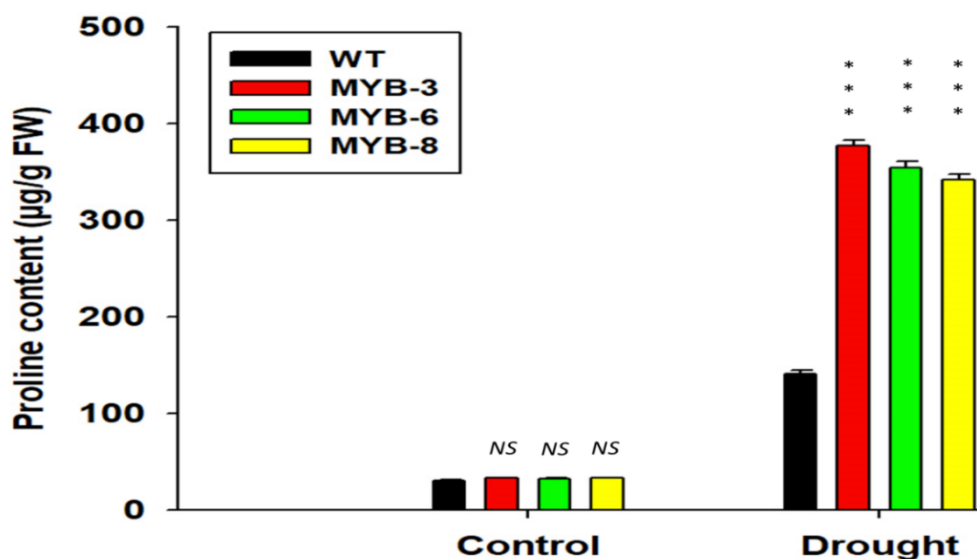
Fig. (8). Proline content of MYB98-3, MYB98-6, and MYB98-8 transgenic lines compared with the wild type under drought stress. The samples were collected in three biological and technical replicates for each line. The data are presented as mean ± SD, n = 3. $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's t tests.

Previous studies have indicated that plants exposed to drought regularly suggested reduction in MDA accumulation (Tang et al., 2019), where, MDA is closely linked with the damage of cell membrane under drought (James et al., 2018). Therefore, the MDA accumulation in OsMYB98 and wild-type plants were further verified. The data displays that no significant differences were recorded under normal conditions, while the OsMYB98 plants showed significantly reduction of MDA content compared with the wild-type plants under drought conditions (Fig. 9). This decrease of MDA content indicate that OsMYB98 can mitigate cell membrane damage by drought stress. Raised proof have confirmed that overexpression of several MYB genes are induced and improved the tolerance of transgenic plants to drought stress (Yang et al., 2012 and Wei et al., 2017). Similarly, these results exhibited that overexpression of OsMYB98 in rice improved transgenic plants tolerance to drought stress (Fig. 6), suggesting OsMYB98 may play key roles in drought. Drought stress has been reported to cause lipid peroxidation, leading to MDA accumulation (James et al., 2018). Data showed that the MDA content was higher in wild-type plants

than OsMYB98 transgenic plants (Fig. 8), which indicate that drought have more damage to wild-type plants compared with transgenic plants.
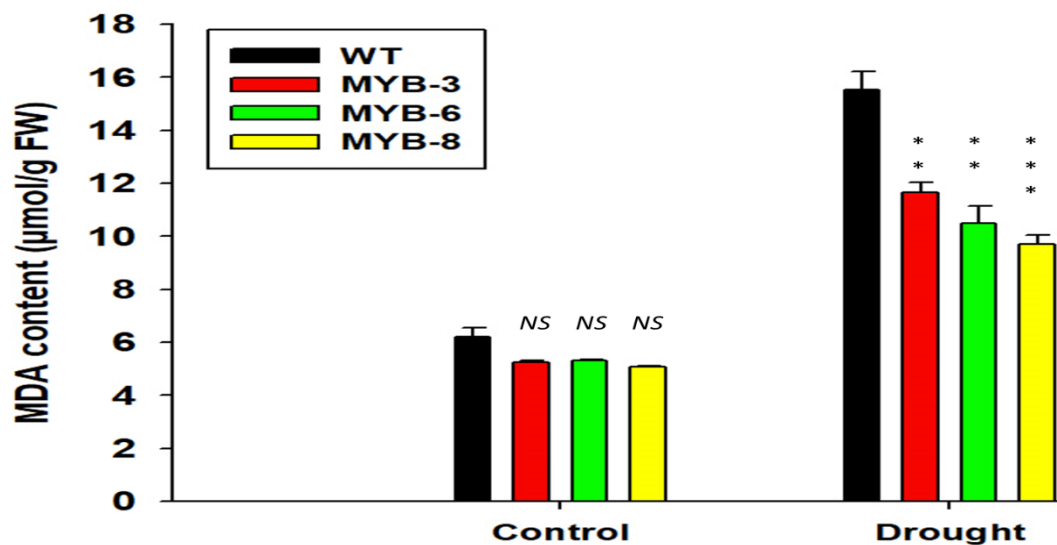

Fig. (9). MDA content of MYB98-3, MYB98-6, and MYB98-8 transgenic lines compared with the wild type under drought stress. The samples were collected in three biological and technical replicates for each line. The data are presented as mean $\pm$ SD, n $=$ 3. $P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Student's t tests.

CONCLUSION

Rice OsMYB98, which was predicted to enhance under drought stress, had amplified from the rice nipponbare variety and functionally characterized and over-expressed in rice Zhonghua11 (ZH11). Over-expression of the OsMYB98 gene resulted in enhancing rice resistance to drought. Here, our comprehension of the function of OsMYB98 in the regulation of drought stress response was enlarged and upgraded, and a nominee gene for the drought-tolerant in rice varieties and other crops was offerd.

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التعريف الوظيفي لجين ال MYB في الأرز

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على الرغم من أن MYB هو عائلة جينات هامة لعوامل النسخ لتطوير النبات والدفاع ضد إجهاد الجفاف، إلا أن فهم مشاركة جينات MYB في نمو الأرز والتكيف مع إجهاد الجفاف لا يزال غير معروف إلى حد كبير. أظهر التحليل الوظيفي لـ MYB98 أن OsMYB98 أنه يتم التعبير عنه بشكل كبير أثناء تطوير البذور ونضجها، ويزداد تعبيره تحت ضغط الجفاف. هنا، تم عزل ال OsMYB98 من صنف الأرز Nipponbare وتم التعبير عنه وظيفيًا بشكل مفرط في صنف (ZH11) Zhonghua11 كنوع بري للتحقق من دور OsMYB98 في ضغوط الجفاف. بناءً على نتائج مستوى النسخ وتحليل التعبير النسبي، تم اختيار ثلاثة خطوط مستقلة لمزيد من التحليل. تعرضت شتلات من OsMYB98 والنوع البري في عمر أسبوعين لـ ٢٠٪ من PEG6000 لإجراء إجهاد الجفاف. أوضحت نتائجنا أن OsMYB98 تم التعبير عنه بشكل تفاضلي وتنظيمي أثناء ضغط الجفاف في جذور OsMYB98-3 وOsMYB98-6 وOsMYB98-8 مقارنة بالنوع البري. يؤدي الإفراط في التعبير عن جين OsMYB98 في الأرز إلى زيادة مقاومة جذور الأرز لضغط الجفاف، حيث تمت زيادة التعبير عن OsMYB98 بسرعة في الجذور وبالتالي رفعه خلال النقطة الزمنية المختبرة ووصل إلى أعلى مستوى في ٢٤ ساعة. زيادة التعبير لـ OsMYB98 أدى إلى تعزيز مقاومة إجهاد الجفاف، ونتج عنه انخفاض محتوى MDA، وانخفاض فقدان المياه، ومحتوى البرولين العالي في نباتات OsMYB98 تحت الجفاف معززًا أن OsMYB98 هو جين مستجيب للإجهاد وقد يكون أساسيًا في المستقبل لتطوير مقاومة الأرز والمحاصيل الأخرى للجفاف.