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RESEARCH PAPER

Membrane proteome profiling of *Mentha arvensis* leaves in response to *Alternaria alternata* infection identifies crucial candidates for defense response

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ABSTRACT

The leaf spot disease of *Mentha arvensis*, caused by *Alternaria alternata*, is a devastating foliar disease worldwide and leads to considerable economic losses. In this investigation, 2-dimensional gel electrophoresis (2-DE) was used to identify the membrane proteins potentially involved in *M. arvensis - A. alternata* interaction. Membrane proteins, isolated from leaves of control and infected plants, were analyzed by 2-DE and identified using mass spectrometry (MALDI TOF–TOF MS/MS). Our analysis identified 21 differentially expressed membrane proteins including several interesting receptors and channel proteins. Of these identified proteins, 34% were found to be involved in plant defense responses. Leucine-rich repeat family protein/ protein kinase family protein which plays critical role in stress response and nucleotide-binding site–leucine-rich repeat (NBS-LRR) which is involved in detecting the advent of pathogen on plant surface were identified to be up-regulated in our study. Interestingly, AKT1-like potassium channel protein which is known to play a crucial role in maintaining ion homeostasis within the cell was also upregulated in the infected sample. In addition, ADP ribolysation factor (ARF)-GTPase activating domain containing protein, a membrane trafficking protein, was also up-regulated in the current study. Protein-protein interaction network analysis followed by functional enrichment revealed that transmembrane ion transport-related proteins represented a major class in this network followed by nucleic acid binding proteins and proteins with kinase activities respectively. Together, our investigation identified several key defense-related proteins which are crucial sensors for detecting pathogen invasion and can serve as a potential resource to understand disease resistance mechanism in mint.

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Alternaria alternata; infection; *mentha arvensis*; membrane proteomics; 2-dimensional gel electrophoresis

Abbreviations: 2-DE, 2 dimensional gel electrophoresis; Arf, ADP-ribosylation factor; ATP, adenosine triphosphate; BSI, Botanical Survey of India; CAT, catalase; Dpi, days post infection; DTT, dithiothreitol; EDTA, ethylene di amino tetra acetic acid; ERF1, ethylene response factor 1; EST, expressed sequence tag; GTP, guanosine triphosphate; HP, hydropathy; Hsfs, heat stress transcription factors; HSP70, heat shock protein 70; IEF, isoelectric focusing; IPG, immobilized pH gradient; LRR-RLKs, Leucine-rich repeat receptor-like kinases; MALDI TOF-TOF MS/MS, matrix assisted laser desorption ionization-time of flight-tandem mass spectrometry; NBS-LRR, nucleotide-binding site–leucine-rich repeat; PCA, principal component analysis; qRT-PCR, quantitative real time-polymerase chain reaction; RLK, receptor-like kinase; TMH, transmembrane helix; TMHMM, transmembrane hidden markov model.

Introduction

Plant-pathogen interaction is a multifaceted process.[1] The plants' response to pathogen attack has long been an area of interest to plant biologists and phytopathologists. Recently, functional genomic strategies, including transcriptomics and proteomics, during pathogen infections have generated enormous datasets.[2] Proteomics provide a direct evidence for identifying target proteins involved in defense responses. Deciphering the interaction of pathogens with their respective hosts on genomic, proteomic, and metabolomic levels is indispensable for designing novel strategies to combat disease. Several model microbial-host pathosystems are being studied in depth but some of the major disease systems remain poorly understood. The genus *Mentha*, also known as 'mint', belongs to family Lamiaceae and has significant impact on the global as well as Indian economy. *Mentha* is known to produce essential oils containing various monoterpenoids, including 3-oxygenated monoterpenes (e.g. menthol), acyclic monoterpenes (e.g., linalool) and 2-oxygenated monoterpenes (e.g. carvone).[3] These constituents are being used in many pharmaceutical industries, cosmetics, and confectionaries. Currently, India is the major global producer of mint oil and its derivatives in the world. However, mint cultivation is threatened by fungal infection which adversely affects both yield and overall quality of mint oil leading to severe economic losses. Fungal pathogens causing most serious loss includes *Alternaria alternata*, *Puccinia menthae*, *Verticillium daliae*, *V. albo-atrum*, *Phoma strasseri*, *Erysiphae cichoracearum* and *Rhizoctonia solani*.[4,5] *A. alternata* causes leaf spot disease in wild mint, *Mentha arvensis* which leads to significant economic losses, by inflicting heavy defoliation of the host.[6] It is reported that *A. alternata* infection reduces the essential oil yield by 55 to 75%, and is sometimes associated with a dual infection with *P. menthae*.[4] The disease is most predominant in tropical countries such as India

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Review Article

Glutathione as a Crucial Modulator of Phytohormone Signalling During Pathogen Defence in Plants

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Plant's resistance to different stress factors is regulated by a complex signalling network which connects the individual signalling pathways, enabling them to fine tune their defence response. For more than last two decades, glutathione (GSH) is gradually gaining importance as a crucial player in this network. The present review summarizes the central role of GSH in modulating plant's defence response to biotic stress, specially emphasizing the molecular mechanism of these regulations. Several transgenic approaches to constitutively enhance GSH levels have been followed and in most cases, these transgenic plants exhibited enhanced biotic stress tolerance. The post 2000 era envisaged a mechanistic approach in this field and GSH has been shown to modulate the defence signalling network by cross-communication with several stress-related phytohormones. GSH imparts stress tolerance against biotrophic infection via NPR1-dependent salicylic acid (SA) mediated pathway. GSH regulates SA accumulation at the level of *isochorismate synthetase* 1 (*ICS*1) expression and can also act in NPR1-independent pathway. A synergistic GSH-ethylene (ET) interplay during necrotrophic infection has also been reported. It has been demonstrated that GSH induces ET biosynthesis by modulating transcriptional and post-transcriptional regulations of its key enzymes. The cross-talk of GSH with jasmonic acid (JA) and abscisic acid (ABA) in alleviating stress has been reported as well. However, mechanistic details of the interaction between GSH and JA or ABA signaling pathways are not elucidated in details.

Keywords: Glutathione; Phytohormone Signalling; Pathogen Defence; Salicylic Acid; Ethylene; Jasmonic Acid

Introduction

Plants in their natural environment are continuously being threatened by a range of stress factors, including invasion by microbial pathogens, herbivorous insects as well as various abiotic stress conditions. Being immobile, plants have to respond to each of these attackers in a rapid and effective way in order to ensure survival. Plant's resistance to different stress factors is a multifaceted regulatory network which links the various signalling pathways thus enabling them to fine tune their defence responses. Previous studies also envisaged that plant's responses to various stress factors are regulated by multiple signalling pathways. A perfect synchronization of these pathways switches on the transcription of appropriate defence related genes and their downstream machinery ultimately helping the system to tide over unfavourable conditions. It has been well-documented that an interconnecting signalling network, comprising the salicylic acid (SA), ethylene (ET) and jasmonic acid (JA) mediated signalling pathways, constitute the basic defence response strategy in plants (Glazebrook, 2005; Klessig *et al.*, 2000; Loake and Grant, 2007; Pieterse *et al.*, 2009; Thomma *et al.*, 1998; van Loon *et al.*, 2006). Glutathione (GSH; γ-glutamylcysteinyl glycine) is a low molecular weight non-protein tripeptide which is found in nearly all prokaryotic as well as eukaryotic cells. GSH represents the major pool of non-protein reduced

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Review

Long non-coding RNAs: Fine-tuning the developmental responses in plants

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Plant developmental biology is associated with various gene regulatory pathways involved in different phases of their life cycle. In course of development, growth and differentiation of different organs in plants are regulated by specific sets of gene expression. With the advances in genomic and bioinformatic techniques, particularly high-throughput sequencing technology, many transcriptional units with no protein-coding potential have been discovered. Previously thought to be the dark matters of genome, long non-coding RNAs (lncRNAs) are gradually gaining importance as crucial players in gene regulation during different developmental phases. Some lncRNAs, showing complementarity to microRNAs (miRNAs), are used as endogenous target mimics of specific miRNA family. A number of lncRNAs can also act as natural antisense transcripts to attenuate the expression of coding genes. Although lncRNA-mediated regulations have extensively been studied in animals, plant lncRNA research is still in its initial phase. The present review highlights the regulatory mechanism and different physiological aspects of lncRNAs in plant development. In plants, lncRNAs are found to be associated with a number of plant developmental functions such as lateral root development, vernalization, photomorphogenesis, pollen development, fiber development and nodulation. Understanding these potent roles of lncRNAs in plant development can further provide novel tools for crop improvement programs in future.

Keywords. Fiber development; long non-coding RNAs; nodule development; photomorphogenesis; pollen development; root development

Abbreviations: ABA, abscisic acid; APOLO, auxin-regulated promoter loop; ARF, auxin response factor; ASCO-RNA, alternative splicing competitor long non-coding RNA; CBP, cap-binding protein; eTM, endogenous target mimic; FLC, FLOWERING LOCUS C; FT, FLOWERING LOCUS T; HID1, hidden treasure 1; LDMAR, long day (LD)-specific male-fertility associated RNA; lincRNA, long intergenic non-coding RNA; lncRNA, long non-coding RNA; miRNA, microRNAs; NAT, natural antisense transcript; ncRNA, non-coding RNA; NMD, nonsense-mediated-mRNA decay; NSR, nuclear speckle RNA-binding protein; PID, PINOID; PIF, phytochrome-interacting factor; PRC1, polycomb recessive complex 1; RBP1, RNA binding protein 1; RdDM, RNA-dependent DNA methylation; siRNA, small interfering RNA; sncRNA, small non-coding RNA; snRNA, small nuclear RNA; UBP1, ubiquitin specific protease 1; UPF, UP-frameshift protein

1. Introduction

The growth and development of plants under particular environmental conditions are delicately regulated by various signaling molecules. These signaling molecules are of two types: external and internal. The external signaling molecules induce a change in the cellular gene expression leading to the development of internal signaling molecules. The internal signaling molecules can then act as an inducer for the second level of gene expression which promotes downstream signaling cascade and initiates a particular developmental phenomenon. The role of sugars, proteins and lipid molecules as signal transducers in numerous signaling pathways has been well deciphered. Nowadays, emerging evidence suggests the role of non-coding RNAs (ncRNAs) as important internal signaling modulators (Morange 2008; Axtell 2013; Dong *et al.* 2016). The ncRNAs can be classified into two groups. One group comprises small non-coding RNAs (sncRNAs) and the other includes long non-coding RNAs (lncRNAs). The sncRNAs are

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RESEARCH PAPER

Rice lectin protein r40c1 imparts drought tolerance by modulating S-adenosylmethionine synthase 2, stress-associated protein 8 and chromatin-associated proteins

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Abstract

Lectin proteins play an important role in biotic and abiotic stress responses in plants. Although the rice lectin protein Osr40c1 has been reported to be regulated by drought stress, the mechanism of its drought tolerance activity has not been studied so far. In this study, it is shown that expression of the *Osr40c1* gene correlates with the drought tolerance potential of various rice cultivars. Transgenic rice plants overexpressing *Osr40c1* were significantly more tolerant to drought stress than the wild-type plants. Furthermore, ectopic expression of the *Osr40c1* gene in tobacco yielded a similar result. Interestingly, the protein displayed a nucleo-cytoplasmic localization and was found to interact with a number of drought-responsive proteins such as S-adenosylmethionine synthase 2 (OsSAM2), stress-associated protein 8 (OsSAP8), DNA-binding protein MNB1B (OsMNB1B), and histone 4 (OsH4). Silencing of each of these protein partners led to drought sensitivity in otherwise tolerant Osr40c1-expressing transgenic tobacco lines indicating that these partners were crucial for the Osr40c1-mediated drought tolerance *in planta*. Moreover, the association of Osr40c1 with these partners occurred specifically under drought stress forming a multi-protein complex. Together, our findings delineate a novel role of Osr40c1 in imparting drought tolerance by regulating OsMNB1B, OsSAM2, and OsH4 proteins, which presumably enables OsSAP8 to induce downstream gene expression.

Keywords: Drought, lectin, histone 4, OsMNB1B, Osr40c1, S-adenosylmethionine synthase 2, stress-associated protein 8.

Introduction

Rice is a staple food crop in most of the countries of Southeast Asia (Cassman *et al.*, 2003; Seck *et al.*, 2012). Generally, rice is cultivated in irrigated land and requires a higher amount of water compared with other crops (Mohanty *et al.*, 2013). Rice cultivation is hampered when the plants are exposed to a period of water deficiency due to an insufficient water supply or uncertainty of rainfall. In addition, plants are always exposed to a plethora of environmental challenges. Among them, drought stress has serious impacts on several physiological functions of plants. It inhibits growth and hampers seed development (Atkinson and Urwin, 2012; You *et al.*, 2014). Therefore, the production of large biomass or high grain yield under water deficit or drought stress conditions has always been a major challenge. During drought, plants regulate several metabolic pathways involved with enzyme activity, alteration in different metabolite levels, and

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Jacalin domain-containing protein *Os*SalT interacts with *Os*DREB2A and *Os*NAC1 to impart drought stress tolerance *in planta*

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ABSTRACT

With the changing climatic conditions, drought has become one of the most threatening abiotic stress factors that adversely affect rice cultivation and productivity. Although the involvement of the jacalin domain-containing protein, *Os*SalT, has been reported in drought and salinity tolerance, its functional mechanism still remains largely unexplored. In this study, expression of the *OsSalT* gene was found to be positively correlated with the drought tolerance potential with its higher transcript abundance in the tolerant *indica* rice cultivar, Vandana, and lower abundance in the susceptible cultivar, MTU1010. Moreover, the overexpression of *OsSalT* gene in rice and its ectopic expression in tobacco significantly improved drought stress tolerance in the transgenic lines. The transgenic lines exhibited significantly improved growth and higher osmolyte accumulation over the wild-type (WT) plants under drought stress. Fascinatingly, the yeast two-hybrid and bimolecular fluorescence complementation (BiFC) analyses confirmed the interaction of *Os*SalT protein with two interesting transcription factors (TFs), *Os*NAC1 and OsDREB2A. *In silico* analysis followed by yeast two-hybrid assay further revealed that the *Os*SalT protein interacted with the C-terminal domains of both *Os*DREB2A and *Os*NAC1 leading to their activation. This triggered the induction of their downstream drought-responsive genes. Together, this study unravelled a novel model for *Os*SalT-mediated regulation of drought tolerance in plants.

1. Introduction

Drought is among the most important abiotic stress factors that affect crop productivity resulting in significant yield losses (Hu and Xiong, 2014). The changing climatic conditions like irregularity and insufficiency of rainfall adversely affect plant growth, development and productivity. Plants have evolved a highly sophisticated adaptive mechanism to combat drought stress. In-depth analysis of drought tolerance mechanism suggests an intricate crosstalk between different signalling cascades in plants (Datta et al., 2020). The drought stress response in plants is regulated by two distinct signalling pathways - the abscisic acid (ABA)-dependent and ABA-independent pathways. Several important transcription factors (TFs) such as myelocytomatosis oncogene (MYC), myeloblastosis oncogene (MYB), basic leucine zipper (bZIP), NAM, ATAF, and CUC (NAC) and dehydration responsive element binding (DREB) function in both the pathways and are highly accumulated in response to drought stress. These TFs are known to modulate downstream osmotic stress-responsive genes to render stress tolerance (Nakashima et al., 2009; Atkinson and Urwin, 2012).

Lectins are a family of carbohydrate-binding proteins that specifically recognize sugar moieties (Van Holle and Van Damme, 2018). Lectins are known to play diverse roles in plant defence, in addition to its involvement in various other physiological phenomena. For example, lectin proteins were found to be induced in response to heat shock in cell suspension cultures (Spadoro Tank and Etzler, 1988; Shahbazova et al., 1996). Another ricin-B containing lectin protein, *Os*r40c1, was reported to impart drought stress tolerance in rice (Sahid et al., 2020). Again, transgenic Thai rice cultivars ectopically expressing snowdrop lectin exhibited resistance to sap-sucking insects (Huesing et al., 2004). ASAL, a novel lectin from garlic, provided resistance against phloem-limited viruses in rice (Saha et al., 2006). In addition, a large number of pattern recognition receptors in plants were reported to contain lectin domains that were essential for pathogen recognition and defence (Kaku et al., 2006; Bellande et al., 2017).

Plant lectins usually constitute a heterogeneous group that has been classified into 7 subfamilies based on their structure and evolutionary

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RESEARCH PAPER

OPEN ACCESS

Glutathione imparts stress tolerance against *Alternaria brassicicola* infection via miRNA mediated gene regulation

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ABSTRACT

Glutathione (GSH) is well known to play a crucial role in imparting resistance against various pathogen invasions. Nevertheless, the role of GSH in regulating miRNA-mediated defense response is yet to be explored. To decipher the GSH-mediated regulation of miRNA expression during necrotrophic infection in *Arabidopsis thaliana*, wild-type Col-0 and *AtECS1*, the transgenic line exhibiting enhanced GSH content, were infected with necrotrophic pathogen *Alternaria brassicicola*. *AtECS1* plants exhibited enhanced resistance as compared to wild-type. MiRNA next-generation sequencing (NGS) was performed to compare the miRNA expression in Col-0 and *AtECS*1 leaves. Under control condition, differentially expressed 96 known miRNAs and 17 novel miRNAs viz. ath-miR8167f, ath-miR1886.3, ath-miR3932b-5p, etc. were identified. However, under infected condition, 73 known and 43 novel differentially expressed miRNAs viz. ath-miR5652, ath-miR160b, ath-miR865-5p, etc. were identified. Functional annotation and enrichment analysis revealed that several miRNAs that target defense-related genes like leucine-rich repeat protein kinase, MYB transcription factors, TCP8, etc. were down regulated in the *AtECS1* line, which, in turn, relieves the repression of their target gene expression, leading to resistance against infection. Together, the present investigation suggests that GSH plays a decisive role in modulating the miRNA-mediated regulation of defense-related genes during pathogen invasion.

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Alternaria brassicicola; *arabidopsis*; glutathione; miRNA; plant defense

Introduction

In plant defense signaling network, glutathione (GSH) is gaining massive importance in disease resistance by playing a central role in regulating the defense signaling network in plants. In the earlier studies, it was shown that over-expression of GSH biosynthesis genes and increased GSH level can induce the expression of different disease-responsive genes, thus imparting stress tolerance.[1–3] Earlier, it was shown that GSH-deficient mutant *pad2.1* was susceptible toward pathogen attack.[3] Exogenous application of GSH can also induce disease resistance in plants by activating NPR1-mediated SA signaling pathway, corroborating the regulatory role of GSH during biotic stress responses in plants.[1,4]

MicroRNAs (miRNAs) are a group of regulatory molecules with 21–24 nucleotides length and help in gene regulation by sequence-specific cleavage or translational repression of their target gene.[5] Several families of miRNAs have been identified from different plant species during pathogen attack, and their disease-responsive role has been investigated.[6–8] However, GSH-mediated regulation of any miRNAs during disease progression in plants is not studied so far. *Alternaria brassicicola* is a devastating necrotrophic fungal pathogen that causes dark spot disease in Brassicaceae family.[9] However, the role of GSH in regulating miRNA-mediated defense strategies in this pathosystem is still unexplored. In this study, our aim is to dissect the role of different miRNAs during *A. brassicola* infection in *Arabidopsis* and their regulation by GSH. Our study identified several stress-responsive miRNAs that are regulated by GSH during pathogen attack.

Materials and methods

Plant material and growth condition and pathogen inoculation

The transgenic *A. thaliana* line (*AtECS1*) overexpressing *Le-γECS* gene and exhibiting enhanced GSH content developed earlier[3] and Columbia-0 (Col-0) served as wild-type were used here. Plants were grown in Murashige and Skoog (MS) media and maintained in a growth chamber (22°C under 16 h light/8 h dark cycles).[10]

Spore suspension of *A. brassicicola* strain MTCC No. 2102, obtained from MTCC Chandigarh, India, was used to inoculate leaves following van Wees et al..[11] For mock treatment, 5 µL droplets of water was used. Infection was scores at 5 dpi.

Trypan blue and DAB staining assay

To determine disease severity in Col-0 and *AtECS1* plants, 4 weeks old Col-0 (CC), infected Col-0 (CI), *AtECS1*, and infected *AtECS*1 (AI) plants were used for trypan blue

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ORIGINAL ARTICLE

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Glutathione regulates transcriptional activation of iron transporters via S-nitrosylation of bHLH factors to modulate subcellular iron homoeostasis

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Abstract

Glutathione (GSH) is known to regulate iron (Fe) deficiency response in plants but its involvement in modulating subcellular Fe homoeostasis remains elusive. In this study, we report that the GSH-deficient mutants, *cad2-1* and *pad2-1* displayed increased sensitivity to Fe deficiency with significant downregulation of the vacuolar Fe exporters, *AtNRAMP3* and *AtNRAMP4*, and the chloroplast Fe importer, *AtPIC1*. Moreover, the *pad2-1* mutant accumulated higher Fe levels in vacuoles but lower Fe levels in chloroplasts compared to wild type (Columbia ecotype [Col-0]) under Fe limited conditions. Exogenous GSH treatment enhanced chloroplast Fe contents in Col-0 but failed to do so in the *nramp3nramp4* double mutants demonstrating that GSH plays a role in modulating subcellular Fe homoeostasis. Pharmacological experiments, mutant analysis, and promoter assays revealed that this regulation involves the transcriptional activation of Fe transporter genes by a GSH–*S*-nitrosoglutathione (GSNO) module. The Fe responsive bHLH transcription factors (TFs), AtbHLH29, AtbHLH38, and AtbHLH101 were found to interact with the promoters of these genes, which were, in turn, activated via *S*-nitrosylation (SNO). Taken together, the present study highlights the role of the GSH–GSNO module in regulating subcellular Fe homoeostasis by transcriptional activation of the Fe transporters *AtNRAMP3*, *AtNRAMP4*, and *AtPIC1* via SNO of bHLH TFs during Fe deficiency.

KEYWORDS

GSNO, Iron deficiency, NRAMP, PIC

1 | INTRODUCTION

Iron (Fe) is an essential micronutrient for plant growth and development. Because of its physicochemical properties, Fe plays a crucial role in regulating numerous cellular responses. This micronutrient coordinates metalloprotein active sites modulating the enzymatic reactions for different metabolic pathways (Briat et al., 2015). Although Fe is found abundantly in the earth's crust, it is usually present in an oxidized form, and its availability to plants is limited. Fe deficiency in plants causes chlorosis and perturbs the photosynthetic and mitochondrial electron transport systems. Therefore, Fe deficiency is a challenging issue for plant growth, development, and productivity.

Soumi Ghosh and Pinki Khan contributed equally to this study.

Soumitra Paul and Riddhi Datta are senior authors.

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ZFP37, C3H, NAC94, and bHLH148 transcription factors regulate cultivar-specific drought response by modulating *r40C1* gene expression in rice

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*Osr*40C1
Drought

ABSTRACT

Lectins are gradually gaining importance in regulating abiotic stress response in plants. Among them, the R40 family plays pivotal role in regulating drought response. The expression of the *Osr40C1* positively correlates with the degree of drought tolerance in *indica* rice cultivars. Although the mechanism of *Osr*40C1-mediated drought tolerance is well characterized, the gene's regulation under drought remains obscure. Here, we demonstrate that the *Osr40C1* promoter is activated under drought to induce its transcription. A number of single nucleotide variations in the *Osr40C1* promoter of the drought-tolerant IR36 and sensitive MTU1010 cultivar are detected. However, promoter swapping analysis reveals that these variations in the promoter sequence do not explain the cultivar-specific expression pattern of this gene. Next, we identified the transcription factors that interact with the *Osr40C1* promoter. Among these transcription factors, *Os*ZFP37, *Os*C3H, *Os*NAC94, and *Os*bHLH148 display significantly higher transcript abundance in IR36 compared with MTU1010. Furthermore, overexpression of each of these transcription factors confers drought tolerance, whereas their silencing leads to drought sensitivity. Interestingly, the *Osr40C1* expression is significantly up-regulated in the transcription factor overexpression lines, while its expression is undetectable in the silencing lines. Together our study highlights how different transcription factors regulate the cultivar-specific expression pattern of *Osr40C1* in rice.

1. Introduction

Drought is a severe threat to plant growth and development. It affects several physiological phenomena, including root development, leaf rolling, stomatal closure, leaf abscission, transpiration rate, water usage efficiency, formation of reactive oxygen species (ROS) and free radicals leading to disruption of cellular equilibrium in plants (Bartels and Sunkar, 2005). To combat drought stress, plants have evolved a plethora of adaptive strategies. Plants, for example, adapt and adjust to water scarcity by accumulating various osmolytes and proteins (Shinozaki and Yamaguchi-Shinozaki, 2007). In addition, plant growth regulators can modulate growth and development via their intricate signalling networks under drought stress (Agarwal et al., 2006; Datta et al., 2023). To withstand water scarcity, abscisic acid (ABA), a master regulator of the drought stress response, modulates stomatal closure and ROS signalling and activates a battery of drought-responsive genes. In response to drought stress, ABA can regulate different transcription factors (TFs) like basic leucine zipper (bZIP), myelocytomatosis oncogenes (MYC, MYB), and zinc finger protein (ZFP) family to induce the downstream drought-signalling genes (Datta et al., 2023). However, other osmotic stress-responsive TFs like NAM ATAF and CUC (NAC) and dehydration-responsive element binding (DREB) factors are activated by ABA-independent signalling pathways and play a key role in regulating drought stress.

Among the different families of TFs, ZFPs play a pivotal role in regulating diverse physiological phenomena, including abiotic and

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