Characteristics of the Aspergillus oryzae GATA Transcription Factor Family and Expression Analysis under Temperature or Salt Stresses

Chunmiao Jiang
Jiangxi Science and Technology Normal University
Gongbo Lv
Jiangxi Science and Technology Normal University
Bin He
Jiangxi Science and Technology Normal University
Zhe Zhang
Jiangxi Science and Technology Normal University
Zhihong Hu
Jiangxi Science and Technology Normal University
Bin Zeng (Zengtx001@aliyun.com)
Jiangxi Science and Technology Normal University https://orcid.org/0000-0002-6151-8343

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Abstract

GATA transcription factors（TFs）are involved in the regulation of diverse growth processes and various environmental stimuli stresses. Although the analysis of GATA TFs involved in abiotic stress has been performed in plants and some fungi, information regarding GATA TFs in *A. oryzae* is extremely poor. Therefore, we identified seven GATA TFs from *A. oryzae 3.042* genome and classified into six subgroups in NJ_tree, including a novel *AoSnf5* with 20-residue between the Cys-X_2-Cys motifs which was found in *Aspergillus* for the first time. Conserved motifs demonstrated that *Aspergillus* GATA TFs with similar motif compositions clustered into one subgroup, which suggests they might have similar genetic functions and further confirms the accuracy of the phylogenetic relationship. Moreover, the expression patterns of seven *A. oryzae* GATA TFs under temperature and salt stresses indicated that *A. oryzae* GATA TFs were mainly responsive to high-temperature and high salt stress. The PPI network of *A. oryzae* GATA TFs proposed some potentially interacting proteins. The comprehensive analysis of *A. oryzae* GATA TFs will be beneficial to understand their functional and evolutionary features and provide useful information for the further analyzing the role of GATA TFs in regulation of distinct environmental conditions in *A. oryzae*.

1. Introduction

GATA transcription factors (TFs) constitute a protein family that is characterized by the presence of one or two highly conserved type-IV zinc fingers and a DNA-binding domain that recognizes the (A/C/T)-G-A-T-A- (A/G) sequence in the promoter sequence of target genes (Scazzocchio 2000). In fungi, diverse roles governed by GATA TFs mainly involved in nitrogen regulation and light responses, regulation of sexual and/or asexual reproduction, and secondary metabolism. GATA TFs *AreB* and *AreA* are not only involved in the nitrogen and carbon metabolism, but also in the control of several complex cellular processes such as transport and secondary metabolism (SM) (Pfannmüller et al. 2017; Chudzicka-Ormaniec et al. 2019). The *SreA* involves in regulation of siderophore biosynthesis and iron uptake (Oberegger et al. 2010; Schrettl et al. 2008), and *NsdD* regulates sexual and/or asexual reproduction and the production of SMs (Lee et al. 2014; 2016; Niehaus et al. 2017). Furthermore, few fungal GATA TFs also play important role in response to the abiotic stresses. *Alternaria alternata SreA* is related with the maintenance of cell wall integrity (Chung et al. 2020), while *Blastomyces dermatitidis SreB* strongly expresses and contributes to filamentous growth at 22 ℃ via lipid metabolism (Marty et al. 2015). Additionally, *GLN3* and *GAT1* have been shown to be involved in salt tolerance in *Saccharomyces cerevisiae* (Crespo et al. 2001). However, there are still very few reports regarding the function of filamentous fungal GATA TFs in response to abiotic stress factors.

Aspergillus oryzae is an important filamentous fungus, which is widely used in East Asian traditional fermented food products (Kitamoto 2015). Simultaneously, *A. oryzae* is exposed to various environmental stress factors during fermentation process. Temperature is the most important environmental factor affecting the growth and activity of microorganisms and can directly affect the activity of enzymes involved in substrate digestion during fermentation process (Chen et al. 2011; Bechman et al. 2012). In

addition, high sodium chloride concentration, which inhibits the growth of spoilage bacteria in soy sauce mash, also affects the growth of *A. oryzae* (Wang et al. 2013; Fernandes et al. 2018). Therefore, the ability of *A. oryzae* to adapt to different temperatures and high salt concentration have attracted the attention of researchers, but the molecular mechanisms underlying their response to these stress factors are still unclear. The previous studies have demonstrated that GATA TFs mainly involved in regulation of various temperature and salt stimuli stress signaling in few fungi (Scazzocchio 2000; Crespo 2001; Marty et al. 2015). Although the Fungal Transcription Factor Database (FTFD) and Tetsuo Kobayashi et al publicized six *A. oryzae* GATA TFs which may involve in nitrogen regulation and light responses, regulation of sexual and/or asexual reproduction, and SM Kobayashi et al. 2007), there is lack of research on a comprehensive analysis of *A. oryzae* 3.042 GATA TF. Therefore, the aim of this study was to analyze *A. oryzae* GATA TF structural characteristics, evolutionary features, and conserved motifs, and the expression patterns of GATA TFs under temperature and salt stresses. Furthermore, the expression patterns and the results of PPI prediction can establish a good foundation for further study on the function and the mechanism of *A. oryzae* GATA TFs involved in abiotic stress responses.

2. Materials And Methods

2.1 Identification of *A. oryzae GATA* transcription factors

The *Aspergillus oryzae 3.042* genome was downloaded from NCBI database (https://www.ncbi.nlm.nih.gov/genome/?term=Aspergillus+oryzae). The BLASTP program with a threshold e-value of 1e-10 was used to predict GATA TFs from the *A. oryzae* genome, using gene sequences from *Aspergillus* as query sequences. All potential *A. oryzae* GATA TF proteins were identified by HMMER3.1 and were predicted if they contained ZnF-GATA domains (PF00320). The sequences that resulted in GATA-type zinc finger genes hits with the GATA zinc-finger domains (PF00320) were considered as GATA TFs. CDD and PFAM databases were used to validate all the potential *A. oryzae* GATA TFs.

To determine the chromosomal locations of the seven identified *A. oryzae* GATA TFs, locus coordinates were downloaded from the *A. oryzae* RIB40 genomics database. The distribution of seven *A. oryzae* TFs on the chromosomes was drawn by MG2C (mg2c.iask.in/mg2c_v2.0/) and visualized using MapChart 2.2 (Voorrips 2002).

2.2 The multi sequences alignment and phylogenetic analysis

ClustalW was used to align *A. oryzae* GATA TF proteins. The protein sequences of known GATA TFs in all other *Aspergillus* were downloaded from fungal transcription factor databases (FTFD, http://ftfd.snu.ac.kr/index.php?a=view). The sequences of GATA TFs between *A. oryzae* and other *Aspergillus* species were also aligned using ClustalW to analyze the phylogenetic relationships of all

Aspergillus GATA TFs. A Neighbor-Joining (NJ) tree was constructed based on aligned results in MEGA6.0 with bootstrap replications of 1000.

2.3 Motif analysis of *A. oryzae* and other *Aspergillus* GATA transcription factors

MEME was used to predict and analyze motifs of *A. oryzae* GATA proteins, which were visualized using TBtools (Chen et al. 2018). The parameters were set to zero or one of a contributing motif site per sequence, and the numbers of motifs were chosen as five; motif widths were set to 6 and 50 (Wu et al. 2016). The other parameters were set to default values. Each motif was individually checked so that only motifs with an e-value of < 1e-10 were retained for motif detection in *A. oryzae* GATA proteins.

2.4 Effects of temperature and salinity treatment on the growth of A. oryzae

A. oryzae 3.042 (CICC 40092), the main fermentation strain used in industry, was selected to test the growth of *A. oryzae* under temperature and salt stress. *A. oryzae* conidia were inoculated in fresh potato dextrose agar (PDA) medium and cultured at 22, 25, 30, 35 and 42 °C for 72 h to investigate the effects of temperature; the optimum growth temperature of *A. oryzae*, 30 °C, was used as the control temperature. PDA media with a final salt concentration of 5.0, 10.0, 12.5 and 15.0 g/100 mL NaCl were prepared to test the effects of salinity stress on *A. oryzae*. Medium without salt was used as the control medium. Two microliters of freshly prepared *A. oryzae* suspension containing 1×10^7 conidia were inoculated on the medium to analyze phenotypes. To test the effect of two abiotic stress on fungal viability, 100 μL 1×10^7 conidia suspension was inoculated on per plate covered with cellophane (Solarbio, Beijing, China); the fungal mycelia were collected at 72 h incubation. Fungal mycelia were then dried overnight, and the biomass was tested. Material for RNA extraction was also collected as the same experimental operation. Three replicates were performed each time for experiments.

2.5 qRT-PCR analysis of *A. oryzae* GATA TFs expression in response to temperature and salinity stress

Total RNA was extracted using an Omega plant RNA kit (Omega Bio-Tek, Georgia, USA) according to the instructions provided by the manufacturer. One microgram of RNA was reverse-transcribed into cDNA using PrimeScript™ RT reagent with the gDNA Eraser kit (TaKaRa, Dalian, China). *A. oryzae* GATA TF primers were designed using the Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast) (Table S1). Gene expression levels were determined by perfoming quantitative real-time polymerase chain reaction (qRT-PCR) on a Bio-rad CFX96 Touch instrument (Bio-Rad, USA) using TB Premix Ex Taq II (TaKaRa) according to the manufacturer's instructions. Data were analyzed using Bio-rad CFX96 software and the $2^{-\triangle\triangle CT}$ method (Livak and Schmittgen 2001).

2.6 Construction of protein-protein interaction network

Protein-protein interaction (PPI) data were obtained from the online database of STRING (https://string-db.org/), which is an open source software for predicting and visualizing complex networks. These interactions were derived from literature of experimental validation including physical interactions and enzymatic reactions found in signal transduction pathways. The PPI networks were visualized in biological graph-visualization tool Cytoscape with the nodes representing proteins/genes (Pathan et al. 2015).

3. Results

3.1 Characteristics of *A. oryzae* GATA TFs

BLASTP analysis was used to check predicted GATA TFs from the *A. oryzae 3.042* genome. All potential *A. oryzae* GATA proteins were used to identify ZnF_GATA domains (PF00320) by HMMER3.1. In total, seven *A. oryzae* GATA TFs were identified, and were named *AoAreA*, *AoAreB*, *AoLreA*, *AoLreB*, *AoNsdD*, *AoSnf5* and *AoSreA* corresponding to the names of fungal orthologs (Table 1). The *A. oryzae* GATA TF amino acid lengths ranged from 313 (*AoAreB*) to 867 aa (*AoAreA*). The details of these *A. oryzae* GATA TFs, such as ZnF_GATA motif type, number domains of ZnF_GATA, sizes of the deduced peptides, and their homologous gene IDs, are listed in Table 1.

The GATA DNA binding domain is a conserved type-IV zinc-finger motif with the form Cys-X_2-Cys-X_{17-20}-Cys-X_2-Cys. The zinc-finger motifs of Cys-X_2-Cys -X_{17-20}-Cys -X_2-Cys among the

Table 1
The characteristics of *A. oryzae* GATA TFs.

Name	Protein ID	Peptide (aa)	ZnF_GATA Motif type	Number domain of ZnF_GATA	Homologous ID	Extra domain
AoSreA	EIT82081.1	567	Cys-X_2-Cys-X_{17}-Cys- X_2-Cys	2	KOC08900.1	TFIIB zinc-binding
AoAreB	EIT79032.1	313	Cys-X_2-Cys-X_{17}-Cys- X_2-Cys	1	XP_002379623.1	TFIIB zinc-binding
AoAreA	EIT72728.1	867	Cys-X_2-Cys-X_{17}-Cys- X_2-Cys	1	RAQ50831.1	AreA_N
AoLreB	EIT79273.1	496	Cys-X_2-Cys-X_{18}-Cys- X_2-Cys	1	RAQ50386.1	PAS
AoNsdD	EIT79449.1	504	Cys-X_2-Cys-X_{18}-Cys- X_2-Cys	1	KOC07076.1	-
AoLreA	EIT77832.1	283	Cys-X_2-Cys-X_{18}-Cys- X_2-Cys	1	XP_002384232.1	PAS
AoSnf5	EIT78280.1	570	Cys-X_2-Cys-X_{20}-Cys- X_2-Cys	1	XP_022385751.1	SNF5/INI1

seven *A. oryzae* GATA proteins showed differences. Six *A. oryzae* GATA domains contained the Cys-X_2-Cys-$X_{17/\ 18}$-Cys-X_2-Cys motif as the reported in other fungi, while the zinc-finger loop of *AoSnf5* had 20-residue between the Cys-X_2-Cys motifs which has rarely been found in fungi (Teakle and Gilmartin 1998; Scazzocchio 2000) (Table 1 and Fig. 1A). Interestingly, *AoSreA* harbored two highly conserved type-IV zinc-finger motifs with Cys-X_2-Cys-X_{17}-Cys-X_2-Cys (Table 1 and Fig. 1A) that two conserved type-IV zinc-finger motifs usually occur in animals. Apart from the ZnF_GATA domain, additional domains such as TFIIB zinc-binding, AreA- N, SNF5/INI1, and PAS were also characterized (Table 1, Fig. 1B). Previous studies have demonstrated that the PAS domain mainly functions in sensing environmental or physiological signals including oxidative and heat stress (Nan et al. 2011; Corrada et al. 2016). Therefore, extra domains presenting in *A. oryzae* GATA may also play the same role in diverse environmental stresses and could facilitate the functional analysis of *A. oryzae* GATA TFs.

In addition, chromosomal location of *A. oryzae* GATA TFs reveals their random distribution in the *A. oryzae* genome. Here, the seven of *A. oryzae* strain 3.042 was mapping to the first complete genome of *A.*

oryzae strain RIB40 chromosomes. The chromosomal distribution of *A. oryzae* GATA TFs was visualized by the MapChart program. Seven *A. oryzae* GATA TFs were randomly distributed on chromosomes 1, 3, 4, and 6 (Fig. 1C). Interestingly, *AoAreB*, *AoSreA*, and *AoSnf5* clustered into the same subgroup in the neighbor-joining tree (Fig. 1B) and were distributed on the same chromosome, which indicates a close evolutionary relationship exists among them. The chromosomal location of *A. oryzae* GATA TFs could help to determine the exact sequence of events.

3.2 Phylogenetic analysis of the *Aspergillus* GATA TFs

A neighbor-joining phylogenetic tree (NJ_tree) was constructed by using MEGA6.0 for the multiple sequence alignment of all *Aspergillus* GATA TFs with 1000 bootstrap replications to analyze phylogenetic relationships between the *A. oryzae* GATA TFs and other *Aspergillus* GATA TFs with the ZnF_GATA domains. All the *Aspergillus* GATA TFs divided into seven subgroups in the NJ_tree based on the number of ZnF_GATA domains and zinc finger motif of GATA domain sequences with other *Aspergillus* GATA TFs from FTFD, including six known subgroups and one unknown function subgroup (Fig. 2). *A. oryzae* GATA TFs were scattered in six subgroups with other *Aspergillus* GATA TFs which functions have been reported, while the novel *AoSnf5* encoding GATA TF also clustered into NSDD subgroups together with *AoNsdD*. The different GATA subgroups perform different functions. For example, the GATA TFs of WC-1 and WC-2 subgroups mainly involve in the regulation of blue- and red-light responses (Purschwitz et al. 2008; Purschwitz et al. 2013). Nitrogen regulation is regulated by the process of nitrogen catabolite repression which controls gene expression through GATA TFs of NIT2 and ASD4 subgroup family in yeasts and filamentous (Pfannmüller et al. 2017; Pomraning et al. 2017; Michielse et al. 2014). Therefore, the *AoLreA*, *AoLreB*, *AoAreA*, and *AoAreB* divided respectively into WC-1, WC-2, NIT2, and ASD4 subgroups might also involve in light responses or nitrogen regulation as the reported. In addition, *NsdD* had been shown not only to affect sexual and asexual reproduction but also secondary metabolism in *Aspergillus* (Lee et al. 2014; 2016), which could help to determine the function of the *AoNsdD* and *Aosnf5* assigned to the NSDD subgroup.

3.3 Analysis of conserved motifs in *A. oryzae* GATA TFs

In order to obtain insights into the diversity of motifs compositions in *A. oryzae* GATA TFs, the *A. oryzae* GATA TFs and other *Aspergillus'* were predicted the conserved motifs Using MEME4.11.4 online software. A total five conerved motifs were identified. The relative location of these motifs within the protein is represented in Fig. 3. The identified consensus sequence of the five motifs is shown in Figure S1. A typical zinc-finger structure which was composed of motif 1 and motif 2 was observed in all *Aspergillus* GATA TFs, but the compositions of GATA TF motifs also had different variable regions. As expected, GATA menbers that had similar motif compositions could be clustered into one subgroup, which suggests they may have similar genetic functions within the same subgroups. In addition, the motif distribution further confirms the accuracy of the phylogenetic relationship of *Aspergillus* GATA TFs. The distribution of motifs in different subgroups implied sources of functional differentiation in GATA TFs in the evolutionary processes.

3.4 Effects of different temperature and salinity treatments on the growth of *A. oryzae*

The temperature and salt concentration are two of the most important environmental factors affecting the growth and fermentation of *A. oryzae* during fermentation process (Chen et al. 2011; Bechman et al. 2012; Wang et al. 2013). Therefore, we investigated the growth of *A. oryzae* under different temperature and salt concentration stresses. The optimum temperature for *A. oryzae* growth usually ranges from 30–35 °C. Low- and high-temperatures significantly inhibited the mycelial growth, especially at the temperature of 22 and 42 ℃ (Fig. 4A, a-e and Fig. 4B). In addition, the high salt concentration also significantly inhibited the hyphal growth and differentiation of *A. oryzae*, and the inhibitory effect increased with the salt concentration (Fig. 4A, f-j and Fig. 4C). Furthermore, the formation and development of *A. oryzae* spores, which shows yellow-green color in the middle of the fungal colony, were also inhibited under low- and high-temperature and high salinity stresses (Fig. 4A).

3.5 Expression patterns of *A. oryzae* GATA TFs in response to temperature and salinity stresses

To determine on the possible roles of *A. oryzae* GATA TFs in response to abiotic stresses, we analyzed the expression level of seven *A. oryzae* GATA TFs by qRT-PCR in *A. oryzae* that grew under different temperatures and salt concentrations (Fig. 5). Seven *A. oryzae* GATA TFs exhibited expression diversity under different temperatures and salt stresses. Except the *AoSnf5*, six *A. oryzae* GATA TFs strongly responded to low- or high-temperatures (Fig. 5A). *AoSreA* and *AoNsdD* showed the same expression trends that they were significantly induced at low-temperature (22 ℃) and inhibited at high-temperature (42 ℃) compared with CK (30 ℃). Besides, *AoAreB*, *AoLreA* and *AoLreB* expression levels were remarkably upregulated under high-temperature stresses compared with 30 ℃(CK),especially *AoAreB* (Fig. 5A). Interestingly, only *AoAreA* was inhibited under both low- and high-temperature stresses. Furthermore, the expression level of *AoAreA*, *AoSreA*, and *AoAreB* was significantly downregulated under high-salt concentration stress, while *AoLreA*, *AoNsdD*, and *AoSnf5* expression level exhibited upregulated under 5.0 and 10.0 g/100 mL NaCl stresses (Fig. 5B). Together, the results demonstrate the importance of *A. oryzae* GATA TFs in response to temperature and high salt stresses and provide a basis information for future studies into the function of *A. oryzae* GATA in abiotic stresses.

3.6 Protein-protein interaction network of *A. oryzae* GATA TFs

To analyze the functions of *A. oryzae* GATA TFs proteins, protein-protein interaction (PPI) network was constructed using the data from STRING database, and only two independent PPI network of *AoAreA* and *AoSreA* proteins was obtained (Fig. 6A and B). Furthermore, we found both *AoAreA* and *AoSreA* proteins interacted with *CreA* that *CreA* deletion mutants show less conidiation than wild type and mutants are sensitive to salt stress (Hou et al. 2018). Therefore, the expression levels of *AoAreA*, *AoSreA*, and *AoCreA* were analyzed under temperature and salt stresses. *AoSreA* and *AoCreA* showed the same expression

patterns under both low- and high-temperature stresses, while the *AoAreA* and *AoCreA* exhibited opposite expression level at the temperature of 22 ℃ (Fig. 6C). Interestingly, three genes showed the same expression patterns under high salt concentration stresses (Fig. 6D), which demonstrates that *AoCreA* may be positively coregulated by both *AoAreA* and *AoSreA* under salt stresses. Additionally, the protein glutathione S-transferase (CADAORAP00007152) that is critical to abiotic stress was also found in the network of *AoAreA* (Favaloro et al. 2000). These results in this study were beneficial to identify more important proteins and biological modules that interacted with *A. oryzae* GATA TFs and understand the roles of *A. oryzae* GATA TFs in response to abiotic stresses. The detailed information of the proteins in the PPI network is listed in Table S2.

4. Discussion

Transcription factors (TFs) regulate expression of genes that mediate growth processes and environmental response and are employed as a principal source of the diversity and change that underlie evolution (Riechmann and Ratcliffe 2000). GATA TFs are transcriptional regulatory proteins that contain a characteristic type-IV zinc finger (Cys-X_2-Cys-X_{17-20}-Cys-X_2-Cys) and a DNA-binding domain recognize the conserved GATA motif in the promoter sequence of target genes (Scazzocchio 2000; Lowry and Atchley 2000). Fungal GATA TFs are mainly involved in the relation of nitrogen metabolism (Michielse et al. 2014; Pfannmüller et al. 2017), light responses (Purschwitz et al. 2008; Fuller et al. 2013), siderophore biosynthesis and mating-type switching (Jung and Kronstad 2011). Few GATA TFs in fungus also take part in response to the abiotic stresses, such as the *SreA*, *SreB*, *LreA*, *LreB*, *GLN3* and *GAT1* (Chung et al. 2020; Crespo et al. 2001; Purschwitz et al. 2008; Fuller et al. 2013; Marty et al. 2015). The number of the GATA TFs is conserved among *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger* and *A. oryaze* that possess six *GATA* TFs, suggesting that filamentous fungi share an identical composition of GATA TFs with each other (Kobayashi et al. 2007). However, in this study, we identified seven *A. oryzae* GATA TFs from the *A. oryzae 3.042* genome using an HMM model. Six known *A. oryzae* GATA TFs, consistent with the report of Kobayashi et al. (2007), were classified into six functional subgroups based on the number of ZnF_GATA domains and zinc finger motif of GATA domain sequences with other *Aspergillus* GATA TFs from FTFD, while the novel *AoSnf5* encoding GATA TF also clustered into NSDD subgroups together with *AoNsdD* (Fig. 2). Conserved motifs demonstrated that GATA TF menbers had similar motif compositions could be clustered into one subgroup (Fig. 3), which suggests they may have similar genetic functions within the same subgroups. In addition, the motif distribution further confirms the accuracy of the phylogenetic relationship of *Aspergillus* GATA TFs. The analyses of phylogenetic tree and conserved motifs demonstrated that the evolution of GATA TFs among different *Aspergillus* was very conservative which might have the same evolutionary events and perform similar function among the *Aspergillus* GATA proteins within the same subgroups.

Although most GATA domains harbor a class-IV zinc-finger motif, this structure differs among kingdoms (Lowry and Atchley 2000). In plants, most GATA domains have a single Cys-X_2-Cys-X_{18}-Cys-X_2-Cys motif, but some harbor more than two zinc-finger motifs or 20-residue within zinc-finger loops (Reyes et a. 2004;

Behringer and Schwechheimer 2015). In animals, the GATA domain harbors two zinc-finger motifs with Cys-X_2-Cys-X_{17}-Cys-X_2-Cys, but only the C-terminal finger is associated with DNA binding (Patient and Mcghee 2002). Fungal GATA TFs are combination of both animal and plant GATA TFs in terms of the amino acid residues present in the zinc-finger loop (Teakle and Gilmartin 1998). The majority of fungal GATA TFs contain a single zinc-finger domain and fall into two different categories: animal-like with 17-residue loops(Cys-X_2-Cys-X_{17}-Cys-X_2-Cys), and plant-like with 18-residue loops (Cys-X_2-Cys-X_{18}-Cys-X_2-Cys) (Teakle and Gilmartin 1998; Scazzocchio 2000; Patient and Mcghee 2002). Nineteen- and 20-residue zinc-finger loops (Cys-X_2-Cys-X_{19-20}-Cys-X_2-Cys) are also found in fungi, albeit rarely (Scazzocchio 2000; Maxon and Herskowitz 2001). Except for the 17- and 18-residue zinc-finger loops in *A. oryzae* GATA TFs, the novel *AoSnf5* contains 20-residue in the zinc-finger loops (Cys-X_2-Cys-X_{20}-Cys-X_2-Cys), which are rarely found in fungi (Table 1 and Fig. 1). To our knowledge, GATA TF with 20-residue zinc-finger loops was found in *Aspergillus* for the first time. In addition, *AoSreA* harbors two ZnF-GATA domains with the form of Cys-X_2-Cys-X_{17}-Cys-X_2-Cys, which is the typical GATA characteristic in animals (. Lowry and Atchley 2000; Patient and Mcghee 2002). Therefore, the features of *A. oryzae* GATA TFs strongly demonstrate that *A. oryzae* GATA TFs might be the combination of both plant and animal GATA TFs, which is consistent with the report that fungal GATA TFs are combination of both plant and animal GATA TFs in terms of the numbers of ZnF-GATA domains and amino acid residues present in the zinc-finger loop.

TFs are one of the key transcriptional regulators governing gene regulation and exhibit different expression profiles under distinct physiological and environmental conditions and act as synchronizing elements between stimuli and response. Many studies have revealed the GATA TFs are involved in the regulation of various abiotic stress responses in plants (Peng et al. 2015; Gupta et al. 2017; Nutan et al. 2019) and few fungi (Crespo et al. 2001; Fulle et al. 2013; Marty et al. 2015; Chung et al. 2020). The temperature and salt concentration are two of the most important environmental factors affecting the growth of *A. oryzae* during fermentation process (Machida et al. 2008; Chen et al. 2011; Bechman et al. 2012; Wang et al. 2013). *AreA* and *AreB* function as positive and negative transcriptional regulators participating in regulating nitrogen metabolism and carbon metabolism in *Fusarium fujikuroi* and *Aspergillus nidulans* (Michielse et al.2014; Pfannmüller et al. 2017; Chudzicka-Ormaniec et al. 2019). The expression level of *AoAreA* and *AoAreB* showed opposite trends at high temperature (42 ℃) compared with CK (30 ℃) in *A. oryzae* (Fig. 5A), which indicated *AoAreA* and *AoAreB* might also act respectively as negative and positive transcriptional regulators under high-temperature stresses. The *AoNsdD* and *AoSnf5*, clustering into NSDD subgroup in the NJ_tree (Fig. 2), were strongly induced under high salt stresses. *NsdD* has been reported as a key repressor affecting the quantity of asexual spores in *Aspergillus* (Lee et al. 2014; 2016), but there is lack of research on *NsdD* in response to adversity stress in *Aspergillus*. Apart from the regulation of siderophore biosynthesis and iron metabolism, *SreA* is also related with the maintenance of cell wall integrity and negatively impacts resistance as Δ*sreA* increases resistance to H_2O_2, calcofluor white, and Congo red (Chung et al. 2020).The expression level of *AoSreA* was significantly downregulated under 42 ℃ and high salt stresses, which indicates *AoSreA* might negatively impact high-temperature and high salt resistance. In contrast, *AoSreA* was significantly

upregulated at 22 ℃, and there is a report that the *SreB* strongly expresses and contributes to filamentous growth at 22 ℃ via lipid metabolism in *Blastomyces dermatitidis* (Marty et al. 2015). *AoSreA* and *SreB* shared the same conserved ZnF_GATA domain (Figure S2), which demonstrates that overexpression *AoSreA* in *A. oryzae* might also enhance the growth of mycelium at 22 ℃. Moreover, *AoCreA*, interacting with *AoSreA* protein within the PPI network, has the same expression patterns as *AoSreA*, which indicates *AoSreA* might positively regulate the *AoCreA* under temperature and high salt stresses. Curiously, the expression level of *AoCreA* was inhibited under high salt stresses in *A. oryzae*, which conflicted with the previous study that Δ*creA* mutants of *Fusarium graminearum* are sensitive to salt stress (Hou and Wang 2018). However, the results provide insights into the critical role of *SreA* in resistance to different temperatures and high salt stresses in *A. oryzae*.

LreA and *LreB*, is the GATA TFs of WC-1 and WC-2 subgroups involve in the regulation of blue- and red-light responses (Purschwitz et al. 2008; Fuller et al.2013). *AoLreA* and *AoLreB*, dividing respectively into WC-1 and WC-2 subgroups in NJ_tree (Fig. 2), acts as a dimer and contain typical PAS dimerization domains that display in Table 1 and Fig. 1B. Previous studies have demonstrated that the PAS domain also functions in sensing environmental or physiological signals including oxidative and heat stress (Nan et al. 2011; Corrada et al. 2016). Therefore, except for the regulation of blue- and red-light responses, the PAS domains presenting in *AoLreA* and *AoLreB* may facilitate the environmental response analysis of *A. oryzae* GATA TFs. Additionally, *LreA* and *LreB* is a regulatory complex of the global regulator *VeA*, while *VeA* plays a critical role in environmental stress responses in *A. cristatus*, and the Δ*veA* mutants are more sensitive to high salt, osmotic pressure, and temperature stress (Calvo 2008; Tan et al. 2018). In our study, *AoLreA* and *AoLreB* was increased under high-temperature (42 ℃) stresses, and *AoLreA* was significantly induced expression under 5.0 and 10.0 g/100 mL NaCl stresses. The results demonstrated that *AoLreA* and *AoLreB* might act as a regulatory complex of the global regulator *VeA* in response to temperature and high salt stresse in *A.oryzae*. Hence, the expression patterns of these *A. oryzae* GATA TFs under distinct environmental conditions provided useful information for the further analysis of *A. oryzae* GATA TFs in regulation of various abiotic stress responses in *Aspergillus.*

Abbreviations

TF: Transcription factors; NJ_tree: Neighbor-joining phylogenetic tree; PPI: Protein-protein interaction network; PDA: Potato dextrose agar.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The genome-wide transcriptome data of *A. oryzae* in different growth stages and salt stress treatment have been submitted to NCBI SRA databases under Bioproject Accession PRJNA407002 and PRJNA383095.

Competing interests

The authors declare that they have no conflict of interests.

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Author’s contributions

This work was completed with the efforts of all authors. Jiang chunmiao was responsible for data analysis, experiment, and manuscript writing. Lv Gongbo and He Bin analyzed the Pfam of *A. oryzae* genes. Zhang Zhe provides the chromosomal location methods. Hu Zhihong provided the methods of phenotypic analysis. Zeng Bin was responsible for the *A. oryzae* materials and research funding. All the authors have read and approved the final manuscript.

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Figures

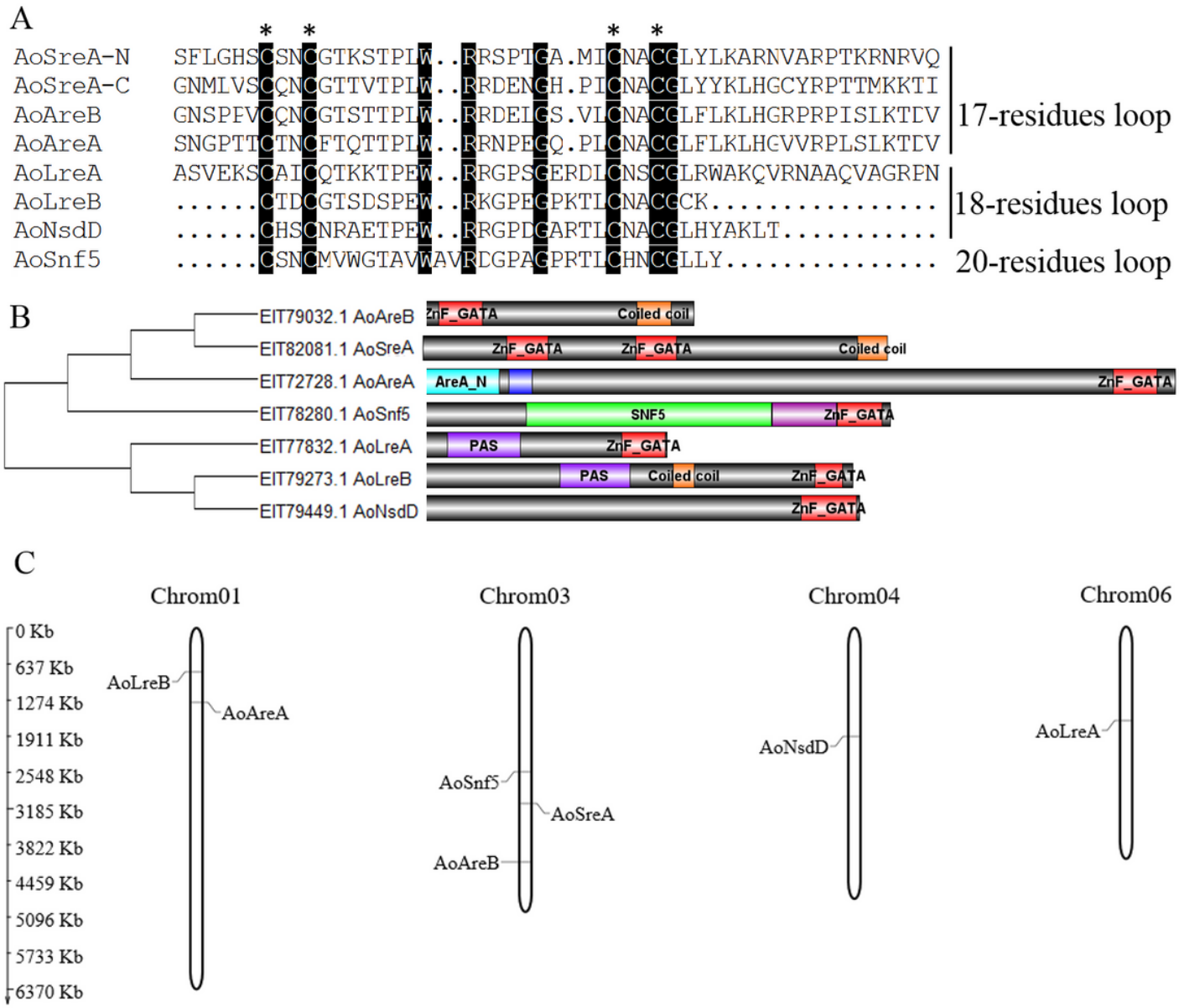

Figure 1

The Conserved domain alignment, prediction of functional domains and chromosomal location of A. oryzae GATA TFs. (A) Alignment of the DNA interacting domain of A. oryzae GATA TFs. Cysteines from the Cys-X2-Cys-X17/18/20-Cys-X2-Cys domain are indicated by an asterisk above the sequence alignment. The 17, 18, and 20 numbers indicate the amino acid residues between Cys-X2-Cys. (B) Seven A. oryzae GATA proteins were aligned and clustered using MEGA6.0, and their ZnF_GATA domains are shown in red on the Neighbor-joining tree. (C) The distribution of A. oryzae GATA TFs on chromosomes. The vertical columns represent chromosomes; gene names are shown at the side of chromosomes.

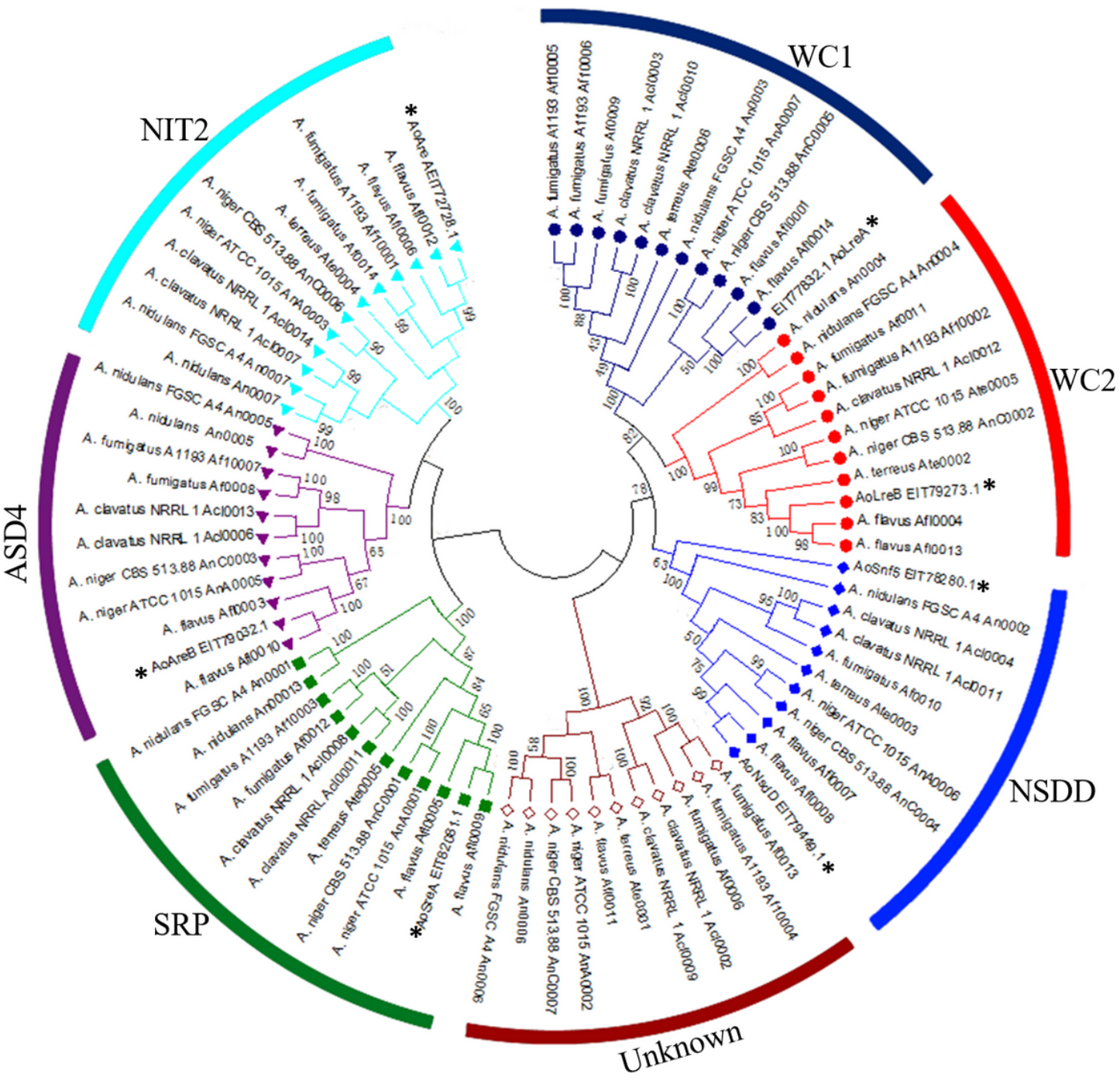

Figure 2

Phylogenetic analysis of A. oryzae and other Aspergillus TFs. GATA protein sequences were aligned using ClustalW in MEGA6.0 software using default parameters. The consensus NJ_tree represent 1, 000 bootstrap replications. Bootstrap values are displayed with nodes. The protein sequences of Aspergillus GATA TFs were downloaded from FTFD. The Aspergillus GATA TFs are classed into seven subgroups in NJ_tree, including one group with unknown function. Seven A. oryzae GATA TFs are scattered in six known subgroups, and the novel AoSnf5 also clustered into NSDD subgroups together with AoNsdD.

Figure 3

The conserved motif arrangement of A. oryzae and other Aspergillus GATA TF proteins based on their phylogenetic relationships. A NJ_tree was predicted from the amino acid sequences of GATA TFs using ClustalW and MEGA6.0 with 1, 000 bootstrap replications. The conserved motifs in the GATA TFs were identified by MEME. In total, five conserved motifs were identified and shown in different colors.

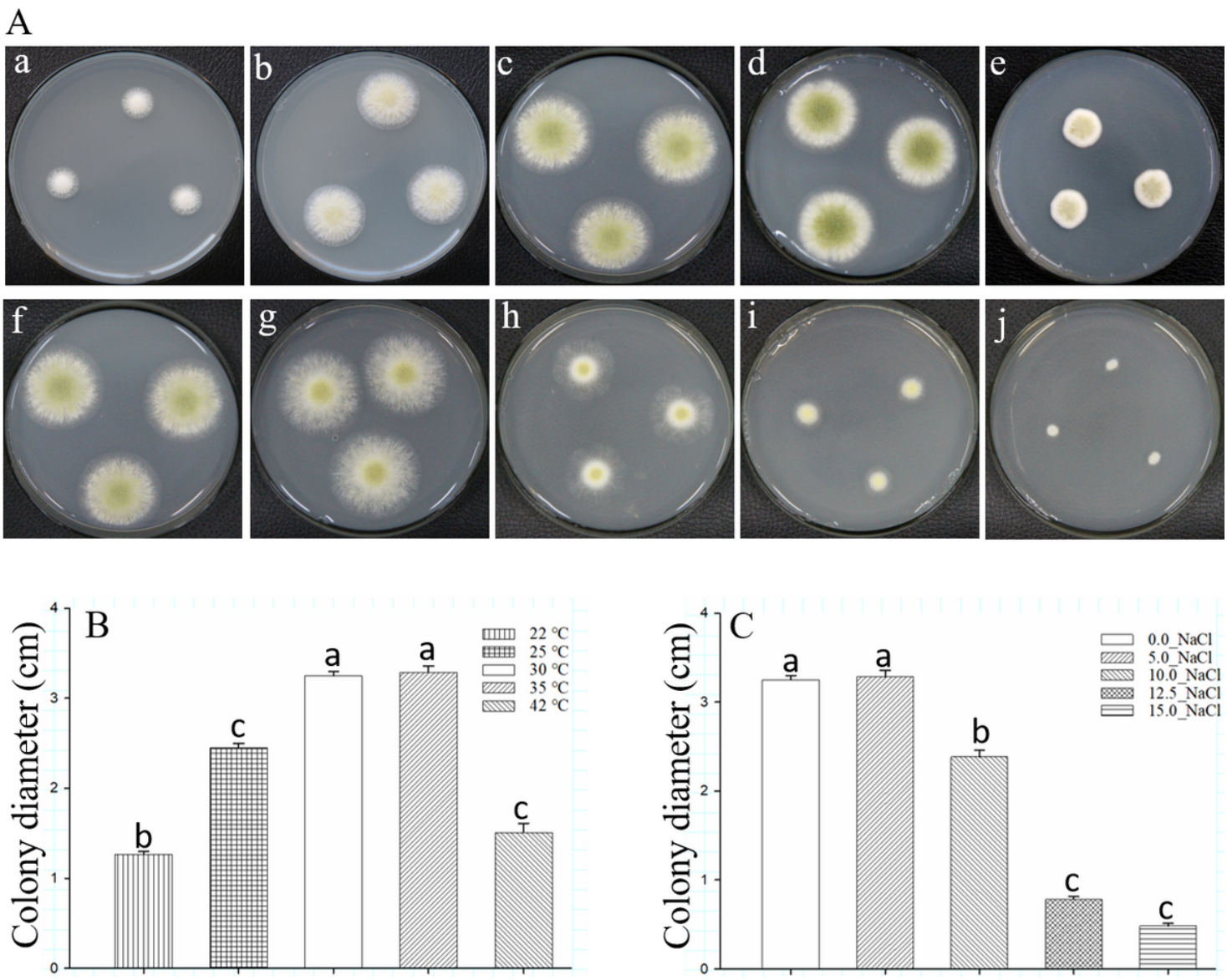

Figure 4

A. oryzae hyphal growth and differentiation under different stress factors for 72 h. (A) The phenotypes of A. oryzae under temperature and salinity stress. (a-e) Phenotypes of A. oryzae exposed different temperature stresses (22, 25, 30, 35, and 42 ℃ from left to right). (f-j) The NaCl concentration of 0.0, 5.0, 10.0, 12.5 and 15.0 g/100 mL were employed for salinity stress. (B) and (C) Colony diameter was determined by measuring diameter under different stress factors. The 30 ℃, the optimum growth temperature of A. oryzae, was used as the control temperature in the experiment. The PDA medium without NaCl used as the control in the experiment of salt treatment. Results represent the average of three repetitions ± SEM (n=3). Different letters in the bar chart represent significant differences ($p<0.01$, Duncan's multiple range test); the same letters in the bar chart represent no significant difference when compared with the control.

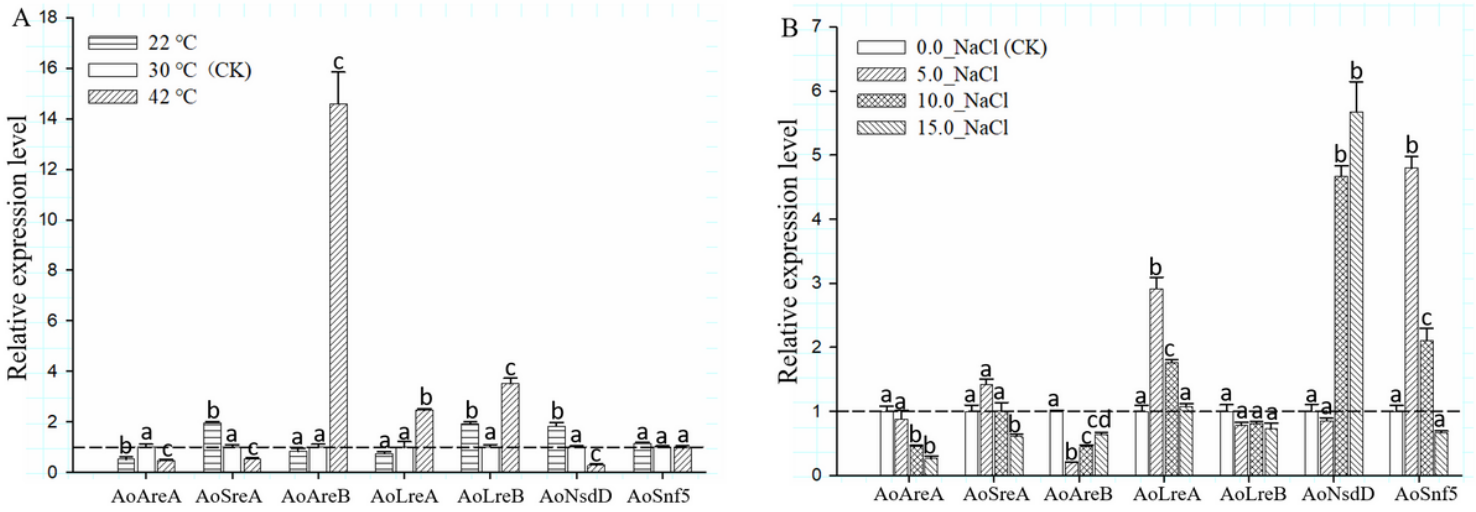

Figure 5

The expression levels of A. oryzae GATA TFs in response to temperature and salt stresses. (A) The relative expression levels of A. oryzae GATA TFs responding to low- and high-temperature stresses. (B) The expression patterns of A. oryzae GATA TFs under different salt concentration stresses. The 30 ℃, the optimum growth temperature of A. oryzae, used as the control temperature (CK) in the experiment. The experiment of PDA medium without NaCl used as the control (CK) under salt stress. Results show the average of three repetitions ± SEM (n=3). Different letters represent significant differences ($p<0.01$, Duncan's multiple range test); the same letters represent no significant difference when compared with the control.

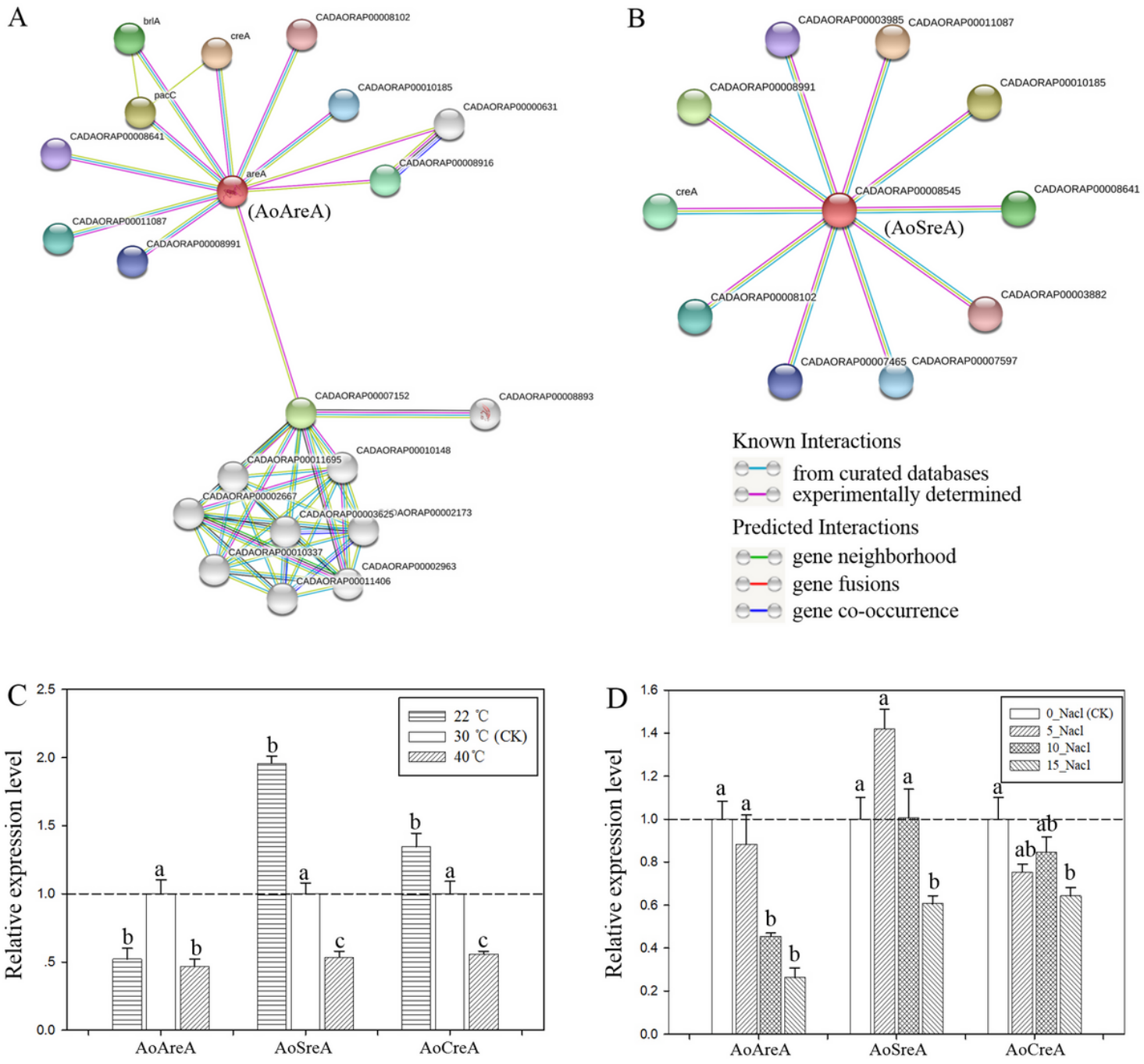

Figure 6

A protein-protein interaction (PPI) network of A. oryzae GATA TFs. (A) and (B) The PPI network of AoAreA and AoSreA proteins. (C) and (D) The relative expression levels of AoAreA and AoSreA were consistent with the interaction protein AoCreA ($p<0.01$, $n=3$). The 30 °C was the control temperature (CK) in the experiment. The experiment of PDA medium without NaCl used as the control (CK) under salt stress. The same letters represent no significant difference compared with the control when assessed using Duncan's multiple range test.

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