

Genome-wide Identification of the *Aspergillus oryzae* GATA Transcription Factor Gene Family and expression Analysis under Temperature or Salt Stresses

Chunmiao Jiang

Jiangxi Key Laboratory of Bioprocess Engineering and Co-Innovation Center for In-Vitro Diagnostic Reagents and Devices of Jiangxi Province, College of Life Sciences, Jiangxi Science and Technology Normal University

Bin He

Jiangxi Key Laboratory of Bioprocess Engineering and Co-Innovation Center for In-vitro Diagnostic Reagents and Devices of Jiangxi Province, College of Life Sciences, Jiangxi Science and Technology Normal University

Zhe Zhang

Jiangxi Key Laboratory of Bioprocess Engineering and Co- Innovation Center for In-Vitro Diagnostic Reagents and Devices of Jiangxi Province, College of Life Sciences, Jiangxi Science and Technology Normal University

Zhihong Hu

Jiangxi Key Laboratory of Bioprocess Engineering and Co-Innovation Center for In-Vitro Diagnostic Reagents and Devices of Jiangxi Province, College of Life Sciences, Jiangxi Science and Technology Normal University

Chuannan Long

Jiangxi Key Laboratory of Bioprocess Engineering and Co-Innovation Center for In-Vitro Diagnostic Reagents and Devices of Jiangxi Province, College of Life, 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

Background

GATA transcription factors (TFs) are transcriptional regulatory proteins that contain a characteristic type-IV zinc finger and recognize the conserved GATA motif in the promoter region. Previous studies demonstrate that GATA 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 have been performed in model plants and some fungi, information regarding GATA TFs in *A. oryzae* is extremely poor.

Results

Therefore, we identified seven GATA TFs from *A. oryzae* 3.042 genome, and named *AoAreA*, *AoAreB*, *AoLreA*, *AoLreB*, *AoNsdD*, *AoSreA* in correspondence to fungal orthologs, including a novel *AoSnf5* with 20-residue between the Cys-X₂-Cys motifs which was found in *Aspergillus* for the first time. Six known *A. oryzae* GATA TFs were classified into six subgroups, while the novel *AoSnf5* also clustered into NSDD subgroups together with *AoNsdD* in the NJ_tree of all *Aspergillus* GATA TFs. Conserved motifs demonstrated that 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 of *Aspergillus* GATA TFs. The expression patterns of seven *A. oryzae* GATA TFs exhibited diversity under temperature and salt stresses. The expression analyses of *AoLreA* and *AoLreB* demonstrates *AoLreA* mainly played role in salt stress and *AoLreB* did under temperature stress. *AoSreA* was shown to positively regulate the expression of *AoCreA* and might act as a negative regulator in temperature and high salt stress response. In addition, the *AoNsdD*, *AoSnf5*, *AoAreB*, and *AoAreA* strongly responded to salt stresses, while *AoAreB* and *AoAreA* showed opposite expression trends at high temperature. Overall, the expression patterns of these *A. oryzae* GATA TFs under distinct environmental conditions provided useful information for the further analysis of GATA TFs in regulation of various abiotic stress in *A. oryzae*.

Conclusion

In conclusion, the comprehensive analysis data of *A. oryzae* GATA TFs will provide insights into the critical role of *A. oryzae* GATA TFs in resistance to temperature and salt stresses in *A. oryzae*.

Background

GATA TFs are widely distributed in fungi, plants, and animals [1]. They constitute a protein family that is characterized by the presence of one or two highly conserved type-IV zinc fingers (Cys-X₂-Cys-X₁₇₋₂₀-Cys-X₂-Cys) and a DNA-binding domain that recognizes the (A/T)-G-A-T-A- (A/G) sequence in the promoter

sequence of target genes [1]. Although most GATA domains harbor a class-IV zinc-finger motif of Cys-X₂-Cys-X₁₇₋₂₀-Cys-X₂-Cys, this structure differs among kingdoms [1]. In plants, most GATA domains have a single Cys-X₂-Cys-X₁₈-Cys-X₂-Cys motif, but some harbor more than two zinc-finger motifs or 20-residue within zinc-finger loops [2, 3]. In animals, the GATA domain harbors two zinc-finger motifs with Cys-X₂-Cys-X₁₇-Cys-X₂-Cys, but only the C-terminal finger is associated with DNA binding [4]. 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 [5]. The majority of fungal GATA TFs contain a single zinc-finger domain and they mostly fall into two different categories: animal-like with 17-residue loops Cys-X₂-Cys-X₁₇-Cys-X₂-Cys (IVa), and plant-like with 18-residue loops Cys-X₂-Cys-X₁₈-Cys-X₂-Cys (IVb) [4, 5, 6]. In addition, nineteen- and 20-residue zinc-finger loops are also found, albeit rarely, in fungi [6]. For example, the *Saccharomyces cerevisiae* *ASH1* of GATA TF contains 20-residue in the zinc-finger loop that binds to the promoter of the HO nuclease gene [7].

The functions of GATA TFs have been widely studied in fungi, animals, and plants. Apart from their active involvement in nitrogen metabolism, flowering growth and development in plants, GATA TFs also play a key role in response to various environmental stimuli stress such as salinity, drought, and temperature stresses [8, 9]. Diverse roles governed by GATA TFs in fungus mainly involved in nitrogen regulation and light responses, regulation of sexual and/or asexual reproduction, and secondary metabolism [10, 11, 12, 13]. Research has demonstrated that the *AreB* and *AreA* GATA TFs are regulators that 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 in filamentous fungi [11, 12]. The *SreA* involves in regulation of siderophore biosynthesis and the control of iron uptake [10, 14], and *NsdD* is a global regulator that regulates sexual and/or asexual reproduction and the production of SMs in *A. nidulans* and *A. fumigatus* [15, 16]. Fungal GATA TFs are a combination of both plant and animal GATA TFs in terms of amino acid residues present in the zinc-finger loop [5, 6, 8]. Thus, few fungal GATA TFs also play important role in response to the abiotic stresses like plant GATA TFs. For example, in *Alternaria alternata*, GATA TF *SreA* is related with the maintenance of cell wall integrity, and the Δ *sreA* increases resistance to calcofluor white, Congo red and H₂O₂ [10]. *SreB* strongly expresses and contributes to filamentous growth at 22 °C via lipid metabolism in *Blastomyces dermatitidis* [17]. Additionally, *GLN3* and *GAT1* of GATA TFs have been shown to be involved in salt tolerance in *Saccharomyces cerevisiae* [18]. 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, that is widely used in East Asian traditional fermented food products, such as soy sauce and sake fermentation [19, 20]. *A. oryzae* secretes synthetic and hydrolytic enzymes, and accumulates flavor compounds, which enhance the nutritional and flavor profile of fermented foods during fermentation [19, 21]. Simultaneously, *A. oryzae* is exposed to environmental stress factors during fermentation process. For example, 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 [22, 23]. In

addition, high sodium chloride concentrations are added to soy sauce mash to inhibit the growth of spoilage bacteria during fermentation process, but high salt concentrations also inhibit the growth of *A. oryzae* [24, 25]. 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 plants and few fungi [6, 8, 9, 18]. Although the 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 secondary metabolism [11, 12, 26], there is lack of research on a comprehensive analysis of GATA TF structural characteristics, evolutionary features, conserved motifs and expression level under different environmental stress factors in *A. oryzae*. Therefore, the aim of this study was to identify the GATA TFs in the whole *A. oryzae* 3.042 genome, to analyze their domain structure, evolutionary features, and conserved motifs, and to provide a basis for the cloning of GATA TFs in *A. oryzae*. Furthermore, the results of protein-protein interaction prediction and the expression patterns of *A. oryzae* GATA TFs under different temperatures and high salt stresses can establish a good foundation for further study on the function and the mechanism of *A. oryzae* GATA TFs involved in abiotic stress responses in *A. oryzae*.

Results

Identification of *A. oryzae* GATA zinc finger 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*, *AoSreA* corresponding to the names of fungal orthologs (Table 1). *A. oryzae* GATA 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.

Table 1 GATA TFs identified from *A. oryzae*.

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

The GATA DNA binding domain is a conserved type-IV zinc-finger motif with the form Cys-X₂-Cys-X₁₇₋₂₀-Cys-X₂-Cys. The zinc-finger motifs of Cys-X₂-Cys -X₁₇₋₂₀-Cys -X₂-Cys among the seven *A. oryzae* GATA proteins showed differences. Six *A. oryzae* GATA domains contained the Cys-X₂-Cys-X_{17/18}-Cys-X₂-Cys

motif as the reported in other fungi, while the zinc-finger loop of *AoSnf5* had 20-residue between the Cys-X₂-Cys motifs which has rarely been found in fungi [5, 6] (Table 1 and Fig.1 A). Interestingly, *AoSreA* harbored two highly conserved type-IV zinc-finger motifs with Cys-X₂-Cys-X₁₇-Cys-X₂-Cys (Table 1 and Fig.1 A) that two conserved type-IV zinc-finger motifs usually occur in animals. Apart from the ZnF_GATA domain, additional domains such as TFIIIB zinc-binding, AreA- N, SNF5/INI1, and PAS were also characterized (Table 1, Fig.1 B). [Previous studies](#) have demonstrated that the PAS domain mainly functions in sensing environmental or physiological signals including oxidative and heat stress [27, 28]. 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. 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.

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 [13, 29]. 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 [12, 30, 31]. 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* [15, 32], which could help to determine the function of the *AoNsdD* and *Aosnf5* assigned to the NSDD subgroup.

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, all the *Aspergillus* GATA TFs were predicted the conserved motifs Using MEME4.11.4 online software. A total

five conserved motifs were identified. The relative location of these motifs within the protein is represented in Fig. 3. The identified consensus sequence of the 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 all GATA TFs also had different variable regions (motif 3, -4, -5). As expected, GATA members 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 GATA TFs. The distribution of motifs in different subgroups implied sources of functional differentiation in GATA TFs in the evolutionary processes.

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 of *A. oryzae* during fermentation process [21, 22, 23, 24, 25]. 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 conditions of 22 and 40 °C (Fig. 4A, a-e; 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; 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).

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 temperature and salt concentration (Fig. 5). Seven *A. oryzae* GATA TFs exhibited expression diversity under different temperatures and salt stresses. Except the *AoLreA* and *AoSnf5*, five *A. oryzae* GATA TFs strongly responded to low- and high-temperatures (Fig. 5A). The expression levels of *AoAreA*, *AoSreA* and *AoNsdD* showed opposite trends, which were significantly induced at low-temperature (22 °C) and inhibited at high-temperature (40 °C) compared with CK (30 °C). Besides, *AoAreB* and *AoLreB* expression levels were upregulated under low- and high-temperature stresses compared with 30 °C(CK), especially under high-temperature (Fig. 5A). Furthermore, the expression level of *AoAreA*, *AoSreA*, and *AoAreB* was significantly downregulated under high-salt stress. In addition, *AoLreA*, *AoNsdD*, and *AoSnf5* expression level exhibited upregulated under 5 and 10 mg/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.

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 [33]. Therefore, the expression levels of *AoAreA*, *AoSreA*, and *AoCreA* were analyzed under temperature and salt stresses. Interestingly, three genes showed the same expression patterns under temperature and salt stresses (Fig. 6C and D), which demonstrates that *AoCreA* may be positively coregulated by both *AoAreA* and *AoSreA* under temperature and salt stresses. Additionally, *AoAreA* protein interacted with CADAORAP00007152 (glutathione S-transferase) that is critical to abiotic stress [34]. 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.

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 [35]. GATA TFs are transcriptional regulatory proteins that contain a characteristic type-IV zinc finger (Cys-X₂-Cys-X₁₇₋₂₀-Cys-X₂-Cys) and a DNA-binding domain recognize the conserved GATA motif in the promoter sequence of target genes [1]. Fungal GATA TFs are mainly involved in the relation of nitrogen metabolism [12, 31], light responses [13, 29], siderophore biosynthesis and mating-type switching [10; 36]. Few GATA TFs in fungus also take part in response to the abiotic stresses, such as the *SreA*, *SreB*, *LreA*, *LreB*, *GLN3* and *GAT1* [10, 13, 17, 18, 29]. The number of the GATA TFs is conserved among *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger* and *A. oryzae* that possess six GATA TFs, suggesting that filamentous fungi share an identical composition of GATA TFs with each other [26]. Here 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.[26], 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*. Conserved motifs demonstrated that GATA TF members 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 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.

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-X₂-Cys-X₁₇-Cys-X₂-Cys), and plant-like with 18-residue loops (Cys-X₂-Cys-X₁₈-Cys-X₂-Cys) [4, 5, 6]. Nineteen- and 20-residue zinc-finger loops (Cys-X₂-Cys-X₁₉₋₂₀-Cys-X₂-Cys) are also found, albeit rarely, in fungi [6, 7]. 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-X₂-Cys-X₂₀-Cys-

X₂-Cys), which are rarely found in fungi. To our knowledge, GATA TF *AoSnf5* 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-X₂-Cys-X₁₇-Cys-X₂-Cys, which is the typical GATA characteristic in animals [1, 4]. 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 [5, 8].

Transcription factors (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 and few fungi [8, 9, 10, 13, 17, 18, 37]. The temperature and salt concentration are two of the most important environmental factors affecting the growth of *A. oryzae* during fermentation process [21, 22, 23, 24, 25]. Therefore, we analysis of the expression levels of *A. oryzae* GATA TFs under different temperature and salt concentration stresses. Seven *A. oryzae* GATA TFs exhibited expression diversity under different temperatures and salt stresses. For example, the expression level of *AoAreB* and *AoAreA* showed opposite trends at high temperature (40 °C) compared with CK (30 °C) in *A. oryzae*, while *AoAreB* and *AoAreA* were inhibited under high salt stresses. *AreA* and *AreB* function as positive and negative transcriptional regulators participating in regulating nitrogen metabolism and carbon metabolism in *Fusarium fujikuroi* and *Aspergillus nidulans* [11, 12, 31], which indicated *AoAreB* and *AoAreA* might also act as positive and negative transcriptional regulators under temperature and salt stresses. The *AoNsdD* and *AoSnf5*, clustering into NSDD subgroup in the NJ_tree (Fig. 2), were strongly induced under high salt stresses. *NsdD* is a key repressor affecting the quantity of asexual spores in *Aspergillus* [32], but there is lack of research on *NsdD* in response to adversity stress in *Aspergillus*. 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*.

Apart from the regulation of siderophore biosynthesis and iron metabolism, GATA TF *SreA* is also related with the maintenance of cell wall integrity and negatively impacts resistance as $\Delta sreA$ increases resistance to H₂O₂, calcofluor white, and Congo red [10, 14]. The expression level of *AoSreA* was significantly downregulated under 40 °C and high salt stresses, which indicates *AoSreA* might negatively impact high-temperature and high salt resistance. In contrast, *AoSreA* was significantly upregulated at 22 °C, and there is a report that the *SreB* strongly expresses and contributes to filamentous growth at 22 °C via lipid metabolism in *Blastomyces dermatitidis* [17]. *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 °C. 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 $\Delta creA$

mutants of *Fusarium graminearum* are sensitive to salt stress [33]. 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 [13, 29]. *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 [27, 28]. 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 ΔveA mutants are more sensitive to high salt, osmotic pressure, and temperature stress [38, 39]. *AoLreA* was significantly induced expression under 5 and 10 mg/mL NaCl stresses, while the expression level of *AoLreB* was increased under low- (22 °C) and high-temperature (40 °C) stresses compared with the CK (30°C). This result demonstrated that *AoLreA* and *AoLreB* might act as a regulatory complex of the global regulator *VeA* in response to different temperature and high salt stresses in *A.oryzae*.

Conclusion

We identified seven GATA TFs from *A. oryzae* 3.042 genome, including the novel *AoSnf5* with 20-residue in the zinc-finger loops (Cys-X₂-Cys-X₂₀-Cys-X₂-Cys), which was found in *Aspergillus* for the first time. Six known *A. oryzae* GATA TFs were classified into six subgroups with other *Aspergillus* GATA TFs, while the novel *AoSnf5* also clustered into NSDD subgroups together with *AoNsdD*. Conserved motifs demonstrated that 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 of *Aspergillus* GATA TFs. Seven *A. oryzae* GATA TFs exhibited expression diversity under different temperature and salt stresses. The *AoNsdD* and *AoSnf5*, clustering into NSDD subgroup in the NJ_tree, were strongly induced under high salt stresses. The expression level of *AoAreB* and *AoAreA* showed opposite trends at high temperature (40 °C) compared with CK (30 °C) in *A. oryzae*, while both them were inhibited under high salt stresses, which indicated *AoAreB* and *AoAreA* might act as negative or positive transcriptional regulators under temperature or salt stresses. *AoLreA* and *AoLreB*, with typical PAS dimerization domains that functions in sensing environmental and heat stress, exhibited different patterns under temperature and salt stresses, which demonstrates *AoLreA* mainly played role in salt stress and *AoLreB* did under temperature stress. *AoSreA* was shown to positively regulate the expression of *AoCreA* regulatory gene and participate in *A. oryzae* response to temperature and high salt stresses. In conclusion, the comprehensive analysis data of *A. oryzae* GATA TFs will be better to further study their functional characterization and evolution of *A. oryzae* GATA TFs and established a foundation for understanding the roles of *A. oryzae* GATA TFs involved in abiotic stress responses.

Methods

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 [40].

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.

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 [41]. 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 [42]. 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.

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 40 °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 (CK). PDA media with a final salt concentration of 5.0, 10.0, 12.5 and 15.0 mg/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 μ L 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.

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 PrimeScriptTM 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 performing 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 $2^{-\Delta\Delta CT}$ method [43].

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 [44].

Abbreviations

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All the necessary data in this study has been provided in the manuscript and the Supplementary files. The software used to infer networks is open source/freely available and has been cited in this study.

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. JC was responsible for data analysis, experiment, and manuscript writing. HB analyzed the Pfam of *A. oryzae* genes. ZZ provide the chromosomal location methods. HZ provided the methods of phenotypic analysis. LC was responsible for revised the manuscript. ZB was responsible for the *A. oryzae* materials and research funding. All the authors have read and approved the final manuscript.

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Not applicable.

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Additional Files

Additional file 1

File contains supplementary tables and figures referenced in this manuscript. **Table S1**. QRT-PCR primerws of *A. oryzae* GATA gene expression in response to abiotic stress. **Table S2**. The detailed information of the proteins in the PPI network. **Figure S1**. The five structural motifs in *A. oryzae* GATA TF proteins. **Figure S2**. The predicted amino acid sequence of AoSreA aligned with SreB. AoSreA and SreB contained several conserved domains including two ZnF_GATA (N-terminal and C-terminal) separated by a cysteine-rich region (CRR) and a conserved C-terminus (CCT) with a predicted coiled-coil domain.

Figures

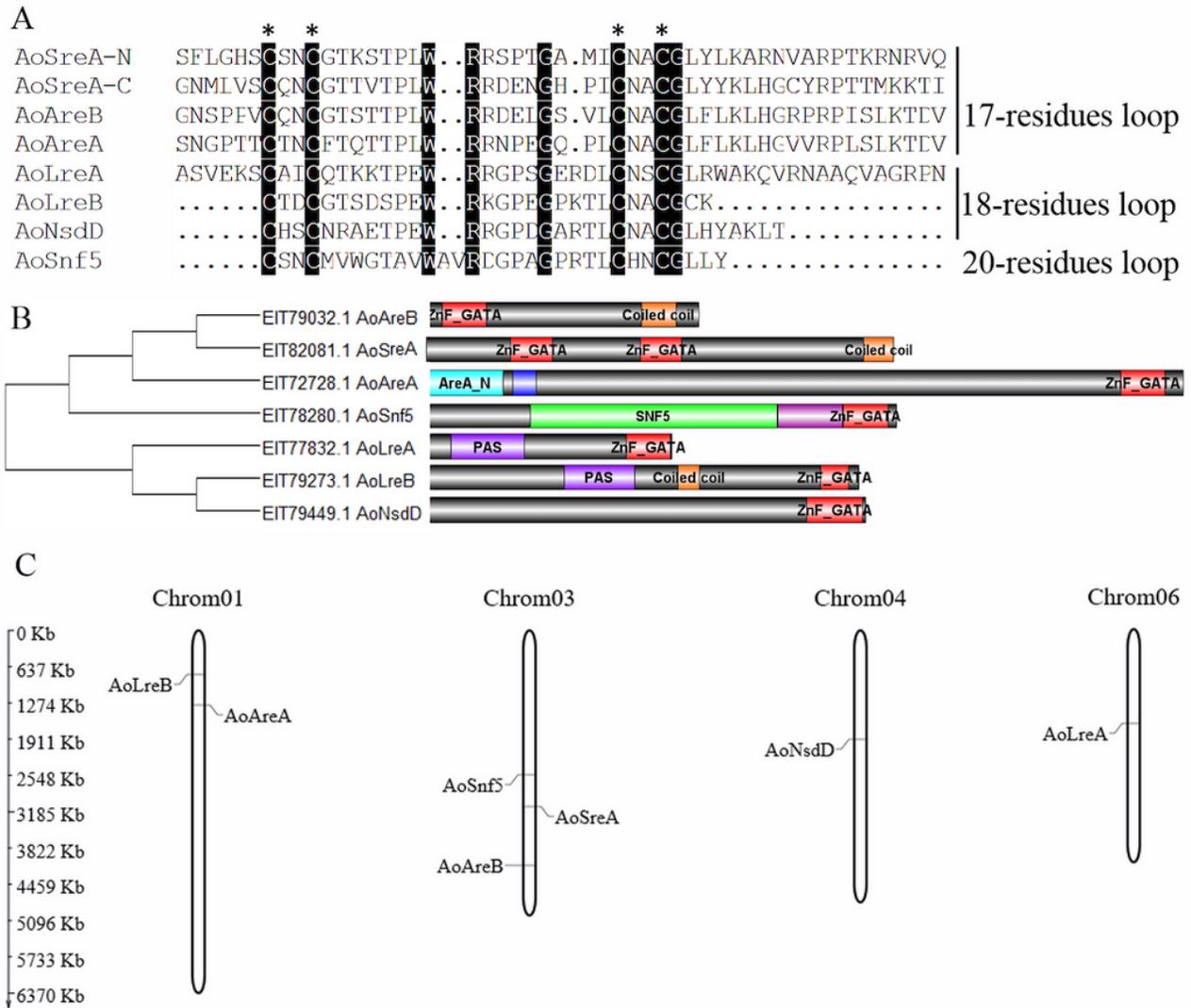


Figure 1

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.

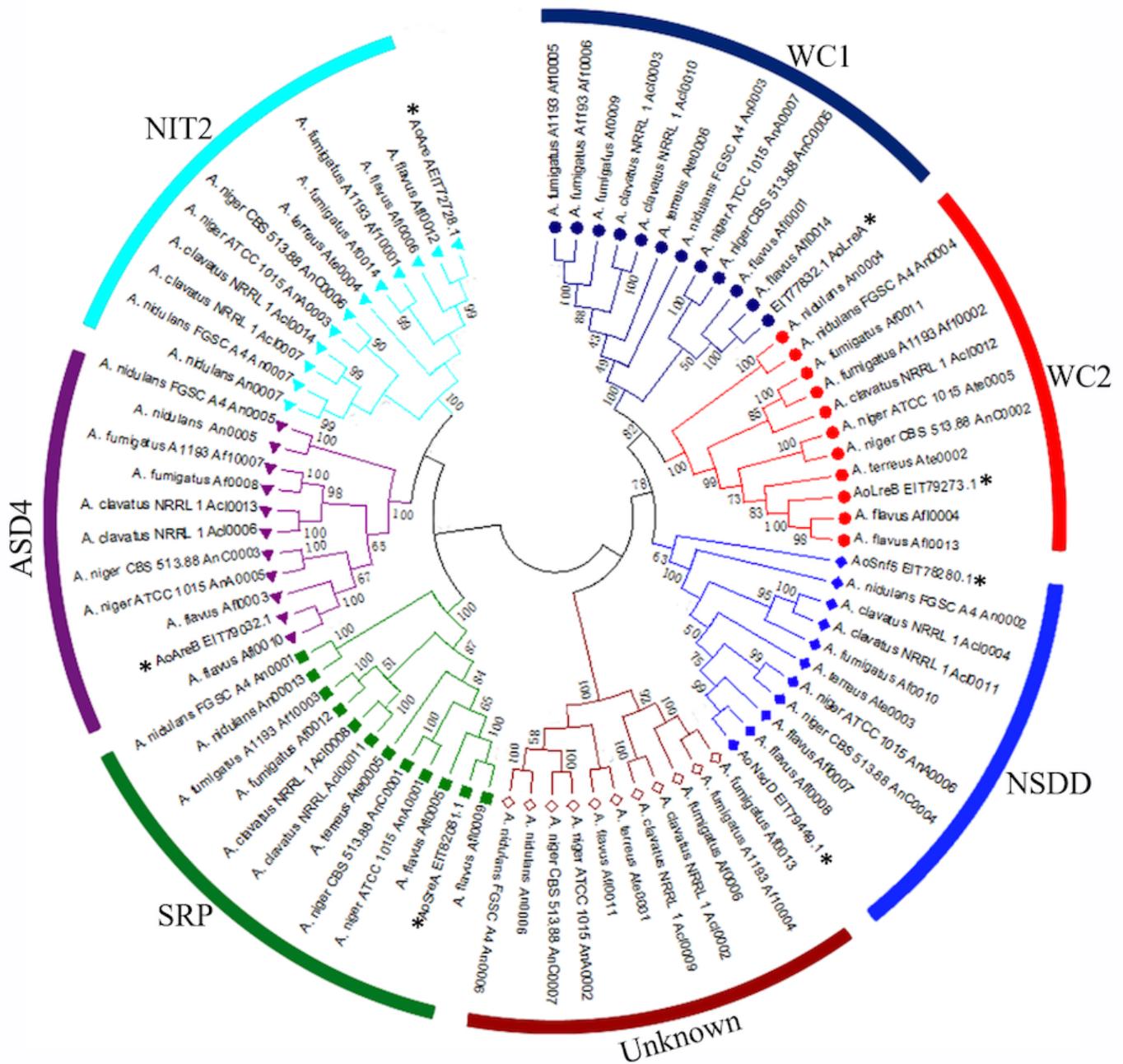


Figure 2

Phylogenetic analysis of *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. All the *Aspergillus* GATA TFs are classed into seven subgroups, including one group with unknown function, while the seven *A. oryzae* GATA TFs are scattered in six subgroups, and the novel AoSnf5 also clustered into NSDD subgroups together with AoNsdD.

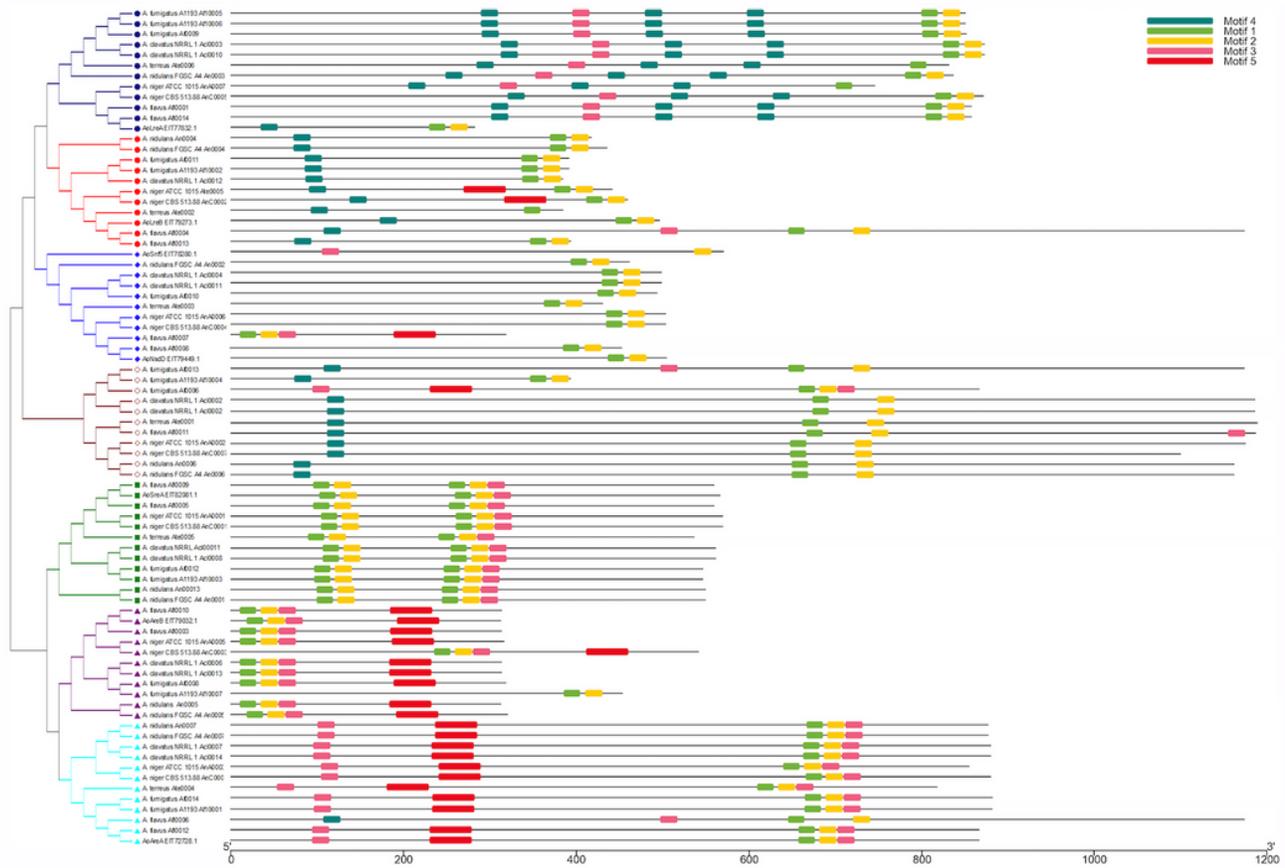


Figure 3

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.

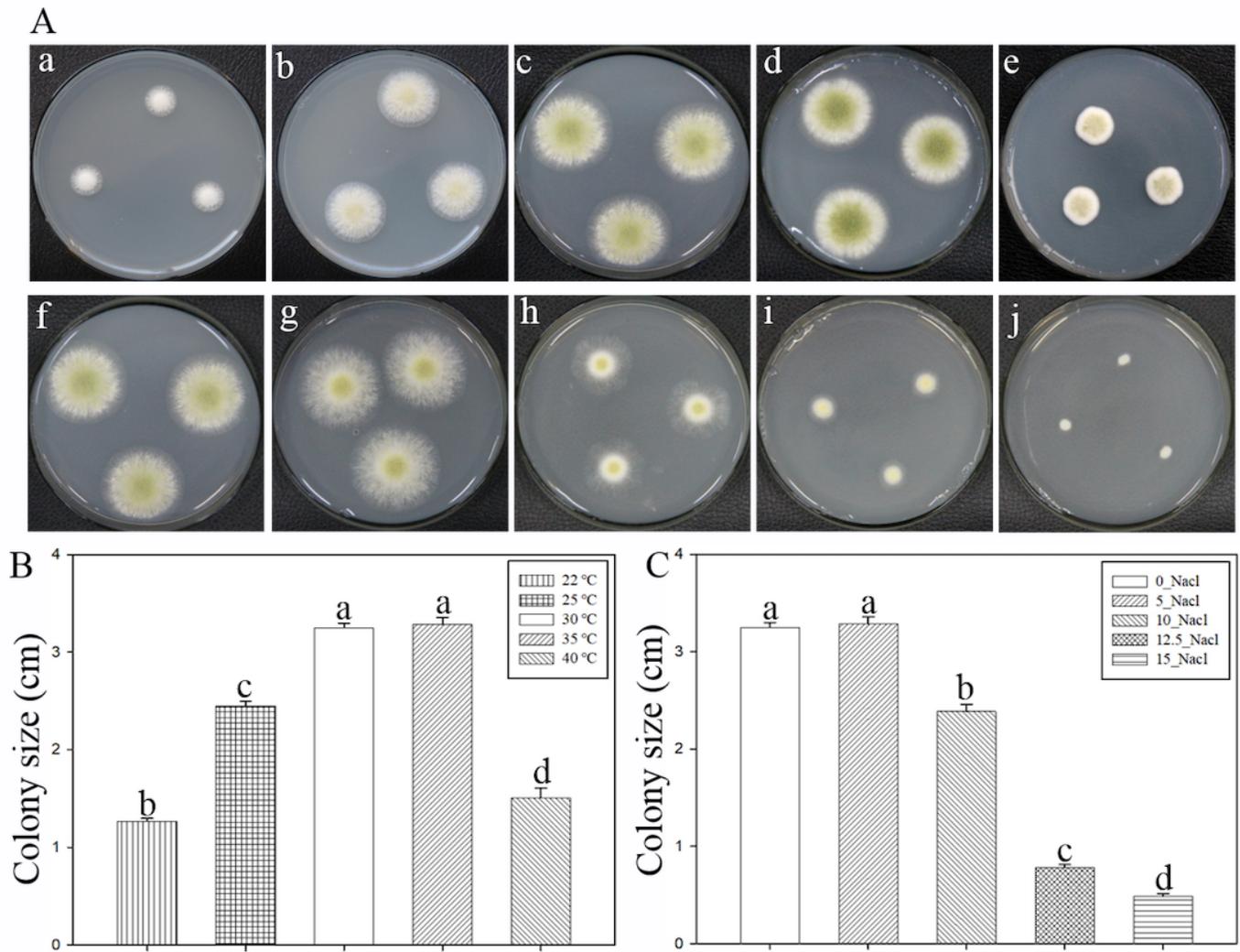


Figure 4

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 40 °C from left to right). (f-j) The NaCl concentration of 0, 5, 10, 12.5 and 15 mg/ mL were employed for salinity stress. (B) and (C) Colony size was determined by measuring diameter under different stress factors. The 30 °C, the optimum growth temperature of *A. oryzae*, was the control temperature (CK) in the experiment. The experiment of PDA medium without NaCl (0 mg/mL) used as the control (CK) under salt stress. Results represent the average of three repetitions \pm SEM. Different letters represent significant differences ($p < 0.01$, $n = 3$); the same letters represent no significant difference when compared with the control; significance was assessed using Duncan's multiple range test.

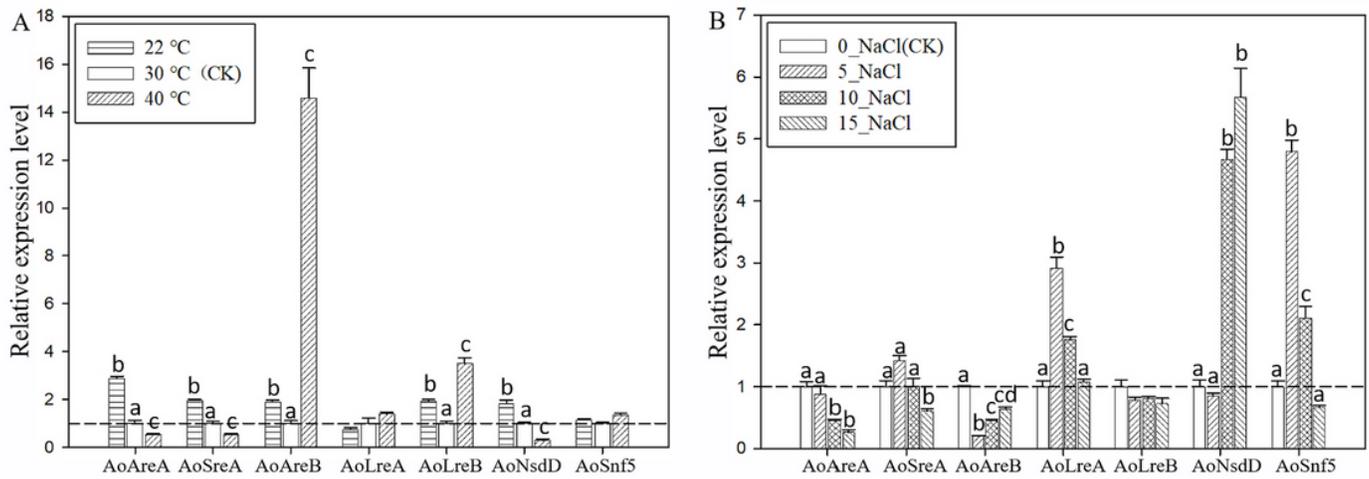


Figure 5

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 °C, the optimum growth temperature of *A. oryzae*, used as the control temperature (CK) in the experiment. The experiment of PDA medium without NaCl (0 mg/mL) used as the control (CK) under salt stress. Results show the average of three repetitions \pm SEM. Different letters represent significant differences ($p < 0.01$, $n = 3$); the same letters represent no significant difference when compared with the control; significance was assessed using Duncan's multiple range test.

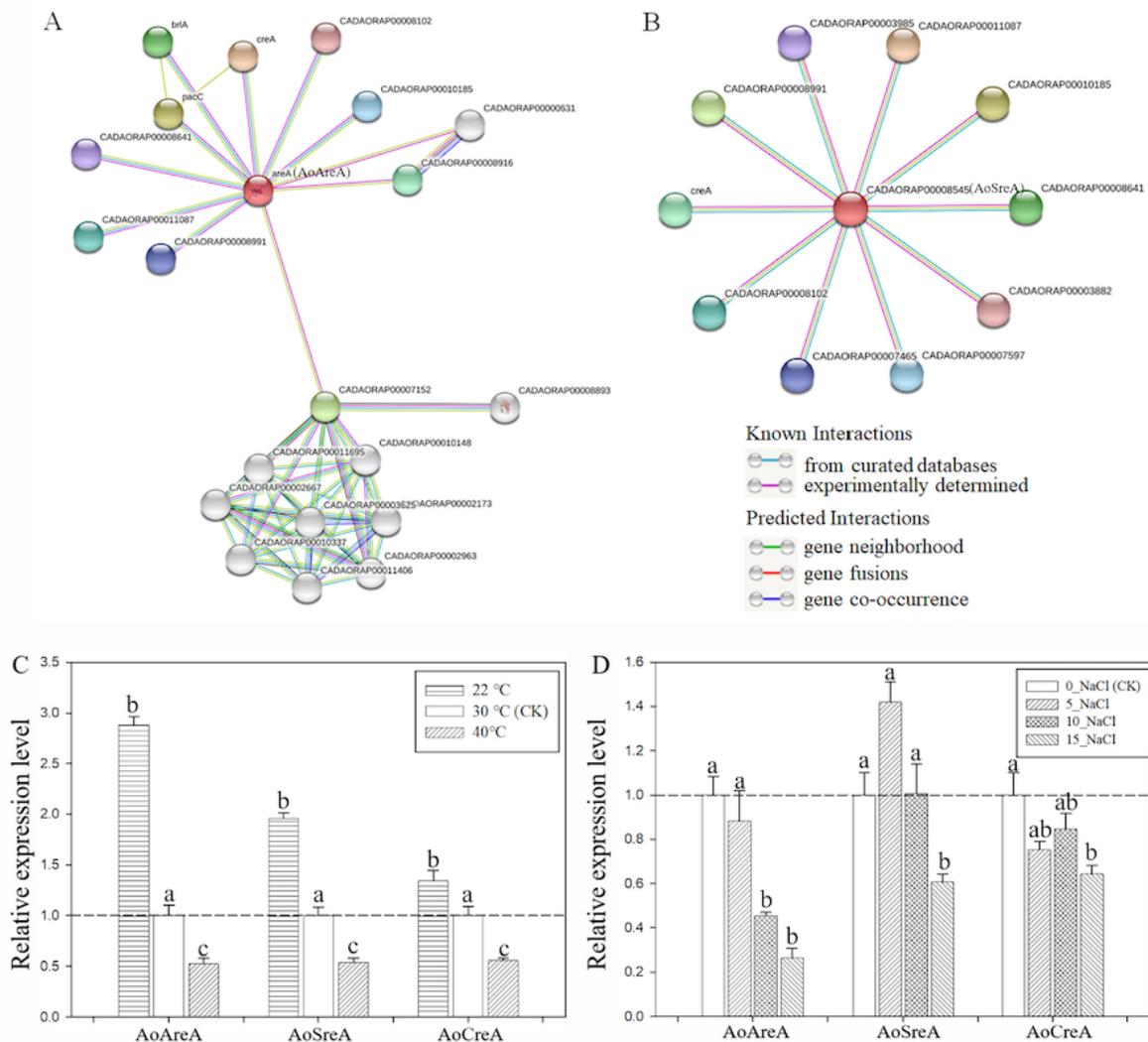


Figure 6

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 (0 mg/mL) 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.

Supplementary Files

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