Plant Omics Journal POJ

POJ 7(1):28-34 (2014) ISSN:1836-3644

Heterologous expression of a rice RNA-recognition motif gene *OsCBP20* in *Escherichia coli* confers abiotic stress tolerance

Chao Zhou†, Rong-Jun Chen†, Xiao-Ling Gao, Li-Hua Li, Zheng-Jun Xu*

Rice Institute, Sichuan Agriculture of University, Chengdu 611130, PR China

***Corresponding author: mywildrice@yahoo.com.cn**

†These authors contributed equally to this work.

Abstract

Diverse RNA-binding proteins (RBPs) have been determined to play a crucial role in post-transcriptional regulation of RNA metabolism during plant response to abiotic stresses. In this report we characterize a rice (*Oryza sativa L.*) protein, named OsCBP20, which is similar to subunits of a nuclear cap-binding protein 20 (CBP20), it contains a canonical RNA recognition motif (RRM). We compared the survivability of *Escherichia coli* (BL21) cells transformed with a recombinant plasmid with control *E. coli* under different concentration NaCl and mannitol. In addition, we also investigated the high temperature (50℃) impact on survival of *E. coil.* The *OsCBP20* gene was isolated from rice (*Oryza sativa* L.) cDNA, encoding protein of 243 amino acids with a calculated molecular mass of 28.6 KDa and a PI of 5.14. On the basis of multiple sequence alignment and phylogenetic analysis, *OsCBP20* is classified in RRM family. Recombinant OsCBP20 protein can be highly expressed in *E. coli*. The OsCBP20 protein can enhance the tolerance of *E. coli* recombinant to high salinity, heat, and dehydration, which suggested that OsCBP20 protein, may play a protective role under stressed conditions. Our work provides new evidence on CBP20 response to abiotic stresses.

Keywords: CBP20, RBPs, Recombinant *E. coli* cell, Abiotic stresses.
Abbreviations: CBC_cap-binding complex; CBP20_cap-binding protein 20; GRP_glycine-rich RNA-binding protein; KH_-K-homology; PAZ_Piwi/ Argonaute/ Zwille; RBD_RNA-binding domain; RBP_RNA-binding protein; RGG_arginine-rich; RNP_heterogeneous ribonucleoprotein; RRM_RNA recognition motif; SR_Serine-Arginine; ZF_zinc finger;

Introduction

Plant growth and development is mainly affected by the abiotic stresses, including cold, drought, high temperature and high salinity. Global climate change, therefore, has a significant impact on rice production. In order to respond to multiple abiotic stress factors, plants make changes of physiological, molecular and biochemical to adapt to the environment. Under stresses, the plants can up-regulate or down-regulate many stresses-related genes to improve stress resistance (Urano et al., 2010). Over the past few years, it has been reported that post-transcriptional gene regulation, such as RNA processing, export, localization, degradation and translational control, plays an important role in the signaling pathways of the complex response of plant to abiotic stress (Ambrosone et al., 2012).

The eukaryotes nuclear cap-binding protein composed of two subunits, which has the function of binding to the cap structure of RNA polymerase II transcripts (Izaurralde et al., 1994).The plant CBC also consists of two subunits: AtCBP20 and AtCBP80. AtCBP20 with a molecular mass of 29.9 KDa has 68 % and 53% identity compared to human orthologue and the yeast protein, respectively. The CBP80 subunit is larger, about 96.5KDa, and lower identity (28 and 50%) to human and yeast orthologue, respectively.AtCBP20 contains a RNA binding domain (RBD) and AtCBP80 contains protein-protein and protein-nucleic acid interaction domain, MIF4G (Kmieciak et al., 2002). Interestingly, neither CBP20 nor CBP80 on its own shows affinity for the cap or, in general, for RNA. Only a cap-binding complex (CBC), comprising both proteins, is able to recognize and bind to capped RNAs (Izaurralde et al., 1994). Moreover, loss of the cbp20 function also confers hypersensitivity to abscisic acid during germination, a significant reduction of stomatal conductance and greatly enhanced tolerance to drought in Arabidopsis thaliana (Papp et al., 2004). Recently, a report shown a new phenotype of the drought-tolerant cbp20 Arabidopsis thaliana mutant have changed epidermal morphology and disturbed miRNA metabolism and mRNA splicing of the mutant is discussed (Jäger et al., 2011).

In plants, RBPs have been identified containing one or more RNA-recognition motif (RRM) at the N-terminus and a variety of auxiliary motifs at the C-terminus. These auxiliary motifs such as glycine- rich, arginine-rich (RGG) and SR (Serine-Arginine) repeats are found in the RBPs (Albà and Pagès 1998). Typical RNA-binding domains including: RNA-binding domain (RBD, also known as RNP domain and RNA recognition motif (RRM)) ;RGG (Arg-Gly-Gly) box; Sm domain; DEAD/DEAH box; zinc finger (ZnF, mostly C-x8-X-x5-X-x3-H); K-homology (KH) domain (type I and type II); the Piwi/Argonaute/ Zwille (PAZ) domain; Pumilio/FBF (PUF or Pum-HD) domain; cold-shock domain; and double stranded RNA-binding domain (dsRBD) (Chen and Varani 2005). Biochemical studies show that RRM motifs are involved in RNA recognition and protein-protein interactions, and finally form the heterogeneous ribonucleoprotein (RNP) complexes (Maris et al., 2005).

Plant RNA-binding proteins (RBPs) have less attention than these of other organism, mainly due to the lack of suitable

plant-derived in vitro system to study the post-transcriptional regulation (Ambrosone et al., 2012). However, approximately 250 RBP genes have been identified in *Oryza sativa* (Cook et al., 2011), while the Arabidopsis thaliana contains more than 200 RBP genes (Lorković 2009).Some plants contain unique RBPs, it is likely to relate to plant specific functions (Bailey-Serres et al., 2009).

Recent studies showed that RBPs respond to many abiotic stresses. First discovered evidence that RBPs responded to stress in plant, MA16, a glycine-rich RNA-binding protein (GRP) induced by dehydration and ABA in maize embryos. MpGR-RBP1 protein belongs to the plant GR-RBP family, which members play important roles in the post-transcriptional regulation of gene expression under various stress conditions (Wang et al., 2011). The EgRBP42 transcript was induced by abiotic stresses, including salinity, drought, cold, heat stress and submergence (Yeap et al., 2012). The transcript level of a glycine-rich RNA-binding protein gene from Malus hupehensis is regulated by ABA (Wang et al., 2012). HnRNP-type nuclear RNA-binding proteins encoded by three UBA2 genes had been found, which involved in a novel wounding signals transduction pathway (Bove et al., 2008). The RNA-binding protein-defense related 1 (AtRBP-DR1) in Arabidopsis thaliana has been demonstrated that it is a positive regulator of SA-mediated immunity, possibly acting on SA signaling-related genes at post-transcriptional level (Qi et al., 2010). An ABA-mediated post-transcriptional regulation protein AKIP1,an RBP interacting with AAPK, an ABA-activated protein kinase, which controls stomata opening/closure (Jiaxu et al., 2002).The three AtRZ-1 family members, zinc finger-containing glycine-rich RNA-binding proteins, showing contribute differently to the enhancement of cold tolerance in Arabidopsis and E. coli (Kim et al., 2010). Tsn1 and tsn2, the RNA-binding protein Tudor-SN null mutants, have been proved that having great influences on germination, seedling growth, survival and fitness under high salinity stress in A. Thaliana (dit Frey et al., 2010).

In this research, a novel abiotic stress response gene *OsCBP20* is cloned from rice (*Oryza sativa* L.). Owing to its similar to CBP20 subunit, we named it OsCBP20. It encodes a putative RBP, and contains a single RRM. OsCBP20 was located in the nucleus in rice protoplast cells. OsCBP20 was induced by abiotic stresses, such as salt, ABA, high-temperature etc. Prokaryotic expression analysis showed that *OsCBP20* could enhance the survival rate of *E. coli* under high salinity, high temperature and osmotic stress. The results provide new evidences that CBP20 has a potential functional to contribute differently to the enhancement of abiotic stress tolerance.

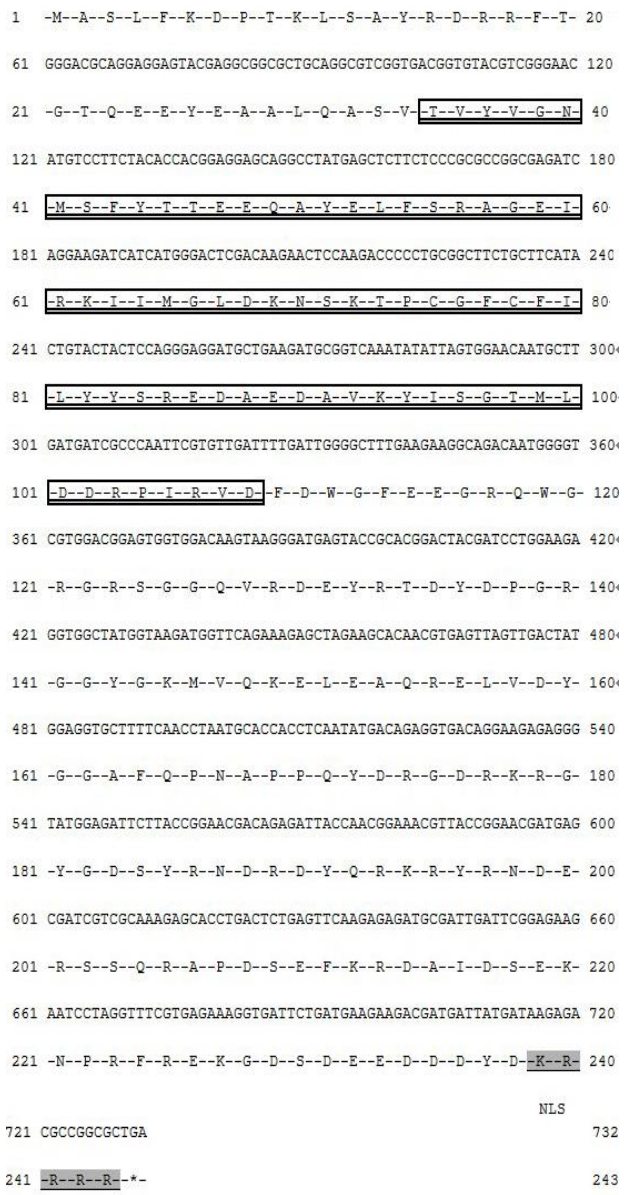

Fig 1. Nucleotide and deduced amino acid sequence of the *OsCBP20*. The stop codon (TGA) is indicated with an asterisk. The RNA-recognition motif (RRM) is *boxed*. The shaded area is forecast a nuclear localization signal (NLS).

Result

Cloning and sequence analysis of OsCBP20 cDNA

In our study, we successfully amplified the OsCBP20 full length cDNA by RT-PCR from rice (*Oryza sativa* L.) total RNA using specific primers. The full length of OsRBP1 cDNA was 1287bp; including a 732bp ORF which encoded a 243 residue polypeptide with a calculated molecular weight of 28.6 kDa and isoelectric point (pI) of 5.14. The OsCBP20 C-terminus contained a basic acid region (KRRRR) which played a role of a nuclear localization signal (NLS) and between 35th and 108th amino acid residues has a single RRM (Fig.1).

We made use of the Plant-CARE software online and predicted the promoter sequences, then analyzed of the DNA sequence of the translation start site upstream of the ATG 1500bp. The promoter sequence contains some putative stress-related *cis-acting elements* such as TATA-box, ABRE, CAAT-element, GC-motif (Table 1). An ABRE (cis-acting element involved in the abscisic acid responsiveness), and an O2-site (cis-acting regulatory element involved in zein metabolism regulation), and two ARE (cis-acting regulatory element essential for the anaerobic induction) , and two ERE (ethylene- responsive element), and two GC-motif (enhancer-like element involved in anoxic specific inducibility), and two TC-rich repeats (cis-acting element involved in defense and stress responsiveness). These stress-related cis-acting elements may be responsive for stress-regulated expression of *OsCBP20*.

Phylogenic analysis of OsCBP20

By query GRAMENE and NCBI database, we found 14

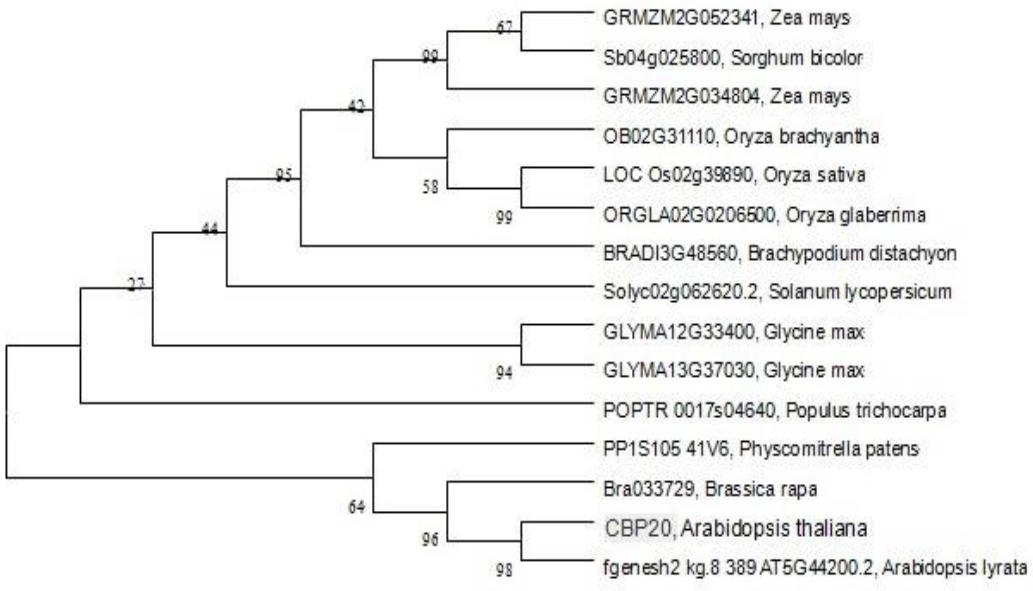

Fig 2. The phylogenetic analysis of the CBP20 from rice and other plant species. The tree was constructed with the MEGA program with amino acid sequences of plant RRM-containing proteins. *Branch numbers* represent a percentage of the bootstrap values in 1,000 sampling replicates.

OsCBP20 homologous proteins. Through the multiple sequence alignment and Phylogenetic analysis; we build a homologous evolutionary tree (Fig. 2). Phylogenetic analysis revealed that OsCBP20 (LOC Os02g39890, *Oryza-sativa*) was clustered closely to *Oryza- glaberrima* RRM-containing protein (ORGLA02G0206500, *Oryza- glaberrima*). In addition, its deduced amino acid sequences show high identity with the sequences of RRM-containing protein of other species, Oryza_brachyantha RRM-containing protein, *Sorghum bicolor* RRM-containing protein and Zea mays RRM-containing proteins. These results indicate that they may have similar functions in different species.

Expression and Analysis of OsCBP20 fusion protein in recombinant E. coli

OsCBP20 was expressed in E. coli and confirmed by SDS-PAGE. The recombinant protein was induced after 2 h of isopropylthio-β-D-galactoside (IPTG) treatment and reached maximum levels at 6 h as confirmed by SDS-PAGE. According to the construction of recombinant plasmid, the size of the proteins deduced from of the BL/pET32 and BL/OsCBP20 cells should be about 21kDa and 49kDa, respectively. Escherichia coli BL21, induced by 1mM IPTG, produced a molecular mass of about 21 kDa of *TrxA* intein protein (Fig. 3, lane 2). A specific band about 50kDa of *TrxA-OsCBP20* fusion protein was detected, which contained a 21kDa band of Trx tag and a 28.6kDa band of *OsCBP20* (Fig. 3, lane 4). These results showed the efficient expression of the *OsCBP20* fusion protein in *E. coli*.

Overexpressed OsCBP20 in E. coli enhanced resistance to abiotic stresses

The recombinant plasmid and pET32a empty vector was transformed into *E. coli* BL21. In order to study the tolerance to high-salinity stress, BL/OsCBP20 and BL/pET32a cells were properly diluted and spotted on plates supplemented with 100 to 600 mM NaCl. The viability of BL/OsCBP20 cells was significantly greater than that of BL/pET32a cells under various NaCl concentrations. Also the viability of BL/OsCBP20 cells was about 3-fold higher than the viability of the control cells in media supplemented with 500 mM NaCl. When NaCl concentration was increased to 600 mM in the mediums, approximately 10% of the BL/OsCBP20 cells survived versus 2 % of the control (Fig. 4A). These results suggest that the expression of *OsCBP20* gene increased the salt tolerance of *E. coli* cells.

To determine the effect of the over-expression of the OsCBP20 protein on the growth of *E. coli* recombinants under high-temperature stresses, cultures of BL/OsCBP20 and BL/pET32a were induced by IPTG, and then spot assay and survival ratio were performed. The results of the spot assay showed that the numbers of BL/OsCBP20 colonies were much greater than those of BL/pET32a at 50℃ (Fig.4B). After 0.5 hour treatment, the BL/pET32a cells and BL/OsCBP20 cells have obviously decreased. Although survival ratio decreased rapidly in both cells upon heat shock, BL/OsCBP20 cells were about 2-fold more than the control cells after 1h heat treatment. After 1.5h treatment, almost all of the BL/pET32a cells were dead, which only 5% of the BL/OsCBP20 cells stayed alive. This result clearly showed that *OsCBP20* gene has significantly induced the tolerance to high-temperature stress.

Similarly, we study the survival ratio of BL/OsCBP20 and BL/pET32a on plates with different concentrations of the mannitol. The result showed the same trend with high- salinity experiment. The survival ratio of BL/OsCBP20 and BL/pET32a was almost no difference at 500 nM, but the significant difference was performance at 1M. This result indicated that *OsCBP20* gene enhanced the tolerance to the dehydration stress.

Experiment evidence here conformed that *OsCBP20* is important to BL/*OsCBP20* cell survival during high saintly, high temperature and osmotic stress.

Discussion

Plant have a series of fine mechanisms for responding to environmental changes, these mechanisms are involved in many aspects of physiology, biochemistry, genetics, development, and molecular biology, in which the adaptive machinery related to molecular biology is the most important (Shao et al., 2007). RBP shows its normal cellular functions, but

Table 1. Distribution and sequences of stress-related cis-acting elements in the promoter region of *OsCBP20*.

	Position	Sequence (CAPITALS: core sequences)
ABRE	-986 to -996	GCAACGTACA
WUN-motif	-523 to -531	AAATTTCCT
ARE	-1276 to -1285	TCATTACGAA
	-876 to -882	TGGTTT
TC-rich repeats	-892 to -898	TGGTTT
	-228 to -237	ATTTTCTCCA
ERE	-480 to -489	GTTTTCTTAC
	-400 to -408	ATTTCAAA
	-896 to -904	ATTTCAAA
GC-motif	-15 to -23	GCCGCGCG
	-115 to -121	CCCCCG
O2-site	-995 to -1004	GATGATATGG
P-box	-535 to -542	CCTTTTG

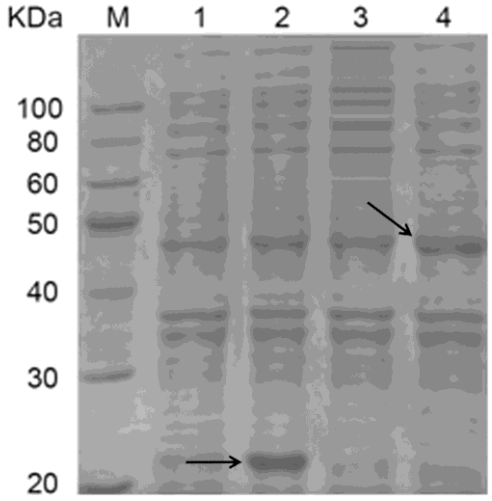

Fig 3. SDS-PAGE analysis of *OsCBP20* overexpression in *E. coli BL21*. *M* marker, *lane 1* untreated BL/pET32a, *lane 2* BL/pET32a induced by IPTG (arrow mark is 21 kD), *lane 3* untreated BL/*OsCBP20*, *lane 4* BL/*OsCBP20* induced by IPTG (arrow mark is about 50 kD).

also reflects the broad post-transcriptional regulatory events that were important to plant respond rapidly to changes in environmental condition. Here, we isolated a putative RBP from rice (*Oryza sativa* L.), subsequently, we analyzed its promoter region and many stress-related cis-acting elements were found. Therefore, we guessed that the gene might be activated by a variety of stresses. The resistance experiments show that the over-expression the *OsCBP20* protein enhanced the tolerance of *E. coli* recombinants to diverse stresses: high-temperature, high-salinity, and osmotic stress.

The most functional studies of RBPs have been focused in bacteria subjected to low temperature. The function of RBPs in cold response/adaptation has been known clearly, but less known on high temperature. Recently, Chandan Sahi and his co-authors reported that a small part of heat-inducible plant GRPs had been identified such as Osgrbp4 (Sahi et al., 2007), and they may be involved in regulating RNA export, stability or translation under stress. In yeast, HRP1 response to hyper-osmotic stress and heat shock (Henry et al., 2003). When plants were subjected to temperature stress, they could rapidly activated splicing mechanisms produce new mRNAs coding protein to adapt to change of environment condition. It has been reported that the expression of Arabidopsis Serine/Arginine-rich (SR) proteins could be altered by heat stress, which modifies the splicing of SR pre-mRNAs (Palusa et al., 2007). Previous report indicated that a cold shock domain protein (CSDP), CSDP1, and a glycine-rich RNA-binding protein (GRP), GRP7, had DNA melting activity and enhanced RNase activity. Moreover, both of them exhibit RNA chaperone activity during the cold adaptation process (Chen et al., 2009). Some review elaborates on the response networks of heat stress in plant, including the Hsf and Hsp response pathways, the response of ROS and the network of the hormone (Qu et al., 2013). In the present study, the *OsCBP20* protein could enhance a survival rate of *E. coil* under a high-temperature stress. We hypothesized that *OsCBP20* could change the Hsf and Hsp pathway, so that the recombinant cells less sensitive to temperature, and the accurate molecular mechanism is not clear enough. On the other hand, a possibly should be that changing RNA translation and creating a novel protein to increase the recombinant cells tolerance to high-temperature. OsCBP20 protein exactly function was still unclear and further investigation will be required on the role of OsCBP20 protein under stress conditions.

It is well known that ABA is an important mediator in triggering the plant response to osmotic stress, and both ABA-dependent and ABA-independent signaling pathway involved in osmotic stress (Verslues et al., 2006, Zhu 2002). Osmotic stress may trigger oxidative stress in plant, generating the formation of reactive oxygen species (ROS). In order to overcome oxidative stress, plants have developed two main antioxidant defense mechanisms that can be classified as non-enzymatic and enzymatic systems (Jaleel et al., 2009). Our experiment showed that OsCBP20 could increase the tolerance of *E. coli* to mannitol. Thus we hypothesized that *OsCBP20* may change the enzymatic systems in *E. coli*, in addition, partly involved in the ABA signaling pathway and plays a marginal role. Future experiment should be committed to exploring the role of the ABA on *OsCBP20*.

Recently study has been reported that the transcript levels of OsDEG10, coding a RNA-binding protein, has been up-regulated under high salt condition (Park et al., 2009). In our result that OsCBP20 apparently enhanced the survival rate of *E. coli* on LB plate supplemented with NaCl. The salt tolerance recombinant bacteria show that the *OsCBP20* gene may be involved in the function of the protective protein, cellular membrane, and cells to avoid damage in the host cells. This may be due to having the common protective mechanisms in eukaryotes and prokaryotes under stress conditions (Yun and

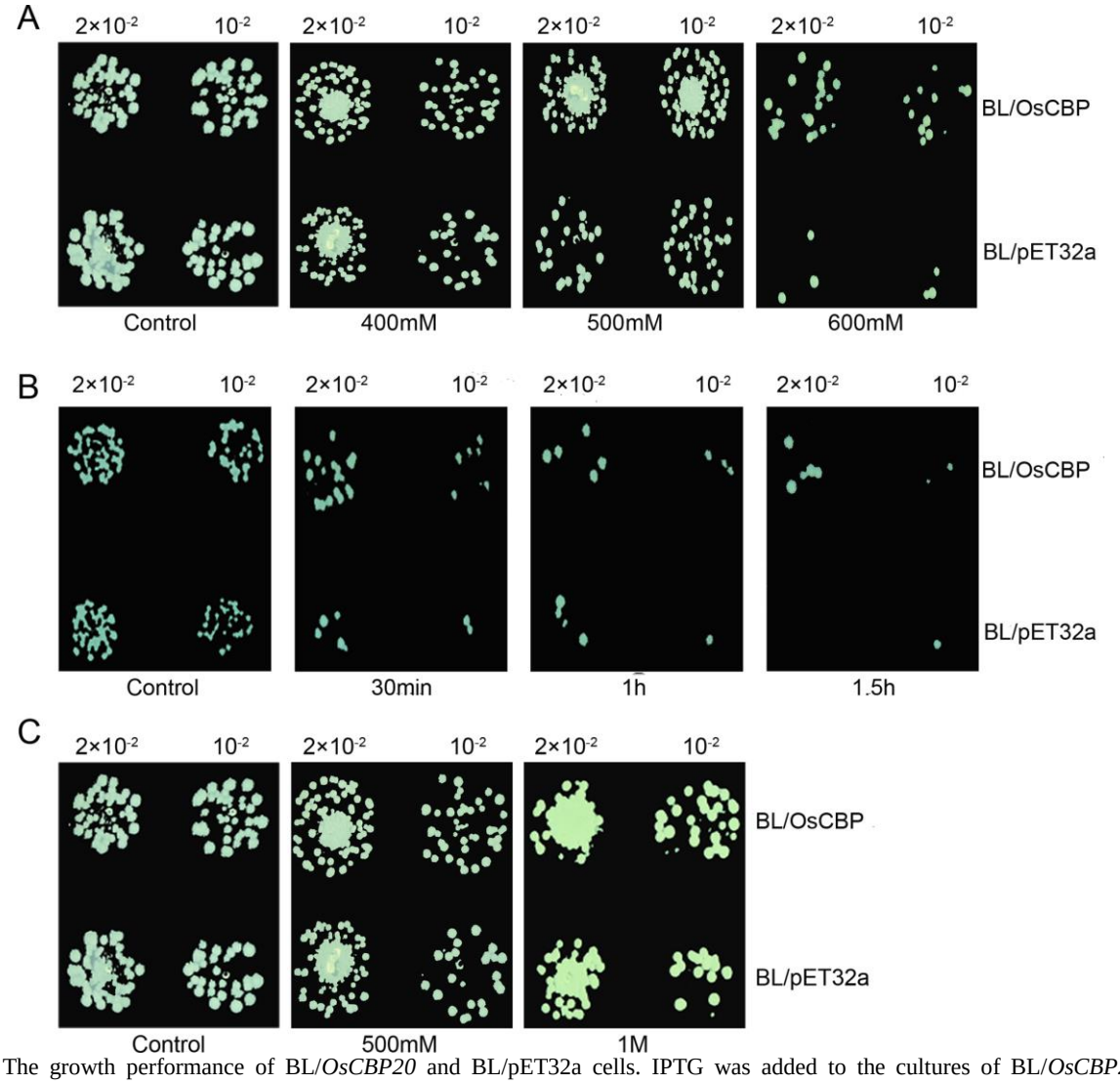

Fig 4. The growth performance of BL/*OsCBP20* and BL/pET32a cells. IPTG was added to the cultures of BL/*OsCBP20* and BL/pET32a to induce the expression of recombinant protein. The cultures were adjusted to OD600 = 1.0. Ten microliters from 2×10-2 to 10-2 dilutions were spotted onto LB plates with 1M IPTG. A) Spot assay of BL/*OsCBP20* and BL/pET32a on the LB plates with different concentration of NaCl. B) The impact of high temperature on the growth of BL/*OsCBP20* and BL/pET32a recombinant cells. When OD600 of the cultures reached 1.0, then 1ml culture was placed in 50℃ water bath for 30min, 1h and 1.5h, respectively. C) To explore the impact of BL/*OsCBP20* and BL/pET32a recombinant cells to osmotic stress. LB plates supplemented with 500mM and 1M concentration of mannitol.

Zheng 2005). Prokaryotes are sensitive to their environment, and their genetic activity is controlled by specific proteins that interact directly with their DNA to quickly adjust to environmental changes. The molecular biology behind transcription and translation is quite different between prokaryotes and eukaryotes. However, classes of gene organization in these organisms may reflect similar mechanisms selecting for their persistence. In some cases, modes of gene expression may be quite different (e.g. transcription of genes of the lac operon versus theβ -globin gene cluster) and the gene cluster may have been arisen by different means，but the selection maintaining the organization is similar. Alternatively, expression of gene clusters may appear to be mechanistically similar (transcription of the polycistronic lac operon in *E. coli* and GDF-1 locus in human), although the selection maintaining these organizations may differ (Lawrence 2002).

The complex mechanisms of plant response to environmental stress have been studied on stress-related gene transcription changes. The largest group of single strand RNA-binding protein is the eukaryotic RNA recognition motif (RRM) family that contains an eight amino acid RNP-1 consensus sequence (Query et al., 1989). RBPs have diverse function in RNA biology. *OsCBP20* has only one RRM motif, and likely through post-transcriptional regulation and sever as molecular chaperones able to regulate the intracellular and molecular fate of RNA partners, and help plant prevent or overcome unfavorable conditions.

Materials and Methods

Plant Material and abiotic treatments

The seeds of rice (*Oryza sativa* ssp. *japonica*) germinated in the incubator at 37℃. After, the seedlings were transferred to hydroponic solutions in culture. The 14 -day-old seedlings were used for the treatment of a variety of abiotic stresses, including salt, cold, heat, desiccation, ABA, GA_3, IAA treatment. The seeding were transformed to hydroponic solutions supplemented with NaCl (250mM), ABA (100μM), GA3

(100μM), IAA (100μM) for 0, 0.5, 1, 2, 4, 8, 16, 24h. For heat stress, the seedlings were transferred to the 42℃ of the incubator and cold stress, the seedlings were transferred to the 4℃ of the incubator for 0, 0.5, 1, 2, 4, 8, 16, 24h.For drought treatment, the seedlings were placed in the test rig for natural drought for 0, 15min, 0.5, 1, 2, 4, 8, 16h.

RNA Isolation and Reverse Transcription (RT)

Total RNA from rice roots, shoots and leaves were extracted from the seedlings of the multi - stress treatment using Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol. Subsequently, the RNA was treated with DNaseI (TaKaRa, Japan) to remove the residual genomic DNA. Preferably with 2 μg of RNA synthesized of first strand cDNA Using RT system (TOYOBO, Japan).

Isolation of OsCBP20

OsCBP20 full-length cDNA sequences obtained from the database GRAMME. The forward primers 5'AAAACCCTAATCGCAAACAGC 3' and reverse primers 5'CACAAAATCCATTCCCAGTCG 3' were designed for isolation of OsRBP1 cDNA from rice (*Oryza sativa* L.). The PCR was performed using cDNA as template, 10pM of primers, 200μM dNTPs, 2.5 U TransStartTM FastPfu DNA Polymerase in 50 μl reaction. PCR condition were 94℃, 3 min, 1 cycle; 94℃, 20 sec; 60℃, 20 sec and 72℃, 30 sec 30 cycles and last 72℃, 5min, 1 cycle. The PCR product was purified and cloned into pEASY-Blunt vector (TransGen, China) for sequencing.

Phylogenetic Analysis

The phylogenetic tree constructed using the neighbor-joining algorithm with 1,000 bootstrap trials by MEGA software (ver. 5.05) by protocol previously described in detail by Jiang YY (Jiang et al., 2012)

Construction of expression vector pET32a-OsCBP20

In order to construct the expression vector pET32a-OsCBP20, We designed primers add *BamH*I and *Hind*III restriction enzyme sites: 5'GGATCCATGGCGTCCCTCTTCAAGGAC 3' and 5'AAGCTTTCAGCGCCGGCGTCTCTTATC 3'. Restriction enzyme recognition sites are underlined. The PCR amplification product was ligated into the pET32a vector at *BamH*I - *Hind*III site to express *OsCBP20* protein fused with Trx•Tag™ thioredoxin at the N terminus. The recombinant plasmid pET32a-OsCBP20 was transformed into *E. coli* BL21. The BL21 cell with pET32a-OsCBP20 plasmid was named as BL/*OsCBP20*.Containing the pET32a plasmid of BL21 cells as blank control was named BL/pET32a.

Expression of OsCBP20 protein in E. coli

Both BL/*OsCBP20* and BL/pET32a were cultured overnight at 37℃ in Luria–Bertani (LB) agar medium supplemented with 100 mg/L ampicillin. Then,a pick single colony was inoculated into fresh LB with 100 mg/L ampicillin with gentle shaking at 37℃. When the optical density at 600nm (OD600) reached 0.5, IPTG was added into cultures to a final concentration of 1mM and continue to grow 4-6hours to induce the target protein in the recombinant cells. The cell cultures were centrifuged at 13,000×g for 2 min, and discarded the supernatant. Precipitate was added PBS and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) sample loading buffer (5×), then placed in a boiling water bath and boiled for 10min. The samples were separated on 12% SDS-PAGE. Finally, gels were stained with coomassie brilliant blue and destaining solution decolorization.

Assay for abiotic stress tolerance of E. coli Transformants

The BL/*OsCBP20* and BL/pET32a were used for stress tolerance assay. IPTG induction and cell culture described the same as above. A final concentration of all induced cultures, OD600 value reached 1.0. For salt tolerance assay, the sample were diluted 50-fold , 100 -fold , 200-fold and then 10 μl of diluted sample was spotted on LB agar plates with 1mM IPTG and 100 mg/L ampicillin, and additional 100nM, 200nM, 300nM, 400nM, 500nM, 600nM concentration gradient of NaCl. For osmotic stress assay, the dilution of the sample and amount of spotting was consistent with the above, added 500nM, 1M concentration of mannitol into LB agar plates. For high temperature tolerance assay, 1-ml sample was placed in 50℃ water bath. The samples were taken every 30 min until 3 hour, and then 10 μl of different dilution fold sample were spotted on ITPG LB agar plates.

Conclusion

In this study, OsCBP20 was isolated and molecularly characterized. The function analyses of the OsCBP20 protein were performed in *E. coli.* The effective prokaryotic recombinant OsCBP20 expression system was established. The OsCBP20 protein was expressed in *E. coli* efficiently. The overexpression of OsCBP20 can improve the tolerance of recombinant *E. coli* under abiotic stresses including high salinity, high temperature and dehydration, suggesting that *OsCBP20* may play an important role in plant to adapt to adverse environments. Our results showed that CBP20 has a potential function to improve abiotic stress tolerance of plant.

Acknowledgments

This research was supported by National Natural Science Foundation of China (31271802) to Zheng-jun Xu.

References

Albà MM, Pagès M (1998) Plant proteins containing the RNA-recognition motif. Trends Plant Sci. 3 (1):15-21

Ambrosone A, Costa A, Leone A, Grillo S (2012) Beyond transcription: RNA-binding proteins as emerging regulators of plant response to environmental constraints. Plant Sci .182 (0):12-18.

Bailey Serres J, Sorenson R, Juntawong P (2009) Getting the message across: cytoplasmic ribonucleoprotein complexes. Trends Plant Sci. 14 (8):443-453.

Bove J, Kim C, Gibson C, Assmann S (2008) Characterization of wound-responsive RNA-binding proteins and their splice variants in Arabidopsis. Plant Mol Biol .67 (1-2):71-88.

Chen Cc, Liang Cs, Kao Al, Yang Cc (2009) HHP1 is involved in osmotic stress sensitivity in Arabidopsis. J Exp Bot .60 (6):1589-1604.

Chen Y, Varani G (2005) Protein families and RNA recognition. FEBS Journal. 272 (9):2088-2097.

Cook KB, Kazan H, Zuberi K, Morris Q, Hughes TR (2011) RBPDB: a database of RNA-binding specificities, vol 39. vol Database issue.

dit Frey N, Muller P, Jammes F, Kizis D, Leung J, Perrot Rechenmann C, Bianchi M (2010) The RNA binding protein Tudor-SN is essential for stress tolerance and stabilizes levels of stress-responsive mRNAs encoding secreted proteins in

Arabidopsis. Plant Cell .22 (5):1575-1591
Henry MF, Mandel D, Routson V, Henry PA (2003) The Yeast hnRNP-like Protein Hrp1/Nab4 Accumulates in the Cytoplasm after Hyperosmotic Stress: A Novel Fps1-dependent Response. Mol Biol Cell.14 (9):3929-3941.
Izaurralde E, Lewis J, McGuigan C, Jankowska M, Darzynkiewicz E, Mattaj IW (1994) A nuclear cap binding protein complex involved in pre-mRNA splicing. Cell. 78 (4):657-668.
Jäger K, Fábián A, Tompa G, Deák C, Höhn M, Olmedilla A, Barnabás B, Papp I (2011) New phenotypes of the drought-tolerant cbp20 Arabidopsis thaliana mutant have changed epidermal morphology. Plant Biol (Stuttg) .13 (1):78-84
Jaleel C, Riadh K, Gopi R, Manivannan P, Inès J, Al Juburi H, Chang-Xing Z, Hong Bo S, Panneerselvam R (2009) Antioxidant defense responses: physiological plasticity in higher plants under abiotic constraints. Acta Physiol Plant. 31 (3):427-436.
Jiaxu L, Toshinori K, Sona P, Carl KYN, Steven PG, Ken ichiro S, Sarah MA (2002) Modulation of an RNA-binding protein by abscisic-acid-activated protein kinase. Nature. 418 (6899):793-797.
Jiang YY, Chen RJ,Dong JL, Xu ZJ, Gao XL (2012). Analysis of GDSL lipase (GLIP) family genes in rice (Oryza sativa). Plant Omics. 5(4):351-358.
Kim WY, Kim JY, Jung HJ, Oh SH, Han YS, Kang H (2010) Comparative analysis of Arabidopsis zinc finger-containing glycine-rich RNA-binding proteins during cold adaptation. Plant Physiol Bioch. 48 (10–11):866-872.
Kmieciak M, Simpson CG, Lewandowska D, Brown JWS, Jarmolowski A (2002) Cloning and characterization of two subunits of Arabidopsis thaliana nuclear cap-binding complex. Gene. 283 (1–2):171-183.
Lawrence JG (2002) Shared Strategies in Gene Organization among Prokaryotes and Eukaryotes. Cell. 110 (4):407-413.
Lorković ZJ (2009) Role of plant RNA-binding proteins in development, stress response and genome organization. Trends Plant Sci .14 (4):229-236.
Maris C, Dominguez C, Allain FHT (2005) The RNA recognition motif, a plastic RNA-binding platform to regulate post-transcriptional gene expression. FEBS Journal. 272 (9):2118-2131.
Palusa SG, Ali GS, Reddy ASN (2007) Alternative splicing of pre-mRNAs of Arabidopsis serine/arginine-rich proteins: regulation by hormones and stresses. Plant J. 49 (6):1091-1107.
Papp I, mur L, Dalmadi á, Dulai S, Koncz C (2004) A mutation in the Cap Binding Protein 20 gene confers drought. Plant Mol Biol .55 (5):679-686.
Park HY, Kang IS, Han JS, Lee CH, An G, Moon YH (2009) OsDEG10 encoding a small RNA-binding protein is involved in abiotic stress signaling. Biochem Bioph Res Co. 380 (3):597-602.
Qi Y, Tsuda K, Joe A, Sato M, Nguyen LV, Glazebrook J, Alfano JR, Cohen JD, Katagiri F (2010) A Putative RNA-Binding Protein Positively Regulates Salicylic Acid–Mediated Immunity in Arabidopsis. Mol Plant Microbe In. 23 (12):1573-1583.
Qu AL, Ding YF, Jiang Q, Zhu C (2013) Molecular mechanisms of the plant heat stress response. Biochem Bioph Res Co. 432 (2):203-207.
Query CC, Bentley RC, Keene JD (1989) A common RNA recognition motif identified within a defined U1 RNA binding domain of the 70K U1 snRNP protein. Cell. 57 (1):89-101.
Sahi C, Agarwal M, Singh A, Grover A (2007) Molecular characterization of a novel isoform of rice (Oryza sativa L.) glycine rich-RNA binding protein and evidence for its involvement in high temperature stress response. Plant Sci. 173 (2):144-155.
Shao HB, Guo QJ, Chu LY, Zhao XN, Su ZL, Hu YC, Cheng JF (2007) Understanding molecular mechanism of higher plant plasticity under abiotic stress. Colloids Surf B Biointerfaces. 54 (1):37-45.
Urano K, Kurihara Y, Seki M, Shinozaki K (2010) 'Omics' analyses of regulatory networks in plant abiotic stress responses. Curr Opin Plant Biol. 13 (2):132-138.
Verslues PE, Agarwal M, Katiyar Agarwal S, Zhu J, Zhu J-K (2006) Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J. 45 (4):523-539.
Wang S, Liang D, Shi S, Ma F, Shu H, Wang R (2011) Isolation and Characterization of a Novel Drought Responsive Gene Encoding a Glycine-rich RNA-binding Protein in Malus prunifolia (Willd.) Borkh. Plant Mol Biol Rep. 29 (1):125-134.
Wang S, Wang R, Liang D, Ma F, Shu H (2012) Molecular characterization and expression analysis of a glycine-rich RNA-binding protein gene from Malus hupehensis Rehd. Mol Biol Rep. 39 (4):4145-4153.
Yeap WC, Ooi T, Namasivayam P, Kulaveerasingam H, Ho C-L (2012) EgRBP42 encoding an hnRNP-like RNA-binding protein from Elaeis guineensis Jacq. is responsive to abiotic stresses. Plant Cell Rep. 31 (10):1829-1843.
Yun L, Zheng Y (2005) PM2, a group 3 LEA protein from soybean, and its 22-mer repeating region confer salt tolerance in Escherichia coli. Biochem Bioph Res Co. 331 (1):325-332.
Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol. 53 (1):247-273.