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Full Length Article

Expression of Aquaporin *BnPIP*-like Gene from Rapeseed (*Brassica napus*) Enhances Salt Resistance in Yeast (*Pichia pastoris*)

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Abstract

A new aquaporin (AQP) *BnPIP-like* gene was isolated and sequenced from rapeseed (*Brassica napus* L.). It encoded a putative protein with 281 amino acids, sharing 95.3% identity with *Arabidopsis* plasma membrane intrinsic proteins (PIP, Genbank: NP_195236.1). Prediction of transmembrane structure showed that *BnPIP-like* gene contained five loops and six transmembrane helices. Online analysis also indicate that *BnPIP-like* protein existed as homo-tetramers. In order to research its functions in eucaryon, *BnPIP-like* gene was fused into the pPIC3.5K and then the recombinant vector, as well as the pPIC3.5K, were induced into methylotrophic yeast (*Pichia pastoris* Strain GS115), respectively. In BMMY media, the BnPIP-like protein was sufficiently expressed after methanol (MeOH) induction for 24 h. The salt stress (300 mM NaCl) treatment was operated after 18 h of normal growth conditions. The OD_{600} values of *BnPIP-like*-transgenic *P. pastoris* and the control were determined every 12 h. The growth curves were obtained and it showed that the growth of both *P. pastoris* with or without BnPIP-like protein was obviously inhibited. However, the concentrations of *BnPIP-like*-transgenic *P. pastoris* was always higher than the control, indicating that the inhibition of growth in *BnPIP-like*-transgenic *P. pastoris* was slighter because of the over-expression of *BnPIP-like* gene. Thus the resistance to salt explained the eukaryotic functions of BnPIP-like protein. Moreover, it provided a theoretical possibility for a more comprehensively industrial utilization of fermenting yeast. © 2016 Friends Science Publishers

Keywords: *Brassica napus*; Aquaporin; Salt resistance; Eukaryotic expression

Introduction

Salt stress usually interrupts the endocellular ionic or osmotic equilibrium, inhibits the growth and metabolisms in higher plants; secondarily it also results in consequent oxidative stress, or even death (Niu *et al*., 1995; Zhu, 2001), among which the inhibition of growth was the most significant. Salt stress is one of the most crucial abiotic stresses in agriculture. There are about 20% of the world's cultivated lands and nearly 50% of all irrigated lands are affected by it (Zhu, 2000; 2001). It lead to the reduction of plant growth and crop production (Munns *et al*., 2006). As to the rapeseed (*Brassica napus* L.), one of the most important oil-crops in the world, soil salinity affects its yield and quality characters significantly (Zadeh and Naeini, 2007; Zamani *et al*., 2010; Jian *et al*., 2014). There were some methods to solve the salinity problems. Besides irrigation with fresh water and improving soil drainage, studying salt tolerance in plant with a view to identify and eventually to manipulate the genes involved in salt resistance is another promising approach (Zhu, 2000).

Higher plants had different regulating metabolism pathways in response to salt stress, such as eliminating the reactive oxygen species (ROS), synthetizing the osmotic regulators, utilizing ATPase to maintain the Na^+ or Cl^- ion concentrations, expressing late embriogenesis abundant protein (LEA) and transporting H_2O molecules by aquaporin (AQP) etc. Aquaporin is a kind of integral membrane proteins, which transport water molecules across membrane selectively and efficiently. It is a member of major intrinsic protein (MIP) super-family (Zardoya, 2005). The first AQP protein, named CHIP28 or AQP1, was isolated from human red blood cell in 1988 (Denker *et al*., 1988); its function as membrane water channel in *Xenopus laevis* oocytes was confirmed in 1992 (Preston *et al*., 1992). One year later, the first plant AQP protein γ-TIP was isolated from *Arabidopsis* (Maurel *et al*., 1993). AQPs from higher plants such as tobacco, spinach, maize, rice, wheat etc. were cloned (Maurel *et al*., 2008; Ludewig and Dynowski, 2009), as well as some non-vascular plant (Danielson and Johanson, 2008). Besides plants, AQPs were also found in creatures: archaebacteria, bacteria, fungus,

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animals etc. Discovery and researches on AQPs overturned the long-standing theory that free diffusion driven by osmotic potential was the only way for water molecules to transport across the membrane. According to their sequence homology and sub-cellular location, plant AQPs could be subdivided in four subgroups: plasma membrane intrinsic proteins (PIPs): PIP1, PIP2 and PIP; tonoplast intrinsic proteins (TIPs), which further subdivided into 5 subgroups: α, β, γ, δ and ε-TIP; nodulin 26-like intrinsic proteins (NIPs); small and basic intrinsic proteins (SIPs, further subdivided into 2 subgroups: SIP1 and SIP2); GlpF-like intrinsic proteins (GIPs). Besides the water molecular transporting and consequent drought or salt tolerance, AQPs also took part in some other biological processes such as photosynthesis (Uehlein *et al*., 2003), flowering (Bots *et al*., 2005), seed germination (Schuurmans *et al*., 2003), seed maturing and so on; it also transports other small molecules like glycerol (Schuurmans *et al*., 2003), H_2O_2 (Bienert *et al*., 2007) and CO_2 (Uehlein *et al*., 2003).

In the present study, a new aquaporin (AQP) *BnPIP-like* gene was first isolated and sequenced from rapeseed. Prediction of transmembrane structure showed that BnPIP-like protein contains five loops and six transmembrane helices and online analysis also indicate that it existed as homo-tetramers. In order to study its functions in eucaryon, *BnPIP-like* gene was fused into the *pPIC3.5K*. After the recombinational plasmid was linearized, *BnPIP-like* gene was then induced into methylotrophic yeast (*Pichia pastoris* Strain GS115). After induced by methanol for 18 h in BMMY media, the BnPIP-like protein was sufficiently expressed. The salt stress (300 mM NaCl) treatment was operated. The OD_{600} values of *BnPIP-like*-transgenic *P. pastoris* and the control showed that both kinds of *P. pastoris* cells (with or without BnPIP-like protein) were obviously inhibited. However, the concentrations of *BnPIP-like*-transgenic *P. pastoris* was always higher than the control, indicating that inhibition of growth in *BnPIP-like*-transgenic *P. pastoris* was slighter because of the over-expression of *BnPIP-like* gene. Thus the resistance to salt explained the eukaryotic functions of BnPIP-like protein. Unlike previous research, here the *BnPIP-like* gene was induced into and expressed in yeast, rather than *Xenopus laevis* oocytes. Thus it also provide a possibility for industrial yeast to enhance their survive rate in tough conditions.

Materials and Methods

Materials and Vectors

Seeds of *Brassica napus* L. (cultivar JR9), eukaryotic expression vector pPIC3.5K and yeast cells (*Pichia pastoris* Strain GS115) were kept in Crop Research Institute, Sichuan Academy of Agricultural Sciences (SAAS). Intermediate cloning vector pEASY-T Simple, competent cell TOP 10 and high fidelity Trans Taq-T DNA polymerase were purchased from Transgen Biotech Company. RNA extraction kit and reverse transcription kit were purchased from TianGen Company. Seeds of *B. napus* were sterilized by 75% ethanol (EtOH) and 0.1% Hg_2Cl_2 successively, and then plated on MS solid medium (Murashige and Skoog, 1962). The aseptic seedlings were grown in climatic chamber at 24°C with 16/8 h of light/dark cycle and 60% humidity.

Cloning and Sequencing of *BnPIP-like* Gene

20 days after germination, the whole aseptic seedlings were used to extract the total RNA. Total RNA was prepared from these 20-days old seedlings using TianGen RNA extraction kit according to the instructions. Reverse transcription was performed with oligo-T primer, by Reverse Transcriptase kit in accordance with the instructions. Thus, the total cDNA was obtained. Then PCR was performed with specific primers PIP-1 and PIP-2 (PIP-1: 5'-ATGTCGAAAGAAGTGAGCGAAGA-3'; PIP-2: 5'-TCAGTTTGTTGCGTTGCTTCGGA-3'). The primers were designed by Primer Premier 5.0 with homologous cloning method, according to the sequence of *Arabidopsis* plasma membrane intrinsic proteins gene (PIP, Genbank : NP_195236.1). The PCR cycling procedure consisted of 2 min at 94°C, 33 cycles for 30 s at 94°C, 30 s at 52°C and 55 s at 72°C, and a final 15-min extension at 72°C. The PCR products were then purified from agarose gel using gel extraction and purification kit. After the fragment was ligated into the pEASY-T simple vetor, recombinant plasmid was induced into *Escherichia coli* DH5α. The positive colonies were then selected and sequenced.

Structural Analysis and Prediction of BnPIP-like Protein

After the fragment was sequenced, physical and chemical properties of nucleic acids and amino acids were analyzed by ProtParam (http://web.expasy.org/protparam/); while relative molecular weights (Mw) and theoretical isoelectric point (pI) were calculated by pI/Mw tool (http://web.expasy.org/compute_pi/); protein secondary structures were analyzed by NPS (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_hnn.html); prediction of transmembrane helices in protein was operated online by bioinformatics tool TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/). Simulative three-dimensional (3-D) model was made by comparative modeling online (http://swissmodel.expasy.org/). Prediction of subcellular location was analyzed online (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/).

Expression of *BnPIP-like* Gene in Rapeseed under Salt Stress

Plump and full seeds of rapeseed were selected to determine the transcriptional expression of *BnPIP-like* gene. After 3

days jarovization at 4°C, the seeds were germinated in soils-mixture of vermiculite: peat (1:1) in a climatic chamber at 25°C with 16/8 h of light/dark cycle and 60% humidity. 18 days after germination, the salt stress treatment was started: 300 mmol/L NaCl solution was sprayed to the seedlings and added into the soil sufficiently. The seedlings at the same size were picked at 0, 2, 4, 6, 8 and 20 h, respectively in order to extract the total RNA of the whole plant. The reverse transcription was performed as described above and then the qPCR was operated. Specific primers to detect the transcriptional expression of *BnPIP-like* gene was designed (Q-P1: 5'-CCCATTACCGGAACTGGAAT-3'; Q-P2: 5'-AACGGACCAACCCAGAAGAT-3') and the *ACTIN* gene (actin-F: 5'-TGGTGAAGGCTGGTTTTGCT-3'; actin-R: 5'-TTCTGACCCATCCCAACCAT-3') was used as an internal control and also amplified simultaneously from each sample. The qPCR cycling procedure consisted of 3 min at 95°C, 27 cycles for 30 s at 95°C, 30 s at 55°C and 30 s at 72°C and a final 5-min extension at 72°C. The qPCR cycling consisted of 1 min at 95°C, 40 cycles of 10 s at 95°C, 40 s at 56°C, 45 s at 72°C (data collection), followed by a melting curve procedure of 1 min at 95°C, 1 min at 56°C, 78 cycles for 56°C ramping to 95°C at the rate of 0.5°C/10 s. The qPCR was performed on a Bio-Rad iCycler fluorescence thermocycler (Bio-Rad, Hercules, CA). The fluorescence master mix reagent for the reaction was Sybr Green (Toyobo). All of the cycle threshold (Ct) values of *BnPIP-like* amplification were normalized by the corresponding *ACTIN* Ct values. Three parallel repeats were done and the results were summarized as averages and the standard deviation (SD). The data were analyzed and plotted using Microsoft Office software.

Expression of *BnPIP-like* gene Induced by Methanol in Yeast

Full-length *BnPIP-like* gene fragment was fused into eukaryotic expression vector pPIC3.5K with restriction sites BamH I and EcoR I. The recombinant plasmid pPIC3.5K-*BnPIP-like* gene was then induce into *E. coli* (TOP 10) competent cells. Colonies were picked up and cultured in LB media with ampicillin, followed by plasmid extraction and restriction enzyme digestion with BamH I and EcoR I in order to screen out the positive ones. Then the recombinant plasmid *pPIC3.5K-BnPIP-like* gene containing fusion genes was then linearized by restriction enzyme Sal 1, followed by electroporation transformation (1500 V, 5 ms) of competent yeast (GS115) cells; as well as the original pPIC3.5K plasmid. The cells were plated on RDB+G418 media at 30°C for 4~5 days and single colonies were respectively picked up and cultured in YPD liquid media with 5 antibiotics (ampicillin, kanamycin, streptomycin, chloramphenicol and tetracycline), followed by extraction of total DNA and consequently PCR in order to screen out the positive colonies. The PCR was performed with specific primers (AOX1-1:5′-GACTGGTTCCAATTGACAAGC-3′; AOX1-2: 5′-GCAAATGGCATTCTGACATCC-3′). The PCR cycling procedure consisted of 5 min at 94°C, 30 cycles for 30 s at 94°C, 30 s at 52°C and 1 min at 72°C, and a final 15-min extension at 72°C. Then the positive colonies were picked up and cultured successively in YPD, BMGY and BMMY (all with 5 antibiotics) media, in order to induce the protein expression with methanol. The protocol was according to Promdonkoy *et al*. (2014).

Determination of the Salt Resistance in *BnPIP-like*-Transgenic Yeast

Positive yeast cells, including *BnPIP-like*-transgenic yeast and control (transformed with original pPIC3.5K fragment integrated into the genome) was respectively cultured in YPD liquid media with 5 antibiotics (described above) at 30°C for 24 h. One mL germ solutions were then added into transitional BMGY media 5 antibiotics at 30°C for 24 h. One mL germ solutions were then added into inducing BMGY media 5 antibiotics at 30°C for 18~24 h, respectively. The OD_{600} values were determined and aseptic NaCl powder was added to 300 mM. The OD_{600} values were then determined every 12 h and the growth curves were obtained. Methanol was replenished every 12 h.

Results

The Sequence of *BnPIP-like* Gene

An 846 bp-length fragment was successfully obtained by RT-PCR and verified by the agarose gel electrophoresis (Fig. 1a) and then followed by extracting and sequencing. Online analysis showed that nucleic acid sequence of this fragment, named *BnPIP-like* gene, shared the highest identity (91.7%) with Arabidopsis plasma membrane intrinsic proteins (PIP, Genbank : NP_195236.1), which belongs to the aquaporin (AQP) family. *BnPIP-like* gene encoded a putative protein with 281 amino acids and their sequence of BnPIP-like protein showed 95.3% identity with *Arabidopsis* PIP (Fig. 1b). The sequences had the conserved domain SGXHXNPAVT of MIP super-family and the conserved domain GGGANXXXXGY and TGINPARSLGAA of PIP family, as well as the conserved domain NPA (Fig. 1b). Further alignment with known AQPs from rapeseed indicated that *BnPIP-like* gene had 64.97%, 65.31%, 31.93% and 74.56% identities with EU487188.1, EU487187.1, AF118381.1 and AF118383.1, respectively (data not shown). Thus, *BnPIP-like* protein was considered a new member of AQPs family.

The formula was $C_{1382}H_{2111}N_{351}O_{366}S_{10}$ with predicted theoretical pI of 8.99 and molecular weight of 29.8197 kDa. The instability index (II) was computed to be 30.39 and it classified the protein as stable. Prediction of transmembrane helices in protein was operated online by bioinformatics tool TMHMM Server v. 2.0, finding *BnPIP-like* gene contained five loops and six transmembrane helices (Fig. 2a). N-

Fig. 1: PCR product and sequence of BnPIP-like
(a) RT-PCR product of BnPIP-like in agarose gel electrophoresis. M: DNA marker DL2000; P: BnPIP-like; (b) Amino acid sequence alignment of BnPIP-like and *Arabidopsis* PIP (Genbank: NP_195236.1). The sequences with red underline stood for the conserved domain SGXHXNPAVT of MIP super-family and the conserved domain GGGANXXXXGY and TGINPARSLGAA of PIP family; red stars stood for conserved domain NPA

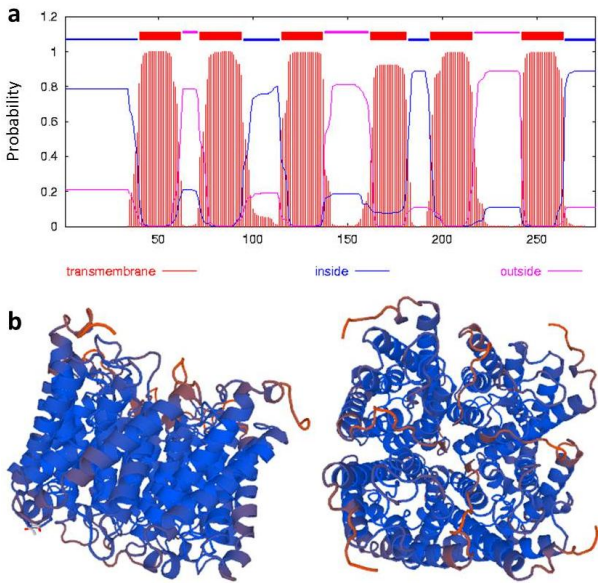

Fig. 2: Predicted structure of BnPIP-like
(a) Prediction of transmembrane helices; (b) 3D structure of BnPIP-like homo-tetramers by comparative modeling online

terminal and C-terminal were both intracellular, as well as loop B and loop D. Loop A, loop C and loop E were extracellular. Moreover, high conserved sequences "Asn-Pro-Ala" were found in both loop B and loop E (data not shown). Three-dimensional (3-D) model was made by comparative modeling online. It showed that BnPIP-like protein, like other PIPs in higher plant, existed as homo-tetramers (Fig. 2b).

Expression of *BnPIP-like* Gene in Rapeseed Seedlings Induced by Salt Stress

Salt stress inhibited the growth of plants and resulted in withering. 2 h after the 300 mMol/L NaCl solution was sprayed to the seedlings and added into the soil, the leaves

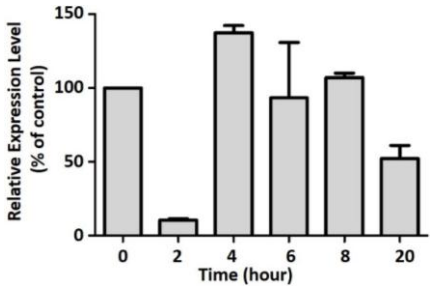

Fig. 3: Relative expression of BnPIP-like on transcriptional level under 300 mmol/L treatment for 0, 2, 4, 6, 8 and 20 h, respectively

and stems started to wither. Then whole seedlings were picked successively and total RNA was extracted and reverse transcription was operated. Relative expression on transcriptional level was determined by qPCR and it showed that the expression significantly suppressed (to about 15% of the control) 2 h after the salt stress started (Fig. 3). But after 4th h, the transcriptional level recovered to normal level as control. As the salt stress continued, the expression of *BnPIP-like* gene decreased again (Fig. 3).

Expression Induced by Methanol of *BnPIP-like* gene in Yeast

Restriction sites BamH I and EcoR I were used to fuse *BnPIP-like* gene fragment into vector pPIC3.5K with mictic tag at N-terminal and His tag at C-terminal (Fig. 4a). The recombinant plasmid pPIC3.5K-BnPIP was then induced into *E. coli* (TOP10) and consequently was extracted from positive clones and checked by double-restriction enzyme (BamH I and EcoR I) digestion. Agarose gel electrophoresis (Fig. 4b) showed that a big fragment about 9 kb and a small fragment about 1 kb were obtained as expected, and sequencing result also indicated it correct (data now shown). Different to *E. coli*, the electroporation transformation of competent yeast cells needed to linearize the expression vector first. So restriction enzyme Sal I was utilized and the cells were treated at 1500 V for 5 ms. Four days after growing on RDB+G418 media at 30°C, clones were picked and cultured in YPD liquid media with 5 antibiotics. Then the PCR was operated and the clones obtaining 1 kb band on agarose gel were considered positive ones (Fig. 4c). The positive yeast cells were cultured in YPD, BMGY and BMMY successively and the target protein was induced by 0.5 methanol after 24 h extracted and then checked on PAGE. The target protein was found successfully expressed (Fig. 4d).

Expression of *BnPIP-like* Gene in Yeast Improved the Cell Survival Ratio under Salt Stress

The transgenic yeast and control shared the same OD_{600} values at the 18th h and then the salt stress treatment started. Under 300 mM NaCl salt stress, both transgenic yeast and

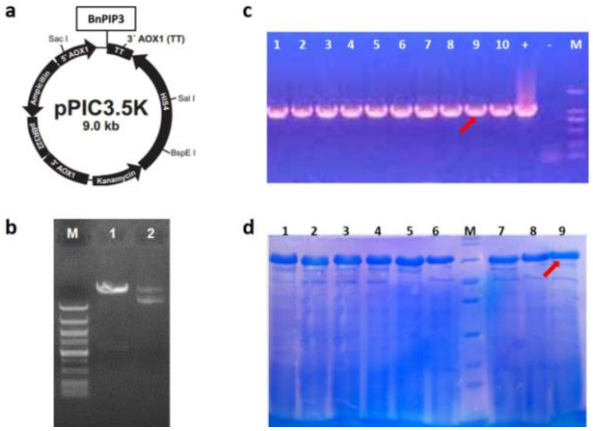

Fig. 4: Expression of BnPIP-like induced by methanol in yeast

(a): Diagrammatic representation of the recombinant plasmid pPIC3.5K-BnPIP; (b): Restriction Digestion Map of pPIC3.5K-BnPIP. M: DNA marker DL5000; 1: plasmid pPIC3.5K-BnPIP digested by BamH I and EcoR I; 2: Plasmid DNA; (c): Determination of positive yeast colonies by PCR. M: DNA marker DL2000; 1~10: 10 independent clones; +: positive control; -: negative control. The target band was pointed by the red arrow; (d): Expression of BnPIP-like induced by methanol in yeast; M: Protein Molecular weight marker SM0431, with 7 bands of 14.4, 18.4, 25, 35, 45, 66.2 and 116 kDa respectively; 1~9: proteins expressed in 9 different conditions. The target protein was pointed by the red arrow

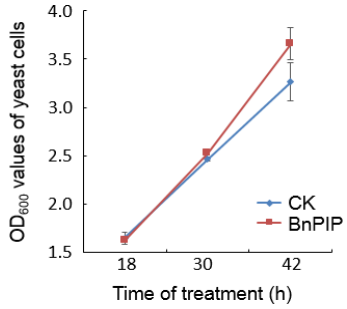

Fig. 5: Growth curves of yeast cells under 300 mM NaCl treatment

control suffered growth inhibition. But from the 30th h on, the *BnPIP-like*-transgenic yeast became stronger than the control. In other words, the transgenic yeast (OD_{600}=2.525) suffered less inhibition than control (OD_{600}= 2.465, Fig. 5). As the salt stress continued, this difference got more significant: on the 42th h, it showed that the OD_{600} value of transgenic yeast got 3.659, while the OD_{600} value of control got only 3.265 (Fig. 5). After that, the difference gradually got smaller; however, the transgenic yeast cells always grew slightly stronger than the control (data not shown).

Discussion

Environment affects higher plant growth greatly. Salinity decreases the germination rate of seeds of plants, depresses the photosynthesis efficiency, inhibits respiration and protein synthesis and disturbs the reactive oxygen species (ROS) or even nucleic acid metabolism (Niu *et al*., 1995; Zhu, 2000; 2001). As to crops, it inhibits the growth and decreases the yield. Besides irrigation and improving soil drainage, studying salt tolerance genes in plant was another promising approach.

There are several genes in different pathways enhancing salt tolerance studies: ROS-eliminating Mn-containing superoxide dismutase *ThMSD* from salt cress (*Eutrema halophilum*) enhanced salt tolerance in transgenic *Arabidopsis* (Xu *et al*., 2014); by regulating the osmotic regulator betaine, a BADH gene from *Atriplex micrantha* could enhance salinity tolerance in transgenic maize plants (Di *et al*., 2015); besides, late embryogenesis abundant (LEA) gene AtEm6 from *Arabidopsis* was also found to enhance salt tolerance in transgenic rice cell lines by regulating expression of Ca^{2+}-dependent protein kinase genes (Tang and Page, 2013). Aquaporin (AQP) as an integral membrane protein, transporting water molecules across membrane efficiently (Denker *et al*., 1988; Zardoya, 2005), was investigated in this study.

A new AQP *BnPIP-like* gene was first isolated from rapeseed and analyzed. Its full-length cDNA had 846 bp (Fig. 1a) and it encoded a putative protein with 281 amino acids. Online sequence alignment showed that in NCBI database, the one which shared highest identity (95.3%, Fig. 1b) with *BnPIP-like* gene was PIP (Genbank: NP_195236.1) from *Arabidopsis*. The theoretical pI of BnPIP-like protein was 8.99 and molecular weight was 29.8 kDa. It had the conserved domain SGXHXNPAVT of MIP super-family and the conserved domain GGGANXXXXGY and TGINPARSLGAA of PIP family, as well as the conserved domain NPA; prediction of transmembrane structure showed that BnPIP-like protein contained five loops and six transmembrane helices (Fig. 2a), existing as homo-tetramers (Fig. 2b), which was in accord with the classical structures for PIP (Chaumont *et al*., 2001; Li *et al*., 2013). Further analysis found that protein EU487188.1, EU487187.1 and AF118383.1 had higher similarity in structure with BnPIP-like protein than AF118381.1 had: AF118381.1 had 7 transmembrane domains and its C-terminal was extracellular (data not shown). This was probably attributed to that they belonged to different subfamilies: protein AF118381.1 belonged to γ-TIP subfamily (located in tonoplast).

To study *BnPIP-like* gene expression model in plants, rapeseed cultivar JR9 was planted and then treated by 300 mmol/L NaCl. It was showed that 2 h after the NaCl solution was sprayed to the seedlings and added into the soil, the plants started to wither, and at this time, the expression was significantly suppressed (to about 15% of the control, Fig. 3). But from the 4th h on, the transcriptional level recovered nearly to level as control. As the salt stress treatment continued, the expression of BnPIP-like gene decline again (Fig. 3).

In order to further investigate functions of BnPIP-like protein, yeast rather than *E. coli* was utilized as an

expression system. Because BnPIP-like protein had 6 transmembrance domains in such a small fragment described as above, it was impossible to be expressed correctly in prokaryotic *E. coli*. It was found inclusion bodies formed in *E. coli* BL21 cells. In our previous experiments, the *BnPIP-like* gene fragment was fused into prokaryotic expression vector pET30a and then induced into *Escherichia coli* BL21 (DE), in order to operate the prokaryotic expression (Xu *et al*., 2014). But the products were expressed mainly as inclusion bodies rather than transmembrane proteins (data not shown). Then yeast was considered, since its eukaryotic expression could complete the posttranscriptional modifications, which were better for eukaryotic gene expression. Moreover, the theoretical pI of BnPIP-like protein was 8.99, far from the pH value of BMMY media (pH 6.0), so it could solve the inclusion body problem efficiently.

This was exactly the reason why we then turned to eukaryotic expression system yeast. Methylotrophic *Pichia pastoris* (Strain GS115) could avoid that problem and determine the protein functions sooner than in model plants. *BnPIP-like* gene was fused into vector *pPIC3.5K* (Fig. 4a). Then the recombinant plasmid *pPIC3.5K-BnPIP* and the original pPIC3.5K were linearized and induced into yeast cells, respectively obtaining the transgenic yeast and the control stains. Positive transgenic yeast was screened out by PCR (Fig. 4c), then the induction conditions were explored and the target protein was successfully expressed (Fig. 4d). After cultured in YPD and BMGY media successively, the yeast cells were induced into BMMY media. The salt stress treatment started on the 18th h in BMMY media with methanol. The OD_{600} values of *BnPIP-like*-transgenic *P. pastoris* and control were determined every 12 h. Although both yeast were obviously inhibited, the concentrations of *BnPIP-like*-transgenic *P. pastoris* was always higher than control (Fig. 5) slightly, indicating that the inhibition of growth in *BnPIP-like*-transgenic *P. pastoris* was slighter because of the over-expression of *BnPIP-like* gene. Thus the resistance to salt explained the eukaryotic functions of BnPIP-like protein. Moreover, it provided a theoretical possibility for a more comprehensively industrial utilization of fermenting yeast. It also established the base for its further functional research in plants.

Conclusion

In this study, *BnPIP-like* gene a new aquaporin gene from rapeseed was isolated and sequenced. Theoretical biochemical characters, especially its transmembrane structure, were analyzed. When induced by methanol, BnPIP-like could enhance the salt resistance in yeast (GS115).

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