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Saline stress regulates water transport functions of *Jatropha curcas* Plasma membrane Intrinsic Proteins JcPIP1 and JcPIP2

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1 SUMMARY

From plasma membrane proteins of Barbados Nut, a 29kD band was isolated belonging to plant plasma membrane intrinsic proteins (PIPs) by LC-MS/MS analysis. Two cDNAs, named *JcPIP1*and *JcPIP2*, were obtained by RT-PCR and RACE. Water transport activity of two PIPs was analyzed by heterologous expression in *Xenopus* oocytes separately. Northern blot analysis revealed that the transcripts of *JcPIP2* expressed in all tested tissues, but *JcPIP1* only expressed in roots of the seedlings. Measurements of the root hydraulic conductance (L_0) of roots of *Jatropha curcas* were carried out and the results were compared to the transcripts of aquaporins presented in seedling roots. L_0 of plants under saline stress was progressively reduced when NaCl concentration increased. In addition, a good correlation between mRNA expression and L_0 was observed in both experiments. Furthermore, the transcripts of *JcPIP1* showed higher expression than *JcPIP2* under same saline stress.

2 INTRODUCTION

Aquaporins, the membrane water channels that play critical roles in controlling the water contents of cells, in plants constitute a large and divergent family with 35 members identified in *Arabidopsis thaliana* (Johanson *et al.*, 2001), 33 members in *Oryza sativa* (Sakurai *et al.*, 2005), and 36 members in *Zea mays* (Chaumont *et al.*, 2001). Based on sequence similarities, plant aquaporins

are typically divided into four subfamilies: plasma membrane intrinsic proteins (PIP), tonoplast intrinsic proteins (TIP), noduline-26 like intrinsic proteins (NIP), and small basic intrinsic proteins (SIP) (Johanson *et al*., 2001; Zardoya, 2005; Mihaela *et al*, 2009).There are strong evidences that aquaporins are central components in plant water relations (Tyerman *et al*., 2002; Chaumont *et al*., 2005). The significances of plant aquaporins functioning in mediating water and/or other small solutes across biomembrane and response to external environmental stresses have been widely reported (Kaldenhoff *et al*., 2006; Jang *et al*., 2007; Zhang *et al*., 2008).

The contribution of aquaporins to plant water transport under salt stress is not well understood. The tobacco stress-induced Aquaporin1 (NtAQP1) functions as both a water and carbon dioxide ($CO_{2)}$ channel. It improved tobacco water use efficiency, hydraulic conductivity and yield production under salt stress (Nir *et al*., 2010). Under salt stress treatment, TaNIP-overexpressing Arabidopsis accumulated higher K^+, Ca^{2+} and praline contents and lower Na^+ level than the wild-type plants. The over expression of TaNIP in transgenic *Arabidopsis* was also up-regulated the expression of a number of stress-associated genes (Gao *et al*., 2010). In poplar (Populus alba×P. tremula var. glandulosa), drought, salt and wounding stress induce the expression of the plasma membrane intrinsic protein 1 gene ((PatPIP1) (Bae *et al*., 2011). In Azospirillum-inoculated barley seedlings, which were inoculated and subjected to 200 mM NaCl for 18 days, the higher putrescine content and lower levels of HvPIP2;1 transcripts in the roots were found(Zawoznik *et al*., 2011). Transgenic Arabidopsis plants that express GFP fusions with AtPIP1;2 and AtPIP2;1, two prototypic PM aquaporins, were used to develop a fluorescence recovery after photobleaching (FRAP) approach. A salt treatment (100 mm NaCl for 30 min) markedly enhanced the cycling of the aquaporin constructs and modified their pharmacological inhibition profile (Luu *et al*., 2012). The dynamic properties of AtPIP2;1 in the PM in normal and salt stress conditions was described by means of single particle tracking (SPT) and fluorescence correlation spectroscopy (FCS) . In salt stress conditions, the rate of AtPIP2;1 cycling was enhanced and endocytosis was cooperated by a membrane raft-associated salt-induced pathway and a clathrin-dependent pathway (Martinière *et al*., 2012).

Jatropha curcas (*J. curcas*), belongs to *Euphorbiaceae* and thrives in many parts of the tropics and sub-tropics. Recently, *J. curcas* draws much attention for its powerful salt and drought tolerance and is a non edible oil crop predominately used to produce bio- diesel. Recent evidence supports the role of aquaporins in plant water relations and provides information on their involvement in salt stress tolerance (Ballesta *et al.* 2003). Early study showed that Aquaporin JcPIP2 is involved in drought responses and played an important role in improving drought tolerance in *Jatropha curcas* (Zhang *et al*., 2007; Wang *et al*., 2008; Jang *et al*., 2011). Aquaporins could play some important roles in the rapid growing of *J. curcas* in salty soil conditions.

In the present study, two *J. curcas* plasma membrane intrinsic proteins (JcPIP1, JcPIP2) were isolated by proteomics technique and the

genes cloned by using RACE. The functional water channels were separately demonstrated by heterologous expression in *Xenopus oocytes*. In this study, the expression pattern of two aquaporins in the whole plant was investigated by northern blot analysis. Furthermore, the correlations between Root hydraulic conductance (L_0) values and transcripts levels of two aquaporins were studied when the seedlings of *J. curcas* were treated with NaCl.

3 MATERIALS AND METHODS

3.1 Plant Growth Conditions: Young roots for protein extraction were collected from *J. curcas* seedlings growing from 100 seeds in a growth room under long-day conditions (16-h-light/8-h-dark cycle) at 28°C. Young roots, stems, leaves, flowers, endosperms and seedcases for RNA isolation were collected in summer from Haikou city of Hainan Province and instantly frozen in liquid nitrogen and stored at -70°C. Young seedlings grown in the greenhouse were prepared for saline stress. Seeds were germinated and cultivated in a 30 L container filled with a substrate composed of a sandy loam soil/turface mixture (1:1). Each container had 20 germinated seeds. The soil contained enough water and nutrients for the plants to grow for two months and no symptoms of nutrient deficiency were shown. Three-week-old seedlings were carefully washed with distilled water and transplanted into five glass containers, and 0.5 L Hoagland's nutrient solution was added into each container. Each glass containers had 10 healthy seedlings. One week later, concentrations of 50, 100, 150, 200, 250, 300, 350 and 400 mM NaCl were added. After24h incubation, roots were treated with NaCl and the control roots were collected for RNA extract and hydraulic conductance measurements. The NaCl treatments were applied to the nutrient solution for the increments of 50 mM each hour to give a final NaCl concentration of 50, 100, 150, 200, 250, 300, 350 and 400 mM NaCl.

3.2 Protein Extraction of Plasma Membrane: Plasma membrane vesicles were purified from roots of 9-day–old plants by aqueous two-phase partitioning in a mixture of PEG 3350 and Dextran T-500, 6.4% (w/v) each, in the presence of 5 mM KCl. Membranes were incubated in 5 mM EDTA, 5 mM EGTA, 4 mol/L urea, and 5 mM Tris-HCl, PH 9.5, for 10 min on ice. After centrifugation for 20 mM NaOH and in 2 mM EDTA, 2 mM EGTA, 100 mM NaCl, and 5mM Tris-HCl, pH 8.0, Membranes were re-suspended in 9 mM KCl, 300 mM Suc, 5mM Na_2EDTA, 5mM Na_2EGTA, 50mM NaF, 5mM DTT, 2 µg/ml eupeptin, and 10mM Tris-borate, pH 8.3, and stored at -80°C before analysis (Larsson *et al.* 1994; Santoni *et al.* 2000; Javot *et al.* 2003).

3.3 Electrophoresis, Immunological analysis and Digestion: Antibodies were directed against short peptide: VGEETQTSHGKC (located at the N-terminus of *Ricinus communis* L. plasma membranes aquaporin) for the immunization of rabbits (Eisenbarth *et al.* 2005). Specific antibody was affinity-purified from the crude blood sera using the peptide described above coupled to CNBr-activated Sepharose 4B (Amersham Biosciences, Freiburg, Germany). The antibodies were allowed to bind the peptide-Sepharose beads for at least 12h at 4°C and eluted using 50 mM glycine (dissolved in water, resulting in a pH of 1.9) at room temperature; the eluted antibodies were immediately neutralized with 0.1 volume of 1% Tris-HCl (pH 8.0) and BSA was added to a final concentration of 1% (w/v). 10 µg

protein extraction of plasma membrane in 100 µL of phosphate buffer (10 mM potassium phosphate, 150 mM NaCl, 1% OG and 10% glycerol pH 7.5) was incubated for 10 minutes at different temperatures (25uC–65uC). After incubation, sample loading buffer (125 mM Tris-HCl pH 6.8, 20% glycerol, 4% SDS, 10% (v/v) 2-mercaptoethanol 0.1% bromophenol blue) was added to the protein and further incubated for 10 minutes at room temperature. SDS- PAGE (12%) of the sample was performed as described by Laemmli (1970). Protein was visualized by staining the gel with Coomassie brilliant blue R250. The separated proteins on a SDS-polyacrylamide gel were transferred electrophoretically in a tank transfer system (Amersham) 10mMCAPS on PvDF membranes for 4 h at 60 V. The membranes were blocked in PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4, pH 7.4) containing 1% (w/v) low-fat milk powder and subsequently incubated with the affinity purified primary antibodies (1:500 dilution). The immunoblot was developed using NBT/BCIP (Roche Diagnostics, Mannheim, Germany) as the substrate for the alkaline phosphatase as recommended by the manufacturer (CDP-Star; Applied Biosystems).

3.4 LC- MS/MS and Database Searching: The tryptic peptide was analyzed by a LCQ DECA XP PLUS ion trap mass spectrometer (Thermo Electron, San Jose, CA, USA) according to manufacturer's directions (Lee *et al.* 2004). Protein spots were excised from the gel, reduced, alkylated, and digested with trypsin. Tryptic-digested peptides were recovered through a series of extraction steps. Extraction with 25mM ammonium bicarbonate and acetonitrile was followed by second extraction step with 5% trifluoroacetic acid and acetonitrile. Extracts were pooled and lyophilized in a vacuum lyophilizer. Lyophilized trypic peptides were re-dissolved in solution containing water, acetonitrile, and trifluoroacetic acid (90:7:3) and bath sonicated for 5 min. For mass spectrometry datum from LCQ, protein identification using MS/MS raw data was performed with the SEQUEST (University of Washington, licensed to Thermo Finnigan) searching program against the National Center for Biotechnology Information (NCBI) protein database (Lee *et al.* 2004).

3.5 RNA Isolation and cDNA Amplification : RNA was extracted from young roots using the TRIZOL reagent (Invitrogen, Karlsruhe, Germany), then purified using the RNAeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. After an initial photometric determination of total RNA concentration, ethidium bromide fluorescence of ribosomal bands on the electrophoresis-gel was used to check the integrity of total RNA and then adjust the amounts. Reverse transcript (RT)-PCR was performed according to the program of Bca Best RNA PCR Kit (Takara Biotechnology Co. Ltd.) with degenerate primers DP1, DP2 derived from Peptide Sequence by LC-MS/MS Analyses belonging to conserved sequence of plant *PIPs*. The specific 5'-RACE and 3'-RACE primers were designed as follows: A11, A12, S11 and S12 for *JcPIP1*; A21, A22, S21 and S22 for *JcPIP2*. The 5' and 3' sequences of cDNAs were obtained by RACE with the 5'- and 3'-Full RACE Core Set (TaKaRa). The PCR products were cloned to PMD18-T vector and sequenced. Based on the nucleotide sequences of the 5'- and 3'-RACE products, primers, GSP11 and GSP12 for *JcPIP1*; GSP21 and GSP22 for *JcPIP2*, were used for the amplification of full-length cDNA sequence of *JcPIPs* (Table 1).

3.6 Oocyte Preparation, in Vitro Complementary RNA Synthesis, and Injection:

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Xenopus laevis oocytes (stage V and VI) were isolated and incubated in Barth's solution [88mM NaCl, 1mM KCl, 2.4 mM $NaHCO_3$, 10 mM Hepes-NaOH, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, pH 7.4] supplemented with penicillin and streptomycin (200 units/ml each) at 16°C (Daniel *et al.* 1996). For oocyte expression, cDNAs was cloned into the Bg*l* II site of a pSP64T-derived Bluescript vector carrying 5' and 3' untranslated sequences of a β-globin gene from *Xenopus*. Capped complementary RNAs (cRNAs) encoding *JcPIPs* was synthesized *in vitro* by using T3 RNA polymerase. The construct was verified by sequencing. After ethanol precipitation, the synthesis products were suspended in diethyrocarbonate-treated H_2O at a final concentration of 1 mg/ml. Capped complementary RNA (10 to 50ng) or an equivalent volume of water was injected into each oocyte using an automatic injector (Drummond, Broomall, USA).

Table 1: Primer pairs used for RT-PCR and RACE

Primers
DP1: 5'-GG(A,T)GG(A,T)GG(A,T)GC(A,G,C,T)AA(C,T)-3'
DP2: 5'-GG(A,T,C)GG(A,T,C)GG(A,T,C)GC(A,G,C,T)AA(C,T)-3'
A11: 5'-GGTGGTGGAGCCAACC-3'
A12: 5'-CTACACTAAGGGTGACG-3'
S11: 5'-CATTTAAGCCCTGCTC-3'
S12: 5'-TACAGAGCAGGAAGAGC-3'
GSP11: 5'- TTTCTTGTTCCTGTACATC-3'
GSP12: 5'-GTCAACGATACGCTACGTAACG-3'
A21: 5'-GGTATGCAGCTGCTGC-3'
A22: 5'-CAGAGCACCGAAGCTC-3'
S21: 5'-CGGCTATAGCAAAGGAAC-3'
S22: 5'-GTGCACGTGACTCACACG-3'
GSP1: 5'-ATCCCCCCGGGCTGCAG-3'
GSP2: 5'-CTTCGTTCTTTCATTTAG-3'

3.7 Osmotic Water Permeability Analysis: Individal oocyte was transferred from Barth`s solution (Osm_{in} is 200 mosmol) to a cuvette perfused with Barth's solution diluted to 40 mosmol (Osm_{out}) at 20°C. Oocyte swelling was followed by video microscopy. Pictures were captured at 5-second interval and cell volume was calculated from the cell section area. Osmotic water permeability(P_f) was determined from the initial slope of the time course of relative cell volume using Equation 1:

$P_f=V_0[d(V/V_0)/dt]/[S\times Vw\times(Osm_{in}-Osm_{out})]$,

where

initial oocyte volume: V_0, is $9\times10^{-4}cm^3$;

initial oocyte surface area: S, is $0.045cm^2$ and

molar volume of water: V_m is $1.8\times10^{-4}cm^3/mol$ (Fetter *et al.* 2004).

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3.8 RNA Gel Blot Analysis: Total RNA were extracted from young roots, young stems, young leaves, flowers, endosperm, and seedcases as above. Total RNA samples (20 µg each) were fractionated by electrophoresis on Hepes-formaldehyd-hyde, 1.5% agarose gel according to established procedures and were transferred to Hybond-N nylon membranes (Amersham, USA) by capillarity using 10x SSC buffer, pH 7.0 (Sambrook *et al.* 1989). Membranes were air-dried and the nucleic acids were bound (fixed) by using UV irradiation from a transilluminator for 5 min. Northern blots were pre-hybridized for 2h at 58 °C with high SDS concentration hybridization solution (Roche; 7% SDS, 50% deionized formamide, 0.1% sodium-lauroylsarcosine, 2% Blocking Reagent). Hybridizations were carried out overnight in the same solution at 58°C with the addition of 25 ng of Digoxigenin-labelled DNA probe. After hybridizations, the membranes were washed at 58 °C for 15min with 2 x SSC buffer plus 1% (W/V) SDS, which were incorporated after the two washes at room temperature. The probe was recovered from PCR products and labeled using DIG High Primer DNA Labeling and Detection Starter Kit II (Roche Ltd.).

3.9 Measurement of L_0: L_0 of roots was measured by pressuring the roots using a SchÖlander pressure chamber (Martinez-Ballesta *et al.* 2000). Briefly, the aerial parts of the seedlings under stress and the control seedlings were removed leaving a separate stem cylinder, which was sealed with silicone grease into a tapered glass tube. In the chamber, a gradual increase of pressure (from 0.1 to 0.5 MPa) was applied to the solution surrounding the detached roots. The solution had the same composition as the one used for the growth of the seedlings. The xylem sap exuded for 4 min at each pressure was collected in Eppendorf tubes and weighed. Finally, the roots were weighed as well. The slope of the linear part of the calculated xylem flow, Jv ($mg \cdot g^{-1} \cdot min^{-1}$), and plotted versus pressure gave the L_0 ($mg \cdot g^{-1} \cdot min^{-1} Mpa^{-1}$) value. A minimum of five seedlings was measured and assayed in each treatment.

3.10 Statistical analysis: Data were analysed using analysis variance (ANOVA) procedures and means separated by Duncan's multiple range test.

4 RESULTS

4.1 Plasma membrane protein Extraction and Electrophoresis: From the plasma membrane proteins of *J. curcas* young roots, a polypeptide band of 28kD was immunodetected with anti-RcPIP2 antibody. The polypeptide was digested by trypsin and generated peptide fragments were subjected for LC-MS/MS analysis.

4.2 Amino Acid Analysis by LC-MS/MS: The collision-induced dissociation spectra showed product ions of the trypic peptide at M/Z 1950.00 from 28kD protein. Fragmentation of the doubly charged precursor ion yielded detectable singly charged b-ion species (b-3 to b-16) and y-ion species (y-3 to y-16) for which the sequence is indicated (Fig. 1). A peptide fragment:

NPYNHLGGGANSVNTGYSK was identified and searched for in SEQUEST database of NCBI with matches to a conserved motif GGGANXXXXGY belonging to all plant PIPs.

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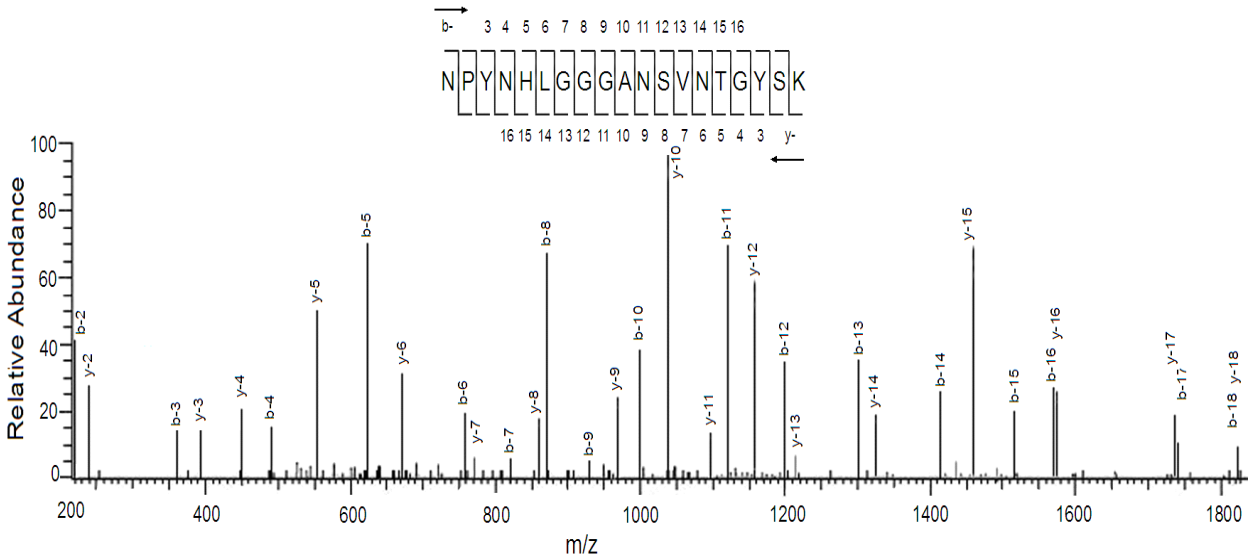

Figure 1: Collision-induced dissociation spectra of tryptic peptide derived from the 28kD protein of the *J.curcas* plasma membrane. The SEQUESR algorithm assigned b-ion and y-ion series to the observed mass spectra based on sequence databases.

4.3 Gene Cloning and sequence analysis of JcPIPs: Peptide fragment NPYNHLGGGANSVNTGYSK was used to design two degenerated primers DP1, DP2 (table 1), with a 64 and 216 fold degree of degeneracy, respectively. According the results of DNA sequences, special primers were designed and two genes were cloned PCR method (Fig. 2). Amino acid sequences were compared with the ClustalW aligment program. GenBank accession No: *JcPIP1*, EU935840, *JcPIP2*, EF030420.

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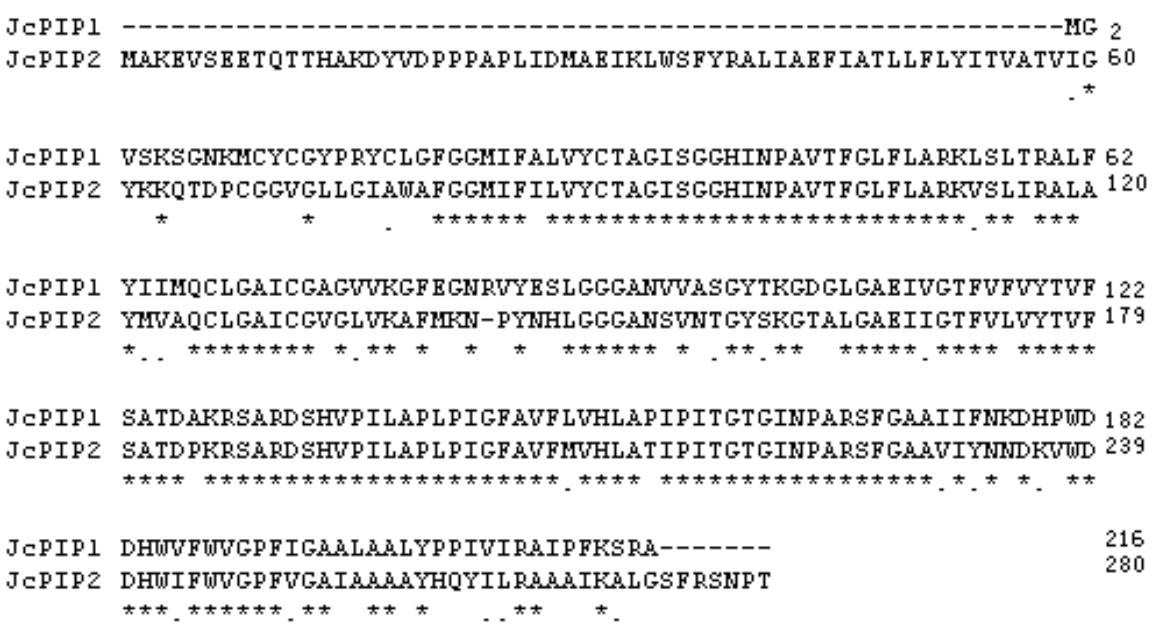

Figure 2: Amino acid sequence alignments of plasma membrane intrinsic (PIP) form *Jatropha curcas* (*JcPIP1* and *JcPIP2*).

4.4 JcPIPs are Functional Water Channel proteins in Xenopus Oocytes: The water channel activity of JcPIPs was assayed by heterologous expression of *JcPIP* cRNAs in *Xenopus oocytes* separately. *JcPIP1* cRNA and *JcPIP2* cRNA injected oocytes exhibited significantly increased osmotic water permeability compared with water-injected oocytes (Fig.3).

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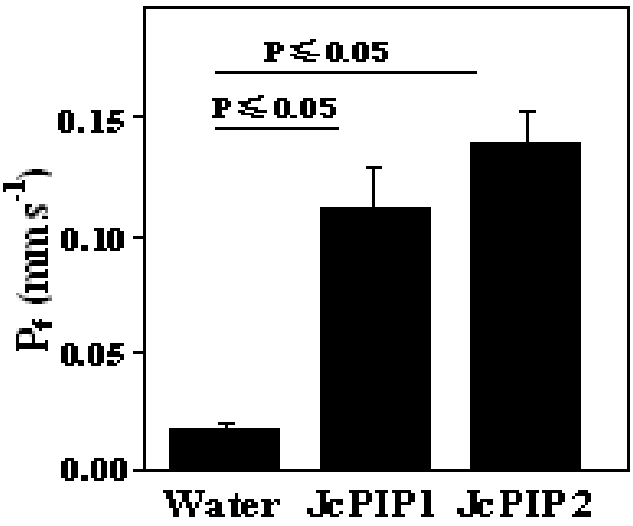

Figure 3: Osmotic water permeability of *Xenopus* oocytes injected with sterile water or with cRNA of *JcPIP1* and *JcPIP2*, respectively. The P_f values are expressed as means±SE(n=10).

4.5 Expression of JcPIPs in Different Tissues of *J. curcas* during development: To characterize the expression pattern of *JcPIPs,* gel-blot analysis of total RNA from different *J. curcar* tissues was performed (Fig. 4). Transcripts of *JcPIP1* only can be found in young roots. However, *JcPIP2* transcripts were observed in all tested tissues such as young roots, stems, leaves, flowers, endosperms and seedcases. The levels of *JcPIP2* mRNA were high in each organ detected.

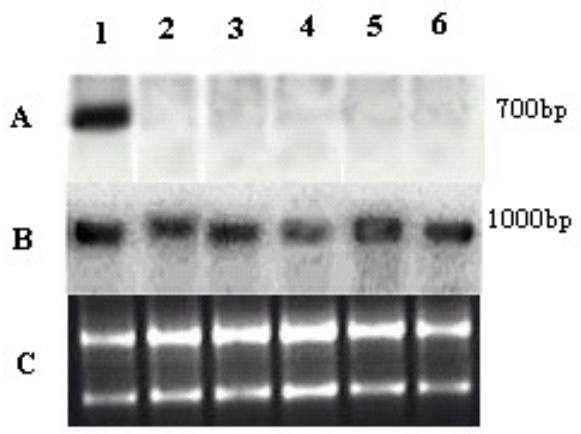

Figure 4: RNA Gel Blot analysis of *J. curcas.*

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Total RNA hybridized with sequence-specific RNA probes synthesized from *JcPIP1* and *JcPIP2* separately. A, corresponding spots of RNA Gel blot of *JcPIP1*, B, corresponding spots of RNA Gel blot of *JcPIP2*, C, the ethidium bromide stained gel of total RNA. 1, young roots; 2, young stems; 3, young leaves; 4, flowers; 5, endosperms; 6, young seedcases.

4.6 Effect of saline stress on L_0: Measurements of L_0 were performed with control plants and plants treated for 24h with different NaCl concentrations (50, 100, 150, 200, 250, 300, 350, 400 mM) using the Scholander pressure chamber. At first, there was an increase of L_0 when the plants treated with NaCl from 0 to 100 mM. Then, no significant differences of L_0 happened between the plants treated with 100 and 150mM NaCl. However, from150 to 400 mM NaCl treatments, L_0 decreased progressively with respect to control plants (Fig. 5).

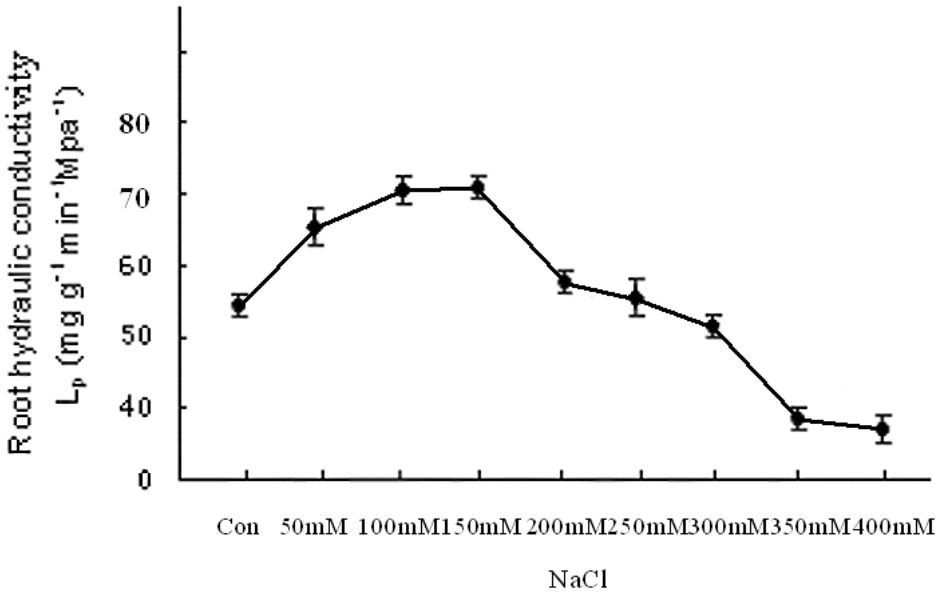

Figure 5: Root hydraulic conductance (L_0) of *J. curcas* control plants and plants treated with different NaCl concentrations (50, 100, 150, 200, 250, 300 and 400 mM) for 24h. Number of plants = 5. Error bars = SE.

4.7 Effect of salinity on gene expression: Total RNA from the control roots and those with different concentrations of NaCl (50, 100, 150, 200, 250, 300 and 400 mM) for 24h was extracted. Transcripts hybridizing to *JcPIP1* and *JcPIP2* cDNA probes were abundant and the amount of mRNA decreased as the concentration of NaCl increased. Furthermore, under same saline stress, the transcripts of *JcPIP1* showed higher expression than *JcPIP2* (Fig. 6).

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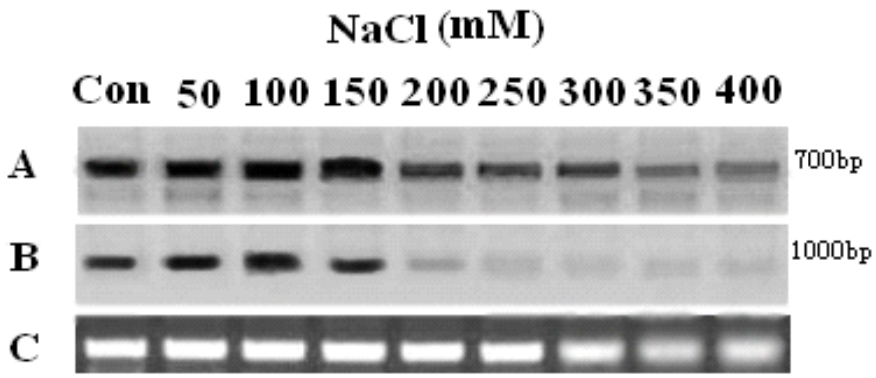

Figure 6: Northern blot of total RNA extracted from roots of J. *curcas* control plants and plants treated with different NaCl concentrations (50, 100, 150, 200, 250, 300 and 400 mM) for 24h. RNA gel blots were hybridized using probes specific for *JcPIP1* (A) and *JcPIP2* (B) respectively. 18sRNA gel (stained with ethidium bromide) is shown in (C).

5 DISCUSSION

In plant, aquaporins are abundant proteins accounting for 5 to 10% of the total proteins in membranes. When intrinsic membrane proteins are subjected to SDS-PAGE, the aquaporins, with molecular masses of 27 to 30kD, usually form the most abundant polypeptide band. Conventional protein purification methods enable the purification of substantial quantities of aquaporins, if a ready supply of membranes is available (Chrispeels *et al.* 1999). In the present study, we isolated a 29kD polypeptide from *J. curcars* plasma membranes band immunodetected with anti-RcPIP2 antibody (Chrispeels *et al.* 1999). From the predicted sequence of *JcPIP* amino acids, conserved amino acid motifs: (Asn-Pro-Ala) characteristic of aquaporin sequence signature, were found same as Fetter *et al* (2004) and Zhang *et al*(2007). A multitude of aquaporin homologs, whose function has not been determined, has been identified. Most of these will probably turn out to function as aquaporins, but so far, not enough is known about water transport specificity to determine function based on sequence data alone. *Xenopus oocytes* provide a convenient system to determine the water transport activity of a single protein expressed as the plasma membrane. Oocytes, injected with *JcPIP1* and *JcPIP2* cRNAs separately, had a swelling rate 10 times higher than that of water-injected oocytes. It is similar to values of other aquporins reported in the literature (Estrella *et al.* 2004; Zhang *et al.* 2008; Ayadi *et al.* 2011). These results indicated that JcPIPs facilitates the transport of water.

Aquaporins are ubiquitous channel proteins and show differential expression Patterns in plants. Early works have revealed that certain aquaporin isoforms show a strict tissue expression pattern, such as seed-specific α-TIP in bean (*phaseolus vulgaris*), foot-specific TobRB7 in tobacco (*Nicotiana tabacum*) (Luu and Maurel 2005), *TaNIP* gene in the root and leaves of wheat (Gao *et al.* 2010) and *JcPIP1* only can

be found in the root of *J. curcas*. It suggests that this JcPIP1 isoform plays important roles, in root water relations. However, other isoforms seem to be expressed in most plant tissues. Some aquarions are constitutively expressed while the expression of other aquarions are regulated by developmental and environmental factors such as drought, salt, cold, ABA treatment, hormones and blue light (Fotiadis *et al.* 2001; Tyerman *et al.* 2002; Zhang *et al.* 2008; Gao *et al.* 2010; Jang *et al.* 2011; Muries *et al.* 2011). Northern blot analysis revealed that, *JcPIP2* seems to be expressed in most plant tissues under salt stress. But in Jang`s research, the *JcPIP2* gene can be found in leaves at 100mM NaCl treatment and was not detected in leaves at 200 and 300 mM NaCl treatments (Jang *et al.* 2011). The little different results could be caused by the treatment methods. However, *JcPIP1* can only be found in the root of *J. curcas*. Aquaporins are regulated both at transcriptional and activity levels. Aquaporin activity is controlled by phosphotylation, which appears to be a short-term response to stresses like drought and salinity (Ouziad *et al.* 2006; Zhang *et al.* 2008; Muries *et al.* 2011). Root hydraulic conductivity might have a direct bearing on the ability of plants to survive and grow under adverse conditions such as heavy salty stress. The reductions in root hydraulic conductivity of salinized plants have been suggested as being due to the hyperosmotic stress and ionic imbalance caused by the high apoplastic concentrations of Na^+ and Cl^-. However, from previous results, it was observed that large reductions in the root hydraulic conductance of salinised plants could be closely related to the decrease in the activity or concentrations in the root plasma membrane (Chrispeels *et al.* 1999;Gao *et al.*2010) , this effect mainly being due to the specific toxicity of Na^+ and Cl^- (Martinez-Ballesta *et al.* 2000). At the same time, in salt stress conditions, the rate of aquaporins cycling was enhanced and endocytosis was cooperated by a membrane raft-associated salt-induced pathway and a clathrin-dependent pathway (Martinière *et al.*2012). The correlation between water permeability in roots and expression of specific aquaporins has not been proven but seems likely (Aroca *et al.* 2005). In ice plants, transcript levels of some aquaporins are down-regulated in the first 30h after exposure to salt stress (Yamada *et al.* 1995). In paprika pepper (Carvajal *et al.* 1999), melon (Carvajal *et al.* 2000) and broccoli (Muries *et al.*2011), NaCl reduces either the activity or abundance of aquaporins as shown. Likewise, in *J. curcas*, L_0 of plants under saline stress increased from 0 to 150mMol NaCl treatment but was progressively reduced when NaCl concentration was increased from 150mMol. In addition, a good correlation between mRNA expression and L_0 was observed in both experiments. Furthermore, the transcripts of JcPIP1 showed higher expression than JcPIP2 under same saline stress. The result showed that JcPIP1 may play more important role than JcPIP2 in root water transport when the seedling living under saline stress. The mechanisms of JcPIP1 and JcPIP2 under salt stress should be researched by further study in the location on the cell.

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