

Aquaporin JcPIP2 is Involved in Drought Responses in *Jatropha curcas*

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Abstract Water channel proteins, aquaporins, play fundamental roles in transmembrane water movements in plants. A new full-length cDNA encoding aquaporin was isolated from the seedlings of *Jatropha curcas*. The gene of the plasma membrane intrinsic protein (PIP) from *J. curcas* (*JcPIP2*) contained an 843 bp open reading frame encoding a protein of 280 amino acids. The amino acid sequence showed 94% identity with *Ricinus communis* PIP. Injection of *JcPIP2* complementary RNA into *Xenopus* oocytes increased 10-fold the osmotic water permeability of the oocytes. Immunodetection of JcPIP2 with anti-JcPIP2 antibody indicated that this protein is ubiquitously located in all tested tissues of the plant. To investigate the relationship between aquaporins and drought resistance in *J. curcas*, the abundance of JcPIP2 was examined in seedlings of two *J. curcas* populations, GaoYou CSC63 and YanBian S1, under water deficit with PEG6000. Under field conditions, those two populations, GaoYou CSC63 was resistant to water deficit, but YanBian S1 was sensitive to water deprivation. With the increasing degree of drought stress, JcPIP2 level increased in seedlings of GaoYou CSC63, whereas there was no significant change in seedlings of YanBian S1. Compared with YanBian S1, GaoYou CSC63 also showed higher root hydraulic conductivity and lower decreasing trend in the seedlings under water deficit. These results indicated that JcPIP2 probably played a role in drought resistance in *J. curcas*.

Keywords *Jatropha curcas*; plasma membrane protein; JcPIP2; root hydraulic conductivity; drought stress

Aquaporins form part of a group of highly conserved membrane proteins called the major intrinsic proteins. In plants, aquaporins present as multiple isoforms. For instance, the genome of *Arabidopsis* encodes 35 aquaporin homologs [1]. The plant aquaporin family can be subdivided into four homology classes that are, to some extent, based on their distinct subcellular localization. The most abundant aquaporins in the vacuolar and plasma membranes belong to the tonoplast intrinsic protein (TIP) and the plasma membrane intrinsic protein (PIP) classes, respectively. The

PIPs are further subdivided into two homology subgroups, PIP1 and PIP2. Nodulin-26 is another aquaporin class that is expressed in the peribacteroid membrane of symbiotic N₂-fixing root nodules. The fourth class of plant aquaporins comprises the small basic intrinsic proteins, found in only a few plant species.

Aquaporins facilitate the diffusion of water molecules across membranes according to a water potential gradient. The amount of active aquaporins will primarily determine the hydraulic conductivity (L_p) of a membrane. The role of plant aquaporins in water transport across membranes has been proven by their expression in *Xenopus* oocytes. The same research system also revealed that some aquaporins could transport other small molecules, such as glycerol, solutes, and ions [2,3].

Aquaporins might exist in all plants, considering the large number of aquaporins and aquaporin homologs in *Arabidopsis* [4]. Aquaporins were thought to be involved in plant adaptation to drought stress. A few aquaporins

Received: March 7, 2007 Accepted: June 19, 2007

This work was supported by the grants from the "Tenth Five Years" Key Program of the Chinese Ministry of Science and Technology (2002BA901A15 and 2004BA411B01), the International Cooperation Project (2003DFB00005), and the Key Project of the Chinese Ministry of Education (307023)

Abbreviations: cRNA, complementary RNA; L_p, hydraulic conductivity; NCBI, National Center for Biotechnology Information; PCR, polymerase chain reaction; PEG6000, polyethylene glycol with an average molecular weight of 6000; P_i, osmotic water permeability; PIP, plasma membrane intrinsic protein; RACE, rapid amplification of cDNA ends; TIP, tonoplast intrinsic protein

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DOI: 10.1111/j.1745-7270.2007.00334.x

and aquaporin homologs were identified by differential hybridization carried out for isolation of drought-induced genes, and alteration in mRNA and protein levels of some aquaporins were the focus of several investigations.

Jatropha curcas belongs to *Euphorbiaceae* and thrives in many tropical and subtropical areas. It can be used to reclaim land and simultaneously produce feedstuff, soap, cosmetics, pesticides, and anticancer medicine [5]. It is easy to establish, grows relatively quickly and is hardy. Being drought tolerant, it can even be planted in desert areas. Recently, *J. curcas* has drawn much attention for its toxicity and high content of seed oil [5–7]. However, no research paper has been published concerning its mechanism of powerful drought tolerance. Recent reports provided direct evidence for aquaporins being involved in drought and salt stress tolerance [8]. Aquaporins are considered to play important roles in the rapid growth of *J. curcas* in dry conditions.

The root is the most important organ for water absorption in plants. The uptake of water from the soil and its delivery to the xylem requires water to move radially across living root tissues. A water channel path was revealed in the roots of a large variety of plant species that contribute to more than 50% of water uptake [9]. It was also proposed that this path might regulate root Lp in response to environmental stimuli, such as drought stress. Furthermore, under solution culture conditions, it was found that the root Lp of maize was higher in a drought-resistant variety than in a drought-sensitive variety [10]. Treated with polyethylene glycol with an average molecular weight of 6000 (PEG6000), there was a significant positive correlation between Lp and the degree of drought resistance in seedlings of maize [11]. Lp has been an important factor in the analysis of plant drought resistance.

In the present work, we describe the molecular cloning of a gene of PIP from *J. curcas* (*JcPIP2*). The function of *JcPIP2* was determined using *Xenopus* oocytes as a heterologous host. Antibody directed at the N-terminus of *JcPIP2* was used in the immunodetection of *JcPIP2* in plants. Furthermore, we investigated the possible role of *JcPIP2* in response to drought stress in two populations of *J. curcas* with different drought resistance under field conditions.

Materials and Methods

Plant materials and growth conditions

The mature seeds were collected from two populations

(GaoYou CSC63 and YanBian S1) of *J. curcas* in Panzhihua City, Sichuan Province, China. GaoYou CSC63 is bred by the Key Laboratory of Bio-resources and Eco-environment at Sichuan University (Chengdu, China), and is resistant to water deficit under field conditions. YanBian S1 thrives on the riverside in the YanBian area of Panzhihua City, and is sensitive to water deprivation under field conditions. GaoYou CSC63 had smaller height, basal diameter, total biomass, total leaf area, fine root/total root ratio and higher net photosynthesis, transpiration, stomatal conductance, instantaneous water use efficiency and abscisic acid content than YanBian S1. Young roots, stems, leaves, flowers, endosperms and seedcases were also collected from GaoYou CSC63 in summer, instantly frozen in liquid N₂ and stored at –70 °C. Young seedlings grown from seeds were cultivated in a greenhouse at 28 °C with a 14 h photoperiod. Seeds were germinated and cultivated in a 30 L container filled with a substrate composed of a sandy loam soil/Turface mixture (1:1). 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 of GaoYou CSC63 and YanBian S1 were carefully washed with distilled water and transplanted into five glass containers, and 0.5 L Hoagland's nutrient solution was added to each container. One week later, to initiate the drought stress, PEG6000 was added into the nutrient solution to make the PEG6000 concentration (V/V) to be 10%, 20%, 30% and 40%. No PEG6000 was added to the control. After 24 h, the treated seedlings were collected for plasma membrane proteins extraction and measurement of Lp. Effects were made to select plants of approximately equal size at the initiation of the stress. To reduce the toxicity to the root, root damage was avoided during the whole treatment with PEG6000.

RNA isolation and cDNA amplification

RNA was extracted from young seedlings using Trizol reagent (Invitrogen, Karlsruhe, Germany), then purified using the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA quality and concentration were checked by agarose gel electrophoresis and spectrophotometry (Sigma, St. Louis, USA), respectively. RNA samples were stored at –70 °C for rapid amplification of cDNA ends (RACE) and real-time polymerase chain reaction (PCR) after removing any DNA.

A cDNA fragment was amplified with degenerate primers DP1, 5'-GG(A/T)GG(A/T)GC(A/G/C/T)AA(C/T)-3' and DP2, 5'-GG(A/T/C)GG(A/T/C)GG(A/T/C)GC(A/G/C/T)AA(C/T)-3' on the basis of the conserved

regions from *Ricinus communis* (GenBank accession No. CAE53883.1) and *Spinach oleracea* (GenBank accession No. AAA99274). Reverse transcription-PCR was carried out using the Bca BEST RNA PCR kit (TaKaRa, Dalian, China). The specific 5'-RACE and 3'-RACE primers were designed as follows: A1 (5'-GGTATGCAGCTGCTGC-3') and A2 (5'-CAGAGCACCGAAGCTC-3'); S1 (5'-CGGCT-ATAGCAAAGGAAC-3') and S2 (5'-GTGCACGTGAC-TCACACG-3'), respectively. The cDNA was obtained by RACE with the 5'- and 3'-full RACE core set (TaKaRa). PCR products were cloned into pMD18-T vector and sequenced. Based on the nucleotide sequences of the 5'- and 3'-RACE products, primers GSP1 (5'-CCT-CTTCTATTTCTAGAG-3') and GSP2 (5'-CTTCGTTC-TTTCATTAG-3') were used for the amplification of the full-length cDNA sequence of *JcPIP2*.

***Xenopus* oocytes preparation, plasmid construction, *in vitro* RNA synthesis and injection**

Oocytes (stages V and VI) were isolated from *Xenopus laevis* and incubated in Barth's solution [88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 10 mM HEPES-NaOH, 0.33 mM Ca(NO₃)₂, 0.41 mM CaCl₂, 0.82 mM MgSO₄, pH 7.4] as described previously [12].

The cDNA of *JcPIP2* was cloned into the *Bgl*III site of pSP64T-derived Bluescript vector carrying 5' and 3' untranslated sequences of the β -globin gene from *Xenopus*. Capped complementary RNA (cRNA) encoding *JcPIP2* was synthesized *in vitro* using T3 RNA polymerase at a final concentration of 1 mg/ml [13]. Capped cRNA (30 ng) or an equivalent volume of water was injected into each oocyte using an automatic injector (Nanoject; Drummond Scientific, Broomall, USA).

Osmotic water permeability analysis

Three days later, individual oocytes were 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 s intervals 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:

$$P_f = V_0 [d(V/V_0)/dt] / [S \times V_w \times (Osm_{in} - Osm_{out})] \quad 1$$

where initial oocyte volume, V_0 , is 9×10^{-4} cm³; initial oocyte surface area, S , is 0.045 cm² and molar volume of water, V_w , is 1.8×10^{-4} cm³ [12,13]. The P_f values were means of the measurements from five cells.

Preparation of *J. curcas* plasma membrane proteins

Purification of plasma membrane proteins was carried out as described previously [14]. The desired tissues were homogenized in homogenization buffer [330 mM sucrose, 100 mM EDTA, 50 mM Tris, 0.05% MES, 5 mM dithiothreitol, and complete proteinase inhibitor cocktail (Roche, Mannheim, Germany), pH 7.5]. Plasma membrane vesicles were purified by aqueous two-phase partitioning in a mixture of PEG3350 and Dextran T-500, 6.4% (*W/W*) each, with 5 mM KCl. Extrinsic membrane proteins were stripped using a procedure adapted from that of Javot *et al.* [8]. Briefly, membranes were incubated in 5 mM EDTA, 5 mM EGTA, 4 M urea, and 5 mM Tris-HCl, pH 9.5, for 10 min on ice. After centrifugation for 30 min at 100,000 g, the membranes were washed successively in 20 mM NaOH and 2 mM EDTA, 2 mM EGTA, 100 mM NaCl, and 5 mM Tris-HCl, pH 8.0. Finally, membranes were resuspended in 10 mM Tris-borate, pH 8.3, containing 9 mM KCl, 300 mM sucrose, 5 mM Na₂EDTA, 5 mM Na₂EGTA, 50 mM NaF, 5 mM dithiothreitol and 2 μ g/ml leupeptin, and stored at -70 °C before electrophoresis.

Antibody preparation and Western blot

The peptide H₂N-MAKEVSEETQTTHAK-CONH₂ was synthesized corresponding to the N-terminal part of *JcPIP2*. The peptide mixed with Imject Freund's Complete Adjuvant (Sigma). A New Zealand white rabbit was injected and boosted three times at 3-week intervals with the same adjuvant. Specific antibody was affinity-purified from the serum using the above peptide coupled with CNBr-activated Sepharose 4B (Amersham Biosciences, Feiburg, Germany) as described previously [17]. The antibody was allowed to bind to the peptide-Sepharose beads for at least 12 h at 4 °C and eluted using 50 mM glycine at room temperature. One-tenth volume of 1 M Tris-HCl (pH 8.0) was used to neutralize the eluted antibody and bovine serum albumin was added to a final concentration of 1 mg/ml.

An equal loading of the protein extracts (20 μ g) in the different lanes was confirmed by staining the gel with Coomassie Brilliant Blue. The proteins were subject to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose. Then the proteins were detected using the rabbit anti-*JcPIP2* antiserum. Goat anti-rabbit immunoglobulin G coupled with horseradish peroxidase (Bio-Rad, Hercules, USA) was used as the second antibody. To quantify the immunoblot signal, the intensity of each band was measured using Adobe Photoshop 7.0 (Adobe Systems, Mountain View, USA), corresponding to Coomassie Brilliant Blue bands.

Measurement of Lp

Lp of roots was measured by pressuring the roots using a SchÖlander pressure chamber [18]. 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, J_v ($\text{mg}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$), and plotted versus pressure gave the Lp ($\text{mg}\cdot\text{g}^{-1}\cdot\text{min}^{-1}\cdot\text{MPa}^{-1}$) value. A minimum of five seedlings was measured and assayed in each treatment.

Statistical analysis

Data were analyzed statistically using SigmaStat 2.03 (SPSS, Chicago, USA). All data were subjected to ANOVA and are shown as the mean \pm SE. Statistical significance was determined using Bonferroni's modification of

Student's *t*-test. Results were considered statistically significant at $P<0.05$.

Results

Gene cloning and sequence comparison

A 504 bp cDNA fragment was generated by reverse transcription-PCR with the degenerate primers, which were designed based on the conserved regions from *R. communis* and *S. oleracea*. The resulting fragment showed 87% and 75% sequence similarity with the *PIP* genes from *R. communis* and *S. oleracea*, respectively, indicating a partial putative *JcPIP2* was isolated from *J. curcas*. Gene-specific primers were further designed to obtain the full-length *JcPIP2*. A 584 bp 5'-RACE product and a 643 bp 3'-RACE product were amplified and subjected to sequence analysis (data not shown), and the full length of cDNA was obtained. Sequence analysis revealed that the cloned *JcPIP2* was 1013 bp in length, and contained an 843 bp open reading frame (GenBank accession No. EF030420).

As it was shown in Fig. 1, the protein encoded by the cloned cDNA had 280 amino acid residues and shared high

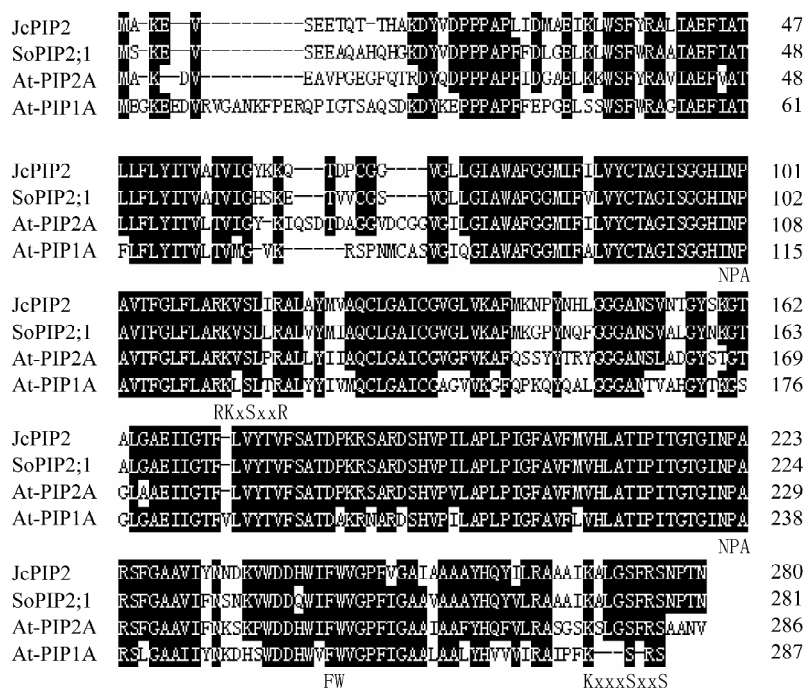


Fig. 1 Amino acid sequence alignments of plasma membrane intrinsic protein (PIP) from *Jatropha curcas* (*JcPIP2*) and other plant PIPs

Amino acid sequences were compared with the ClustalW multiple alignment program. Accession numbers for the sequences are as follows: At-PIP1A (NP_191702, *Arabidopsis thaliana*); At-PIP2A (NP_190910, *A. thaliana*); *JcPIP2* (ABM54183, *J. curcas*); SoPIP2;1 (AAA99274, *Spinach oleracea*). Identical amino acid residues common to at least three sequences are shaded. The numbers refer to the respective amino acid sequence. The conserved motifs are marked with bold letters.

homology with PIP2s from higher plants. Conserved motifs NPA, FW, RKxSxxR, and KxxxSxxS exist in the putative protein (Fig. 1). These conserved motifs might be candidates for the location determinant to PIP proteins and/or the regulation of water channel activity.

Based on BLASTP 2.2.3 program analysis (<http://www.ncbi.nlm.nih.gov/BLAST/>), the JcPIP2 amino acid sequence showed a high identity with PIPs from *R. communis* (GenBank accession No. CAE53883.1) (94%), *Mimosa pudica* (GenBank accession No. BAD90701.1) (91%), *Bruguiera gymnorhiza* (GenBank accession No. ABL76066.1) (90%), *S. oleracea* (GenBank accession No. AAA99274) (86%) and *Arabidopsis thaliana* (GenBank accession No. NP_190910) (78%). This result indicated that the putative protein might be localized at the plasma membrane.

Functional analysis of JcPIP2 in *Xenopus* oocytes

Oocytes injected with JcPIP2 cRNA had a swelling rate up to 10 times higher than that of water-injected oocytes, confirming that JcPIP2 is a water channel protein. The mean swelling rate of 0.102 units/min corresponded to an osmotic membrane permeability coefficient, P_f , of 1.35×10^{-2} cm/s (Fig. 2).

Abundance of JcPIP2 in different tissues during development

The antibody, anti-JcPIP2, clearly binds with the 29 kDa antigen in the purified plasma membrane proteins (Fig. 3). The signals of JcPIP2 were observed in all tested tissues: young roots, young stems, young leaves, flowers, endosperms and young seed cases. The relatively higher amounts of JcPIP2 were shown in young roots than in

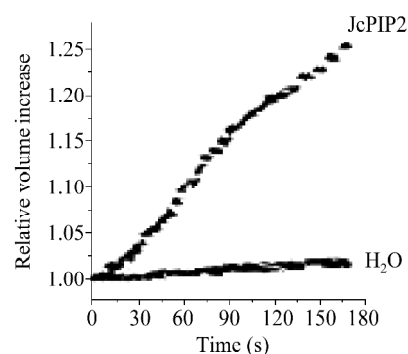


Fig. 2 Time-courses of changes in the relative volume increases of *Xenopus* oocytes injected with 30 ng of complementary RNA or RNase-free water

JcPIP2, plasma membrane intrinsic protein from *Jatropha curcas*. $n=10$.

any other tested tissues (Fig. 3).

Effect of drought stress on Lp

Measurement of Lp was carried out on seedlings of the two populations. Lp changed with the increasing degree of drought stress both in GaoYou CSC63 and YanBian S1 seedlings (Fig. 4). As the concentration of PEG6000 increased in the nutrient solution, Lp decreased progressively with respect to the control seedlings. But seedlings of GaoYou CSC63 showed a higher Lp and a lower decreasing trend than the YanBian S1 seedlings when treated with PEG6000.

Abundance of JcPIP2 in seedlings under drought stress

As shown in Fig. 5, the protein levels of JcPIP2 in seedlings of GaoYou CSC63 significantly increased when the plants

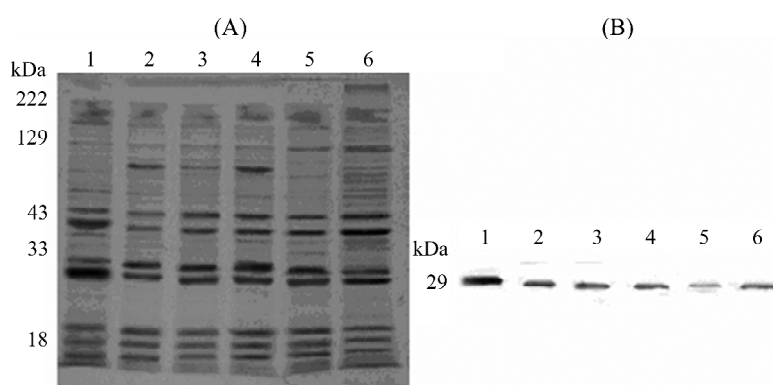


Fig. 3 SDS-PAGE and Western blot analysis of membrane intrinsic protein from *Jatropha curcas* (JcPIP2) in plant tissues

Plasma membrane protein extracts from tested tissues were fractionated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (A). The blot was developed with anti-JcPIP2 antibody (B). Samples of equal protein were loaded in each lane. Molecular mass markers are indicated on the left. 1, young roots; 2, young stems; 3, young leaves; 4, flowers; 5, endosperms; 6, young seed cases.

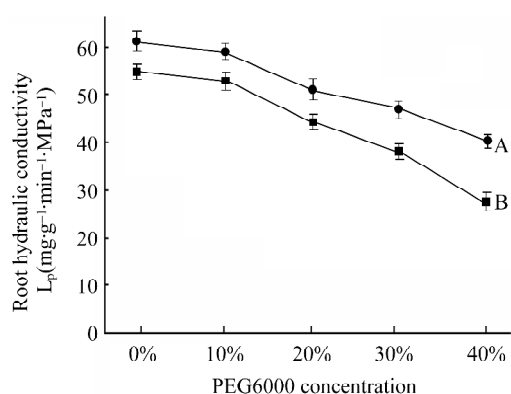


Fig. 4 Hydraulic conductivity of *Jatropha curcas* seedlings under drought stress

A, hydraulic conductivity (L_p) of the seedlings of GaoYou CSC63 under different degrees of drought stress ($n=5$); B, L_p of the seedlings of YanBian S1 under different degrees of drought stress ($n=5$). Error bars=SE. PEG6000, polyethylene glycol with an average molecular weight of 6000.

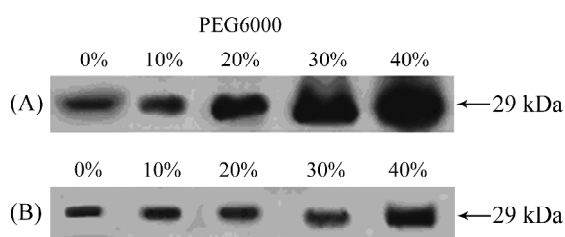


Fig. 5 Drought stress response of plasma membrane intrinsic protein from *Jatropha curcas* (JcPIP2)

Plasma membrane protein extracts of seedlings treated with polyethylene glycol with an average molecular weight of 6000 (PEG6000) were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose. The blots were developed with anti-JcPIP2 antibody. Samples of equal protein were loaded in each lane. Molecular mass markers are indicated on the left. (A) GaoYou CSC63. (B) YanBian S1.

were treated with a higher PEG6000 concentration for 24 h. But no significant increase of JcPIP2 was seen in YanBian S1 seedlings except those treated with 40% PEG6000.

Discussion

The discovery of water channel proteins in the membranes of plant cells allowed the formulation of new mechanisms for water transportation and osmotic adjustment in plants [19]. Previous reports showed that aquaporins were related to drought tolerance in many plant species. The molecular bases of drought resistance in

J. curcas have not been studied. Many genes encoding aquaporins were isolated from different plant species, however, until now no genes have been identified from *J. curcas*. Here, we cloned a *JcPIP2* cDNA from *J. curcas*.

Aquaporins, with varied molecular masses from 27 to 34 kDa, usually form the most abundant polypeptide [20]. Appropriate protein purification methods providing a ready supply of plasma membranes is available [21]. *JcPIP2* was isolated from the plasma membrane proteins in *J. curcas* and immunodetected with anti-JcPIP2. Using the same method, aquaporins were also isolated from *A. thaliana* [21], *Raphanus* [22] and *R. communis* L. [17]. The antibodies were produced by the short peptide sequence selected from the deduced amino acid sequence of the corresponding aquaporin gene.

Based on the amino acid sequences, PIPs can be divided into two major groups, PIP1 and PIP2. Compared with PIP1 proteins, PIP2 proteins possess a shorter N-terminal extension and a longer C-terminal end containing putative phosphorylation sites [23]. The NPA motifs located on the N-terminal half and C-terminal half show an internal homology of the amino acid sequence. The FW motif is conserved in the sixth transmembrane helix and possibly contributes to aquaporin substrate specificity [24]. Two other conserved motifs, RKxSxxR and KxxxSxxS, exist in the putative protein of *JcPIP2* which are recognized by several protein kinases [4].

Many aquaporin molecules have been identified, but their functions have not been fully determined. Most of them probably function as water channel proteins. But it is not enough to determine the function of water transport activity based only on sequence data. *Xenopus* oocytes provide a convenient system to determine the water transport activity of a single protein. Therefore, the water transport activities of plant PIPs [17,21,25] have been individually determined. In this report, oocytes injected with *JcPIP2* cRNA had a swelling rate 10-fold higher than that of oocytes injected with water. Similar results have been reported for other aquaporins [26]. The results in the present study indicated that *JcPIP2* also highly facilitates the transport of water, as with all other tested plant PIP2s [23].

Aquaporins are ubiquitous channel proteins that show different distribution patterns in plants. Previous work has revealed that certain aquaporin isoforms show organ-, tissue-, and cell type-specific manners, such as seed-specific α -TIP in bean (*Phaseolus vulgaris*), root-specific TobRB7 in tobacco (*Nicotiana tabacum*), γ -TIP in zones of cell enlargement of *Arabidopsis* [4], and MIP-MOD in the stigma epidermis of *Brassica* [27]. However, other aquaporin isoforms seem to be located in most plant tissues

[1]. JcPIP2 is also ubiquitously located in most plant tissues from the results based on immunodetection. The cross-reactivity of antibodies with several close aquaporin homologs can be observed [23], particularly in the PIP subfamily, which share high identity (>95%). Isoform-specific antibodies should be raised against the most divergent amino acid sequence [28]. In contrast to immunodetection techniques, mass spectrometry analysis of aquaporins can provide an accurate distinction of very close aquaporin homologs [29]. However, this approach remains mostly qualitative and does not provide precise information on protein abundance.

Recently, water stress-associated proteins, aquaporin, dehydrin, leafy cotyledon1-like protein, and fructose-1,6 bisphosphatase have been studied in many plant species in order to investigate the drought resistant mechanism. It has been revealed that land plants evolved to cope with rapid changes in the availability of water by regulating all PIPs. During drought stress, evidence for strong regulation of PIPs was found both at the transcriptional and post-transcriptional level, such as phosphorylation [30]. Root hydraulic conductivity might have a direct bearing on the ability of plants to survive and grow under adverse conditions such as water shortage. A large body of Lp data existing on the regulation of root water transport activity during development and under stress conditions was found in the studies of plant aquaporins [31]. The effect of drought on the regulation of *PIP* gene expression and abundance in *P. vulgaris* plants was measured [32]. The regulation of arbuscular mycorrhizal symbiosis in *P. vulgaris* plants of root hydraulic properties and root PIP was evaluated under drought stress [33]. Those results indicated that aquaporins could account for 35%–80% of root Lp under wet conditions, and for 60%–80% of root Lp in drying or rewetted soil. Early evidence also showed the water permeability in the tonoplast was much higher than in the plasma membrane [21]. The most likely reason is that the activity of the plasma membrane proteins determines the hydraulic conductivity in a tissue.

In *J. curcas*, drought stress, as expected, negatively affected root hydraulic conductivity. It can also be found in other plant species that the drought-resistant variety showed a higher Lp than the drought-sensitive variety [34]. At the same time, the abundance of JcPIP2 in seedlings of drought-resistant populations clearly increased compared with drought-sensitive populations under water deficit. The abundance of JcPIP2 was induced by heavy drought stress, which resulted in higher levels of the Lp value and a lower decreasing trend in GaoYou CSC63 than in YanBian S1. Therefore, JcPIP2 probably played a role in drought

resistance in *J. curcas*. However, when treated with 10% and 20% PEG6000, there was no difference in Lp between GaoYou CSC63 and YanBian S1 (**Fig. 4**). Therefore, the increase in abundance of JcPIP2 only has a possible effect in relatively increasing Lp for higher levels of stress. Furthermore, due to the high identity between plant PIPs, it is possible that some other PIP proteins in *J. curcas* were being recognized by the antibody. It might derive from some or all plasma membrane proteins in the membrane.

Acknowledgements

Drs. Yingze NIU and Nianhui ZHANG are acknowledged for their generous help during the preparation of the manuscript.

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