Research Article

The Tonoplast Intrinsic Protein Gene *KvTIP3* is Responsive to Different Abiotic Stresses in *Kosteletzkya virginica*

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In higher plants, aquaporin proteins (AQPs) play important roles in the uptake of water across cell membranes. However, their functions in halophytes are still largely unknown. In this work, we isolated, cloned, and identified *KvTIP3*, a tonoplast intrinsic protein gene from *Kosteletzkya virginica*. Bioinformatic analyses demonstrated that *KvTIP3* encoded a tonoplast protein with the common properties of AQPs. Further multiple sequence alignment and phylogenetic analyses showed that KvTIP3 shared 65%–82% homology with other AQPs from *Arabidopsis*, cotton, polar, and cocoa. Quantitative real-time PCR (qPCR) analyses revealed that *KvTIP3* was ubiquitously expressed in various tissues such as leaves, stems, and roots, with a predominant expression in roots. In addition, *KvTIP3* transcript was strongly induced by NaCl, low temperature, and ABA in *K. virginica*. Our findings suggest that *KvTIP3* encodes a new AQP possibly involved in multiple abiotic stress responses in *K. virginica*, and *KvTIP3* could be used as a potential candidate gene for the improvement of plants resistant to various abiotic stresses.

1. Introduction

Water is one of the essential requirements for all the organisms living on Earth [1, 2]. As a principal member of the biological kingdom, plants have evolved a series of mechanisms to absorb, transport, and utilize water. Unlike animals, plants cannot escape away from the adverse environment, such as drought and salt stress. Therefore, they must ensure the balance of water at both cellular and whole plant levels. AQPs are a family of channel proteins existing in all living organisms and play crucial roles in regulating the movement of water across the cell membrane [3]. For example, 13 AQPs were identified in human genome [4]. 35 and 36 AQPs were identified in *Arabidopsis* and maize, respectively [5, 6]. In addition, the number of AQPs isolated was 28 in *Vitis vinifera* L. [7], 28 in *Beta vulgaris* [8], 55 in *Populus trichocarpa* [9], 41 in *Solanum lycopersicum* [10], 63 in bamboo [11], 66 in *Glycine max* [12], and 71 in cotton [13].

AQPs are integral membrane proteins working in the form of tetramers and each monomer acts as an independent water channel [14]. According to the phylogenetic positions and subcellular localizations, plant AQPs are subdivided into four groups: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin 26-like intrinsic proteins (NIPs), and small basic intrinsic proteins (SIPs) [15]. PIPs and TIPs are the most abundant proteins located in the plasma membrane and tonoplast, respectively [15]. Recently, three more groups, uncharacterized intrinsic proteins (XIPs), GlpF-like intrinsic proteins (GIPs), and hybrid intrinsic proteins (HIPs), were also proposed [16, 17]. Therefore, AQPs consist of seven subfamilies. TIPs are AQPs predominantly located at the tonoplast [15]. Structurally, TIPs possess the conserved domains and motifs of AQPs, the six transmembrane helices with N-terminus and C-terminus located in the cytosol, and two NPA (asparagine-prolinealanine) motifs [7, 12]. The transmembrane helices constitute the channel for water and other substrate molecules. The conserved NPA motifs determine the substrate specificity [18]. In addition to transporting water across the membrane, TIPs can also facilitate the transport of other small molecules such as CO₂, glycerol, NH₃, arsenite, and silicon [19, 20]. Therefore, TIPs not only play a key role in water uptake but also function in many other aspects during the growth and development of plant.

Recently, an increasing number of researches revealed that TIPs played important functions in plant response to abiotic stresses. In Arabidopsis, TIP3;1, TIP3;2, and TIP4;1 were found to be involved in seed dormancy and germination in response to water stress [21]. In the halophyte Thellungiella salsuginea (salt cress), TsTIP1;2 was involved in multiple stresses responses [22]. Recently, AQPs were reported to be involved in abiotic stress response in the halophyte Eutrema salsugineum [23]. In Mesembryanthemum crystallinum, osmotic stress can induce the relocation of McTIP1;2 from the tonoplast to other membranes [24]. Consistently, expression of CsTIP2;1 from citrus in tobacco improved the growth and antioxidant capacity of transgenic plants under stress conditions [25]. Similarly, expression of tomato SlTIP2;2 and jojoba ScPIP1 enhanced the tolerance of transgenic plants to various abiotic stresses [26]. However, the functions of TIPs are multifaceted. The same environmental stimulus can lead to both upregulated and downregulated expression of different TIPs. Similarly, overexpression of TIPs does not always cause a positive effect on transgenic plants. For example, expression of GsTIP2;1, which was responsive to abiotic stresses, depressed salt and dehydration resistance in Arabidopsis [27]. Therefore, AQPs could function in various environmental stress responses.

K. virginica, a typical halophyte, is a new species with fine salt-tolerant characteristics at both physiological and molecular levels [28]. As an extremophile plant, *K. virginica* is very tolerant to adverse environmental stresses. It could grow and reproduce successfully in soil containing 0.3 to 2.5% sodium [29]. Previously, we examined the global gene expression profiles of *K. virginica* in response to salt stress [28]. Many gene expressions and pathways showed discrepancy. In this work, we isolated and identified a tonoplast aquaporin encoding gene KvTIP3 from *K. virginica*, which showed the most significant gene expression variation as exhibited by transcriptomic analyses. Our results demonstrate that KvTIP3 is a typical TIP encoding gene with a responsive expression to different abiotic stresses.

2. Materials and Methods

2.1. Plant Materials. K. virginica seeds were collected from the Yellow River Delta, Shandong Province, China. Seeds were surface sterilized and seedlings were cultivated as described before [30]. Two-week-old seedlings with the same morphology were collected and used for the following experiments as described previously [31, 32].

2.2. Abiotic Stress Treatments. Two-week-old seedlings were subjected to high temperature, low temperature, high salinity, osmotic stress, and ABA treatments as described previously [30, 33].

2.3. RNA Isolation and cDNA Synthesis. Total RNA was extracted and analyzed as described previously [30]. The RNA samples were used to synthesize cDNA with TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgen, China). Primers used for cDNA synthesis were random primer and anchored oligo $(dT)_{18}$. Reaction was carried out by incubating the reaction mixture at 42°C for 30 min, and then at 85°C for 5 min. Finally, cDNA was examined with Agilent 2100 BioAnalyzer to confirm its quality and quantity.

2.4. Cloning and Bioinformatic Analyses of KvTIP3. The full length of KvTIP3 gene was amplified by RACE technique according to the user's manual (SMART[™] RACE cDNA Amplification Kit). Gene-specific primers (GSP-F: 5'-TAGTAAACCG CACGGACCACAGA-3' and GSP-R: 5'-CCAACATCTTAGTAGGCGGACCATT -3') and nested (NGSP-F: 5'-TTCCTCCAAGCAAGGCAprimers CCGAAG-3' and NGSP-R: 5'-GCCTTGGTAGGTTG-GAGGTGGGA-3') were designed according to the sequence of cDNA fragments in our previous transcriptomic sequencing data, which was deposited in Transcriptome Shotgun Assembly (TSA) Sequence Database with the accession number-GCJL00000000 [29]. The obtained gene fragment was ligated into the pEASY-T5 Zero Cloning Vector (Transgen, China) after purification and sequencing.

2.5. Quantitative Real-Time RT-PCR (qPCR) Analyses. qPCR was carried out with the instrument ABI Prism-SYMBOL 0 \f "Times New Roman" \s 127500 FAST (Applied Biosystems, Foster City, CA). The reaction system was performed using the SYBR Green Real-Time Selected Master Mix (Applied Biosystems by Life technologies), and the reaction condition was performed following the user's manual. cDNA was diluted to the required concentration (<50 ng/ μ L), and gene-specific primers were designed with the Beacon Designer (BD) 7.0 software based on the RACE results. Gene-specific primers used for qPCR are 5'-ATGCCAACTCGTAGATAC-3' and 5'-CTAGTAATCTT-CAGGAG C-3'. The reaction volume was $20 \,\mu\text{L}$ with a composition of 2 μ L diluted cDNA template, 10 μ L 2 × SYBR Master Mix, $1.5 \,\mu\text{L}$ primers, and $6.5 \,\mu\text{L}$ double distilled water. A reference gene, 18SrRNA, which was the most stably expressed gene among the four selected reference genes (18SrRNA, ACT, TUA, and EF) under salt stress condition, was used as an internal control [34]. qPCR amplification was performed as following: initial denaturation at 95°C for 2 min, 40 cycles of 15 s at 95°C for denaturation, and 1 min at 60°C for annealing and extension. Fluorescence signal was conducted at the temperature between 60°C and 90°C. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiment, was strictly followed throughout our experiment [35]. In addition, to guarantee the accuracy of the experiment, the primer specificity of qPCR was insured by the typical melting curve, amplification plot, and the single product of RT-PCR. cDNA was diluted to suitable concentrations to guarantee the Cq values in the optimum range. For all the experiments, each sample was performed in triplicates and three biological replicates were performed.

2.6. Data Analysis. All the data were mean values \pm SD of three experiments. For qPCR analysis, the $\Delta\Delta$ Cq method was used. ANOVA was applied to analyze the significant differences, and the *P* value was kept below 0.05. Sigma Plot 12.0 was used for plotting.

3. Results

3.1. Isolation and Sequence Analysis of KvTIP3. To understand the possible function of AQPs in abiotic stress tolerance in halophyte, a *K. virginica* homolog of tonoplast intrinsic protein gene, *KvTIP3*, was isolated (GenBank accession no. KT732779). The 1084-bp *KvTIP3* consists of a 154-bp 5' untranslated region (UTR), a 768-bp encoding region, and a 161-bp 3' UTR, encoding a 256 amino acid protein (KvTIP3) with a calculated molecular mass of 27 kD and isoelectric point of 6.58, as predicted by ProtParam (http://www.expasy.org/tools/).

3.2. Bioinformatic Analysis of KvTIP3. We further performed bioinformatic analyses. PsortII (http://www.psort. org/) prediction revealed that KvTIP3 protein was located in the secreted pathway with a probability of 94.4%. Further topological prediction with TMHHM (http://www.cbs.dtu. dk/services/TMHMM-2.0/) and TMPred (http://www.ch. embnet.org/software/TMPRED_form.html) demonstrated that KvTIP3 contained six transmembrane helices with its N-terminus and C-terminus located in the cytoplasm (Figures 1(a) and 1(b)). It is well known that the activities of AQPs are regulated by their phosphorylation status. For example, the Snf1-related protein kinase 2.6 (SnRK2.6) was able to phosphorylate a cytosolic PIP2;1 peptide at Ser-121 to regulate the response of guard cells to ABA signaling [36]. Similarly, Cys116 and Cys118 have been proved to be the phosphorylation sites in TIP proteins [37]. We also examined the possible phosphorylation sites in KvTIP3 with NetPhos 2.0 (http://www.cbs.dtu.dk/services/NetPhos/) and found that KvTIP3 also contained 7 potential phosphorylation sites, including 4 Ser, 2 Thr, and 1 Tyr (Figure 1(c)). Moreover, we constructed a phylogenetic tree including the typical TIPs in Arabidopsis and other closely related organisms. We observed that KvTIP3 was clustered to the Arabidopsis AtTIP3 subgroup and was 82% homologous to TIP3-2 in Gossypium hirsutum, one of the most closely related organisms to K. virginica (Figure 2(d)). In addition, KvTIP3 shared 65%-78% amino acid sequence homology

with tonoplast aquaporins from other higher plants (Figure 2). KvTIP3, like TIPs from other higher plant, contained two NPA motifs and formed the cavity of AQPs to act as a selective barrier for the transport of water or small molecules [38].

3.3. KvTIP3 Was Induced by Salt Stress in K. virginica Seedlings. As a first step to know the expression profile of KvTIP3 in K. virginica, we examined its relative expression levels in different tissues of two-week-old seedlings under normal growth condition by qPCR (Figure 3(a)). KvTIP3 was ubiquitously expressed in various tissues including leaves, stems, and roots, with a predominant expression in the roots (Figure 3(b)). Based on the information extracted from the transcriptomic sequencing database (accession number-GCJL0000000) that KvTIP3 cDNA fragments were significantly accumulated upon salt stress treatment, we performed qPCR to investigate the transcript levels of KvTIP3 in K. virginica under both normal and salt stress conditions. Two-week-old seedlings were treated with 0, 200, 300, or 400 mM NaCl for 24 h. Although no significant phenotype change was observed after different treatments, salt stress significantly induced the expression of KvTIP3 in a concentration-dependent manner, with the most significant induction with 300 mM NaCl (Figure 3(c)). When treated with 300 mM NaCl for different time periods, the transcript level of KvTIP3 increased after 6 h and reached a maximum level at 12h followed by a gradual decrease at 24h (Figure 3(d)).

3.4. Effects of Various Abiotic Stresses on the Expression of KvTIP3. For the past few years, an increasing number of reports have revealed the important roles of AQPs in abiotic stress responses [39-41]. Thus, we tested the expression of KvTIP3 under a few abiotic stresses and ABA treatments (Figures 4(a)-4(d)). Similar to the salt stress treatment, both low temperature and ABA treatments upregulated the expression of KvTIP3. The expression of KvTIP3 increased after the seedlings were kept at 4°C for 24 hours (Figure 4(a)), whereas the expression of KvTIP3 increased within 6 hours after the seedlings were treated with $100 \,\mu M$ ABA (Figure 4(b)). Different from these observations, the expression of KvTIP3 was downregulated when the seedlings were kept at 42°C or treated with 15% PEG (Figures 4(c) and 4(d)). All these results suggest that KvTIP3 is an abiotic stress and ABA inducible or responsive gene.

4. Discussion

Previous studies have demonstrated that AQPs play important roles in plant growth, development, and in plant resistance to adverse environmental stresses. In Tulip (*Tulipa gesneriana*), both TgTIP1;1 and TgTIP1;2 were involved in petal development by regulating the absorption and transport of water [42]. Under osmotic or salt stress condition, AQPs in cucumber seedlings were able to regulate root and leaf hydraulic properties to respond to the environmental stimulus [41]. In this work, we isolated a novel AQP



FIGURE 1: The secondary structure, phosphorylation sites, and phylogenetic analysis of KvTIP3. (a, b) Transmembrane domain analysis of KvTIP3 with TMHHM and TMPred. (c) Phylogenetic analyses of KvTIP3 with other closely related TIPs proteins. All the sequences were extracted from the NCBI database. The phylogenetic tree was constructed by Mega 7.0 program using the neighbor-joining (NJ) method. (d) The potential phosphorylation sites of KvTIP3 predicted with NetPhos 2.0.

encoding gene *KvTIP3* from *K. virginica*. Acting as multifunctional membrane channels, AQPs have very highly conserved structure [7, 18]. Sequence analyses of KvTIP3 revealed that it was one of the homologs of AQPs. The molecular weights of the reported AQPs ranged from 23 to 31 kDa (243–302 amino acid residues). We found that KvTIP3 gene encoded a 27 kDa AQP that consisted of 256 amino acid residues.

ACIIFI-1	MPIRNIAIG. RPDEAIRPDALKAALADTISILIPVVA GSGSGMAFNKLIENGAIIPSGLVAAAVAHAFGLEV	/ 1
AtTIP1-2	MPTRNIAICGVQEEVYHPNALRAALAEFISTLIFVFACSGSGIAFNKITDNGATTPSGLVAAALAHAFGLFV	72
AtTIP1-3	MPINRIALG, TEGEASREDATRAAFARESMUTEVEA GOGSGMAYGKI TGDGPATPAG IVAASISHAFALEV	71
A+TTP2-1	MAGUARG SEDDSESTAST DAVI APATSTILLEVEA CUCSATA VARITSDAAT DTDC	60
ACTIF2 1		0.0
AUTIF2-2	BVKIEIG.SVGDSFSVASLAAILSEFIAILSEFIAILSEVGAGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	05
AtTIP2-3	MVKIEVG.SVGDSFSVSSLKAYLSEFIATLLEVFAGVGSAVAFAKLTSDGALDPAGLVAIAIAHAFALFV	69
AtTIP3-1	MATSARRAYGFG.RADEATHPDSIRATLAEFLSTFVFVFAAEGSILSLDKLYWEHAAHAGTNTPGGLILVALAHAFALFA	79
AtTIP3-2	MATSARRAYGFG.RADEATHPDSIRATLAEFLSTFVFVFAGEGSILALDKLYWDTAAHTGTNTPGGLVLVALAHALALFA	79
AtTIP4-1	MKKIELG.HHSEAAKPDCIKALIVEFITTFLFVFAGVGSAMATDSIVGNTLVGLFAVAVAHAFVVAV	66
AtTIP5-1	MRRMIPTSFSSKFQGVLSMNALRCYVSEFISTFFVLA AVGSVMSSRKLMAGDVSGPFGVLIPAIANALALSS	73
GaTIP3-2	MPPRRYOFG, RADEATHPDSMRATLAE FLST I IFVFA GEGS TLALDKMYKDTSATPAR IVMTALAHGI SI FA	71
GrTTP3-2	MEDDEVOKS BAREATHERSMEATLAFELSTITEVEA GEGSTLATERWYVETSATEAD IVMTALAHGISTEA	71
DETTD	MEDDATE DAREATHERSMEAT AF SUSTEMENTAL SCOULAR PREVENCES COMMUNICATION AND AND AND AND AND AND AND AND AND AN	70
TeTTD		/
ICIIP	MPPRRIARG.RSDEATHPDSMRATLAEHLSTLIFVRAGEGSTLALDKMYKDASTTPAGLVMVALAHALSLFS	71
KVTIP3	MPTRRYEVG.RADEATHPDSMRATLADELAAIIEVFAGEGSILAIDKMYKDTSTTPARLVMVALAHGLALFA	71

AtTIP1-1	AV SYGAN ISCCT WIPAWITEGAFICENITLLEGILWITACL LCSVVACLTUKFATCGLAVPAFGLSACVCVLNAFVFDTVM	151
AtTTP1-2	AV SVGANTSGGEUNPAVTEGVLIGENTTLERGTIVE TACLIGSVAACETISEATGGEPTPAEGISAGVGSINALVEETVM	152
AtTID1_3	AUSUCANUS COLUMDAUT FOR TICONITI I DATI VITACI I CAUNACITI PUST CONFIANTS SU SUCUT DUNAU UT TUM	151
ACTIPI-5		101
AtiiP2-1	AVAIGANISGG, WPAVIFGLAVEG IIVIIGVFWIAGLIGSIAACFIIKIVIGGLAVPINSVAAGLGSIEGVVPPIII	149
AtTIP2-2	GVSIAANISEEFINPAVILELAVEENITVITEFPYVIACCLESIVACLIIVFVINEESVPIHEVAAGLEAIEGVVMPIVV	149
AtTIP2-3	GVSIAANISGGELNPAVTLGLAIGENITLIIGFFXWIACCLGSIVACLLUVFVINGKSVPIHGVSAGLGAVEGVVMPIVV	149
AtTIP3-1	AVSAAINVSGGHVNPAVTFGALVGGRVTAIRAIYYVIACL LGAILACLLIRLTINGMRPVGFRLASGVGAVNGLVLDIIL	159
AtTIP3-2	AVSAAINVSGGHVNPAVTFAALIGGRISVIRAIYYNVAGL IGAILACLLIRLAINGLRPVGFHVASGVSELHGLLMDIIL	159
AtTIP4-1	MISAG.HISGGHINFAVTLGLLLCCHISVFRAFLYWIDCL LASSAACFLISYLTGGMGTFVHTLASGVSYTCGIIWPIIL	145
AtTIP5-1	SVYISWNVSGGHVNFAVTFAMAVACRISVFTAMFYWTSOM IASVMACLVHKVTVMECHVFIYKIAGEMTGFGASVIFGVL	153
GaTTP3-2	AVASSYNVSGGHVNPAVTEGALLGGETSLVBALYVTACL IGSTVACLETRLTA, GMRPGGEALASGVGELRGLTIATVL	150
GETTP3-2	AVASSUNUSGEUNPAVTEGALI GEPTSLUPAL VV TACLI GSTVACLI DI TA GMPDGGEALASGVGFLOGI TE TVL	150
DETTD	AVASSTNISCO-UNDAVITESI VCOLSUIDAVVAUACI LOSUAATI DI VINCADDUCEHVOSCVCEVECI INDAT	150
TeTID		150
ICIIP	GVAVSENVSGG VNPAVTEGALLGERISFIRAVITYVALLGATVACLETLVIA.GMRPAGFIVASGVGELRGLVIATVL	150
KALIB3	AVASSMAVSEER VAPAVIEGALLEERISVVRAVYWVAQLEGALVMCLEDRLTA. EMRPAGETVASGVGELRCMIDEIVL	150

AtTIP1-1	TEGLVYTYYADA IDEKNESLGTIAEIAICFIVGAN ILAGS AESCASMNDAVAECPAVVSWTWTNHWVWWAGELWEGGTAG	231
AtTIP1-2	TECTUVTUVA TANDEVNOST CTTALETATORTUCANTE ACCARTECTIVE CONTRACTOR	
	TECTAL TATENED TO TTELET A CONTRACTOR ON A DAMAGE A DEPARTMENT	232
AtTTP1-3		232
AtTIP1-3	TEGLIVYTWYATAVDERKKEDIGIIAELAIGUVGANILKAG DESGESMAPPAVERAVVSWIWINHWVWAGELIEGGIAG	232
AtTIP1-3 AtTIP2-1	TFGLVYTWYATAADFREGIGIIAFLAIGIIVGATIAG <u>FISGSSMNFFVAFEAVVSWIWINHWVWAGTIEGG</u> IAG TFGLVYTWYATAVDFKKCDIGIIAFLAIGLIVGATILVGG FIGASMNFFVSFCEAVVSWIWINHWVWAGFIGAATAA TFALVYTWYATAADFKKCSIGTIAFLAIGLIVGATILAAG FISGSSMNFFRSFCEAVAGGFSCHWVYWGFLIGGGIAG	232 231 229
AtTIP1-3 AtTIP2-1 AtTIP2-2	IFGLVYTVYATANDERKCDIGIIAFLAIGLIVGAN IAAG AFSGASMNENVSFGERVVSNIWINHW VNAGELIGGIAG IFGLVYTVYATANDERKCDIGIIAFLAIGLIVGAN ILAAG FESGGSMNENVSFGERVVSNIWINHW VNAGELIGGGIAG IFALVYTVYATANDERKCSLGIIAFLAIGLIVGAN ILAAG FESGGSMNENSFGERVAAGDFSGHWVW VGELIGGGIAG IFALVYTVYATANDERKCSLGIIAFLAIGFIVGAN ILAAG FESGGSMNENSFGERVAAGDFSGHWVW VGELIGGGIAG	232 231 229 229
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3	IFGLVYIVYAFEAVDEKKEDIGIIAELAIGLIVGANILAG HESGASMNENVEFEAVVSWIWINHWVWAGEDIGGIAG TFGLVYIVYAFADDEKKESIGIIAELAIGLIVGANILAAG HESGASMNENVEFEAVVSWIWINHWVWAGEDIGGIAG TFALVYIVYATADDEKKESIGIIAELAIGLIVGANILAAG FESGGSMNENSFEERVAAGEDESGHWVYAVGELIGGIAG TFALVYIVYATADDEKKESIGIIAELAIGFIVGANILAAG FESGGSMNENSFEERVVSGDESQIWIYAVGELVEGALAG TFALVYIVYATADDEKKESIGIIAELAIGFIVGANILAAG FESGGSMNENSFEERVVSGDESQIWIYAVGELVEGALAG	232 231 229 229 229
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1	IFGLVYIVYATAVDEKKEDIGIIAELAIGLIVGANILAG HESGASMNENVEFEHVVSWIWINHWVWAGEDIGGIAG IFGLVYIVYATAVDEKKESIGIIAELAIGLIVGANILAG HESGASMNENSEERVVAVGESGEWVVVGEFIGAIAA IFALVYIVYATADDEKKESIGIIAELAIGLIVGANILAAG HESGGSMNENSEERVAAGDESGHWVVVGELIGGIAG IFALVYIVYATADDEKKESIGIIAELAIGFIVGANILAAG HESGGSMNENSEERVVSGDESGIWIYAVGELVGGALAG IFALVYIVYATADDEKKESIGIIAELAIGFIVGANILAAG HESGGSMNENSEERVVSGDISGIWIYAVGELVGGALAG IFALVYIVYATADDEKKESIGIIAELAIGFIVGANILAAG HESGGSMNENSEERVVSGDISGIWIYAVGELVGGALAG IFALVYIVYATADDEKKESIGIIAELAIGFIVGANILAAG HESGGSMNENSEERVVSGDISGIWIYAVGELVGGALAG	232 231 229 229 229 239
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-2	TFGLVYTWYATAVDFKKCDIGTIAFLAIGLIVGAN ILAG HSGASMNPAVFERAVVSWIWINHWVWAGFELGGLAG TFGLVYTWYATAVDFKKCSLGTIAFLAIGLIVGAN ILAG FSGGSMNPAVSFERAVVSWIWTNHWVWAGFELGGAIAG TFALVYTWYATAADFKKCSLGTIAFLAIGLIVGAN ILAG FSGGSMNPASFERAVSGDFSGIWIYWVGFELGGALAG TFALVYTWYATAADFKKCSLGTIAFIAIGFIVGAN ILAG FSGGSMNPASFERAVSGDFSGIWIYWVGFELGGALAG TFALVYTWYATAADFKKCSLGTIAFIAIGFIVGAN ILAG FSGGSMNPASFERAVSGDFSGIWIYWVGFELGGALAG TFGLVYWYSTLIDFKCSLGIIAFIAIGFIVGAN ILAG FSGGSMNPASFERAVSGDFSGIWIYWVGFFIGSALAA TFALVYWYSTLIDFKCSLGIIAFIAIGFIVGAN ILAG FSGASMNPARAFERAVSGNLSGIWIYWVGFFIGSALAA	232 231 229 229 229 239 239 239
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-2 AtTIP4-1	IFGLVYTVYATAVDFKKCOLGIIAFLAIGLIVGANILAG MESGASMNPAVSFGAVVSWIWINHWVWAGELLGGIAG IFGLVYTVYATAVDFKKCSLGIIAFLAIGLIVGANILAG MESGASMNPAVSFGAVVSWIWINHWVYWGEFIGAATAA IFALVYTVYATAADFKKCSLGIIAFLAIGFIVGANILAAG FESGGSMNPARSFGAVVSGDFSCHWVWVGELLGGGIAG IFALVYTVYATAADFKKCSLGIIAFLAIGFIVGANILAAG FESGGSMNPARSFGAVVSGDFSCHWVWVGELLGGGIAG IFGLVYVYYSTATADFKKCSLGIIAFLAIGFIVGANILAAG FESGGSMNPARSFGAVVSGDLSCHWVWVGELVGGALAG IFGLVYVYYSTATADFKKCSLGIIAFLAIGFIVGANILAAG FESGASMNPARSFGAVVSGDFSCHWVWVGELVGGALAG IFGLVYVYYSTATADFKKCSLGIIAFLAIGLIVGANILAAG FESGASMNPARSFGALVGKRWSNHWIYVGFFIGGALAA IFSLLFTYYATIVDFKKCSLGIIAFLAIGLIVGANILVGG FESGASMNPARSFGALVGKRWSNHWIYVVGFFIGGALAA IFSLLFTYYATIVDFKKCSLGIIAFLAIGLIVGANILVGG FESGASMNPARSFGALVSGNWTHWVWVGGLIGGGLGAG	232 231 229 229 229 239 239 239 239
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-2 AtTIP3-2 AtTIP4-1 AtTIP5-1	IFGLVYIVYIVIATAVDEKKCDIGIIAELAIGLIVGANILAG ABSGASMNEZVEFGAVVSWIWINHWVWAGEDLGGIAG IFGLVYIVYIVIATAVDEKKCSLGIIAELAIGLIVGANILAG BESGGSMNEZVEFGAVVSWIWINHWVWAGEDLGGIAG IFALVYIVYIATAVDEKKCSLGIIAELAIGLIVGANILAAG BESGGSMNEZSEGAVVSGDESGIWVYVGELUGGIAG IFALVYIVYIATAVDEKKCSLGIIAELAIGLIVGANILAAG BESGGSMNEZSEGAVVSGDESGIWIYVGELUGGIAG IFALVYIVYITATAPEKKCSLGIIAELAIGLIVGANILAAG BESGGSMNEZSEGAVVSGDESGIWIYVGELUGGAIAG IFALVYIVYITATAPEKKCSLGIIAELAIGLIVGANILAAG BESGGSMNEZSEGAVVSGDESGIWIYVGELUGGAIAG IFALVYIVYITATAPEKKCSLGIIAELAIGLIVGANILAAG BESGGSMNEZSEGAVVSGDESGIWIYVGELUGGAIAG IFALVYIVYITILDEKKCSLGIIAELAIGLIVGANILAG BESGSSMEZAEGEAVVSGDESGIWIYVGELUGGAIAG IFALVYIVYISILIDEKKCSLGIIAELAIGLIVGANILAG BESGSSMEZAEGEAUVSGNWIDHWIYVGEFIGGAIAA IFALVYVYIYITIDEKKCSLGIIAELAIGLIVGANILAG BESGGSMEZAEGEAUVSGNWIDHWIYVGEFIGGAIAA AFVLVYIVYITIDEKKCSLGIFFIGFUCANILAG ASSGASMEZAEGEAUVSGNWIDHWIYVGEFIGGAIAA	232 231 229 229 239 239 239 239 225 232
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-2 AtTIP4-1 AtTIP5-1 GaTIP3-2	IFGLVYIVYATAADEKKEDIGIIAELAHLEELUGAN ILAG ABSGASMPRAVSEEAVUSWIWINHWVWAGELUGGIAG IFGLVYIVYATAADEKKESLGIIAELAHGLIVGAN ILAG BESGGSMPRASEGRAVUSWIWINHWVWAGELUGGIAG IFALVYIVYATAADEKKESLGIIAELAHGLIVGAN ILAG BESGGSMPRASEGRAVUSGDESGIWIYWVGELUGGIAG IFALVYIVYATAADEKKESLGIIAELAHGIUVGAN ILAG BESGGSMPRASEGRAVUSGDISQIWIYWVGELUGGIAG IFALVYIVYATAADEKKESLGIIAELAHGIUVGAN ILAG BESGGSMPRASEGRAVUSGDISQIWIYWVGELUGGIAG IFALVYIVYATAADEKKESLGIIAELAHGIUVGAN ILAG BESGGSMPRASEGRAVUSGDISQIWIYWVGELUGGIAG IFALVYIVYATAADEKKESLGIIAELAHGIUVGAN ILAG BESGGSMPRASEGRAVUSGDISQIWIYWVGELUGGIAG IFALVYIVYSTAIDEKKESLGIIAELAHGIUVGAN ILAG BESGSMPRASEGRAVUSGRWDHWIYWVGELUGGIAG IFSLUYVYYSTAIDEKKESIGIIAELAHGIUVGAN ILAG BESGSMPRASEGRAVUSGENUYWYYYYYYYY IFSLIFIVYAYIVATGIDEKKESLGGAN ILAG BESGSMPRASEGRAVUSSEKNQAVYWVGELUGGIAG AFVLUYYYYATGIDEKKESVGTIAELAHGIUVSAN ILVGG BESGSMPRASEGRAVVSSEKNQAVYWVGELUGGIAG IYGLVYTYYATGIDEKKESVGTIAELAHGIUVSAN ILVG BEGGSMPRASEGRAVVSSEKNQAVYWVGELUGGIAG	232 231 229 229 239 239 239 225 232 232
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-2 AtTIP4-1 AtTIP5-1 GaTIP3-2 GrTIP3-2	IFGLVYTVYATAADFRKCOLGIIAFLAIGLIVGAN TLAG MESGASMNEZVSFGEAVVSNIWINAW VAGELLGGIAG IFGLVYTVYATAADFRKCSLGIIAFLAIGLIVGAN TLAG FESGGSMNEZVSFGEAVVSNIWINAW VAGELLGGIAG IFALVYTVYATAADFRKCSLGIIAFLAIGFIVGAN TLAG FESGGSMNEZSFGEAVVSGLSGIWIYV VGELUGGAIAG IFALVYTVYATAADFRKCSLGIIAFLAIGFIVGAN TLAG FESGGSMNEZSFGEAVVSGLSGIWIYV VGELUGGAIAG IFALVYTVYATAADFRKCSLGIIAFLAIGFIVGAN TLAG FESGGSMNEZSFGEAVVSGLSGIWIYV VGELUGGAIAG IFALVYTVYATAADFRKCSLGIIAFLAIGLIVGAN TLAG FESGGSMNEZSFGEAVVSGLSGIWIYV VGELUGGAIAA IFSLYTVYVYSTATADFRKCSLGIIAFLAIGLIVGAN TLAG FESGGSMNEZSFGEAVVSGLVGGUGAIAG IFALVYVYYSTATADFRKCSLGIIAFLAIGLIVGAN TLAG FESGASMNEZSFGEAVVSGLVGWWHHWIYV VGELUGGAIAA IFSLLFTVYATIVDFKCSLGIGIFFIGFVGAN TLAG FESGASMNEZSFGEAVVSGWVDHWVYVGELIGGIAA IFSLLFTVYATIVDFKCSLGFGELLIGFVGAN TLAG FESGASMNEZSFGEAVVSGWVDHWVYVGELIGGIAA IFSLLFTVYATGIDFRCSVGTIAFLAIGLIVGAN TLAG FESGASMNEZSFGEALVGWWWHWIYVGELIGGATAA IYGLVYVYATGIDFRCSVGTIAFLAIGLIVSAN TLAG FESGEAMNEZAFGEALVGWWWHWVYVGELIGGATAA	232 231 229 229 239 239 239 232 230 230
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-2 AtTIP3-2 AtTIP4-1 AtTIP5-1 GaTIP3-2 GrTIP3-2 PtTIP	IFGLVYTVYLATAVDFRKCOLGIIAFLAIGLIVGAN ILAG MESGASMNPRVSFGRVVSWIWINHWVWAGHLIGGIAG IFGLVYTVYLATAVDFRKCSLGIIAFLAIGLIVGAN ILAG MESGASMNPRVSFGRVVSWIWINHWVWAGHLIGGIAG IFALVYTVYLATAVDFRKCSLGIIAFLAIGFIVGAN ILAG FESGGSMNPRSFGRVVSGDFSGIWVWVGHLIGGIAG IFALVYTVYLATAVDFRKCSLGIIAFLAIGFIVGAN ILAG FESGGSMNPRSFGRVVSGDLSGIWIYWVGHLIGGIAG IFALVYTVYLATAVDFRKCSLGIIAFLAIGFIVGAN ILAG FESGGSMNPRSFGRVVSGDLSGIWIYWVGHLIGGIAG IFALVYTVYLATAVDFRKCSLGIIAFLAIGFIVGAN ILAG FESGGSMNPRSFGRVVSGDLSGIWIYWVGHLIGGIAG IFALVYTVYLATAVDFRKCSLGIIAFLAIGFIVGAN ILAG FESGASMNPRSFGRVVSGDLSGIWIYWVGHLIGGIAG IFALVYTVYLGIDFRKCSLGIIAFLAIGLIVGAN ILAG FESGASMNPRSFGRVVSGDLSGIWIYWVGHLIGGIAG IFALVYVYSTAIDFRKCSLGIIAFLAIGLIVGAN ILAG FESGASMNPRSFGRVVSGDLSGIWIYWVGHJGGIGGIAG IFSLLFTVIATIVDFKKCSLDGFGHLIGFVVGAN ILAG FESGASMNPRSFGRVVSGNWTHWVYVGHJGGGIAG AFVLVYTVF.TASDFRRCLPLAVGHIFIGFVAGAN VLAG FESGGSMNPRAFGRLVSGNWTHWVYVGHLIGGIAG IFGLVYVYYATGIDFRKCSVGTIAFLAIGLIVSAN ILVGG FEGAZMNPRAFGCRLVGWRWNHWWIYVGHLIGGGIAG AFVLVYTVF.TGIDFRKCSVGTIAFLAIGLIVSAN ILVGG FEGAZMNPRAFGCRLVGWRWNHWWIYVGHLIGGGIAG IFGLVYVYYATGIDFKCSVGTIAFLAIGLIVSAN ILVGG FEGAZMNPRAFGCRLVGWRWNHWWIYVGHLIGGGIAG IFGLVYVYYATGIDFKCSVGTIAFLAIGLIVSAN ILVGG FEGAZMNPRAFGCRLVGWRWNHWWIYVGFLIGGGIAG IFGLVYVYYATGIDFKCSVGTIAFLAIGLIVSAN ILVGG FEGAZMNPRAFGCRLVGWRWNHWWIYVGFLIGGGIAG	232 231 229 229 239 239 239 230 230 230
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-2 AtTIP3-2 AtTIP4-1 AtTIP5-1 GaTIP3-2 GrTIP3-2 PtTIP TcTIP	IFGLVYIVYLATAVDEKKCDIGIIAELAIGLIVGANILAG MESGASMNERVSFGERVVSWIWINHWVWAGEDLGGIAG IFGLVYIVYLATAVDEKKCSLGIIAELAIGLIVGANILAG MESGASMNERVSFGERVVSWIWINHWVWAGEDLGGIAG IFALVYIVYLATADEKKCSLGIIAELAIGLIVGANILAG FESGGSMNERSFGERVVSGELSGIWIYNVGELLGGGIAG IFALVYIVYLATADEKKCSLGIIAELAIGLIVGANILAAG FESGGSMNERSFGERVVSGELSGIWIYNVGELLGGGIAG IFALVYIVYLATADEKKCSLGIIAELAIGLIVGANILAAG FESGGSMNERSFGERVVSGELSGIWIYNVGELUGGALAG IFALVYIVYLATADEKKCSLGIIAELAIGLIVGANILAAG FESGGSMNERSFGERVVSGELSGIWIYNVGELUGGALAG IFALVYIVYLATADEKKCSLGIIAELAIGLIVGANILAAG FESGGSMNERSFGERVVSGELSGIWIYNVGELUGGALAG IFALVYIVYSTILDEKKCSLGIIAELAIGLIVGANILAG FESGASMNERAFGERLVSGWWENWUYNVGEFIGGALAA IFALVYIVYTYTIDEKKCSLGIAELAIGLIVGANILAG FESGASMNERAFGERLVSGWWENWUYNVGEFIGGALAA IFALVYIVYTTIDEKKCSLGIGFLIGFVCANILAG FESGASMNERAFGERLVSGWWENWUYNVGEFIGGALAA IFALVYIVYTTIDEKKCSLGIGFLIGFVCANILAG FESGGSMNERAFGERLVSGWWENWUYNVGEFIGGALAA IFALVYIVYTTIDEKKCSLGIGFLIGFVCANILAG FESGGSMNERAFGERLVSGWWENWUWYGEFIGGALAA IFGLVYTVYTTIDEKKCSUGIIAELAIGLIVSANILAG FESGGSMNERAFGERLVSGWWENWUWYGELIGGALAA IFGLVYTVYTATGIDEKKCSVGIIAELAIGLIVSANILAG FEGGAMNERAFGERLVGWWWNHWIYNVGELIGGGIAG AFVLVYTVYTATGIDEKCSVGIIAELAIGLIVSANILVGG FEGAZMNERAFGERLVGWWWNHWIYNVGELIGGGIAA IFGLVYTVYTATGIDEKCSVGIIAELAIGLIVSANILVGG FEGAZMNERAFGERLVGWWWNHWIYNVGELIGGGIAA IFGLVYTVYTATGIDEKCSVGIIAELAIGLIVSANILVGG FEGAZMNERAFGERLUGWWWNHWIYNVGELIGGGIAA IFGLVYTVYTATALDEKCSUGIIAELAIGLIVSANILVGG FEGAZMNERAFGERLUGWWWNHWWIYNVGELIGGGIAA	232 231 229 229 239 239 239 232 230 230 230 230
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-1 AtTIP3-2 AtTIP4-1 AtTIP5-1 GaTIP3-2 GrTIP3-2 PtTIP TcTIP KvTIP3	IF SU YI WATA VERKEDIGI IA HAIGI VASA HAG ASSASSAN PROFERVOWIWINAW WAGELIGGIAG IF GLVY WATA VERKEDIGI IA HAIGI VASA HAG ASSASSAN PROFERVOWIWINAW WAGELIGGIAG IF ALVY WATA AD FKKCSIGI IA HAIGI VASA HAG ASSASSAN PROFERVOWIWINAW WAGELIGGIAG IF ALVY WATA AD FKKCSIGI IA HAIGI VASA HIAG FSGGSMNPARSFERVOWIGELIGGIAG IF ALVY WATA AD FKKCSIGI IA HAIGI VASA HIAG FSGGSMNPARSFERVOWIGELIGGIAG IF ALVY WATA AD FKKCSIGI IA HAIGI VASA HIAG FSGGSMNPARSFERVOWIGELIGGIAG IF ALVY WATA AD FKKCSIGI IA HAIGI VASA HIAG FSGGSMNPARSFERVOWIGELIGGIAG IF ALVY WATA AD FKKCSIGI IA HAIGI VASA HIAG FSGGSMNPARSFERVOWIGELIGGIAG IF ALVY WATA AD FKKCSIGI IA HAIGI VASA HIAG FSGGSMNPARSFERVOWIGELIGGIAG IF ALVY WATA AD FKKCSIGI IA HAIGI VASA HIAG FSGGSMNPARSFERAL VAG WWOWIGH IGSIA IF ALVY WATA AD FKKCSIGI IA HAIGI VASA HIAG FSGGSMNPARSFERAL VAG WWOWIGH IGSIA IF ALVY WATA ID FKKCSIGI FFGGILIG FVGAN HIAG FSGGSMNPARSFERAL VAG WWWOWIGH IGSIG AFVLVY WATA ID FKCSIGI FFGGILIG FVGAN HIAG FSGGSMNPARSFERAL VAG WWWOWIGH IGSIG AFVLVY WATA ID FKCSIGI FFGGILIG FVGAN HIAG FSGGSMNPARSFERAL VAG WWWWOWIGH IGGIAG AFVLVY WATAID FKCSIGI FFGGILIG FVGAN HIAG FSGGSMNPARSFERAL VAG WWWWWOH FIGGIAA TYGLVY WATAID FKCSIGI FFGGILIG FVGAN HIAG FFGGAMNPARSFERAL VAG WWWWWWGHIGGIAG AFVLVY WATAID FKCSIGI FFIGFAL FIGFVAAN HIAG FFGCWYWYATAID FKCSIGI FFGGILIG FVGAN HIAG FFGCWYWYATAID FKCSIGI FFGGILIG FVGAN HIAG FFGCWYWYATAID FKCSIGI FFIGFVAN HIAG FFGCWYWYATAID FKCSIGI FFGGILIG FVGAN HIAG FFGCWYWYATAID FKCSIGI FFIGFVAN HIAG FFGCWYWYATAID FKCSIGI FFIGFVAN HIAG FFGCWYWYATAID FKCSIGI FFGGLIG FFGGAN HIAG FFGCWYWYATAID FKCSIGI FFGUARA HIAG FFGCWYWYATAID FKCSIGI FFGGLIAFLANG FFGCWYWYATAID FKCSIGI FFGUARA HIAG FFGCWYWYATAID FKCSIGI FFGGLIAFLANG FFGUYYWYATAID FKCSIGI FFGGLIAFLANG FFGUYYWYATAID FKCSIGI FFGGUARA HIAG FFGUYYWYATAID FKCSIGI FFGUARA HIAG FFGCWYWYATAID FKCSIGI FFGGUARA HIAG FFGUYYWYATAID FKCSIGI FFGGUARA HIAG FF	232 231 229 229 239 239 239 239 230 230 230 230
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-2 AtTIP3-2 AtTIP4-1 AtTIP5-1 GaTIP3-2 GTIP3-2 PtTIP TcTIP KvTIP3	IFGLVYTVYATAVDFKKCDIGIIAFLAIGLIVGAN TAGO HSGAGNNPAVEGEAVVSNIWINHWVYAGELIGGIAG IFGLVYTVYATAVDFKKCDIGIIAFLAIGLIVGAN TIAG HSGAGNNPAVSFGEAVVSNIWINHWVYAGELIGGGIAG IFALVYTVYATAVDFKKCSLGTIAFLAIGLIVGAN TIAG HSGAGNNPASFGEAVVSGDFSGHWVYAGELIGGGIAG IFALVYTVYATAADFKKCSLGTIAFLAIGFIVGAN TIAG HSGGGNNPASFGEAVVSGDFSGIWIYAUGELIGGGIAG IFGLVYVYYSTATADFKKCSLGTIAFLAIGLIVGAN TIAG HSGGSMNPASFGEAVVSGDFSGIWIYAUGELIGGGIAG IFGLVYVYSTATADFKKCSLGTIAFLAIGLIVGAN TIAG HSGGSMNPASFGEAVVSGDFSGIWIYAUGELIGGGIAG IFGLVYVYSTATADFKKCSLGTIAFLAIGLIVGAN TIAG HSGGSMNPASFGEAVVSGDFSGIWIYAUGELIGGGIAG IFGLVYVYSTATADFKKCSLGIIAFLAIGLIVGAN TIAG HSGGSMNPASFGEAVVSGDFSGIWIYAUGELIGGAIAA IFSLLFTVYATATDFKCSLGIIAFLAIGLIVGAN TIAG HSGGSMNPASFGEAVVSGVYVYGELIGGAIAA IFSLLFTVYATTVDFKCSLGFGELLIGFVVGAN TIAG HSGGSMNPASFGEALVGGNWSNHWIYAVGFLIGGAIAA IFSLLFTVYATGIDFKCSUGIGILAFLAIGLIVGAN TIAG HSGGSMNPASFGEALVGGNWSNHWIYAVGFLIGGAIAA IFSLLFTVYATGIDFKCSUGIGILAFLAIGLIVGAN TIAG HSGGSMNPASFGEALVGGNWSNHWIYAVGFLIGGAIAA IFSLLFTVYATGIDFKCSUGILAFLAIGLIVGAN TIAG HSGGSMNPASFGEALVGGNWYGSFKNGAVYAVGFLIGGAIAA IFGLVYVYYATGIDFKCSUGILAFLAIGLIVAN TIAG HSGASMNPASFGEALVGWRWNHWIYAVGFLIGGAIAA IFGLVYTVYATGIDFKCSUGILAFLAIGLIVAN TIAG HSGASMNPASFGEALVGWRWNHWIYAVGFLIGGIAA IFGVYTVYATGIDFKCSUGIIAFLAIGLIVAN TIAG HSGASMNPASFGALVGWRWNHWIYAVGFLIGGIAA IFGVYTVYATATDFKCSLGIIAFLAIGFIVGAN TIAG HSGASMNPASFGALVGWRWNHWIYAVGFLIGGIAA IFGVYTVYATATDFKCSLGIIAFLAIGFIVGAN TIAG HSGASMNPASFGALVGWRWNHWIYAVGFLIGGIAA	232 231 229 229 239 239 239 230 230 230 230 230 230
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-1 AtTIP3-2 AtTIP4-1 AtTIP5-1 GaTIP3-2 GrTIP3-2 GrTIP3-2 PtTIP TcTIP KvTIP3	IFGLYYIVLATAVDERKCOLGIIAELAIGLIVGANILAG ASGASONPYAEGAVVSNIWINAWVAGELLGGIAG IFGLYYIVLATAVDERKCSLGIIAELAIGLIVGANILAG ASGASONPYAEGAVVSNIWINAWVAGELLGGIAG IFALVYIVLATADERKCSLGIIAELAIGLIVGANILAAG ESGGSMNPARSFGEAVVSGDESGHWVYAVGELLGGIAG IFALVYIVLATADERKCSLGIIAELAIGFIVGANILAAG ESGGSMNPARSFGEAVVSGDESGIWIVAVGELLGGIAG IFALVYIVLATADERKCSLGIIAELAIGFIVGANILAAG ESGGSMNPARSFGEAVVSGDESGIWIVAVGELLGGIAG IFALVYIVLATADERKCSLGIIAELAIGLIVGANILAAG ESGGSMNPARSFGEAVVSGDESGIWIVAVGELLGGIAG IFALVYIVLATADERKCSLGIIAELAIGLIVGANILAAG ESGGSMNPARSFGEAVVSGDESGIWIVAVGELLGGIAG IFALVYVVSTALDERKCSLGIIAELAIGLIVGANILAG ESGASMNPARSFGEAVVSGDESGIWIVAVGELLGGIAA IFALVYVVSTALOERKCSLGIIAELAIGLIVGANILAG ESGASMNPARSFGEAVVSGDESGIWIVAVGELLGGIAA IFSLLFTVIATIVDERKCSLGFGELLIGEVVGANILAG ESGASMNPARSFGEAVVSGVAVVGELLGGIAA IFSLLFTVIATIVDERKCSLDGFGELLIGEVVGANILAG ESGASMNPARSFGEAVVGSFKNQAVVVGELLGGIAA IFSLLFTVIATIVDERKCSLDGFGELLIGEVVGANILAG ESGASMNPARSFGEAVVGSFKNQAVVVGELLGGIAA IFGLVYVVYATGIDERKCSVGTIAELAIGLIVGANILAG ESGGSMNPARSFGEAVVGSFKNQAVVVGELLGGIAA IFGLVYVVYATGIDERKCSVGIIAELAIGLIVGANILAG ESGGSMNPARSFGEALVGRWNHHWIVVGELLGGIAA IFGLVYVVYATAIDERKCSVGIIAELAIGLIVGANILAG ESGGSMNPARSFGEALVGRWNHHWIVVGELLGGIAA IFGLVYVVYATAIDERKCSVGIIAELAIGLIVGANILVGG EIGGAMNPARSFGEALVGRWNHHWIVVGELLGGIAA IFGLVYTVYATAIDERKCSUGIIAELAIGLIVGANILVGG EIGGAMNPARSFGEALVGRWNHHWIVVGELLGGIAA IFGLVYTVYATAIDERKCSLGIIAELAIGLIVGANILVGG EIGGAMNPARSFGEALVGRWNHHWIVVGELLGGIAA IFGLVYTVYATAIDEKCSLGIIAELAIGLIVGANILVGG EIGGAMNPARSFGEALVGRWNHHWIVVGELLGGIAA IFGLVYTVYATAIDEKCSLGIIAELAIGLIVGANILVGG EIGGAMNPARSFGEALVGWRWNHHWIVVGELLGGIAA IFGLVYTVYATAIDEKCSLGIIAELAIGLIVGANILVGG EIGGAMNPARSFGEALVGWRWNHHWIVVGELLGGIAA IFGLVYTVYAAAIDEKKSLGIIAELAIGLIVAANILVGG EIGGAMNPARAFGALVGWRWNHWIVVVGELLGGIAA IFGLVYTVYAAAIDEKKSLGIIAELAIGLIVAANILVGG EIGGAMNPARAFGAALVGWRWNHWIVVVGELLGGIAA IFGLVYTVYAAAIDEKKSLGIIAELAIGLIVAANILVGG EIGGAANNPARAFGAALVGWRWNHWIVVVGELLGGIAA	232 231 229 229 239 239 239 232 230 230 230 230 230
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-2 AtTIP4-1 AtTIP5-1 GaTIP3-2 GrTIP3-2 GrTIP3-2 PtTIP TCTIP KvTIP3 AtTIP1-1	IFGLVYIVYLATAVDERKCOLGIIAELAIGLIVGANILAG MESGASMNERVSEGEVVOSNIWINHWVWAGELIGGIAG IFGLVYIVYLATAVDERKCOLGIIAELAIGLIVGANILAG MESGASMNERVSEGEVVOSNIWINHWVWAGELIGGIAG IFALVYTVATAADEKKCSLGIIAELAIGLIVGANILAAG FESGGSMNERSEGEVVSSDESGIWVVGELIGGIAG IFALVYTVATAADEKKCSLGIIAELAIGLIVGANILAAG FESGGSMNERSEGEVVSSDESGIWVGELIGGIAG IFALVYTVATAADEKKCSLGIIAELAIGLIVGANILAAG FESGGSMNERSEGEVVSSDESGIWVGELIGGIAG IFALVYTVATAADEKKCSLGIIAELAIGLIVGANILAAG FESGGSMNERSEGEVVSSDESGIWVGELIGGIAG IFALVYTVATAADEKKCSLGIIAELAIGLIVGANILAG FESGGSMNERSEGEVVSSDESGIWVGELIGGIAG IFALVYTVATAADEKKCSLGIIAELAIGLIVGANILAG FESGSSMNERSEGEVVSSDESGIWVEWVGELIGGIAG IFALVYTVATADEKKCSLGIIAELAIGLIVGANILVGG FEGGSSMNERSEGEVVSSDESGIWVEWVGELIGGIAG AFVLVYTVATIDEKKCSLGIGELIGEVVGANILAG FESGSSMNERSEGEVVSSDESGIWVEWVGELIGGIAG AFVLVYTVF.TASDERKCSVGIIAELAIGLIVGANILAG FESGGSMNERSEGEVVSSDESKNQAVYWGELIGGIAG AFVLVYTVF.TASDERKCSVGIIAELAIGLIVGANILAG FESGGSMNERSEGEVVSSDESKNQAVYWGELIGGIAG AFVLVYTVF.TASDERKCSVGIIAELAIGLIVSANILVGG FEGGASMNERSEGEVVSSDESKNQAVYWGELIGGIAG AFVLVYTVF.TASDERKCSVGIIAELAIGLIVSANILVGG FEGGASMNERAFGEALVGWRWNHHWIYWGELIGGGIAG AFVLVYTVYATGIDEKKCSVGIIAELAIGLIVSANILVGG FEGGASMNERAFGEALVGWRWNHHWIYWGELIGGGIAG IFGLVYTVYATGIDEKKCSVGIIAELAIGLIVGANILVGG FEGASMNERAFGEALVGWRWNHWIYWGELIGGGIAG IFGLVYTVYATGIDEKKCSLGIIAELAIGLIVGANILVGG FEGASMNERAFGEALVGWRWNHWIYWGELIGGGIAG IFGLVYTVYATAIDEKCSLGIIAELAIGLIVGANILVGG FEGASMNERAFGEALVGWRWNHWIYWGELIGGIAG IFGLVYTVYATAIDEKCSLGIIAELAIGLIVGANILVGG FEGASMNERAFGEALVGWRWNHWIYWGELIGGIAG IFGLVYTVYATAIDEKCSLGIIAELAIGLIVAANILVGG FEGASMNERAFGEALVGWRWNHWIYWGELIGGIAG IFGLVYTVATAIDEKCSLGIIAELAIGLIVGANILVGG FEGASMNERAFGEALVGWRWNHWIYWGELIGGIAG IFGLVYTVATAIDEKCSLGIIAELAIGLIVAANILVGG FEGASMNERAFGEALVGWRWNHWIYWGELIGGIAG IFGLVYTVATAIDEKCSLGIIAELAIGLIVAANILVGG FEGASMNERAFGEALVGWRWNHWIYWGELIGGIAG IFGLVYTVAAGELIGGIAG IFGLVYTVAAGENCESLGIIAELAIGLIVAANILVGG FEGASMNERAFGEALVGWRWNHWIYWGELIGGIAG IFGLVYTVAAGENCESLGIIAELAIGLIVAANILVGG FEGASMNERAFGEALVGWRWNHWIYWGELIGGIAG IFGLVYTVAAGENCESLGIIAELAIGLIVAANILVGG FEGASMNERAFGAALVGWRWNHWIYWGELIGGIAG IFGLVYTVAA	232 231 229 229 239 239 225 232 230 230 230 230 230
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-2 AtTIP3-1 AtTIP3-1 AtTIP3-2 AtTIP4-1 AtTIP3-2 GaTIP3-2 GrTIP3-2 GrTIP3-2 PtTIP TcTIP KvTIP3 AtTIP1-1 AtTIP1-2 2-2770 2	IFGLYYTYLYATAD FRKEDIGIIAFLAIGLIVGAN TAGO AS GAS MARYAFEAV VNHEGAV VNHWYTYWATAD FRKEDIGIAG IFGLYYTYLYATAD FRKEDIGIIAFLAIGLIVGAN TAGO AS GAS MARYAFEAV VNHEGAV VNHWYTYW VAGELIGGGIAG IFALVYTYLYATAD FRKESIGTIAFLAIGLIVGAN TAAG FESGGSMAPARSFGAVVSATWINHWYTYWGFLIGGGIAG IFALVYTYLYATAD FRKESIGTIAFLAIGFIVGAN TAAG FESGGSMAPARSFGAVVSATWINHWYTYWGFLIGGIAG IFALVYTYLYATAD FRKESIGTIAFLAIGFIVGAN TAAG FESGGSMAPARSFGAVVSACUSGISGIWIYWGFLIGGAIAG IFALVYTYLYATAD FRKESIGTIAFLAIGLIVGAN TAAG FESGGSMAPARSFGAVVSACUSGISGIWIYWGFLIGGAIAG IFALVYTYLYATAD FRKESIGTIAFLAIGLIVGAN TAAG FESGGSMAPARSFGAVVSACUSGISGIWIYWGFLIGGAIAA IFSLLFTVLYTYLYATAD FRKESIGTIAFLAIGLIVGAN TAAG FESGGSMAPARSFGAVVSACUSGISGIWWWHWWYWGFLIGGAIAA IFSLLFTVLYTYLATAD FRKESIGTIAFLAIGLIVGAN TAAG FESGGSMAPARSFGALVGARWSANW TAYNGFFIGGAIAA IFSLLFTVLATIVDFKCSIGTIAFLAIGLIVGAN TAAG FESGGSMAPARSFGALVGARWSANW TAYNGFFIGGAIAA IFSLLFTVLATIVDFKCSIGTIAFLAIGLIVGAN TAAG FESGGSMAPARSFGALVGARWNANW TAYGFFIGGAIAA IFSLLFTVLATIVDFKCSIGTIAFLAIGLIVGAN TAAG FESGGSMAPARSFGALVGARWNANW TAYGFFIGGGIAG AFVLVYTYLATGIDFRKCSVGTIAFLAIGLIVSAN TAVGG FEGAAMAPARSFGALVGWRWNHW TAYGFFIGGIAA IFGVYTYLATGIDFRKCSIGTIAFLAIGLIVSAN TAVGG FEGAAMAPARSFGALVGWRWNHW TAYGFFIGGIAA IFGVYTYLATAIDFKRCSIGTIAFLAIGFIVGAN TAVGG FEGAAMAPARSFGALVGWRWNHW TAYGFFIGGIAA IFGVYTYLATAIDFKRCSIGTIAFLAIGLIVSAN TAVGG FEGAAMAPARSFGALVGWRWNHW TAYGFFIGGIAA IFGVYTYLATAIDFKRCSIGTIAFLAIGLIVSAN TAVGG FEGAAMAPARSFGALIGWRWNHW TAYGFFIGGIAA IFGVYTYLATAIDFKRCSIGTIAFLAIGLIVGAN TAVGG FEGAAMAPARSFGALIGWRWNHW TAYGFFIGGIAA IFGVYTYLATAIDFKRCSIGTIAFLAIGLIVAN TAVGG FEGAAMAPARSFGALIGWRWNHW TAYGFFIGGIAA IFGVYTYLATAIDFKCSIGTIAFLAIGLIVAN TAVGG FEGAAMAPARSFGALIGWRWNHW TAYGFIGGGIAG IFGUYTYLATAIDFKCSIGTIAFLAIGLIVAN TAVGG FEGAAMAPARSFGALIGWRWANNHW TAYGFIGGGIAG IFGUYTYLATAIDFKCSIGTIAFLAIGLIVAN TAGG FEGAAMAPARSFGALIGWRWANNHW TAYGFIGGGIAG IFGUYTYLATAIDFKCSIGTIAFLAIGLIVAN TAGG FEGAAMAPARSFGALIGWRWANNHW TAYGFIGGGIAG IFGUYTYLATAIDFKCSIGTIAFLAIGLIVAN TAYGG FEGAAMAPARSFGALIGWRWANNHW TAYGFIGGGIAG IFGUYTYLAFTAN FERTAFUAFT	232 231 229 229 239 239 225 230 230 230 230 230 230
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-2 AtTIP3-2 AtTIP4-1 AtTIP5-1 GaTIP3-2 PtTIP TcTIP KvTIP3 AtTIP1-1 AtTIP1-2 AtTIP1-3	IFGLVYIVLATAVDFRKCDLGIIAFLAIGLIVGANILAGO ASGASONPENERVSFGEAVVSNIWINAWVKNAGELIGGIAG IFGLVYIVLATAVDFRKCSLGIIAFLAIGLIVGANILAGO ASGASONPENERSFGEAVVSNIWINAWVKNAGELIGGIAG IFALVYIVVATAADFRKCSLGIIAFLAIGFIVGANILAAG FESGGSMNERSFGEAVVSGDFSQIWIWVNGELIGGIAG IFALVYIVVATAADFRKCSLGIIAFLAIGFIVGANILAAG FESGGSMNERSFGEAVVSGDFSQIWIWVNGELIGGIAG IFALVYIVVATAADFRKCSLGIIAFLAIGFIVGANILAAG FESGGSMNERSFGEAVVSGDFSQIWIWVNGELIGGIAG IFALVYIVVATAADFRKCSLGIIAFLAIGFIVGANILAAG FESGGSMNERSFGEAVVSGDFSQIWIWVNGELIGGIAG IFGLVYVVYSTAIDFRKCSLGIIAFLAIGLIVGANILAAG FESGGSMNERSFGEAVVSGDFSQIWIWVNGELIGGIAA IFALVYVVYSTAIDFRKCSLGFGELLIGFIVGANILAG FESGGSMNERSFGEAVVSGDFSQIWIWVNGELIGGIAA IFSLLFTWATIVDFKCSLGFGELLIGFVAGANILAG FESGGSMNERSFGEAVVSGEKNQAVVNGELIGGIAA IFSLLFTWATIVDFKCSLGFGELLIGFVAGANILAG FESGGSMNERSFGEALVSGNWTDHWVWNGELIGGIAA AFVLWYTVF.TASDFRRCLFLAVGFFFGFVAGANILAG FESGGSMNERAFGEALVGRWNNHWIWVGFLIGGGIAA YGLVYTVYATGIDFKRCSVGTIAFLAIGLIVSANILVG FEGGAMNERAFGEALVGRWNNHWIWVGFLIGGIAA YGLVYTVYATGIDFKRCSUGTIAFLAIGFIVGANILVG FEGAZMNERAFGEALUGWRWNHWIWVGFLIGGCIAA YGLVYTVYATAIDFKCSLGIIAFLAIGLIVSANILVG FEGAZMNERAFGEALUGWRWNHWIWVGFLIGGIAA YGLVYTVYATAIDFKCSLGIIAFLAIGFIVGANILVG FEGAZMNERAFGEALUGWRWNHWIWVGFLIGGIAA YGLVYTVYATAIDFKCSLGIIAFLAIGLIVAANILVG FEGAZMNERAFGALUGWRWNHWIWVGFLIGGIAA YGLVYTVYATAIDFKCSLGIIAFLAIGLIVAANILVG FEGGZMANILVG FEGAZMNERAFGALUGWRWNHWIWVGFLIGGIAA YGLVYTVYATAIDFKCSLGIIAFLAIGLIVAANILVG FEGGZMANILVG FEGAZWNERAFGALUGWRWNHWIWVGFLIGGIAA	232 231 229 229 239 225 232 230 230 230 230 230
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-1 AtTIP3-2 AtTIP4-1 AtTIP5-1 GaTIP3-2 GrTIP3-2 GrTIP3-2 PtTIP TcTIP KvTIP3 AtTIP1-1 AtTIP1-2 AtTIP1-3 AtTIP2-1	IFGUVYIVINATAUPERKOLGIIAHLAIGLIVGAALLAG PESGAGNIPKIGEAVVSNIWINAHWVNAGELLGGIAG IFGUVYIVINATAUPERKOSLGIIAHLAIGLIVGAAILAG PESGGSMIPKSEGAVVSNIWINAHWVNAGELLGGIAG IFALVYIVINATAUPERKOSLGIIAHLAIGLIVGAAILAAG PESGGSMIPKSEGAVVSNIWINHWVNAGELLGGIAG IFALVYIVINATAUPERKOSLGIIAHLAIGLIVGAAILAAG PESGGSMIPKSEGAVVSKUWINHWVNAGELLGGIAG IFALVYIVINATAUPERKOSLGIIAHLAIGEIVGAAILAAG PESGGSMIPKSEGAVVSGDESQIWIWVGELLGGIAG IFALVYIVINATAUPERKOSLGIIAHLAIGEIVGAAILAAG PESGGSMIPKSEGAVVSGDESQIWIWVGELLGGIAG IFALVYIVINATAUPERKOSLGIIAHLAIGEIVGAAILAAG PESGASMIPKSEGAVVSGDESQIWIWVGELLGGIAG IFALVYIVINATAUPERKOSLGIIAHLAIGLIVGAAILVGG PESGASMIPKSEGALVGSRWEMENWIWVGELLGGIAG IFALVYVYSTIDPEKCSLGGGELLIGEVVGANILVGG PESGASMIPKSEGALVGSRWEMENWIWVGELLGGIAA IFSLIFTVIATIVDEKKOSLDGGELLIGEVVGANILVGG PESGASMIPKSEGALVGSRWEMENWIWVGELLGGIAA IFSLIFTVIATIVDEKKOSLDGFGELLIGEVVGANILVGG PESGASMIPKSEGALVGSWEMENWIWVGELLGGIAA IFSLIFTVIATIVDEKKOSLDGFGELLIGEVVGANILVGG PESGASMIPKSEGALVGSWEMENWIWVGELLGGIAA IFSLIFTVIATIVDEKKOSLDGFGELLIGEVVGANILVGG PESGASMIPKSEGALVGKWEMENWIWVGELLGGIAA IFSLIFTVIATIVDEKKOSLGIAHLAIGLIVGANILVGG PESGASMIPKSEGALVGKWEMENWIWVGELLGGIAA IFGLVYVVVATGIDEKKOSUGIIAHLAIGLIVGANILVGG PESGASMIPKSEGAUVGKWEMENHWIVVGELLGGIAA IFGLVYVVVATGIDEKKOSLGIIAHLAIGLIVGANILVGG PESGASMIPKSEGALVGKWEMENHWIVVGELLGGIAA IFGLVYVVVATGIDEKKOSLGIIAHLAIGLIVGANILVGG PESGASMIPKSEGALVGKWEMENHWIVVGELLGGIAA IFGLVYVVVATGIDEKKOSLGIIAHLAIGLIVANILVGG PESGASMIPKSEGALVGWWEMENHWIVVGELLGGIAA	232 231 229 229 239 239 232 230 230 230 230 230
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-2 AtTIP3-1 AtTIP3-2 AtTIP3-2 AtTIP4-1 AtTIP5-2 GrTIP3-2 GrTIP3-2 GrTIP3-2 GrTIP3-2 AtTIP1-1 AtTIP1-1 AtTIP1-3 AtTIP2-1 AtTIP2-2	IFGLVYTV1ATADFRKCDLGIIAFLAIGLIVGAN TAGO ASGASMNEYWHEFHVON WN WAGELIGGIAG IFGLVYTV1ATADFRKCSLGIIAFLAIGLIVGAN TLAG ASGAMNEYWHEFHVON WN WW WGELIGGIAG IFALVYTV1ATADFRKCSLGIIAFLAIGFIVGAN TLAG FSGGSMNERSFERVOSNIWTNHWVYWGFLIGGATAA IFALVYTV1ATADFRKCSLGIIAFLAIGFIVGAN TLAG FSGGSMNERSFERVOSNIWTNHWVYWGFLIGGATAA IFALVYTV1ATADFRKCSLGIIAFLAIGFIVGAN TLAG FSGGSMNERSFERVOSGDFSGIWIWW WGFLIGGATAA IFALVYTV1VATADFRKCSLGIIAFLAIGFIVGAN TLAG FSGGSMNERSFERVOSGDSCJIWIWWGFLIGGATAA IFALVYTV1V1ATADFRKCSLGIIAFLAIGFIVGAN TLAG FSGGSMNERSFERVOSGDLSGIWWWWWGFLIGGATAA IFALVYTV1V1TATADFRKCSLGIIAFLAIGLIVGAN TLVG FSGGSMNERSFEGAVVSGDLSGIWWWWWGFLIGGATAA IFALVYVV1STAIDFRKCSLGFGELLIGFVVGAN TLVG FSGGSMNERSFEGAVVSGDLVGMWHWWWWGFLIGGATAA IFSLFTVVTV1STAIDFRKCSLGFGELLIGFVVGAN TLVG FSGGSMNERSFEGALVGMWWSMWWWWWGFLIGGATAA IFSLFTVVTV1V1TAGTDFKCSLGFGELLIGFVVGAN TLVG FSGGSMNERSFEGALVGMWWSMWWWWWGFLIGGATAA IYGLVYTV1ATGTDFKCSLGFGELLIGFVVGAN TLVG FSGGSMNERSFEGALVGMWWHWWWWGFLIGGATAA IYGLVYTV1ATGTDFKCSLGFTAFLAIGLIVSAN TLVG FSGASMNERAFGFALVGWWWHWWWWGFLIGGATAA IYGLVYTV1ATGTDFKCSLGTTAFLAIGLIVSAN TLVG FSGASMNERAFGFALVGWRWNHWWWWWGFLIGGATAA IYGLVYTV1ATGTDFKCSLGTTAFLAIGLIVSAN TLVG FSGASMNERAFGFALVGWRWNHWWWWWGFLIGGATAA IYGLVYTV1ATATDFKCSLGTTAFLAIGLIVGAN TLVG FSGASMNERAFGFALVGWRWNHWWWWWGFLIGGATAA IYGLVYTV1ATATDFKCSLGTTAFLAIGLIVGAN TLVG FSGASMNERAFGFALVGWRWNHWWWWWGFLIGGATAG IFGLVYTV1ATATDFKCSLGTTAFLAIGLIVGAN TLVG FSGASMNERAFGFALVGWRWNHWWWWWGFLIGGATAG	232 231 229 229 239 239 239 230 230 230 230 230
AtTIP1-3 AtTIP2-1 AtTIP2-2 AtTIP2-3 AtTIP3-1 AtTIP3-2 AtTIP3-2 AtTIP4-1 AtTIP3-2 GTTIP3-2 GTTIP3-2 FTIP TcTIP KvTIP3 AtTIP1-1 AtTIP1-2 AtTIP1-3 AtTIP2-1 AtTIP2-3	IFGLVYTVLATAVDFRKCDIGIIAFLAIGLIVGAN LAGO HSGASONPEVSFGFVVSWIWINHWVVWAGELIGGIAG IFGLVYTVLATAVDFKKCDIGIIAFLAIGLIVGAN ILAGO HSGASONPEVSFGFVVSWIWINHWVVWAGELIGGIAG IFALVYTVVATAVDFKKCSLGTIAFLAIGLIVGAN ILAG HSGGSMNPENSFGFVVSWIWINHWVVWAGELIGGIAG IFALVYTVVATAPDFKCSLGTIAFLAIGFIVGAN ILAG HSGGSMNPENSFGFVVSGDFSQIWIWVWGELIGGIAG IFALVYTVVATAPDFKCSLGTIAFLAIGFIVGAN ILAG HSGGSMNPENSFGFVVSGDFSQIWIWVWGELIGGIAG IFALVYTVVATAPDFKCSLGIIAFLAIGFIVGAN ILAG HSGGSMNPENSFGFVVSGDFSQIWIWVWGELIGGIAG IFGLVYVVSTIDFKCSLGIIAFLAIGLIVGAN ILAG HSGGSMNPENSFGFVVSGDFSQIWIWWWWWGELIGGALAG IFALVYTVVATAPDFKCSLGIIAFLAIGLIVGAN ILAG HSGASMNPENSFGFVVSGDFSQIWIWWWWGELIGGALAG IFALVYVVSTIDFKCSLGIIAFLAIGLIVGAN ILAG HSGASMNPENFGFVSGDFVGWWHHWWIWVGELIGGALAG IFALVYVVSTIDFKCSLGIIAFLAIGLIVGAN ILAG HSGASMNPENFGFVSGNWSNWWIWWWWGELIGGALAG IFSLLFTVVATIVDFKCSLGIGIAFLAIGLIVGAN ILAG HSGASMNPENFORFGFLVGWWWHWWIWVGELIGGALAG IFSLLYVVVSTUTATAGIDFKCSUGIAFLAIGLIVGAN ILAG HSGASMNPENFORFGFLVGWWWNHWWIWVGELIGGALAG IFSLLYVVVVSTUTATAGIDFKCSUGIAFLAIGLIVGAN ILAG HSGASMNPENFORFGFLVGWWWNHWWIWVGELIGGALAA IYGUVYTVATAGIDFKCSUGIAFLAIGFINGAN ILAG IFGVVYTVATAGIDFKCSUGIAFLAIGLIVGAN ILAG IFGCVYTVATAGIDFKCSUGIAFLAIGLIVAN IFGCVYTVATAGIDFKCSUGIAFLAIGLIVAN IFGCVYTVATAGIDFKCSUGIAFLAIGLIVAN IFGCVYTVATAGIDFKCSUGIAFLAIGENAN IFGCVYTVATAGIDFKCSUGIAFLAIGENAN IFGCVYTVATAGIDFKCSUGIAFLAIGENAN	232 231 229 229 239 225 232 230 230 230 230 230
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FIGURE 2: Amino acid sequence alignment of KvTIP3, AtTIP1-1, AtTIP1-2, AtTIP1-3, AtTIP2-1, AtTIP2-2, AtTIP2-3, AtTIP3-1, AtTIP3-2, AtTIP4-1, and AtTIP5-1 from Arabidopsis thaliana, GaTIP3-2 from Gossypium arboreum, GrTIP3-2 from Gossypium raimondii, PtTIP from Populus trichocarpa, and TcTIP from Theobroma cacao. Different colors in background display the different degrees of conserved sequences.

Similar to the AQPs from other plant species, KvTIP3 also contained six membrane-spanning α -helices, as predicted with both TMHHM and TMPred software programs (Figures 1(a) and 1(b)). The water channel activities of AQPs were activated and deactivated via the phosphorylation and dephosphorylation of their phosphorylation sites [43]. The Snf1-related protein kinase 2.6 (SnRK2.6) was reported to be able to phosphorylate AQPs [36]. The

C-termini of several PIPs were also discovered to be phosphorylated by a sugar-induced receptor kinase [44]. We found that KvTIP3 contained 7 potential phosphorylation sites, implying that it may also play its functions via phosphorylation mechanism (Figure 1(c)). Further analyses indicated that KvTIP3 was homologous to and shared amino acid sequence homology with other plant AQPs (Figure 1(d)). It is well known that the conserved NPA



FIGURE 3: Expression analyses of KvTIP3. (a) Phenotype of a two-week-old *K. virginica* seedling used for salt stress or ABA treatment. (b) Expression analysis of KvTIP3 in the leaves, stems, and roots of two-week-old *K. virginica* seedlings grown under normal growth condition. The expression level in leaves was set as the standard value 1. (c) Expression profiles of KvTIP3 under different salt stress conditions. Two-week-old *K. virginica* seedlings were treated with 0, 200, 300, and 400 mM NaCl for 24 hours. (d) Expression profiles of KvTIP3 under salt stress condition. Two-week-old *K. virginica* seedlings were treated with 300 mM NaCl for 0, 6, 12, and 24 hours. The mean values of three biological replicates were used for data analysis. The results of qPCR were normalized with the internal control gene, 18SrRNA. Error bar indicates SD (n = 3).

motif in AQPs functions as a selective filter for the substrate specificity [44]. Sequence analysis showed that KvTIP3 contained two NPA motifs, indicating that the KvTIP3 was a typical AQP (Figure 2).

The expression pattern of AQPs may affect their biological functions in the growth, development, and response to environmental stress of plants. We observed that the expression level of KvTIP3 in roots was over 270-folds higher than in leaves and stems (Figure 3(b)). Similar expression pattern of AQPs was also observed in Arabidopsis [45], rice [46], grape [47], and barley [48]. In addition, the KvLEA gene which we reported previously also showed a similar expression pattern, indicating that these genes play important roles in roots of plants to improve salt resistance [30]. Staying the same with our transcriptome analysis, KvTIP3 could be induced obviously by NaCl treatment [28]. However, different from KvLEA gene [30], 300 mM NaCl led to the most drastic accumulation to KvTIP3 rather than 400 mM (Figure 3(c)). As for the different time of duration treatments, KvTIP3 gave the

same expression profiles with *KvLEA*, proving that both *KvTIP3* and *KvLEA* were salt stress responsive genes of *K. virginica* (Figure 3(d)).

We also examined the expression levels of KvTIP3 in response to different abiotic stresses and ABA treatment. *KvTIP3* was upregulated by low temperature and ABA but downregulated by high temperature and osmotic stress (Figures 4(a)-4(d)). This is consistent with previous studies that the responses of AQPs under adverse environment were diverse [49]. The responses of KvTIP3 under high temperature and osmotic stress were not obvious (Figures 4(c) and 4(d)). Therefore, we speculated that KvTIP3 may not be involved in high temperature response. However, TIPs were reported to take part in the response to osmotic stress [12]. In this research, the result showed that there should be other KvTIPs that participate in osmotic stress response in K. virginica rather than KvTIP3. Besides, ABA treatment gave rise to the most drastic accumulation to KvTIP3 among these treatments (Figure 4(b)). For instance, the expression of KvTIP3 reached as much as 180-



FIGURE 4: Expression profiles of KvTIP3 upon different stresses or ABA treatments. (a) Expression profiles of KvTIP3 upon low temperature treatment. (b) Expression profiles of KvTIP3 upon ABA treatment. (c) Expression profiles of KvTIP3 upon high temperature treatment. (d) Expression profiles of KvTIP3 upon osmotic stress treatment. Error bar indicates SD (n = 3).

fold after 6h ABA treatment. And the expression of *KvTIP3* increased rapidly as soon as after 2 h ABA treatment; hence, we speculated that *KvTIP3* may be involved in ABA-mediated stress responses in *K. virginica*. Actually, the intersections between ABA and AQPs have already been reported in other species. In *Arabidopsis*, AQPs functioned in ABA-triggered stomatal closure [36], and the vacuolar aquaporins were able to maintain seed longevity under the control of ABA [50]. In addition, ABA also could influence the phosphorylation of PIPs to regulate their activities [51]. Therefore, the exact mechanisms between *KvTIP3* and ABA deserve further investigation in the following research.

5. Conclusions

In this report, we characterized a novel tonoplast intrinsic protein gene KvTIP3 from K. *virginica*. Bioinformatic analyses revealed that KvTIP3 is a typical AQP with conserved transmembrane helices and NPA motifs. Gene expression profiles displayed that it was predominately expressed in roots and was responsive to various abiotic stresses. Our study on KvTIP3 will enrich the gene resources and lay foundation for further study on AQPs. KvTIP3 could be used as a potential candidate gene for the improvement of plants resistant to various abiotic stresses in the future.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Xiaohua Liu and Jieshan Cheng contributed equally to this work.

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