Characterization and tissue-specific as well as heat-stress expression analysis of CBL-interacting protein kinase genes in Dimocarpus longan Lour

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Abstract: The CBL-interacting protein kinase (CIPK) gene family plays important roles in plant growth, development, and responses to abiotic stresses. To date, the CIPKs have been extensively identified and characterized in various plants. However, there have been few studies on CIPKs in Dimocarpus longan Lour (D. longan). In this study we identified eight putative CIPKs in D. longan (DlCIPKs) according to RNA-seq data. The physicochemical properties, subcellular localization, conserved motifs, phylogenetic relationships, glycosylation and phosphorylation sites, and gene ontology annotation were analyzed using bioinformatics tools. The tissue-specific and heat-stress-responsive expressions were systematically investigated as well. Ten motifs widely maintained by DlCIPKs were identified. All eight DlCIPKs identified from D. longan and the 50 CIPKs selected from Zea mays, Oryza sativa, Sorghum bicolore, Vitis vinifera, Populus trichocarpa, and Arabidopsis thaliana were categorized together into seven groups; group 7 was the largest and contained 20 CIPKs. Localization of these eight DlCIPKs were predicted in the plasma membrane, cytoplasm, chloroplast thylakoid membrane, or nucleus and were predicted to differ. All eight DlCIPKs were predicted to be phosphorylated but with different numbers of phosphorylation sites. The quantitative real-time polymerase chain reaction (qRT-PCR) analysis revealed that among the eight DlCIPKs, only DlCIPK1, DlCIPK5, and DlCIPK8 were differently expressed; notably, between root and leaf tissues. All eight DlCIPKs responded to heat treatments. The expressions of DlCIPK1, DlCIPK4, DlCIPK5, and DlCIPK6 in D. longan leaves were upregulated to the maximum level after 1 h of heat treatment. In contrast, the expression levels of DlCIPK2, DlCIPK3, DlCIPK7, and DlCIPK8 increased significantly after 4 h of heat treatment. The results of this study will enrich our knowledge of DlCIPKs and establish a foundation for enhancing tolerance to abiotic stresses through genetic engineering in D. longan.

Key words: Dimocarpus longan Lour, CIPK, bioinformatics analysis, gene expression, abiotic stresses

1. Introduction

Calcium ions (Ca\(^{2+}\)) as second messenger are widely involved in plant growth, development, and regulation of external environmental stresses (Hepler, 2005). The concentration of Ca\(^{2+}\) is affected in the cytoplasm by changes in physiological conditions (Jorg et al., 2010) including soil salinity, drought, low temperature, high pH, and low potassium ion (K\(^{+}\)) concentrations (Li et al., 2009). When the concentration of Ca\(^{2+}\) is varied, the Ca\(^{2+}\) signatures are perceived and transduced through three kinds of Ca\(^{2+}\) sensors including calmodulin (CaM), Ca\(^{2+}\)-dependent protein kinase (CDPK), and the calcineurin B-like (CBL) protein family (Kudla et al., 2018). Ca\(^{2+}\) sensors are divided into sensor responders and sensor relays (Sanders et al., 2002). Calcineurin B-like, a kind of sensor relay, needs to interact with CBL-interacting protein kinases (CIPKs) to transduce the Ca\(^{2+}\) signals and regulate the expression of stress-responsive genes (Kudla et al., 1999; Wang et al., 2011).

CBL-interacting protein kinases belong to the serine–threonine kinase family (Shi et al., 1999; Liu et al., 2000). All the CIPKs found in Arabidopsis thaliana had a kinase catalytic domain at the N-terminal and a regulatory domain at the C-terminal (Li et al., 2009). The C-terminal regulatory domain consisted of a highly conserved NAF motif and a PPI motif. The NAF motif was capable of specifically binding to CBL proteins, forming a CBL–CIPK complex (Albrecht et al., 2001; Lee et al., 2007). To date, CIPKs have been identified from many plants including Oryza sativa (Kolukisaoglu et al., 2004), Triticum aestivum (Tai et al., 2013), and Zea mays (Sun et al., 2015). Related studies have clearly demonstrated that CIPKs play a pivotal role in plant growth and responses to abiotic stresses. In Arabidopsis thaliana, the CBL–CIPK complex is involved in salt resistance (Qu et al., 2002; Qu et al., 2004). Overexpression of OsCIPK3 in rice could significantly enhance resistance to low temperatures (Xiang et al., 2007). Overexpression of SiCIPK6 enables Setaria italica
to survive under high-temperature conditions (Yu et al., 2016).

Functions of the CIPKs involved in abiotic stresses have been extensively studied in a variety of plants including Manihot esculenta (Yan et al., 2018), Populus (Yu et al., 2007), and Sorghum (Li et al., 2010). However, there were few reports of the involvement of CIPKs in plant development and abiotic stresses in D. longan, which is widely cultivated in southern China and Southeast Asia as a medicinal and food plant (Chen et al., 2015). It has become a very popular fruit in China due to its sweet taste and nutrient-rich profile (Sudjaroen et al., 2012).

In addition to the delicious pulp, the roots and leaves of D. longan are both of important medicinal value, as they contain a large amount of active ingredients such as flavonoids, phenols, polysaccharides, and organic acids, which are antitumor, antioxidation, and aid in nervous system regulation (Zhang et al., 2012).

Consumer demand for D. longan is increasing due to its tasty pulp and medicinal value, and improving its resistance to adverse circumstances will support an increase in production. In this study we identified eight DlCIPKs from D. longan using RNA-seq data (accession no.: SRP155595) and explored their physicochemical properties, secondary structures, motif composition, gene ontology (GO) annotation, and phylogenetic relationships. Additionally, the tissue-specific expression of DlCIPKs and their expressions in response to high-temperature stress were investigated. Our findings will enrich the understanding of the CIPK family and form the basis for further research on the biological functions of DlCIPKs in D. longan. In addition, this study will be beneficial for improving survival rates under abiotic stresses through genetic engineering in D. longan.

2. Materials and methods

2.1. Plant materials and heat treatments

The D. longan plants were cultivated in a chamber at the Harbin University of Commerce, Harbin, China. Temperature and humidity were maintained at 25 °C and 50%, respectively. Root and leaf tissues were obtained from mature D. longan plants after two months of cultivation. For heat treatments, the D. longan was cultured in a growth chamber at 38 °C for 1, 4, 8, and 24 h, respectively. The leaves were then harvested at specified time points during heat treatment. The entire experiment was repeated three times.

2.2. Identification and physicochemical properties of DlCIPK genes

According to the D. longan RNA-seq data (accession no.: SRP155595), eight DlCIPKs were identified and further confirmed using the NCBI conserved domain search tool (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). All eight DlCIPKs were explored in detail. The ExPasy ProtParam (http://web.expasy.org/protparam/) was applied to calculate protein sequence length, isolectric point, molecular weight, instability index, aliphatic index, and grand average of hydropathicity (GRAVY).

2.3. Analysis of secondary structure, conserved motifs, multiple sequence alignment, and phylogenetic tree

The secondary structure of the CIPKs was predicted using SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html). The multiple expectation maximization for motif elicitation (MEME) system (http://meme-suite.org/tools/meme) was used to predict the conserved motifs of DlCIPKs, and BioEdit 7.1 software was used to perform multiple sequence alignment of DlCIPKs. The phylogenetic tree was constructed by MEGA 5.0 using the neighbor-joining method with a bootstrap of 1000 iterations. The NCBI accession numbers of CIPKs from Zea mays, Oryza sativa, Sorghum bicolor, Vitis vinifera, Populus trichocarpa, and Arabidopsis thaliana are listed in Table 1.

2.4. Prediction of subcellular localization, glycosylation sites, and phosphorylation sites

The subcellular localizations of DlCIPKs were predicted by WoLF PSORT (http://wolfpsort.org/). The NetNGlyc server 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/) was used to predict the N-glycosylation sites of DlCIPKs. The phosphorylation sites of DlCIPKs were predicted and analyzed by the NetPhos 3.1 server (http://www.cbs.dtu.dk/services/NetPhos/).

2.5. Gene ontology (GO) annotation

The Blast2GO v3.0 was used to analyze the functional classification of DlCIPKs. Genes were categorized into three GO terms, inclusive of biological processes, molecular functions, and cellular components.

2.6. RNA isolation and qRT-PCR analysis

The RNA was isolated from the roots and leaves under normal cultivation conditions and from leaves after high temperature treatments for 1, 4, 8, and 24 h using the cetyltrimethylammonium bromide (CTAB) method (Jaakola et al., 2001). TransScript One-Step gDNA removal and cDNA Synthesis SuperMix kits were used to synthesize the first-strand cDNAs. The expression patterns of DlCIPKs were determined by qRT-PCR using TransStart Top Green qPCR SuperMix (TransGen Biotech, Beijing, China). The reaction conditions were as follows: total reaction volume was 20 μL, containing 10 μL 2× TransStart Top Green qPCR SuperMix, 1 μL cDNA template, 7 μL ddH2O, 1 μL upstream primer, and 1 μL downstream primer. The D. longan tubulin gene was used as the reference gene. Primers used for qRT-PCR are listed in Table 2. All experiments were performed in three replicates. The qRT-PCR reaction conditions were as follows: 95 °C for 1 min, 95 °C for 5 s, 60 °C for 30 s,
726 °C and 72 °C for 30 s. Relative gene expression was evaluated by the relative quantification method \((2^{-\Delta\Delta Ct})\) (Schmittgen and Livak, 2008).

3. Results

3.1. Identification of CIPKs from *D. longan* and analysis of their physicochemical properties and secondary structures

In this study, eight *DlCIPK* were identified from *D. longan* by RNA-seq data and further confirmed using the NCBI conserved domain search tool. All eight *DlCIPK* were renamed, *DlCIPK1* to *DlCIPK8*, according to the order in which they were identified from the RNA-seq data.

The detailed physicochemical properties of *DlCIPK* include protein length, theoretical isoelectric point, aliphatic index, molecular weight, instability index (II), grand average of hydropathicity (GRAVY), alpha helices, extended strands, beta turns, and random coils, were calculated by ExPASy-ProtParam and are shown in Table 3. The lengths of the encoded proteins ranged from 175 to 493 amino acids (AA). The molecular weights of the *DlCIPK* were between 32228.91 and 55096.14 Da. The theoretical isoelectric points ranged from 8.31 to 9.48. The instability indices varied, with *DlCIPK1*, *DlCIPK2*, *DlCIPK3*, and *DlCIPK5* being <40. The GRAVY values of all eight *DlCIPK* were negative.

The secondary structures of *DlCIPK* predicted by SOPMA were mainly composed of α-helix, extended chain, β-turn, and random coil. Among them, α-helix was predominant, accounting for 35.95%, followed by random coils (35.50%), extended chain (18.67%), and β-turn (9.89%) (Table 3).

3.2. Conserved motifs of *DlCIPK*

The putative conserved motifs of eight *DlCIPK* were predicted by MEME. The results identified 10 motifs (Figure 1), which were displayed in the locations where they were found in *DlCIPK* (Figure 2). All 10 identified motifs were widely maintained in *DlCIPK*.

3.3. Multiple sequence alignments and phylogenetic analysis

The multiple sequence alignment performed in this study showed that all *DlCIPK*, except *DlCIPK6*, contained an NAF domain (Figure 3). In order to investigate the
evolutionary relationships of DICIPKs in *D. longan*, the full-length amino acid sequences from a total of 50 CIPKs taken from *Zeae mays*, *Oryza sativa*, *Sorghum bicolor*, *Vitis vinifera*, *Populus trichocarpa*, and *Arabidopsis thaliana* were selected. These, together with the eight DICIPKs, were used to construct the phylogenetic tree (Figure 4). In the phylogenetic tree, CIPKs with bootstrap values above 50 were clustered together. A total of 58 CIPKs were classified into seven groups. Group 7 contained the largest number (20 CIPKs), followed by group 1 (16 CIPKs), group 6 (8 CIPKs), group 3 (5 CIPKs), group 2 (4 CIPK), group 4 (3 CIPKs), and group 5 (2 CIPKs).

3.4. Subcellular localizations, glycosylation sites, and phosphorylation sites of DICIPKs

We used WoLF PSORT to predict the subcellular localization of DICIPKs (Table 4). DICIPK2, DICIPK4, DICIPK5, DICIPK7, and DICIPK8 were predicted to be located in the plasma membrane. DICIPK6 was located in the cytoplasm; DICIPK1 and DICIPK3 were located in the chloroplast thylakoid membrane and nucleus, respectively.

NetPhos 3.1 server was used to predict the phosphorylation sites of DICIPKs. The results showed that all DICIPKs had multiple phosphorylation sites (Table 4). However, the locations and quantities of their phosphorylation sites differed significantly. Overall, the quantities of serine phosphorylation in DICIPKs were the greatest, followed by threonine phosphorylation and tyrosine phosphorylation, respectively.

DICIPKs were also evaluated for N-glycosylation sites using NetNGlyc server 1.0. The results suggested that the N-glycosylation sites were found only in DICIPK2, DICIPK4, and DICIPK7, each of which contained only 1 N-glycosylation site.

3.5. Gene ontology (GO) annotation

Each of the eight DICIPKs identified from *D. longan* were analyzed and categorized into biological process, molecular function, and cellular component by Blast2GO v3.0 (Figure 5). The results showed that DICIPKs were assigned four terms in biological process. All eight DICIPKs were predicted to be involved in protein phosphorylation, one DICIPK in abscisic acid response, one DICIPK in calcium-mediated signaling, and one DICIPK in seed trichome elongation. Molecular function analysis showed that all eight DICIPKs participated in ATP binding, and six of these were likely related to protein serine–threonine kinase activity. There was only one DICIPK classified as an integral part of the cellular component.

3.6. Expression patterns of DICIPKs in root and leaf tissues

Quantitative RT-PCR was used to analyze the tissue-specific expression patterns of DICIPKs in root and leaf tissues (Figure 6). All eight DICIPKs could be expressed in both root and leaf tissues. The expression levels of DICIPK1, DICIPK5, and DICIPK8 differed significantly between root and leaf tissues. The expression level of DICIPK1 in the root was nearly 7-fold higher than in the leaf, whereas the expression levels of DICIPK5 and DICIPK8 in the leaf were roughly 12- and 50-fold higher than in the root, respectively.

3.7. Expression patterns of DICIPKs in leaf tissue under heat treatments

In addition to explorations of gene expression patterns in root and leaf tissues, all eight DICIPKs from the leaves were subjected to qRT-PCR analysis after high temperature (38 °C) treatments for 1, 4, 8, and 24 h, respectively. The results showed that all of them responded to heat treatments but had different expression patterns (Figure 7). Four genes, including DICIPK1, DICIPK4, DICIPK5, and DICIPK6, were obviously up-regulated to their maximum levels after 1 h of heat treatment. The expression patterns of the other four genes, DICIPK2, DICIPK3, DICIPK7, and DICIPK8, gradually increased with the extension of stress time, reaching their maximum levels after 4 h of heat treatment.

### Table 3. Physicochemical properties of DICIPKs.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene ID</th>
<th>ORF (AA)</th>
<th>PI</th>
<th>Aliphatic index</th>
<th>MW(Da)</th>
<th>Instability index (II)</th>
<th>GRAVY</th>
<th>Alpha helix</th>
<th>Extended strand</th>
<th>Beta turn</th>
<th>Random coil</th>
</tr>
</thead>
<tbody>
<tr>
<td>DICIPK1</td>
<td>c32533.graph_c0</td>
<td>449</td>
<td>8.68</td>
<td>86.99</td>
<td>51265.02</td>
<td>29.61</td>
<td>-0.306</td>
<td>37.86%</td>
<td>18.26%</td>
<td>9.58%</td>
<td>34.30%</td>
</tr>
<tr>
<td>DICIPK2</td>
<td>c33236.graph_c1</td>
<td>463</td>
<td>8.92</td>
<td>78.75</td>
<td>52506.21</td>
<td>38.90</td>
<td>-0.366</td>
<td>34.99%</td>
<td>19.01%</td>
<td>9.50%</td>
<td>36.50%</td>
</tr>
<tr>
<td>DICIPK3</td>
<td>c27042.graph_c0</td>
<td>437</td>
<td>9.26</td>
<td>90.37</td>
<td>49468.34</td>
<td>24.83</td>
<td>-0.330</td>
<td>35.93%</td>
<td>18.08%</td>
<td>10.76%</td>
<td>35.24%</td>
</tr>
<tr>
<td>DICIPK4</td>
<td>c28150.graph_c0</td>
<td>493</td>
<td>8.31</td>
<td>85.07</td>
<td>55096.14</td>
<td>41.15</td>
<td>-0.308</td>
<td>36.31%</td>
<td>16.63%</td>
<td>9.53%</td>
<td>37.53%</td>
</tr>
<tr>
<td>DICIPK5</td>
<td>c33943.graph_c0</td>
<td>442</td>
<td>9.28</td>
<td>80.20</td>
<td>50518.54</td>
<td>29.00</td>
<td>-0.364</td>
<td>35.97%</td>
<td>19.00%</td>
<td>9.73%</td>
<td>35.29%</td>
</tr>
<tr>
<td>DICIPK6</td>
<td>c43079.graph_c0</td>
<td>175</td>
<td>9.48</td>
<td>94.06</td>
<td>19917.24</td>
<td>21.95</td>
<td>-0.274</td>
<td>34.29%</td>
<td>25.71%</td>
<td>13.71%</td>
<td>26.29%</td>
</tr>
<tr>
<td>DICIPK7</td>
<td>c27252.graph_c0</td>
<td>466</td>
<td>9.09</td>
<td>87.21</td>
<td>52086.16</td>
<td>37.05</td>
<td>-0.303</td>
<td>34.55%</td>
<td>19.31%</td>
<td>9.23%</td>
<td>36.91%</td>
</tr>
<tr>
<td>DICIPK8</td>
<td>c34561.graph_c0</td>
<td>284</td>
<td>8.68</td>
<td>75.56</td>
<td>32228.91</td>
<td>48.95</td>
<td>-0.391</td>
<td>37.68%</td>
<td>13.38%</td>
<td>7.04%</td>
<td>41.90%</td>
</tr>
</tbody>
</table>
4. Discussion
CBL-interacting protein kinase, the specific target protein kinase of CBL, is a unique family of protein kinases with serine–threonine kinase activity that is found in plants (Luan et al., 2002). The biological functions of CIPKs have been investigated extensively in a variety of plants. However, there are few studies of the molecular function of CIPKs in D. longan. In this study, we identified eight DlCIPKs from D. longan. Physicochemical properties, subcellular localization, conserved motifs, phylogenetic analysis, glycosylation sites, phosphorylation sites, GO annotation, tissue-specific expression, and abiotic stress expression of all eight DlCIPKs were systematically analyzed.

The lengths of DlCIPKs from D. longan varied from 175 to 493 AA. In maize, the longest and shortest CIPKs were about 400 and 500 AA, respectively (Wang et al., 2007; Zhao et al., 2009). This suggests that the lengths of CIPKs vary among different species. The molecular weights of DlCIPKs ranged from 19917.24 Da to 55096.14 Da, which was significantly different from those in tomato (47600 Da to 57800 Da) (Wang et al., 2018). Proteins with low GRAVY scores are likely to be soluble (Kyte and Doolittle, 1982). The GRAVY scores of the eight DlCIPKs were predicted to be negative, suggesting that they are soluble proteins. Furthermore, we predicted the subcellular localization of DlCIPKs using WoLF PSORT, demonstrating that most DlCIPKs were likely located in the plasma membrane, followed by the cytoplasm and nucleus. This result was quite similar to results in Dendrobium officinale (Li et al., 2018). This may be because CIPK locations ultimately depend on CBL locations (D’Angelo et al., 2006).

Next, we predicted the conserved motifs of the DlCIPKs using MEME and found 10 conserved motifs, which were widely distributed in the DlCIPKs. The result implied that these motifs may be closely related to CIPK functions. The multiple sequence alignment of DlCIPKs identified from D. longan indicated that nearly all DlCIPKs, except DlCIPK6, contained an NAF motif at the C-terminal that functioned in conjunction with CBLs. The NAF motif was absent in DlCIPK6 from D. longan and in VvCIPK10 from Vitis vinifera (Lu et al., 2017), suggesting that absence of the NAF motif does not completely affect the biological function of CIPKs. Furthermore, we evaluated the evolutionary relationships of CIPKs from Zea mays, Oryza sativa, Sorghum bicolor, Vitis vinifera, Populus trichocarpa, Arabidopsis thaliana, and D. longan. In total, 58 CIPKs were categorized into seven groups, showing the genetic relationship among Zea mays, Oryza sativa, Sorghum bicolor, Vitis vinifera, Populus trichocarpa, Arabidopsis thaliana, and D. longan. As seen in Figure 4, DlCIPK1, DlCIPK2, DlCIPK3, DlCIPK4, DlCIPK5, and DlCIPK7 showed a high evolutionary relationship with...
Figure 2. Phylogenetic relationships and motif distributions of 8 DLCPKs. MEGA 5.0 was used to construct the phylogenetic tree. Each colored box represents a motif, and nonconserved sequences are displayed as black lines.

Figure 3. Sequence alignment of DLCPK sequences. Note: red box indicates the NAF domain.
Figure 4. Phylogenetic tree of CIPKs from *Zea mays*, *Oryza sativa*, *Sorghum bicolor*, *Vitis vinifera*, *Populus trichocarpa*, *Arabidopsis thaliana*, and *D. longan*. The tree was constructed using MEGA 5.0 with the neighbor-joining method (1000 bootstraps). Colors show groups where CIPKs exist.

Table 4. Subcellular localization and numbers of phosphorylation and glycosylation of DlCIPKs.

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Phosphorylation sites</th>
<th>Glycosylation sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serine</td>
<td>Threonine</td>
</tr>
<tr>
<td>DlCIPK1</td>
<td>chloroplast thylakoid membrane</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>DlCIPK2</td>
<td>plasma membrane</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>DlCIPK3</td>
<td>Nucleus</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>DlCIPK4</td>
<td>plasma membrane</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>DlCIPK5</td>
<td>plasma membrane</td>
<td>29</td>
<td>12</td>
</tr>
<tr>
<td>DlCIPK6</td>
<td>Cytoplasm</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>DlCIPK7</td>
<td>plasma membrane</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>DlCIPK8</td>
<td>plasma membrane</td>
<td>24</td>
<td>9</td>
</tr>
</tbody>
</table>
CIPKs from dicotyledon. This result was consistent with findings in maize, which showed that almost all ZmCIPKs are clustered with CIPKs from dicotyledon (Chen et al., 2011). Therefore, we speculated that the CIPKs from dicotyledon have a close evolutionary relationship.

Next, we examined the putative glycosylation sites and phosphorylation sites of DlCIPKs using NetNGlyc server 1.0 and NetPhos 3.1 server, respectively. The results showed that phosphorylation and glycosylation varied significantly among all eight DlCIPKs. Phosphorylation and glycosylation are two important routes of protein post-translational modifications and affect protein structure, localization, and biological activity, thereby regulating growth, development, and responses to abiotic stresses in plants (Wang et al., 2017). For instance, salt stress could induce protein phosphorylation in rice root, enhancing its tolerance to salt stress (Chitteti et al., 2007). Therefore, we believe that the phosphorylation sites predicted in DlCIPKs will provide an important beginning for further investigation into phosphorylation sites that may be closely related to abiotic stress resistance.

Finally, we explored the tissue-specific expression of DlCIPKs by qRT-PCR in D. longan. The results showed that DlCIPK1, DlCIPK5, and DlCIPK8 had significantly different expression levels between root and leaf, which are likely involved in various tissue-specific developments. Dimocarpus longan is a subtropical plant, whose growth and development may be dramatically inhibited by high temperatures, which negatively affects production. In this study, qRT-PCR was performed in order to investigate the relationships between DlCIPK expression and high-temperature stress. The results indicated that all eight
DICIPKs respond to high temperature stress at 38 °C; at this temperature DICIPK1, DICIPK4, DICIPK5, and DICIPK6 showed high expressions in leaf after 1 h of heat treatment. High expressions of DICIPK2, DICIPK3, DICIPK7, and DICIPK8 occurred in leaf after 4 h of heat treatment. The expression patterns of CIPKs have been

Figure 6. Expression patterns of 8 DICIPKs in root and leaf tissues of D. longan.
studied extensively in many plants, revealing their close relationship with abiotic stresses (Kolukisaoglu et al., 2004; Yu et al., 2007; Li et al., 2009). In *Vitis vinifera*, overexpression of CIPKs may enhance survival at high temperatures (Xi et al., 2017). Similarly, tolerance to high temperatures in soybean was greatly strengthened when...
In conclusion, this study showed that DICIPK1, DICIPK5, and DICIPK8 had significantly different expression levels in root and leaf tissues. All eight DICIPKs responded to high temperature stress (38 °C), with maximum expression levels occurring after either 1 h or 4 h of heat treatment. These results will enrich our understanding of CIPKs in plants and provide a foundation for further investigation of the function and application of DICIPKs in terms of plant growth and abiotic stress response in D. longan.

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Contributions of authors
Wei ZHENG and Xueming DONG contributed equally to this work.

References


Chen L, Ren F, Zhou L, Wang QQ, Zhong H et al. (2012). The expression levels of NnCIPK6 identified from Nelumbo nucifera significantly increased under salt treatment, indicating its involvement in salt stress (Liu et al., 2014). Therefore, we believe that the eight DICIPKs identified from D. longan may be related to other abiotic stresses including cold and salt stresses, which will be studied in the future.

In conclusion, this study showed that DICIPK1, DICIPK5, and DICIPK8 had significantly different expression levels in root and leaf tissues. All eight DICIPKs responded to high temperature stress (38 °C), with maximum expression levels occurring after either 1 h or 4 h of heat treatment. These results will enrich our understanding of CIPKs in plants and provide a foundation for further investigation of the function and application of DICIPKs in terms of plant growth and abiotic stress response in D. longan.

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References


