

# 荔枝 *CDPK* 基因家族鉴定及其在霜疫病胁迫下的表达分析

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**摘要:**【目的】鉴定荔枝 (*Litchi chinensis* Sonn.) 钙依赖蛋白激酶 (*CDPK*) 基因家族, 并分析其不同组织和荔枝霜疫病胁迫下的表达模式。【方法】基于荔枝基因组数据, 利用生物信息学方法鉴定 *CDPK* 家族基因, 并对其序列特征、基因结构、启动子顺式作用元件、染色体定位和进化关系等进行分析。依赖 276 份荔枝种质材料, 筛选高抗霜疫病的优良荔枝品种。另外, 通过 RNA-Seq 和 qRT-PCR 方法检测高抗病荔枝品种中 *LcCDPK* 基因家族成员在荔枝霜疫病胁迫处理下的表达模式。【结果】从 276 份荔枝自然群体材料中鉴定到荔枝高抗霜疫病品种裕荣 1 号 (YR1)。从荔枝基因组中共鉴定出 19 个 *LcCDPK* 基因家族成员, 分布于 11 条染色体上。根据保守结构域和系统发育分析将其分为 4 个亚家族。基因结构分析表明, *LcCDPKs* 外显子数量为 7~19。蛋白结构分析发现, 所有 *LcCDPK* 蛋白均具有 1~4 个 EF-hand 结构域。顺式作用元件分析表明, *LcCDPKs* 成员拥有大量的生物和非生物胁迫响应元件。组织表达分析发现, *LcCDPK* 基因在荔枝不同组织存在组织特异性。另外, 表达分析发现在高抗霜疫病荔枝裕荣 1 号 (YR1) 中, *LcCDPK5*、*LcCDPK17* 和 *LcCDPK19* 在霜疫病胁迫后急剧上调表达, *LcCDPK3* 和 *LcCDPK8* 急剧下调表达。【结论】裕荣 1 号 (YR1) 是一种高抗霜疫病的荔枝品种。荔枝基因组中共鉴定出 19 个 *CDPK* 基因家族成员, 具有明显的组织特异性, *LcCDPK5*、*LcCDPK17*、*LcCDPK19*、*LcCDPK3* 和 *LcCDPK8* 可能在荔枝抗霜疫病过程中发挥重要作用。研究结果为进一步探究荔枝 *CDPK* 基因家族提供了参考。

关键词: 荔枝; 钙依赖蛋白激酶 (*CDPK*); 荔枝霜疫病; 表达分析

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## Identification of *LcCDPKs* and analysis of their expression patterns in response to downy mildew stresses in lychee

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**Abstract:** 【Objective】Lychee (*Litchi chinensis* Sonn.) is a delicious and nutritional fruit widely accepted by consumers. However, the fruits are highly susceptible to various diseases. Lychee downy blight is one of the major diseases in lychee. The calcium dependent protein kinases (*CDPKs*) play vital roles in regulating plant growth, development and response to abiotic or biotic stresses. However, the potential role of *CDPK* gene family in lychee (*LcCDPK*) have not been reported. Here, we aimed to identify and analyze the *CDPK* gene family from the lychee genome with bioinformatic technology, and investigate the phylogeny, protein structure, expression patterns and response of *CDPK* gene in lychee under lychee downy mildew stress. This study would provide a basis foundation for further functional characterization of the lychee *CDPK* genes. 【Methods】The lychee genome was used to identify and analyze the

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*LcCDPK* genes by bioinformatics using a local BLASTP search of TBtool software. All putative candidates were manually verified with the InterProScan program to confirm the presence of the protein kinase domain and the CaM domain. The length of sequences, isoelectric point (pI), molecular weights (MW) and predication subcellular location of the *LcCDPKs* were estimated by ExpASy Compute pI/Mw tool. The multiple alignments of amino acid sequences were performed using MEGA-X program. The neighbor-joining phylogenetic tree was constructed via applying the MEGA6 program, with bootstrapping set at 1000 replications. The gene structure of the *LcCDPKs* were analyzed using the Gene Structure Display Server (GSDS) program with default settings. The *LcCDPK* genes were mapped to lychee chromosomes based on physical location from the database of the lychee genome using MapChart. The *cis*-acting regulatory elements located in a region upstream 2 kb to the start codon of *LcCDPK* genes were identified using the PlantCARE database, and visualized with TBtools. The germplasm used in this study contained 276 lychee landraces from different geographic areas. This population was selected for the present study because the collection was representative of the diverse genetic variation in lychee. These 276 germplasm materials were provided by Institute of Fruit Trees, Guangdong Academy of Agricultural Sciences and were planted in National Fruit Tree Germplasm, Guangzhou Lychee Nursery. The pathogen of *P. litchii* was provided by South China Agricultural University. *P. litchii* were cultured in juice agar (CJA) medium at 27 °C. The spore suspension was filtrated using double sterile layer and adjusted to  $10^4$  spores  $\text{mL}^{-1}$  for inoculation. For transcription analysis, the leaves of Yurong 1 (YR1) lychee were inoculated with 5  $\mu\text{L}$  sporangia suspension of *P. litchii* ( $10^4$  spores  $\cdot \text{mL}^{-1}$ ) or a mock suspension (sterile water), and then the leaves were collected at the indicated time points. All tissues were immediately placed in liquid nitrogen and stored at  $-80$  °C. The sequencing data of lychee were available from NCBI Sequence Read Archive database under the accession number PRJNA747875. The expression levels of the *LcCDPK* genes were analyzed in various tissues, including female flowers ovary, root, pericarp, aril, embryo, epicarp, male flowers anther, leaf and seed. The heat map with hierarchical clustering of the *LcCDPK* genes were constructed using MeV4.9 software by average linkage with Euclidean distance method, to visualize the expression levels in nine tissues based on the  $\log_{10}(\text{FPKM}+1)$  values of the *LcCDPK* genes. Based on the transcriptome data, the expression of the *CDPK* was analyzed and verified by qRT-PCR. **【Results】** This study identified and comprehensively analyzed the *CDPK* family genes based on the whole genome data of lychee. The sequence characteristics, gene structure, promoter *cis*-acting elements, chromosome localization, evolutionary relationships and expression patterns in different tissues under the stress of lychee downy mildew were analyzed. A total of 19 *CDPK* family members were identified from lychee genome, which were distributed on 11 chromosomes. According to conserved domain and phylogenetic analysis, the 19 *CDPK* family members were divided into four subfamilies. The gene structure analysis indicated that the number of exon-intron ranged in the *LcCDPKs* from 7 to 19. The protein structure domain analysis showed that all *LcCDPK* proteins had 1-4 EF-hand domains. The promoter *cis*-acting element analysis exhibited that *LcCDPKs* had a large number of biological and abiotic stress response elements. Tissue-specific expression analysis showed that many *LcCDPKs* genes could be detected in all tissues, while a few of *LcCDPKs* genes were tissue specific. The differential gene expression analysis of the *LcCDPK* genes in response to lychee downy mildew stresses helped us identify the *LcCDPK5*, *LcCDPK17* and *LcCDPK19* as the candidate genes for disease resistance in lychee, whose relative transcript abundance rapidly increased after lychee downy mildew infection. **【Conclusion】** A total of 19 *CDPK* family genes were identified in lychee genome. The expression of the *LcCDPK* genes in different tissues and their expression patterns in the response to

lychee downy mildew stresses elucidated that the *LcCDPK5*, *LcCDPK17*, *LcCDPK19*, *LcCDPK3* and *LcCDPK8* might play an important role in the resistances of lychee to lychee downy mildew. Our study would provide critical foundation for further functional characterization of the lychee *CDPK* gene family.

**Key words:** *Litchi chinensis* Sonn.; Calcium dependent protein kinase; Lychee downy mildew; Expression analysis

钙离子( $\text{Ca}^{2+}$ )作为第二信使参与植物细胞的信号转导,在植物生长发育以及抗逆境胁迫中均发挥着重要作用<sup>[1]</sup>。在植物中,钙离子结合蛋白( $\text{Ca}^{2+}$ -binding proteins)分为2类,分别是 $\text{Ca}^{2+}$ 传感蛋白和 $\text{Ca}^{2+}$ 受体蛋白<sup>[2]</sup>。其中,钙依赖性蛋白激酶(Calcium dependent protein kinases, CDPKs)是植物细胞中最常见的一类 $\text{Ca}^{2+}$ 受体蛋白,在植物的生长发育、种子萌发、生物及非生物胁迫应答、离子通道运输等信号转导过程中起着重要作用<sup>[3-5]</sup>。

CDPK是一种广泛存在于植物中具有特殊结构特征的蛋白<sup>[6]</sup>,结构主要由4部分组成,包括N端可变结构域(variable N-terminal domain)、Ser/Thr激酶结构域(Ser/Thr kinase domain)、自抑制连接结构域(auto-inhibitory junction domain)和类钙调素结构域(Calmodulin-like domain)<sup>[7-8]</sup>。其中,N端可变区对其结合的底物有识别作用,结构域长度变化差异大,对CDPKs的亚细胞定位有重要作用<sup>[9]</sup>。Ser/Thr激酶区域具有高度保守的催化序列,在不同物种间具有较高的同源性<sup>[10]</sup>。自抑制域高度保守一般由20~30个氨基酸组成,通过假底物机制调节CDPKs的激酶活性<sup>[11]</sup>。类钙调素结构域包含1~4个EF-hands,用于与 $\text{Ca}^{2+}$ 结合<sup>[12]</sup>。 $\text{Ca}^{2+}$ 与EF-hands结合可诱导CDPK构象变化,从而导致激酶结构域活性位点的折叠和暴露,激活CDPK使一系列底物磷酸化<sup>[13]</sup>。

CDPK蛋白于1982年首次在豌豆中被报道<sup>[14]</sup>,然而直到1991年才在大豆中被克隆和鉴定<sup>[15]</sup>。CDPK在植物生长发育以及生物和非生物胁迫方面均发挥着关键作用。在拟南芥中AtCPK2、AtCPK11、AtCPK17、AtCPK20、AtCPK24、AtCPK34被报道广泛参与花粉管的萌发<sup>[16-17]</sup>;AtCPK1、AtCPK3、AtCPK4、AtCPK5、AtCPK6、AtCPK8、AtCPK10、AtCPK11、AtCPK12和AtCPK21参与环境对植物非生物胁迫<sup>[18-21]</sup>;AtCPK28调控植物的生长发育<sup>[22]</sup>。在水稻中,OsCPK7、OsCPK13、OsCPK17和OsCPK24被报道参与低温胁迫<sup>[23-25]</sup>;而OsCPK4、OsCPK9、Os-

CPK12、OsCPK13和OsCPK21被研究发现参与干旱和盐胁迫<sup>[26-28]</sup>。除生长发育和非生物胁迫外,CDPK在生物胁迫中也发挥着广泛作用。前人研究表明拟南芥CPK28与质膜相关的BIK1相互作用并磷酸化,从而负调控植物PTI免疫反应<sup>[29]</sup>;拟南芥AtCPK5、AtCPK6和AtCPK11通过调控乙烯生物合成酶ACS基因的表达控制乙烯的产生,来参与灰霉病感染免疫应答<sup>[30]</sup>。马铃薯StCDPK4及StCDPK5通过磷酸化NADPH氧化酶促进ROS产生,同时调控病原胁迫下相关基因的表达,从而激活防御机制<sup>[31]</sup>。在小麦中,TaCDPK2迅速响应小麦叶锈病的胁迫,在小麦抗叶锈病中发挥重要作用<sup>[32]</sup>。在番茄Cf-9抗性蛋白转基因烟草中发现,当Cf-9与枝孢霉Avr9互作后激活了CDPK的表达,并作为抗原诱导反应中重要的钙传感器发挥功能。将CDPK基因沉默后发现植物丧失Cf-Avr9诱导的特异性超敏反应,表明CDPK对于介导Cf-9/Avr9诱导的植物防御体系不可或缺<sup>[33]</sup>。另外,玉米ZmCPK11被报道参与机械损伤胁迫<sup>[34]</sup>。在烟草中,NaCDPK4和NaCDPK5被报道参与机械损伤和食草动物啃食胁迫<sup>[35]</sup>。

目前,CDPK家族基因在多个物种中被克隆和鉴定。在拟南芥中被发现有34个CDPK基因<sup>[36]</sup>;在水稻中被发现有31个CDPK基因<sup>[37]</sup>;在玉米中被发现有40个CDPK基因<sup>[38]</sup>;在小麦中被发现有20个CDPK基因<sup>[32]</sup>;在大豆中被鉴定有39个CDPK基因<sup>[8]</sup>。CDPK基因的研究在荔枝中还未见报道。栽培荔枝品种妃子笑全基因组序列已被释放<sup>[39]</sup>,这使得荔枝基因家族鉴定成为可能。中国是全球最大的荔枝生产国,同时荔枝在食品、医药和化妆品等方面起着重要作用<sup>[40]</sup>。然而,荔枝在生长发育过程中,受到各种病害的影响。其中,荔枝霜疫病是危害荔枝的一种重要病害<sup>[41]</sup>。荔枝霜疫病是由荔枝霜疫霉菌(*Peronophthora litchi*)侵染荔枝所引起的,严重危害着荔枝的产量和品质<sup>[42]</sup>。笔者针对荔枝霜疫病这一问题,结合CDPK在植物生长以及抗

病中的重要作用,对荔枝CDPK成员进行了全基因组家族鉴定,并对其基因结构、蛋白结构、染色体定位,以及组织表达和在荔枝霜疫病胁迫条件的表达模式进行分析,为进一步研究荔枝CDPK功能提供参考。

## 1 材料和方法

### 1.1 植物材料及生长条件

试验所用276份荔枝种质材料由广东省农业科学院果树研究所提供。荔枝种质资源种植于国家果树种质广州荔枝资源圃。荔枝病原菌材料荔枝霜疫霉菌由华南农业大学提供。

### 1.2 *LcCDPK*基因家族的鉴定

所用的荔枝基因组序列均下载于荔枝基因组数据库<sup>[39]</sup>。为鉴定荔枝CDPK基因家族成员,笔者在本研究中使用拟南芥数据库TAIR(<https://www.arabidopsis.org/>)下载AtCPK氨基酸序列作为参考,比对荔枝基因组数据库筛选得到候选基因。搜索到的候选基因利用InterProScan program(<http://www.ebi.ac.uk/interpro>)进行确认蛋白激酶区域是否同时存在Protein kinase结构域和EF手性结构。最后,所有确认的蛋白序列利用Pfam(<http://pfam.sanger.ac.uk/search>)和SMART(<http://smart.embl-heidelberg.de/>)工具进行重新评估。

### 1.3 *LcCDPK*序列及进化分析

利用Clustal X2.1对荔枝、拟南芥和水稻的CDPK氨基酸序列进行多重序列比对。使用MEME(<http://meme-suite.org/tools/meme>)工具分析LcCDPK蛋白保守Motif;利用GSDS 2.0(<http://gsds.cbi.pku.edu.cn>)进行基因结构分析;从荔枝基因组数据库中分别提取CDPK基因上游2000 bp的序列,利用PlantCARE(<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>)分析启动子顺式作用元件<sup>[43]</sup>。最后,使用TBtools对保守Motif、基因结构、顺式作用元件和染色体定位进行可视化。利用PROSITE(<https://web.expasy.org/myristoylator/>)网站预测LcCDPK的N-myristoylation位点;利用wolfsort(<https://wolfsort.hgc.jp>)对荔枝CDPK蛋白亚细胞定位进行预测。在MEGA5.0软件中使用Neighbor-joining构建荔枝CDPK家族进化树,设置参数为:距离模型,Bootstrap法,重复1000次<sup>[44]</sup>。

### 1.4 荔枝霜疫病处理方法

荔枝霜疫霉菌的培养和孢子悬浮液配置参考Xing等<sup>[45]</sup>的方法。简要为荔枝霜疫霉菌培养于萝卜琼脂培养基(胡萝卜200 g,琼脂粉20 g,水1000 mL),培养温度为27℃。荔枝霜疫霉菌孢子释放于灭菌水中,用血球计数板计算并调整孢子悬浮液的浓度。

对于荔枝成熟果实的荔枝霜疫病的处理方法,笔者在参考Sun等<sup>[41]</sup>的方法基础上并稍作修改。简要方法为,在接种荔枝霜疫霉菌孢子前用无菌水清洗荔枝成熟果实3次。后将每个荔枝果实用移液器接种5 μL荔枝霜疫霉菌孢子悬浮液(孢子个数为 $1 \times 10^4$ 个·mL<sup>-1</sup>);接种后的果实转移至保鲜盒中并放置恒温培养箱培养,培养温度为27℃,昼夜各12 h。3次重复,每个重复10粒荔枝果实。对于荔枝叶片的荔枝霜疫病的处理方法,笔者在参考Xing等<sup>[45]</sup>的方法基础上并稍作修改。具体为,采集荔枝新鲜叶片,平铺于培养皿中,每个培养皿中放入4枚荔枝叶片,并加入10 mL无菌水,用移液器接种5 μL荔枝霜疫霉菌孢子悬浮液(孢子个数为 $1 \times 10^4$ 个·mL<sup>-1</sup>);盛有叶片的培养皿转移至恒温培养箱培养,培养温度为27℃,昼夜时间各12 h。3次重复,每个重复10枚荔枝叶片。

荔枝叶片和成熟果实病级参考Xing等<sup>[45]</sup>的方法。病情指数(Disease index, DI)的计算参考Sun等<sup>[41]</sup>的方法。

RNA-Seq以及RT-PCR所用的荔枝材料为裕荣1号(YR1)。所用荔枝霜疫病的处理方法同前文一致。简要为分别用5 μL荔枝霜疫霉菌孢子悬浮液(孢子个数为 $1 \times 10^4$ 个·mL<sup>-1</sup>)和5 μL灭菌水(对照组,Mock)接种荔枝新鲜叶片。接种后的材料转移至恒温培养箱中培养,培养条件同前文一致。在处理后的24 h收集并液氮保存目标材料,各材料3个生物学重复用于后续分析。

### 1.5 转录组数据分析

植物总RNA的提取使用Plant RNA Kit(R6827, Omega)试剂盒完成。使用Ultra RNA样本制备试剂盒(Illumina)构建RNA-seq文库。RNA测序由北京奥维森基因科技有限公司完成,双端测序(Paired-End, Illumina HiSeq 4000)。计算RPKM作为基因的表达量,使用R语言heatmap函数进行数据可视化。

### 1.6 试验数据

本文中所用荔枝组织特异性表达原始数据来自SRA(NCBI Sequence Read Archive)数据库,登录

号为 PRJNA747875。共 9 种组织：雌花子房 (female flowers ovary)、根 (root)、果皮 (pericarp)、假种皮 (aril)、胚 (embryo)、外种皮 (episperm)、雄花花药 (male flowers anther)、叶 (leaf) 和种子 (seed)。

本文中荔枝霜疫霉菌处理荔枝叶片转录组数据已经上传至 GEO (NCBI Gene Expression Omnibus database) 数据库, 登录号为 GSE201243。

### 1.7 实时荧光定量分析

使用 qRT-PCR 进一步分析荔枝响应荔枝霜疫霉菌胁迫下的候选基因。植物叶片提取试剂盒为 Plant RNA Kit (R6827, Omega)。RNA 反转录试剂盒为 SYBR Green master mix (Vazyme, Cat# Q711-02)。使用 LightCycler480 thermal cycler (Roche) 进行 qRT-PCR 反应。每个样品 3 个生物学重复。使用引物见表 1。以荔枝 *Actin* 基因为内参基因<sup>[41]</sup>。基因相对表达量用  $2^{-\Delta\Delta C_t}$  计算<sup>[46]</sup>。

表 1 文中所用引物序列

Table 1 Sequences of primers used in this study

引物名称 Primer name	序列(5'-3') Sequence (5'-3')	目的 Purpose
Actin_F	CAACTGGTATTGTCTTGGATTCTG	qRT-PCR
Actin_R	TCATCAAGGCATCGGTTAGA	qRT-PCR
LcCDPK3F	CGGATTTTCGGCTACGCAAAG	qRT-PCR
LcCDPK3R	GCAACACGATCTCCATTGGC	qRT-PCR
LcCDPK5F	TGGCAAGAAACTGGGTCAAGGT	qRT-PCR
LcCDPK5R	TGCTGGTGCTCAGACAGATGGT	qRT-PCR
LcCDPK8F	GATGCTTCAAAGGGACCCGA	qRT-PCR
LcCDPK8R	CGACAGTACGGCAGAATCCA	qRT-PCR
LcCDPK17F	GCAGCACAGTTTTCTTGCGA	qRT-PCR
LcCDPK17R	AGACTGGCATTGTACACCGTT	qRT-PCR
LcCDPK19F	TGGAGTACCGCCATTTGGG	qRT-PCR
LcCDPK19R	TCCTGACTAGTCTCTTGCA	qRT-PCR

## 2 结果与分析

### 2.1 *LcCDPK* 基因家族成员鉴定及其系统发育分析

为了鉴定荔枝 *CDPK* 基因家族成员, 笔者在本研究中使用拟南芥的 *CDPK* 氨基酸序列作为参考序列, 然后在荔枝全基因组数据库中进行搜索比对, 共筛选到 21 个候选基因。随后, 对候选基因进行蛋白结构分析, 以确定其具有 Protein kinase 和 EF-hand 结构域 (图 1), 最终得到 19 个荔枝 *CDPK* 基因家族成员, 将其命名为 *LcCDPK1*~*LcCDPK19* (表 2)。19 个 *LcCDPK* 中, 除 *LcCDPK13* 外, 其余基因的 CDS 为 1491~2556 bp, 编码 496~851 个氨基酸 (表 2), 蛋

白质分子质量为 55.65~95.41 ku, 等电点为 5.22~9.13 (表 2)。*LcCDPK13* 比较特殊, 其 CDS 为 4047 bp, 编码 1348 个氨基酸, 蛋白质分子质量为 151.44 ku, 等电点为 8.70。利用 Wolf psort 对 *LcCDPK* 亚细胞定位进行预测, 如表 2 所示, *LcCDPK1* 和 *LcCDPK4* 定位在叶绿体中; *LcCDPK3*、*LcCDPK6* 和 *LcCDPK17* 定位在细胞核中; *LcCDPK13* 定位在质膜中; 其余定位在细胞质中。*CDPK* 的 N 末端含有 N-myristoylation (豆蔻酰化位点), 能促进蛋白间的相互作用。此研究用 <https://web.expasy.org/myristoylator/> 网站预测的 19 个 *LcCDPK* 基因中, 有 9 个在其 N 末端具有豆蔻酰化位点, 而其余 10 个不具有豆蔻酰化位点 (表 2)。

为更好地研究荔枝中 *CDPK* 家族进化关系, 笔者使用已知 34 个拟南芥 *AtCPK* 和 31 个水稻 *OsCPK* 氨基酸序列, 以及笔者在本研究中所鉴定到的 19 个 *LcCDPK* 氨基酸序列, 共 84 个 *CDPK* 序列进行了进化分析。如图 2 和图 4-A 所示, 19 个 *LcCDPK* 可分为 4 个亚家族, 其中 I~IV 亚家族中分别含有 6、5、4 和 4 个 *LcCDPK* 基因。

### 2.2 *LcCDPK* 染色体分布

基于 *LcCDPK* 基因的物理位置 (表 2), 笔者对这 19 个 *LcCDPK* 基因在 15 条染色体上进行了定位 ( $2n = 30$ , 图 3)。结果发现, 第 11 号染色体包含最多的 *LcCDPK* 基因, 第 4、6、12 和 14 号染色体没有发现 *LcCDPK* 基因。多数 *LcCDPK* 位于染色体臂上。如图 3 所示, *LcCDPK1* 和 *LcCDPK2* 被定位在 Chr5 上; *LcCDPK3*、*LcCDPK4* 和 *LcCDPK5* 被定位在 Chr11 上; *LcCDPK6* 被定位在 Chr7 上; *LcCDPK7* 和 *LcCDPK8* 被定位在 Chr8 上; *LcCDPK9* 被定位在 Chr2 上; *LcCDPK10* 被定位在 Chr1 上; *LcCDPK11* 和 *LcCDPK12* 被定位在 Chr15 上; *LcCDPK13* 和 *LcCDPK14* 被定位在 Chr10 上; *LcCDPK15* 和 *LcCDPK16* 被定位在 Chr13 上; *LcCDPK17* 和 *LcCDPK18* 被定位在 Chr3 上; *LcCDPK19* 被定位在 Chr9 上。在拟南芥中 34 个 *CDPK* 基因分布于 5 条染色体上<sup>[6]</sup>, 在水稻中 31 个 *CDPK* 基因分布于 12 条染色体上<sup>[37]</sup>, 表明 *CDPK* 基因在植物基因组中分布广泛。

### 2.3 *LcCDPK* 基因结构和保守 Motif 分析

为了探索 *LcCDPK* 基因结构的保守性和多样性, 笔者对 *LcCDPK* 基因进行了结构分析, 发现 *LcCDPK* 分别含有 7~19 个外显子, 以及 6~18 个内

LcCDPK1	REDHENYIVMELCGGELLDRIISLR--GKYSEDDAKAVLVQIINWVAFCHIQGVVHRDLKPENFLYTSKDN--SRLKATDFGLSDVRSRDERLNDI/VGSAMY	318
LcCDPK2	YEDSLYVHVMELCGGELLDRIIQE--GHYSEFAAEATKTIIVGVWEACHSLVGMHRDLKPENFLVNKDD--FSLKATDFGLSVFKPQIETTVVGSPLY	264
LcCDPK3	REDDSYVYIVMELCGGELLDRIISLKKDSRYTERDAAVVWOMLKAQAQHLHLVHRDMKPENFLKSTKD--SPLKATDFGLSDIITPKKQRDI/VGSAMY	267
LcCDPK4	YEDQENYIVMELCGGELLDRIISLR--GKYTEDDAKAVMIQIINWVAFCHIQGVVHRDLKPENFLFTSKDN--SRLKATDFGLSDVRFPRDERLNDI/VGSAMY	339
LcCDPK5	YEDRLFVHVMELCGGELLDRIIVR--GHYSEFAAEATKTIIVGVWEACHSLVGMHRDLKPENFLFLGADSDSALDFGLSVYKPKGTFPCIVVGSPLY	200
LcCDPK6	YEDDHAHVMELCGGELLDRIIVR--GHYTERAAAWAKTIVEVWQMHKGVGMHRDLKPENFLFANKKET--AALKATDFGLSVFKPGERETETI/VGSPLY	226
LcCDPK7	YEDRQHDHVMELCGGELLDRIISK--GSYSEFAEASTCROIINWVHVFHFMVGMHRDLKPENFLVSKDRA--APTKATDFGLSVIETCKACRDI/VGSAMY	253
LcCDPK8	YEDSVFVHVMELCGGELLDRIIVAK--GHYSEFAAEATKTIIVGVWEACHSLVGMHRDLKPENFLFDSPGDD--AKLKATDFGLSVYKPKGTFPCIVVGSPLY	195
LcCDPK9	YEDRQSHVVMELCGGELLDRIITAK--GHYSEFAEASTCRAIINWVHSHFMVGMHRDLKPENFLLSKDN--ALLKATDFGLSVIETCKEYRDI/VGSAMY	245
LcCDPK10	YEDKEAHYIVMELCGGELLDRIIVR--GHYTERAAAWAKTIVEVWQMHKGVGMHRDLKPENFLFADANSH--SPLKATDFGLSVIETCKEYRDI/VGSPLY	229
LcCDPK11	YEDSVAVHVMELCGGELLDRIIVK--GHYTERAAAWAKTIVEVWQMHKGVGMHRDLKPENFLVNEED--SPKATDFGLSVFKPKGTFPCIVVGSPLY	324
LcCDPK12	YEDAVAVHVMELCGGELLDRIIVR--GHYTERAAAWAKTIVEVWQMHKGVGMHRDLKPENFLVSHDD--SPLKATDFGLSVFKPKGTFPCIVVGSPLY	294
LcCDPK13	YEDKXSNLIMELCGGELLDRIIVR--GHYSEFAEASTCROIINWVHSHFMVGMHRDLKPENFLFLSTD--SPLKATDFGLSVFKKCDI/FRDL/VGSAMY	236
LcCDPK14	YEDSENHVMELCGGELLDRIIVR--GHYTERAAAWAKTIVEVWQMHKGVGMHRDLKPENFLFANKKES--SPLKATDFGLSVFKPKGTFPCIVVGSPLY	242
LcCDPK15	YEDRQSHVVMELCGGELLDRIITAK--GHYTERAAAWAKTIVEVWQMHKGVGMHRDLKPENFLLNKKN--APLKATDFGLSVYKPKGTFPCIVVGSPLY	247
LcCDPK16	YEDDSAVHVMELCGGELLDRIIVR--GHYTERAAAWAKTIVEVWQMHKGVGMHRDLKPENFLFANKKET--SALKATDFGLSVFKPKGTFPCIVVGSPLY	542
LcCDPK17	CEDTGTVHVMELCGGELLDRIIVR--YSEFAEASTCROIINWVHSHFMVGMHRDLKPENFLFLNSTD--SPLKATDFGLSVFKPKGTFPCIVVGSPLY	203
LcCDPK18	YEDAMAVHVMELCGGELLDRIIQE--GHYTERAAAWAKTIVEVWQMHKGVGMHRDLKPENFLFISQED--SLKATDFGLSVFKPKGTFPCIVVGSPLY	293
LcCDPK19	YEDRQSHVVMELCGGELLDRIITAK--GHYTERAAAWAKTIVEVWQMHKGVGMHRDLKPENFLLSKDN--AKLKATDFGLSVIETCKEYRDI/VGSAMY	263
LcCDPK1	VAPEVLR--SYGLDADVWSIGVIAIYLLCGSRPFWARTSGFRAVLKADPSDEGFWESLSTEAKDFVRLLNKLSKRRNTAACALESHPWIRNY--NGVKVEL	419
LcCDPK2	VAPEVLR--HYGPEADVWVAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	366
LcCDPK3	VAPEVLR--RSEPPSDVWSIGVIAIYLLCGSRPFWARTSGFRAVLKADPSDEGFWESLSTEAKDFVRLLNKLSKRRNTAACALESHPWIRNY--NGVKVEL	369
LcCDPK4	VAPEVLR--SYGLDADVWSIGVIAIYLLCGSRPFWARTSGFRAVLKADPSDEGFWESLSTEAKDFVRLLNKLSKRRNTAACALESHPWIRNY--NGVKVEL	440
LcCDPK5	VAPEVLR--HYGPEADVWVAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	302
LcCDPK6	VAPEVLR--NYGPEADVWSAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	328
LcCDPK7	VAPEVLR--NYGPEADVWSAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	355
LcCDPK8	VAPEVLR--HYGPEADVWVAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	297
LcCDPK9	VAPEVLR--RSEPPSDVWSIGVIAIYLLCGSRPFWARTSGFRAVLKADPSDEGFWESLSTEAKDFVRLLNKLSKRRNTAACALESHPWIRNY--NGVKVEL	347
LcCDPK10	VAPEVLR--NYGPEADVWSAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	331
LcCDPK11	VAPEVLR--HYGPEADVWVAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	426
LcCDPK12	VAPEVLR--HYGQACDQVWSAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	395
LcCDPK13	VAPEVLR--NYGPEADVWSAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	338
LcCDPK14	VAPEVLR--NYGPEADVWSAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	344
LcCDPK15	VAPEVLR--HYGQACDQVWSAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	349
LcCDPK16	VAPEVLR--NYGPEADVWSAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	644
LcCDPK17	VSPBALSDRITTKSDMWSLGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	305
LcCDPK18	VAPEVLR--RSEPPSDVWSIGVIAIYLLCGSRPFWARTSGFRAVLKADPSDEGFWESLSTEAKDFVRLLNKLSKRRNTAACALESHPWIRNY--NGVKVEL	395
LcCDPK19	VAPEVLR--SYGKEDIWSAGVILYLLSGVPPFWAETBGMFQAVLKGHLDSDSDPWLISDSAKDILIRKMLCSQSDRTAHEVLCHEWICENGVA/PRRSL	365
LcCDPK1	DILIFKMLSYMRSSTRNAALKALSKT-----LTVDEIFYLKEQFALLEPKNESSTLESIKKALMNATDVKESRPEFTSSIDALQY	505
LcCDPK2	DPVWLSRLKOFSAANKKLMALRVAER-----LSEEBTAGLRMFKAMTDNSCATFDELKAGLRR--YESTLKDETRDLMDADVNS	451
LcCDPK3	DISVLLNMRFVRYSRKQFALRLAST-----INEGESLDRDFDAILVYKNGSLSLEEMQALARDLPWKESRVLVLEQADISTD	455
LcCDPK4	DILVFKMLSYMRSSTRNAALKALSKT-----LTVDEIFYLKEQFALLEPKNESSTLESIKKALMNATDVKESRPEFTSSIDALQY	526
LcCDPK5	DSAVLSRMKOFSAANKKLMALRVAER-----LSEEBTAGLRMFKAMTDNSCATFDELKAGLRR--YESTLKDETRDLMDADVNS	387
LcCDPK6	GETVRAHLKOFVANKKRRALRVAEH-----LSVEEVAGIKGFRIMTGNRCKINIDELVGLHR--LHQIPDSELQILMDAGVDR	413
LcCDPK7	DSAVLSRMKOFSAANKKLMALRVAER-----LSEEBTAGLRMFKAMTDNSCATFDELKAGLRR--YESTLKDETRDLMDADVNS	440
LcCDPK8	DSAVLSRLKOFSAANKKLMALRVAER-----LSEEBTAGLRMFKAMTDNSCATFDELKAGLRR--YESTLKDETRDLMDADVNS	382
LcCDPK9	DSAVLSRMKOFSAANKKLMALRVAER-----LSEEBTAGLRMFKAMTDNSCATFDELKAGLRR--YESTLKDETRDLMDADVNS	432
LcCDPK10	GEHWRTIKQFCLMSFKKALRVAEN-----LSDDEAAGLKMFTNITNSSETTYDELTGLAR--LSEKTEAEVQDLMDADVNS	416
LcCDPK11	DSAVLSRLKOFSAANKKLMALRVAER-----LSEEBTAGLRMFKAMTDNSCATFDELKAGLRR--YESTLKDETRDLMDADVNS	511
LcCDPK12	DSAVLSRLKOFSAANKKLMALRVAER-----LSEEBTAGLRMFKAMTDNSCATFDELKAGLRR--YESTLKDETRDLMDADVNS	480
LcCDPK13	DIAVLTSMKOFSAANKKLMALRVAEN-----LSEEBTAGLRMFKAMTDNSCATFDELKAGLRR--YESTLKDETRDLMDADVNS	423
LcCDPK14	GDIWRTLRKOFVANKKRRALRVAEH-----LSVEEVAGIKGFRIMTGNRCKINIDELVGLHR--LHQIPDSELQILMDAGVDR	429
LcCDPK15	ENAVLGRKOFKAMNFKKALRVAAG-----LSEEBTAGLRMFKAMTDNSCATFDELKAGLRR--YESTLKDETRDLMDADVNS	434
LcCDPK16	GETVYKALKOFVANKKRRALRVAEH-----LSVEEVAGIKGFRIMTGNRCKINIDELVGLHR--LHQIPDSELQILMDAGVDR	729
LcCDPK17	DAEVVSRLOQSNARRKLRALASCCSTVFLRTRKLLKLLGSYDLEEEFENLRIFHKIKCANGD--NASLSEFEVYKA--MMSLVLVLAAPRIFDLFNNR	406
LcCDPK18	DSAVLSRLKOFSAANKKLMALRVAER-----LSEEBTAGLRMFKAMTDNSCATFDELKAGLRR--YESTLKDETRDLMDADVNS	480
LcCDPK19	DNAVLSRMKOFSAANKKLMALRVAEN-----LSEEBTAGLRMFKAMTDNSCATFDELKAGLRR--YESTLKDETRDLMDADVNS	450
LcCDPK1	RRMCFEFCAAASVHQLBALG--RWEQHARCFYEIFEDGNRAIVIBELASELG-----LGPSIPVHVLHDWIRHTDGLKSLG/VKLIH--GASS	594
LcCDPK2	GTIDYGEFFAATVHNLKLEREE-----HVAFAQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	540
LcCDPK3	GLVDFSEFAATLHVHQLBEHSDSKWQORSRDAFERFIDRDRGYITPELRTHTG-----LKGSL--DFLLEADILKDGKISLSEFRRLRTASISS	546
LcCDPK4	RRMDFEFCAAASVHQLBALG--RWEQHARCFYEIFEDGNRAIVIBELASELG-----LGPSIPVHVLHDWIRHTDGLKSLG/VKLIH--GASS	615
LcCDPK5	GTIDYGEFFAATVHNLKLEREE-----HVAFAQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	476
LcCDPK6	CYLDYGEFFAIVSVHRLKMGND-----HWKAFQFDFDQNGYIEMBELRDLADEV-----DTS-EVVTAIMHDVDTKDKGRISWDFEAMMKAGTDWR	505
LcCDPK7	CSIDYEFHTATMHRHKLKEE-----HYKAFQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	530
LcCDPK8	GTIDYGEFFAATLHNLKIEREE-----HVAFAQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	471
LcCDPK9	GTIDYEFHTATMHRHKLKEE-----HYKAFQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	522
LcCDPK10	GTIDYEFHTATMHRHKLKEE-----HYKAFQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	508
LcCDPK11	GTIDYGEFFAATLHNLKIEREE-----HVAFAQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	600
LcCDPK12	GTIDYGEFFAATLHNLKIEREE-----HVAFAQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	564
LcCDPK13	GTIDYEFHTATMHRHKLKEE-----HYKAFQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	513
LcCDPK14	CYLDYGEFFAIVSVHRLKMGND-----HWKAFQFDFDQNGYIEMBELRDLADEV-----DTS-EVVTAIMHDVDTKDKGRISWDFEAMMKAGTDWR	520
LcCDPK15	GTIDYEFHTATMHRHKLKEE-----HYKAFQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	524
LcCDPK16	CSLNYGEFFAIVSVHRLKMGND-----HWKAFQFDFDQNGYIEMBELRDLADEV-----DTS-EVVTAIMHDVDTKDKGRISWDFEAMMKAGTDWR	820
LcCDPK17	GTIDYGEFFAATLHNLKIEREE-----HVAFAQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	503
LcCDPK18	GTIDYGEFFAATLHNLKIEREE-----HVAFAQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	569
LcCDPK19	GTIDYEFHTATMHRHKLKEE-----HYKAFQYFDKDGSGYITDELQACQTEHN-----MTD-AFEDHIREVDQNSDGRIDYGEFFVAMKQNGMGI	540

红色的直线表示 Ser/Thr 蛋白激酶区域,黑色的直线分别表示 4 个 EF-hands 区域。

Red line indicate the Ser/Thr kinase domain, black lines indicate the four EF-hands.

图 1 LcCDPK 氨基酸序列多重序列对比

Fig. 1 Multiple sequence alignment of LcCDPK protein in lychee

表 2 荔枝 *CDPK* 基因家族成员特征信息  
Table 2 The characteristics of 19 *LcCDPK* genes

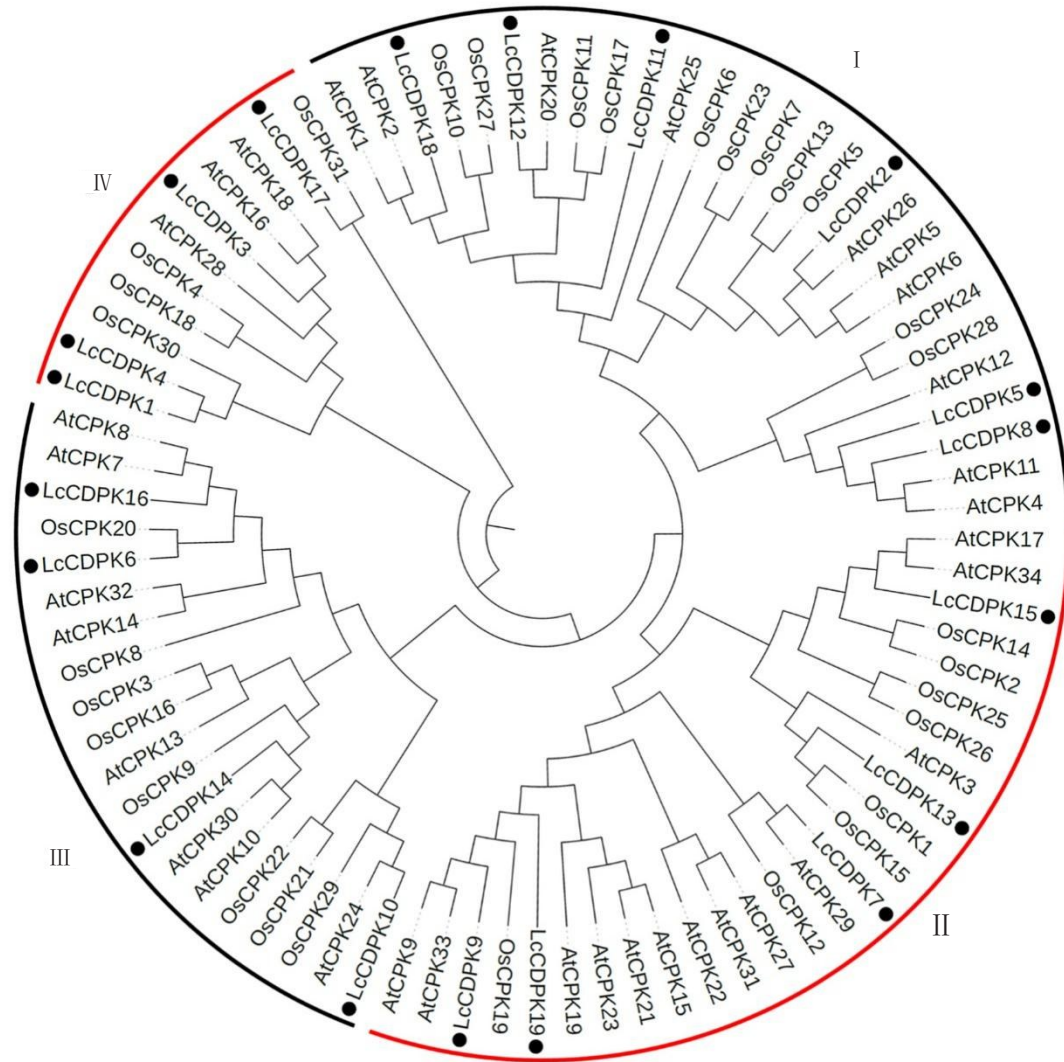
基因名称 Gene name	基因编号 Gene ID	CDS 长度 CDS Length/ bp	氨基酸 长度 Amino acids/aa	染色体 Chromo- some	EF Hands	等电点 pI <sup>a</sup>	分子质量 Molecular mass/ku	起始位置 Start	终止位置 End	亚细胞定位 Subcellular localization	N-Myris- toylation
<i>LcCDPK1</i>	LITCHI001674	1806	601	Chr5	1	8.67	67.68	29 446 798	29 453 620	Chloroplast	Y
<i>LcCDPK2</i>	LITCHI002195	1683	560	Chr5	4	5.80	62.90	34 128 272	34 132 785	Cytoplasm	N
<i>LcCDPK3</i>	LITCHI006474	1689	562	Chr11	4	9.13	63.80	1 569 144	1 578 338	Nucleus	Y
<i>LcCDPK4</i>	LITCHI006812	1869	622	Chr11	1	8.87	69.22	4 705 922	4 712 563	Chloroplast	Y
<i>LcCDPK5</i>	LITCHI007334	1503	500	Chr11	4	5.27	56.29	11 613 237	11 618 541	Cytoplasm	N
<i>LcCDPK6</i>	LITCHI008906	1557	518	Chr7	4	6.61	58.81	19 312 107	19 317 474	Nucleus	N
<i>LcCDPK7</i>	LITCHI010062	1608	535	Chr8	4	5.86	60.12	1 857 782	1 862 347	Cytoplasm	Y
<i>LcCDPK8</i>	LITCHI011639	1491	496	Chr8	4	5.59	55.65	24 775 847	24 780 830	Cytoplasm	N
<i>LcCDPK9</i>	LITCHI014137	1581	526	Chr2	4	6.10	58.81	39 048 466	39 057 334	Cytoplasm	Y
<i>LcCDPK10</i>	LITCHI015851	1644	547	Chr1	4	6.14	62.20	19 870 410	19 873 267	Cytoplasm	Y
<i>LcCDPK11</i>	LITCHI019005	1866	621	Chr15	4	5.48	69.86	11 587 821	11 593 634	Cytoplasm	N
<i>LcCDPK12</i>	LITCHI019021	1722	573	Chr15	4	5.34	63.48	11 743 007	11 748 387	Cytoplasm	N
<i>LcCDPK13</i>	LITCHI022010	4047	1348	Chr10	4	8.70	151.44	2 965 362	2 976 577	Plasma Membrane	N
<i>LcCDPK14</i>	LITCHI022230	1674	557	Chr10	4	6.55	63.34	4 555 317	4 559 219	Cytoplasm	N
<i>LcCDPK15</i>	LITCHI023733	1611	536	Chr13	4	5.46	59.19	5 194 965	5 198 136	Cytoplasm	Y
<i>LcCDPK16</i>	LITCHI023923	2556	851	Chr13	4	6.57	95.41	8 788 218	8 801 045	Cytoplasm	Y
<i>LcCDPK17</i>	LITCHI027028	1542	513	Chr3	3	5.58	57.11	16 195 697	16 204 228	Nucleus	N
<i>LcCDPK18</i>	LITCHI027050	1770	589	Chr3	4	5.22	65.51	16 459 442	16 465 948	Cytoplasm	N
<i>LcCDPK19</i>	LITCHI029637	1641	546	Chr9	4	6.33	61.70	27 392 060	27 399 369	Cytoplasm	Y

含子。如图 4-B 所示, *LcCDPK* 的第 I 亚家族成员中均含有 7 个外显子和 6 个内含子, 表明同一亚家族中的 *LcCDPK* 基因具有高度保守的基因结构。与之相似的是第 II 亚家族, 除 *LcCDPK13* 含有 19 个外显子和 18 个内含子外, 其余家族成员均含有 8 个外显子和 7 个内含子。相反, 在第 III 和第 IV 亚家族中呈现较大的基因结构多样性, 这两个家族中含有多个外显子和内含子, 例如, 在第 III 亚家族中 *LcCDPK6* 与 *LcCDPK10* 含有 8 个外显子和 7 个内含子, *LcCDPK14* 仅含有 7 个外显子和 6 个内含子, *LcCDPK16* 含有 13 个外显子和 12 个内含子。第 IV 亚族中, *LcCDPK1* 与 *LcCDPK4* 含有 11 个外显子和 10 个内含子, *LcCDPK3* 含有 12 个外显子和 11 个内含子, *LcCDPK17* 含有 7 个外显子和 6 个内含子。

随后使用 *LcCDPK* 的氨基酸序列进行了保守 Motif 分析。一共鉴定出 5 个保守的 Motifs, Motif1~Motif5 (图 4-C)。19 个 *LcCDPK* 均包含这 5 个 Motif。值得注意的是, *LcCDPK16* 较为特殊, 拥有两组 Motif1、Motif2 和 Motif4。

#### 2.4 *LcCDPK* 顺式作用元件分析

顺式作用元件在植物生长发育和抗逆境胁迫中均执行着不同的功能。植物激素例如水杨酸 (SA)、茉莉酸 (JA)、乙烯 (ET) 和脱落酸 (ABA) 等通过诱导转录因子与其相应的顺式作用元件相互作用, 在植物生长和逆境胁迫中发挥着重要作用<sup>[47]</sup>。为了探索荔枝中 *LcCDPK* 基因的转录调控, 笔者在本研究中利用 Plant CARE 数据库对 *LcCDPK* 基因家族成员起始密码子上游 2000 bp 启动子区域的潜在顺式作用元件进行了分析。如图 5 所示, 8 个潜在的顺式作用元件被鉴定, 包含激素响应相关顺式作用元件 (ABRE、CGTCA-motif 和 TCA-element)、光响应顺式作用元件 (AE-box 和 G-box) 以及逆境胁迫顺式作用元件 (ARE、MBS 和 TC-rich repeats)。*LcCDPK18* 启动子只包含两个顺式作用元件 (TC-rich repeats 和 AE-box), 其余 *LcCDPK* 家族成员均含有多个顺式作用元件 (图 5)。TC-rich repeats 是一种植物防卫和胁迫的顺式作用元件<sup>[48]</sup>, 分析发现在 *LcCDPK2* 和 *LcCDPK6* 的启动子中均包含这一顺式作用元件 (图 5)。TCA-element 是一种植物响应水杨酸 (SA) 应对植物病原胁迫的重要顺式元件<sup>[49]</sup>, 分析发现 *LcCDPK1*、



利用荔枝、拟南芥和水稻 CDPK 的全长氨基酸序列进行系统发育分析。CDPK 家族分为 4 个主要亚组(I~IV)。黑点表示荔枝 CDPK。

The full-length amino acid sequences of CDPKs from Lychee (Lc), Arabidopsis (At) and rice (Os) were used for phylogenetic analysis. The CDPK family is divided into four major subgroups ( I -IV). Black dots indicate the lychee CDPK.

图 2 LcCDPK 氨基酸序列的系统发育分析

Fig. 2 Phylogenetic of LcCDPK sequences

LcCDPK5、LcCDPK10、LcCDPK12、LcCDPK14、LcCDPK17和LcCDPK19这7个基因的启动子中均包含这一顺式作用元件。

### 2.5 LcCDPK组织特异性分析

CDPK在植物不同发育阶段均发挥着重要作用<sup>[9]</sup>。为了研究LcCDPK基因在荔枝发育中的表达模式,笔者在本研究中利用RNA-Seq数据对LcCDPK基因在9个不同荔枝组织(根、叶、雄花花药、雌花子房、胚、外种皮、假种皮、果皮和种子)中进行了表达分析(图6)。结果发现,虽然19个LcCDPK基因在9个组织中均有表达,但在不同组织中表达却存在巨大差异。聚类分析发现,19个LcCDPK分为

3组(图6)。第I组包含8个基因(LcCDPK16、LcCDPK7、LcCDPK4、LcCDPK19、LcCDPK5、LcCDPK9、LcCDPK6和LcCDPK18),主要在根和叶组织中大量表达,而在假种皮中呈现低表达。第II组包含9个基因(LcCDPK14、LcCDPK17、LcCDPK1、LcCDPK8、LcCDPK11、LcCDPK12、LcCDPK10、LcCDPK15和LcCDPK3),大部分基因在雄花花药中大量表达。第III组包含两个基因(LcCDPK13和LcCDPK2),在根、雌花子房和果皮中高表达,而雄花花药中显示低表达。归纳发现,LcCDPK16、LcCDPK7、LcCDPK4、LcCDPK9、LcCDPK6、LcCDPK18、LcCDPK14、LcCDPK17、LcCDPK11和LcCD-

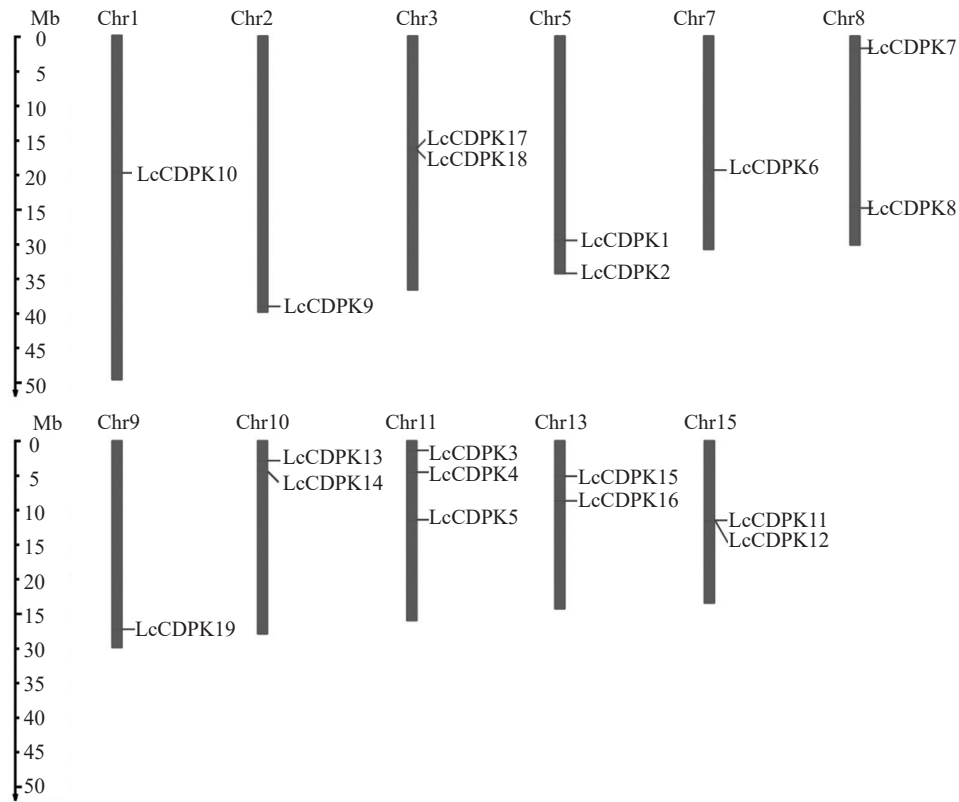
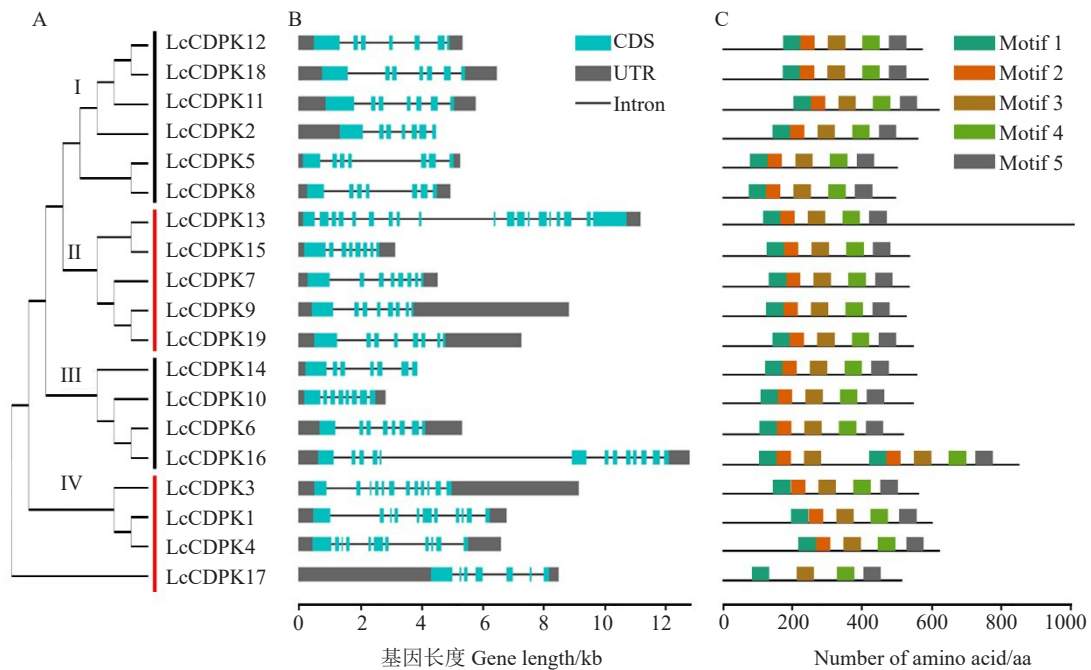


图 3 *LcCDPK* 染色体定位

Fig. 3 Distribution of *LcCDPK* on lychee chromosomes

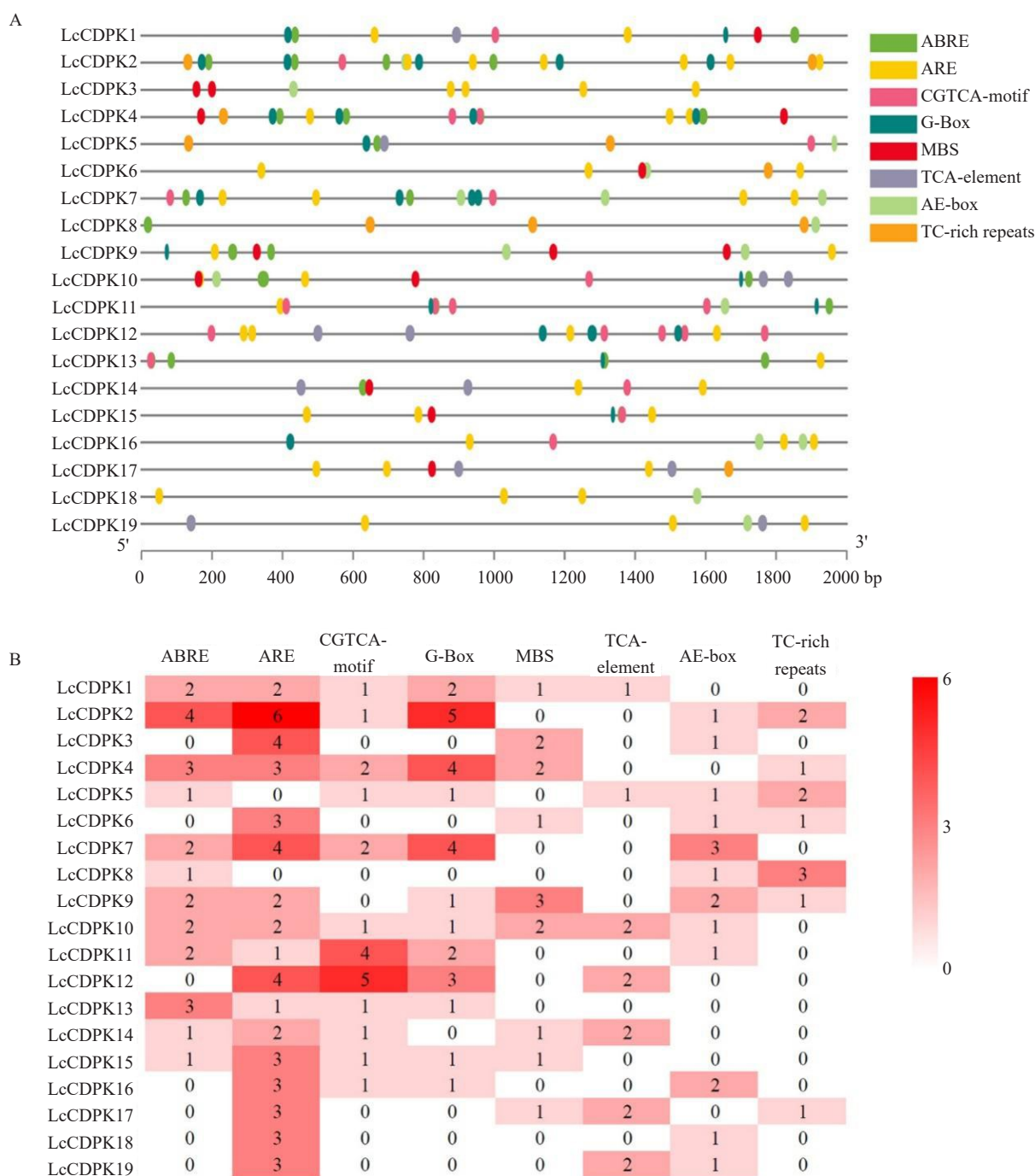


A. 利用 *LcCDPK* 氨基酸全长序列, 采用 Neighbor-Joining(NJ)方法构建系统进化树; B. *LcCDPK* 基因家族成员的基因结构; C. *LcCDPK* 基因家族成员保守基序分布。

A. The phylogenetic tree was constructed using full-length protein sequences by the Neighbor-Joining (NJ) method; B. Gene structure of *LcCDPK* family members; C. Distribution of conserved motifs of *LcCDPK*.

图 4 *LcCDPK* 基因家族成员进化树、基因结构和保守基序分布

Fig. 4 Phylogenetic tree, gene structure and distribution of conserved motifs of Lychee *CDPKs*



A. *LcCDPK* 基因家族成员启动子顺式作用元件种类和位置;B. *LcCDPK* 基因家族成员启动子顺式作用元件种类和数量。

A. The locations of cis-acting elements in promoters of *LcCDPK* family members; B. Types and numbers of cis-acting elements in promoters of *LcCDPK* family members.

图 5 *LcCDPK* 顺式作用元件分析

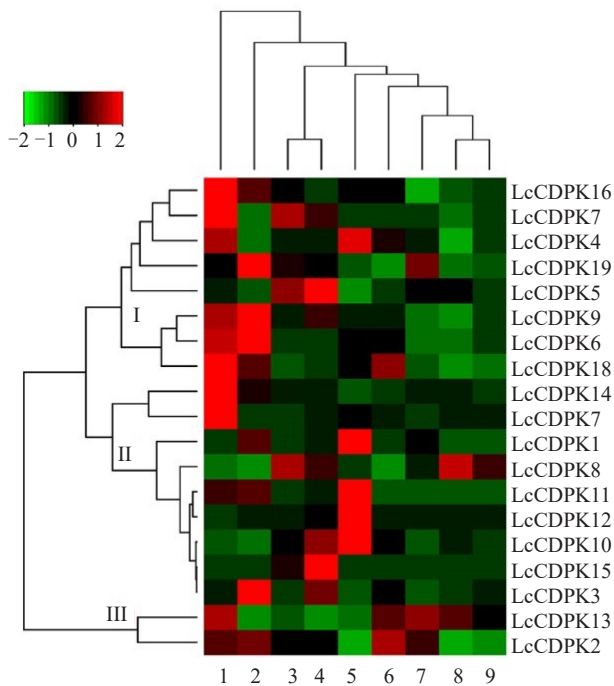
Fig. 5 Analysis of cis-regulatory elements in the promoters of *LcCDPK* genes

*PK13* 在根中表现显著的高表达,可能与植物根系生理有关;*LcCDPK19*、*LcCDPK9*、*LcCDPK6* 和 *LcCDPK3* 在叶中表现显著高表达,可能与叶片中的营养、抗病等生理功能有关;*LcCDPK1*、*LcCDPK11*、*LcCDPK12* 和 *LcCDPK10* 在雄花花药中表现显著高表达,

可能与生殖生理有关。

## 2.6 荔枝高抗霜疫病材料的鉴定

荔枝霜疫病,是由荔枝霜疫霉菌感染荔枝所引起的、发生在荔枝上的一种最为严重的病害<sup>[41]</sup>。该病流行于荔枝的整个生长发育过程中,尤其在嫩叶、

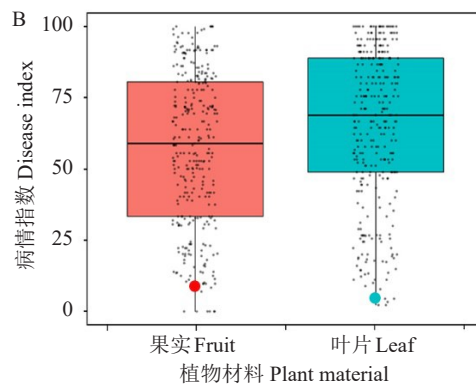
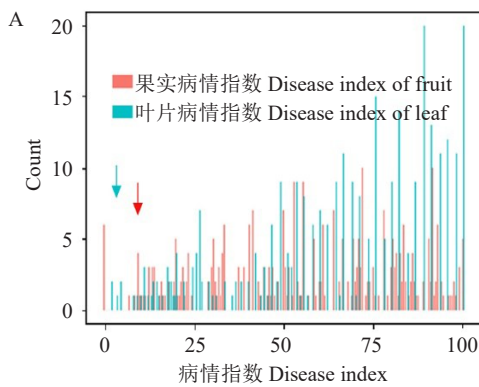


LcCDPK 基因在 9 个不同荔枝组织中的表达。1. 根;2. 叶;3. 外种皮;4. 胚;5. 雄花花药;6. 雌花子房;7. 果皮;8. 假种皮;9. 种子。

The heatmap of CDPK genes was constructed using R. Color key represents the relative transcript abundance of the LcCDPK genes in nine lychee tissues. 1. Root; 2. Leaf; 2. Episperm; 4. Embryo; 5. Male flowers anther; 6. Female flowers ovary; 7. Pericarp; 8. Aril; 9. Seed.

图 6 LcCDPK 基因组织表达分析

Fig. 6 Heatmap of lychee LcCDPK gene expressions in nine tissues



A. 276 份荔枝叶片和成熟果实对霜疫病抗性的病情指数频率分布;B. 荔枝叶片和成熟果实病情指数的表型分析。分别以 276 份荔枝自然群体的叶片和成熟果实为材料筛选出对霜疫病高抗品种裕荣 1 号(YR1)。青色箭头和圆点均表示裕荣 1 号(YR1)叶片的病情指数,红色箭头和圆点均表示裕荣 1 号(YR1)成熟果实病情指数。黑色小点表示每个材料的病情指数。荔枝叶片病情指数包含 2021 和 2022 年 2 个生物学重复,荔枝果实病情指数包含 2021 年 1 个生物学重复。

A. Frequency distributions of DI based on the means of the traits of 276 lychee accessions; B. Phenotypic analysis of DI values in lychee leaves and mature fruits. Leaves and mature fruits of 276 lychee germplasm materials were used as materials to screen the high resistance cultivar YR1. The cyan arrow and dot indicated the DI values of YR1 leaves, and the red arrow and dot indicated the DI values of YR1 mature fruits. The data of lychee leaf DI with two biological repeat, and the data of lychee fruit DI with one biological repeat.

图 7 荔枝抗霜疫病材料的筛选鉴定

Fig. 7 Screening and Identification of lychee cultivar YR1 with high-resistance to lychee downy mildew

花期和果实成熟期,甚至在采后运输贮藏过程中也能发生严重的危害<sup>[50]</sup>。为探索 LcCDPK 家族成员在荔枝霜疫病胁迫下的表达模式,笔者在本研究中以 276 份荔枝自然群体的叶片和成熟果实为材料筛选高抗霜疫病荔枝品种(表 3)。其中 2021 年使用荔枝叶片所鉴定的材料有 256 份,病情指数最大值为 100,最小值为 2.22,平均值为 68.28;2022 年使用叶片所鉴定的材料有 242 份,病情指数最大值为 100,最小值为 2.59,平均值为 68.47。使用荔枝成熟果实鉴定荔枝抗霜疫病材料只有 2021 年的 147 份材料,病情指数最大值为 100,最小值为 0,平均值为 57.64(表 3)。综合分析荔枝叶片和成熟果实病情指数的条形图和箱线图(图 7),结果表明裕荣 1 号(YR1)荔枝在荔枝叶片和成熟果实的病情指数均小于 25,按照前人的荔枝抗病分级标准<sup>[41]</sup>,裕荣 1 号(YR1)是一种高抗荔枝霜疫病的荔枝品种。

2.7 荔枝霜疫病胁迫下的 LcCDPK 家族成员表达

表 3 荔枝自然群体病情指数的描述性统计

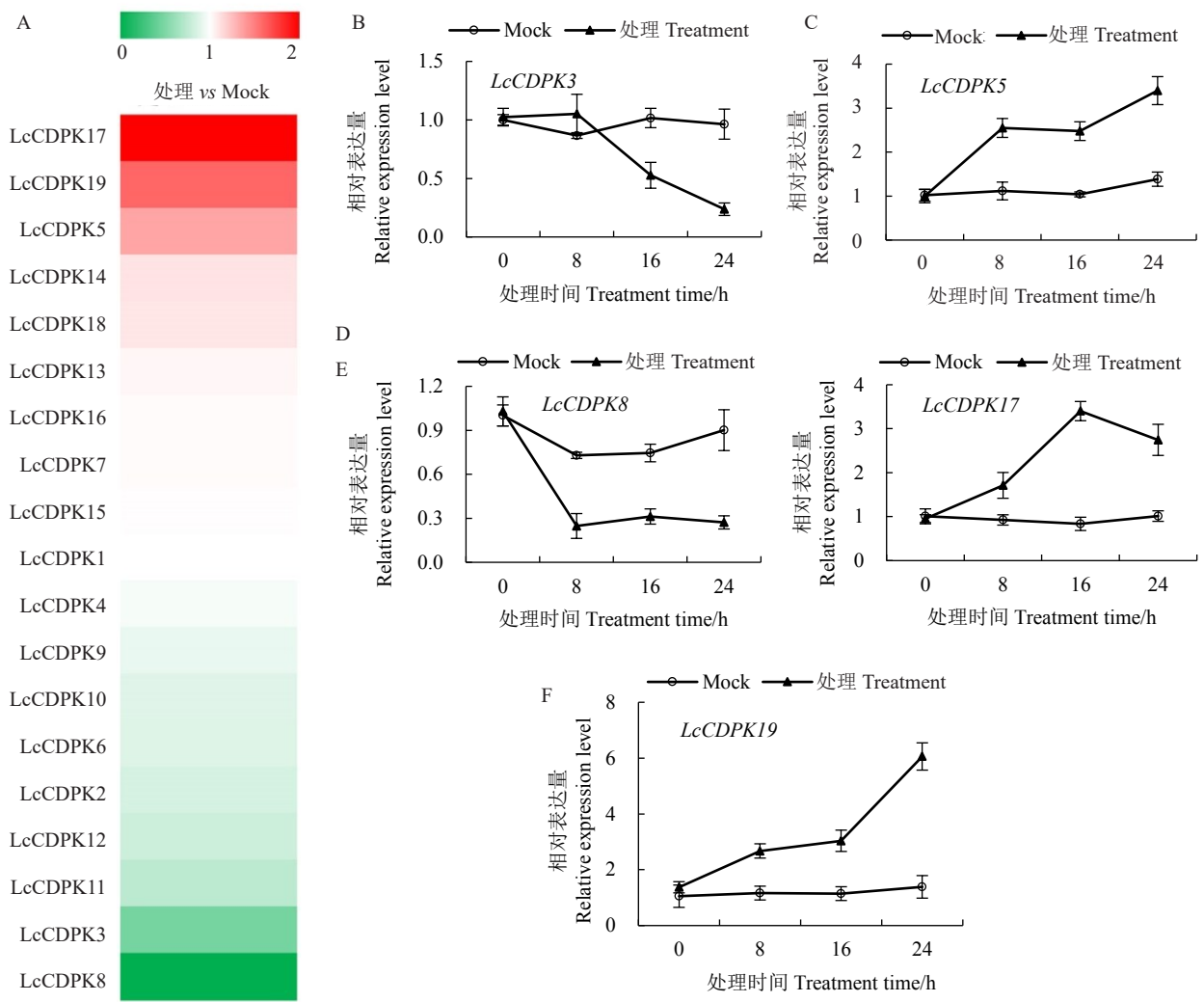
Table 3 Descriptive statistics explained by the population structure for disease index (DI) values in lychee accessions

类别	数目	年份	平均值	标准差	中值	最小值	最大值	极差
Trait	N	Year	Mean	SD	Median	Min	Max	Range
叶片 Leaf	256	2021	68.28	23.31	73.33	2.22	100	97.78
叶片 Leaf	242	2022	68.47	21.63	71.85	2.59	100	97.41
果实 Fruit	147	2021	57.64	24.60	68.83	0.00	100	100.00

分析

在筛选到的高抗荔枝霜疫病材料裕荣1号(YR1)的基础上,笔者利用RNA-Seq分析了荔枝霜疫病胁迫下的*LcCDPK*家族成员表达模式(图8-A)。与对照相比较,*LcCDPK17*,*LcCDPK19*和*LcCDPK5*在荔枝霜疫病处理后显著上调表达(图8-A);*LcCDPK3*和*LcCDPK8*在病处理下显著下调表达(图8-A)。为了进一步检验这一结果,挑选这5个基因在荔枝霜疫病处理后分时间点取样进行qRT-PCR验证(图8-B~F)。与RNA-Seq结果一致的是

(图8-B~F),在荔枝霜疫病胁迫后,*LcCDPK19*在原菌胁迫24 h后达到最高点,表达量是Mock处理的6倍左右(图8-F);*LcCDPK17*在病处理后16 h达到最大值(图8-E);*LcCDPK5*在病处理后24 h达到最大值,表达量大约是Mock处理的3.5倍(图8-E)。另外,*LcCDPK3*和*LcCDPK8*在病处理后迅速下调表达,均低于Mock处理(图8-B,8-D)。这些结果表明这些基因可能在荔枝抗霜疫病过程中发挥重要作用。



A. 热图表示荔枝霜疫病与 Mock 处理荔枝叶片 24 h 后 *LcCDPK* 基因表达量的比值。RNA-seq 所使用材料为裕荣 1 号(YR1)荔枝叶片,不同颜色表示表达量比值的差异,红色表示上调表达,绿色表示下调表达;B~F. 基于 A 中 RNA-seq 所得到的候选基因进行 qRT-PCR 验证。荔枝 *Actin* 作为内参,所用数据为 3 个生物学重复的(平均值  $\pm$  SD)。

A. The heatmap exhibit the ratio of the expression levels of lychee *LcCDPK* genes between lychee downy mildew treatment and Mock condition. RNA-seq data using leaf samples of the high-resistant lychee cultivar YR1. B-F. Relative expression levels of five lychee *LcCDPKs* in response to a simulated lychee downy mildew stress. The lychee *Actin* was used as the internal control. The data represent (the mean  $\pm$  SD) of three replicates.

图 8 *LcCDPKs* 在荔枝霜疫病胁迫下的表达分析

Fig. 8 Expression pattern analysis of *LcCDPKs* under the infection of lychee downy mildew

### 3 讨 论

目前, *CDPK* 基因家族成员已在众多植物中被鉴定并被广泛研究。在模式植物拟南芥中共有 34 个 *CDPK* 基因被鉴定; 在禾本科中, 水稻中有 31 个 *CDPKs*<sup>[37]</sup>、小麦中有 20 个 *CDPKs*<sup>[32]</sup>、玉米中有 40 个 *CDPKs*<sup>[38]</sup>; 在葫芦科中, 黄瓜中有 19 个 *CDPKs*<sup>[51]</sup>、甜瓜中有 18 个 *CDPKs*<sup>[52]</sup>; 在水果中, 柑橘基因组含有 29 个 *CDPKs*<sup>[53]</sup>、菠萝有 17 个 *CDPKs*<sup>[54]</sup>。笔者利用生物信息学等方法, 从荔枝中鉴定出 19 个 *LcCDPK* 基因家族成员。荔枝妃子笑主要含 15 条假染色体序列, 基因组大小约 470 Mb<sup>[39]</sup>。这说明 *CDPK* 基因家族成员数量与物种基因组大小没有线性关系, 推测可能是在荔枝进化过程中, 由于自然选择的压力, 有些 *LcCDPK* 基因功能丧失, 逐渐演化消亡或演变成其他基因。

在植物中 *CDPK* 具有明显的结构特征。笔者在本研究中发现 19 个 *LcCDPK* 成员 N 端可变区的长短不一; 催化区蛋白激酶区域同源性较高, 含有典型的 Ser/Thr 蛋白激酶催化保守序列; 调控区含有 1~4 个 EF-hands 结构。综合前人研究进展, 推测 *CDPK* 基因可能来自于蛋白激酶和 CaM 基因的融合。然而, 基因结构分析发现 *LcCDPK* 各成员的内含子及外显子数量差异比较大。这可能是 *LcCDPK* 基因家族成员在植物中承担各种不同功能角色的重要原因。

*CDPKs* 在植物的生长发育及应对生物、非生物胁迫中均发挥着重要的作用<sup>[55]</sup>。*CDPKs* 在不同组织中基因表达差异可能暗示着其功能的分化。在对荔枝 9 个不同组织的表达模式分析时发现, 19 个 *LcCDPK* 基因在 9 个组织中均有表达, 但在不同组织中表达情况却存在巨大差异。第 I 组成员主要在根和叶中大量表达, 这暗示它们可能在根和叶中发挥重要作用。第 II 组大部分基因在雄花花药中大量表达, 这些基因可能与雄花的发育有关。例如, 牵牛花 *PnCDPK1* 是花形态建成生殖生长信号转导中的重要组成部分, 转录水平在叶芽转变成花芽后迅速升高<sup>[56]</sup>。第 III 组包含两个成员 (*LcCDPK13* 和 *LcCDPK2*), 在根、雌花子房和果皮中高表达。这两个基因在不同组织中可能发挥不同的功能。

荔枝霜疫病是由荔枝霜疫霉菌感染所引起的、发生在荔枝上的一种严重病害。为了探讨 *LcCDPK* 对荔枝霜疫病的应答, 笔者在本研究中以 276 份荔

枝自然群体叶片和成熟果实为材料, 筛选出荔枝高抗品种裕荣 1 号 (YR1)。基因表达数据表明, *LcCDPK17*、*LcCDPK19* 和 *LcCDPK5* 在荔枝霜疫病处理后显著上调表达。顺式作用分析表明, *LcCDPK17* 和 *LcCDPK19* 启动子区域分别包含两个 TCA-element; *LcCDPK5* 启动子区域包含一个 TCA-element。前人研究表明 TCA-element 是一种植物响应水杨酸 (SA) 应对植物病原胁迫的重要顺式作用元件<sup>[49]</sup>。这 3 个基因可能参与荔枝 SA 通路响应病原微生物的胁迫。TC-rich repeats 是一种参与植物防卫和胁迫的顺式作用元件<sup>[48]</sup>。基因表达分析表明, *LcCDPK8* 在荔枝霜疫病处理下显著下调表达。顺式作用元件分析表明, *LcCDPK8* 启动子区域包含 3 个 TC-rich repeats, 这表明 *LcCDPK8* 可能负调控植物病原微生物胁迫。

笔者在全基因组水平对荔枝 *CDPK* 家族基因进行了鉴定和详细的生物信息学分析。另外, 在筛选到高抗霜疫病品种裕荣 1 号 (YR1) 的基础上, 对荔枝 *CDPK* 家族成员进行了荔枝霜疫病胁迫相关研究, 为进一步研究荔枝 *CDPK* 基因家族功能提供了基础。

### 4 结 论

从荔枝基因组中共鉴定出 19 个 *LcCDPK* 基因家族成员, 分为 4 个亚家族, 分布于 11 条染色体上。在 276 份荔枝自然群体中鉴定出一份高抗霜疫病荔枝材料裕荣 1 号。荔枝霜疫病胁迫表达分析表明 *LcCDPK5*、*LcCDPK17*、*LcCDPK19*、*LcCDPK3* 和 *LcCDPK8* 可能在荔枝抗病过程中发挥着重要作用。

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