小桐子*MAPKKKK*基因家族的全基因组鉴定及表达分析

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摘要: 促分裂原活化蛋白激酶激酶激酶激酶(mitogen-activated protein kinase kinase kinase kinase, MAPKKKK)属Ser/Thr类蛋白激酶, 参与典型MAPK级联系统与其它信号转导途径, 从而调节植物光周期、花器官生长及抗逆性等生物学过程。本研究基于序列比对的方法, 在全基因组水平对小桐子*MAPKKKK*基因家族进行鉴定, 并对其基因结构、系统进化、表达特性及潜在功能进行了解析。结果表明, 小桐子基因组中共鉴定到6个*MAPKKKK*基因, 主要定位细胞核, 蛋白序列长度分布在513~812 aa, 等电点分布在5.12~6.72。序列比对都发现激酶结构域及保守基序-TFVGTPxWMAPEV-, 除JcMAPKKKK4激酶结构域位于序列中部外, 其它成员都位于N端。与拟南芥、葡萄MAPKKKK共聚类与系统进化分析显示, MAPKKKK聚类为8个亚组, 且各亚组内成员存在蛋白序列长度、外显子数量、等电点特异性。基因结构分析表明, 除*JcMAPKKKK6*仅含有1个外显子, 其它5个小桐子*MAPKKKK*基因包含18~22个外显子。表达分析显示, *JcMAPKKKK1*、*JcMAPKKKK2*及*JcMAPKKKK5*在叶片中表达量较高, 而*JcMAPKKKK3*与*JcMAPKKKK6*在根中表达量较高。同时, *JcMAPKKKK3*与*JcMAPKKKK6*是响应小桐子抗冷性的主要基因。蛋白互作分析表明, 小桐子MAPKKKK与WNK、Mo25及Mob家族蛋白具有广泛的互作关系, 并可能参与植物极性生长、细胞分裂及ABA信号转导等过程。以上结果为开展小桐子*MAPKKKK*基因的功能鉴定与信号转导机制研究提供了参考。

关键词: 小桐子; 蛋白激酶; *MAPKKKK*; 基因家族; 表达分析

可逆性的蛋白质磷酸化/去磷酸化过程在大部分的细胞代谢中发挥核心作用, 该过程由蛋白激酶(protein kinase)与蛋白磷酸化酶(phosphoprotein phosphatases)负责完成。其中, 蛋白激酶催化将三磷酸核苷酸(nucleotide triphosphates, NTP) (通常为ATP)的γ-磷酸基团转移至目标蛋白的1个或数个氨基酸残基上, 从而调节该蛋白质的功能或催化活性(Hanks等1988)。根据磷酸化的氨基酸残基的不同, 蛋白激酶分为Ser/Thr蛋白激酶(serine/threonine-protein kinase)、Tyr蛋白激酶(tyrosine-protein kinase)及双特异性蛋白激酶(dual specificity protein kinase) (Chuang等2015)。促分裂原活化蛋白激酶级联系统都属Ser/Thr类蛋白激酶, 广泛参与细胞信号转导途径, 且从酵母到人类都较为保守(Frideman和Perrimon 2006)。该级联系统包含依次磷酸化的三级蛋白激酶, 即促分裂原活化蛋白激酶激酶激酶(mitogen-activated protein kinase kinase kinase, MAPKKK)、促分裂原活化蛋白激酶激酶(mitogen-activated protein kinase kinase, MAPKK)、促分裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK) (Pedley和Martin 2005)。

研究表明, MAPKKK也受到上游激活因子的调节, 通常为GTP结合蛋白(GTP-binding protein), 但在部分信号途径中为促分裂原活化蛋白激酶激酶激酶激酶(mitogen-activated protein kinase kinase kinase kinase, MAPKKKK) (Chuang等2015)。目前, *MAPKKKK*基因家族在哺乳动物中研究较为详细, 人类已经鉴定到6个成员, 即MAPKKKK1 (HPK1)、MAPKKKK2 (GCK)、MAPKKKK3 (GLK)、MAPKKKK4 (HGK/NIK)、MAPKKKK5 (KHS/GCKR)及MAPKKKK6 (MINK), 隶属于哺乳动物Ser/Thr类蛋白激酶的Ste20类家族(Ste20-like family), 重点参与人类免疫与炎症信号转导途径(Chuang等2015; Delpire 2009)。而在植物中该家族研究还较

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少, 只在少数物种中进行了鉴定及聚类分析, 如拟南芥与葡萄分别鉴定到10个(Champion等2004)、7个(Çakır和Kılıçkaya 2015), 同时, 研究显示MAPKKKK参与的信号途径在植物激素信号及抗逆信号转导中也发挥重要作用(Bogre等2000; Popescu等2009)。哺乳动物MAPKKKK家族蛋白聚类分析显示, 该家族包含N端激酶结构域(N-terminal kinase domain)、富Pro基序(proline-rich motifs)及C端Citron同源结构域(C-terminal citron-homology domain), 且在N端激酶结构域有较高的序列保守性(Chuang等2015)。

小桐子(*Jatropha curcas*)属大戟科(Euphorbiaceae)麻疯树属(*Jatropha*)多年生落叶小型乔木, 起源于中南美洲热带地区(林娟等2004), 现广泛分布于世界各热带与亚热带地区及我国南方诸省的干热河谷地带(Yang等2012)。作为重要的木本油料植物, 小桐子种子含油量高达35%~60%, 适应各种柴油发动机, 且关键技术指标达到了欧四标准。另外, 小桐子抗逆性强、生长快速、易于繁殖, 还可作为干旱贫瘠地区的生态造林树种, 榨油的油饼富含蛋白质, 经过脱毒后可作为高营养的动物饲料, 具有广阔的开发利用前景(Makkar和Becker 2009)。目前, 研究者已经对拟南芥与葡萄等植物在基因组范围内进行了*MAPKKKK*基因家族的鉴定, 而对于小桐子*MAPKKKK*基因的鉴定及分析还未见报道。本研究基于小桐子基因组数据(Sato等2011), 利用生物信息学方法鉴定到6个小桐子*MAPKKKK*基因, 并对其理化性质、基因结构、系统进化及表达特性进行了分析, 以期为研究小桐子*MAPKKKK*基因家族的功能及在抗逆信号转导中的作用机制奠定基础。

1 材料与方法

1.1 小桐子*MAPKKKK*基因家族的鉴定

从GenBank下载小桐子(*Jatropha curcas* L.)最新注释蛋白质数据库, 利用NCBI的Makeblastdb程序将该数据库本地化。根据Champion等(2004)、Çakır和Kılıçkaya (2015)鉴定的拟南芥与葡萄*MAPKKKK*基因家族登录号, 从拟南芥TAIR数据库(https://www.arabidopsis.org/)与葡萄基因组数据库(https://phytozome.jgi.doe.gov/ pz/portal.html)下载拟南芥10个与葡萄7个*MAPKKKK*基因的蛋白质序列。利用NCBI Blast程序对小桐子蛋白质数据库进行本地BlastP相似性比对(阈值$E<1^{-10}$, 序列相似性>50%), 得到初步筛选的小桐子MAPKKKK蛋白质序列。通过序列自对比(self-Blast)去除重复序列, 将非冗余的候选序列利用Pfam (http://pfam.sanger.ac.uk/)与CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi)在线工具分析蛋白激酶结构域(protein kinase domain)做进一步筛选, 得到小桐子MAPKKKK家族蛋白序列。同时下载其对应的基因序列与mRNA序列用于后续基因结构分析。

1.2 小桐子*MAPKKKK*基因家族序列分析

利用ProtParam工具(http://web.expasy.org/protparam/)对小桐子MAPKKKK进行氨基酸数目、理论分子量(Mw)、等电点(pI)等基本参数的分析。亚细胞定位利用CELLO (http://cello.life.nctu.edu.tw/)进行分析。染色体定位以Wu等(2015)构建的小桐子遗传连锁图谱进行锚定。根据MAPKKKK的编码区序列(coding sequence, CDS)得到各*MAPKKKK*基因起始密码子ATG上游1 500 bp的调控序列, 并通过PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/)对其顺式作用元件进行鉴定。将鉴定的小桐子MAPKKKK蛋白序列与拟南芥、葡萄的MAPKKKK蛋白序列利用ClustalX (Version2.0)进行序列相似性比对, 然后用MEGA 6.0软件通过邻接法(NJ)构建系统进化树。同时, 利用GenDOC软件对ClustalX比对结果进行MAPKKKK蛋白保守结构域分析。另外, 通过mRNA序列与基因序列比对以确定*MAPKKKK*基因内含子与外显子的结构, 并利用GSDS (Gene Structure Display Server, http://gsds.cbi.pku.edu.cn/)绘制基因结构图。利用MEME (http://meme-suite.org/tools/meme)在线分析工具对MAPKKKK蛋白序列进行motif分析。利用STRING (http://string-db. org)进行MAPKKKK蛋白的功能互作网络分析。

1.3 小桐子*MAPKKKK*基因家族的表达分析

选取饱满的小桐子种子, 按照Ao等(2013)的方法进行发芽处理与幼苗培养。之后将生长14 d的小桐子幼苗置于相对湿度75%、12°C、16 h/8 h光周期的低温培养箱中进行低温处理, 分别取低温处理12、24和48 h与对照(CK, 正常培养)的小桐

子第2片真叶与根, 以及吸涨24 h的种子, 液氮速冻后保存于–80°C冰箱中用于后续RNA的提取。利用TriZol试剂(Invitrogen公司)提取各器官材料的总RNA, 用DNase I (Thermo公司)消化残余基因组DNA, 分别取3 μg总RNA, 以混合逆转录引物[Random Primer 0.1 μg·μL^{-1}与Anchored Oligo(dT)$_{18}$ Primer 0.5 μg·μL^{-1}各占50%], 利用RevertAid First Strand cDNA Synthesis Kit (Thermo公司)合成第一链cDNA。以*GAPDH*为内参(Zhang等2013), 进行小桐子*MAPKKKK*基因的实时荧光定量PCR (quantitative real-time polymerase chain reaction, qRT-PCR)表达分析, 所用仪器为Bio-Rad CFX Connect, 试剂为Power SYBR Green PCR Master Mix (Thermo公司), 20 μL反应体系, 每个样品重复3次。所用引物序列见表1, 扩增条件为: 95°C预变性3 min; 95°C变性10 s, 55°C退火20 s, 72°C延伸20 s, 45个循环, 之后增加溶解曲线程序(65~95°C连续检测信号5 s, 0.5°C增量)。采用$2^{-\Delta\Delta C_t}$法进行相对表达量分析。器官差异表达分析以叶片的表达量为基准, 低温差异表达分析以对照(CK)的表达量为基准。

2 实验结果

2.1 小桐子*MAPKKKK*的鉴定及系统进化分析

基于拟南芥与葡萄已经鉴定的17个MAPKKKK蛋白序列对小桐子蛋白质数据库进行BLAST检索, 结合Pfam与CDD分析Ser/Thr蛋白激酶结构域, 共鉴定到6个小桐子*MAPKKKK*基因(*JcMAPKKKK1*~*JcMAPKKKK6*) (表2)。性质分析表明, 除*JcMAPKKKK6* (1 865 bp)较短外, 基因长度分布在8 450 (*JcMAPKKKK1*)~1 4682 (*JcMAPKKKK4*) bp之间; 蛋白质长度分布在513 (JcMAPKKKK6)~812 (JcMAPKKKK4) aa之间; 等电点都偏酸性, 分布在5.12 (JcMAPKKKK4)~6.72 (JcMAPKKKK5)之间。CELLO推测表明, 除JcMAPKKKK6定位细胞质之外, 其它5个JcMAPKKKK都定位细胞核。另外, 小桐子第2、3、4、9号染色体各定位1个*JcMAPKKKK*基因, 而第1号染色体定位2个*JcMAPKKKK*基因。

通过PlantCARE分析了小桐子6个*MAPKKKK*基因启动子中的顺式作用元件。结果表明, 小桐子6个*MAPKKKK*基因各自响应不同的植物激素与

表1 qRT-PCR实验中所用的引物序列

Table 1 Primers used in the qRT-PCR experiment

引物名称	上游引物(5′→3′)	下游引物(5′→3′)
JcMAPKKKK1	GGTGCAAATAAAGGGGCGAT	TAGCGGTGATCCCTGTGAAG
JcMAPKKKK2	TTGACACGGGAGATAGGCAG	CATGGGCAAGTTCAAGAGCA
JcMAPKKKK3	TCGAGATGTTAAGGCGGGAA	CTCATGCGTTGCCTATCACC
JcMAPKKKK4	GAACCCGAAACCAAAACCCA	AGGAGGGAGAGAGTCTTCGT
JcMAPKKKK5	TCCTAGATCACGCCAAGCAA	CGCACAACTGATCCTTCTGG
JcMAPKKKK6	TATGTCTGCTGGTTCCCTCC	AAGTGGCCTTGGTTGTGAAG
GAPDH	TGAAGGACTGGAGAGGTGGA	ATCAACAGTTGGAACACGGAA

表2 小桐子MAPKKKK蛋白的理化性质

Table 2 Physicochemical property of *MAPKKKK* in *J. curcas*

基因名称	登录号	基因长度/bp	mRNA长度/bp	蛋白质长度/aa	外显子数目	等电点	激酶结构域	亚细胞定位	染色体定位*
JcMAPKKKK1	105645574	8 450	3 021	728	21	6.41	18~279	细胞核	9
JcMAPKKKK2	105641495	12 171	3 543	688	21	6.13	16~277	细胞核	3
JcMAPKKKK3	105630271	9 183	2 823	694	21	6.09	13~274	细胞核	2
JcMAPKKKK4	105630562	14 682	2 728	812	18	5.12	240~494	细胞核	4
JcMAPKKKK5	105637555	13 000	2 720	690	22	6.72	15~267	细胞核	1
JcMAPKKKK6	105632224	1 865	1 865	513	1	5.94	20~299	细胞质	1

*表示根据Wu等(2015)构建的小桐子遗传连锁图谱进行基因的染色体定位分析。

非生物胁迫信号, 其中, *JcMAPKKKK1*、*JcMAPKKKK3*、*JcMAPKKKK4*及*JcMAPKKKK5*鉴定到脱落酸应答元件; *JcMAPKKKK1*、*JcMAPKKKK2*、*JcMAPKKKK4*及*JcMAPKKKK6*鉴定到茉莉酸甲酯应答元件; *JcMAPKKKK3*与*JcMAPKKKK5*鉴定到乙烯应答元件, 而*JcMAPKKKK6*高响应水杨酸、*JcMAPKKKK5*高响应赤霉素。在鉴定的非生物胁迫元件中, 除*JcMAPKKKK2*外, 都包含高温应答元件; 除*JcMAPKKKK3*外, 都包含植物病程应答元件; 而只有*JcMAPKKKK2*与*JcMAPKKKK3*鉴定到低温响应元件, 可能参与小桐子的抗冷性形成过程(表3)。

将鉴定的小桐子JcMAPKKKK蛋白序列与拟南芥、葡萄的MAPKKKK进行序列比对并构建系统进化树(图1)。结果显示, 23个MAPKKKK聚类为8个亚组, 且各亚组内成员存在蛋白序列长度、外显子数量、等电点特异性, 如亚组VIII的蛋白序列长度都分布在688~706 aa之间, 而亚组VI分布在453~513 aa之间。亚组IV与VIII都有21~22个外显子, 而亚组VI包含1~4个外显子, 同时, 除亚组II的VvMAPKKKK2 (9.31)与亚组V的AtMAPKKKK7 (8.88)之外, 其它所有的MAPKKKKs的等电点都偏酸性。另外, 除亚组VII成员的激酶结构域位于蛋白序列的中部外(包括小桐子JcMAPKKKK4位于240~494 aa之间), 其它所有的MAPKKKKs的激酶结构域都位于多肽链的N端(图1)。

2.2 小桐子*MAPKKKK*的基因结构与基序特征分析

基于鉴定到的6个小桐子*MAPKKKK*基因, 利用GSDS在线工具分析其基因结构。结果显示, 小桐子6个*MAPKKKK*基因都包含5′-UTR与3′-UTR区域。其中, *JcMAPKKKK6*仅含有1个外显子, 且基因较短, 单独聚类为一支, 而其它5个*MAPKKKK*基因都包含18 (*JcMAPKKKK4*)~22 (*JcMAPKKKK5*)个外显子, 且都具有较长的内含子序列(图2-A)。利用MEME进行基序分析表明, 聚类关系越近的JcMAPKKKKs都具有相似的保守基序类型与分布特征, 如JcMAPKKKK1/JcMAPKKKK2/JcMAPKKKK3 (图2-B)。

将小桐子的6个MAPKKKK蛋白进行多序列比对发现, 除激酶结构域序列(长度240~260 aa)较为保守外, 其它区域如N末端与C末端序列变化都较大。小桐子MAPKKKK激酶结构域序列比对结果显示, 鉴定到MAPK级联基因家族的典型催化基序-TFVGTPxWMAPEV- (图3)。

2.3 小桐子*MAPKKKK*基因的差异表达分析

通过荧光定量qRT-PCR得到小桐子6个*MAPKKKK*基因在叶片、根及种子的表达数据。结果表明, 6个*MAPKKKK*基因在小桐子各器官中都有表达, 但存在表达特异性。其中, *JcMAPKKKK3*与*JcMAPKKKK6*在根中表达量较高(P<0.01), 推测是参与小桐子根中MAPK级联信号转导的主要*MAPKKKK*基因。另外, *JcMAPKKKK6*在种子中表达量较其它*MAPKKKK*基因都高, 可能在种子萌发过程起主要作用。同时, *JcMAPKKKK1*、*JcMAPKKKK2*及*JcMAPKKKK5*表现出相同的器官表达差异性, 都在叶片中表达量最高, 其次为根, 而在种子中表

表3 小桐子*MAPKKKK*基因调控元件

Table 3 Gene *cis*-acting elements of *MAPKKKK* in *J. curcas*

基因名称	脱落酸应答元件	赤霉素应答元件	乙烯应答元件	水杨酸应答元件	茉莉酸甲酯应答元件	高温应答元件	低温应答元件	抗病性应答元件
JcMAPKKKK1	+	−	−	+	+	++	−	+
JcMAPKKKK2	−	−	−	−	+	−	+	+
JcMAPKKKK3	+	−	+	+	−	+++	+	−
JcMAPKKKK4	++	−	−	+	+	++	−	+
JcMAPKKKK5	++	++++++	++	−	−	++	−	+
JcMAPKKKK6	−	+++	−	+++	+	+	−	+

脱落酸应答元件(ABA/ABRE, CACGTG); 赤霉素应答元件(GARE-motif, AAACAGA/TCTGTTG); 乙烯应答元件(ERE, ATTTCAAA); 水杨酸应答元件(TCA-element, TCAGAAGAGG); 茉莉酸甲酯应答元件(MeJA, TGACG); 高温应答元件(HSE, AAAAAATTTC); 低温胁迫应答元件(LTR, CCGAAA); 抗病性应答元件(W-box, TTGACC); “+”表示有, “−”表示无, “+”数量表示鉴定到的元件数量。

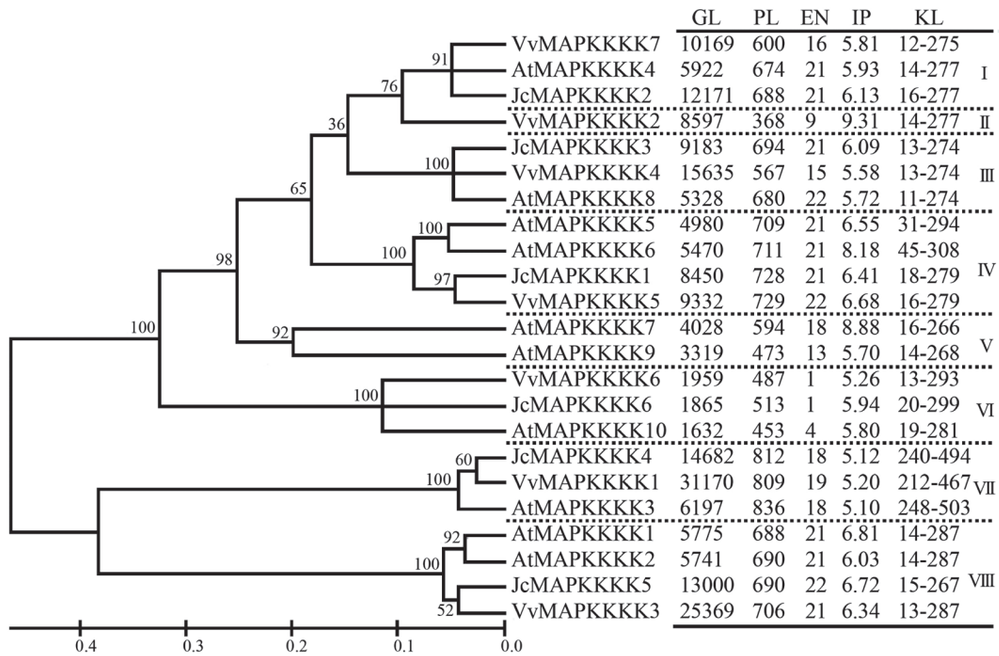

图1 小桐子与拟南芥、葡萄*MAPKKKK*基因家族的系统进化分析

Fig.1 Phylogenetic relationship of *J. curcas* with *A. thaliana* and *V. vinifera MAPKKKK* gene families

GL: 基因长度; PL: 蛋白质长度; EN: 外显子数量; IP: 等电点; KL: 激酶结构域位置。序列登录号如下, 拟南芥(*Arabidopsis thaliana*, *At*): *AtMAPKKKK1* (At1g53165)、*AtMAPKKKK2* (At3g15220)、*AtMAPKKKK3* (At1g69220)、*AtMAPKKKK4* (At5g14720)、*AtMAPKKKK5* (At4g24100)、*AtMAPKKKK6* (At4g10730)、*AtMAPKKKK7* (At1g70430)、*AtMAPKKKK8* (At1g79640)、*AtMAPKKKK9* (At1g23700)、*AtMAPKKKK10* (At4g14480); 葡萄(*Vitis vinifera, Vv*): *VvMAPKKKK1* (CBI27303.3)、*VvMAPKKKK2* (CBI28527.3)、*VvMAPKKKK3* (CBI20268.3)、*VvMAPKKKK4* (CBI25246.3)、*VvMAPKKKK5* (CBI34578.3)、*VvMAPKKKK6* (CBI23577.3)、*VvMAPKKKK7* (CBI34913.3)。

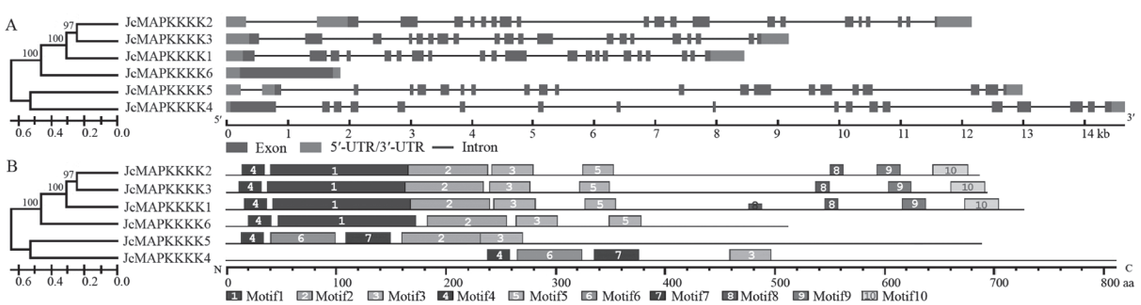

图2 小桐子*MAPKKKK*基因家族的基因结构及motif分析

Fig.2 Gene structure features and conserved motifs distribution of *MAPKKKK* family in *J. curcas*

A: 小桐子*MAPKKKK*基因内含子/外显子结构, 线条表示内含子, 框代表外显子; B: 小桐子MAPKKKK蛋白保守motif分析。

达量较低, 且较叶片中表达差异达到极显著水平(P<0.01) (图4)。低温处理下, 在叶片中*JcMAPKKKK1*、*JcMAPKKKK2*、*JcMAPKKKK4*及*JcMAPKKKK5*都呈整体下降表达的趋势; 而*JcMAPKKKK3*与*JcMAPKKKK6*随着低温处理时间的延长, 表达量逐渐上调, 且分别在低温处理48 h时, 较对照上调表达3.27倍(P<0.01)与2.78倍(P<0.05) (图5)。相反, 低温条件下根中*JcMAPKKKK1*~*JcMAPKKKK6*

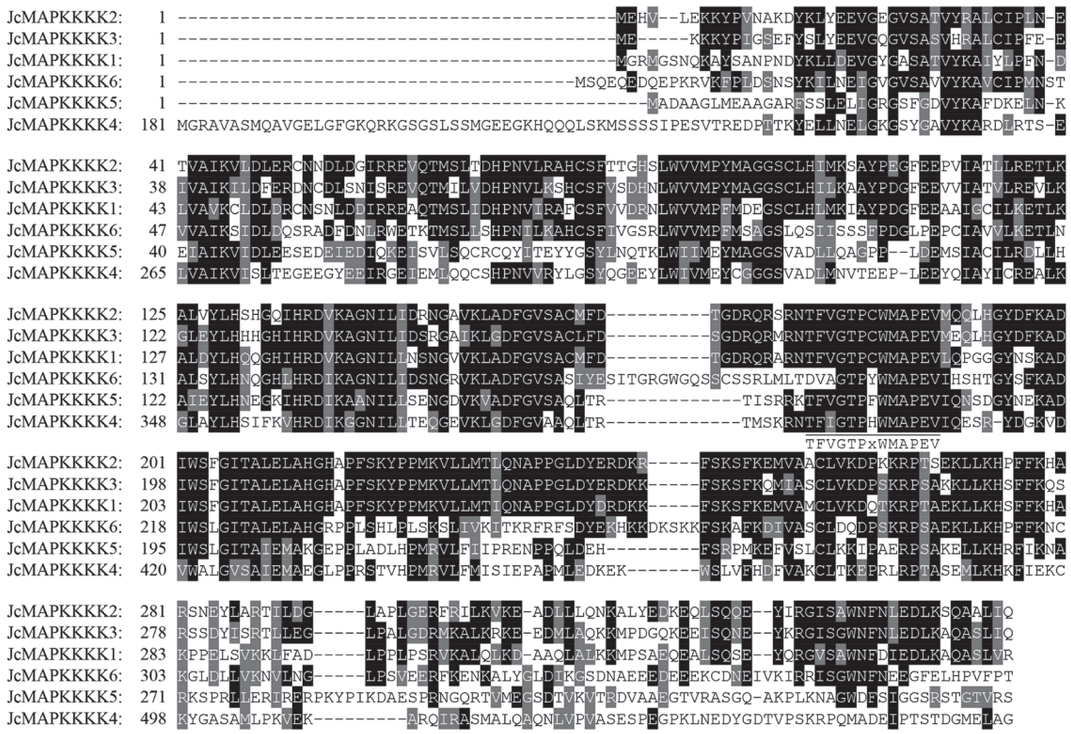

图3 小桐子MAPKKKK激酶结构域序列比对

Fig.3 Sequence alignment of kinase domain of MAPKKKK in *J. curcas*

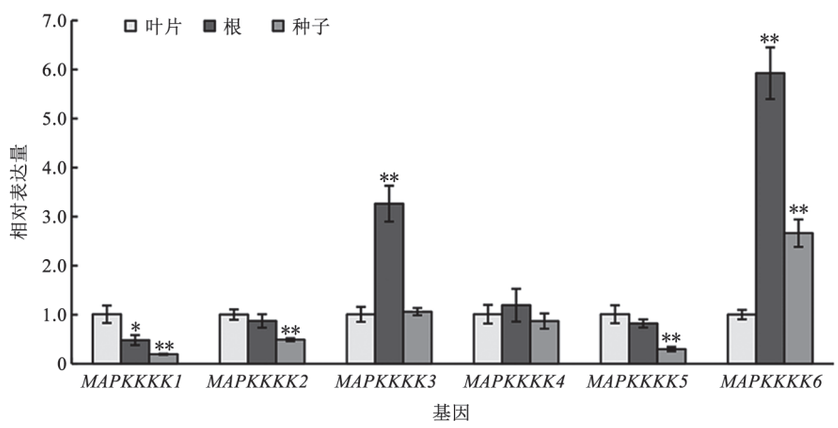

图4 小桐子*MAPKKKK*家族基因的器官差异表达分析

Fig.4 Differential expression of *MAPKKKK* family genes in different tissues of *J. curcas*

**与*分别表示不同器官的表达量较叶片在$P<0.01$与$P<0.05$水平的差异显著性。

都呈上调表达趋势, 其中*JcMAPKKKK3*在低温处理48 h时, 较对照上调表达6.71倍($P<0.01$); *JcMAPKKKK6*在低温处理24 h时达到最大表达量, 较对照上调表达2.35倍($P<0.01$) (图6)。以上结果表明, *JcMAPKKKK3*与*JcMAPKKKK6*是低温处理下的高响应基因, 与小桐子抗冷性的形成直接相关。

2.4 小桐子*MAPKKKK*基因的功能解析

基于小桐子与拟南芥MAPKKKK同源蛋白, 通

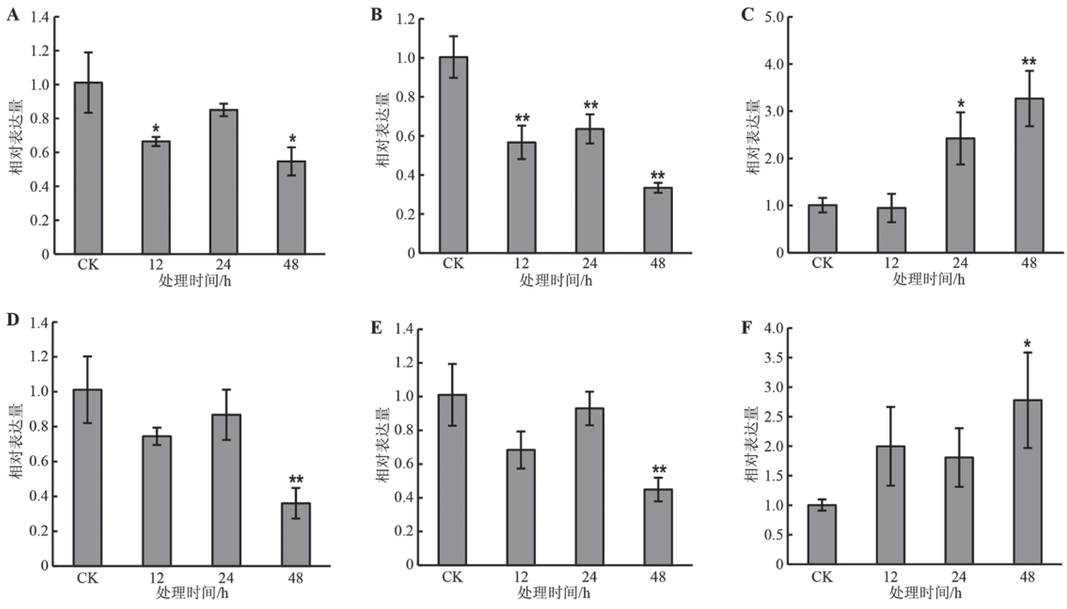

图5 低温处理下小桐子叶片中*MAPKKKK*基因的表达分析

Fig.5 Relative expression levels of *MAPKKKK* genes in leaves of *J. curcas* under cold treatment

A: *JcMAPKKKK1*; B: *JcMAPKKKK2*; C: *JcMAPKKKK3*; D: *JcMAPKKKK4*; E: *JcMAPKKKK5*; F: *JcMAPKKKK6*。**与*分别表示不同时间的表达量与对照在$P<0.01$与$P<0.05$水平的差异显著性, 下图同此。

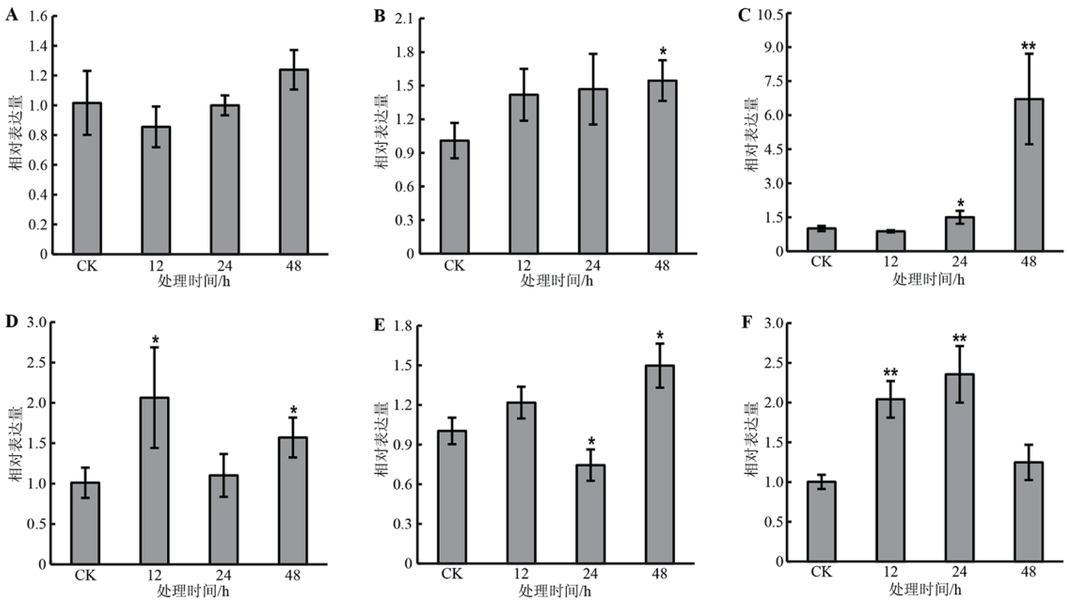

图6 低温处理下小桐子根中*MAPKKKK*基因的表达分析

Fig.6 Relative expression levels of *MAPKKKK* genes in roots of *J. curcas* under cold treatment

A: *JcMAPKKKK1*; B: *JcMAPKKKK2*; C: *JcMAPKKKK3*; D: *JcMAPKKKK4*; E: *JcMAPKKKK5*; F: *JcMAPKKKK6*。

过STRING 10.5进行蛋白互作网络分析, 以解析其可能的潜在功能与参与的信号转导或代谢途径, 结果表明, 小桐子JcMAPKKKK1 (拟南芥同源蛋白At4g24100)、JcMAPKKKK2 (At5g14720)及JcMAPKKKK3 (At1g79640)都主要与无赖氨酸激酶(with no lysine kinase, WNK)互作, 参与植物光周期及花期的调节; 另外, 还可与Mo25家族蛋白互作从而调节细胞极性生长过程。同时, JcMAPKKKK5 (At3g15220)与Mo25、Mob家族蛋白具有广泛互作关系, 此外, 还受到MAPK磷酸酶1 (MAPK phosphatase 1, MIKP1)、呼吸爆发氧化酶D (respiratory burst oxidase D, RBOHD)的调节, 并作用于MOB类蛋白从而调节细胞分裂与根系的生长过程。JcMAPKKKK6 (At4g14480)除了与Mo25家族蛋白互作外, 还可以作用于SnRK2.2 (sucrose non-fermenting-1 related protein kinase 2.2)与OST1 (open stomata 1)蛋白, 从而参与ABA信号转导途径。而只有JcMAPKKKK4 (At1g69220)直接与MEKK类MAPKKK互作, 参与典型MAPK级联系统的信号转导过程(图7)。

3 讨论

根据目前鉴定的动植物MAPKKKK构建系统进化树显示, MAPKKKK蛋白序列结构、功能结构域或基序并没有显著的聚类特征(Dan等2001), 说明动植物的MAPKKKK在分化之前属于相近的类群, 推测可能是在动植物分化之后各自单独进化形成的。Champion等(2004)将动植物MAPKKKK聚类为8个组, 即Ste20/PAK、PAK、PAK-related、TAO、MST、GCK、SOK以及ScNRK1, 部分组如SOK的MAPKKKK成员并不参与典型的MAPK级联信号系统, 可以直接磷酸化激活MAPKKs或参与其它信号途径。但MAPKKKK在动物与植物之内单独聚类却具有明显的亚组特征, 如人类鉴定的6个MAPKKKK(属于Ste20-like类Ser/Thr激酶)可以聚类为2个亚组, 与哺乳动物Ste20-like可分为PAK (p21-activated kinase)与GCK (germinal center kinase)两类一致(Chuang等2015; Strange等2006)。另外, Çakır和Kılıçkaya (2015)将拟南芥与葡萄的MAPK-

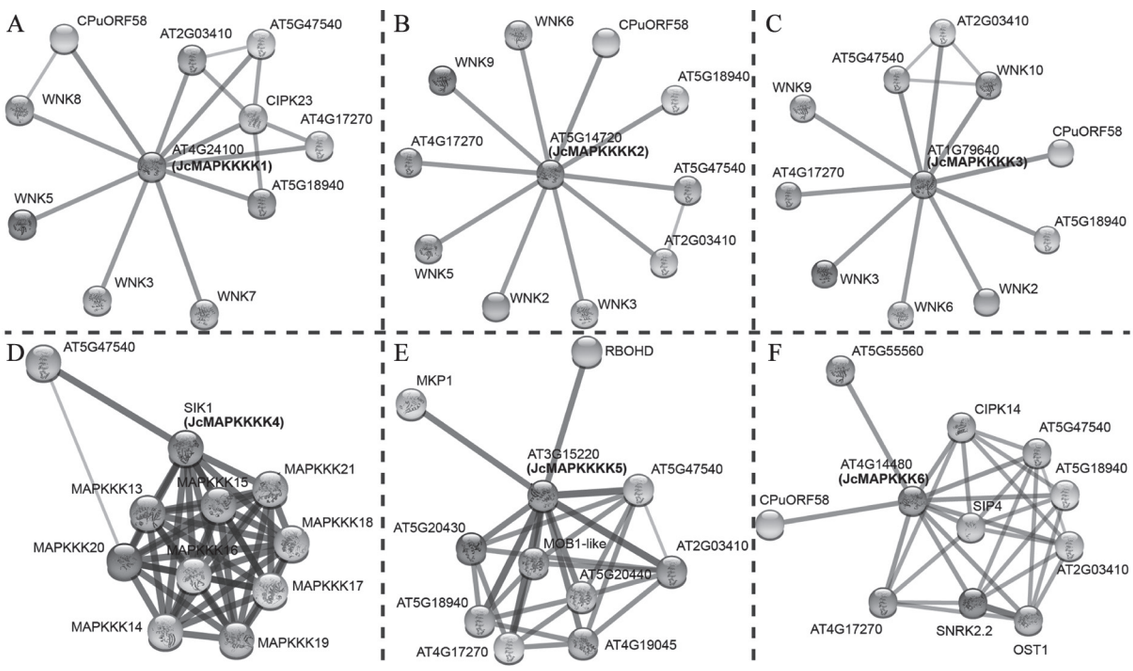

图7 小桐子与拟南芥相关MAPKKKK蛋白的互作网络分析

Fig.7 Interaction network analysis of MAPKKKK proteins identified in *J. curcas* and *Arabidopsis*

A: *JcMAPKKKK1*; B: *JcMAPKKKK2*; C: *JcMAPKKKK3*; D: *JcMAPKKKK4*; E: *JcMAPKKKK5*; F: *JcMAPKKKK6*。

KKK聚类为明显的8个亚组, 而本研究中进一步将小桐子鉴定的6个MAPKKKK与拟南芥、葡萄进行聚类与进化树构建, 得到了同样的分支特征(8个亚组), 且在蛋白质长度、外显子数量及等电点等参数存在显著的亚组特征(图1)。

目前, 在植物全基因组范围内鉴定并进行功能研究的MAPKKKK还较少, 且植物MAPKKKK家族成员较动物存在较低的序列相似性(Chuang等2015), 如葡萄7个MAPKKKK序列相似性(similarity)在18%~74%之间, 而小桐子6个MAPKKKK仅存在25.0%~60.8%相似性。但不同植物之间MAPKKKK存在较多的直系同源(ortholog)序列, 如Çakır和Kılıçkaya (2015)在拟南芥与葡萄MAPKKKK中鉴定到4对。而本研究中, 3种植物聚类结果也鉴定到6对, 如JcMAPKKKK1/VvMAPKKKK5 (序列相似性86.4%)、JcMAPKKKK2/AtMAPKKKK4 (79.5%)、JcMAPKKKK3/AtMAPKKKK8 (78.3%)、JcMAPKKKK4/VvMAPKKKK1 (75.4%)、JcMAPKKKK5/VvMAPKKKK3 (87.3%)及JcMAPKKKK6/AtMAPKKKK10 (70.4%)。

已报道, 动物的MAPKKKK家族蛋白的氨基酸序列相似性较高, 结构域分析表明都包括N端激酶结构域、富Pro基序(如人类MAPKKKK1包含4个; MAPKKKK3与MAPKKKK6包含3个; MAPKKKK2、MAPKKKK4及MAPKKKK5包含2个)以及C端Citron同源结构域, 且在部分MAPKKKK如人类的MAPKKKK1中还鉴定到半胱天冬酶(caspase)酶切位点(-DDVD-)(Chuang等2015)。但根据Champion等(2004)的聚类结果, Ste20/PAK与PAK类MAPKKKK却存在C端激酶结构域, 另外, 部分植物的MAPKKKK的激酶结构域还定位在多肽链的中部, 如拟南芥的AtMAPKKKK3 (248~503位)、葡萄的VvMAPKKKK1 (212~467位) (Çakır和Kılıçkaya 2015) (图1), 而本研究中鉴定到的小桐子JcMAPKKKK4也属于此类, 其激酶结构域位于240~494位。同时, 在植物的MAPKKKK中未发现富Pro基序与Citron同源结构域, 也表明动物与植物的MAPKKKK在分化之后形成了不同的类群, 但在植物如小桐子的MAPKKKK中都鉴定到了MAPKKK典型级联系统激酶, MAPKKK为-G(T/S)Px(W/Y/F)MAPEV- (MEKK亚家族), -GTxx(W/Y)MAPE- (RAF亚家族), -GTPEEMAPE(L/V/M)(Y/F/L)- (ZIK亚家族) (Ichimura等2002); MAPKK为-VGTxxYMSPER- (Hamel等2006); MAPK为-T(E/D)YVxTRWYRAPE(L/V)- (Camps等1999); 催化基序-TFVGTPxWMAPEV-的核心序列-(M/R)(A/S)PE- (图3)。

目前, 对于*MAPKKKK*基因的功能研究在哺乳动物尤其是人类中开展较多, Chuang等(2015)对人类鉴定的6个*MAPKKKK*基因在免疫与炎症系统中的功能及参与的信号转导途径等进行了详细的综述。在植物中, Gu等(2010)与Wang等(2014)分离到玉米的一种*MAPKKKK*基因*ZmSIMK1*, 转化研究表明其可以显著提高拟南芥的抗盐性、烟草的抗旱性与系统抗逆性。另外, Major等(2009)在野生马铃薯中发现了GCK亚组的*ScMAP4K1*基因, 在其胚珠发育、种子与果实成熟中发挥重要作用。本研究鉴定的6个小桐子MAPKKKK, 只有JcMAPKKKK4位于MAPK级联系统的上游, 参与MEKK类MAPKKK的调节, 而其它成员则直接参与其它代谢或信号转导途径, 与Chuang等(2015)在动物中关于MAPKKKK的功能研究与参与的信号转导途径一致。同时, 根据小桐子转录组表达数据显示(Wu等2015; Wang等2018), 小桐子WNK家族蛋白的WNK3、WNK5及WNK11在叶片中表达量较其它器官高, 而主要与其互作的JcMAPKKKK1与JcMAPKKKK2也在叶片中高表达(图4), 表明小桐子可能通过WNK3/WNK5/WNK11-JcMAPKKKK1/JcMAPKKKK2互作途径调节叶片感受光周期的过程(图7-A和B)。另外, 与小桐子JcMAPKKKK5互作的RBOHD蛋白在根中表达量较高, 推测其通过JcMAPKKKK5-RBOHD互作途径参与小桐子细胞分裂与根系发育等生理过程(图7-E)。

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Genome-wide identification and expression analysis of *MAPKKKK* gene family in *Jatropha curcas*

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Abstract: Mitogen-activated protein kinase kinase kinase kinase, as a Ser/Thr protein kinase, function as the biological processes of photoperiod, flowering time, and stress resistance, which plays vital roles in classical MAPK cascade and other signalling transduction pathways. For insight into the characteristics of *MAPKKKK* in *Jatropha curcas*, the *MAPKKKK* gene family was identified from *J. curcas* based on the BLAST method, and then the gene structure, phylogenetic relationship, expression profile and potential function were analyzed. The results showed that the *J. curcas* genome identified 6 *MAPKKKKs* and the predicted MAPKKKK proteins mainly located in nucleus with protein length ranged from 513 to 812 aa and pI value distributed from 5.12 to 6.72. Kinase domain and conserved motif of -TFVGTPxWMAPEV- were found in all the *J. curcas* MAPKKKK depends on sequence alignment. Except for JcMAPKKKK4 located in the middle of polypeptide, all other members located at N-terminal. Based on the phylogenetic relationship, MAPKKKKs from *J. curcas* with *Arabidopsis thaliana* and *Vitis vinifera* were classified into 8 subgroups and owned subgroup-specificity in protein length, exon number, and electrical point. Furthermore, gene structure analysis showed that except for only one exon in *JcMAPKKKK6*, the other five *J. curcas MAPKKKK* genes contained 18–22 exons. Expression analysis revealed that *JcMAPKKKK1*, *JcMAPKKKK2*, and *JcMAPKKKK5* were highly expressed in leaves, but *JcMAPKKKK3* and *JcMAPKKKK6* possessed high expression levels in roots. In addition, *JcMAPKKKK3* and *JcMAPKKKK6* were the main genes that respond to the cold resistance in *J. curcas*. Protein interaction analysis indicated that *J. curcas* MAPKKKKs had extensive interaction with WNKN, Mo25, and Mob family proteins, and were involved in plant polarity growth, cell division, and ABA signal transduction. The results of this study might lay a significant foundation for further studies on the gene function and signalling transduction mechanism of *MAPKKKK* gene family in *J. curcas*.

Key words: *Jatropha curcas*; protein kinase; *MAPKKKK*; gene family; expression analysis

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