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香蕉*WAT1*基因的鉴定及表达分析

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摘 要:【目的】基于香蕉基因组数据筛选*Walls are thin 1* (*WAT1*)基因,分析它们的序列及表达特性。**【方法】**以拟南芥*WAT1*为参考序列,通过本地Blast筛选获得香蕉*WAT1*基因,分析其核苷酸、启动子及编码蛋白特性,并利用实时定量PCR技术研究其在不同组织部位、不同激素和逆境胁迫处理下的表达情况。**【结果】**筛选获得5个香蕉*WAT1*基因(命名为*MaWAT1-1~5*)。蛋白亚细胞定位预测结果显示,MaWAT1-1、MaWAT1-2、MaWAT1-4主要定位在液泡和细胞膜上,MaWAT1-3主要定位在细胞质和细胞膜,MaWAT1-5定位在细胞膜和叶绿体。基因结构分析和系统进化树分析均将MaWAT1s分为两组,*MaWAT1-1*、*MaWAT1-2*、*MaWAT1-4*聚为一组(含6个外显子和5个内含子),*MaWAT1-3*和*MaWAT1-5*归为一组(含7个外显子和6个内含子)。启动子顺式作用元件分析结果显示:*MaWAT1s*启动子含有大量激素和胁迫响应相关元件。实时定量PCR结果显示,*MaWAT1-4*在叶片中表达量最高,*MaWAT1-1*在根和假茎中表达量最高,其余均在根中表达量最高;大多数*MaWAT1s*的表达受GA_3、SA、盐胁迫和干旱等显著诱导,受高温显著抑制,同时部分成员的表达受IAA、ABA、JA、低温、机械损伤和枯萎病影响显著。**【结论】***MaWAT1s*的表达受多种激素和逆境影响显著,可能在香蕉生长发育和抗逆防御反应中发挥着重要作用。

关键词:香蕉;*MaWAT1*;基因结构;顺式作用元件;基因表达

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Identification and expression analysis of *WAT1* genes in banana

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Abstract:【Objective】*WAT1* gene, a member of the *Medicago truncatula NODULIN 21* (*MtN21*) gene family, encodes a plant-specific protein that plays an important role in the secondary cell wall formation, and the transportation of the nutrients, amino acids, hormones and other substances in plant cells. Recently, its roles in the process of plant resistances to stresses were also widely identified. Up to now, however, no research has been reported on the *WAT1* gene in banana. This study aims to reveal the sequence characteristics and expression pattern of the *WAT1* genes in banana (*Musa nana* Lour.) under different phytohormones and stress treatments.【Methods】By using the *Arabidopsis thaliana WAT1* gene as reference sequence, the *WAT1* genes of banana were screened and selected from the banana genome data by local Blast. Then, their gDNA, CDS nucleotide sequences and encoded protein sequences were subjected to series of bioinformatics analysis. Moreover, their expressions under different phytohormones (including GA_3, MeJA, ABA, SA and IAA) and stresses (including salt, low temperature, drought, high temperature, wounding and *Fusarium* wilt) were studied using quantitative real time PCR (qRT-PCR). 【Results】Totally, the five *WAT1* genes (respectively located in chromosomes 1, 3, 6, 7, and 10, and named as the *MaWAT1-1* to *MaWAT1-5*) were identified from banana. Their gDNA and CDS

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length respectively ranged from 1 954 bp to 2 877 bp and from 1 086 bp to 1 164 bp. Their encoded proteins respectively contained 361-387 aa with relative molecular weight of 39-42 kD, and an isoelectric point of 8.5-9.2. All these MaWAT1s were found to be hydrophobic proteins with 10 transmembrane structures but without signal peptide. The subcellular localization prediction result showed that the *MaWAT1-1*, the *MaWAT1-2* and the *MaWAT1-4* were mainly located in the vacuole and cell membrane, the *MaWAT1-3* was mainly located in cytoplasm and cell membrane, and the *MaWAT1-5* was mainly located in cell membrane and chloroplast. Gene structure analysis showed that the banana *MaWAT1-1*, *MaWAT1-2* and *MaWAT1-4* owned 6 exons and 5 introns, while the *MaWAT1-3* and the *MaWAT1-5* had 7 exons and 6 introns. Conserved domain prediction revealed that all the MaWAT1s had two conserved EamA domains covering more than 70% of the whole proteins. The MaWAT1s were divided into two subgroups by the phylogenetic tree analysis. One subgroup was consisted of the MaWAT1-1, the MaWAT1-2 and the MaWAT1-4 (with higher similarity to the Arabidopsis WAT1), and the other subgroup was consisted of the MaWAT1-3 and MaWAT1-5. Promoter *cis*-acting element analysis showed that all the promoters of the *MaWAT1s* contained many phytohormone and stress responsive elements, indicating that the expression of the *MaWAT1s* might be regulated by these factors. Furthermore, we also found that the number and types of phytohormone responsive cis-acting elements varied a lot among these promoters. Quantitative real-time PCR (qRT-PCR) was conducted to look at the expression patterns of the *MaWAT1s* in different tissues and organs and in response to the different phytohormones. Results showed that the expression of *MaWAT1s* varied in different tissues and organs, the *MaWAT1-4* showed the highest expression in the leaves, the *MaWAT1-1* showed the highest expression in the roots and pseudostemes, while the expression of other the *MaWAT1s* was the highest in the roots. The ABA responsive elements were found in the promoters of the *MaWAT1-1*, the *MaWAT1-2* and the *MaWAT1-4*. Consistently, their expression was found to be induced by the ABA treatment. Notably, under the ABA treatment, the expression of the *MaWAT1-2* at all the treatment time points was found to be higher than that in the control. The GA responsive element was found in the *MaWAT1-1*, the *MaWAT1-2* and the *MaWAT1-3* promoters. The expression of the *MaWAT1-1* was about 6.7 times higher than that of the control at 48 h post the GA_3 treatment, and the expression of the *MaWAT1-2* at 12 h post the GA_3 treatment was about 54.0 times higher than that of the control. The *MaWAT1-1* to *4* promoters contained the MeJA responsive elements, and their expression after the MeJA treatment was found to be significantly induced at some time points after the MeJA treatment. The WAT1 was recognized as an auxin transporter. Under the IAA treatment, the expression of the *MaWAT1-1*, whose promoter contained an auxin responsive element, was 18.2 and 11.8 times higher than that of the control at 3 h and 6 h, respectively. The SA treatment showed a certain induction effect on the expression of the *MaWAT1s*. The expression of the *MaWAT1-3* to *5*, whose promoters contained SA responsive elements, was significantly induced by the SA treatment at some time points. However, the expression of the *MaWAT1-1* and the *MaWAT1-2*, whose promoters contained no such elements but several other stress responsive elements, was more significantly induced by the SA treatment and the highest expression was respectively 7.7-fold and 10.0-fold of the control. Besides, many stress responsive elements, such as drought-, low temperature-, high temperature-, anaerobic-, fungal induction-, defense stress-responsive elements, were found in the *MaWAT1s* promoter. The qRT-PCR results also showed that the expression of the *MaWAT1s* was significantly affected by the stress treatments and their expression patterns differed. The expression of the *MaWAT1s* was significantly induced by the salt stress. For example, the expression level of the *MaWAT1-1* was about 4.8 times higher than that of the control at 48 h post salt treatment, and the ex-

pression of the *MaWAT1-2* at 4 h and 48 h post salt treatment was respectively 15.9 and 15.4 times of the control. Notably, the expression of the *MaWAT1-3* at 48 h post salt treatment was about 103.6 times of the control. Under the drought stress, the expression of the *MaWAT1-1* to *4* was mostly induced. The expression of the *MaWAT1-2* was significantly higher (9.7 times) than that of the control after 4 days of drought treatment. The expression of all the *MaWAT1s* was significantly suppressed by the 38 ℃ high temperature stress treatment. Under the low temperature treatment, the expression levels of the *MaWAT1-1* and the *MaWAT1-2* were significantly increased at 24 h and 12 h, respectively, while the expression of the *MaWAT1-3* to *5* was down-regulated by the low temperature treatment. Under the wounding treatment, the expression patterns of the *MaWAT1s* also varied. The expression of the *MaWAT1-1* to *3* was induced to different extents after the wounding treatment, while the expression of the *MaWAT1-4* was significantly down-regulated in the early stages (0.5 d and 1 d) but significantly increased 3 d after the treatment, and the expression of the *MaWAT1-5* after the wounding treatment was lower than that of the control. Under the Fusarium wilt treatment, the expression levels of the *MaWAT1-2* and the *MaWAT1-3* were significantly higher than that of the control at some time points, while the others were lower or significantly lower than that of the control.【Conclusion】Results obtained in this study revealed that the expression of the *MaWAT1s* was greatly influenced by several phytohormones and stresses, indicating that they might function in the development and stress responses in banana.

Key words: Banana; *MaWAT1*; Gene structure; *Cis*-acting element; Gene expression

结瘤素基因在根瘤植物根瘤形成和根系固氮过程中扮演重要角色[1-2]，然而，它们在粮食作物、果蔬等植物体中表达量很低甚至不表达[3]。近些年的研究发现，非结瘤植物中存在大量的类结瘤素基因，它们不再参与根瘤形成，而是主要参与调控植物生长、转运生物活性物质等过程[4-5]。类结瘤基因包含七个家族，分别为 *MtN3/ saliva/SWEET* 家族、*MtN21/EamA-like/UMAMIT* 家族、*Early Nodulin-Like* 家族、*Major Facilitator* 超家族、*Sec14p-nodulin domain protein* 家族、*NOD26-like intrinsic protein* 家族和 *Vacuolar Iron Transporter/ nodulin-like* 家族[4]。其中，*MtN21* 家族因与蒺藜苜蓿（*Medicago truncatula*）*NODULIN 21* 基因高度同源而得名[6]。MtN21 主要参与氨基酸和生长素转运，受到生长素、细胞分裂素等激素调控，在植物次生壁生成、离层和不定根形成等过程发挥重要作用[7-8]。

Walls Are Thin 1 基因（*WAT1*）属于 *MtN21* 家族，拟南芥 *WAT1* 基因的功能最先被揭示[9]。Ranocha 等[10]研究发现，*WAT1* 在拟南芥所有组织器官中均有表达，在茎和下胚轴表达量最高，在带有次生壁的细胞比例高的器官中高表达。编码次生壁相关 NAC 结构域蛋白 SND1 和 NAC 次生壁增厚促进因子 NST1 的基因在植物纤维次生壁形成过程中发挥重要调控作用，利用 RNAi 沉默这 2 个基因会导致纤维次生壁厚度严重降低[11-12]。而拟南芥 *WAT1* 突变体中，*SND1* 和 *NST1* 以及它们的靶标基因 *KNAT7*、*MYB46* 和 *MYB103* 的表达显著降低[10]，说明 WAT1 可能是 SND1 和 NST1 的调控因子[13]，在植物纤维次生壁生成过程中发挥重要作用。拟南芥 *WAT1* 突变体植株木质素含量降低、茎纤维次生细胞壁厚度降低、植株矮化，茎中生长素含量显著降低、生长素运输受阻[10, 14]。同时，研究还表明 WAT1 是生长素局部液泡膜的转运体[15]，在植物形态建成中发挥着重要作用[10, 15-16]。Ju 等[17]发现节间较长、植株结构疏松的陆地棉品种‘L28’中，*WAT1* 基因的表达高于节间较短且结构紧凑的品种‘XLZ77’，而且在‘L28’中 *WAT1* 基因的表达随着结果枝节间的增长而增加，说明 *WAT1* 基因在陆地棉节间发育过程发挥作用。近些年的研究还发现，*WAT1* 除参与调控植物生长发育过程外，还在植物抗病防御反应中发挥作用[15-16]。Ye 等[18]研究发现，沉默 *GhWATs* 的棉花黄萎病抗性显著升高。Denancé 等[15]发现拟南芥 *WAT1* 突变体对青枯病、黄萎病和一些维管束病原体的抗性增强，同时他们还指出 WAT1 可以通过调节水杨酸（SA）和色氨酸代谢影响拟南芥的抗病性。SA 是植物抗逆防御反应的

关键激素，而色氨酸代谢可以产生生长素合成的前体物质[19-20]。说明 *WAT1* 通过调控生长素、SA 等激素的代谢在植物生长发育和抗逆防御反应方面发挥着举足轻重的作用。

香蕉是世界上重要的水果和作物，香蕉产业亦是我国华南地区重要的经济支柱之一[21]。然而，栽培香蕉抗逆能力较弱，近些年来受寒潮和香蕉枯萎病等影响，我国香蕉种植面积呈减少趋势，产量和品质也有所下降[22]。因此，开展香蕉抗性育种研究势在必行。鉴于 *WAT1* 基因在植物生长发育及抗逆防御反应中的作用，笔者对香蕉 *WAT1* 基因进行了鉴定和系列生物信息学分析，同时研究它们在不同激素和胁迫处理下的表达模式。预期本研究可为研究香蕉 *WAT1* 基因的功能及其在香蕉抗性育种研究中的应用奠定基础。

1 材料和方法

1.1 实验材料及处理

本研究所用移栽后 2 个月的‘天宝蕉’和香蕉枯萎病菌 *Foc*TR4 菌株均由福建农林大学园艺植物生物工程研究所提供。对植株分别进行 IAA、GA_3、SA、ABA、MeJA、干旱、盐、低温（4 ℃）、高温（38 ℃）、机械损伤和接种枯萎病菌 *Foc*TR4 处理，具体步骤参照孙雪丽等[23]的方法。收集健康‘天宝蕉’叶片、假茎、球茎和根样品以及各种激素和逆境处理后的样品（除 FocTR4 处理取根外，其余均为第 2 枚真叶）迅速液氮中预冷，之后保存于−80 ℃冰箱中，用于 RNA 提取。

1.2 香蕉 *WAT1* 基因的筛选

从香蕉基因组数据库（http://banana-genome-hub.southgreen.fr/）下载获得香蕉基因组 gDNA、CDS 和蛋白序列。以拟南芥 *WAT1* 基因（At1g75500）为参考序列，通过本地 blast 筛选获得香蕉 *WAT1* 基因，筛选标准为：Score >100，E 值<$1e^{-010}$。

1.3 生物信息学分析

参照孙雪丽等[23-24]的方法利用 ExPASy（https://web.expasy.org/protparam/）预测香蕉 MaWAT1 蛋白的基本理化性质；利用 InterProScan（http://www.ebi.ac.uk/interpro/search/sequence-search）分析 MaWAT1 结构域；利用 TMHMM Server v. 2.0（http://www.cbs.dtu.dk/services/TMHMM/）分析 MaWAT1 蛋白跨膜结构；利用 PSORT（https://www.genscript.com/）预测 MaWAT1 蛋白亚细胞定位情况；利用 SignalP 4.1 Server（http://www.cbs.dtu.dk/services/SignalP/）预测 MaWAT1 蛋白信号肽；利用 GSDS-2.0（http://gsds.cbi.pku.edu.cn/）分析 *MaWAT1s* 基因结构；使用 MapInspect 进行 *MaWAT1s* 基因染色体定位分析；使用 MEME（http://meme- suite.org/）在线分析 MaWAT1 蛋白保守基序（基数设为 20，其余为默认参数）。从香蕉基因组网站下载 *MaWAT1s* 基因转录起始位点上游 2 000 bp 的序列，使用 Plantcare（http://bioinformatics.psb.ugent.be/）预测 *MaWAT1s* 启动子顺式作用元件。通过 MEGA 7.0 软件将拟南芥、水稻、大豆和香蕉 WAT1s 氨基酸序列采用邻接法（Neighbor-Joining，NJ）在默认参数下构建进化树。

1.4 实时定量 PCR（qRT-PCR）

参考冯新等[25]的方法，利用 RNAprep Pure Plant Kit（TIANGEN, CHINA）试剂盒提取所有样品 RNA，利用 PrimerScript™ RT Reagent Kit (Perfect Real Time)试剂盒（TaKaRa，Japan）反转录获得 cDNA。利用 DNAMAN 软件根据 *MaWAT1s* 基因 CDS 序列设计定量引物（表 1）。qRT-PCR 反应在 Roche Light-Cycler480 上进行，以 *CAC* 基因作为内参基因。25 μL qRT-PCR 体系包含：Dream Taq™Green PCR Master (2×) 12.5 μL，ddH_2O 9.5 μL，模板 cDNA 1 μL，上下游引物各 1 μL。反应条件为：95 ℃变性 5 s，60 ℃

表1 本研究所用 qRT-PCR 引物

Table 1 Information of the qRT-PCR primers used in this study

基因 Gene	基因ID Gene ID	正向引物（F） Forward primer（F）	反向引物（R） Reverse primer（R）
MaWAT1-1	Ma01_g02070	CTCCTCGTCCCGTTTGCCTA	CTGGTTCGCGGTAATACCAC
MaWAT1-2	Ma03_g14340	TTGATCCTGCTCGTTCCCT	GAGCCCAAGCAGATAGAATCCC
MaWAT1-3	Ma06_g11040	TCTCCAGATTTGGTGCATT	AAAGTAGAGGCCAAACACA
MaWAT1-4	Ma07_g18150	CCGGCTTTGACACTATCGTTC	TCCGCACCTTCTCTATCCTG
MaWAT1-5	Ma10_g20140	GATCCATTCTGATTGTGCTTG	CAGCGTTTTCTTTATGCAA
CAC		CTCCTATGTTGCTCGCTTATG	GGCTACTACTTCGGTTCTTTC

退火 30 s，72 ℃延伸 15 s；40（*MaWAT1*、*MaWAT2* 和 *MaWAT4*）/48（MaWAT3 和 MaWAT5）个循环。采用 $2^{-\Delta\Delta ct}$ 法计算不同组织器官和不同处理下 *MaWAT1s* 基因相对表达情况。利用 SPSS 软件进行显著性分析，利用 GraphPad Prism 5 软件作图。

2　结果与分析

2.1　*MaWAT1* 基因基本信息和蛋白质理化性质分析结果

通过本地 blast 从香蕉基因组中筛选获得 5 条香蕉 *WAT1* 基因，染色体定位结果显示它们分别位于 1 号、3 号、6 号、7 号和 10 号染色体上（图 1），按照它们在染色体上的分布依次命名为 *MaWAT1-1~MaWAT1- 5*。它们与拟南芥 *WAT1* 基因（At1g75500）的相似度分别为 64.5%、66.3%、57.1%、65.9%和 55.2%，编码蛋白与拟南芥 *WAT1* 相似度分别为 71.6%、72.5%、58.4%、73.5%和 55.8%。5 条 *MaWAT1s* gDNA 长度为 1 954~2 877 bp，CDS 长度为 1 086~1 164 bp，预测分别编码包含 361~387 个氨基酸、相对分子质量 39~42 kD、等电点 8.61~9.12 的碱性疏水蛋白。其中，MaWAT1-2 和 MaWAT1-3 是不稳定蛋白，其余 3 个是稳定蛋白（表 2）。跨膜结构预测结果显示，所有 MaWAT1s 都含有 10 个跨膜螺旋。蛋白信号肽预测结果显示，MaWAT1s 均不具信号肽，不属于分泌蛋白[26]。亚细胞定位预测结果显示：MaWAT1-1、MaWAT1-2 和 MaWAT1-4 与拟南芥 WAT1 一样主要定位在液泡和细胞膜上[27]，而 MaWAT1-3 主要定位在细胞质和细胞膜，MaWAT1-5 主要定位在细胞膜和叶绿体。

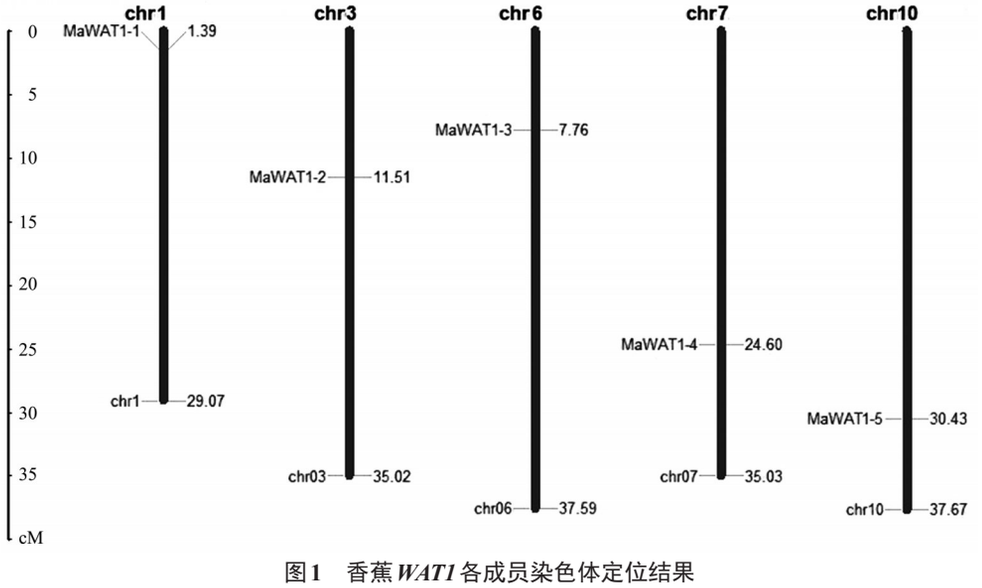


图1　香蕉*WAT1*各成员染色体定位结果

Fig. 1　Chromosome mapping results of banana *WAT1* genes

表2　*MaWAT1s*及其编码蛋白的基本信息

Table 2　Basic information of *MaWAT1s* and their encoded proteins

基因名称 Gene name	gDNA 长度 gDNA length/bp	编码区长度 CDS length/bp	染色体位置 Chromosome location	蛋白质 Protein					信号肽 Signal peptide	跨膜结构 Transmembrane domain	亚细胞定位 Protein subcellular localization prediction
				长度 Length/aa	分子质量 Molecular weight/Da	等电点 pI	不稳定指数 II	亲水性 GRAVY			
MaWAT1-1	2 067	1 140	chr01:1 385 043~1 386 831(-)	379	40 957.30	8.61	35.7	0.662	NO	10	液泡、细胞膜 Vacuole, Cytomembrane
MaWAT1-2	2 830	1 164	chr03:11 509 344~11 511 865(-)	387	41 671.01	8.86	42.83	0.641	NO	10	液泡、细胞膜 Vacuole, Cytomembrane
MaWAT1-3	1 954	1 086	chr06:7 760 278~7 761 996(+)	361	39 267.34	8.93	41.24	0.614	NO	10	细胞质、细胞膜 Cytoplasm, Cytomembrane
MaWAT1-4	2 877	1 164	chr07:24 596 704~24 598 950(+)	387	41 839.22	9.09	38.64	0.583	NO	10	液泡、细胞膜 Vacuole, Cytomembrane
MaWAT1-5	2 255	1 089	chr10:30 426 222~30 427 946(+)	362	39 468.64	9.12	37.34	0.601	NO	10	细胞膜、叶绿体 Cytomembrane, Chloroplast

2.2 香蕉 *WAT1* 基因结构分析结果

基因结构分析结果显示，*MaWAT1-1*、*MaWAT1-2* 和 *MaWAT1-4* 和拟南芥 *WAT1* 一样都具有 6 个外显子和 5 个内含子[27]，而 *MaWAT1-3* 和 *MaWAT1-5* 均具 7 个外显子和 6 个内含子（图 2）。

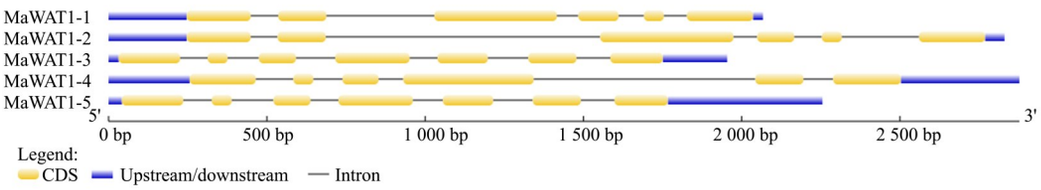
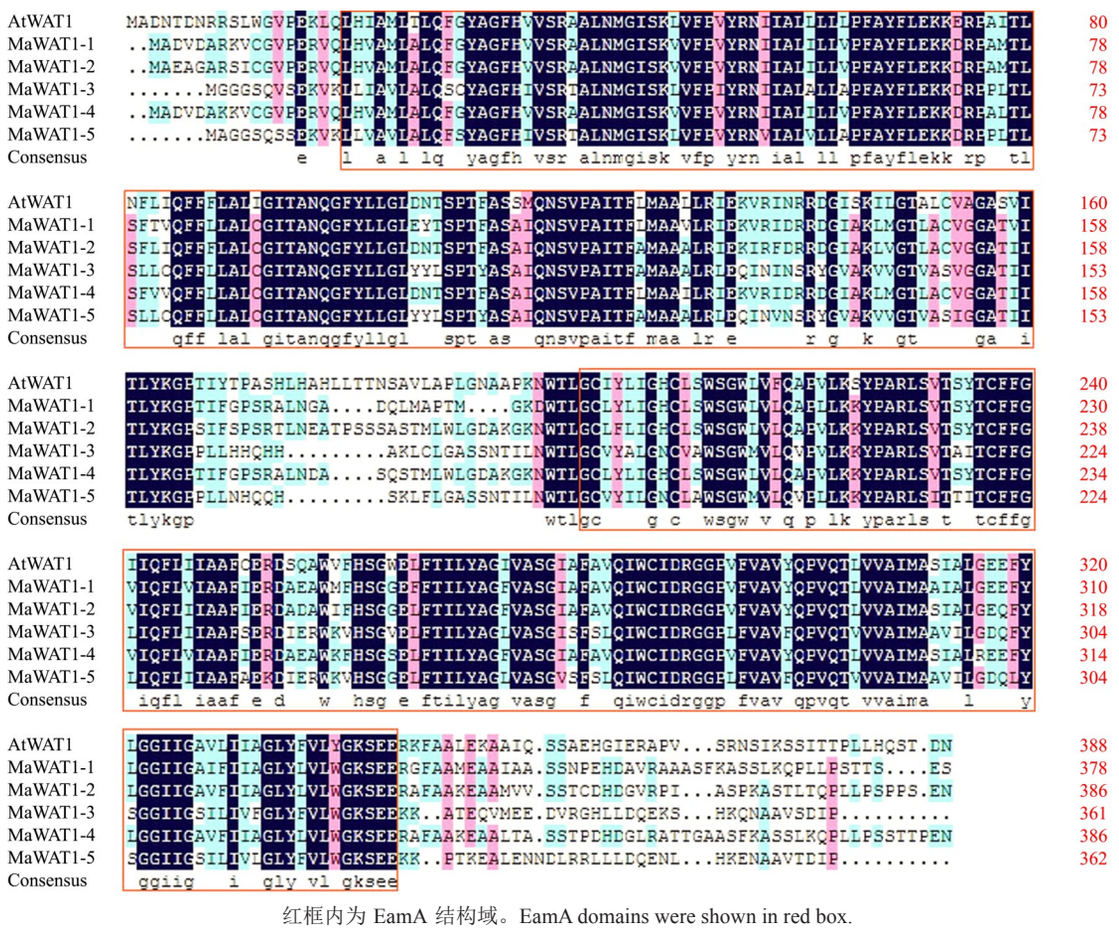


图2 香蕉 *WAT1* 基因结构

Fig. 2 Gene structure of banana *WAT1* genes

2.3 MaWAT1s结构域和保守基序分析结果

结构域预测结果显示，与拟南芥 WAT1 一样，MaWAT1s 都具有两个保守的 EamA 结构域，长度分别为 141 和 144 个氨基酸（图 3）。保守基序预测结果显示，MaWAT1s 共含有 18 种保守基序，其中 9 种保守基序在 5 个 MaWAT1s 中均有存在，2 种只存在于 MaWAT1-1、MaWAT1-2 和 MaWAT1-4 中，7 种仅存在于 MaWAT1-3 和 MaWAT1-5 蛋白中（图 4）。



红框内为 EamA 结构域。EamA domains were shown in red box.

图3 香蕉与拟南芥WAT1蛋白序列比对结果

Fig. 3 Sequence alignment of WAT1s from banana and *Arabidopsis thaliana*

2.4 系统进化分析结果

通过对香蕉、拟南芥、水稻和大豆 WAT1 蛋白序列构建进化树发现，MaWAT1s 可以分为两组，其中 MaWAT1-1、MaWAT1-2 和 MaWAT1-4 聚为一组，与拟南芥 WAT1 亲缘关系较近；MaWAT1-3、MaWAT1-5 聚为另一组（图 5）。

2.5 *MaWAT1* 基因启动子顺式作用元件分析结果

启动子顺式作用元件分析结果显示，*MaWAT1s* 各成员启动子顺式作用元件数目存在较大差异（图 6），如：*MaWAT1-4* 启动子中 TATA-box 和 Enhancer 元件数目显著多于其他成员的启

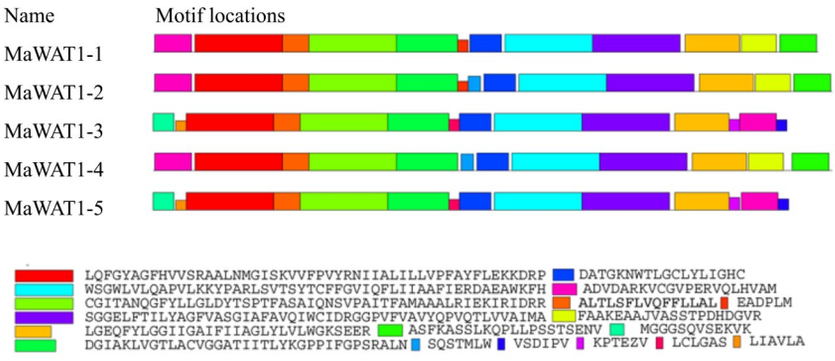


图4　香蕉WAT1蛋白保守基序分析

Fig. 4　Motif prediction of banana WAT1s

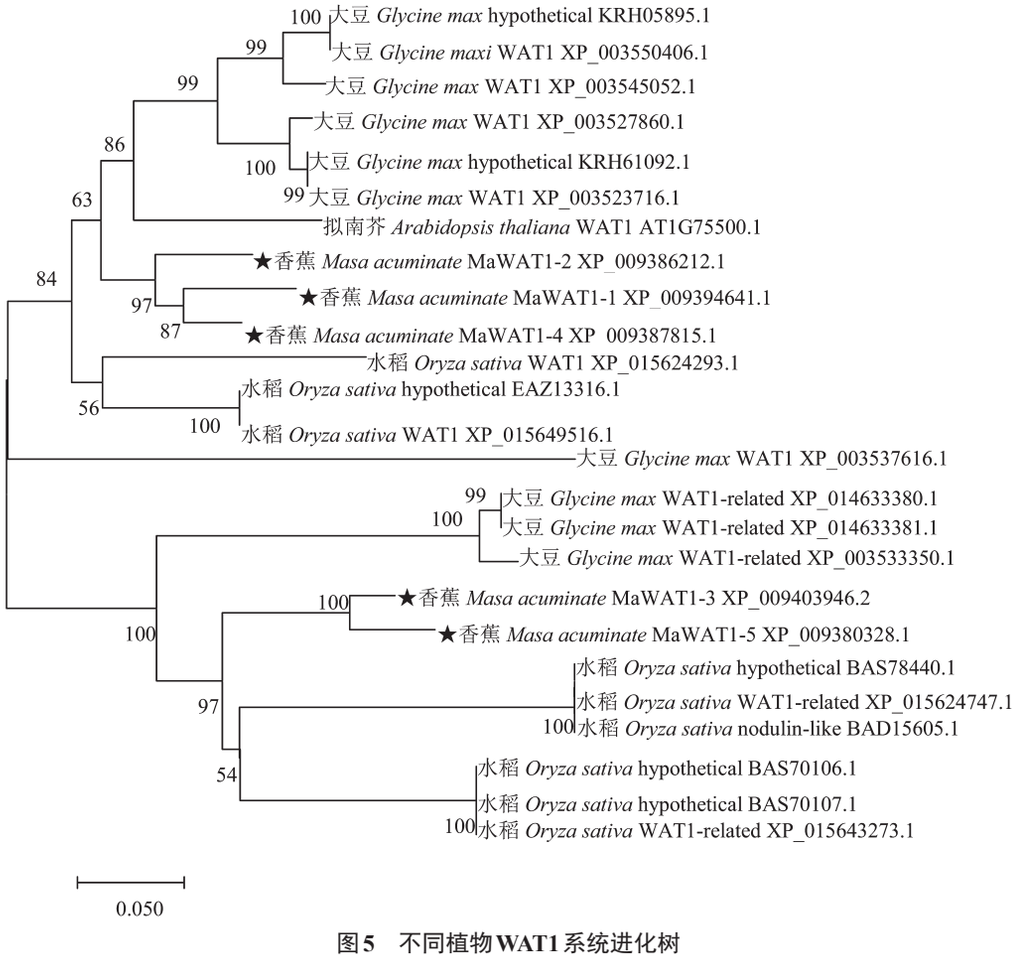


图5　不同植物WAT1系统进化树

Fig. 5　Phylogenetic tree of WAT1s from different plants

动子。这些基因的启动子除均含有较多的 CAAT-box、TATA-box 等核心作用元件和光响应元件外，还存在大量的激素（ABA、MeJA、GA_3、IAA、SA 等）响应和逆境响应（干旱、低温、高温厌氧、真菌诱导、防御胁迫等）相关元件（图 6），暗示 MaWAT1 基因在香蕉响应这些激素或逆境过程中发挥作用。*MaWAT1* 各成员的启动子顺式作用元件类型存在一定差异，如 *MaWAT1-1* 含有 ABA、MeJA、GA_3、IAA、高温胁迫、真菌诱导、防御胁迫响应等元件；*MaWAT1-2* 含有 ABA、MeJA、GA_3、高温胁迫、厌氧诱导、寒害与脱水等元件；*MaWAT1-3* 含有 MeJA、GA_3、SA、低温胁迫、高温胁迫、厌氧诱导等元件；*MaWAT1-4* 含有 ABA、MeJA、SA、防御胁迫响应等元件；*MaWAT1-5* 含有 IAA、SA、厌氧诱导、防御胁迫响应等元件，说明 *MaWAT1* 基因各成员的功能可能存在一定的差异。

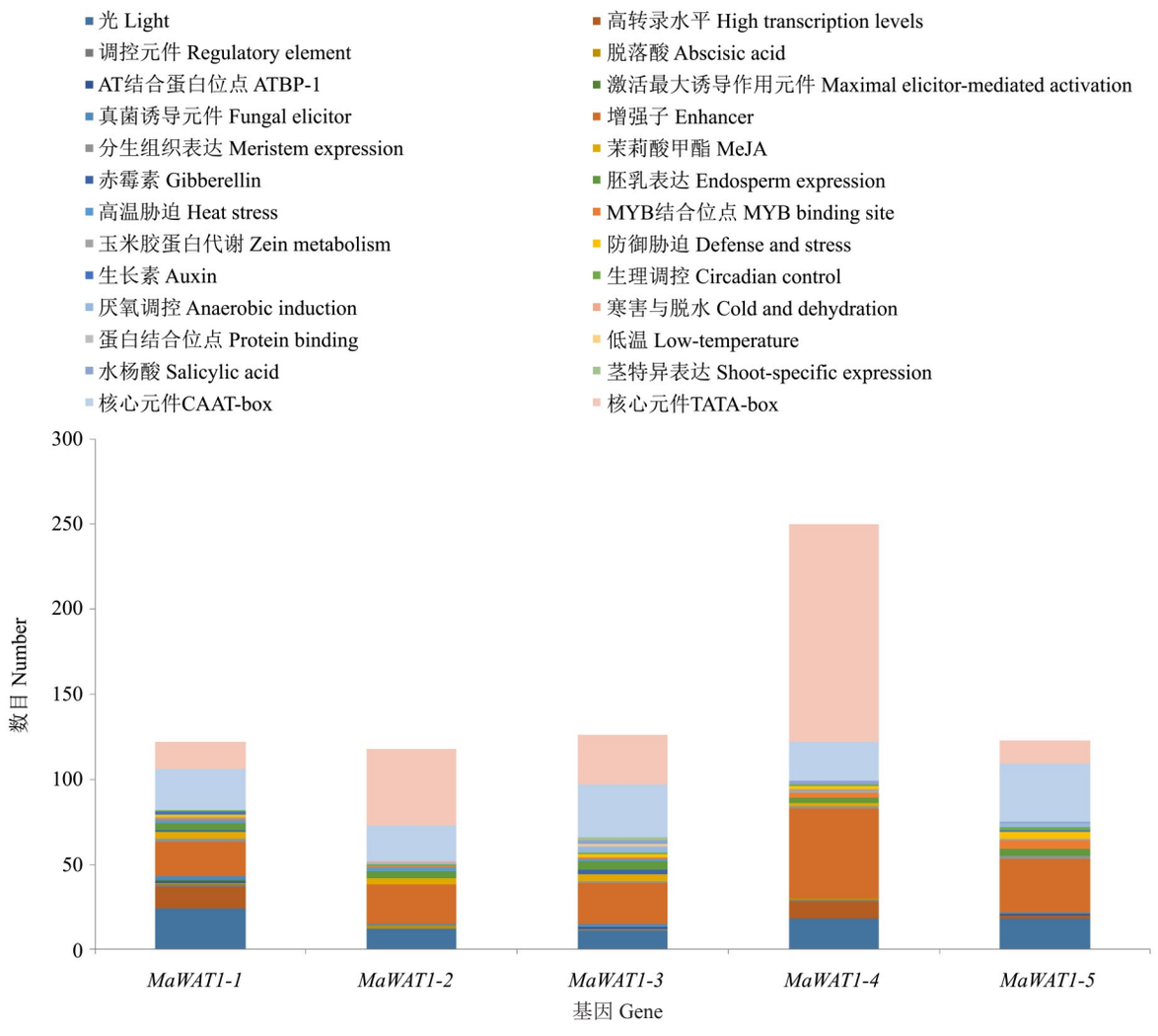


图6 香蕉*WAT1*基因各成员启动子顺式作用元件种类和个数

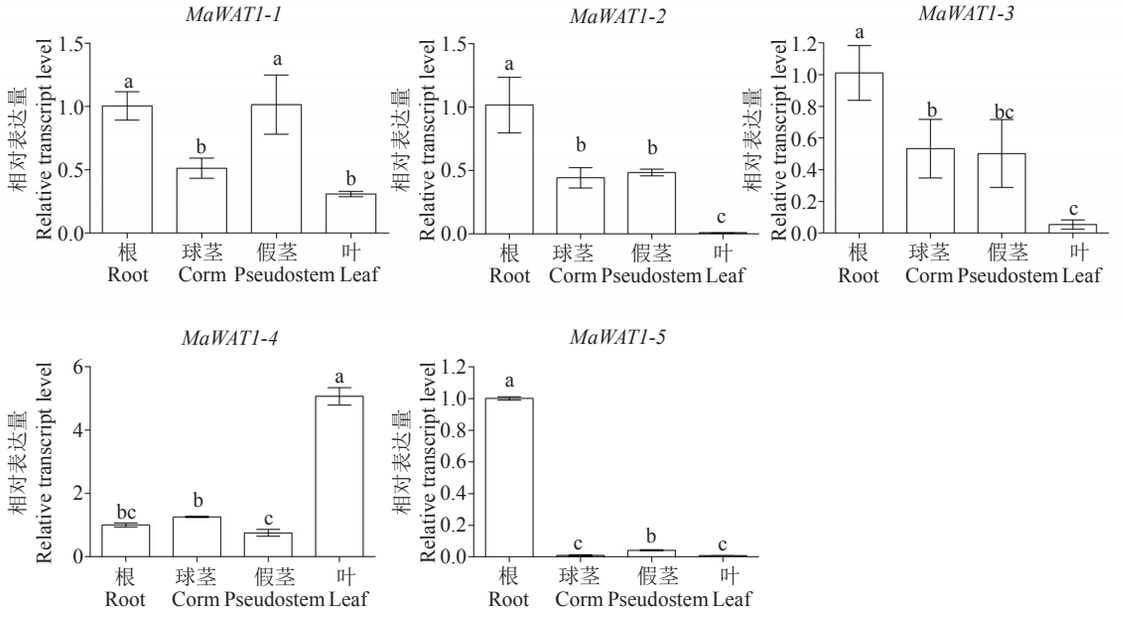
Fig. 6 Types and numbers of *cis*-acting elements in promoters of banana *WAT1* genes

2.6 *MaWAT1s*在不同组织器官中的表达情况

比较了 *MaWAT1s* 基因在不同组织部位中的相对表达情况(图 7),发现 *MaWAT1-1* 在根和假茎中表达水平接近且表达量最高,在球茎和叶中的表达量分别约为根的 51.0%和 30.6%。*MaWAT1-2* 和 *MaWAT1-3* 在根中的表达量最高,其次为假茎和球茎,其在叶中表达量最低且显著低于其他部位(分别仅为根的 0.9%和 5.3%)。*MaWAT1-4* 在叶中的表达最高,分别是根、球茎和假茎的 5.1、4.0 和 6.7 倍。*MaWAT1-5* 在根中的表达量最高且显著高于其他部位,球茎、假茎和叶中该基因的表达量仅为根的 0.9%、4.2%和 0.9%。

2.7 *MaWAT1s*在不同激素处理下的表达情况

qRT-PCR 结果(图 8)显示,在不同激素处理下,*MaWAT1s* 的表达模式差异较大。ABA 处理下,*MaWAT1-1* 和 *MaWAT1-4* 相对表达量呈波动变化,*MaWAT1-1* 在处理 8 h 和 24 h 时的表达量显著高于对照,而在其他时间点均显著低于对照;*MaWAT1-4* 在 24 h 后表达量最高且显著高于对照,但在 4 h 和 12 h 时的表达量显著低于对照;*MaWAT1-2* 在处理后的表达模式呈“升-降”的趋势且均显著高于对照,在处理后 12 h 时的表达量最高;*MaWAT1-3* 的表达在处理 48 h 时显著高于对照,但其余时间点均显著低于对照;*MaWAT1-5* 的表达在 ABA 处理后呈‘降-升’的趋势,4 h 时显著低于对照,但在 48 h 时显著高于对照。GA_3处理下,*MaWAT1-1* 在处理 4 h、24 h 和 48 h 时的表达显著高于对照,48 h 时的表达量最高,约为对照的 6.7 倍;*MaWAT1-2* 在处理 12 h、24 h 和 48 h 时的表达显著高于对照,12 h 时的表达量最高,为对照的 54.0 倍;*MaWAT1-3* 的表达呈先降后升的趋势,但均低于对照;*MaWAT1-4* 的表达呈‘升-降-升’的变化趋势,且在处理 24 h 时的表达量显著

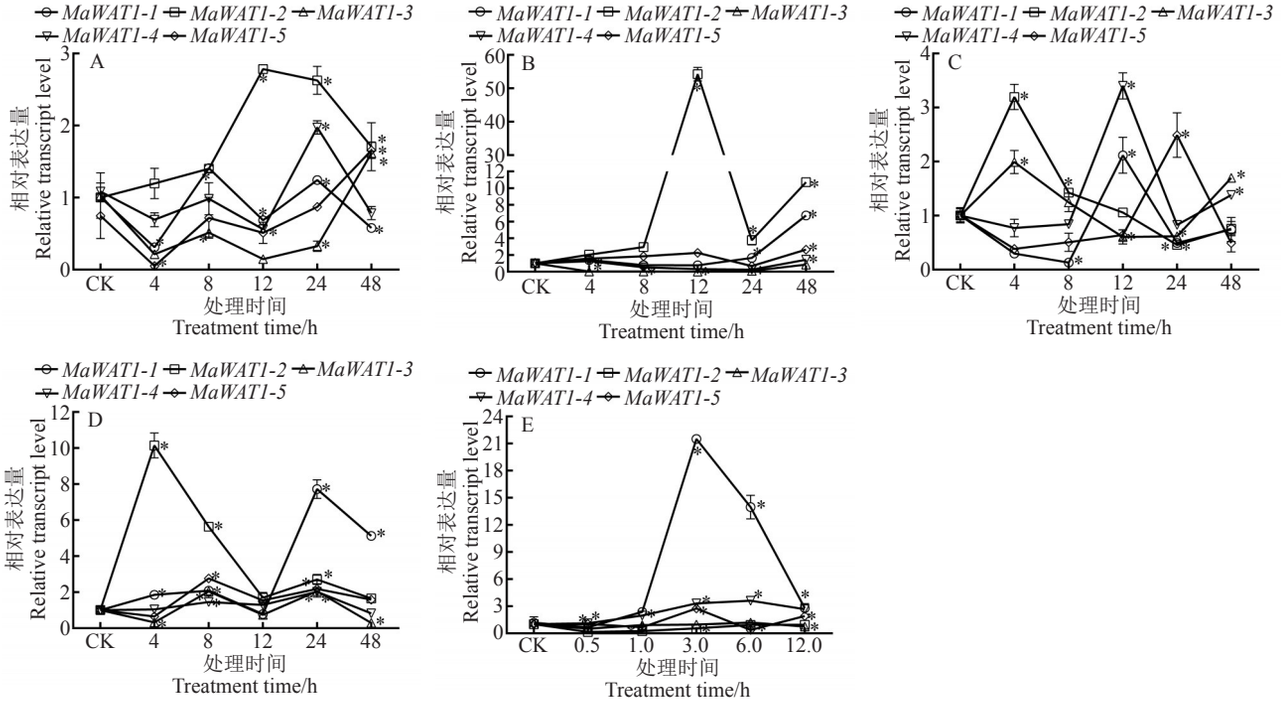


不同小写字母代表在 p <0.05 差异显著。下图同。

Different small letters indicate significant difference at 0.05 level. The same for the following figures.

图7　*MaWAT1* 基因在不同组织器官中的表达情况

Fig. 7　Relative expression of *MaWAT1* genes in different tissues and organs



A~E 分别为 ABA、GA_3、MeJA、SA 和 IAA 处理。

A-E is ABA, GA_3, MeJA, SA and IAA treatment, respectively.

图8　*MaWAT1s* 基因在5种激素处理下的表达模式

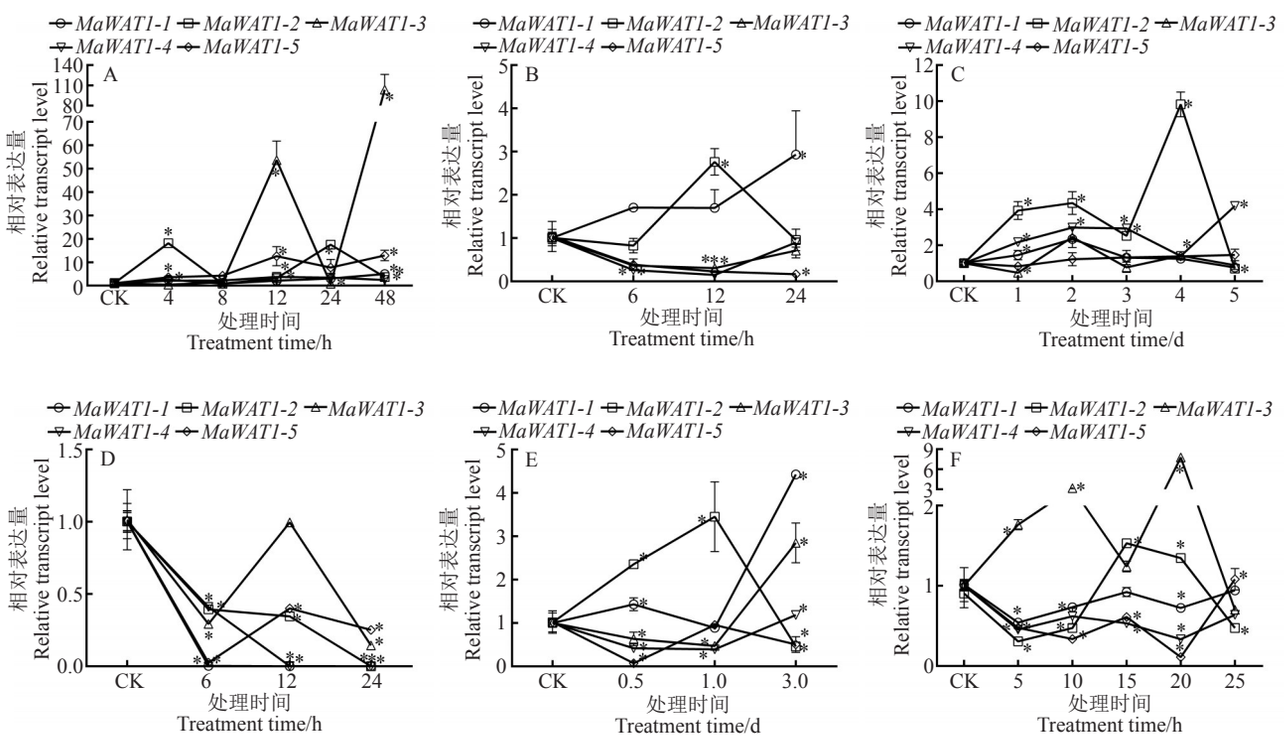
Fig. 8　Expression patterns of *MaWAT1* genes under five phytohormone treatments

低于对照；在处理前期具有一定诱导作用，在处理8~24 h之间具有显著抑制作用，在48 h具有显著诱导作用；*MaWAT1-5* 的表达除在处理24 h时显著低于对照外，其余均高于对照。MeJA处理下，*MaWAT1-1* 的表达呈‘降-升-降-升’的变化趋势，在处理后12 h的表达量显著高于对照，其余时间点均

低于或显著低于对照；*MaWAT1-2* 和 *MaWAT1-3* 均呈‘升-降-升’的变化趋势，且在处理 4 h 时的表达量均最高且显著高于对照；*MaWAT1-4* 在处理 12 h 和 48 h 的表达量显著高于对照，12 h 时的表达量最高；*MaWAT1-5* 在处理 24 h 的表达量显著高于对照，其余时间点与对照差异不显著。SA 处理下，除 *MaWAT1-3* 在处理 4 h 和 48 h 时显著低于对照外，其余 *MaWAT1s* 的表达大多显著高于对照，尤其是 *MaWAT1-1* 和 *MaWAT1-2* 表达量最高时分别是对照的 7.7 倍和 10.0 倍。IAA 处理下，*MaWAT1-1* 的表达呈‘降-升-降’的变化趋势，在处理 3 h 和 6 h 的表达量分别为对照的 18.2 倍和 11.8 倍；*MaWAT1-2* 和 *MaWAT1-3* 的表达分别呈‘降-升’和‘降-升-降’的变化趋势；*MaWAT1-4* 的表达呈‘升-降’的变化趋势，在处理 1 h、3 h、6 h 和 12 h 时的表达量均显著高于对照；*MaWAT1-5* 的表达在处理 3 h 和 12 h 的表达量显著高于对照，但在 6 h 时显著低于对照。

2.8 *MaWAT1* 基因在不同逆境处理下的表达情况

qRT-PCR 结果（图 9）显示：盐胁迫下，*MaWAT1s* 的表达均受盐胁迫诱导显著，且上调倍数较大，如：*MaWAT1-1* 在处理 48 h 时的表达量约为对照的 4.8 倍，*MaWAT1-2* 在处理 4 h 和 48 h 的表达量分别为对照的 15.9 倍和 15.4 倍；*MaWAT1-3* 在处理 48 h 的表达更是约为对照的 103.6 倍。低温处理下，*MaWAT1-1* 和 *MaWAT1-2* 分别在处理 24 h 和 12 h 的表达显著高于对照，分别为对照的 2.9 倍和 2.7 倍；而 *MaWAT1-3*、*MaWAT1-4* 和 *MaWAT1-5* 的表达则均受低温抑制。干旱胁迫下，除 *MaWAT1-5* 表达变化不显著外，其余 4 个 *MaWAT1s* 的表达大多受干旱胁迫诱导，如：*MaWAT1-2* 在干旱处理 4 d 后的表达量约为对照的 9.7 倍。38 ℃高温胁迫下所有 *MaWAT1s* 的表达均受到显著抑制。机械损伤处理下，*MaWAT1-1*、*MaWAT1-2* 和 *MaWAT1-3* 的表达受到不同程度的诱导；*MaWAT1-4* 的表达呈‘降-升’的变化趋势，处理 0.5 d 和 1 d 时显著低于对照，处理 3 d 时显著高于对照；*MaWAT1-5* 的表达均低于对照，在处理 0.5d 显著低于对照，仅约为对照的 7.1%。枯萎病处理下，*MaWAT1-1*、*MaWAT1-4* 和 *MaWAT1-5* 的表达受到显著抑；*MaWAT1-2* 的表达呈‘降-升-降’的变化趋势，在处理后 5 h、10 h 和 25



A~F 分别为盐胁迫、低温、干旱、高温、机械损伤和枯萎病处理。

A-F is salt, low temperature, drought, high temperature, wounding and Fusarium wilt treatment, respectively.

图9 *MaWAT1s* 基因在不同胁迫处理下的表达情况

Fig. 9 Expression of *MaWAT1* genes under different stress treatments

h的表达量显著低于对照，但在15 h和20 h时显著高于对照；*MaWAT1-3*的表达则呈‘升-降-升-降’的变化趋势，且在处理后5 h、10 h和20 h的表达量显著高于对照。

3　讨　论

本研究以拟南芥*WAT1*（At1g75500）为参考序列，利用本地Blast从香蕉基因组鉴定获得5条香蕉*WAT1*基因（*MaWAT1-1*~5），比较了它们的核苷酸、启动子和编码蛋白的序列特性，同时研究了它们在不同组织器官和不同逆境处理下的表达情况。

3.1　*MaWAT1s*编码蛋白均具有保守的EamA结构域，但它们的功能存在一定差异

5条*MaWAT1s*基因编码蛋白均含有10个跨膜结构域，其中2个为MtN21家族蛋白典型结构域EamA，长度分别为141 aa和144 aa，占比大于总蛋白的70%。该结构域命名来源于对大肠杆菌中氨基酸输出具有药物或代谢产物转运结构域的蛋白[28-29]，在植物活性物质运输中发挥着重要作用[30]。含有EamA结构域的蛋白可能具有内在膜蛋白的生物特性[31-32]。暗示该结构域对于MaWAT1s的功能至关重要。

基因结构和系统进化树分析结果均将*MaWAT1s*分为两组：一组包括*MaWAT1-1*、*MaWAT1-2*和*MaWAT1-4*，与拟南芥*WAT1*（At1g75500）相似度均高于64%，含有6个外显子和5个内含子，编码蛋白长380 aa左右，编码蛋白主要定位在液泡和细胞膜上；另一组包含*MaWAT1-3*和*MaWAT1-5*，与拟南芥*WAT1*（At1g75500）相似度分别为57.1%和55.2%，均具有7个外显子和6个内含子，编码蛋白长360 aa左右，*MaWAT1-3*主要定位在细胞质和细胞膜，*MaWAT1-5*主要定位在细胞膜和叶绿体上。qRT-PCR结果显示*MaWAT1-4*在香蕉叶片中的表达量最高，*MaWAT1-1*在香蕉根和假茎中均有较高的表达量，而其他三个成员均在根中的表达量最高。以上结果表明*MaWAT1s*在组织器官水平和亚细胞水平的表达均存在差异，说明它们的功能可能会有所不同[33-34]，同时也说明基因结构对基因编码蛋白的进化和功能影响较大[26,35]。

3.2　*MaWAT1s*广泛参与香蕉激素和逆境应答反应

拟南芥WAT1被证实是生长素转运蛋白[16,36-37]，其蛋白活性影响生长素的动态平衡[38]。拟南芥*WAT1*突变体茎部纤维次生细胞壁厚度降低、茎高降低、茎中生长素含量减少及转运受阻，而外源生长素处理可以使拟南芥*WAT1*突变体生长素运输及次生壁恢复到正常水平[16]。本研究发现：IAA处理下，*MaWAT1-1*在处理3 h和6 h时的表达量分别为对照的18.2倍和11.8倍；*MaWAT1-4*在IAA处理1 h、3 h、6 h和12 h时的表达量均显著高于对照；*MaWAT1-5*的表达在处理3 h和12 h的表达量显著高于对照。说明它们可能与拟南芥*WAT1*一样参与生长素运输[16]。

Denancé等[15]的研究表明，SA可以诱导拟南芥*WAT1*的表达。本研究中SA处理对*MaWAT1s*的表达均表现出一定的诱导作用，启动子上存在SA响应元件的*MaWAT1-3~5*的表达均在某些时间点受到SA处理的显著诱导。然而启动子上不含该元件的*MaWAT1-1*和*MaWAT1-2*的表达受SA的诱导程度更高，最高时分别是对照的7.7倍和10.0倍，这可能与它们的启动子上存在有大量的抗逆防御相关元件有关，同时也说明所有*MaWAT1s*均在香蕉SA信号传导过程中发挥作用[15]。

研究显示拟南芥和棉花*WAT1*基因的下调表达或不表达有助于提高植株抗病性[15, 18]。本研究中在枯萎病处理下，除*MaWAT1-2*和*MaWAT1-3*在一些时间点的表达量显著高于对照外，其余均低于甚至显著低于对照。表明*MaWAT1s*表达量的降低可能有助于提高香蕉对枯萎病的抗性。

*MaWAT1s*启动子存在大量的激素响应相关元件，qRT-PCR结果也显示*MaWAT1s*的表达受多种激素处理影响显著。此外，*MaWAT1s*的启动子激素应答相关顺式作用元件类型和数目存在一定差异。*MaWAT1-1*、*MaWAT1-2*和*MaWAT1-4*启动子上存在ABA响应元件，它们的表达在一定程度上受ABA诱导，*MaWAT1-2*在ABA处理后所有时间点的表达量均高于对照。*MaWAT1-1*、*MaWAT1-2*和*MaWAT1-3*启动子上存在GA_3响应元件，*MaWAT1-1*在GA_3处理48 h时的表达量为对照的6.7倍，*MaWAT1-2*在GA_3处理12 h时的表达量约为对照的54.0倍。说明*MaWAT1s*在不同激素处理下的表达模式与启动子顺式作用元件类型存在一定相关性[23]。

SA是植物抵御非生物逆境和生物逆境的重要调控因子[39-40]。前期的研究发现WAT1参与植物

SA 代谢的调控[15]，暗示其在植物非生物逆境反应中也可能起到重要作用。本研究表明 *MaWAT1s* 启动子上存在大量逆境响应（干旱、低温、高温、厌氧、真菌诱导、防御胁迫等）元件，且它们的表达受多种逆境处理影响显著。盐胁迫下，MaWAT1s 的表达显著上调，且上调倍数较大；干旱胁迫下，*MaWAT1-1~4* 的表达大多上调。38 ℃高温胁迫下所有 *MaWAT1s* 的表达均受到显著抑制；低温和机械损伤对 *MaWAT1s* 的表达也存在显著影响。以上结果表明 *MaWAT1s* 在香蕉响应非生物逆境过程中也扮演着重要角色。

4 结 论

MaWAT1s 成员间存在一定的进化和功能差异，基因结构差异和启动子顺式作用元件类型和数目的差异可能与这些差异息息相关。*MaWAT1s* 的表达受多种植物激素和逆境影响显著，说明它们在香蕉生长发育和抗逆防御反应中均发挥着重要作用。

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