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无核白及红地球葡萄*VvMADS46*基因的克隆及其无核调控功能分析

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摘 要:【目的】探究*VvMADS46*与葡萄无核性状产生的关系。**【方法】**采用实时荧光定量PCR分析*VvMADS46*在葡萄不同组织器官的表达,分析克隆*VvMADS46*基因序列,构建系统发育树,进行*VvMADS46*基因的亚细胞定位分析及过量表达番茄性状观察,研究其功能。**【结果】***VvMADS46*在无核白和红地球的根、茎、叶、花序、花、卷须、果实等不同组织中均有表达,在花中的表达量较高,在果实中的表达量次之,而在茎、叶和卷须中的表达量较低;花后33、36、39、42 d 4个时期无核葡萄无核白、火焰无核*VvMADS46*的表达量都显著高于有核葡萄红地球、巨峰;构建了*VvMADS46*基因的亚细胞定位载体,通过农杆菌侵染洋葱,发现了*VvMADS46*定位在细胞核上;克隆了*VvMADS46*基因,在NCBI数据库进行序列比对,得到其同源基因为*AP3*基因,筛选得到AtAP3、AeAP3、CmAP3、LjAP3、LrAP3、PhAP3、AcAP3和PtAP3,并构建了系统发育树;构建了*VvMADS46*基因的植物过量表达载体,采用农杆菌介导法转化模式植物番茄,发现转基因番茄植株明显矮小且种子显著变小。**【结论】***VvMADS46*与葡萄胚珠败育、无核性状发生存在密切联系。

关键词:葡萄;*VvMADS46*;基因克隆;无核调控

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Cloning and function analysis of *VvMADS46* in Red Globe and Thompson Seedless for seedless regulation

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Abstract:【Objective】Grapevine (*Vitis vinifera* L.) as one of the most economically important fruit crop is widely cultivated all over the world. Seedlessness of the fruit is one of the most desired traits for table grape consumers. The *MADS-box* genes, which generally comprise large families, encoded transcription factors (TFs) with a highly conserved MADS domain, and played diverse roles in plant embryo development, flowering time, floral meristem, and fruit ripening regulation. In this study, *VvMADS46* was expressed in the different grape cultivated species tissues and ovule developmental stages. Gene cloning, sequence alignment and overexpression in the Micro-Tom tomato were analyzed for *VvMADS46* gene related grape seedless function identification, in order to provide foundation for further research of *VvMADS46* participating in regulation of seedless grapes.【Methods】Grape tissues and organs, including roots, stems, leaves, tendrils, inflorescences, flowers in the fully opening stage and fruits on 33 days after flowering (DAF) were harvested from the seedless grape Thompson Seedless and the seeded grape

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Red Globe, respectively. Ovules were collected from the two seeded grape cultivars (Kyoho and Red Globe) and the two seedless grape cultivars (Thompson Seedless and Flame Seedless) on 33, 36, 39 and 42 DAF. Total RNA extraction and first strand cDNA synthesis were accomplished using EZNA Plant RNA Kit (R6827-01, Omega Bio-tek, USA) and PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa Biotechnology, Dalian, China), respectively. *VvMADS46* expression in the different grape tissues and organs was made by quantitative real-time PCR. *VvMADS46* cDNA sequences, alignment and phylogenetic tree were analyzed by homologous clone. The sub-cellular localization and the overexpression of *VvMADS46* were analyzed in the onion and the model plant Micro-Tom tomato.【Results】*VvMADS46* was expressed in all the different tissues and organs of the Thompson Seedless and the Red Globe. Obviously, there was no significant difference in the roots, stems, leaves and tendrils between the seedless and seeded grapes, but the expression of *VvMADS46* in the inflorescences, flowers and fruits were significantly higher than those in four vegetative growth organs, also higher in the Thompson Seedless than that in the Red Globe. In four researched grape ovule development stages, the expression levels of *VvMADS46* on 33 and 36 DAF were higher than those on 39 and 42 DAF. Additionally, the expression levels of *VvMADS46* in the seedless grape Thompson Seedless and Flame Seedless were significantly higher than those the in seeded grape Red Globe and Kyoho in all these four stages. The full-length cDNA sequence of *VvMADS46* was 681 bp, encoding 226 amino acids, with a complete MADS conservative structure. The sequence alignment showed that mRNA sequences and amino acid sequences of *VvMADS46* cDNA in the Thompson Seedless and Red Globe were similar to the Pinot Noir (cv. PN40024), with 99.8% and 99.1% similarity, respectively. According to the homologous alignment, 8 AP3 proteins (AtAP3, AeAP3, CmAP3, LjAP3, LrAP3, PhAP3, AcAP3 and PtAP3) were screened from the NCBI database, the phylogenetic tree was constructed using MEGA 5.0. Additionally, the subcellular localization vector PBI221-*VvMADS46*-GFP and the overexpression vector pCAMBIA2300-*VvMADS46* were constructed, following *Agrobacterium tumefaciens* mediated infection in the onion surface cells and transformation into the Micro-Tom tomatoes, respectively. GFP signaling was observed by the inverted fluorescence microscope and localized in the grape nucleus. The growth and development phenomenon of the overexpression tomatoes, the empty vector transgenic tomatoes and the wild type tomato lines were identified and compared on more than 30 DAF with the same condition. The empty vector transgenic tomatoes and the wild type tomato lines had no significantly growth phenomenon difference, however, the height of the *VvMADS46* overexpression tomatoes were significantly shorter than that of the empty vector transgenic tomatoes and the wild type. Moreover, the fruit size and seed number of the empty vector transgenic tomato lines and the wild type tomato fruits showed no significantly difference, but the fruit size of the *VvMADS46* overexpression tomatoes was significantly smaller than that of the empty vector transgenic tomatoes and the wild type, also the seed number was significantly decreased, with nearly no healthy and plump seeds in the *VvMADS46* overexpression tomato lines. All these researches suggested that a possible function of *VvMADS46* in development and formation of seedless fruits in grape.【Conclusion】*VvMADS46* was a reproductive growth organ induction gene in grapevine, and possibly played an important role in grape seedless character regulation through inhibiting the ovule development.

Key words: Grapevine; *VvMADS46*; Gene cloning; Seedless regulation

葡萄（*Vitis vinifera* L.）是一种重要的经济果树，在国内外栽培面积很广。无核葡萄具有食用方便、口味极佳、营养丰富等优点，深受消费者青睐，在鲜食、制干方面具有广阔的市场[1]。近年来无核葡萄在世界鲜食葡萄中消费量有逐年上升的趋势，世界各葡萄生产大国也逐渐加强对无核葡萄的研究与开发[2]，我国自20世纪50年代开始，多个科研院所开始鲜食无核优质葡萄育种及调控机制等研究工作[3]，开展葡萄无核基因发掘及其功能研究具有重要理论指导及产业发展意义。

多年来，葡萄无核调控基因水平的研究仍然相对较少，Hanania等[4]从无核及有核Thompson株系中筛选得到差异表达基因*Chloroplast Chaperonin 21*（*ch-Cpn21*），本氏烟草和番茄中*ch-Cpn21*的抑制表达显著影响种子的生长；Costenaro-da-Silva等[5]分析鉴定了无核葡萄结果后不同生长时期关键调控基因的差异表达，发现*VvRIP1*通过ABA信号途径参与Sultanine种子及果实成熟调控，在结果后4周高水平表达，*ch-Cpn21(VvCPN21)*在胚乳发育的关键时期（DS0−DS4）表达量特别低，导致Sultanine无核化现象的发生，与Hanania等[4]的研究结果一致，*ch-Cpn21*基因的抑制表达可以有效促使无核葡萄种子败育现象的发生；*VvCBP*同源沉默的番茄植株果实中种子的数量显著减少，说明*VvCBP*正向调控葡萄种子的发育[6]；*VvAGL11*基因的SNPs和INDELs变异可以促使葡萄种子和果实质量表现多样性，说明*VvAGL11*与葡萄无核调控存在一定关系[7]。

研究表明，MADS-box类植物转录因子与葡萄的花器官调控及无核性状的发生存在密切的联系[7-9]。欧洲葡萄赤霞珠中AP3（LOC：NP_001267960）可以参与雄蕊和花瓣等生殖器官的发育过程[8]，此外，黑比诺中54个MADS-box基因得到鉴定，赤霉素处理后MADS-box家族基因存在差异表达，说明赤霉素处理可以诱导MADS-box家族基因参与的葡萄无核调控，*VvMADS28*和*VvMADS39*在有核葡萄（红地球、巨峰、红乳）和无核葡萄（无核寒香蜜、昆香无核、无核白）胚珠发育过程中存在极显著差异表达，这也间接反映了这2个MADS-box基因与葡萄无核调控存在关系[9]。

本研究正是在课题组前期有核和无核葡萄杂交后代转录组研究分析[10]及葡萄MADS-box转录因子家族研究[9]的基础上，从无核白和红地球中克隆*VvMADS46*基因，并在番茄中过量表达，以探究*VvMADS46*基因参与的葡萄无核调控，为深入了解MADS-box转录因子参与的葡萄无核调控机制奠定基础，同时也为葡萄无核性状改良提供理论依据。

1　材料和方法

1.1　材料

巨峰、红地球、无核白和火焰无核葡萄材料均保存在西北农林科技大学园艺场葡萄种质资源圃，Micro-Tom番茄野生型材料保存于课题组实验室。

1.2　方法

1.2.1　*VvMADS46*在7个组织器官、4个葡萄品种胚珠发育关键时期的表达分析　通过E.Z.N.A.® Plant RNA Kit试剂盒提取红地球及无核白葡萄根、茎、叶、花序、花、卷须、果实7个不同器官及红地球、巨峰、无核白、火焰无核4个葡萄材料胚珠不同发育时期的RNA，利用TaKaRa试剂盒反转录得到cDNA，通过美国伯乐Bio-Rad iQ5定量PCR仪分析*VvMADS46*在不同组织器官和不同葡萄品种胚珠发育不同时期的表达情况，内参基因为*Actin1*（GeneBank登录号：AY680701），所有试验都进行3次生物学及技术重复。

1.2.2　*VvMADS46*基因的克隆及序列分析　以1.2.1中提取有核葡萄材料红地球及无核葡萄材料无核白的cDNA为模板，设计基因克隆引物（F：GCTCTAGAATGGCTAGAGGAAAGATTGAGATCA，R：GGGGTACCCTACTCGAGCAAAGTAAAGGTCAAA）克隆得到*VvMADS46* cDNA序列；通过氨基酸序列分析，得到VvMADS46的保守结构信息；在NCBI数据库中筛选得到拟南芥（*Arabidopsis thaliana*，AF115814）、毛花猕猴桃（*Actinidia eriantha*，HQ1133591）、杭菊（*Chrysanthemum* × *morifolium*，AAO22985）、百脉根（*Lotus japonicus*，AAX13301）、百合（*Lilium regale*, BAB91550）、矮牵牛（*Petunia* × *hybrida*, X69946）、中华猕猴桃（*Actinidia chinensis*，HQ113358）和毛白杨（*Populus tomentosa*，AY3596061）中VvMADS46的同源蛋白，利用ClustalX进行序列比对分析，构建VvMADS46在不同物种间的系统发育树。

1.2.3　*VvMADS46*基因过量表达及亚细胞定位载体的构建　对课题组保存的pCAMBIA2300、PBI221-GFP载体及含有目的基因*VvMADS46*的T-easy重组

质粒载体进行*Xba* Ⅰ和*Kpn* Ⅰ限制性内切酶双酶切，通过电泳检测、产物回收获得目的基因及各载体片段，经DNA连接酶连接，鉴定获得VvMADS46过表达重组载体pCAMBIA2300-*VvMADS46*及亚细胞定位重组载体PBI221-*VvMADS46*-GFP，并转化土壤农杆菌GV3101。

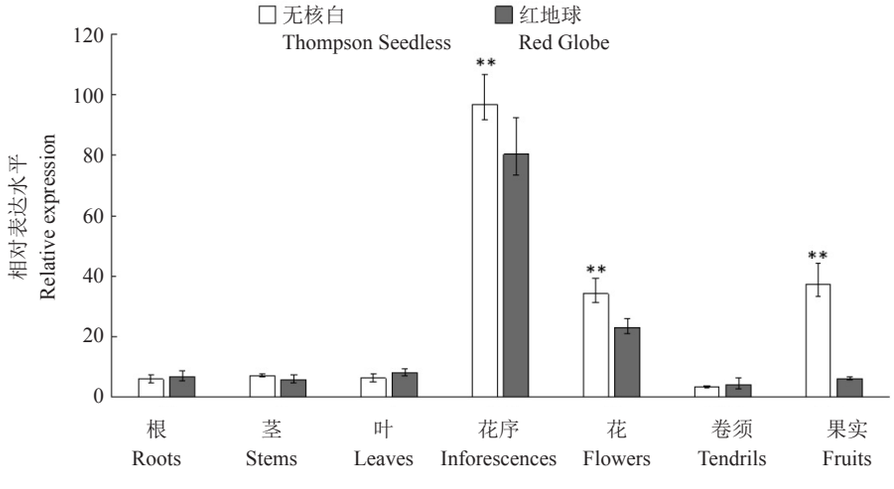
1.2.4 农杆菌介导的Micro-Tom番茄遗传转化及表型观察 使用土壤农杆菌介导法将*VvMADS46*过量表达载体转化Micro-Tom番茄[11]，通过卡那霉素抗性筛选获得阳性株系，并从得到的阳性株系上摘取叶片提取DNA，以此为模板，通过*VvMADS46*克隆引物进行PCR扩增，分析鉴定已获得转基因阳性株系是否真实转入目的基因片段，淘汰假阳性株系。筛选含有*VvMADS46*目的基因的番茄株系，在整个生长周期，观察比较其与野生型及转化pCAMBIA2300空载体的转基因番茄株系生长状态及果实发育阶段果实种子的差异。

1.2.5 *VvMADS46*的亚细胞定位分析 选取肉质厚的白色洋葱，根部在水中浸泡1 d，剥去外皮置于70%乙醇消毒10 min，用无菌水清洗4~5次，然后用手术刀在洋葱表皮划出方块撕下内表皮，置于1/2MS培养基中黑暗处理，28 ℃培养箱预培养1 d。将已活化的含有PBI221-GFP对照载体及PBI221-*VvMADS46*-GFP重组载体的GV3101土壤农杆菌菌液倒入预培养洋葱表皮的培养基中，浸泡30 min，用滤纸擦干，转入新的1/2MS培养基中，于25 ℃培养箱中培养2 d，取出培养后的洋葱表皮，制片于倒置荧光显微镜下，观察绿色荧光的分布。

2 结果与分析

2.1 *VvMADS46*基因在无核白和红地球葡萄不同组织器官的表达分析

基因在不同组织器官的选择性表达可以反映基因在特异植物组织器官生长发育过程中的作用，笔者采用实时荧光定量PCR方法分析了*VvMADS46*基因在无核白和红地球不同组织器官内的表达情况（图1）。无核白和红地球葡萄根、茎、叶、卷须4种器官中*VvMADS46*基因的表达量不存在显著性差异，而在花序、花、果实中*VvMADS46*基因表达量明显高于前4种营养生长的器官，且无核材料无核白中*VvMADS46*在花序、花及果实的表达量显著高于有核葡萄材料红地球。



*表示在 $p < 0.05$ 差异显著，**表示在 $p < 0.01$ 差异极显著。下同。

* indicates significant difference at $p < 0.05$, ** indicates extremely significant difference at $p < 0.01$. The same below.

图1 *VvMADS46*基因在无核白和红地球7个组织器官中的表达差异分析

Fig. 1 Differential expression analysis of *VvMADS46* in seven tissues of Thompson seedless and Red Globe

2.2 *VvMADS46*基因在不同葡萄品种胚珠发育不同时期的表达分析

葡萄无核性状的形成与胚珠败育存在密切的联系，笔者在本研究中分析了无核葡萄材料无核白、火焰无核以及有核葡萄材料红地球、巨峰花后33、36、39、42 d（胚珠败育前后4个关键时期）*VvMADS46*的表达情况，*VvMADS46*在花后33 d和36 d的表达量高于花后39 d和42 d，并且4个时期无核葡萄材料无核白、火焰无核*VvMADS46*的表达量均显著高于有核葡萄材料红地球、巨峰（图2）。

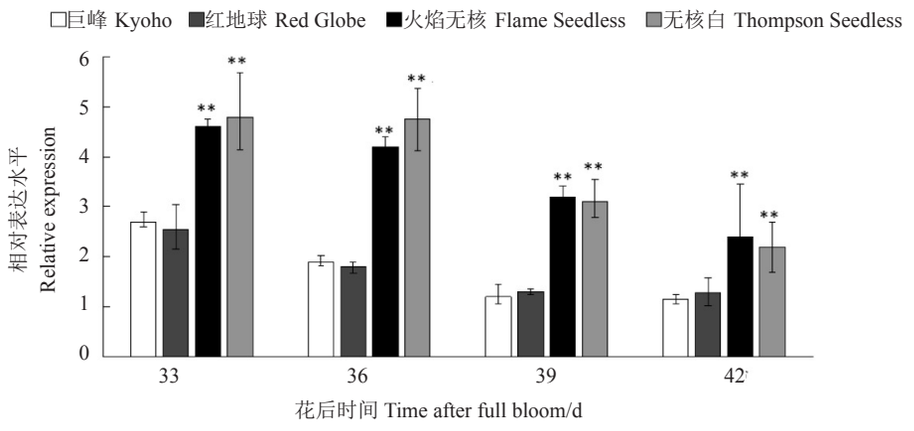


图 2　*VvMADS46* 基因在 4 个葡萄品种胚珠发育关键时期的表达差异分析

Fig. 2　Differential expression analysis of *VvMADS46* in four ovule development stages of four grape species

2.3　*VvMADS46* 基因的同源克隆与序列的相似性分析

采用同源克隆方法从无核白和红地球中扩增得到 *VvMADS46* 的 cDNA 序列，测序结果显示 *VvMADS46* 的 cDNA 序列全长为 681 bp，编码 226 个氨基酸，并具有完整的 MADS 保守结构（图 3）。经序列比对可以发现，*VvMADS46* 的 cDNA 及氨基酸序列在无核白、红地球中与葡萄基因组网站公布的黑比诺葡萄（PN40024）序列相似，分别具有 99.8% 和 99.1% 的序列相似率（图 4）。

2.4　*VvMADS46* 同源序列比对及系统发育树构建

笔者在本研究中依据 VvMADS46 克隆信息，在 NCBI 上序列比对，查阅相关文献，寻找到拟南芥（*Arabidopsis thaliana*，*AF115814*）、毛花猕猴桃（*Actinidia eriantha*，*HQ1133591*）、杭菊（*Chrysanthemum* × *morifolium*，*AAO22985*）、百脉根（*Lotus japonicus*，*AAX13301*）、百合（*Lilium regale*，*BAB91550*）、矮牵

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1    ATGGCTAGAGGAAAGATTGAGATCAAGAGGATAGAGAACTCGACGAACAGGCAGGTCACC
1     M  A  R  G  K  I  E  I  K  R  I  E  N  S  T  N  R  Q  V  T
61   TACTCCAAGAGACGAAATGGTATCTTCAAGAAGGCCAGTGAGCTCACTGTTCTTTGTGAT
21    Y  S  K  R  R  N  G  I  F  K  K  A  S  E  L  T  V  L  C  D
121  GCTAAGGTTTCTATCATCATGCTCTCCAGTACTGGAAAGCTCCATGAATACATCAGTCCT
41    A  K  V  S  I  I  M  L  S  S  T  G  K  L  H  E  Y  I  S  P
181  TCCACTACAACGAAACAAATATTTGATCAGTACCAGAACACTCTAGGAGTGGATCTATGG
61    S  T  T  T  K  Q  I  F  D  Q  Y  Q  N  T  L  G  V  D  L  W
241  AGCTATCACTATGAGAGAATGCAAGAAAACCTGAAGAAACTGAAAGATGTGAACAAGAAT
81    S  Y  H  Y  E  R  M  Q  E  N  L  K  K  L  K  D  V  N  K  N
301  CTCAGGAAGGAGATTAGGCAGAGGATGGGTGAACATTTGAGCGATTTGAGCGTTGAGGAA
101   L  R  K  E  I  R  Q  R  M  G  E  H  L  S  D  L  S  V  E  E
361  CTGCGAGATCTTGAACAAGAGATGGAGAGTTCTTTGAAGATGGTTCGTGATAGGAAGTAC
121   L  R  D  L  E  Q  E  M  E  S  S  L  K  M  V  R  G  R  K  Y
421  CAGGTGATCAATAATCAGATTGAAACTTTCAAGAAAAAGGTAAGGAATGTGGAACAAATA
141   Q  V  I  N  N  Q  I  E  T  F  K  K  K  V  R  N  V  E  Q  I
481  CACAAAAACCTCCTACATGAATTTGATGCAAGGGACAGAGATCAACACTATGGGCTAGTG
161   H  K  N  L  L  H  E  F  D  A  R  D  R  D  Q  H  Y  G  L  V
541  GACAATGGAGGGGATTACGAATCTGTTCTTGGATTCTCAAATGGAAGCTCTCCGGTATTT
181   D  N  G  G  D  Y  E  S  V  L  G  F  S  N  G  S  S  P  V  F
601  GCCCTAAGCTTGCAGCCTAACCCGCCTAATGATCTTCACTCGGGTGTGGGCTCTGATTTG
201   A  L  S  L  Q  P  N  P  P  N  D  L  H  S  G  V  G  S  D  L
661  ACCTTTACTTTGCTCGAGTAG
221   T  F  T  L  L  E  *
```

方形框 ATG、TAG 分别为起始、终止密码子，下划线部分代表 MADS 保守结构。

The boxed sequences indicate the start and the stop codon，The underlines indicate MADS like domain.

图 3　*VvMADS46* 的序列信息

Fig. 3　The sequences information of *VvMADS46*

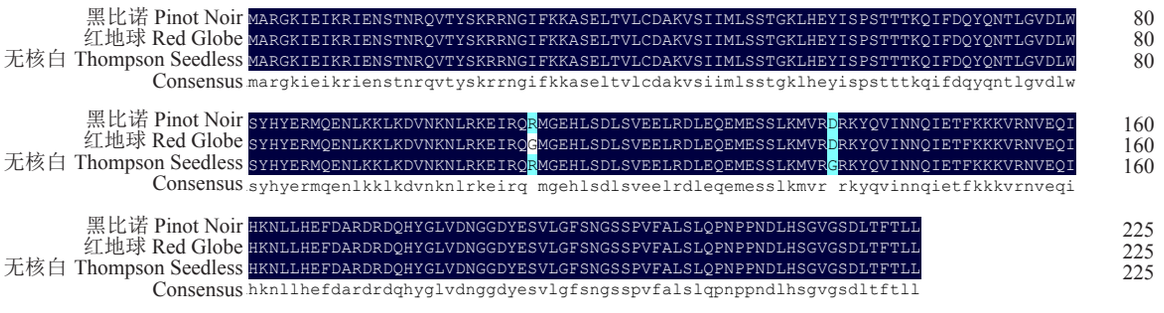


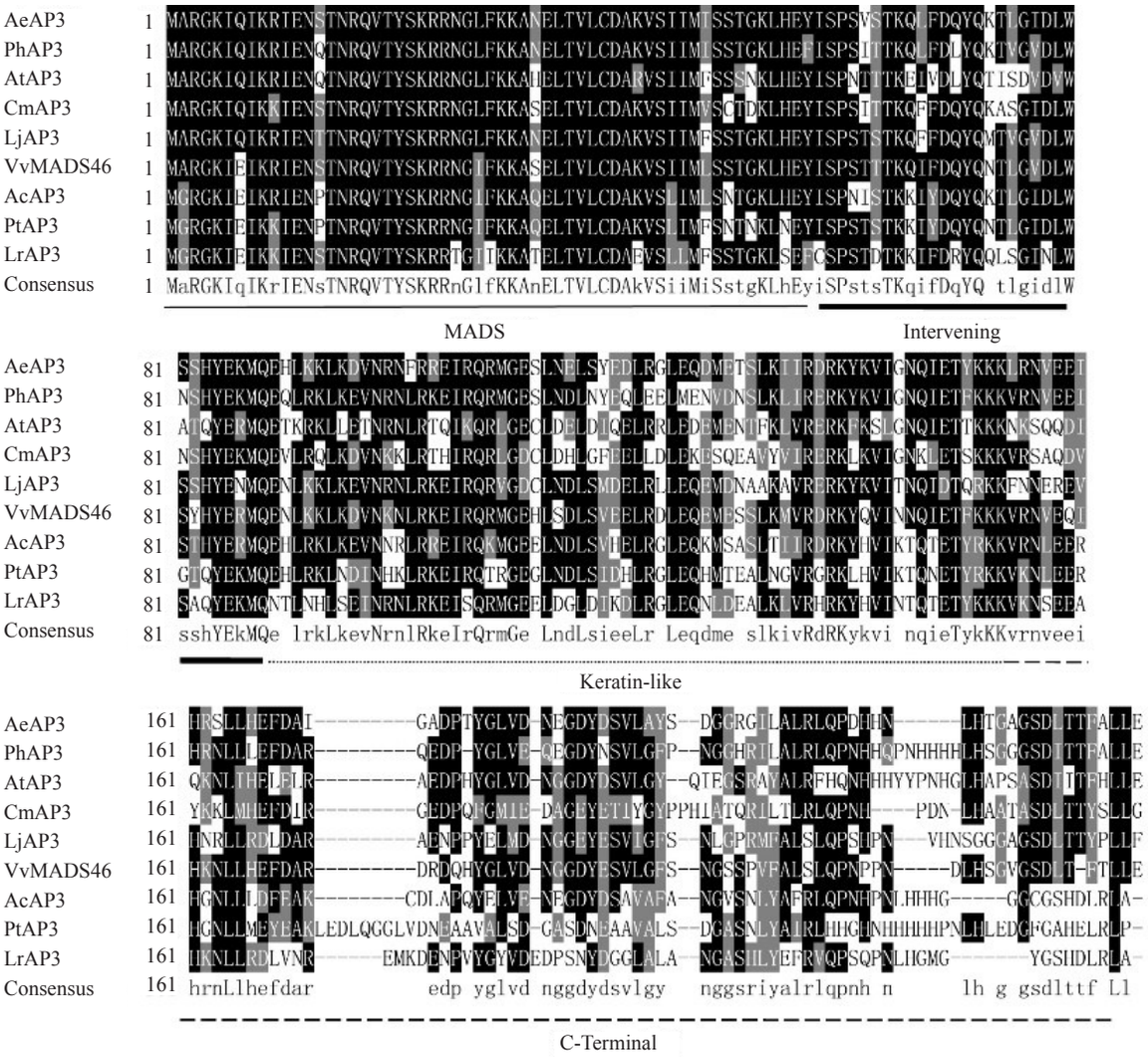
图 4　红地球、无核白与欧洲葡萄黑比诺 *VvMADS46* 氨基酸序列比对

Fig. 4　The amino acid sequences alignment of *VvMADS46* from Thompson Seedless, Red Globe and *Vitis vinifera* 'Pinot Noir'

牛(*Petunia* × *hybrida*,*X69946*)、中华猕猴桃(*Actinidia chinensis*,*HQ113358*)和毛白杨(*Populus tomentosa*,*AY3596061*)中的同源基因。通过Clustal X进行了序列比对,结果如图5所示,MADS、Intervening、Keratin-like及C-Terminal结构进行了对应标注,MADS-box区域相对来说保守性最强,并通过MegAlign 5.0构建了这9个氨基酸序列的系统发育树(图6)。

2.5 过量表达 *VvMADS46* 对番茄植株生长及果实发育的影响

将*VvMADS46*构建的过量表达载体转化Micro-Tom番茄,相同条件下培养至花后1个多月,果实转色成熟。观察比较pCMBIA2300 -*VvMADS46*过量表达载体、空载体转基因番茄及野生型番茄植株的生长情况,空载体转基因番茄和野生型番茄植株生长势不存在显著差异,但*VvMADS46*转基因植株株型明显矮小(图7);观察比较3种材料番茄成熟果实大小和种子数量,空载体转基因番茄和野生型番茄果实大小及种子数量无明显差别,但*VvMADS46*转基因番茄果实明显偏小且果实中基本没有健康饱满的种子,胚珠种子败育现象明显(图8)。



At. 拟南芥(AF115814);Ae. 毛花猕猴桃(HQ1133591);Cm. 杭菊(AAO22985);Lj. 百脉根(AAX13301);Lr. 百合(BAB91550);Ph. 矮牵牛(X69946);Ac. 中华猕猴桃(HQ113358);Pt. 毛白杨(AY3596061)。图 6 同。

At. *Arabidopsis thaliana* (AF115814); Ae. *Actinidia eriantha* (HQ1133591); Cm. *Chrysanthemum* × *morifolium* (AAO22985); Lj. *Lotus japonicas* (AAX13301); Lr. *Lilium regale* (BAB91550); Ph. *Petunia* × *hybrida*(X69946); Ac. *Actinidia chinensis* (HQ113358); Pt. *Populus tomentosa* (AY3596061). The same Fig. 6.

图 5 VvMADS46 与其他 9 个物种 AP3 蛋白同源序列比对分析

Fig. 5 The alignment of VvMADS46 with other nine homology AP3 proteins

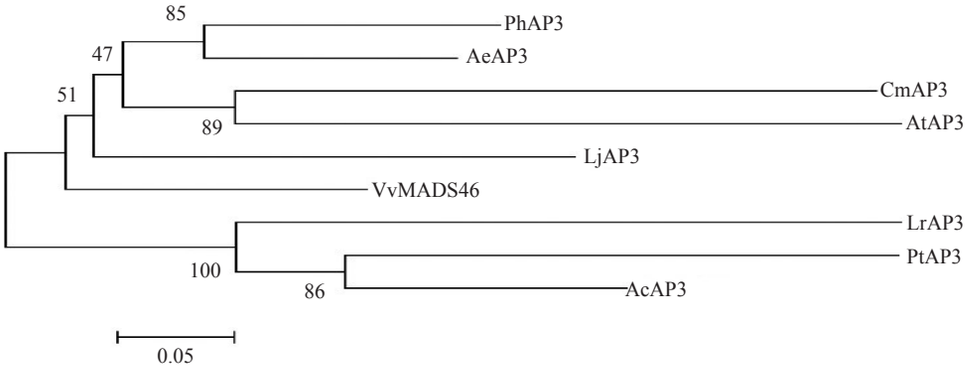


图 6　*VvMADS46* 与其他 9 个物种 AP3 同源蛋白系统发育树

Fig. 6　Phylogenetic tree analysis of *VvMADS46* with homology AP3 proteins in other nine plants

W.T. 野生表现型；E.T. 空载体转化表现型；O.T. *VvMADS46* 过量表达表现型。图 8 同。

W.T. Wild type; E.T. Empty vector type; O.T. *VvMADS46* overexpression type. The same Fig. 8.

图 7　*VvMADS46* 过量表达、空载体及野生型番茄植株及果实生长状况

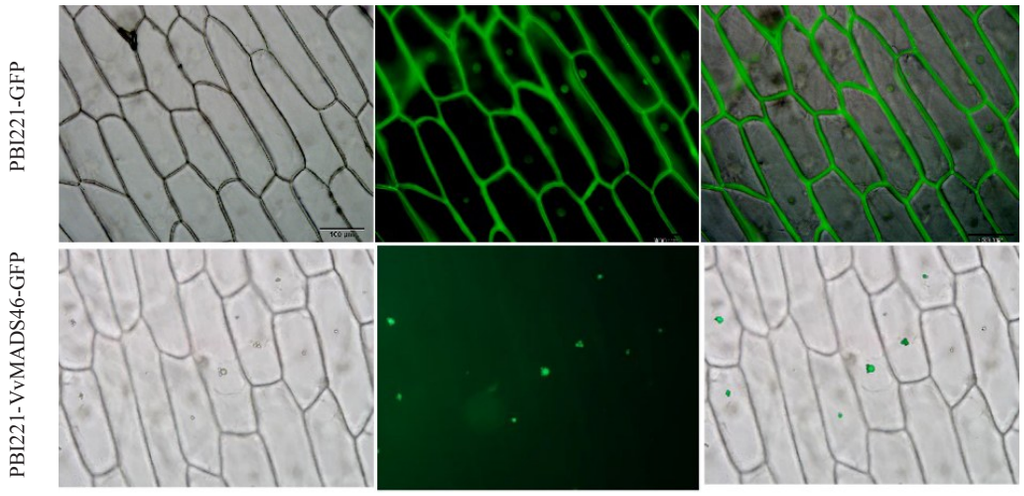
Fig. 7　Phenotype observation of *VvMADS46* overexpression and wild Miro-Tom tomato

图 8　*VvMADS46* 过量表达、空载体及野生型番茄植株种子发育观察

Fig. 8　Seed development observation of *VvMADS46* overexpression and wild Miro-Tom tomato

2.6 *VvMADS46*亚细胞定位

通过农杆菌转化洋葱表皮分析VvMADS46的蛋白定位信息。转化PBI221-*VvMADS46*-GFP后的洋葱表皮细胞，使用倒置荧光显微镜观察，显示GFP荧光信号主要集中并重叠在细胞核中，说明VvMADS46蛋白定位在植物细胞的细胞核中（图9）。



明场和GFP分别表示PBI221-*VvMADS46*-GFP及对照载体PBI221-GFP转化洋葱表皮细胞后明场视野和488 nm激发下GFP信号的观察结果，合并表示明场视野和GFP信号的融合结果。

Bright and GFP indicated bright field and 488 nm excited GFP signal of PBI221-*VvMADS46*-GFP and PBI221-GFP transgenic onion surface cells respectively; merged indicated the mergence of bright field and GFP signal.

图9 *VvMADS46*亚细胞定位观察

Fig. 9 The sub-cellular localization observation of *VvMADS46*

3 讨 论

MADS-box家族是指含有MADS结构域的一类转录因子，分成type Ⅰ（SRF-like）和type Ⅱ（MEF2-like）两大类，在真核生物中具有重要且广泛的功能[12-16]，大量研究表明，type Ⅱ MIKC^{C}类MADS-box基因可以参与植物胚发育的调控[17-19]，在拟南芥中发现的*AGL11*（*STK*）、*SHP1*（*AGL1*）及*SHP2*（*AGL5*）等胚珠身份基因均属于MADS-box家族基因[19]，矮牵牛MADS-box家族的*FBP7*（*FLORAL BINDING PROTEIN7*）和*FBP11*（*FLORAL BINDING PROTEIN11*）基因也被认定为胚珠发育调控基因[20-21]。

笔者分别在无核葡萄材料无核白和有核葡萄材料红地球中克隆了*VvMADS46*基因，探究了*VvMADS46*与无核白葡萄无核调控的关系，序列比对结果及系统发育树分析显示，无核白和红地球的*VvMADS46*都是葡萄*AP3*基因[8]，与其他物种的*AP3*基因高度相似，*AP3*基因在多个研究报道中已证实与植物花器官及胚正常发育存在密切联系[8,22]，预测*VvMADS46*可能参与无核葡萄胚珠的形成调控，导致无核白葡萄无核化现象的发生。

不同于*ch-Cpn21*及*VvCBP*等基因在无核葡萄胚珠败育关键时期出现显著负调控表达，从而影响胚珠及种子的生长，诱导葡萄无核现象的发生[4,6]，*VvMADS46*在无核葡萄无核白和火焰无核花后33、36、39、42 d（胚珠败育前后4个关键时期）表达量均显著高于有核葡萄红地球和巨峰，说明*VvMADS46*可以响应胚珠发育及花器官生长发育变化的诱导，并通过一系列的信号反应，表现出对葡萄胚珠发育的抑制，诱导无核现象的发生；*VvMADS46*基因在Micro-Tom番茄的过量表达，促使转基因番茄果实明显偏小且果实中基本没有健康饱满的种子，种子败育现象明显，这同样是对*VvMADS46*可以诱导无核葡萄胚珠败育、促进无核现象发生的有力验证，但是*VvMADS46*参与葡萄胚珠败育的具体上下游调控还有待进一步研究。

4 结 论

从无核白和红地球中克隆得到*VvMADS46*基

因，并通过进化同源分析、亚细胞定位及模式植物番茄中过量表达研究预测其功能，结果表明，*VvMADS46* 定位在细胞核内，并在无核葡萄胚珠发育的关键时期大量诱导表达，预测 *VvMADS46* 蛋白参与一系列调控反应，参与抑制葡萄胚珠细胞的正常发育，诱导葡萄胚珠败育及无核现象的发生。

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