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苹果*sMdCAX11*基因的功能分析与鉴定

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摘 要:【目的】深入分析苹果*sMdCAX11*(去掉NRR区域的*MdCAX11*)基因功能。**【方法】**分别利用蜜脆苹果果实和拟南芥材料,采用过表达*sMdCAX11*的试验方法,观察果实、叶片等各组织的表型,并测定不同组织的矿质元素含量,同时对苹果*MdCAX11*基因启动子区域进行预测分析以及启动子转录活性分析。**【结果】**瞬时过表达*sMdCAX11*的苹果果肉颜色变褐,并出现皱缩;同时过表达*sMdCAX11*的苹果果肉和拟南芥叶片的总Ca含量明显下降,且元素比值(K+Mg)/Ca明显升高。**【结论】***sMdCAX11*基因过表达可导致植株组织的元素分配不均,不同元素间的比例失衡。

关键词:苹果;Ca^{2+}/H^{+}反向转运体(CAX);基因功能;矿质元素;钙含量

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Functional analysis and characterization of the *sMdCAX11* gene in apple

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Abstract:【Objective】Ca^{2+}/H^{+} antiporter (CAX) is a type of low-affinity and high-capacity transporter that mainly relies on the transmembrane proton gradient to complete the transport of Ca^{2+}. This protein may be related to the occurrence of plant calcium deficiency. It is known that Ca^{2+}/H^{+} reverse transporter proteins (CAXs) in model plants like *Arabidopsis thaliana* and tomato play important roles in regulating intracellular Ca^{2+} distribution and allocation, and maintaining intracellular calcium homeostasis, and overexpression of the *CAXs* genes in different plants could cause calcium deficiency symptoms in the plants. In the preliminary stage of this study, Honeycrisp apple bitter pit disorder fruits with different degrees of incidence were used as test materials, and the differences in mineral element contents and expression patterns of calcium transport-related genes in disordered fruits were analyzed. The key regulatory genes *MdCAX5* and *sMdCAX11* (*MdCAX11* with the N-terminal autoinhibitory region removed) involved in the development of bitter pit disorder were then identified. However, the function of MdCAX11 protein in apple was still unclear. Meanwhile, it was still unclear whether Ca^{2+}/H^{+} reverse transporter proteins were involved in the development of bitter pit disorder in fruit.【Methods】The gene functions of *sMdCAX11* were analyzed using experimental methods like genetic transformation. In this study, we first utilized the transient transformation of Honeycrisp apple fruits to verify the calcium transport function of the sMdCAX11 protein. Fruits transiently transformed with the *sMdCAX11* gene were sectioned to observe the changes in the flesh tissue near the injection hole, and the mineral element content of the flesh tissue was also determined. Next, we stably transformed *sMdCAX11* into the Arabidopsis Col-0 and successfully obtained positive T4 generation transgenic plants. The PCR tests at DNA lev-

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el and RNA level verified that all obtained were positive plants, and the leaves were analyzed for mineral element detection. In this study, various types of elements contained in the 1500 bp promoter region upstream of the start codon of the apple *MdCAX11* gene were also predicted and analyzed. Meanwhile, in order to investigate the effect of the ProCAX11 promoter on the response to Ca^{2+}, tobacco leaves sprayed with different concentrations (0, 10, 20 and 40 mmol · L^{-1}) of $CaCl_2$ were infiltrated by using Agrobacterium proCAX11::GUS. The effect of calcium ion on the transcriptional activity of *MdCAX11* gene promoter was verified by GUS staining and GUS protein activity analysis.【Results】The total calcium content in apple flesh tissues overexpressing the *sMdCAX11* gene significantly decreased and continued to decrease with the extension of storage time. By analyzing the total Mg and K contents in the flesh tissues, these two elements showed a rapid increase after a transient decrease at the 3rd day after infestation, reaching the highest value at the 5th day. Further analysis of the elemental ratios of the flesh tissues revealed that the total mineral elements (K+Mg)/Ca significantly increased in the flesh of transiently transformed *sMdCAX11* and continued to rise with the extension of storage time. At the 5th day of infestation, (K+Mg)/Ca ratio of water-soluble mineral elements was significantly higher in the flesh tissues of transiently transformed *sMdCAX11* genes, while there was no significant change in the elemental ratios of the control. By analyzing the leaf mineral element contents of the four *sMdCAX11* transgenic Arabidopsis lines, consistent with the apple flesh material transiently transformed with *sMdCAX11*, the (K+Mg)/Ca ratios of the total elements, and the ratios of the water-soluble elements appeared to be marked increase and significantly different in the positive plants. A large number of *cis*-acting elements responsive to external environmental conditions were present in the promoter region of the MdCAX11, such as ARE, an element involved in anaerobic induction, as well as the light-responsive elements ATCT-motif, Box 4, G-box, GT1-motif, TCCC-motif and chs-CMA2a. In addition, the MdCAX11 promoter region was characterized by the presence of several transcription factor binding sites, such as the WRKY transcription factor binding sites WBOXNTERF3, WBOXATNPR1 and WRKY710S, as well as the binding sites of transcription factors like MYB. By analyzing the GUS protein activity of tobacco leaves, it was found that ProCAX11 initiation significantly increased in a high calcium environment. The GUS protein activity significantly increased when they were sprayed with different concentrations of $CaCl_2$, and the difference was significant compared with the control. Simultaniously, the GUS protein activity reached the highest value when they were sprayed with 20 mmol · L^{-1} $CaCl_2$. The transcriptional activity of MdCAX11 promoter was significantly affected by Ca^{2+}.【Conclusion】The calcium transport capacity of the *MdCAX11* gene was influenced by the N-terminal autoinhibitory region, and the *sMdCAX11* gene was equipped to transport calcium ions. Overexpression of the *sMdCAX11* gene significantly reduced the calcium content in plants and disrupted the balance of mineral element ratios. *sMdCAX11* transgenic *Arabidopsis thaliana* leaves contained significantly lower total Ca content as well as water-soluble Ca content compared with the wild type, and the (K+Mg)/Ca ratio of the total and water-soluble mineral elements was significantly higher than that of the control. In conclusion, these findings provided further evidence that overexpression of the *sMdCAX11* gene can cause uneven distribution of elements in plant tissues and imbalance in element proportions.

Key words: Apple; Ca^{2+}/H^{+} antiporter (CAX); Gene function; Mineral elements; Calcium content

Ca^{2+}/H^{+}反向转运体（CAXs）是一类低亲合、高容量的转运蛋白，即使胞质中Ca^{2+}水平较低时也可以发挥其生理功能。近年来，在拟南芥、水稻、葡萄等植物中已克隆出多个*CAX*基因，这些基因编码的蛋白不仅可以运输Ca^{2+}，还能运输Mn^{2+}[1-2]。将拟南芥的*AtCAX4*基因超表达载体转入到番茄中，获得转基因

番茄苗，其中转基因植株对钙离子的吸收增强，植株体内的钙元素含量增加，甚至延长果实的货架期[3]。

研究CAXs的蛋白结构发现，几乎所有的Ca^{2+}/H^{+}反向转运蛋白都有着相似的结构（图1），400 aa左右的氨基酸，均含有11个跨膜区域（TMD），都存在着一个Ca^{2+}的结合区（CaD），以此调节CAXs蛋白转运钙离子的能力[4]。Ca^{2+}/H^{+}反向转运蛋白的N端有亲水性自抑制区（NRR），N末端自抑制区存在于细胞质中，该段序列可直接影响CAX蛋白的活性[5]。利用酵母突变体K667菌株进行功能互补实验时发现，Ca^{2+}的转运受CAXs蛋白N-末端自抑制区的调控，全长的*CAXs*基因编码的蛋白无法有效地转运Ca^{2+}[6-7]。*CAXs*的这种转运特性存在于大多数植物的Ca^{2+}/H^{+}反向转运蛋白中，例如拟南芥*AtCAX1*、*AtCAX2*、*AtCAX3*、*AtCAX4*[8]，棉花*GhCAX1*、*GhCAX3*[9]，水稻*OsCAX1*、*OsCAX3*和*OsCAX4*[10]。

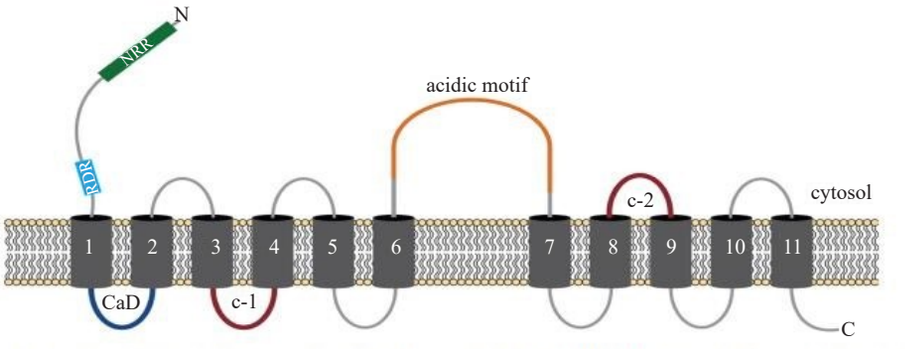


图1　CAXs保守功能域的拓扑结构

Fig. 1　Proposed topological model of CAXs displaying conserved features

蛋白的调控机制在不同植物品种及不同的成员之间不尽相同。例如，绿豆中去NRR的sVCAX1可以弥补K667酵母菌株转运Ca^{2+}的缺陷，同时转化全长MdCAX2L-2的K667菌株也可以正常转运Ca^{2+}[11]。相似的情况也多有报道，白菜全长BrCAX蛋白和苹果全长MdCAX5蛋白在酵母菌株K667中均可弥补突变株的钙转运缺陷[12-13]。研究去除N-末端自抑制区sPutCAX1的基因功能时发现，与星星草的全长PutCAX1基因相比，sPutCAX1的钙转运能力明显下降[14]。同时也有文献表明，CAXs的N-末端自抑制区在不同的植物体细胞中有各自不同的调节功能，但目前对这些相关调节机制尚不明了。通过对CAXs蛋白多样性的研究可以发现，不同物种之间的CAXs蛋白存在着较大的差别。

在前期研究中发现*sMdCAX11*（去NRR的MdCAX11）也表现出较强的钙转运能力，与*MdCAX5*的基因功能相似[12]。同时sMdCAX11蛋白作为一价阳离子和二价阳离子的转运体，既可以转运Ca^{2+}，也可以转运Na^{+}。因此在本研究中将重点研究*sMdCAX11*的基因功能，通过观察过表达sMdCAX11试验材料的表型及分析不同组织的矿质元素含量，研究sMdCAX11蛋白在植物体内所起到的关键作用。

1　材料和方法

1.1　试验材料

用于瞬时转化的蜜脆苹果果实采收自陕西省宝鸡市西北农林科技大学千阳试验站，选取无病害、机械损伤的苹果，样品采集后迅速带回实验室，1 ℃贮藏冷库存放。用于瞬时转化的本氏烟草放置于光照培养箱进行培养（培养条件：22 ℃/20 ℃，16 h光照/8 h黑暗），培养至6~8枚叶时用于试验。用于稳定转化的拟南芥为Col-0生态型，光照培养条件为16 h光照（22 ℃）和8 h黑暗（20 ℃）。

克隆载体pMD19-T Simple vector购自TaKaRa公司，植物表达载体pVBG2307、pC0390GUS等均由实验室保存。大肠杆菌*E.coli* DH5α购自天根公司，农杆菌菌株GV3101感受态购自上海唯地生物有限公司。

1.2　苹果的瞬时转化

克隆*sMdCAX11*基因CDS序列（去掉终止密码子），将得到的片段插入到融合载体GFP蛋白的N端，得到新的融合载体35S::sMdCAX11-GFP。将获得的融合载体通过冻融法转入农杆菌GV3101感受态细胞，获得阳性农杆菌。苹果瞬时转化的方法参

考Jiang等[15]方法进行，在果实瞬时转化的第3天、第5天、第9天采样并液氮速冻后保存于−80 ℃冰箱。

1.3 拟南芥的稳定转化方法

利用方法1.2中获得的含有35S::sMdCAX11-GFP的农杆菌用于拟南芥的稳定转化，采用浸花序法获得阳性拟南芥植株。

1.4 总Ca、Mg、K、N和P含量的测定

1.4.1 总Ca、Mg和K含量的测定 称取3.00 g果肉冻样置于70 ℃烘箱中烘至恒质量，称取1.00 g烘干样品并放置于100 mL消解管中，同时加入3 mL高氯酸和12 mL硝酸，浸泡过夜后进行高温消解，对消解样品赶酸、定容后稀释一定倍数，利用原子吸收光谱仪（ZA3000）测定样品的总Ca、K和Mg含量。

1.4.2 总N和P含量的测定 称取0.20 g烘干样品与8 mL硫酸混合后放入100 mL消解管中浸泡过夜，经高温消解、赶酸、定容、稀释后利用连续流动化学分析仪测定总N、P含量。

总矿质元素含量以干质量表示，每项测定均包括3次生物学重复。

1.5 水溶性Ca、Mg、K、N和P含量的测定

水溶性矿质元素的测定方法参照Pavicic等[16]的报道并有所改动。称取6 g冻样置于研钵中，加20 mL去离子水充分研磨，将研磨后的匀浆10 000 r·min^{-1}离心30 min。收集上清液，将离心管的沉淀用20 mL去离子水重悬后，如上所述再次离心。收集两次离心后的上清液经多次滤纸过滤后定容到50 mL，稀释至一定倍数后利用原子吸收光谱仪测定水溶性Ca、Mg和K含量，利用连续流动化学分析仪测定水溶性N和P含量。水溶性矿质养分含量以鲜质量表示。每项测定均包括3次生物学重复。

1.6 ProCAX11启动子的克隆及顺式作用元件分析

采用植物基因组DNA提取试剂盒（AG21011）提取植物总DNA。以*MdCAX11*全长在苹果基因组数据库中比对，获得起始密码子上游1500 bp左右的核苷酸序列。随后设计引物，以蜜脆叶片DNA为模板，克隆*MdCAX11*基因启动子序列。利用Plant CARE和PLACE在线网站预测ProCAX11启动子存在的转录因子结合位点及顺式作用元件。

1.7 GUS染色方法及蛋白活性分析

克隆*MdCAX11*基因启动子ProCAX11序列，将得到的片段插入到载体pC0390GUS，得到新的融合载体ProCAX11::GUS，对照为pC0390GUS空载。将获得的融合载体采用冻融法转入农杆菌GV3101感受态细胞。PCR鉴定阳性的菌液瞬时侵染本氏烟草。GUS染色、GUS粗蛋白提取及浓度测定、GUS蛋白荧光值测定等方法参照Chen等[17]的报道。

1.8 ProCAX11启动子对不同浓度$CaCl_2$的响应分析

为了探究ProCAX11启动子对$CaCl_2$的响应效果，配制浓度为0、10、20和40 mmol·L^{-1}的$CaCl_2$溶液。在侵染前24 h时，对本氏烟草植株喷洒不同浓度的$CaCl_2$溶液，叶片的正反面均匀喷洒，直至叶片两面均被打湿且不断滴水为止，然后放回原来的培养条件下继续培养。利用转化有融合载体ProCAX5::GUS的农杆菌侵染烟草叶片。侵染48 h后对侵染的烟草叶片进行GUS染色及GUS蛋白活性分析。

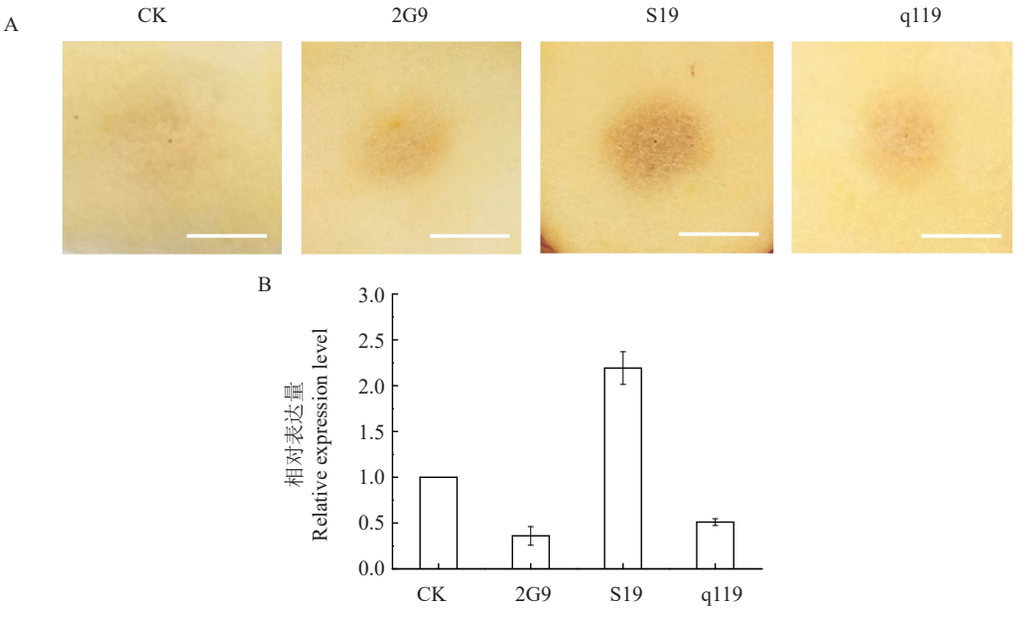
2 结果与分析

2.1 *sMdCAX11*瞬时过表达在苹果果实的表型鉴定及元素分析

利用瞬时转化蜜脆苹果果实的方法，来验证sMdCAX11蛋白的钙转运功能。对瞬时转化*sMdCAX11*基因的果实进行切片，观察注射孔附近的果肉组织，发现在侵染第9天时果肉组织明显变褐，果肉组织皱缩，与对照组相比差异显著（图2-A）。基因相对表达量分析检测侵染第9天时果肉的*sMdCAX11*基因表达量显著上调，这也直接说明了瞬时转化试验效果良好，可以用于进一步的分析检测（图2-B）。

2.2 *sMdCAX11*瞬时过表达在苹果果实的元素分析

分析瞬时转化*sMdCAX11*基因的果肉组织总矿质元素及水溶性矿质元素的含量，对照组为瞬时转化空载的果肉组织，结果发现过表达*sMdCAX11*基因的果肉组织总钙含量显著下降，且随着贮藏时间的延长而不断降低（图3-A）。在侵染后第3天时，水溶性Ca含量与对照组相比出现了短暂的上升，但随着贮藏时间的延长而显著下降（图3-B）。分析果肉组织的总Mg、K含量，这两种元素在侵染后第3天时出现短暂降低之后迅速升高，在第5天时达到最高值。而水溶性Mg、K含量则与总Mg、K含量的变化趋势相反（图3）。进一步分析果肉组织的元素比



A. 贮藏 9 d 后的苹果注射孔附近果肉的表型变化；B. 贮藏 9 d 后的苹果果肉 *sMdCAX11* 基因相对表达量。CK 为注射无菌侵染液；2G9 为注射空载农杆菌；S19 为注射携带 35S::sMdCAX11 农杆菌；q119 为注射携带 35S::MdCAX11 农杆菌。比例尺=1 cm。

A. The phenotypic changes of the apple flesh near the injection hole after 9 days of storage; B. The relative expression level of *sMdCAX11* gene in the apple flesh after 9 days of storage. CK for injection sterile infection solution; 2G9 for injection of empty vector; S19 is injection carrying 35S::sMdCAX11 *Agrobacterium*; q119 is injection carrying 35S::MdCAX11 Agrobacterium. Scale bar = 1 cm.

图 2　瞬时过表达 *sMdCAX11* 在苹果果实中的表型鉴定

Fig. 2　Phenotype identification of transiently overexpressed *sMdCAX11* in apple fruits

值发现，总矿质元素（K+Mg）/Ca 比值在瞬时转化 *sMdCAX11* 的果肉中显著增加，且随着贮藏时间的延长而不断升高。在侵染第 5 天时，水溶性矿质元素的（K+Mg）/Ca 比值在瞬时转化 *sMdCAX11* 基因的果肉组织中明显升高，同时对照组的元素比值无显著变化（图 3）。

2.3　过表达 *sMdCAX11* 拟南芥的元素分析

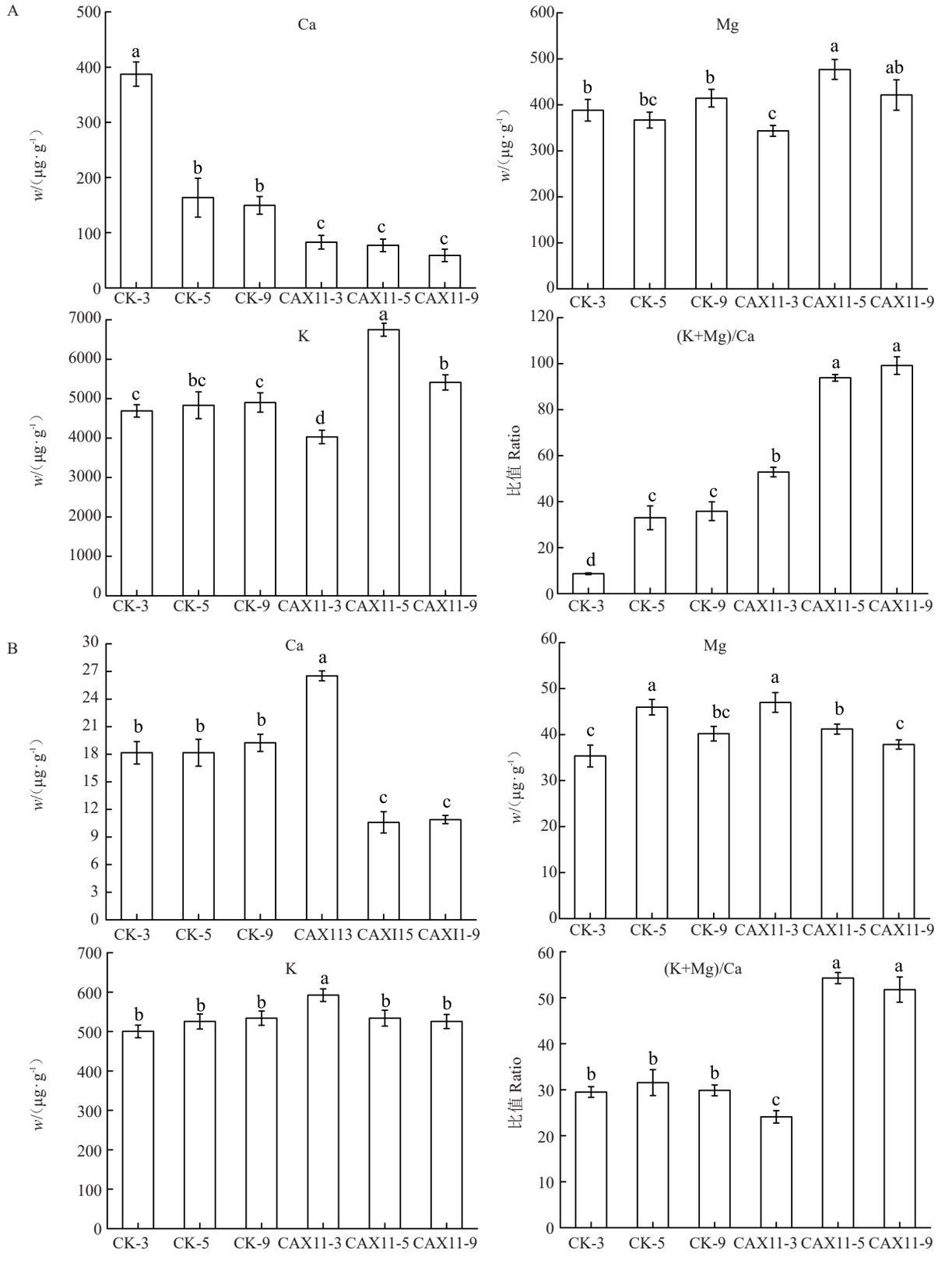
为进一步验证 *sMdCAX11* 参与钙转运的功能，将 *sMdCAX11* 稳定转化拟南芥 Col-0 生态型，并成功获得阳性 T_4 代转基因植株。通过 DNA 水平及 RNA 水平的 PCR 检验，验证所获得的均为阳性植株，并开展后续试验。对拟南芥叶片进行矿质元素检测分析，结果发现 4 个过表达 *sMdCAX11* 拟南芥株系的叶片总 Ca 含量与水溶性 Ca 含量均出现显著下降，差异极显著（图 4）。同时 4 个株系的阳性样本间差异不显著，说明转基因植株间的表型稳定，不存在特异性。分析 4 个转基因拟南芥株系叶片的总 Mg 与水溶性 Mg 含量，发现与野生型对照组相比差异不显著（图 4）。然而 4 个转基因拟南芥株系的总 K 含量与水溶性 K 含量均高于野生型且差异显著，但在 4 个株系间差异不显著（图 4）。与瞬时转化 *sMdCAX11* 的苹果果肉材料相一致的是元素的（K+Mg）/Ca 比值，总元素的比值与水溶性元素的比值在阳性植株中均出现明显增大，且差异显著（图 4）。

2.4　ProCAX11 启动子顺式作用元件分析

利用 Plant CARE 等在线网站，对苹果 *MdCAX11* 基因起始密码子上游 1500 bp 启动子区域所包含的各类元件进行预测分析（表 1）。结果表明，该基因启动子区存在大量响应外界环境条件的顺式作用元件，如参与厌氧诱导的元件 ARE，以及参与光响应的元件 ATCT-motif、Box 4、G-box、GT1-motif、TCCC-motif 和 chs-CMA2a（表 1）。该基因的启动子也存在参与激素应答的调控元件，如参与脱落酸的 ABRE（表 1）。另外，ProCAX11 启动子区域还存在着多个转录因子结合位点，如 WRKY 转录因子结合位点 WBOXNTERF3、WBOXATNPR1 和 WRKY710S，以及 MYB 等转录因子的结合位点（表 1）。

2.5　ProCAX11 启动子转录活性及钙元素响应分析

为探究 ProCAX11 启动子对 $CaCl_2$ 的响应效果，利用转化融合载体 ProCAX11::GUS 的农杆菌侵染喷洒过不同浓度（0、10、20、40 mmol·L^{-1}）$CaCl_2$ 的烟草叶片，并采用 GUS 染色及 GUS 蛋白活性分析的方

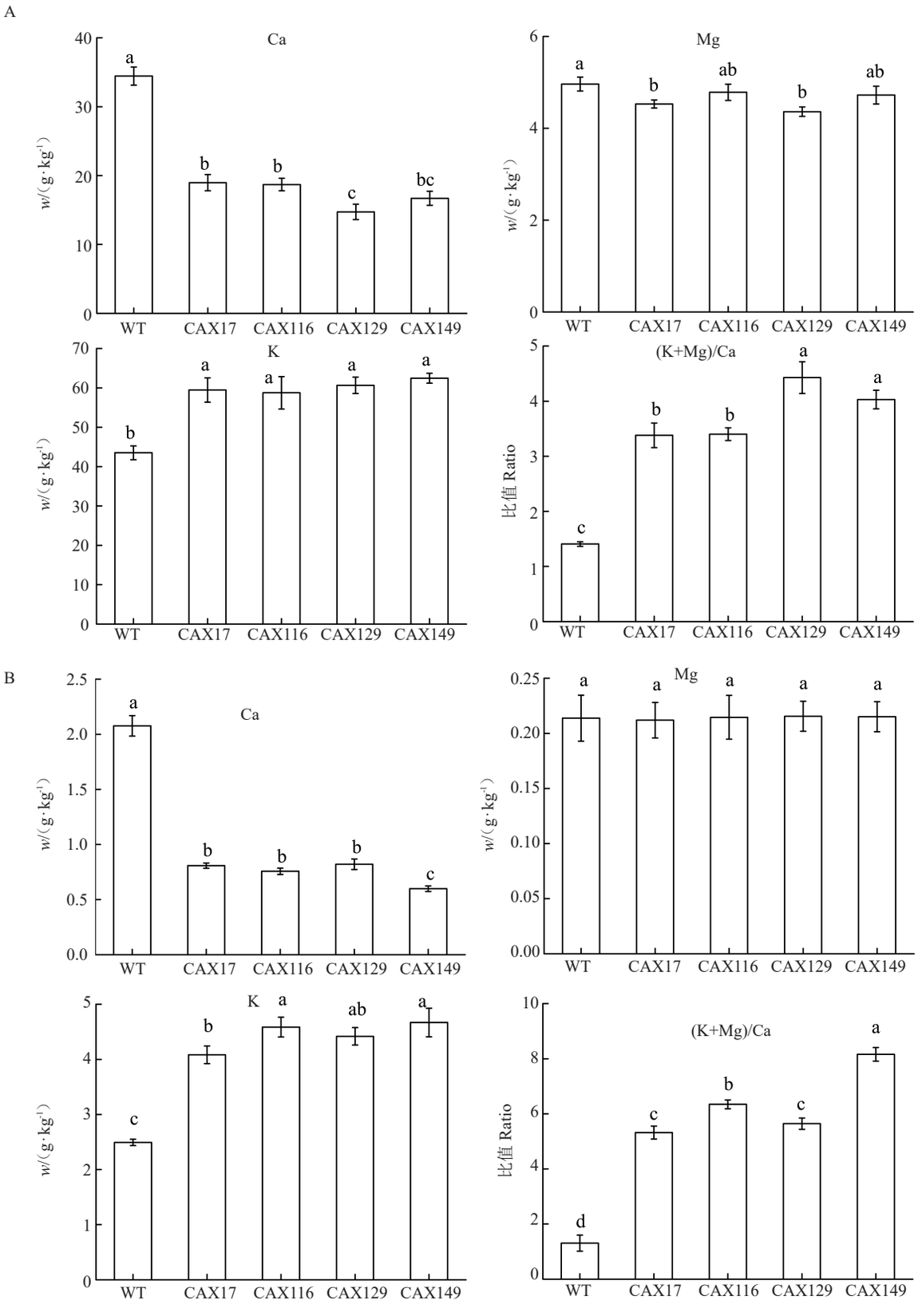


A. 总矿质元素含量；B. 水溶性矿质元素含量。CK-3、5、9 分别为注射空载农杆菌的果实贮藏 3 d、5 d 和 9 d，CAX11-3、5、9 分别为瞬时转化 *sMdCAX11* 的果实贮藏 3 d、5 d、9 d。

A. Total mineral element content; B. Water-soluble mineral element content. CK-3, 5, 9 are stored for 3 d, 5 d, and 9 d after injection of unloaded Agrobacterium, and CAX11-3, 5, and 9 are the apple overexpressed *sMdCAX11* storage 3 d, 5 d, 9 d, respectively.

图 3 瞬时过表达 *sMdCAX11* 在苹果果实中的元素分析

Fig. 3 Element analysis of transiently overexpressed *sMdCAX11* in apple fruits



A. 总矿质元素含量；B. 水溶性矿质元素含量。WT. 野生型拟南芥；CAX17~149. 阳性植株的不同株系。

A. Total mineral element content; B. Water-soluble mineral element content. WT. Wild-type *Arabidopsis thaliana*; CAX17-149. The different lines of positive plants.

图 4　稳定过表达 *sMdCAX11* 的拟南芥叶片元素分析

Fig. 4　Elemental analysis of *Arabidopsis thaliana* leaves overexpressing *sMdCAX11*

表1 ProCAX11启动子顺式作用元件与功能分析

Table 1 Functional analysis of *cis*-elements of ProCAX11 promoter

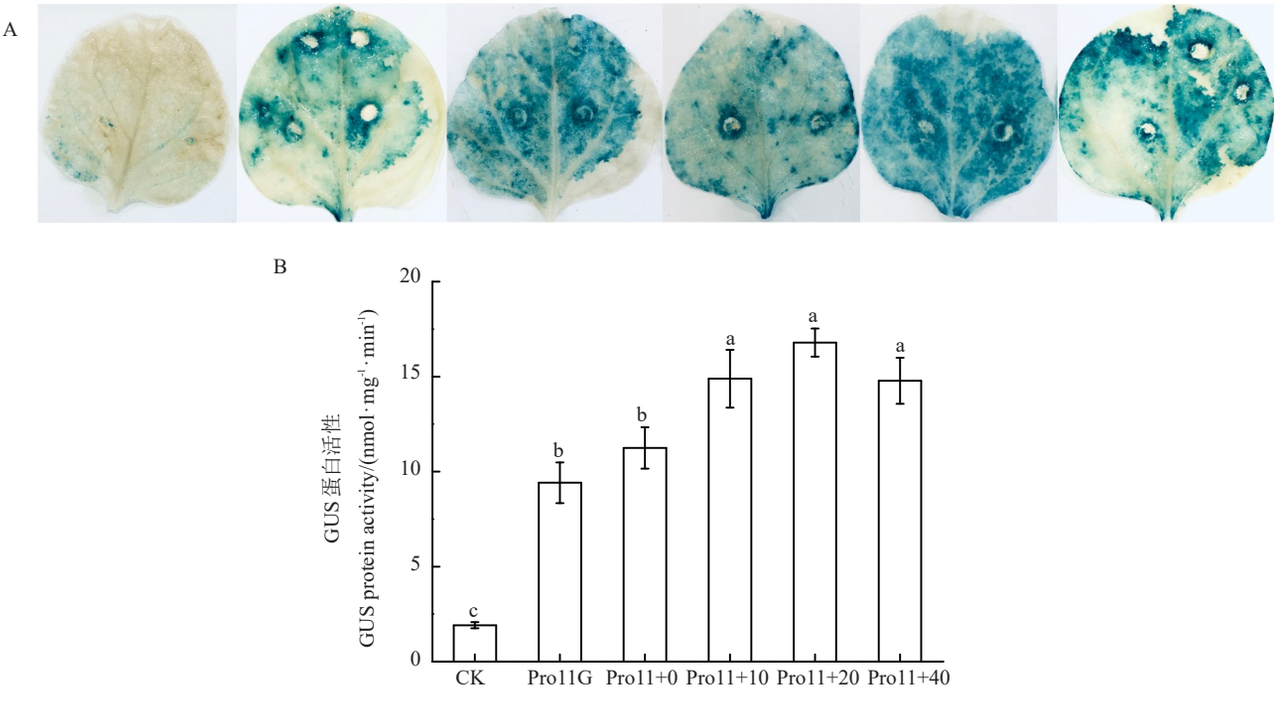
顺式作用元件 *Cis*-element	功能 Function	顺式作用元件个数 Number of *cis*-element
ABRE	参与脱落酸反应的顺式作用元件 *Cis*-acting element involved in the abscisic acid responsiveness	3
ARE	厌氧诱导所必需的顺式调控元件 *Cis*-acting regulatory element essential for the anaerobic induction	4
ATCT-motif	参与光反应的保守DNA模块部分 Part of a conserved DNA module involved in light responsiveness	1
Box 4	参与光反应的保守DNA模块部分 Part of a conserved DNA module involved in light responsiveness	1
Box III	蛋白质结合位点 Protein binding site	1
CAAT-box	启动子和增强子区的常见顺式作用元件 Common cis-acting element in promoter and enhancer regions	30
G-box	参与光反应的顺式调控元件 *Cis*-acting regulatory element involved in light responsiveness	3
GT1-motif	光响应元件 Light responsive element	3
MBS	MYB结合位点参与干旱诱导 MYB binding site involved in drought-inducibility	1
MYB	MYB结合位点 MYB binding site	2
MYB-like sequence	MYB结合位点 MYB binding site	2
Myb	MYB结合位点 MYB binding site	1
Myc	MYB结合位点 MYB binding site	1
STRE	未知 Unknown	2
TATA-box	转录起始−30 bp左右的核心启动子元件 Core promoter element around −30 bp of transcription start	26
TCCC-motif	光响应元件部分 Part of a light responsive element	1
WRE3	未知 Unknown	1
WBOXATNPR1	WRKY转录因子结合位点 WRKY transcription factor binding site	3
WBOXNTERF3	WRKY转录因子结合位点 WRKY transcription factor binding site	2
WRKY710S	WRKY转录因子结合位点 WRKY transcription factor binding site	4
chs-CMA2a	光响应元件部分 Part of a light responsive element	1

法来验证钙离子对*MdCAX11*基因启动子转录活性的影响（图5）。GUS染色发现喷洒10 mmol·L^{-1}与20 mmol·L^{-1} $CaCl_2$的烟草叶片颜色相比于其他组明显更深，CK为注射空载农杆菌烟草叶片（图5-A）。通过分析烟草叶片的GUS蛋白活性，发现在高钙的环境下*MdCAX11*启动效果明显增强。在喷洒不同浓度的$CaCl_2$时，GUS蛋白活性显著提高，与对照组相比差异显著。同时在喷洒20 mmol·L^{-1} $CaCl_2$时，GUS蛋白活性达到最高值（图5-B）。ProCAX11启动子转录活性受Ca^{2+}的显著影响。然而，随着钙离子浓度的增加，GUS蛋白的活性并没有随之增高（图5）。

3 讨 论

在前期研究中已经证实*sMdCAX11*（去NRR的MdCAX11）可以表现出较强的钙转运能力[12]。同时sMdCAX11蛋白作为一价阳离子和二价阳离子的转运体，既可以转运Ca^{2+}，也可以转运Na^{+}。为验证sMdCAX11蛋白的功能，笔者在本研究中分别利用了苹果果实和拟南芥材料，采用瞬时过表达和稳定过表达*sMdCAX11*的试验手段，观察果肉组织的表型，并测定不同组织的矿质元素含量。

在白菜[13]、番茄[5]和土豆[18]中过表达液泡CAX转运蛋白，植株出现了类似缺钙的症状。瞬时过表达*sMdCAX11*的苹果在侵染第9天时注射孔附近的果肉颜色变褐，并出现皱缩，有明显的死细胞，而这一症状与苦痘病发病部位的果肉表型十分相像。分析瞬时转化*sMdCAX11*基因的果肉组织总矿质元素及水溶性矿质元素的含量。过表达*sMdCAX11*基因的果肉组织相比于对照组总钙含量显著下降。分析果肉组织的元素比值发现，总矿质元素及水溶性矿质元素（K+Mg）/Ca比值在瞬时转化*sMdCAX11*的果



A. GUS 染色；B. GUS 蛋白活性分析。CK 为注射空载农杆菌烟草叶片；Pro11G. ProCAX11-GUS；0~40 分别代表 0、10、20、40 mmol·L^{-1} 的 $CaCl_2$。

A. GUS staining; B. GUS protein activity analysis. CK. Inject empty vector tobacco leaves; Pro11G. ProCAX11-GUS; 0-40 represents 0, 10, 20, 40 mmol·L^{-1} $CaCl_2$.

图 5　ProCAX11 启动子转录活性及对不同浓度 $CaCl_2$ 的响应分析

Fig. 5　ProCAX11 promoter transcriptional activity analysis and response analysis to different concentrations of $CaCl_2$

肉中显著增大，且随着贮藏时间的延长而不断升高。这一结果与苦痘病果实中不同矿质元素的分布及比例极其相似。苦痘病果实中的水溶性Ca含量显著低于健康果实，且(K+Mg)/Ca比值显著高于对照果实[19-20]。

稳定过表达*sMdCAX11*的拟南芥叶片在矿质元素含量及比值的检测结果上与苹果果实相一致，总Ca含量与水溶性Ca含量在阳性植株的叶片中明显下降，且(K+Mg)/Ca比值在阳性植株中显著升高。这一结果也证实了*sMdCAX11*基因过表达可导致植株组织的元素分配不均，不同元素间的比例失衡的结论。但这一结果与先前研究报道并不完全相符[21]。这可能是因为笔者在本研究中对果实的Ca含量检测时并未对细胞膜、细胞质等分别进行检测，所以Ca含量与先前研究不一致。

在先前的研究报道中也表明WRKY转录因子可能参与了苦痘病的发生与发展[18,22]。对苹果*MdCAX11*基因启动子区域进行预测分析，发现基因启动子区存在着WRKY转录因子结合位点WBOXNTERF3、WBOXATNPR1和WRKY71OS。因此在下一步的工作中将开展WRKY转录因子与*MdCAX11*基因启动子的互作分析，以期明确WRKY转录因子与苦痘病发生的相关性。

4　结　论

*MdCAX11*基因的钙转运能力受N末端自抑制区的影响，*sMdCAX11*基因的过表达显著降低植物体内钙含量，打破了矿质元素比例的平衡。

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