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钙对苹果果实钙调蛋白含量和Ca^{2+}-ATPase活性及其基因表达的影响

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Effects of calcium on content of calmodulin, activity of Ca^{2+}-ATPase and their gene expressions in apple (*Malus pumila* Mill.) fruits

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摘要 研究不同浓度钙（0、1和10 mmo1/L $CaCl_2$；5 mmo1/L EGTA）对苹果果实钙调蛋白（CaM）含量和Ca^{2+}-ATPase活性及其基因表达的影响。利用同源克隆方法分离*CaM*和*Ca^{2+}-ATPase*基因，采用荧光定量PCR方法研究它们表达特征。结果表明，果实切片外源补钙，可溶性Ca^{2+}及CaM含量在高钙处理12 h达到高峰；高钙处理12 h质膜Ca^{2+}-ATPase活性显著增加，与胞内CaM含量增加时间一致；高钙处理24 h液泡膜Ca^{2+}-ATPase活性显著增加；随着质膜和液泡膜Ca^{2+}-ATPase活性显著增加，可溶性Ca^{2+}含量在加钙处理48 h显著下降。研究基因表达发现，加钙处理6 h苹果*CaM*基因的表达量显著增加；加钙处理12 h苹果*Ca^{2+}-ATPase*基因的表达量显著增加，与CaM含量及质膜Ca^{2+}-ATPase活性变化一致。果实缺钙处理显著增加*CaM*基因表达量，而苹果*Ca^{2+}-ATPase*基因的表达量没有显著变化。上述研究表明，苹果补钙可以提高细胞内可溶性Ca^{2+}和CaM含量以及*CaM*基因的表达量，有效启动钙信使系统；质膜及液泡膜Ca^{2+}-ATPase是调控胞内Ca^{2+}关键的酶，通过提高质膜及液泡膜Ca^{2+}-ATPase的活性及*Ca^{2+}-ATPase*基因的表达量，维持胞内Ca^{2+}的稳态水平。

关键词： 钙 钙调蛋白 Ca2 +-ATPase 基因表达 荧光定量PCR 苹果

Abstract: Effects of calcium on calmodulin (CaM) content, Ca^{2+}-ATPase activity and their gene expressions in apple (*Malus pumila*) fruits were investigated under the hydroponic condition with 0，1，10 mmo1/L $CaCl_2$ and 5 mmo1/L EGTA. *CaM* and *Ca^{2+}-ATPase* gene were isolated by homologous cloning and their expression patterns were analyzed by real–time PCR. Addition of 10 mmo1/L$CaCl_2$ to the culture solutions with 12 h exposure significantly increased soluble Ca^{2+} and CaM contents in apple fruits. With the CaM content increasing, plasma and tonoplast membrane Ca^{2+}-ATPase activities were enhanced after 12 and 24 h Ca exposure respectively, resulting in the decrease in the soluble Ca^{2+} content after 48 h Ca exposure. In molecular level, the abundances of *CaM* and *Ca^{2+}- ATPase* genes were up-regulated after 6 h and 12 h Ca exposure, respectively, in accordance with change of the CaM content and plasma membrane Ca^{2+}-ATPaseactivity. Addition of 5 mmo1/L EGTA to the culture solutions induced up-regulated the expression of the *CaM* gene, however, did not markedly change the expression of the *Ca^{2+}-ATPase* gene. It was concluded that external calcium might increase soluble Ca^{2+} and CaM contents and the expression of the *CaM* gene to stimulate an intracellular Ca^{2+}/calmodulin signaling system. Plasma and tonoplast membrane Ca^{2+}-ATPase are of major physiological importance to expel Ca^{2+} from eukaryotic cells and to maintain overall Ca^{2+} homoeostasis by enhancing their activities and gene expression.

Keywords: Calcium Calmodulin Ca2 +-ATPase Gene expression Real-time PCR Apple

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