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NO参与铝诱导蚕豆保卫细胞死亡的调控

Involvement of NO in aluminum-induced guard cell death in detached epidermis of *Vicia faba* leaves

关键词：$AlCl_3$ NO 蚕豆保卫细胞 细胞程序性死亡

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摘要：铝(Al)是地壳中含量最丰富的金属元素,是酸性土壤中导致植物生长抑制和作物产量下降的一个主要因素,但铝毒性作用机制尚不清楚.本文以蚕豆叶表皮为材料,研究铝胁迫对气孔保卫细胞活性的影响,探讨NO在铝诱导细胞死亡中的作用.结果表明,一定浓度的$AlCl_3$可诱导气孔保卫细胞活性降低,部分细胞死亡,且随着铝浓度的增高细胞死亡率增高.死细胞呈现核固缩、核崩解、凋亡小体等典型凋亡特征,且凋亡抑制剂Z-Asp-CH_2-DCB能阻止$AlCl_3$诱发的细胞死亡.用NO清除剂c-PTIO、NO合酶抑制剂L-NAME或硝酸还原酶抑制剂NaN_3降低铝处理组胞内NO后,细胞死亡率显著降低,胞内ROS、Ca^{2+}水平同期降低;NaN_3还能降低铝处理组中具有程序性死亡特征的细胞比率.用ROS清除剂AsA清除铝处理组胞内ROS后,细胞死亡率显著降低,胞内Ca^{2+}和NO水平亦显著降低;铝处理液中加入Ca^{2+}通道抑制剂$LaCl_3$后,细胞死亡率低于铝单独处理组,胞内ROS和NO水平无明显改变。研究结果表明,铝胁迫引起的胞内NO合成增加通过Ca^{2+}信号途径介导了保卫细胞的程序性死亡.

Abstract：Aluminum (Al) is the most abundant metallic element in the earth's crust. It has been found that Al is a major factor reducing plant growth and crop production in acid soils. However, the mechanism of aluminum toxicity is still not completely clear. In this study, effect of aluminum on guard cell viability was investigated in *V. faba* leaves. Epidermal strips were obtained by peeling off the lower epidermis from young fully expanded leaves, incubated in 2-(N-morpholino) ethanesulfonic acid (MES) buffer containing some chemicals ($AlCl_3$ with or without some alleviants) for 3 h, and then stained with fluorescein diacetate (FDA) to show cell viability. Alternative staining agents in this experiment include 2',7'-dichlorofluorescein diacetate (DCFH-DA), 3-Amino,4-aminomethyl-2',7'-difluorescein diacetate (DAF-FM DA) and fluo-3 acetomethoxyester (Fluo-3AM) to indicate intracellular ROS, NO and Ca^{2+} levels. The results showed that aluminum treatment significantly decreased cell viability at concentrations of 0.2 to 10 mmol • L^{-1}. A synchronous increase in cell death rate and intracellular NO, ROS and Ca^{2+} levels occurred in *V. faba* guard cells exposure to aluminum. Typical features of programmed cell death including nuclear condensation, fragmentation and apoptotic bodies were found in Al-treated guard cells, and could be reversed by caspases inhibitor Z-Asp-2,6-dichlorobenzoyloxymethylketone (Z-Asp-CH_2-DCB), demonstrating a programmed cell death caused by aluminum toxicity. After application of NO scavenger c-PTIO, NO synthase inhibitor L-NAME or nitrite reductase inhibitor NaN_3 to inhibit the elevation of intracellular NO in guard cells evoked by aluminum, Al-induced cell death rate significantly decreased, and the intracellular ROS and Ca^{2+} levels in guard cells also decreased. NaN_3 can also decrease PCD rate in aluminum treatment groups. Antioxidant substance ascorbic acid (AsA) decreased Al-induced cell death and also the levels of intracellular NO and Ca^{2+} levels in aluminum treatment groups. Application of $LaCl_3$, a plasma membrane Ca^{2+} channel blocker, significantly decreased Al-induced cell death. Our results suggest that Al-induced cell death in *V. faba* guard cells could be one kind of programmed cell death mediated by NO generation and activation of cytosolic Ca^{2+} signaling.

Key words：$AlCl_3$ NO *V. faba* guard cell programmed cell death

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