**Toxin3-induced PCD requires the synergistic effect of Protein1 and Protein2**

 **2.1 Phenotypic analysis of Toxin3-induced PCD in Protein1 and Protein2 overexpression and mutants**

 To analyze the functions of Protein1 and Protein2 in Toxin3-induced programmed cell death (PCD), we hybridized *protein1* and *protein2* single mutants and obtained *protein1 protein2* double mutants. For the leaves of wild-type Col-0, *protein1*, *protein2* and *protein1 protein2*, respectively, 10 mmol/L MgCl 2 or 10 μmol/L Toxin3 (Toxin3 was added to 10 mmol/L MgCl 2) was injected. After 72 h of treatment, the leaves were taken and photographed to observe the occurrence of PCD. The results are shown in Figure 2A. Compared with the wild-type Col-0, the degree of PCD in the *protein1 protein2* double mutants was significantly reduced.

On the other hand, we constructed transgenic plants overexpressing *Protein1* and *Protein2* and selected two strains from them (*Protein1ox5*, *Protein1ox6,* *Protein2ox7* and *Protein2ox8*). The leaves were treated with the Col-0 control group. 10 mmol/L MgCl 2 or 10 μmol/L Toxin3 (Toxin3 was added to 10 mmol/L MgCl 2) was injected. After 72 h of treatment, the leaves were photographed, and the occurrence of PCD was observed. The results showed that compared with the wild-type control Col-0, both Protein1- and Protein2-overexpressing plants had more severe Toxin3-induced PCD, as shown in Figure 2A.

 Combining the results of Toxin3 induction of PCD in mutants and overexpression plants, these results indicate that Protein1 and Protein2 both play a positive regulatory role in the process of Toxin3-induced PCD. To further prove this conclusion, we conducted two subsequent experiments: quantitative analysis of electrical conductivity and statistical analysis of the sensitivity of PCD in seedlings and the proportion of PCD changes in seedlings.

 **2.2** **Statistical analysis of Toxin3 sensitivity in Protein1 and Protein2 overexpression and mutants**

 To further analyze the positive regulatory relationship of Protein1 and Protein2 in the process of Toxin3 induction of PCD, we performed statistical analysis of the sensitivity using the overexpression of Protein1 and Protein2 and the mutants to Toxin3. First, Col-0, *protein1*, *protein2*, *protein1 protein2*, *Protein1ox5*, *Protein1ox6*, *Protein2ox7,* and

**2 Toxin3诱导的PCD需要Protein1和Protein2的协同作用**

**2.1 Protein1和Protein2过表达和突变体中Toxin3诱导PCD的表型分析**

为了分析Protein1和Protein2在Toxin3诱导程序性细胞死亡（PCD）中的功能，我们将*protein1*、*protein2*单突变体进行杂交并获得*protein1 protein2*双突变体，对野生型Col-0、*protein1*、*protein2*和*protein1 protein2*的叶片分别以10 mmol/L MgCl2或10 μmol/L Toxin3（Toxin3添加于10 mmol/L MgCl2中）进行注射，经处理72 h后取叶片进行拍照，观察PCD的发生情况。结果如图2A所示，与野生型Col-0相比，在*protein1 protein2*双突变体中发生PCD的程度明显减弱。

另一方面，我们构建了*Protein1*和*Protein2*的过表达转基因植株，并从中各选出两个株系（*Protein1ox5*、*Protein1ox6*和*Protein2ox7*、*Protein2ox8*），与Col-0对照组一起对叶片分别以10 mmol/L MgCl2或10 μmol/L Toxin3（Toxin3添加于10 mmol/L MgCl2中）进行注射，经处理72 h后分别取叶片进行拍照和观察PCD的发生情况。结果表明，与野生型对照Col-0相比，无论是Protein1还是Protein2的过表达植株，其发生Toxin3诱导PCD的程度更为加重，如图2A所示。

综合突变体和过表达植株中Toxin3诱导PCD的结果，表明Protein1和Protein2在Toxin3诱导PCD过程中均发挥正调控作用。为了进一步证明这一结论，我们进行了后续的两方面实验：采用电导率的定量分析和幼苗发生PCD的敏感性及其变化比例的统计分析。

**2.2 Protein1和Protein2过表达和突变体中Toxin3敏感性的统计分析**

为了进一步分析Protein1和Protein2在Toxin3诱导PCD过程中的正调控关系，我们采用Protein1和Protein2过表达和突变体对Toxin3的敏感性进行了统计分析。先将培养一周的Col-0、*protein1*、*protein2*、*protein1 protein2*、*Protein1ox5*、*Protein1ox6*、*Protein2ox7*、*Protein2ox8*幼苗分别转移到含有2 μmol/L Toxin3的新的MS培养基中培养6天，然后根据它们对Toxin3的敏感性程度将其分为超敏感、敏感和不敏感三种类型，如图2B所示。对于每种生态型的植株，我们都按照图2B中所示进行超敏感、敏感和不敏感的分类计数，并且计算各种敏感性类别在该生态型中所有植株（*n*>50）中的比率，得到图2C所示的统计结果。与Col-0相比，*protein1 protein2*对Toxin3不敏感的比例大大升高，而*Protein1ox5*、*Protein1ox6*、*Protein2ox7*、*Protein2ox8*对Toxin3不敏感的比例有很大幅度的降低，发生超敏的比例大大升高。该Toxin3敏感性的统计结果也同样表明，Protein1和Protein2在Toxin3诱导PCD过程中发挥正调控作用。