

Screening and effect analysis of specific small molecule inhibitors of programmed death molecules in plant

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Abstract. objective to conduct a screen for small molecule inhibitors that can inhibit programmed death of plant molecules and test their inhibitory effects. Methods small molecule drugs with inhibitory or alleviating effects on programmed death of plant cells were selected by screening a large number of literatures, and the indexes of plant root length and fresh weight, Results H₂S could reduce the inhibition of root length and fresh weight of Cucumber Seedlings by Cd in a concentration dependent effect. CD induced the accumulation of endogenous H₂S, indicating that H₂S is involved in the response to CD. H₂S reduces ROS accumulation and membrane lipid peroxidation by activating antioxidant systems. H₂S reduces apical cell death by decreasing oxidative damage. Conclusions elevated endogenous H₂S levels negatively regulate programmed death molecules in plants.

Keywords: procedural death; Gas signal molecules; H₂S.

1. Preface

Gluchzman first proposed the concept of Programmed Cell Death (PCD) in 1952, that is, the autonomous and orderly cell death behavior of cells themselves, which is a universal life phenomenon existing in biological cells. The initial main research content was to observe and describe the abnormal behavior of amphibians tissues in animals. The research on programmed cell death of plant cells started late.

1.1 Morphological and molecular biological characteristics of programmed cell death in plants

In terms of morphology, recent studies have shown that programmed cell death in plant and animal cells has many similar characteristics, including changes in cell morphology and DNA degradation. followed by the molecular mechanism of programmed cell death and signal transduction in plants.

1.2 Methods for detecting programmed cell death in plants

The morphological characteristics of programmed cell death are the basis for determining whether programmed cell death has occurred or not. and it has been further confirmed at the molecular level that the short male inflorescence of chestnut belongs to the phenomenon of programmed plant cell death[7].

1.3 Molecular mechanisms of programmed cell death in plants

In recent years, as the research on programmed cell death in animals has intensified, ced-9-transformed tobacco and tomato plants were shorter and produced less or almost no fruit. The main protein kinases are protein kinase A (PKA), namely Zn²⁺-dependent nucleic acid endonucleases and Ca²⁺-dependent nucleic acid endonucleases. ZENI and BENI are two typical examples of Zn²⁺-dependent nucleic acid endonucleases, the former with a molecular weight of 43 kD obtained from the developing ductal tissue of Cichorium leaves,

1.4 Programmed death molecular signaling in plant cells

Ca²⁺ and calmodulin as signaling molecules are very important for programmed cell death in many plants. It has been found that the elevation of Ca²⁺ can directly activate calcium dependent endonucleases, RNase and DNase activities in petal senescence were promoted by Ca²⁺ and inhibited by EDTA, are both important in the regulation of programmed cell death in plants, as supported by numerous experimental findings.

1.5 Screening of small molecule inhibitors of programmed death in plants

Through a summary of the extensive literature, hydrogen sulfide (H₂S) is found to have received extensive research and attention as the third gaseous signaling molecule discovered in recent years, cell physiology, and molecular level assays, and analyzed the effects of changes in endogenous H₂S content under NaHS induction on the inhibition of programmed cell death in plants [28].

2. Materials and methods

2.1 Experimental materials

For the experiment, cucumber cultivars of Xinchun 4 were selected, and seeds with consistent size and full grain were soaked for 6-8 h, after which the seeds were placed in Petri dishes for 48 h of dark germination and the temperature was set at 28 ° C.

2.2 Experimental methods

The first part was to screen the suitable concentration of H₂S, and a total of 6 treatments were set up in the test. Control: Cultured in distilled water for 4D; they were further treated with 100 μ M NaHS for 24 h and finally incubated with distilled water for 48 h; NaHS pre-treatment + CD: seeds were germinated for 24 h and then continued with 100 μ M NaHS were treated for 24 h and finally replaced with 200 μ M MCD were subjected to stress treatment for 48h.

2.3 Detection indicators

After the operation of above experimental methods, the plant's root length and fresh weight, relative electrical conductivity, MDA, root vigor, endogenous H₂S content, hydrogen peroxide and superoxide anion content, and apical cell death number were measured. The specific assay method was as follows.

put in the incubator at 37 ° C under dark heat

2.4 data statistics and analysis

Statistics and mapping of the data were performed using Excel 2010, and SPSS 19.0 was used for one-way ANOVA of the data ($P < 0.05$) with Duncan's for multiple comparisons.

3. Results

3.1 Effects of different concentrations of NaHS on root length and fresh weight of Cucumber Seedlings under Cd Stress

To select out NaHS concentrations suitable for subsequent trials, set 1, 10, 100, and 200 μ M concentrations were pretreated on cucumber seedlings which were germinated for 24h. With increasing NaHS concentration, cucumber root length and fresh weight first increased but then decreased. Versus 200.0 μ M MCD treated Mm NaHS (100 μ M) Can promote the root elongation of Cucumber Seedlings under Cd stress, so the test used 100 μ M NaHS for further experiments. As shown in Fig.1.

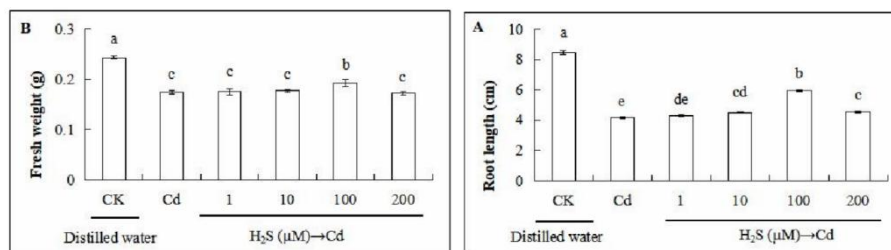


Fig. 1: Effects of NaHS on root length and fresh weight of Cucumber Seedlings under Cd stress.

3.2 Effect of Exogenous NaHS on Endogenous H2S Content in Cucumber Seedling Roots

In order to study the relationship between endogenous H2S and Cd stress in cucumber seedling roots, while exogenous NaHS induction could further increase the accumulation of endogenous H2S to promote the transmission of endogenous H2S signals. As shown in Figure 2.

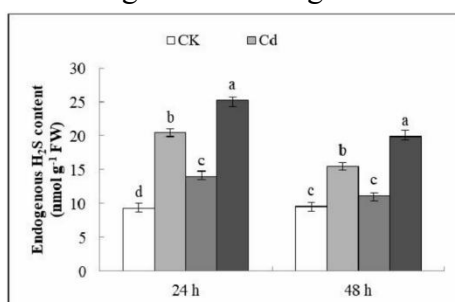


Fig. 2: Effects of different treatments on endogenous H2S content of cucumber seedlings.

3.3 Effects of H2S on relative conductivity, MDA, root activity, H2O2 and O2 content of cucumber seedlings under Cd stress

It can be seen from Table 3-1 that Cd stress increases the relative conductivity and MDA content of cucumber seedling roots, It decreased by 37.21%. There was no significant difference between NaHS pretreatment alone and the control. As shown in Table 1.

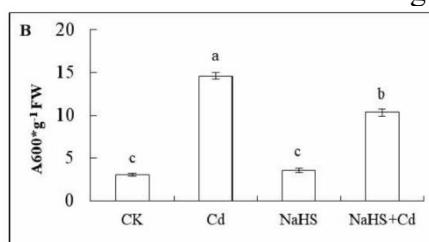
Treatmens	ELF (%)	MDA (umol:g-1 FW)	Root system activity (mg*g-1*h-1)	H2O2 (umol*g-1 FW)	O2- (nmol*g-1FW)
CK	37.44±11.54 c	1.15±0.23c	49.21±7.29a	4.40±0.33	0.07±0.02
Cd	80.15±3.03 a	2.21±0.11a	23.01±0.77b	7.33±0.44	0.44±0.06
NaHS	41.88±1.71	1.22±0.10c	43.59±3.99a	4.56±0.41	0.13±0.01
NaHS+Cd	61.21±4.23 bc	1.88±0.09b	29.88±2.67a	6.09±0.10	0.25±0.04

Table 1: Effect of H2S on relative conductivity, MDA, root activity, H2O2 and O2 content of cucumber seedlings under Cd stress

3.4 Effect of H2S on root tip cell death under Cd stress

Cd stress induced the death of root tip cells to inhibit root elongation. To explore whether H2S could reduce cell death, Evans blue staining was used to detect. However, NaHS+Cd treatment significantly reduced the Evans blue abundance by 29.22%, indicating that NaHS pretreatment can reduce Cd induced root tip cell death and ensure root elongation.

Figure 3: Effect of NaHS on Cell Death of Cucumber Seedling Root Tip under Cd Stress



4. Discussion

the effect is concentration dependent. Cd induced the accumulation of endogenous H₂S, indicating that H₂S participated in the response to Cd. H₂S can reduce the accumulation of ROS and membrane lipid peroxidation by activating the antioxidant system. H₂S can reduce the death of root tip cells by reducing oxidative damage.

4.1 Guess about the application of programmed cell death in crop stress resistance engineering

Although the application of programmed cell death is still rare, we found that it has great development space in crop stress resistance engineering.

4.2 The conjecture of the application of programmed cell death in improving crop shape

The phenomenon of programmed cell death is widespread in plants under different conditions, and the internal gene sequence is more inclined to the internal original sequence, that is, such genes begin to express at the early stage of plant development, and their status is more important on chromosomes. this conjecture will have great potential in the future.

Reference

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