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得獎獎項	二等獎 義大利科學博覽會 FAST

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關鍵詞	<u>localized stress、signaling mechanism、 substance allocation</u>
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作者簡介



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我是呂家瑋同學，目前就讀國立台南第一高級中學科學班。我因為入高中後發覺自己對生物以及科學研究有興趣而選擇做科展，並希望自己能在比賽過程中發揮所長，分享研究內容。

我是賴楚元，就讀國立臺南第一高級中學科學班。享受在實驗中探索未知，經過不斷的嘗試完成研究，也對在國際科展分享成果十分期待。

2024年臺灣國際科學展覽會

研究報告

區 別：

科 別：植物學科

作品名稱：大「逆」不道—局部逆境下植物體內傳訊與物質分配機制

關 鍵 詞：localized stress、signaling mechanism、substance allocation

編 號：

Abstract

When a leaf of a plant encounters stress, how does the plant convey the stress signal to other tissues and manage nutrient distribution? This field of study has been largely unexplored. However, the unique interconnected frond structure of *Lemna trisulca*, along with the use of a divided Petri dish, is very suitable for handling localized stress and investigating the mechanisms of intracellular signaling and nutrient distribution.

Research has shown that when the mother leaf experiences localized stress, it releases healthy daughter leaves to minimize collateral damage to the daughter leaves. Conversely, when the daughter leaves face localized stress, the mother leaf chooses to retain them and continues supplying them with nutrients to support their survival. In-depth studies revealed that stressed daughter leaves accumulate Reactive Oxygen Species (ROS), triggering nutrient distribution by sending a distress signal to the mother leaf. This prompts the mother leaf to use Ca^{2+} as a signaling molecule to deliver nutrients to the daughter leaves.

Selective detachment is regulated and triggered by the interaction between Ca^{2+} and ROS within the mother leaf. When the mother leaf undergoes stress, Ca^{2+} acts upstream to induce ROS accumulation at the nodes, sending a unidirectional detachment signal to the daughter leaves. This causes ROS accumulation at the daughter leaf nodes, inducing detachment and thereby reducing the collateral damage the daughter leaf could experience due to the mother leaves.

I. Introduction

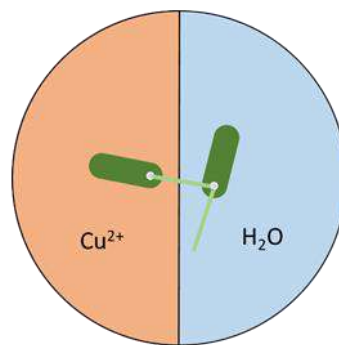
i. Research Motive and Background

When a single leaf of a plant encounters stress, how does the plant transmit this stress signal to other tissues and distribute nutrients? We observed that the aquatic plant *Lemna trisulca* has a unique frond connection structure, where the daughter fronds are connected to the nodes of the mother frond by long stalks, creating a large, interconnected network. This sparked an idea! Isn't this unique connection just perfect for creating localized stress using a split plate setup? By leveraging the characteristics of *Lemna trisulca* and this device design, we can delve deeply into the mechanisms of inter-tissue signal transmission and nutrient distribution under localized stress in plants. Thus, we used the split plate to subject *Lemna trisulca* to localized stress, hoping to uncover the mysteries behind this process.



第一作者拍攝

Figure 1-1. Interconnected structure of *Lemna trisulca*



第二作者繪製

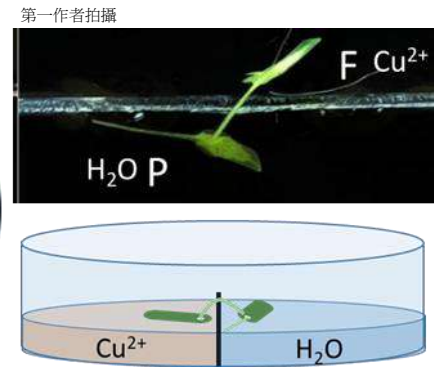


Figure 1-2. Diagram of Divided Petri Dish Setup to Localized Stress

ii. Research Purpose

- (1) Select the appropriate type and concentration of metal ion stress for *Lemna trisulca*.
- (2) Subject *Lemna trisulca* to localized stress and observe its morphological and physiological responses.
- (3) Investigate the initiation site, signaling direction, and underlying physiological significance of the selective detachment phenomenon in *Lemna trisulca*.

- (4) Explore the proximal and distal signaling mechanisms involved in the selective detachment process of *Lemna trisulca* under localized stress.
- (5) Explore the mechanism of nutrient allocation in *Lemna trisulca* under localized stress.
- (6) Explore the proximal and distal signaling mechanisms in the nutrient allocation process of *Lemna trisulca* under localized stress.
- (7) Explore the interactions between signaling molecules in *Lemna trisulca* under localized stress.

iii. Literature Review

Nutrient allocation within plants is the process by which nutrients are effectively distributed to different tissues and organs to support growth and development, such as transferring nutrients to mature fruits or reallocating nutrients from aging leaves to new leaves. This process involves complex signaling pathways, including the role of cytokinin (CK) (Moustafa Fathy, 2022).

Due to the widespread use of copper-containing pesticides and the discharge of copper ion pollutants into the environment, especially in streams, copper pollution has harmful effects on plants. In plants, excessive copper ions can induce toxicity through the generation of Reactive Oxygen Species (ROS) and the disruption of enzyme and protein structures (Ru-Xin Wang, 2024). To cope with changes in copper levels in the environment, plants precisely regulate copper ion homeostasis through various mechanisms. This article describes the impact of copper ions on biological functions, the toxicity of excessive copper ions to plants, the transport processes of copper ions in plants, and the mechanisms of plant detoxification and tolerance to copper stress. Furthermore, potential research directions are suggested for better understanding the molecular regulatory mechanisms of plants under copper stress.

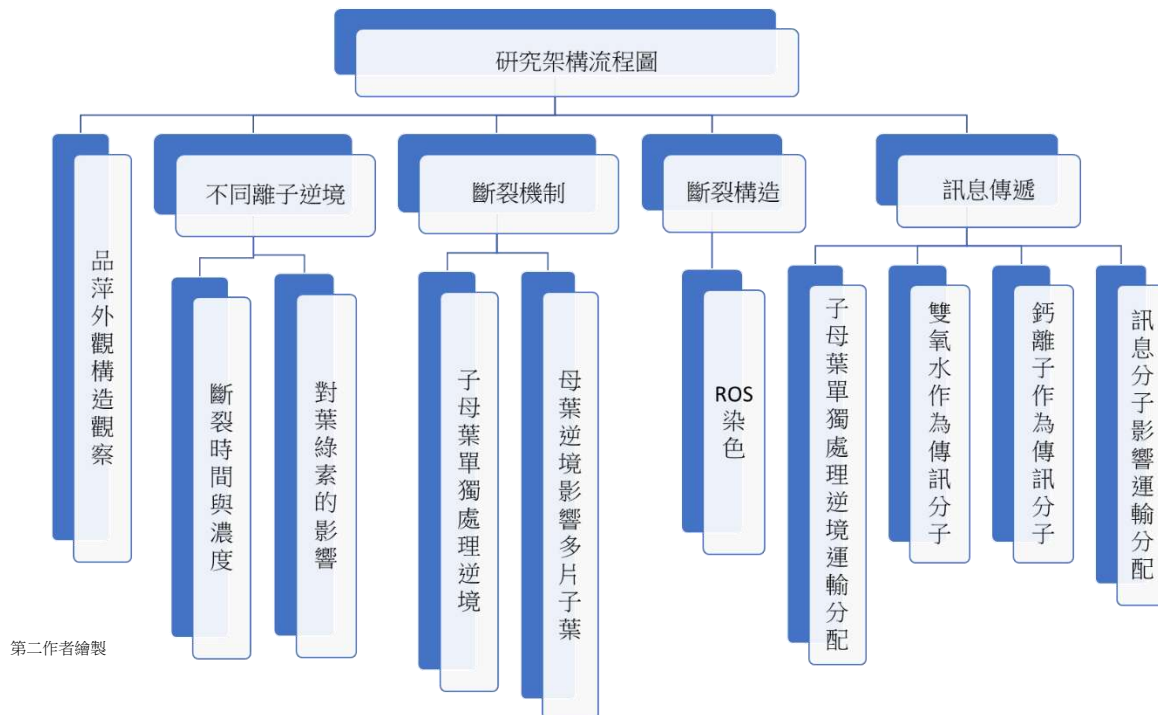
Most plants, when facing stress, utilize the increased reactive oxygen species (ROS) and Ca^{2+} in their system, with the help of the RBOH enzyme system, to create a positive feedback loop, transmitting a unidirectional ROS wave. This allows for long-distance transmission of stress signals, enabling the plant to initiate stress responses to maximize survival (Takamitsu Kurusu, 2015).

Combining the above concepts, we aim to explore if plants allocate nutrients under stress and how signal transduction relates to this process. Using *Lemna trisulca* with its unique leaf structure, we will investigate internal signal transduction and nutrient allocation under stress using a split plate setup.

II. Materials and Instruments Used

- i. **Plant Materials:** *Lemna trisulca*
- ii. **Experimenting Materials:** CuCl₂, KCl, NaCl, DAB, MES buffer (pH 6.8), NAC, Ethanol, H₂O₂, EDTA, EGTA
- iii. **Experimenting Instruments:** Glass Beaker (100ml), Electronic balance, Petri dish, Divided Petri dish, Electronic Oscillator, Quartz Tube, Spectrophotometer, Micropipette, Dissecting microscope, Compound microscope.

III. Research Method



- i. **Cultivation of *Lemna trisulca***

- (1) Purchase *Lemna trisulca* from aquatic plant suppliers and cultivate it in a water tank.
- (2) Change the water every week regularly.

ii. Chlorophyll Concentration Measurement

- (1) Place *Lemna trisulca* into test tubes containing 95% EtOH.
- (2) Heat the test tubes to 80°C and maintain this temperature for 15 minutes.
- (3) Remove the plant, leaving the chlorophyll-EtOH solution.
- (4) Measure the absorbance of the solution using a spectrophotometer (A_{645} / A_{663}).
 - (i) Conc. Chlorophyll a : $13.7 \times A_{663} - 5.76 \times A_{645}$
 - (ii) Conc. Chlorophyll b : $25.8 \times A_{645} - 7.60 \times A_{663}$
- (5) Chlorophyll conc. = Chlorophyll a conc. + Chlorophyll b conc.

iii. Connection Rate Calculation

- (1) Select 10 consecutive *Lemna trisulca* leaves, noting that the number of connections is 9, and record the initial connection number as C_0 .
- (2) Determine the connection number C from the experimental results.
- (3) Connection Rate = $\frac{C}{C_0}$

iv. Reactive Oxygen Species (ROS) Staining 【DAB Staining】

- (1) Dissolve 0.2 g of DAB in 100 ml of MES buffer solution (pH 6.8).
- (2) Place the samples and DAB solution in a petri dish.
- (3) Apply a vacuum for 10 minutes, then incubate in pure darkness for 8 hours.
- (4) Place the samples in 99% EtOH to decolorize the chlorophyll.
- (5) Observe the stained leaf samples under a microscope to observe staining results

v. Definition of Daughter/Mother Leaves

Remove the duckweed's bifoliate, with the connected one called the mother leaf and the connecting one called the daughter leaf.

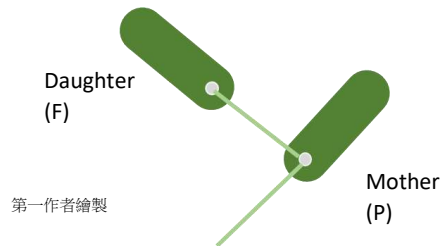


Figure 1-1. Daughter and Mother leaf diagram

vi. Divided Petri Dish Experiment

Cut a 1cm-by-1cm hole in the center of a 9cm Petri dish divider. Place the daughter and mother leaves of the duckweed on opposite sides of the Petri dish and add 10ml of different solutions.

- (1) Investigation of detachment: Add 2 ml of 10 mM Cu^{2+} solution to one side of the dish and H_2O to the other, observing it with a dissecting microscope.
- (2) Investigation of substance distribution: Add 2 ml of red ink to one side of the dish in localized stress, observing it with a dissecting microscope.
- (3) Investigation of signaling mechanism:
 - (i) Add 2 ml of 10 mM H_2O_2 solution along with 10 mM NAC to one side of the dish.
 - (ii) Potential DAB staining for further exploration.

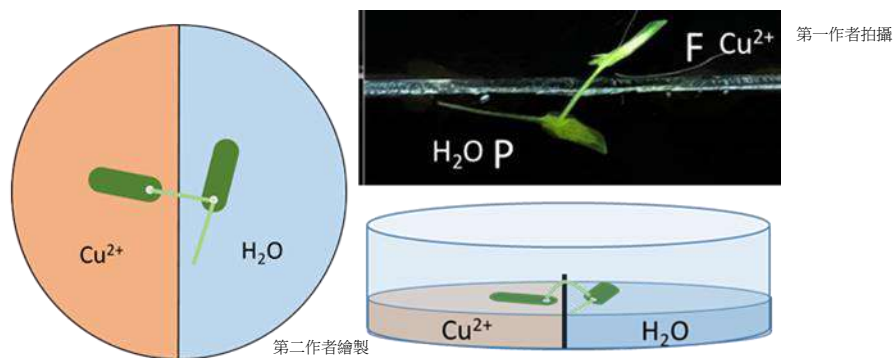


Figure 1-2. Diagram of creating localized stress condition using a divided Petri dish

vii. Calcium Ion Signaling

- (1) Place 10 connected leaves in 50 ml beaker and treat them with 50 ml 10 μM Cu^{2+} solution.
- (2) Add Ca^{2+} at concentrations of 5 μM , 10 μM , and 15 μM to observe the abscission.
- (3) Add 0.1 mM EGTA to chelate Ca^{2+} and confirm the role of Ca^{2+} in the signaling process.
- (4) Observe the results when Ca^{2+} is chelated.

viii. ROS Signaling

- (1) Place 10 connected leaves in a 50 ml beaker and treat them with 50 ml of 10 mM H_2O_2 solution to induce oxidative stress.
- (2) Observe the abscission. Add 1 mM, 5 mM, and 10 mM of NAC to observe the effect of ROS clearance.

IV. Result

i. Morphological Observation

Among various duckweeds, *Lemna trisulca* has a particularly unique structure! Under the microscope, *Lemna trisulca* exhibits a connected growth pattern. The new daughter leaves emerge from the node of the mother leaf and are attached by a long stalk, as shown in Figure 1-1. Each mother leaf can bear one or two daughter leaves,

and as these daughter leaves mature, they can act as mother leaves, giving rise to new daughter leaves from their nodes, continuously extending and forming an intricate, interwoven structure, as shown in Figure 1-2.



Figure 1-1. The appearance of *Lemna trisulca* under a compound microscope. (100x) **Figure 1-2. The appearance of *Lemna trisulca* under a dissecting microscope (10x) / (40x)**

In addition, *Lemna trisulca* is a rare submerged type of duckweed. As shown in Figure 1-3, only the top layer of *Lemna trisulca* floats on the water surface, while the rest remain submerged. This growth pattern presents the issue of light obstruction! The lower layer of *Lemna trisulca* is shaded by the upper layer, and the light underwater is significantly reduced compared to the surface. As a result, the lower plants should have lower photosynthesis and poorer growth. However, observations reveal that the submerged *Lemna trisulca* remains vibrant green, with no noticeable difference in leaf size or shape compared to the unshaded surface plants. We speculate that there is a nutrient distribution mechanism among the *Lemna trisulca*! The upper, unshaded plants may be transporting photosynthetic nutrients to the shaded, lower plants.



Figure 1-3. Growth of *Lemna trisulca* underwater.

After the above observations, we had a sudden idea! *Lemna trisulca* can likely distribute nutrients internally, and its unique frond connection structure is well-suited for creating localized stress on one side using a split-dish setup. This makes it an ideal material for exploring how plants transmit signals and allocate nutrients under localized stress conditions. Therefore, we selected *Lemna trisulca* and designed a split-dish apparatus for further in-depth research.

ii. Selection of Suitable Metal Ion Stress

Before conducting in-depth research on signal transmission and nutrient distribution under localized stress in plants, it is crucial to first select the type of stress. The freshwater habitats of *Lemna trisulca* are often polluted by metal ions. Therefore, we aim to identify which metal ions cause stress to *Lemna trisulca*. We selected common metal ions (K^+ , Na^+ , Mg^{2+} , Mn^{2+} , Cu^{2+}) and a control group (H_2O). Ten interconnected leaves were immersed in these solutions at concentrations of 1, 5, 10, 15, and $20\mu M$, allowing them to grow for 24 hours. We then observed their growth conditions, measured chlorophyll content, and calculated the connection rate and chlorophyll concentration.

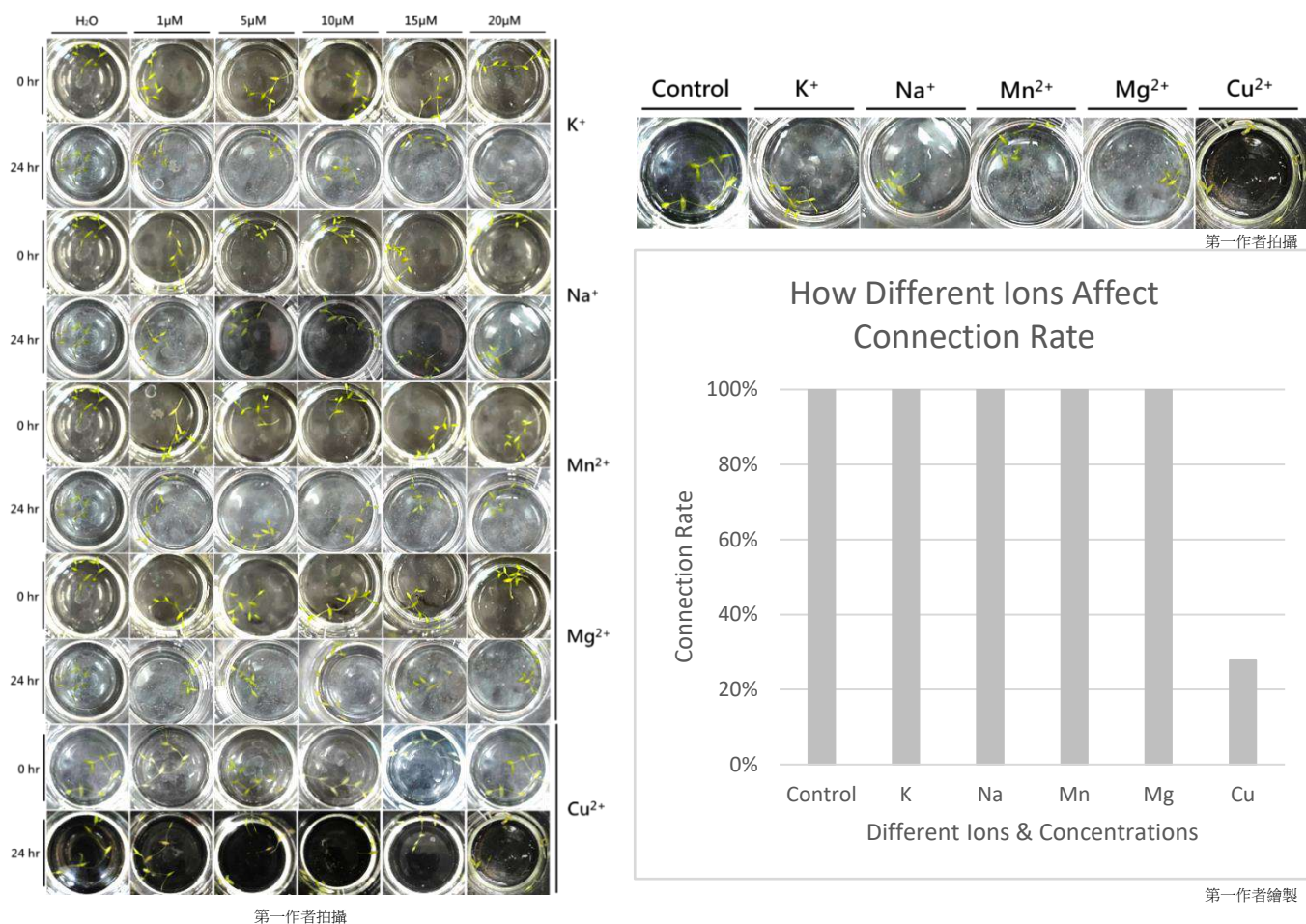


Figure 2-1. The effects of each metal ion on *Lemna trisulca* growth, growth condition, connection rate of *Lemna trisulca* in 10 μ M of each metal ion

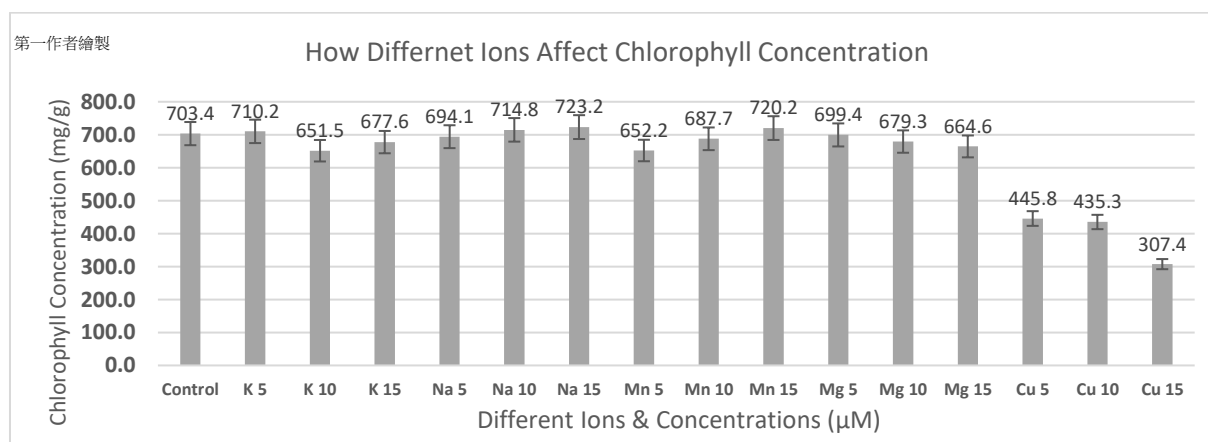


Figure 2-2. The chlorophyll concentration of *Lemna trisulca* in 5 μ M, 10 μ M, and 15 μ M of each metal ion

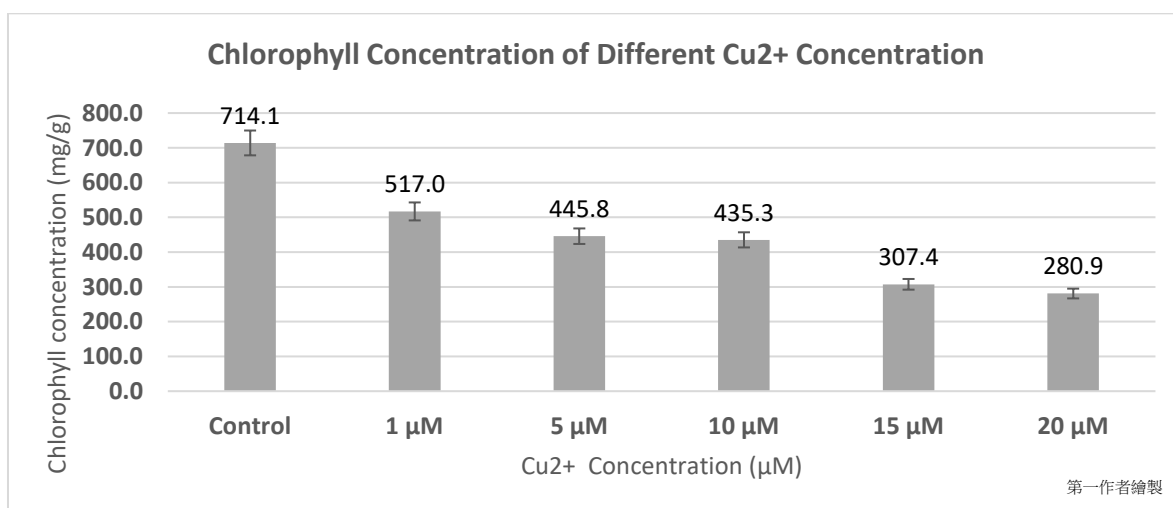
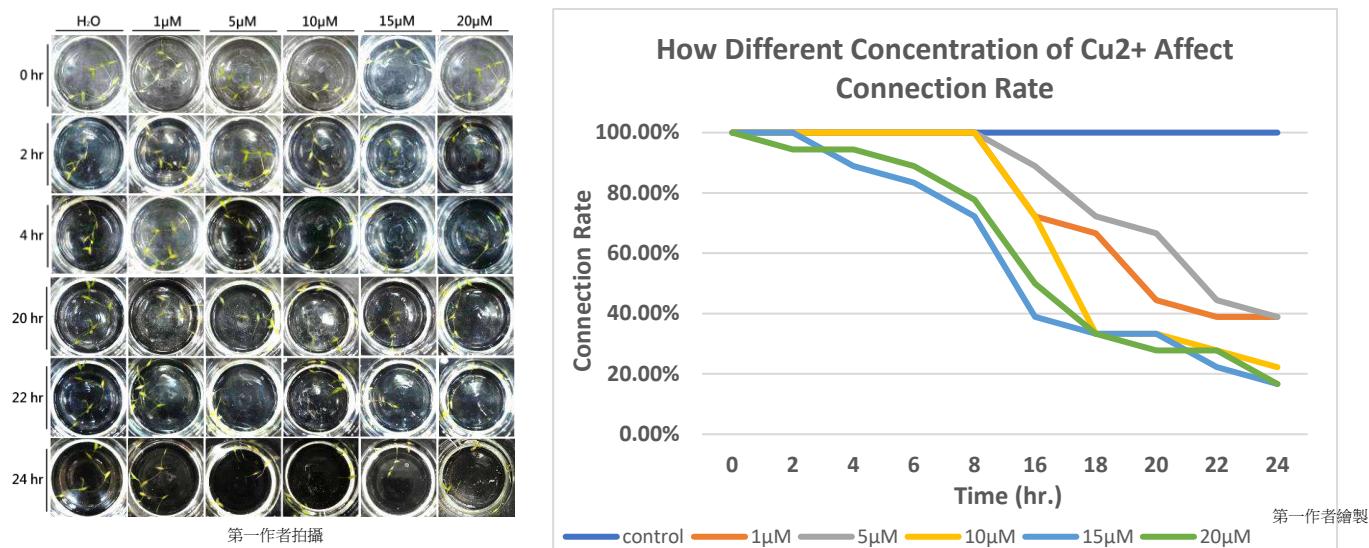
Based on the above charts, *Lemna trisulca* showed no bleaching or fragmentation in the presence of K^+ , Na^+ , Mn^{2+} , Mg^{2+} , with an 100% connection rate, meaning all 9 connections remained intact, and the chlorophyll concentration stayed the same. Thus, it is inferred that **15 μ M concentrations of K^+ , Na^+ , Mn^{2+} , Mg^{2+} do not harm *Lemna trisulca*.** In contrast, after 24 hours in a Cu^{2+} environment, 60% of the connected structures fractured, and chlorophyll concentrations significantly dropped, leading to bleaching and wilting. Therefore, it is concluded that **15 μ M Cu^{2+} causes stress damage to *Lemna trisulca*.**

Based on the two pieces of evidence above, it is inferred that **15 μ M Cu^{2+} constitutes a stress condition for *Lemna trisulca*, resulting in detachment, bleaching, and wilting.** Therefore, **Cu^{2+} will be used as the treatment condition for local stress in subsequent experiments.**

iii. Finding Optimal Cu^{2+} Stress Concentration

(1) Detailed observation of the detachment of *Lemna trisulca* at fixed time intervals.

Previous experiments confirmed that Cu^{2+} induces stress in *Lemna trisulca*. To further **explore the optimal Cu^{2+} concentration for local stress experiments**, we decided to investigate the relationship between different Cu^{2+} concentrations and the time required for detachment and reduced connectivity. Thus, we immersed 10 connected *Lemna trisulca* leaves in Cu^{2+} with concentrations of 1, 5, 10, 15, and 20 μ M, allowing them to grow for 24 hours. Every two hours we observed and recorded the number of connections to calculate the connection rate.



Based on the above data, *Lemna trisulca* exhibited a significant decline in connection rate under all concentrations of Cu²⁺, with it dropping to below 40% after 24 hours—leaving only about three or fewer connections out of nine—compared to the control group (H₂O). Examining the relationship between concentration and time in more detail, at 15 and 20 μM of Cu²⁺, *Lemna trisulca* began to show detachment after just 2 hours, with continuous detachment reducing the connection rate to around 16.7% after 24 hours. Under 10 μM Cu²⁺, no

detachment occurred in the first 8 hours, but after 18 hours, the connection rate quickly dropped to around 33.3%, and by 24 hours, it had decreased to approximately 22.2%. At 1 and 5 μM of Cu^{2+} , the detachment was less severe, with a higher connection rate of around 38.9% after 24 hours.

From the chlorophyll concentration data, it is evident that after 24 hours in a Cu^{2+} environment, *Lemna trisulca* experienced a significant decline in chlorophyll levels. The higher the Cu^{2+} concentration, the lower the chlorophyll concentration, with bleaching and wilting becoming more severe at 10, 15, and 20 μM .

Based on the above evidence, it is inferred that within the Cu^{2+} concentration range of 1-20 μM , the higher the Cu^{2+} concentration, the lower the connection rate and chlorophyll concentration in *Lemna trisulca*, indicating a more pronounced stress response. To ensure clear stress and detachment effects in subsequent experiments without causing excessive bleaching or death, a Cu^{2+} concentration of 10 μM —where both the connection rate and chlorophyll concentration drop by about half—was chosen as the treatment concentration for localized stress experiments.

iv. Mechanism of Detachment Under Copper Stress in Daughter/Mother Leaves

(1) Mother/Daughter leaf detachment in localized stress

From the above experiments, we learned that *Lemna trisulca* undergoes detachment under copper stress. This led us to wonder whether this detachment mechanism is initiated by the mother leaf or the daughter leaf. To investigate, we used connected *Lemna trisulca* pairs—one mother leaf and one daughter leaf—and placed them in a divided Petri dish, treating the daughter leaf with a 10 μM Cu^{2+} stress while keeping the mother leaf in H_2O , then observed the detachment.

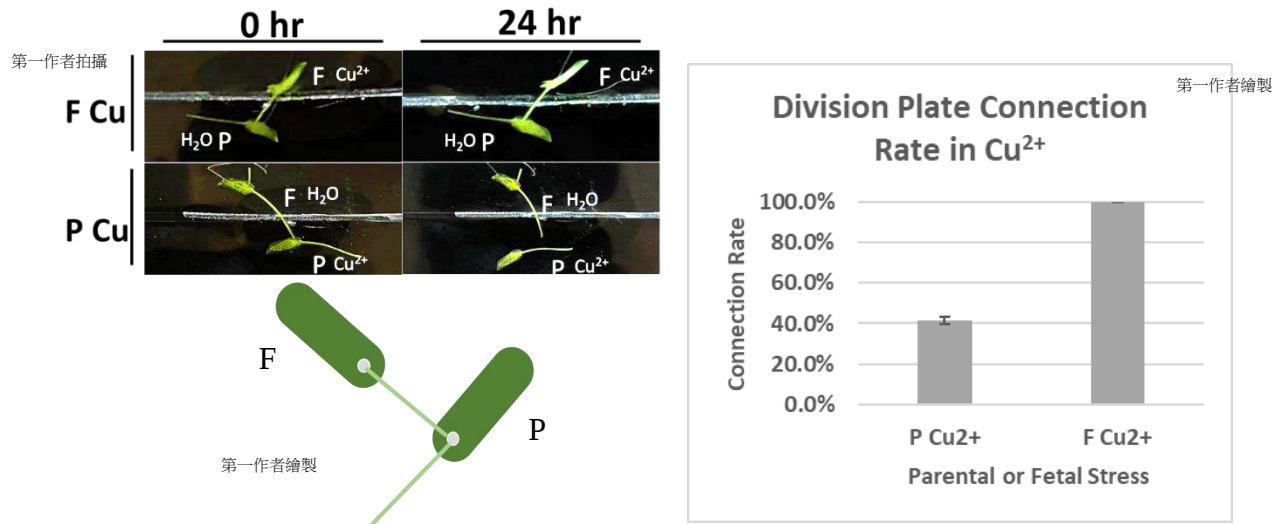


Figure 4-1. The detachment, connection rate, and diagram of the *Lemna trisulca* where daughter/mother leaf is treated with local Cu²⁺ stress.

As shown in Figure 4-1, when the mother leaf is exposed to stress alone, there is only about 40% connection rate between the *Lemna trisulca* pair. However, when the daughter leaf is treated with stress alone, no detachment occurs. We hypothesize that the selective detachment phenomenon is **initiated by the mother leaf**, and only when the mother leaf is stressed will it send a detachment signal to the daughter leaf, inducing detachment in the daughter leaf. This mechanism helps reduce the risk of healthy daughter leaves being affected by the stressed mother leaf. Conversely, when the daughter leaf is exposed to local stress, the mother leaf does not release the daughter leaf, suggesting that **the detachment signal is not sent back from the stressed daughter leaf to the healthy mother leaf**. This phenomenon helps the healthy mother leaf continue to provide nutrients to support the survival of the stressed daughter leaf.

Based on the above research, we hypothesize that **in the Cu²⁺ stress condition, the detachment signal in *Lemna trisulca* is initiated by the mother leaf and is transmitted unidirectionally! The detachment signal is only transmitted from the stressed mother leaf to the healthy daughter leaf**, and the stressed daughter leaf does not send a signal back to the healthy mother leaf.

After understanding that the detachment signal in *Lemna trisulca* is initiated by the mother leaf and transmitted unidirectionally, we became curious about **how many healthy daughter leaves the stressed mother leaf can transmit the detachment signal to**. Therefore, we treated the mother leaf at P1 with 10 μM Cu^{2+} stress on one side of the Petri dish, while treating 1, 2, 3, or 4 daughter leaves with H_2O on the other side, and observed at which leaf detachment occurs in *Lemna trisulca*.

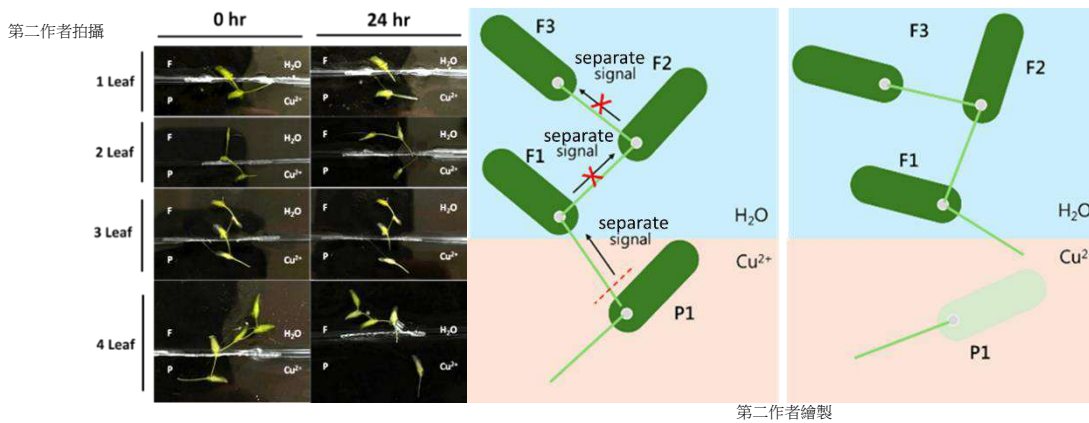


Figure 4-2. The detachment of multiple daughter leaves by the stressed mother leaf.

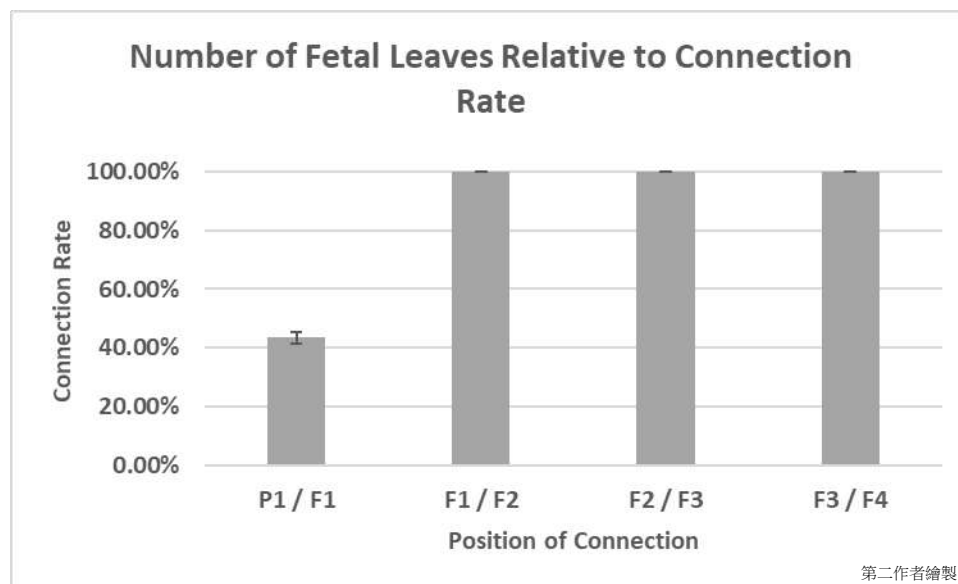


Figure 4-3. The connection rate of multiple daughter leaves (F1, F2, F3, F4) by the stressed mother leaf (P1).

As shown in Figure 4-3, when a mother leaf is connected to multiple daughter leaves and treated with stress, only the first daughter leaf (F1) experiences detachment from the mother leaf (P1), with a connection rate of around 40%. No detachment occurs among other daughter leaves. We infer that **the detachment signal from the stressed mother leaf can only be transmitted to the closest daughter leaf (F1) and does not propagate further to the second daughter leaf (F2)**, likely to protect the other daughter leaves and ensure maximum survival rate.

Next, we wanted to see if the **detachment signal truly only travels unidirectionally from the mother leaf to the daughter leaves**. To evaluate this, we created three separate water environments using two divided Petri dishes, with the middle exposed to copper stress. We placed the daughter leaf closest to the mother leaf (P1) in a 10 μM copper stress, while the other leaves (P2 and F1) were kept in H_2O , observing whether the detachment signal would return to the mother leaf.

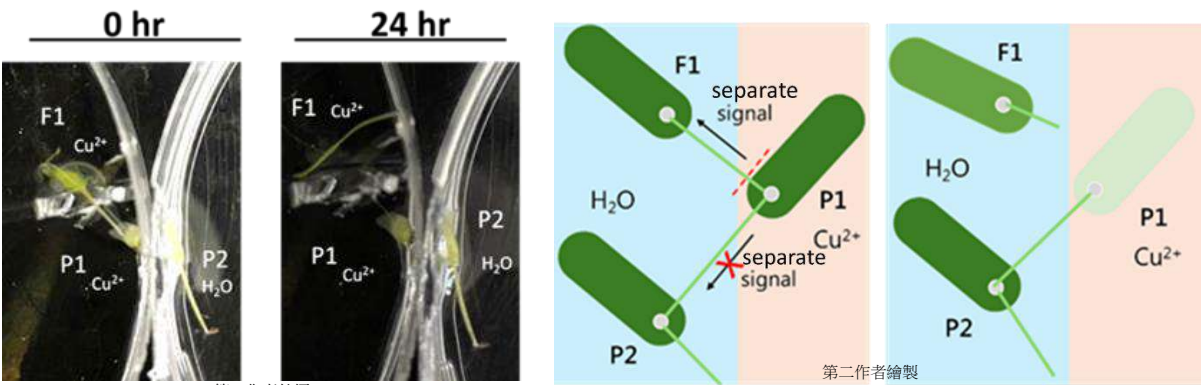


Figure 4-4. Detachment behavior of middle daughter leaf (P1)

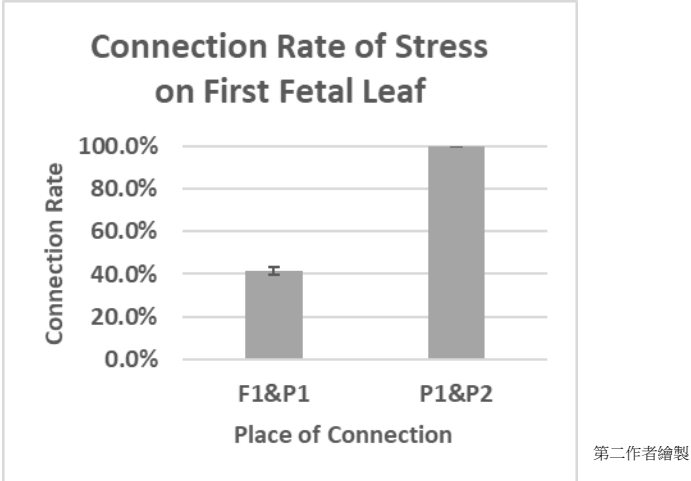


Figure 4-5. Connection rate of middle daughter leaf (P1)

As shown in Figure 4-5, we observed a notable detachment between the first daughter leaf (F1) and the middle leaf (P1), allowing F1 to remain unaffected by stress. However, the connection between F1 and the mother leaf (P) stayed intact with no detachment occurring. This confirms that **the detachment signal does not travel back to the healthy mother leaf, aligning with our earlier findings that the signal is transmitted unidirectionally from P1 to F1**. This mechanism enables the mother leaf to continue supporting the stressed daughter leaf.

v. Chlorophyll Concentration Under Whole-plant Stress Treatment and Individual Stress Treatment.

Based on the above experiments, we discovered that when a leaf encounters stress, the detachment signal cannot be transmitted back to the healthy parent leaf, leaving the stressed leaf still attached to the healthy one. What benefits could this bring? We hypothesize that maintaining the connection allows the healthy parent leaf to continue transporting substances to support the stressed leaf, enhancing its survival rate. To verify this assumption, we aim to test the plant's chlorophyll content as evidence for our hypothesis.

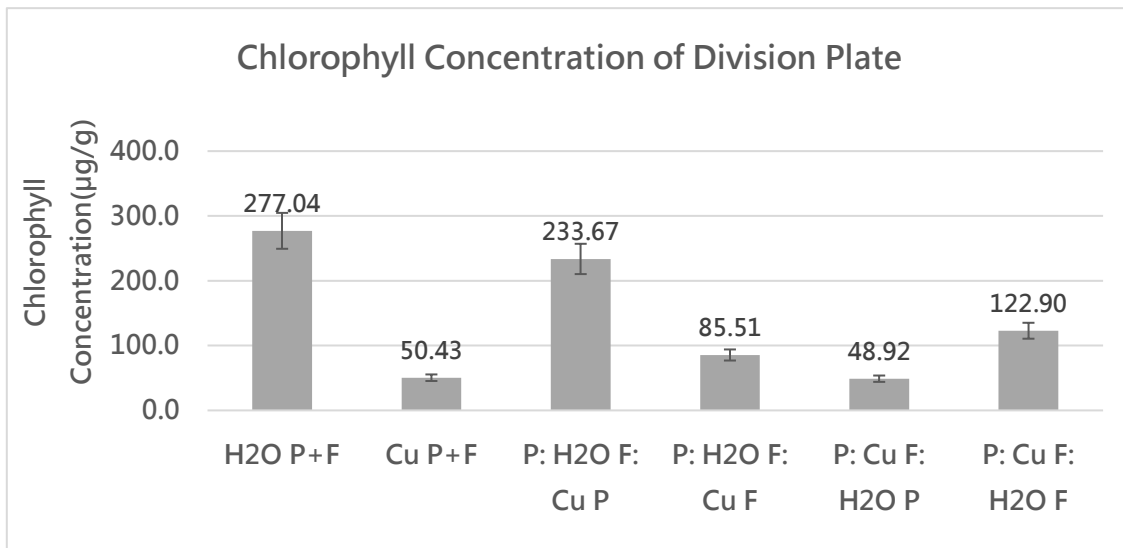


Figure 5-1. Chlorophyll content under individual copper stress treatment

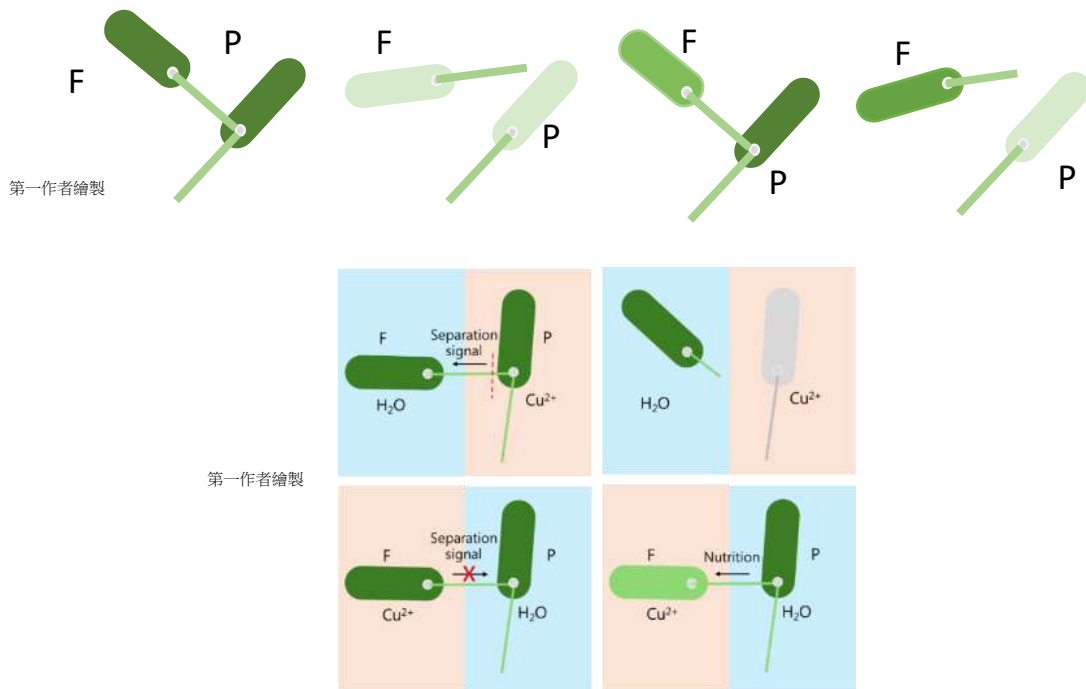


Figure 5-2. Diagram of results from the four experiments

The experimental results show that when only the mother leaf is exposed to stress, its chlorophyll content is similar to that of both leaves being treated under stress. The daughter leaf also shows a reduction in chlorophyll but is less affected, with its levels around 72.5 $\mu\text{g/g}$, higher than the 50.4 $\mu\text{g/g}$ observed when both leaves are exposed to Cu^{2+} stress. When only the daughter leaf is treated with stress, its chlorophyll decreases, but remains approximately 35.1 $\mu\text{g/g}$ higher compared to the full-plant stress treatment, while the mother leaf's chlorophyll content remains unchanged compared to the control group.

From the results, we conclude that **if the mother leaf is subjected to adversity alone, it will result in detachment, reducing the impact of the stressed mother leaf on the healthy daughter leaves**, allowing them to remain green and increasing their survival chances. Conversely, **if only the daughter leaf is subjected to adversity, detachment will not occur, allowing the daughter leaf to remain attached to the mother leaf. This enables the mother leaf to transport nutrients to support the growth of the daughter leaf**, reducing its wilting and enhancing its survival probability.

vi. **ROS is the Detachment Signal that is Unidirectionally Transmitted from the Mother Leaf to the Daughter Leaf**

After researching, we found that copper stress induces the accumulation of ROS in plants, which can cause harm but also function as signaling molecules to activate the plant's stress response mechanisms. Therefore, we were curious **whether ROS participates in the substance distribution and selective detachment mechanisms under local stress in *Lemna trisulca*, and what role it plays.** To investigate this, we conducted a series of studies.

(1) Observing the detachment phenomenon in *Lemna trisulca* under H_2O_2 stress.

First, we need to **confirm that ROS indeed participates in the detachment mechanism of *Lemna trisulca*.** We placed 10 connected leaves in solutions of 1 mM, 5 mM, and 10 mM H_2O_2 to observe the detachment phenomenon.

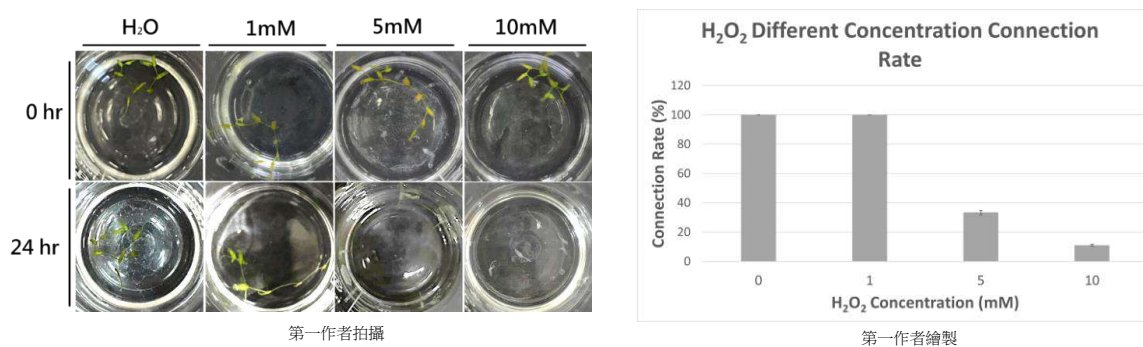


Figure 6-1. Detachment and connection rate of *Lemna trisulca* under H_2O_2 stress

(1) Observing the detachment phenomenon in *Lemna trisulca* under H_2O_2 stress.

As shown in Figure 6-1, **5 mM H_2O_2 can induce detachment in *Lemna trisulca*,** resulting in a connection rate of approximately 33.3%. In 10 mM H_2O_2 , *Lemna trisulca* exhibits significant detachment, with a connection rate of only about 16.7%. This indicates that **ROS do indeed participate in the detachment mechanism of *Lemna trisulca*.**

(2) *Lemna trisulca* treated with Cu^{2+} and added NAC to observe detachment.

The experiments above confirmed that ROS can initiate the detachment mechanism in *Lemna trisulca*, but **it does not guarantee that ROS induced by Cu^{2+} stress will promote the detachment mechanism.** To investigate this, we designed an experiment in which 1 mM, 5 mM, and 10 mM of NAC was added to the 5 μM and 10 μM Cu^{2+} stress to block the accumulation of ROS under Cu^{2+} stress, and we observed the detachment.

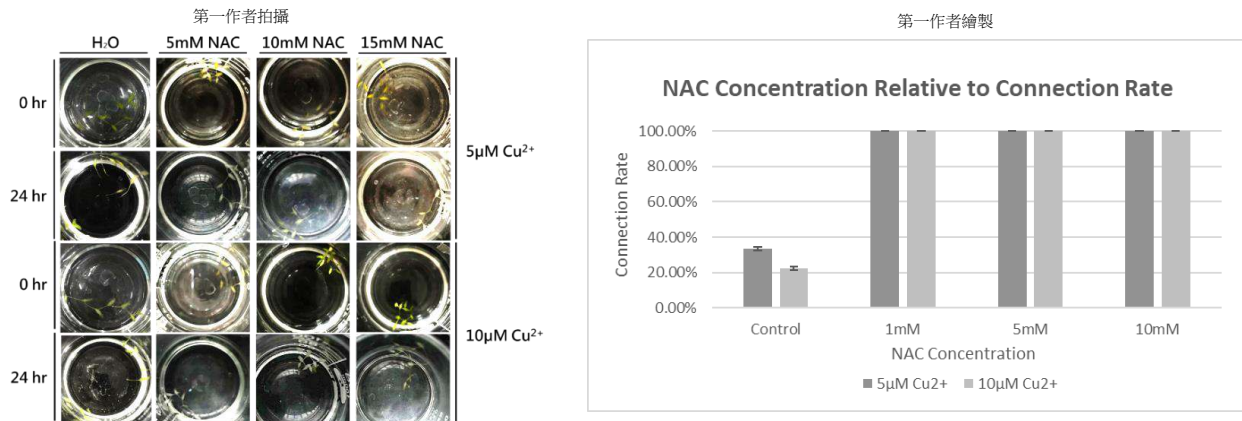


Figure 6-2. Detachment and connection rate of *Lemna trisulca* under Cu^{2+} stress and added NAC

As shown in Figure 6-2, although copper stress still causes *Lemna trisulca* to become chlorotic, **as long as NAC is added to remove the ROS induced by Cu^{2+} stress, *Lemna trisulca* will not exhibit any detachment!** The connection rate is 100.0%, which also confirms that the **ROS generated under Cu^{2+} stress indeed participates in the detachment mechanism of *Lemna trisulca*.**

- (3) ROS serves as a key signaling molecule for selective detachment of *Lemna trisulca* under localized stress.

The above experiments have confirmed that the ROS produced under Cu^{2+} stress indeed participates in the detachment mechanism of *Lemna trisulca*. Next, we are curious **whether ROS is the key signaling molecule that induces selective detachment of *Lemna trisulca* under localized stress.** To clarify this, we again used a divided Petri dish to treat either the daughter leaf or the mother leaf with 10 mM H_2O_2 and observed the detachment situation.

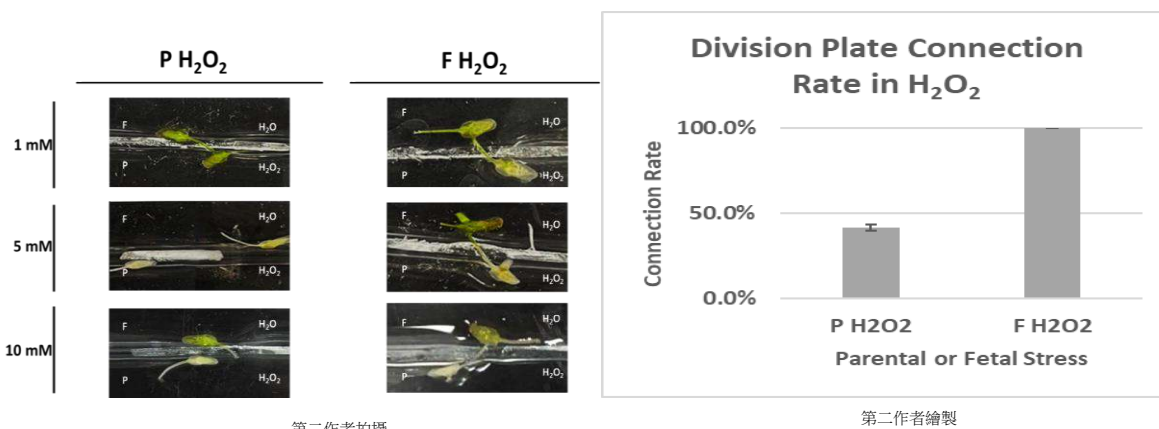


Figure 6-3. Detachment and connection rate of mother and daughter leaf under individual H_2O_2 stress

As shown in Figure 6-3, only the group where the mother leaf was individually treated with H_2O_2 exhibited detachment, with a connection rate of approximately 41.2%. In contrast, the daughter leaf individually treated with H_2O_2 showed no detachment, maintaining a connection rate of 100.0%. The experimental results are consistent with the previous findings under localized Cu^{2+} stress. Therefore, we speculate that **ROS acts as the key signaling molecule for selective detachment of *Lemna trisulca* under localized stress.**

(4) DAB staining of *Lemna trisulca* treated with Cu^{2+} stress

From the above experiments, we deduce that *Lemna trisulca* utilizes ROS as a signaling molecule for selective detachment under Cu^{2+} stress, and that the **detachment signal is unidirectionally transmitted (only from the mother leaf to the daughter leaf).** Therefore, we are curious **whether ROS is the unidirectionally transmitted detachment signal.** To clarify this, we first need to determine **where ROS accumulates and its distribution in *Lemna trisulca* under Cu^{2+} stress.** We used DAB staining to observe the accumulation sites of ROS under Cu^{2+} stress.

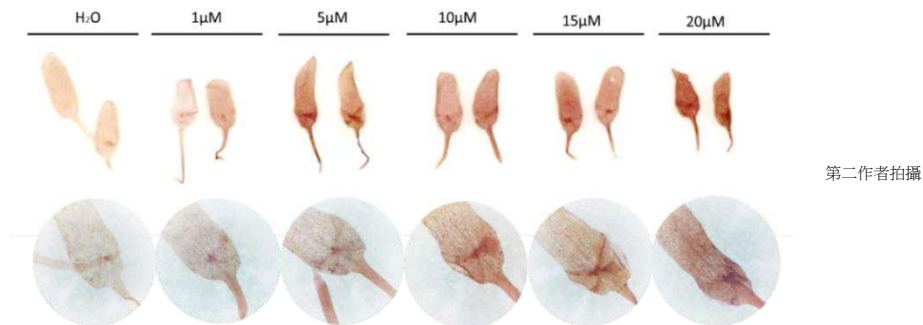


Figure 6-4. DAB staining of *Lemna trisulca* under Cu^{2+} stress

First, we treated the entire *Lemna trisulca* plant with Cu^{2+} stress and performed DAB staining. As shown in Figure 6-4, **the higher the concentration of Cu^{2+} , the more evident the accumulation of ROS at the nodes, which are also the location of detachment.** This result supports the hypothesis that ROS is related to the detachment mechanism in *Lemna trisulca*. Next, we used a divided Petri dish to allow *Lemna trisulca* to be treated with localized stress and observed the accumulation of ROS, as shown in Figure 6-5.

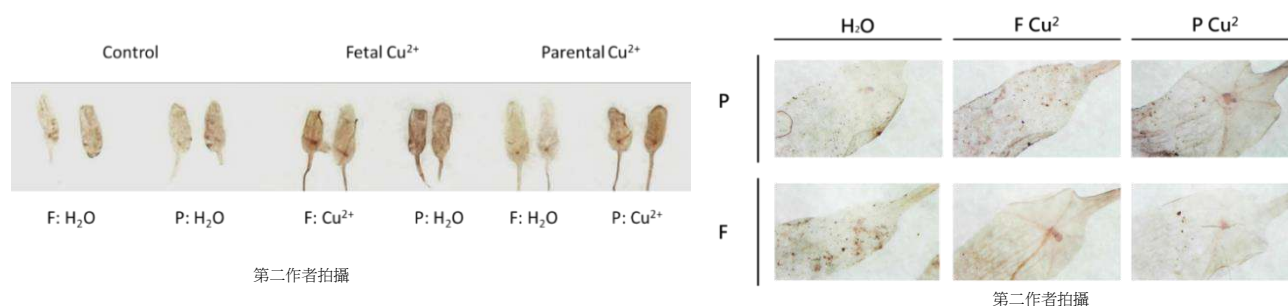


Figure 6-5. DAB staining of mother and daughter leaf under localized Cu^{2+} stress

When the daughter leaf of *Lemna trisulca* is treated with copper stress alone, ROS accumulates at its nodes, while the **mother leaf shows no accumulation, indicating that the stressed daughter leaf does not transmit a detachment signal back to the mother leaf.** In contrast, when the mother leaf is treated with Cu^{2+} stress, ROS accumulates in both nodes. This suggests that the **detachment signal from the stressed mother leaf is transmitted unidirectionally to the healthy daughter leaf**, inducing ROS accumulation, and leading to detachment. Thus, we

conclude that the stressed mother leaf transmits a detachment signal to the healthy daughter leaf, prompting ROS accumulation at its nodes.

- (5) ROS serves as the signaling molecule for long-distance communication within *Lemna trisulca* under localized stress conditions

Previous experiments suggest that the detachment signal from stressed mother leaves is transmitted unidirectionally to healthy daughter leaves, triggering ROS accumulation at the nodes. However, is the ROS accumulation in the daughter leaves the key factor inducing detachment? Would blocking ROS accumulation with NAC disrupt the transmission of the detachment signal, preventing detachment? To assess this hypothesis, we designed the following experiment.

We used a divided Petri dish to treat the mother leaf with Cu^{2+} stress while adding NAC to the daughter leaves to block ROS accumulation and disrupt the detachment signal transmission. The results are shown in Figure 6-6.

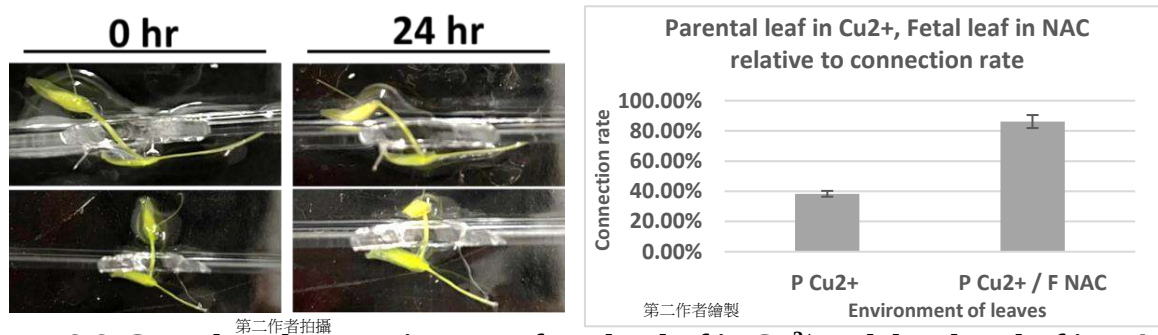


Figure 6-6. Growth and connection rate of mother leaf in Cu^{2+} and daughter leaf in NAC

When the mother leaf is subjected to stress while the daughter leaf is treated with NAC, the detachment rate significantly decreases, and the connection rate of *Lemna trisulca* increases to about 86%. This indicates that blocking the unidirectional transmission of ROS from the mother leaf to the daughter leaf prevents the activation of the detachment mechanism. The results demonstrate that the mother leaf transmits detachment signals to the daughter leaf, inducing ROS accumulation at the daughter leaf's nodes. The detachment mechanism is activated only when both the daughter and mother leaf nodes accumulate ROS. Since the detachment signal cannot be transmitted back from the daughter leaf to

the mother leaf, the daughter leaf cannot induce ROS accumulation at the distant mother leaf's nodes under stress. Therefore, the daughter leaf remains attached to the mother leaf, benefiting from the mother to enhance its survival under stress.

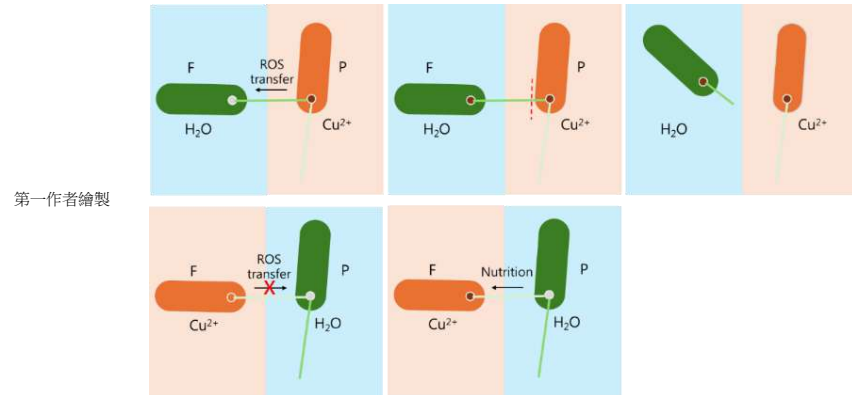


Figure 6-7. Diagram of signaling in *Lemna trisulca* under localized stress

vii. Substance Transport and Allocation Under Localized Stress

(1) Allocation in *Lemna trisulca* under localized Cu^{2+} stress

In the above experiments, we found that the mother leaf under Cu^{2+} stress induces the healthy daughter leaf to detach, reducing collateral damage, while the daughter leaf remains connected to the mother leaf to enhance its survival rate when it is under stress. **But does the healthy mother leaf actually transport substances to the stressed daughter leaf to help it resist stress?** To confirm the existence of the substance allocation mechanism within the plant under localized stress, we used **a divided Petri dish to create localized stress and added red dye**

to observe substance transport within the plant. As shown in Figure 7-1, we examined the samples using a dissecting microscope after 24 hours.

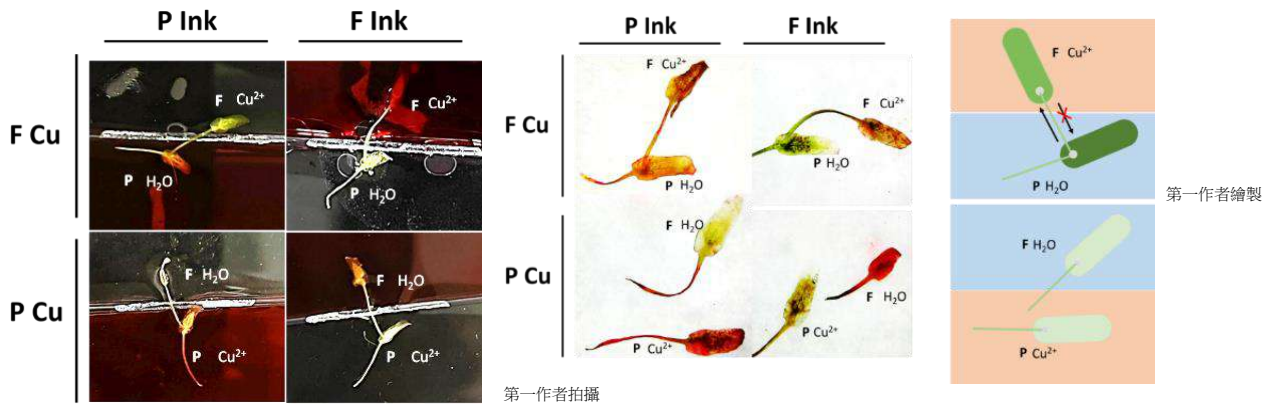


Figure 7-1. Mother and Daughter leaf under localized Cu^{2+} stress and red dye conditions, observed under a dissecting microscope (40x)

(1) Substance allocation in *Lemna trisulca* under localized Cu^{2+} stress

In Figure 7-1, when the daughter leaf experiences Cu^{2+} stress and the mother leaf is treated with red dye, the daughter leaf turns red after 24 hours. If the daughter leaf is treated with both Cu^{2+} stress and red dye, the mother leaf shows no color change, indicating that **the mother leaf supplies substances to the stressed daughter leaf, but not vice versa.**

When the mother leaf is treated with Cu^{2+} stress and the daughter leaf is treated with red dye, the mother leaf again shows no color change. However, if the mother leaf is treated with both Cu^{2+} stress and red dye, minimal color change occurs in the daughter leaf due to detachment. These results confirm that **a healthy mother leaf can supply substances to a stressed daughter leaf while preventing the return of toxic substances, reducing collateral damage to the mother leaf.**

(2) Substance allocation in *Lemna trisulca* under localized H_2O_2 stress

From previous experiments, we know that ROS acts as a signaling molecule for long-distance communication between tissues under localized stress in plants. Now, we wonder **whether ROS also serves as a signaling molecule for nutrient transport within *Lemna trisulca* under localized Cu^{2+} stress.** To verify if ROS

participates in coordinating nutrient distribution under localized stress, we conducted an experiment where either the daughter or mother leaf was treated with H_2O_2 , along with the addition of red dye. After 24hr, we used a dissecting microscope to observe transport within the plant.

Figure 7-2 shows that when the daughter leaf is treated with H_2O_2 and the mother leaf with red dye, the daughter leaf turns red, but the mother leaf does not. When the mother leaf is treated with H_2O_2 , detachment occurs, and red dye is seen in the daughter leaf. This suggests that ROS in the daughter leaf under Cu^{2+} stress signals the mother leaf to transport materials, but the daughter leaf does not send substances back to the mother leaf. Combined with previous findings, we conclude that ROS accumulated in the daughter leaf under copper stress serves as a signal for the mother leaf to transport materials to the daughter leaf.

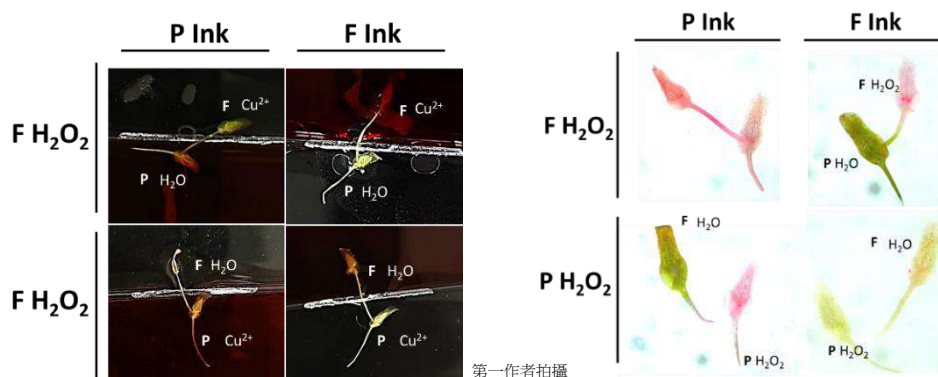


Figure 7-2. Mother and Daughter leaf under localized H_2O_2 stress and red dye conditions, observed under a dissecting microscope (40x)

(3) Substance allocation in *Lemna trisulca* under localized Cu^{2+} stress adding NAC

From the above experiments, we have learned that ROS affects the transport mechanism. Next, we treated either the daughter leaves or mother leaves separately with $10\mu M$ Cu^{2+} stress and $10mM$ NAC, adding red ink to explore

whether the ROS induced by Cu^{2+} stress affects the material transport mechanism.

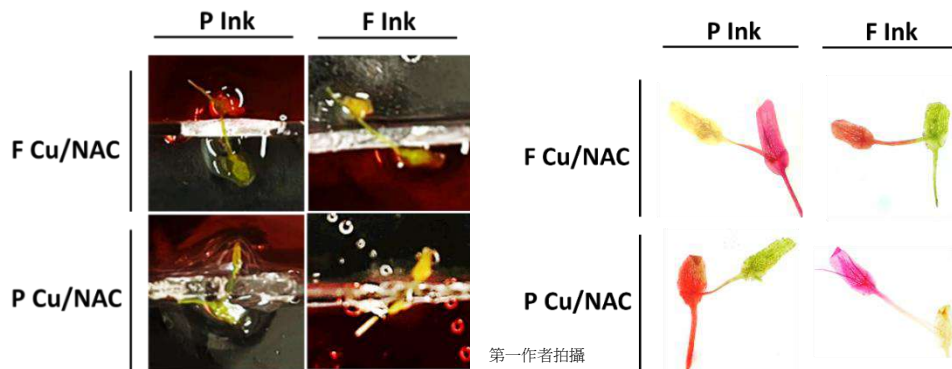


Figure 7-3. Mother and Daughter leaf under localized Cu^{2+} stress, NAC, and red dye conditions, observed under a dissecting microscope (40x)

As shown in Figure 7-3, when the daughter and mother leaves of *Lemna trisulca* are exposed to stress conditions with NAC present, no detachment occurs between them, and no substance transportation takes place between the daughter and mother leaves. Each absorbs substances from its own environment without transporting substances to one another. This confirms that ROS induced by localized Cu^{2+} stress in daughter leaves activates the plant's internal substance allocation mechanism.

viii. Ca^{2+} as Signaling Molecules in the Detachment Mechanism of *Lemna trisulca*

It is known that the detachment mechanism of *Lemna trisulca* involves communication and signaling between leaves, with ROS being an important signaling molecule. Next, we wanted to explore whether calcium ions (Ca^{2+}), a common signaling molecule in plants, could also function as a signaling molecule in *Lemna trisulca*. Thus, we designed the following experiments.

(1) Adding Ca^{2+} as a signaling molecule in Cu^{2+} stress.

We took 10 connected *Lemna trisulca* leaves and treated them with 10 μ M Cu²⁺, adding 5, 10, and 15 μ M Ca²⁺, allowing them to grow for 24 hours while observing for any detachment and calculating the connection rate.

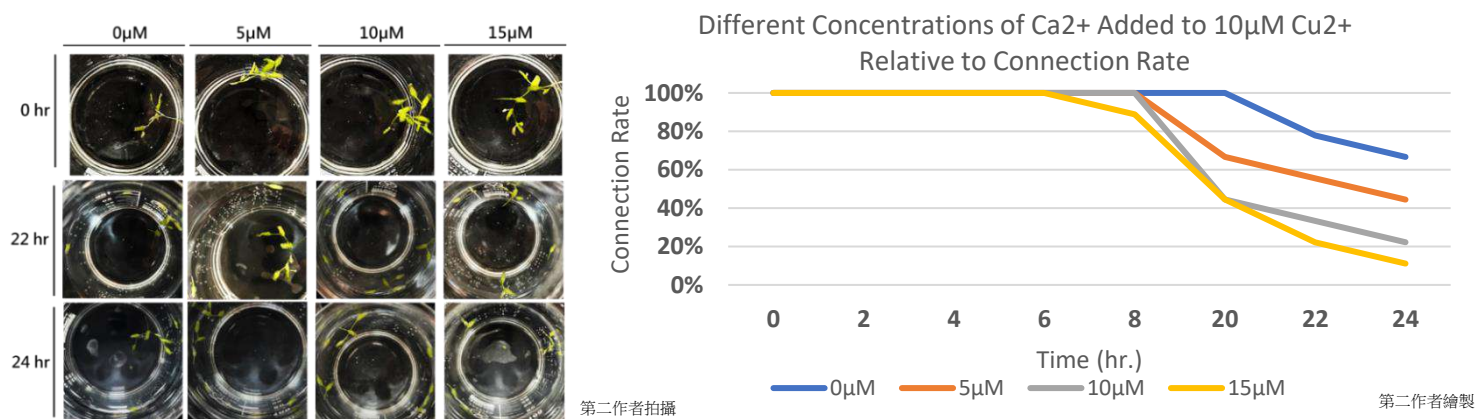


Figure 8-1. Detachment and connection rate under Cu²⁺ stress and adding Ca²⁺

From the charts above, Ca²⁺ increased the detachment in *Lemna trisulca* under Cu²⁺ stress. After 8 hours, the connection rate of 15 μ M Ca²⁺ dropped to about 88.9%, while the control group remained at 100.0%. All groups with added Ca²⁺ had lower connection rates than the control, indicating that **Ca²⁺ enhance detachment, indicating their involvement in the detachment mechanism.**

(2) Adding EDTA to chelate metal ions in Cu²⁺ stress.

We were still **concerned that the increase in Ca²⁺ concentration in the environment might affect detachment, rather than the detachment being induced by the Cu²⁺ stress in *Lemna trisulca*.** To confirm that Ca²⁺ is a necessary signaling molecule within *Lemna trisulca*, we added EDTA to chelate Ca²⁺ under Cu²⁺ stress. We took 10 connected leaves and treated them with 10 μ M Cu²⁺ in multiple cups, adding EDTA at concentrations of 0.1, 0.2, and 0.3 mM, allowing them to grow for 24 hours, after which we observed and calculated the connection rates.

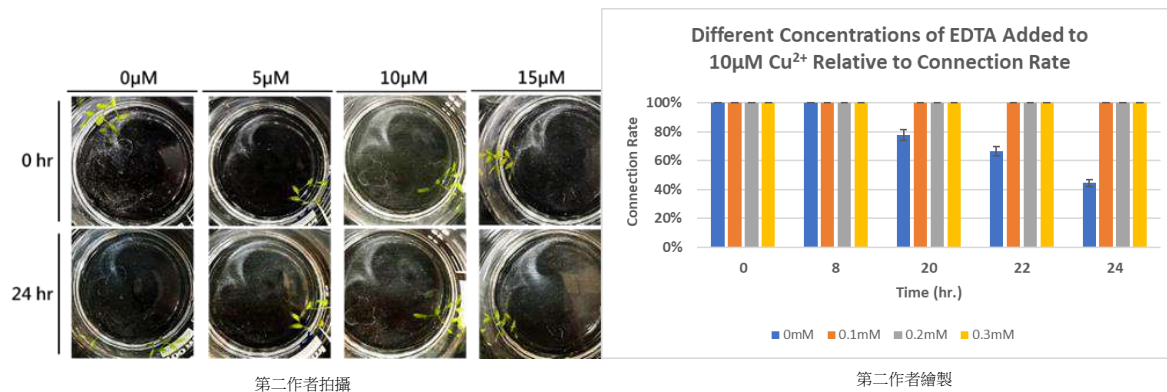


Figure 8-2. Detachment and connection rate under Cu^{2+} stress and adding EDTA

Summarizing the charts above, compared to previous experiments with added Ca^{2+} , the chelation of Ca^{2+} resulted in the complete disappearance of detachment in *Lemna trisulca*, maintaining a connection rate of 100.0%. This suggests that Ca^{2+} participate in the breakage mechanism under Cu^{2+} stress. However, since **EDTA can also chelate Cu^{2+}** , we conducted the following experiment.

(3) Adding EGTA to chelate Ca^{2+} in Cu^{2+} stress.

Due to the possibility of EDTA chelating Cu^{2+} , we used **EGTA, which selectively chelates Ca^{2+} , to observe the detachment**. We treated ten consecutive *Lemna trisulca* leaves with 10 μM Cu^{2+} and added concentrations of 0.1, 0.2, and 0.3 mM EGTA, allowing the plants to grow for 24 hours before observing any detachment and calculating the connection rates.

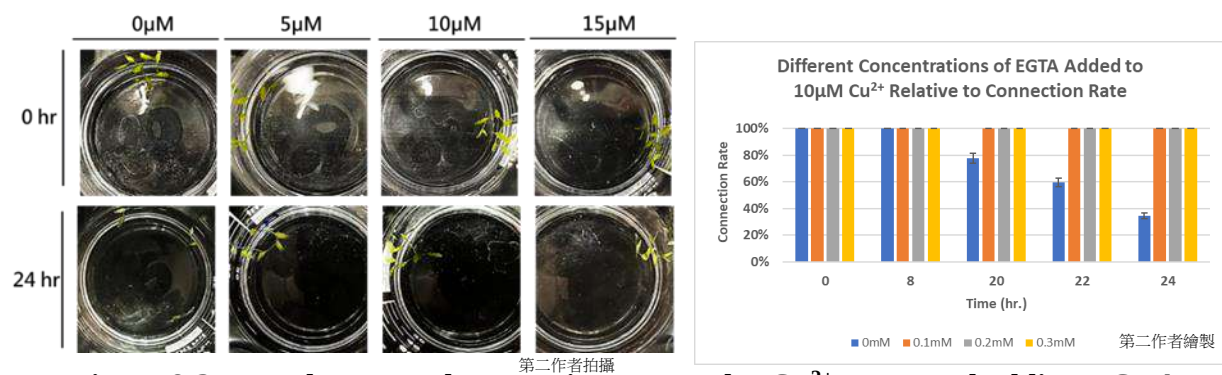


Figure 8-3. Detachment and connection rate under Cu^{2+} stress and adding EGTA

Summarizing the charts above, we observed that when only Ca^{2+} were chelated, detachment did not occur and resulted in a 100.0% connection rate. This confirms that Ca^{2+} participate in the detachment mechanism under stress and are essential signaling molecules that induce detachment in response to Cu^{2+} stress.

ix. **The Interaction Mechanism Between H_2O_2 and Ca^{2+} under Cu^{2+} Stress in *Lemna trisulca***

Since we know that NAC and Ca^{2+} are necessary signaling molecules during detachment in *Lemna trisulca*, we want to investigate the interaction of these two on the mechanism of stress-induced detachment in *Lemna trisulca*. We aim to determine the signaling pathways involved, identifying which is upstream, which is downstream, and which is the primary substance causing the fractures.

Before conducting the experiment, we aimed to explore the potential signaling pathways to confirm the correct mechanism, establishing the upstream and downstream relationship between Ca^{2+} and ROS. Initially, we hypothesized that ROS is upstream and Ca^{2+} is downstream, inducing detachment. Therefore, we designed the following experiment.

To confirm our hypothesis, we treated the plants with 0.1 mM EGTA + 10 mM H_2O_2 , 10 mM H_2O_2 solution, and 10 μM Ca^{2+} solution for 24 hours. We then observed their growth and calculated the connection rates. If our hypothesis holds true, the connection rate of the H_2O_2 experiment should be greater than that of the EGTA + H_2O_2 experiment.

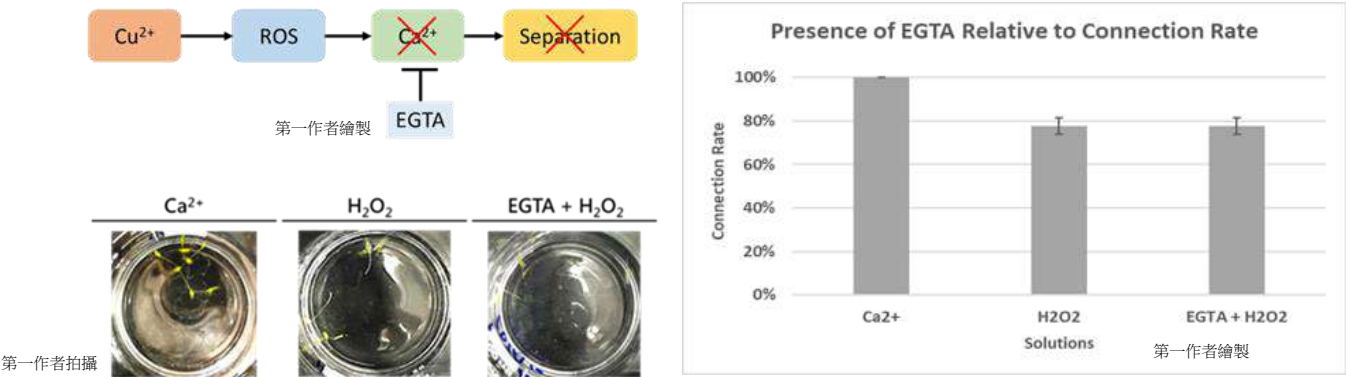


Figure 9-1. Growth and connection rate after 24 hr in Ca^{2+} , H_2O_2 , EGTA + H_2O_2

Summarizing the charts above, the detachment of *Lemna trisulca* in H_2O_2 and $EGTA + H_2O_2$ are similar, with connection rates of approximately 77.8% in both cases. Therefore, our initial hypothesis was incorrect. We infer that the stress response of *Lemna trisulca* to ROS is independent of Ca^{2+} ; Ca^{2+} does not influence the detachment caused by ROS, only the detachment caused by Cu^{2+} . We hypothesize that Ca^{2+} is upstream, while ROS are downstream.

To verify this hypothesis, we treated *Lemna trisulca* with $10\mu M Cu^{2+} + 10mM NAC + 10\mu M Ca^{2+}$, $10mM H_2O_2 + 10mM NAC + 10\mu M Ca^{2+}$, and $10\mu M Cu^{2+} + 10\mu M Ca^{2+}$, and observed their growth conditions and calculated the connection rates after 24 hours. If this hypothesis is correct, then the experiments with NAC should not show any detachment, as ROS would be eliminated, and even with Ca^{2+} , they should not promote increased detachment.

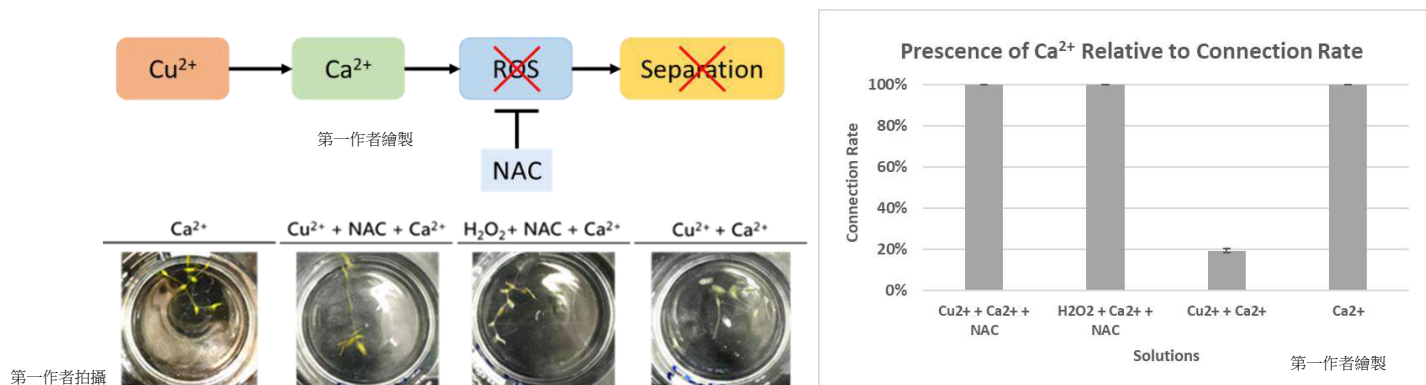


Figure 9-2. Growth and connection rate in $Cu^{2+} + NAC + Ca^{2+}$, $H_2O_2 + NAC + Ca^{2+}$, Ca^{2+}

The experimental results align with our hypothesis that the groups with NAC added showed no detachment, confirming that the upstream signaling molecule is Ca^{2+} , while the downstream signaling molecule is ROS, inducing detachment.

Next, we were curious whether there is a positive feedback mechanism between Ca^{2+} and ROS, where both might promote each other's accumulation. Therefore, we treated *Lemna trisulca* in solutions of $10\mu M Ca^{2+} + 10mM H_2O_2$, $10mM H_2O_2$, and $10\mu M Ca^{2+}$, and treated them for 24 hours, observing their growth and calculating the connection rates. If a positive feedback mechanism exists, the connection rate in the $Ca^{2+} + H_2O_2$ group should be lower than that in the H_2O_2 group, indicating that the detachment is increased.

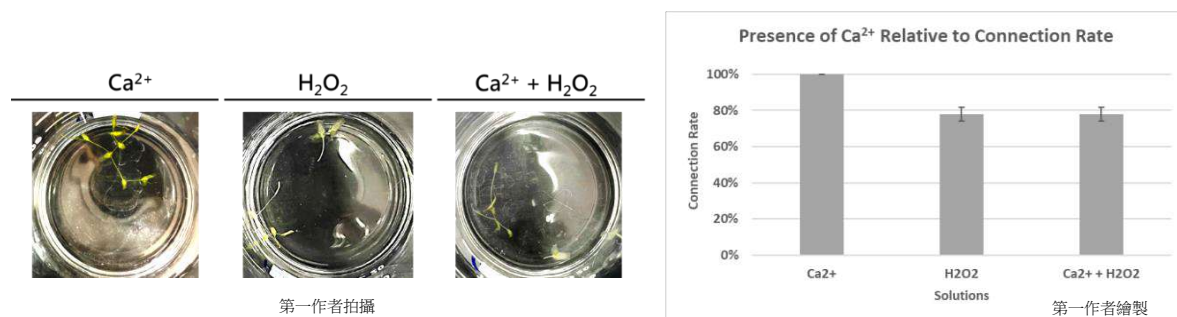
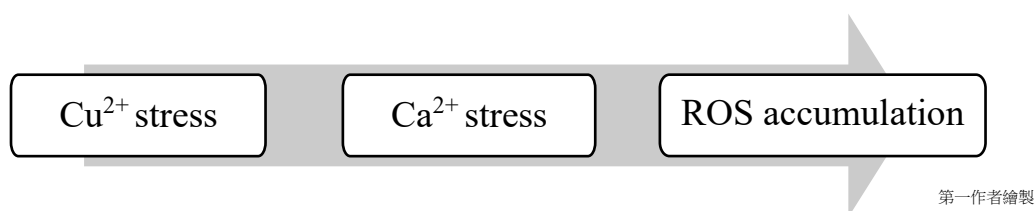


Figure 9-3. Growth and connection rate in Ca^{2+} , $\text{H}_2\text{O}_2 + \text{Ca}^{2+}$, H_2O_2

The experimental results revealed that the connection rates in the groups with $\text{Ca}^{2+} + \text{H}_2\text{O}_2$ were the same as those in the H_2O_2 group. Therefore, it can be inferred that there is **either no positive feedback mechanism between Ca^{2+} and H_2O_2 that induces each other's accumulation, or the concentration of Ca^{2+} has reached a threshold, minimizing detachment increase within *Lemna trisulca*.**

Thus, we conclude the detachment mechanism in *Lemna trisulca*: the plant senses Cu^{2+} stress and uses Ca^{2+} as a signaling molecule to transmit the stress signal throughout the plant, leading to the production of ROS that trigger detachment.



x. Ca^{2+} Signaling Mechanism in *Lemna trisulca* Under Localized Cu^{2+} Stress

(1) Ca^{2+} as a signaling molecule in stressed tissue

We know that Ca^{2+} is an important signaling molecule in *Lemna trisulca* and understand its relationship with Cu^{2+} and H_2O_2 . Next, we are curious whether **Ca^{2+} can serve as a signaling molecule for communication within stressed tissues or for long-distance signaling.** Therefore, we placed two leaves in a divided Petri dish and conducted two experiments: one with the mother leaf treated with Cu^{2+} stress and EGTA, and the other with the mother leaf treated with Cu^{2+} stress and the daughter leaf treated with EGTA, observing detachment.

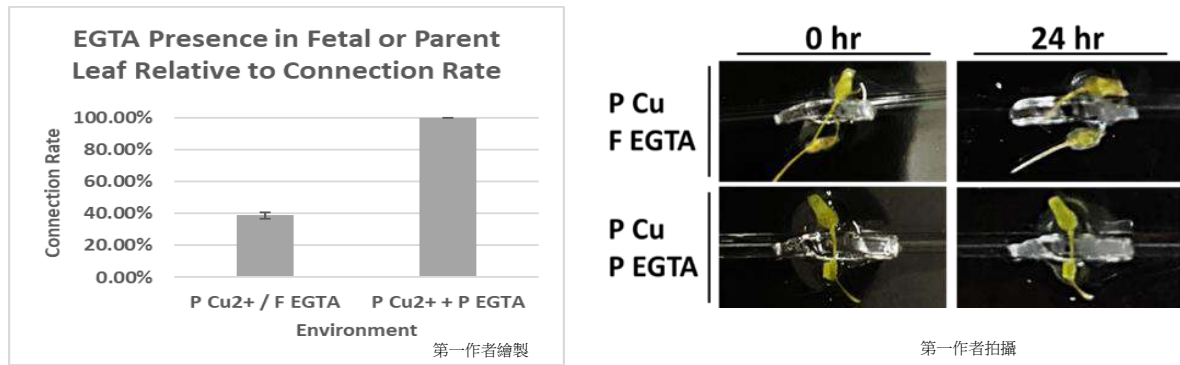


Figure 10-1. Growth and connection rate of mother leaf treated with Cu²⁺/EGTA and mother leaf treated with Cu²⁺ while daughter leaf treated with EGTA

As shown in Figure 10-1, when the mother leaf is treated with Cu²⁺ stress and EGTA, the connection rate is 100.0%, with no signs of fracture. This indicates that **Ca²⁺ must be involved in the signaling of the stressed mother leaf to induce the detachment mechanism**. In contrast, when the mother leaf is treated with Cu²⁺ stress and the daughter leaf is treated with EGTA, the connection rate drops to around 40%. This suggests that the chelation of Ca²⁺ in the daughter leaf does not affect the connection rate. We propose that the stressed mother leaf must use Ca²⁺ to assist in the unidirectional transmission of detachment signals to the healthy daughter leaf, triggering its fracture and release. This process does not involve Ca²⁺ within the daughter leaf. In summary, the **influence of Ca²⁺ on the selective detachment mechanism in *Lemna trisulca* occurs only in the injured mother leaf, not in the daughter leaf**.

(2) Ca²⁺ does not act as a signaling molecule for long-distance communication

From previous experiments, we learned that the signaling of detachment is transmitted unidirectionally from the mother leaf to the daughter leaf, leading to the accumulation of ROS at both the mother and daughter leaf nodes, which triggers the detachment of the daughter leaf. Although Ca²⁺ is involved in the selective detachment mechanism induced by local stress, its effect is limited to the mother leaf. Additionally, **earlier experiments revealed an interactive relationship between ROS and Ca²⁺, and literature indicates that their interaction can generate ROS waves for inter-tissue signaling in plants**. Therefore, we are

curious whether the Ca^{2+} in the mother leaf interacts with ROS in a similar manner to generate ROS waves that are transmitted unidirectionally to the daughter leaf, thereby inducing the accumulation of ROS at the daughter leaf nodes and triggering detachment. To confirm this hypothesis, we treated the mother leaf with Cu^{2+} stress and individually treated both the child and mother leaves with EGTA in *Lemna trisulca*, followed by DAB staining to observe the accumulation of ROS under conditions where Ca^{2+} is chelated.

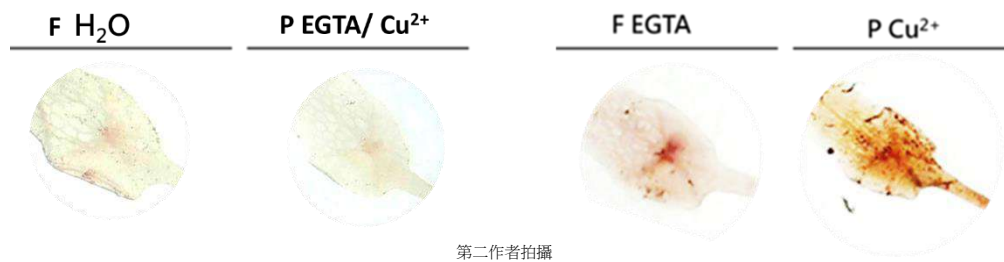


Figure 10-2. DAB staining of mother in Cu^{2+} and both individually in EGTA (40x)

As shown in Figure 10-2, when the mother leaf is treated with Cu^{2+} stress and the daughter leaf with EGTA, both leaves accumulate ROS at the nodes, indicating that the **unidirectional transmission of the detachment signal is not affected by the chelation of Ca^{2+} in the daughter leaf**. In contrast, when the mother leaf is treated alone with Cu^{2+} stress and EGTA, neither the daughter nor the mother leaf shows significant ROS accumulation. This suggests that the accumulation of ROS in the stressed mother leaf is influenced by Ca^{2+} , which plays a key role in activating the unidirectional transmission of the detachment signal from the mother leaf to the daughter leaf. We hypothesize that **the interaction between Ca^{2+} and ROS in the stressed mother leaf generates an ROS wave, serving as a unidirectional breaking signal that promotes ROS accumulation in the daughter leaf, leading to its eventual detachment.**

xi. Ca^{2+} as a Signaling Molecule in *Lemna trisulca* Substance Allocation Mechanism Under Localized Stress

From the previous experiments, we found that when the daughter leaf is treated individually with Cu^{2+} stress, it accumulates ROS, allowing the healthy mother leaf to transport substances to the stressed daughter leaf for survival, while the stressed daughter leaf does not return toxins to the mother leaf, reducing collateral damage. We were curious **whether this transport and allocation mechanism is regulated by Ca^{2+}** . The healthy mother leaf will transport substances to assist the stressed daughter leaf, indicating that the daughter leaf should have a signaling mechanism to convey a stress signal to the mother leaf. However, since the stressed daughter leaf cannot induce ROS accumulation in the healthy mother leaf, **could it be that the stressed daughter leaf triggers Ca^{2+} signaling within the mother leaf to initiate substance distribution?** Therefore, we treated two *Lemna trisulca* leaves with Cu^{2+} stress and EGTA in the daughter leaf while the mother leaf was treated with red ink; in another group, the daughter leaf was treated with Cu^{2+} stress while the mother leaf was treated with EGTA and red ink, and after 24 hours, we observed the transport and allocation using a dissecting microscope.

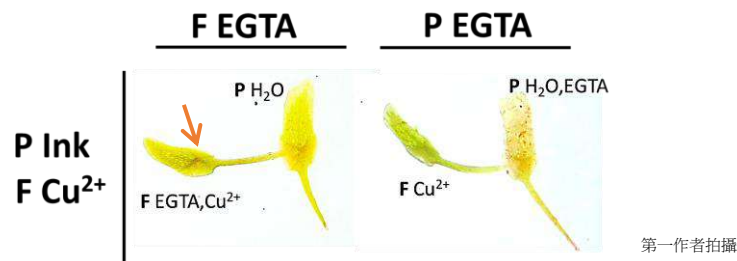


Figure 11-1. Daughter leaf under Cu^{2+} stress and both leaves under EGTA and red dye conditions, observed under a dissecting microscope (40x)

According to Figure 11-1, when the daughter leaf is treated with Cu^{2+} stress and EGTA while the mother leaf is treated with red dye, the daughter (indicated by the red arrow) turns red, showing that the mother leaf still transports substances to assist the daughter leaf in overcoming stress. However, when the daughter leaf is treated with Cu^{2+} stress while the mother leaf is treated with EGTA and red dye, no substance substance allocation occurs. It is inferred that **under stress, the daughter**

leaf accumulates ROS and sends out a stress signal to the mother leaf. Subsequently, the mother leaf utilizes Ca^{2+} for internal communication, initiating the mechanism to transport substances to assist the daughter leaf.

V. Further Work and Application

i. Substance Allocation Mechanism of *Lemna trisulca*

It is known that cytokinin (CK) can affect nutrient distribution. We hypothesize that when only the daughter leaf is treated with stress, the plant utilizes CK to distribute nutrients, allowing the mother leaf to increase the nutrient supply to the daughter leaf, thereby maintaining its growth and maximizing its survival probability. Under stress, the increased ROS can influence the activity of ion channels within *Lemna trisulca*, regulating the signals and pathways related to nutrient deficiency. In this context, the CK levels in the plant will increase, directing nutrients to areas where they are lacking, specifically from the mother leaf to the daughter leaf.

ii. H_2O_2 , Ca^{2+} , RBOH Interaction and Detachment Mechanism of *Lemna trisulca*

ROS, such as singlet oxygen ($\text{O}_2^{\cdot}$), superoxide ($\text{O}_2^{\cdot-}$), or hydrogen peroxide (H_2O_2), act as signaling molecules that can regulate a wide range of biological processes, from developmental and growth regulation to responses to biotic or abiotic stimuli. This network enables cells to maintain a non-toxic level of ROS while allowing for transient accumulation of ROS at specific cellular locations as signals. These pathways include fundamental cellular processes such as photosynthesis, respiration, and photorespiration, which produce ROS as unavoidable byproducts. A key participant in this ROS-generating enzymatic network is the respiratory burst or NADPH oxidase. In this mechanism, Ca^{2+} often acts as another signaling molecule, creating a feedback mechanism between the two. Therefore, we believe that *Lemna trisulca* can utilize the RBOH enzyme system to perform signaling within the plant.

The RBOH enzyme operates by allowing plants to sense local stress, leading to the production of Ca^{2+} signaling factors within cells. This triggers the RBOH enzyme to unidirectionally transmit ROS to other tissues, a phenomenon known as the ROS

wave. In *Lemna trisulca*, we hypothesize that when the mother leaf senses stress, it causes an increase in both ROS and Ca^{2+} concentrations. Through the ROS wave, ROS is unidirectionally sent to the daughter leaf, leading to the accumulation of ROS at the nodes. After mutual communication between the two, this results in the occurrence of detachment.

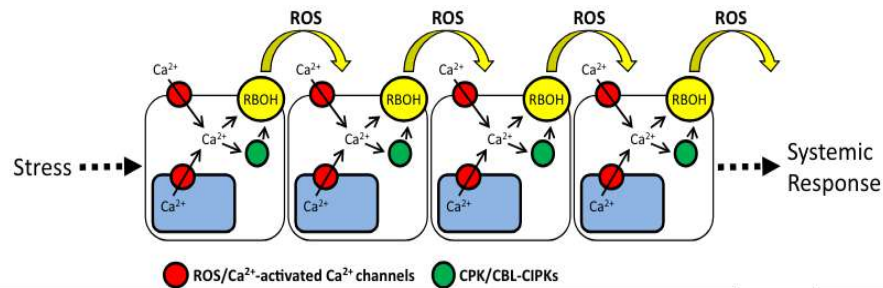


Figure 12-1. Diagram of ROS wave in plants

出處：Reactive oxygen species, abiotic stress and stress combination, Feroza K. Choudhury, The Plant Journal (2016)

iii. The Ecological Significance of Substance Allocation to *Lemna trisulca*

The substance allocation in *Lemna trisulca* primarily occurs under stress conditions. In its ecology, *Lemna trisulca* responds to heavy metal stress in its growth environment, such as Cu^{2+} , by implementing a similar nutrient allocation mechanism. This involves distributing nutrients from older leaves to younger leaves and adjusting cell division to ensure the continuous reproduction of the species. This strategy aims to maximize the survival rate of the species and prevent extinction.

VI. Conclusion

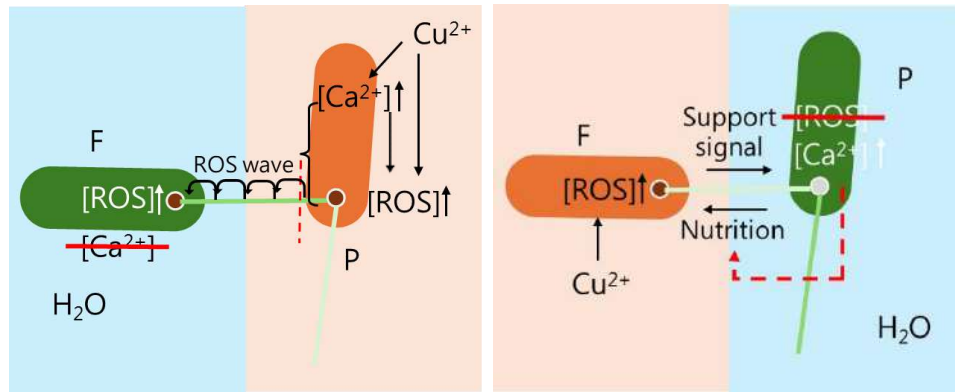
In the experimental results, we found that Cu^{2+} is a stress molecule for *Lemna trisulca*, where under stress conditions, the mother leaf induces a fracture to reduce the impact of stress on the daughter leaves. In response, the mother leaf assists the daughter leaves in coping with the stress, effectively aiding their growth. We confirmed that *Lemna trisulca* produces ROS as a necessary signaling molecule under Cu^{2+} stress, which triggers the fracturing process. Furthermore, we observed that ROS accumulates at the nodes of *Lemna trisulca* under Cu^{2+} stress, and that ROS signals from stressed daughter leaves do not return to healthy mother leaves. In contrast, ROS signals from stressed mother leaves

can unidirectionally transmit to the nodes of healthy daughter leaves, inducing detachment. It was also determined that both sides must accumulate ROS through mutual communication to trigger the detachment.

Next, we found that Ca^{2+} influences the detachment mechanism of *Lemna trisulca* under stress, serving as a necessary signaling molecule for inducing detachment. Regarding the detailed detachment mechanism, we deduced that *Lemna trisulca* perceives Cu^{2+} stress and uses Ca^{2+} as a signaling molecule to transmit the stress signal throughout the plant, ultimately leading to the production of peroxides that trigger fractures and alter the allocation of substances, with may or may not any positive feedback involved.

Additionally, we discovered that both Ca^{2+} and ROS affect the selective detachment and substance allocation in *Lemna trisulca*. In the selective detachment mechanism, the mother leaf accumulates ROS in response to Cu^{2+} stress, with Ca^{2+} participating in this process. We hypothesize that they altogether assist in generating a unidirectional ROS wave that transmits from the mother leaf to the daughter leaf, resulting in ROS accumulation at the nodes of the daughter leaves. Ultimately, this communication leads to detachment. The unidirectional nature of ROS transmission is a key reason for the selective detachment produced under local stress in *Lemna trisulca*, allowing the mother leaf to release healthy daughter leaves to prevent further damage.

In terms of substance transport and allocation, the daughter leaves induce ROS accumulation in response to Cu^{2+} stress, which we speculate alters the hormonal concentration gradient within the plant, changing hormone distribution. As illustrated, the daughter leaves send out stress signals to the mother leaf, which subsequently induces Ca^{2+} accumulation at the nodes of the mother leaf. At this point, the mother leaf modifies the internal transport and allocation of substances, providing nutrients and materials to the daughter leaves, thereby increasing and maximizing their survival rate.



第二作者繪製

Figure 13-1. Diagram of detachment and substance allocation mechanism under localized stress of *Lemna trisulca*

VII. References

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1. 本研究以品萍(*Lemna trisulca*)為材料，探討在銅離子逆境下母葉與子葉之間的體內傳訊與物質分配機制。結果發現當母葉遭遇局部逆境時會將健康子葉釋放，而子葉遭遇局部逆境時，母葉會選擇保留子葉並持續送物質助其生存。
2. 從提出問題、假說、實驗證實、討論，邏輯清晰。
3. 本研究所得結果與分析皆合理，但其他環境逆境如高鹽及溫度逆境是否會有類似的結果值得探討。