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The Regulatory Effect of Salicylic Acid on the Physiology of Mentha under Aluminum Stress and Its Alleviation of DNA Damage

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Abstract

Aluminum stress is an important factor limiting plant growth in acidic soil, and has significant effects on the growth, development and physiological metabolism of *Mentha canadensis*. However, the exogenous addition of Salicylic acid significantly alleviated the negative effects of aluminum stress on *Mentha canadensis*. In order to reveal the mechanism of Salicylic acid in alleviating aluminum stress, this study used the different concentrations of *Mentha canadensis* plants under aluminum stress, and measured the growth of the plants, and analyzed the degree of DNA damage. This study not only revealed the mechanism of Salicylic acid in alleviating aluminum stress, but also provided theoretical basis and practical guidance for cultivation. Future studies will further explore the molecular mechanisms of Salicylic acid regulation of Al stress resistance and how the resistance can be improved by genetic engineering means.

Subject Areas

Life Sciences

Keywords

Aluminium Stress, *Mentha canadensis*, Salicylic Acid, DNA Damage, Alleviation Effect, Physiological Regulation

1. Introduction

In recent years, with the aggravation of environmental pollution, especially the increase in heavy metal pollution in soil, aluminum, one of the common soil pollutants, has garnered attention for its impact on plant growth. Aluminum stress can lead to enhanced oxidative stress reactions in plants, affecting photosynthesis respiration, thereby inhibiting the growth and development of plants. Salicylic acid, a type of plant hormone, has been found to effectively improve plants' tolerance to various adverse conditions.

Scholars, both domestically and internationally, have made significant achievements in studying the response mechanisms of plants under aluminum stress. However, further exploration is required regarding how Salicylic acid regulates the physiological state of plants and alleviates DNA damage under aluminum stress. Research has shown that Salicylic acid can alleviate the aluminum stress on plants by activating the antioxidant enzyme system, regulating ion balance, changing membrane permeability, and signal transduction pathways. It can also reduce the free radicals by regulating gene expression, thereby lowering the extent of DNA damage caused by aluminum stress.

Nevertheless, there is still a lack of systematic analysis and on the specific mechanisms of how Salicylic acid regulates the physiology of *Mentha canadensis* and alleviates DNA damage under aluminum stress. Therefore, this study aims to delve into how Salicylic acid regulates the physiology of *Mentha canadensis* and alleviates DNA damage under aluminum stress, revealing its potential mechanisms. This will provide theoretical support for understanding molecular mechanisms of plant response to aluminum stress and offer references for developing new plant protection strategies.

This study not only helps to deepen the understanding of the response mechanisms under aluminum stress but also holds significant importance for improving crop yield and quality, especially in areas with severe heavy metal pollution. Future research can focus more on the interactions of Salicylic acid and other plant hormones or environmental factors, as well as the differences in plant responses to salicylic acid, to provide more scientific guidance for applications in agricultural production.

2. The Effect of Aluminum Stress on *Mentha canadensis*

2.1. Overview of Aluminum Stress

Aluminum is the most abundant metal element in the earth's crust, but under natural conditions, it usually exists in insoluble silicates or oxides without direct toxicity to plants. However, in acidic soils, aluminum ions (Al^{3+}) Will dissolve heavily and enter the soil solution, being toxic to the roots of plants. These aluminum ions are able to bind to the negative charge on the cell wall, destroying the cell wall structure, which in turn affects the normal function of the cells. Aluminum ions can also enter the cell interior and combine with nucleic acids, proteins and other important biological molecules in the cell, interfering with their normal metabolic activities.

The effect of aluminum stress on plant growth is multifaceted. Aluminum stress will inhibit root elongation and lateral root formation, resulting in root dysplasia and reducing the ability of the plant to take up water and nutrients. This directly affects the plant growth rate and biomass accumulation. Aluminum stress also interferes with the photosynthesis process in plants. Photosynthesis is an important basis for plant growth and development, and aluminum stress will destroy the chloroplast structure and reduce the photosynthetic pigment content, and then affect the efficiency of photosynthesis. This resulted in leaf yellowing and reduced accumulation of photosynthetic products, which further weakened the plant growth potential.

In addition to its direct effects on plant growth, aluminum stress also induces a series of physiological and biochemical changes in plants. For example, aluminum stress can stimulate the oxidative stress response in plants, leading to the accumulation of harmful substances such as reactive oxygen species, which can then damage cell membranes and biological macromolecules. In response to the oxidative damage caused by aluminum stress, plants initiate antioxidant systems to remove reactive oxygen species, but this also consumes considerable energy and material resources.

The effects of aluminum stress on plant growth are comprehensive. From root development to photosynthesis, physiological and biochemical processes, will be affected to different degrees. Therefore, further study of the mechanism of aluminum stress and exploring effective mitigation measures are important to improve the stress resistance and production capacity of plants in acidic soil.

2.2. Response of Mentha to Aluminum Stress

The growth performance of *Mentha canadensis* under Al stress showed a significantly inhibited state. As a plant sensitive to environmental changes, its physiological response under aluminum stress is particularly remarkable. Root growth of *Mentha canadensis* was significantly inhibited under aluminum stress conditions. The accumulation of Al ions in soil causes toxic effects on root tip cells, which in turn hinder root elongation and branching. This inhibition was more pronounced at higher aluminum concentrations, manifested by short roots, dark color, and significantly reduced root vitality. Since the root system is the main organ for absorbing water and nutrients, its growth obstruction will directly affect the overall growth status of *Mentha canadensis*.

In addition, aluminum stress can lead to yellowing and wilting of *Mentha canadensis* leaves. This is due to aluminum ions interfering with nutrient uptake and transport in the plant, preventing leaves from obtaining adequate nutrient supply. It can also trigger the oxidative stress response in the plant body, resulting in damage to the cell membrane and then affecting the normal physiological function of the leaves. These symptoms are particularly severe when aluminum stress lasts longer or at higher aluminum concentrations and may even lead to the death of *Mentha canadensis* plants.

In addition to growth inhibition, physiological changes in *Mentha canadensis* under aluminum stress include reduced photosynthesis efficiency. Aluminum ions can destroy the chloroplast structure and affect the synthesis and stability of photosynthetic pigments, thus reducing the photosynthesis ability of *Mentha canadensis*. This will further reduce the accumulation of organic matter in the plants and weaken their stress resistance. It is noteworthy that aluminum stress also has an effect on the osmoregulatory system of *Mentha canadensis*. Under aluminum stress conditions, the content of osmoregulatory substances such as proline and soluble sugars in *Mentha canadensis* will change to cope with the adverse effects of the external environment. However, when aluminum stress exceeds a certain limit, the synthesis and accumulation of these osmoregulatory substances will be hindered, resulting in a decline in the osmoregulatory ability of *Mentha canadensis* and a failure to effectively maintain the water balance and steady state in the cell.

2.3. DNA Damage Caused by Aluminum Stress

Aluminum ions are able to bind to the phosphate groups in DNA molecules, leading to the distortion and breakage of DNA chains, which can cause the disorder of the genetic information of cells. Aluminum stress poses a serious threat to the DNA structural stability of plant cells, and as a common medicinal plant, the DNA damage under aluminum stress is particularly noteworthy.

Under aluminum stress conditions, DNA damage in *Mentha canadensis* plants was mainly characterized by single-strand breaks and double-strand breaks. These lesions can be effectively detected by molecular biology techniques such as the comet assay. The experimental data showed that the degree of DNA breakage in the *Mentha canadensis* leaf cells also showed a significant increasing trend with the increasing concentration of aluminum ions. In particular, with a high concentration of aluminum ion treatment, the degree of DNA fragmentation is intensified, and the genetic integrity of cells is severely damaged.

In addition to direct DNA strand breaks, aluminum stress also induces an excessive accumulation of reactive oxygen species (ROS) in menthol cells. These highly reactive molecules are capable of attacking DNA bases, initiating base mismatches and the formation of DNA adducts. This type of damage is more insidious, but it also has a long-term impact on the normal function of the cell. By comparing the data from different aluminum stress time points, we can find that the oxidative DNA damage caused by ROS also gradually accumulated with the increasing stress time.

At the molecular level, DNA damage caused by aluminum stress can trigger the DNA damage response mechanisms in menthol cells. This includes activation of DNA repair proteins and regulation of cell cycle checkpoints. However, these response mechanisms may fail when the strength of aluminum stress exceeds the cell's ability to self-repair, ultimately leading to cell death or accumulation of genetic variation.

Damage to *Mentha canadensis* DNA by Al stress is a complex and multilayered process involving direct physical damage and indirect oxidative damage. These injuries not only affect the growth and production of *Mentha canadensis*, but also their medicinal quality through changes in genetic information.

3. Exploration of the Physiological Regulation of Salicylic Acid on Plants

3.1. The Physiological Characteristics of Salicylic Acid

Salicylic acid, chemically named o-hydroxybenzoic acid, is a simple phenolic compound that is widely found in higher plants. It has a variety of biological activities and plays an important role in plant growth and development and stress resistance. In plants, Salicylic acid can act as a signaling molecule involved in the regulation of various physiological processes.

The basic properties of Salicylic acid include its solubility and stability. It is easily soluble in ethanol, ether, and other organic solvents, but it is also soluble in water, especially boiling water. This solubility allows Salicylic acid to move freely in the plant body to play its physiological role [1]. Salicylic acid is relatively stable at room temperature and is not easy to decompose, which ensures its long-term effect on plants.

In plants, the role of Salicylic acid is mainly reflected in the following aspects: Salicylic acid can regulate the growth and development of plants, improve the stress resistance of plants. Exogenous application of Salicylic acid can promote seed germination, root growth and leaf expansion, and can also activate the plant defense system, induce the expression of related stress resistance genes, and enhance the resistance to stress when plants suffer from adverse environments such as pathogen infection, high temperature, drought or heavy metal stress [2]. For example, under aluminum stress conditions, Salicylic acid can reduce the damage of aluminum ions and improve the aluminum resistance of plants by regulating the antioxidant system in plants.

Salicylic acid also interacts with other signaling molecules in plants. In plants, signaling molecules such as Salicylic acid, Jasmonic acid and ethylene together constitute a complex signaling network. Through cooperation or antagonism, they can jointly regulate the growth and development of plants. This signaling interaction allows plants to respond more flexibly to changes in the external environment.

3.2. The Physiological Effects of Salicylic Acid

Salicylic acid is able to promote plant seed growth and root growth. Studies have shown that treatment with Salicylic acid at appropriate concentration can increase the germination rate, shorten the germination time, and promote root development and increase root hair number and length, and thus enhancing the ability of plants to absorb water and nutrients. This role is particularly important for improving plants ability to survive in adverse environments.

Salicylic acid also showed a significant promotion effect for leaf growth. Through exogenous application of Salicylic acid, we can increase the chlorophyll content of leaves and improve the efficiency of photosynthesis, which can promote plant growth and accumulation of dry matter. This not only contributes to the normal growth and development of plants, but also improves their resistance, especially in the face of extreme environments such as drought and high temperatures.

Salicylate also induces systemic acquired resistance (SAR) in plants, a broad-spectrum disease resistance response. As a signaling molecule of SAR, Salicylic acid is able to activate the plant defense system and enhance the plant's resistance to pathogens and insect pests. This finding provides new ideas and methods for pest control in agricultural production.

It is worth mentioning that the role of Salicylic acid in improving plant stress resistance has also attracted much attention. Under adverse environmental conditions such as salt stress and drought stress, Salicylic acid can reduce the damage caused by stress by regulating the osmotic pressure and ion balance in plants [3]. Salicylic acid also induces the activity of antioxidant enzymes in plants, thus removing excessive reactive oxygen radicals and protecting cells from oxidative damage.

3.3. Response of Salicylic Acid to Aluminum Stress

Under aluminum stress, a series of changes occur in the physiological environment in plant cells, including disruption of ion balance, inhibition of enzyme activity, and an increase in oxidative stress response [4]. These changes can all adversely affect the normal growth and metabolism of plants. Salicylic acid can regulate the metabolic process of plants through various ways, so as to help plants better cope with aluminum stress.

Salicylic acid can induce the improvement of antioxidant enzyme activity in plants, such as superoxide dismutase, catalase, etc., which can effectively remove the excessive reactive oxygen free radicals produced by aluminum stress, and reduce the damage of oxidative stress to cells [5]. Salicylic acid can also promote the synthesis of proline osmotic substances such as proline, enhance the osmotic regulation ability of cells, and maintain the water balance in cells, so as to maintain the normal physiological function of cells under aluminum stress.

Salicylate is also able to respond to aluminum stress by regulating signaling pathways in plants. It can interact with plant endogenous hormones, such as ethylene and Jasmonic acid, and jointly regulate plant growth and development and stress resistance reactions. Under aluminum stress conditions, Salicylic acid can inhibit the excessive production of ethylene, reduce its inhibitory effect on plant growth, and promote the synthesis of stress-related hormones such as Jasmonic acid, and enhance the stress resistance of plants.

In addition, it reduces the toxic effect of aluminum stress on plants by affecting the absorption and transport of aluminum by plants. It can regulate the growth and development of plant roots, reduce the uptake of aluminum, and promote the redistribution and compartmentalization of aluminum in plants, and reduce the toxic effect of aluminum on sensitive tissues and cells.

3.4. The Physiological Regulation of *Mentha canadensis* by Salicylic Acid

3.4.1. The Effect of Salicylic Acid on the Growth of *Mentha canadensis*

Mentha canadensis growth changed significantly after Salicylic acid treatment. These changes are mainly reflected in the overall growth trend of the plant, the morphology and color of the leaves, and the development of the root system.

In terms of overall plant growth, the Salicylic acid-treated *Mentha canadensis* showed a more vigorous growth force [6]. The treated group had significantly increased *Mentha canadensis* plant height, more robust stalks and more abundant branches compared to the untreated controls. This overall growth advantage suggests a positive role of salicylate in promoting the vegetative growth of *Mentha canadensis*.

In terms of leaf shape and color, the *Mentha canadensis* leaves are wider and thicker, and the leaf color is more green. This change not only improves the ornamental value of *Mentha canadensis*, but more importantly, reflects the positive effect of Salicylic acid in promoting photosynthesis and material accumulation in *Mentha canadensis* leaves. Wide and thick leaves can provide more photosynthetic area, thus enhancing the photosynthetic capacity of *Mentha canadensis*, and providing more sufficient energy and material basis for their growth and metabolism.

In terms of root development, the treated *Mentha canadensis* had more developed roots with increased root length and more dense root hairs. The root is an important organ for plants to absorb water and nutrients, and its development directly affects the growth and resistance of plants. Therefore, salicylic acid, which promotes *Mentha canadensis* root development, not only enhances its ability to absorb water and nutrients but also enhances its resistance to external environmental stress.

However, the promotion effect of Salicylic acid on *Mentha canadensis* growth is not linear, but there is a certain concentration effect. Salicylic acid can significantly increase the growth of *Mentha canadensis*; however, it may cause some growth inhibition or toxicity of *Mentha canadensis*. Therefore, in practical application, it is necessary to choose the appropriate Salicylic acid concentration according to the specific situation to achieve the best growth promotion effect.

3.4.2. The Impact of Salicylic Acid on the Metabolic Pathway of *Mentha canadensis*

As an important plant hormone, Salicylic acid plays a key role in plant growth and development and stress resistance response. Under aluminum stress conditions, Salicylic acid can affect the metabolic process of *Mentha canadensis* in various ways and improve its stress resistance.

Salicylic acid is able to induce the enhanced activity of various defense enzymes in *Mentha canadensis*, including superoxide dismutase, catalase, etc., which together constitute the antioxidant system of *Mentha canadensis*. Under aluminum stress, the activity of these enzymes is often inhibited, leading to the accumulation of reactive oxygen free radicals in *Mentha canadensis*, which can then trigger cell damage. The intervention of Salicylic acid can effectively activate the activity of these enzymes and accelerate the removal of reactive oxygen free radicals, thus reducing the oxidative damage of menthol cells caused by aluminum stress. It is also able to regulate the osmotic balance in *Mentha canadensis*, which is another important mechanism to improve its stress resistance. Under aluminum stress conditions, plant cells often face the problem of osmotic pressure imbalance, which affects the normal physiological function of the cells. Salicylic acid can help menthol cells maintain a stable osmotic pressure by promoting the synthesis of proline and other osmotic substances, ensuring that the cells can still maintain normal physiological activities under aluminum stress.

The effect of Salicylic acid on the metabolic pathway of *Mentha canadensis* is also reflected in its regulation of secondary metabolites. Secondary metabolites are a class of defensive compounds produced by plants in response to environmental stresses. Salicylic acid can stimulate the synthesis of secondary metabolites in *Mentha canadensis*, such as phenols and flavonoids. These compounds have multiple biological activities such as antioxidant, antibacterial and antiviral in plants, and can effectively enhance the resistance of *Mentha canadensis* to aluminum stress.

It can also improve the resistance of *Mentha canadensis* by affecting its root development. The root system is an important organ for plants to absorb water and nutrients, and it is also a sensitive site for plants to feel environmental stress. Salicylic acid can promote the growth and branching of *Mentha canadensis* root, increase the contact area of root and soil, and improve the efficiency of water and nutrient absorption. Under aluminum stress conditions, good root development can help *Mentha canadensis* better cope with environmental stress and maintain the healthy growth of the plant.

3.5. The Molecular Mechanism behind the Alleviating Effect of Salicylic Acid

1. Enhancing Antioxidant Enzyme Activity: Salicylic acid can enhance the activity of antioxidant enzymes within plants, such as superoxide dismutase, catalase, and ascorbate peroxidase. These enzymes are capable of scavenging reactive oxygen species and alleviating oxidative. 2. Regulating Root Exudates: Treatment with salicylic acid can increase the content of organic acids in plant root exudates, particularly citric. This helps to chelate aluminum ions in the soil, reducing the toxicity of aluminum to the plant. 3. Influencing Gene Expression: Salicylic regulates the expression of a series of aluminum tolerance genes, such as the aluminum-tolerant transcription factor GnART1, citrate synthase gene GmCS citrate transporter gene GmMATE, malate transporter gene GmAlCT, and the aluminum-related transporter genes GmAlS1 and GmIP1; 2. This enhances the plant's ability to tolerate aluminum. 4. Modulating Endogenous H2S Signaling: Research has shown that sallic acid can alleviate aluminum stress by modulating endogenous hydrogen

sulfide signaling. H2S, as a gaseous signaling molecule, can work in concert with salicylic acid to further enhance the plant's ability to tolerate aluminum.

4. Research on Plant DNA Damage under Adverse Conditions

4.1. Exploration of DNA Damage and Repair Processes

Plants can damage cellular DNA due to long external environment, biological or physicochemical stress or external factors such as ionizing radiation, reactive oxygen species, metabolic by-products and metal stress [7], which initiates cell death [8]. The corresponding detection and repair mechanism (DDR) is ubiquitous in all kinds of organisms, as shown in Figure 6. This mechanism can reconstruct DNA, restore genetic information, and maintain genetic stability through base repair and nucleotide repair. Otherwise, plant MMR genes stimulate DNA repair and reduce DNA methylation and DNA adduct, and play a key role in DDR [9], While some intracellular substances and exogenous compounds also have promoting effects on DNA repair. We have shown that glutathione plays an important role in cell cycle regulation in Arabidopsis leaves, increasing the number of DNA templates, increasing the internal replication index, helping to fight cadmium stress and maintaining genome integrity [10]. Taken together, these findings provide necessary theoretical support to explore crop DNA damage under adversity.

4.2. The Impact of Aluminum Pollution on Plant DNA Damage

In adverse environments, plants can initiate their own response to alleviate external stress. Recently, with the rapid development of molecular biology techniques, multiple aluminum-tolerant gene expression mechanisms and functions of encoding proteins have been identified, including the ALMT, MATE protein family [11], GST, SOD and other antioxidant enzymes [12], ABC, and the transporter protein [13] And the Nramp transport family [14] class. Aluminum will enter the root cells and bind to the target DNA main chain. When the aluminum toxicity exceeds the acceptable threshold, the DNA will suffer irreversible damage and cause peroxidation of plant membrane lipids, which will inhibit photosynthesis and respiration, causing cell damage and even death. Compared with histones and non-histones, aluminum tends to bind noncovalently to DNA and causes its conformational change, and the double helix rigidity increases, thus curbing DNA replication [15]. In mitosis, aluminum not only induces chromosome fragmentation, bridging and adhesion, but also affects the spindle-driven chromosome movement disorder [16], Inhibiting the progression of cell division. Differential expression of genes induced by aluminum can affect various metabolic pathways and then cause physiological changes.

4.3. Discussion on Alleviation of Aluminum Stress in Plants under Salicylic Acid Treatment

The role of Salicylic acid in alleviating aluminum stress is mainly reflected in its ability to reduce aluminum damage to plant DNA. Aluminum stress causes single- or double-strand breaks, base mismatches or formation of DNA adducts in plant cells, and these lesions can affect the normal physiological function of plants and even lead to cell death. As an important plant hormone, salicylate plays a key role in plant stress resistance. Under the condition of aluminum stress, Salicylic acid reduces DNA damage through various ways: 1. Activation of antioxidant system: Salicylic acid can improve the activity of antioxidant enzymes (such as superoxide dismutase SOD, catalase CAT, etc.), effectively remove excessive reactive oxygen species (ROS) caused by aluminum stress, and reduce the oxidative damage of ROS to DNA. 2. Adjust the ion balance: Salicylic acid can regulate the ion balance in cells, reduce the accumulation of aluminum ions in cells, and reduce the direct damage of aluminum to DNA. 3. Induced DNA repair proteins: Salicylic acid can induce plants to produce proteins with DNA repair function and accelerate the repair process of damaged DNA.

5. Prospect

Aluminum stress has a significant negative impact on the growth and physiological processes of *Mentha canadensis*. Under Al stress, photosynthesis of *Mentha canadensis* is inhibited, and its water absorption ability decreases, leading to slow growth and yellowing of leaves. Al stress also triggers a series of disorders in *Mentha canadensis*, including a decrease in antioxidant enzyme activity and the accumulation of substances like malondialdehyde and proline, which further exacerbate the stress more severely, Al stress can cause damage to the DNA of *Mentha canadensis*, which may threaten its genetic stability and long-term survival. However, Salicylic acid can alleviate the effects of Al toxicity, significantly improving the growth condition of *Mentha canadensis*, enhancing its photosynthetic efficiency and water absorption ability, and reducing physiological disorders caused by Al stress.

Based on current research, future studies on the physiological regulation of *Mentha canadensis* and the alleviation of DNA damage by SA under Al can be explored from multiple perspectives. On the one hand, the mechanism of how SA affects the physiological response of *Mentha canadensis* can be further refined. Although we haveinarily understood the positive impact of SA in alleviating Al stress on the growth and physiological indicators of *Mentha canadensis*, the specific pathways and molecular mechanisms need to be elucidated. On the other hand, focusing on the alleviation of DNA damage by SA, future research can delve into the specifics of the DNA repair mechanism. It has been established that SA can reduce the DNA damage caused by Al stress, but the exact process of how it promotes DNA repair is still unclear. Therefore, experiments should be designed to explore whether SA activates certain specific DNA repair enzymes or regulates the expression of repair-related genes to achieve its protective effect.

Conflicts of Interest

The authors declare no conflicts of interest.

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