Journal of
**Advances in Agronomy and Crop Science**

Research Article Open Access

# Salt Tolerance of Two Different Varieties of Tobacco Under NaCl Stress

**Yuelin Wang[#], Hanjun Zhou[#], Liang Xu[#] and Xiefeng Ye[*]**

Tobacco Science College of Henan Agricultural University, National Tobacco Cultivation & Physiology & Biochemistry Research Centre, Key Laboratory for Tobacco Cultivation of Tobacco Industry, Zhengzhou 450046, China
Yuelin Wang, Hanjun Zhou, and Liang Xu contributed equally to this work.

[*]**Corresponding Author:** Xiefeng Ye, Tobacco Science College of Henan Agricultural University, National Tobacco Cultivation & Physiology & Biochemistry Research Centre, Key Laboratory for Tobacco Cultivation of Tobacco Industry, Zhengzhou 450046, China, E-mail: yexiefeng@henau.edu.cn

**Received Date:** June 10, 2024 **Accepted Date:** July 10, 2024 **Published Date:** July 13, 2024

**Citation:** Yuelin Wang, Hanjun Zhou, Liang Xu, Xiefeng Ye (2024) Salt Tolerance of Two Different Varieties of Tobacco Under NaCl Stress. J Adv Agron Crop Sci 3: 1-22

## Abstract

**Background:** In the context of increasing global soil salinization, reducing the damage caused by soil salinity and improving the salt tolerance of crops has become an urgent issue in modern agriculture. However, few studies had been reported on the different salt tolerance performances of different varieties of tobacco under salinity stress due to the gradual evolution of geographical separation.

**Results:** The aim of this study was to investigate the different responses of Basma (Spice tobacco) and K326(Flue-cured tobacco) under NaCl stress to identify more effective method to improve salt tolerance in tobacco. In this study, Basma and K326 were treated with a 150 mM NaCl solution and included a blank control treatment. Physiological indicators such as root length growth, antioxidant enzyme activity, ion concentration, hormone content, and related gene expression were measured to assess. In the plate medium, the root length growth of K326 under NaCl stress treatment was only 60% of that of Basma, which had higher salt tolerance. The reasons for this difference mainly concentrated on three aspects. Firstly, in Basma, the accumulation of reactive oxygen species caused by salt stress is scavenged by increasing the activity of antioxidant enzymes, and the damage to the plasma membrane is decreased. Secondly, Basma alleviates ion toxicity by upregulating the SOS1 gene in the roots, balancing intracellular osmotic pressure and maintaining ion homeostasi. Lastly, the expression of *PIN4*, a pivotal factor in growth hormone transport, is upregulated to obtain antigravity transport of growth hormone and increase IAA content in roots, thereby promoting root growth and enhancing the plant's resistance to salt stress.

**Conclusion:** In this study, it was found that oriental tobacco Basma demonstrated greater resistance to salt stress compared

to flue-cured tobacco K326. This was primarily observed in root growth and activity, as well as significant differences in physiological indices such as the accumulation of reactive oxygen species (ROS), regional ion distribution, and hormone content distribution. The experimental results suggest that Basma achieves strong salt tolerance through three main mechanisms: increasing the activity of antioxidant enzymes to eliminate ROS accumulation, expressing *NtSOS1* to adapt to ion regionalization under salt stress, regulating sodium-potassium ratio, and increasing the expression of IAA polar transporter gene *NtPIN4* to maintain a balance of IAA content. In the cultivation of crops like tobacco, the salt tolerance can be effectively enhanced by improving reactive oxygen species scavenging, modifying root structure, and applying IAA.

**Keywords**: Tobacco; Variety; Salt Tolerance; IAA; SOS1

**Abbreviations**

ROS: Reactive oxygen species; pro (Proline); IAA: Auxin; NBT: Nitrotetrazolium blue chloride; DAB: Diaminobenzidine; APX (Ascorbate peroxidase); GSH: Glutathione; $H_2O_2$: Hydrogen peroxide; $O_2^-$: Superoxide anion; CAT: Catalase; SOD: Superoxide dismutase; POD: Peroxidase; ASA: Ascorbic acid; DAB: Diaminobenzidine; MDA: Malondialdehyde; TTC: 2, 3, 5-Tripheyl Tetrazolium Chloride.

## Introduction

Soil salinization refers to the phenomenon that plants cannot grow normally due to the migration of salt ions in the soil, which accumulates on the surface or lower layer, resulting in high soil salt content [1]. Currently, soil salinization has become one of the most important factors limiting the sustainable development of modern agricultural economies. It is estimated that about $11 \times 10^8$ ha of global soil are under the threat of soil salinization, which is about 7% of the world's land area [2], close to 20% of irrigated soil is threatened by salinization, and this percentage is increasing, 58% of which occurs in irrigated agricultural areas [3]. Excessive salt stress can induce crop dormancy, accelerate senescence, and decrease yields during cultivation, significantly impairing the overall productivity of plants [4]. The World Food and Agriculture Organization suggested that salinization of soils, limited arable land area, and sudden natural disasters make the development of modern agriculture an unprecedented challenge [5].

High salt stress in the environment after plant germination often leads to the increase of cell membrane permeability, the accumulation of reactive oxygen species (ROS), and the disorder of cell metabolism, which is difficult to reach the normal level of physiological metabolism and seriously endangers the growth of restricted plants. High concentration of the salt solution will cause osmotic stress and ionic toxicity of plants, lead to excessive accumulation of $Na^+$ and $Cl^-$, and affect the normal growth and development of plants [6,7]. Under salt stress conditions, a large influx of ions into plant cells may lead to the reduction of oxygen elements or the abnormal ordering of oxygen atoms, thereby leading to the formation of unstable ROS. Excessive accumulation of ROS can damage various components and macromolecules in the cell, including cell membrane, plasma membrane, nucleic acids, and proteins, ultimately leading to cell death [8,9]. During their long evolution, plants have developed complex and sophisticated adaptive mechanisms to adapt to salt stress. In their evolutionary response to salt stress, plants have adopted a fundamental strategy of minimizing the exposure of their tissues to saline environments. The enzymatic and non-enzymatic ROS protection systems of plants under stress are capable of promptly and efficaciously neutralizing excessive ROS, so as to ensure the normal growth and development of plants [10]. In the face of ion toxicity under salt stress, plants are also alleviated by the SOS pathway (salt oversensitivity). The SOS signaling pathway consists of three main proteins, SOS1, SOS2, and SOS3. SOS1 protein is the first $Na^+/H^+$ reverse transporter found by human beings, which can expel $Na^+$ out of the cell, thus reducing the intracellular concentration of $Na^+$ [11].

Phytohormones, as important small molecule chemicals in plants, are involved in plant growth, development, and response to environmental stresses. IAA (auxin) is one of the important hormones in plant growth, and have garnered increasing attention for their implicated roles in the plant's response to abiotic stressors. High levels of IAA regulated the expression of several abiotic stress-related genes and altered the activity of antioxidant enzymes. In comparison, the diminished expression levels of IAA in the yuc1 yuc2 yuc6 triple mutant exhibit an exacerbation in ROS accumulation, concurrently attenuating the plant's drought tolerance [12]. Sarwat Saleem et al [13] found that colonization of roots with IAA-producing ST bacterial in roots can improve some physiological functions, such as reducing osmotic stress, increasing $K^+$ uptake, reducing $Na^+$ uptake, maintaining proline (pro) content, relative water content, and relative conductivity in inoculated plants, and improving seed germination and growth parameters. In the study of Amirbakhtiar et al [14] sequenced the root transcriptome of the Iranian salt-tolerant wheat variety Arg and found that genes related to plant hormone signal transduction play an important role in the corresponding high salt stress. Salt stress gradually changed the growth and development of Arabidopsis lateral roots, the number of lateral roots, the elongation of primary roots, and the gravitropism of roots by regulating the concentration of growth hormone [15]. Xia et al [16] found that overexpression of *OsmiR393* in rice inhibited the signaling of growth hormones and weakened the tolerance of rice to drought and high salt. Under salt stress conditions, the synthesis of endogenous IAA in plants is inhibited, which affects normal plant growth and development, When this restriction is removed, the salt tolerance of plants increases [17].

Plants have evolved in different environments and have diversified their germplasm resources through the expression of genetic diversities over time. Under high salt stress, different varieties of the same species show varying levels of salt tolerance. The salt-tolerant rice variety Pokkali has been reported to contain a more active antioxidant defense system that effectively scavenges $H_2O_2$ (hydrogen peroxide) compared to salt-sensitive rice varieties [18]. There are few reports on the response of different tobacco varieties to salt stress. In order to to elucidate the salt tolerance mechanisms, we evaluated 34 tobacco varieties at the budding stage and categorized them into four classes based on their salt tolerance [19]. Utilizing the preliminary data, we selected Basma, the most salt-tolerant spice variety, and K326, the second most tolerant main cultivar of roasted tobacco, for further study. We applied a 150 mM NaCl solution, determined to be the optimal concentration for limiting root elongation in our initial laboratory experiments [20,21], as the stressful conditions.

## Materials and Methods

### Cultivation of Different Varieties of Tobacco

Seeds of flue-cured tobacco K326 and oriental tobacco Basma were selected (The seeds were provided by Yuxi Zhong Yan Seed Co., Ltd China and Yunnan Baoshan aromatic Tobacco Co., Ltd China), entire experimental manipulation of tobacco cultured on plate medium was completed on an ultra-clean bench. The seeds were soaked in a 1% potassium permanganate solution for 30 min to ensure effective removal of surface contaminants and then washed with sterilized ultrapure water to remove residual potassium permanganate reagent from the seed surface. After 10 washes, the seeds were evenly sown on solid MS medium using autoclaved toothpicks. After 7days, healthy and uniformly grown plants on the plates were selected for stress and control treatments. A part of them was transferred from blank MS medium to MS medium plates supplemented with 150 mM NaCl [20], and samples were taken for the determination of various indexes after 0-5 days of treatment. The other part was transferred to hydroponic medium after 7 days of incubation in plate medium without stress treatment. The hydroponic medium was gradually transitioned from 1/4 Hoagland solution to full Hoagland nutrient solution culture. After 14 days of incubation in Hoagland solution, it was transferred to a 150 mmol NaCl solution for stress treatment, and samples were taken within 0-72 hours for the determination of various indexes.

Plate culture and hydroponic culture were carried out in an artificial climatic chamber (RXZ-500, Ningbo Jiangnan Instrument Co., Ltd.) at a temperature of 26°C, a humidity of 60%, a light intensity of 4950 lux, and a day-night alternation of 12 h. The incubation was carried out in an artificial climatic chamber.

### Measurement of Root Length Changes and Peroxide Staining During the Seedling Stage

The root length of seedlings was measured with ruler after 5 days of the stress treatment. The seedlings transferred to blank MS medium were used as control, and the root length growth of two varieties under salt stress treatment were measured. Staining experiments on roots and leaves of 5-day-old tobacco seedlings stress treated on culture medium were carried out.

The endogenous $H_2O_2$ content in different tissues of seedlings was detected using the 3,3-diaminobenzidine (DAB) staining method. The tissues to be tested were immersed in a 0.1% DAB-hydrochloric acid solution system (pH 3.8) for 20 mins, and the dark brown polymerization product obtained from the staining was the amount of $H_2O_2$ in the plants [71]. After rinsing with deionized water, the stain was photographed under an ultra-deep field microscope (SteREO Discovery.V8, ZEISS, Oberkochen, Germany).

Nitroblue tetrazolium (NBT) staining was used to detect endogenous superoxide anion ($O_2^-$) in plant tissues. The NBT reagent was added to the sodium citrate buffer at the concentration of 10mM and diluted to 1mM (pH6.0) and plant tissues from the stress-treated and control-treated groups were immersed in the staining solution for 20 min at 25°C and protected from light, and the dark blue insoluble material obtained was the amount of $O_2^-$ accumulated in the plants [72]. After rinsing with deionized water, the staining solution was placed under an ultra-deep field microscope (SteREO Discovery.V8, ZEISS, Oberkochen, Germany) and photographed.

Cell death staining of root tips and leaves was performed by Trypan blue reagent staining method, where treated seedlings were transferred to Trypan blue solution (25°C, light) for 6 h and then washed with deionized water to remove floating colors; the darker blue polymers obtained, the more severe the cell death. Plant leaf tissues were boiled with 75% ethanol to remove the green color, rinsed with deionized water, and then observed with an ultra-deep field microscope and photographed (SteREO Discovery.V8, ZEISS, Oberkochen, Germany) [73].

### Determination of ROS Content, Antioxidant Enzyme Activity, Root Morphology, Root Activity at Seeding Stage

To determine the quantification of ROS and antioxidant enzyme activity in tobacco tissues, $H_2O_2$, $O_2^-$, CAT and POD all were determined by spectrophotometry. Weigh 0.1 g of tissue was ground into a homogenate on an ice bath, and then the extract was added and measured according to the detailed steps of the the kit produced by Suzhou Comin Reagent Co.

Different varieties of tobacco samples were subjected to salt stress treatment at 0, 6, 12, 24, and 72 hours, cutting the tobacco roots from the root base of the samples with scissors, washing the roots of different varieties of tobacco separately with deionized water, and placing the samples on the scanner for panoramic scanning (Epson Perfection V8000 Photo) of the root system. After the scanning was completed, the total projected area, total root length, and root tip number of different varieties were analyzed by the system of WinRHIZO (Regent Instuments Ins8, QC, Canada) software under salt stress at different times.

The root activity was determined by TTC (2, 3, 5-Tripheyl Tetrazolium Chloride) method, weighing 0.5 g of plant roots in a 20 mL test tube, adding 5 mL of 0.4% TTC solution and 5 mL of phosphate buffer to the test tube, and keeping it in a dark environment at 37°C. After 24 h, 1-2mL of 1 mol/L $H_2SO_4$ was added to the test tube to stop the reaction, the roots were taken out, drained, and 10 mL of ethyl acetate reagent was added and the root viability values were measured at 485 nm.

### Determination of Pro Content, Soluble Sugar Content, Endogenous Hormones, Elemental Content of K and Na at Seeding Stage

Pro and soluble sugar content was by spectrophotometry. Weigh 0.1 g of tissue added and measured according to the detailed steps of the the kit produced by Suzhou Comin Reagent Co.

The content of ABA and IAA in aboveground (leaves and stems) and underground (roots) parts of tobacco were determined by enzyme-linked immunoassay, re-

spectively.

The samples were divided into above-ground and underground parts, dried, ground, passed through a 20-mesh sieve, nitrated, and then the K, Na, contents were determined by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) (MPX, Aglient Technologies, Palo Alto, CA, USA) inductively coupled emission spectrometer.

### RNA Extraction and Relative Gene Expression Determination

Total RNA was extracted from tobacco using FOREGENE Plant Total RNA Isolation Kit, and the first-strand cDNA was synthesized using the All-in-One First-Strand Synthesis MasterMix kit from Kemix. Top Green qPCR SuperMix from Conway Biotechnology was used, primers are shown in Table S1. Fluorescent quantitative PCR was performed on ABI Step One Plus fluorescent quantitative PCR instrument (Applied Biosystems, CA, USA), and the results were analyzed and calculated using the 2-ΔΔCT method.

## Statistical Analysis

The data were represented by the TE mean ±standard deviation (SD) of 3 repeats. After univariate analysis of variance, t-test was used for statistical analysis. In an experiment, the least square method (LSD) was used to test the significant differences among different treatments ($P < 0.05$ $P<0.01$).

## Results

### Root Length Growth of Tobacco Seedlings

The normal MS culture medium was used as control. After 5 d of sowing, the tobacco seedlings with uniform growth were selected and transferred to the 150 mM NaCl treatment substrate and the control substrate, and the changes in root length were counted on the 5th day of treatment. From Figure 1 a and b, it can be seen that salt stress significantly limited the root length growth of the two varieties, the root length of the Basma variety under the control treatment was significantly higher than that of the K326 variety and increased by about 28% compared with the K326 variety, which may be due to the variety difference. After 5 days of culture, the increase in root length of the Basma variety under salt stress was almost equal to that of the K326 variety under control conditions. Specifically, the increase in root length of the Basma variety under salt stress was 1.17 times that of the K326 variety under control conditions. Furthermore, compared to the root length increase of the K326 variety under salt stress, the increase in root length of the Basma variety was 1.73 times as much. The root length growth of Basma and K326 under salt stress was 0.76 times and 0.46 times that of the control condition, respectively.

### NBT Staining, DAB Staining, and Trypan Blue Staining of Tobacco Seedlings

Superoxide anion free radical, also known as superoxide anion ($O_2^-$), is one of the metabolites of oxygen molecules in organisms, with strong redox properties, excessive accumulation will cause cellular tissue damage. Nitrotetrazolium Blue chloride (NBT) can react with $O_2^-$ to form a blue flocculent complex, which is insoluble in water. From Figure 1d, it can be concluded that Basma has strong salt tolerance and the accumulation of $O_2^-$ in leaves and root tip tissues under salt stress is significantly less than that of K326, indicating that Basma can reduce the tissue damage caused by salt stress by reducing the level of $O_2^-$.

$H_2O_2$ is a common ROS in plant tissues, and it reacts with colorless diaminobenzidine (DAB) to oxidize DAB to a brown insoluble precipitate. The content of $H_2O_2$ in leaf and root tip tissues of different salt-tolerant species was determined at 5 d of salt stress treatment, and the DAB staining method was used. It can be seen from Fig. 1e that salt stress treatment increased the accumulation of $H_2O_2$ in leaf and root tip tissues, but the accumulation of $H_2O_2$ in all tissues of the highly salt-tolerant Basma variety was significantly lower than that of K326 under salt stress.

Trypan blue was used to test cell death in tobacco roots and leaves. When the cell membrane structure is damaged, it can be stained blue by Trypan Blue due to the increased permeability of the cell membrane. As seen in Fig. 1f, the cell membrane integrity of K326 and Basma was significantly damaged under salt stress, but the cell membrane structural integrity of Basma was better than that of K326 under salt stress treatment, indicating that salt stress caused

more damage to the cell membrane structure of K326 than Basma.

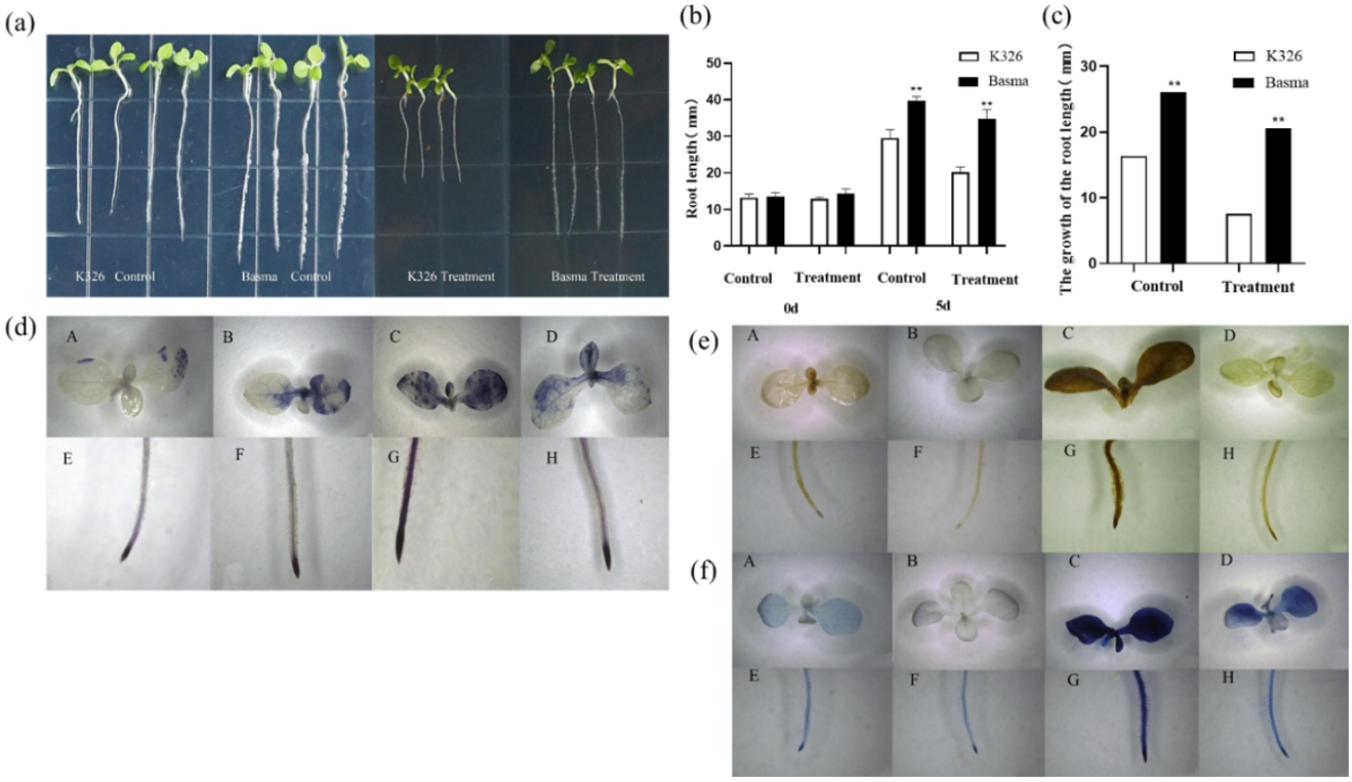


**Figure 1:** Comparison of root length and reactive oxygen staining of different tobacco varieties after salt stress treatment: root length performance of two varieties under control and stress treatments (a). Root length growth statistics of two varieties under control and stress treatments at different stages (b, c). NBT staining (d), DAB staining (e), and Trypan blue staining (f) of two varieties under control and stress treatments. * mean $P<0.05$, ** mean $P<0.01$. The same below.

(A: leaf staining of K326 control treatment B: leaf staining of Basma control treatment

C: leaf staining of K326 stress treatment D: leaf staining of Basma stress treatment

E: Root tip staining of K326 control treatment F: Root tip staining of Basma control treatment

G: Root tip staining of K326 stress treatment H: Root tip staining of Basma stress treatment)

## Related Genes Expression of Tobacco Seedlings

According to the results of the ROS staining in Figure 1, the Basma variety, known for its high salt tolerance, exhibited significantly lower levels of reactive oxygen species accumulation compared to the K326 variety. Consequently, the expression of functional genes associated with the antioxidant pathway, including antioxidant enzymes and non-enzymatic components, was assessed under salt stress and control conditions for 72 h. The gene expression levels in the K326 variety under control conditions were normalized to a value of 1.0. As illustrated in Figure 2, the expression of antioxidant enzymes was generally upregulated following salt stress treatment, with the exception of the *NtCAT*, which showed downregulation on the 72h. Specifically, the expression of the *NtSOD* gene was 1.16-fold and 1.66-fold higher in the K326 and Basma varieties, respectively. The expression of the *NtPOD1* gene increased by 2.04--fold in the K326 variety and by 3.58-fold in the Basma variety, with both increases being statistically significant. Conversely, the expression of the *NtCAT* gene decreased to 60% and 76% of control levels in the K326 and Basma varieties, respectively, with these decreases also being statistically significant. The expression of all genes in antioxidant non-en-

zymatic pathway was up-regulated compared with the control, in which *NtAPX* (Ascorbate peroxidase) gene increased 2.32-fold in Basma and increasd 2.58-fold in K326, and *NtGST* (Glutathione) gene, as a key enzyme gene in GSH synthesis pathway, increased 1.17-fold in K326 and 1.69-fold in Basma. The expression of key genes in antioxidant pathway showed that compared with the control, the number of up-regulated genes in Basma varieties was higher than that in K326 varieties.

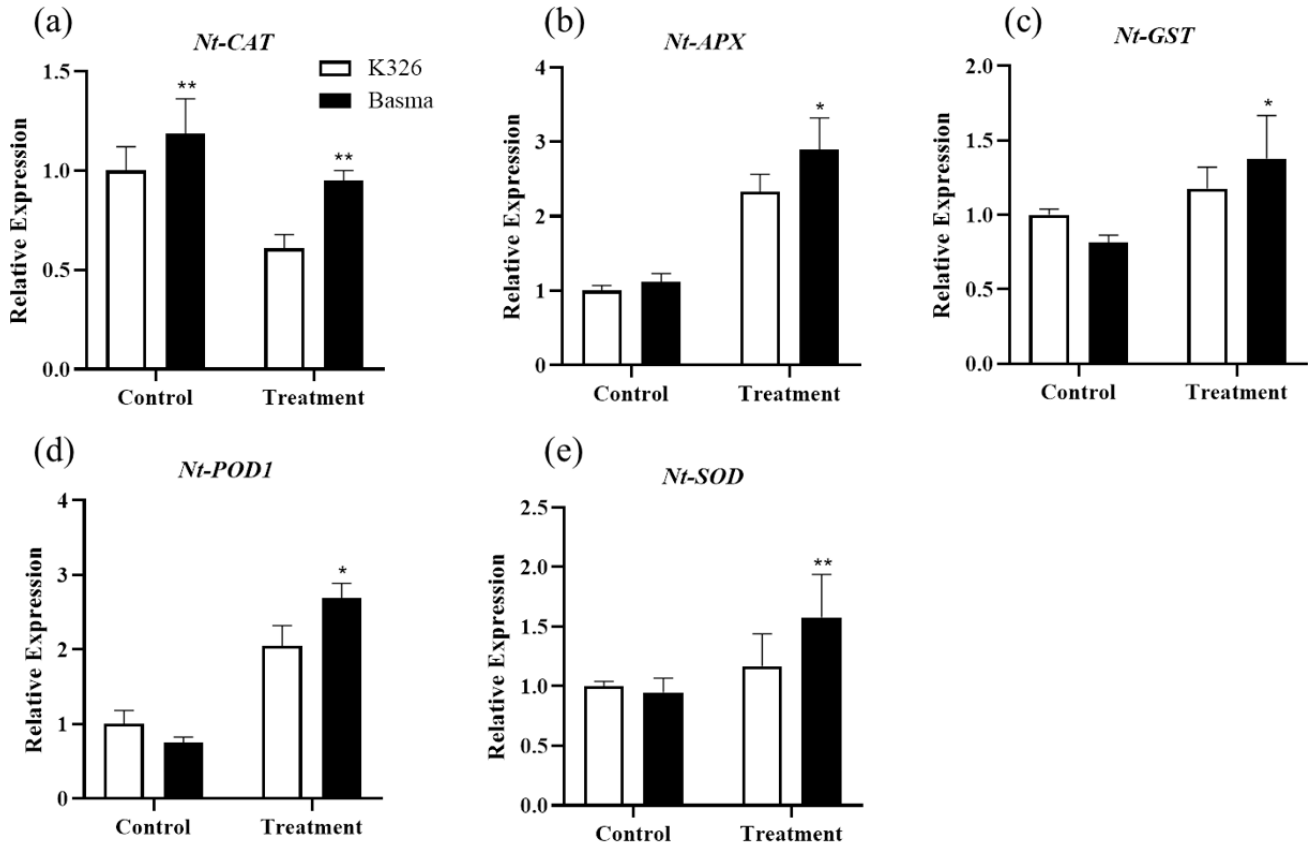


**Figure 2:** Comparison of gene expression of different varieties after 5 days of salt stress: Expression of *NtCAT* (a) after stress in different varieties, *NtAPX* (b), *NtGST* (c), *NtPOD1* (d), and *NtSOD* (e) after stress in different varieties. The asterisk indicates that the mean value was significantly different between K326 and Basma. * mean $P<0.05$, ** mean $P<0.01$.

## Root Development and Root Vigor of Tobacco at Seedings

Root scanning analysis was carried out at 0 h, 6 h, 12 h, 24 h, 48 h, and 72 h after salt stress treatment. The results showed that with the extension of salt stress time, the root development of the two varieties was inhibited, but the root development of Basma with strong salt tolerance was better than that of K326. According to the results of the root scan analysis (Table 1), the total root length, root surface area, root volume, and root tip number of Basma increased, and the average diameter decreased, showing a "thin and long" shape. Except for the average root diameter, the root scanning data of K326 decreased gradually under salt stress, and was significantly lower than that of Basma, showing a "thick and short" shape. The growth and activity of roots directly affected the growth of shoots, and salt stress will cause serious damage to the growth of plant roots. Samples were taken at 0 h, 6 h, 12 h, 24 h, 48 h, and 72 h after salt stress, and the results were shown in Figure 3. With the extension of salt stress time, the root activity of different salt--tolerant varieties decreased gradually, and the root activity of Basma decreased by about twice as much as that of K326 decreased, but the root activity of Basma was still significantly higher than that of K326.

**Table 1:** Changes of root scanning indexes of different varieties under salt stress

| Time after treatment (h) | Variety | Root length （mm） | Root surface area （$cm^2$） | Root volume （$cm^3$） | Mean root diameter （mm） | Root tip number |
|---|---|---|---|---|---|---|
| 0 | K326 | 45.62±1.15b | 5.90±0.24b | 0.03±0.00b | 0.38±0.12a | 52.67±3.33b |
| | Basma | 79.48±5.87a | 7.66±0.17a | 0.08±0.00a | 0.33±0.01b | 63.5±2.50a |
| 12 | K326 | 41.75±0.70b | 4.63±0.16b | 0.03±0.01b | 0.36±0.01a | 49.33±0.67b |
| | Basma | 79.33±4.41a | 7.52±0.55a | 0.05±0.01a | 0.30±0.01b | 64.75±5.25a |
| 24 | K326 | 41.82±1.95b | 5.02±0.40b | 0.03±0.00b | 0.33±0.01a | 47.33±2. 67b |
| | Basma | 83.29±6.99a | 9.26±0.96a | 0.07±0.01a | 0.29±0.01b | 67.75±5.25a |
| 48 | K326 | 42.05±2.50b | 4.01±0.35b | 0.03±0.00b | 0.31±0.00a | 46.30±1.70b |
| | Basma | 85.50±4.38a | 9.36±1.42a | 0.07±0.01a | 0.31±0.00a | 72.00±3.50a |
| 72 | K326 | 37.41±1.42b | 3.09±0.22b | 0.02±0.00b | 0.31±0.01a | 43.50±3.00b |
| | Basma | 87.65±6.81a | 11.20±1.44a | 0.10±0.01a | 0.31±0.01a | 80.25±6.75a |


**Figure 3:** Changes of root development of different tobacco varieties after salt stress at seedings stage (a). Changes of root activity after salt stress at seedling stage (b). * mean $P<0.05$, ** mean $P<0.01$.

## ROS, Antioxidant Enzymes, Pro, and Soluble Sugar Content at the Tobacco Seedings

Both $H_2O_2$ and $O_2^-$ are the main manifestations of ROS in plant tissues, and this peroxide is produced by plants under abiotic stresses, impairing the normal life activities of cells. To investigate the changes in activation levels under salt stress treatment in two tobacco varieties with different salt tolerance, the accumulation of ROS was measured at six time periods after salt stress treatment, 0 h, 6 h, 12 h, 24 h, 48 h, and 72 h, and the results are shown in Figure 4 a and b. The reactive oxygen content in the tissues of different salt-tolerant varieties under salt stress treatment showed a trend of increasing and then decreasing, and the reactive oxygen content of the highly salt-tolerant variety Basma was lower than that of K326 at all time periods of salt stress treatment. At the highest of accumulation, the accumulation of the $O_2^-$ of the Basma variety was 16.5% lower than that of the K326 variety.

CAT and POD were the main scavengers of $H_2O_2$. As shown in Figure 4， d and e, the peak antioxidant enzyme activities of the two different salt-tolerant tobacco varieties were staggered during the six time periods of salt stress treatment, and the antioxidant enzyme activities of the highly salt-tolerant variety Basma were significantly higher than those of K326 at the late stage of salt stress incubation, and at 72 h of salt stress treatment, the antioxidant enzyme activities of Basma were 4.79 times in CAT and 1.40 times in POD of K326, respectively.

Pro is a protein widely present in the free state in plants and can be used under conditions of adversity stress not only as a major osmotic substance to alleviate osmotic stress but also as a protective substance for membranes and enzymes and as a free radical scavenger. As can be seen from Figure 4 c, the results of the osmotic substance content assay for six time periods of different salt-tolerant varieties under salt stress treatment showed that pro started to accumulate in large amounts after 24 h of salt stress, and the accumulation of pro in Basma was greater than that of the K326, which was 1.4 times greater than that of the K326. Soluble sugar is an important osmotic regulator in plants and an important regulator of plant growth, development and gene expression. As shown in Figure 4 f the accumulation of soluble sugar content of the two varieties under salt stress was also significantly different after 24 hours. The soluble sugar content of Basma variety was significantly higher than that of K326 variety, which was 2.05 times higher than that of K326 variety.

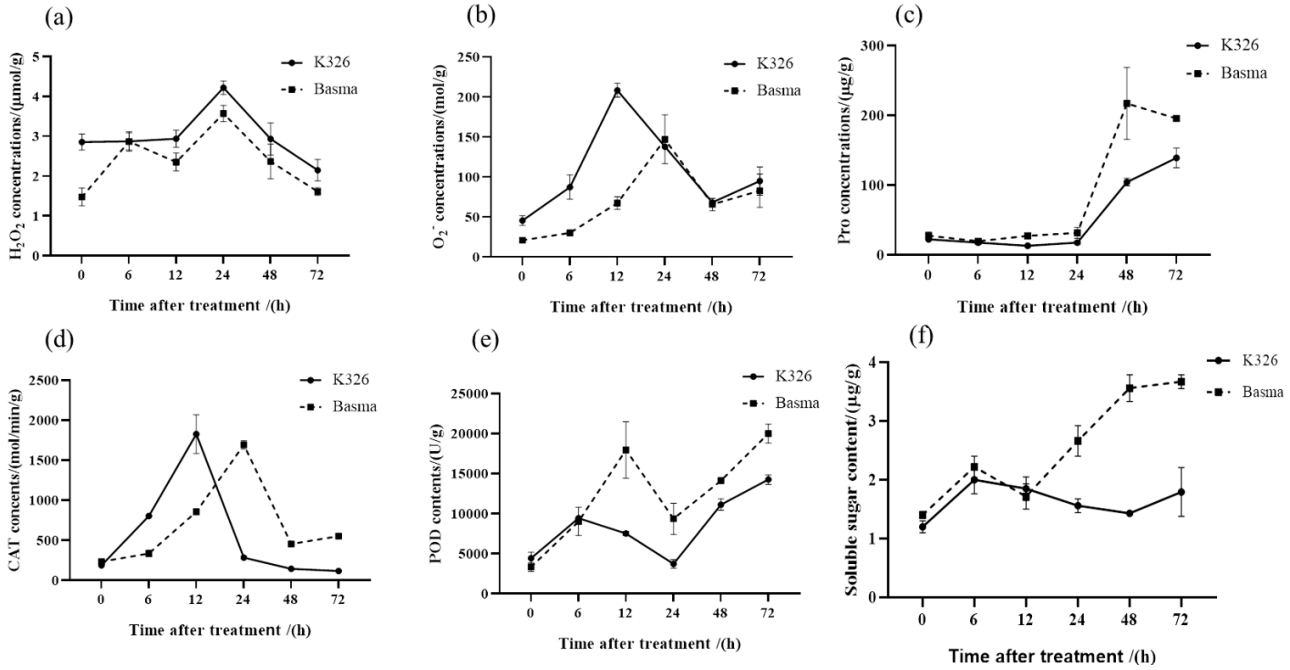


**Figure 4:** Comparison of ROS, antioxidant enzymes, and Pro content in different tobacco species at the seeding stage after salt stress treatment: Changes in $H_2O_2$ concentration (a), $O_2^-$ concentration (b), Pro concentration (c), CAT content (d), POD content (e), soluble sugar content (f) after stress treatment of different species.

## K, Na Elements Content, and *SOS1* Gene of Tobacco Varieties at the Seedings

Ion toxicity is an important mechanism of plant damage caused by salt stress. The large accumulation of inorganic ions leads to an increase in salt concentration, which disrupts the plasma membrane structure of plants and also inhibits the uptake of other mineral elements, resulting in nutrient imbalance. The results are shown in Figure 5 illustrate a substantial increase in the accumulation of Na in both the aboveground and underground parts of Basma plant tissues under salt stress treatment, compared with control. The increase was 21.01-22.90 times in the above--ground part and 12.84-14.15 times in the underground part. The accumulation of elemental K was significantly reduced compared to the control treatment, with reductions to 26.05% for Basma and 14.82% for K326 in the aboveground part and 33.80% for Basma and 40.59% for K326 in the underground part. After salt stress, the sodium-potassium ratio value of both varieties increased, but under salt stress, the sodium-potassium ratio of the aboveground part of K326 was 26.90 times that of Basma, and the sodium-potassium ratio of the underground part of K326 was 19.40 times that of Basma. After salt stress, the accumulation of Na in K326 was much higher than that of Basma.

The SOS1 protein is located on the cell membrane and can pump sodium ions outward, which playing a key role in the study of salt tolerance in plants. A decrease in the expression level of *NtSOS1* may lead to a decrease in the SOS1 protein content, resulting in an increase in the intracellular Na content under salt stress and a certain a substantial toxic effect on the growth of cells and plants. As shown in Figure 5 d and h, the expression of *NtSOS1* in the aboveground and underground parts of the two varieties at the seeding stage was examined at four time periods of 0 h, 6 h, 12 h, and 24 h, and the expression of its in the aboveground part of the K326 variety was calculated as the control. The *NtSOS1* expression in the underground part of Basma variety was significantly higher than that of K326 at 0 h. At 6 h, the relative expression of the aboveground part of K326 variety increased to 2.27-fold. And the relative expression of *NtSOS1* of Basma variety above ground increased 4 times at 12 h of salt stress treatment compared with that at 0 h, especially the gene expression of Basma underground part was 6.42-fold higher than that of K326 at the same time. At 24h of salt stress treatment, the relative expression of *NtSOS1* gene in the aboveground part of Basma variety was not significantly different from that of K326, while the underground part was 1.91-fold higher than that of K326.

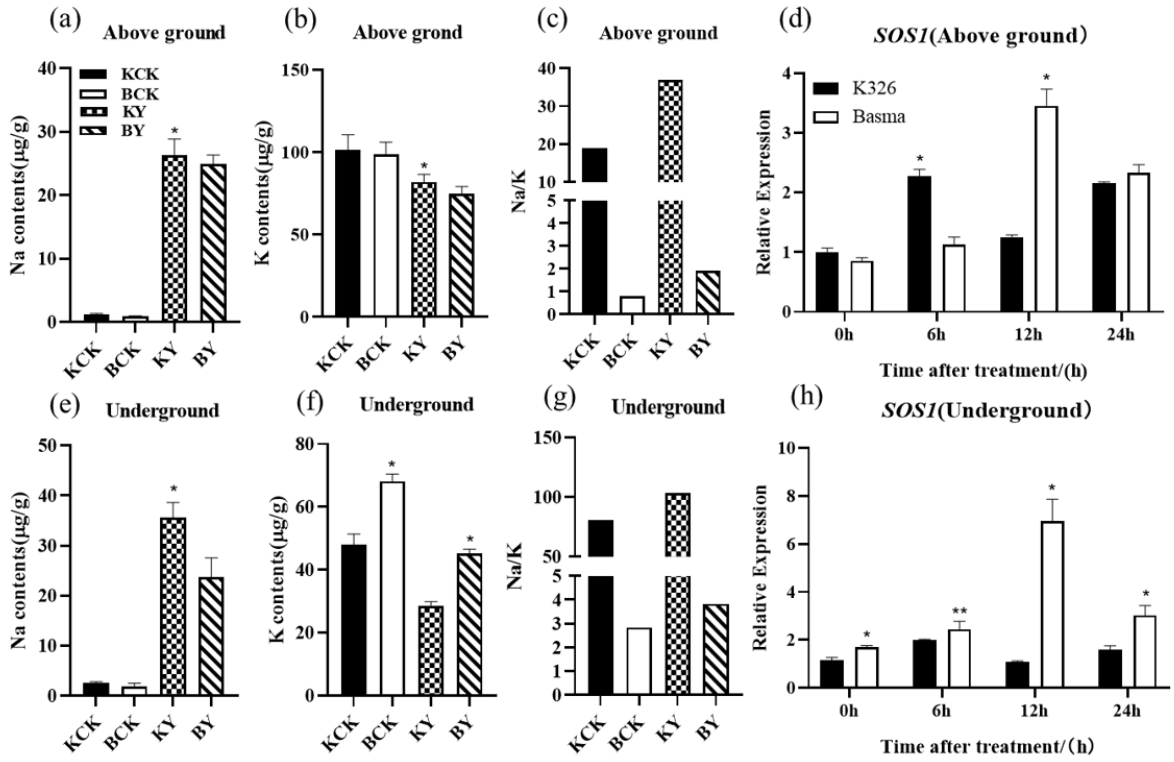


**Figure 5:** Comparison of K, Na content, and SOS1 Gene expression in different Tobacco varieties at Seeding stage after salt stress treatment: Na content (a), K content (b), sodium-potassium ratio (c), and *SOS1* expression (d) in aboveground part of different varieties control treatment and stress treatment. Na content (e), K content (f), sodium-potassium ratio (g), and *NtSOS1* expression (h) in the underground part of different varieties control treatment and stress treatment. *mean $P<0.05$, **mean $P<0.01$.

## Hormone Content of Tobacco at the Seedings

When plants are subjected to environmental stress, ABA and IAA contents change rapidly which not only modulate their immediate stress-responsive mechanisms but also regulate long-term growth and developmental processes. The ABA and IAA contents of the two varieties under salt stress for 0 h, 12 h, 24 h, and 72 h were determined, and the results are presented in Figure 6. As shown in Figure 6 a-c, the content of ABA in the aboveground parts of the two varieties increased rapidly from 0 to 24 h after salt stress, but decreased sharply after 24 h. The ABA content in

the underground part of Basma increased continuously, while that of K326 increased from 0 to 12 h and then decreased. At 72 h, the content of ABA in the underground part of Basma was significantly higher than that of K326. As shown in Fig. 6 d-f, the total IAA content of different salt--tolerant varieties decreased at first and then increased under salt stress. The IAA content in the aboveground parts of K326 decreased after 12 hours of salt stress, while Basma's increased. At 72 hours, K326's IAA content was 1.5 times that of Basma. The results of the determination of IAA content in underground part showed that the IAA content in underground part of two varieties decreased at first and then increased under salt stress. After 24 h of salt stress, the content of IAA in underground part of Basma was significantly higher than that of K326. The results showed that in the later stage of salt stress, there was no significant difference in the total amount of IAA between the two varieties, but the IAA accumulation in the underground part of Basma was significantly higher than that in the aboveground part.

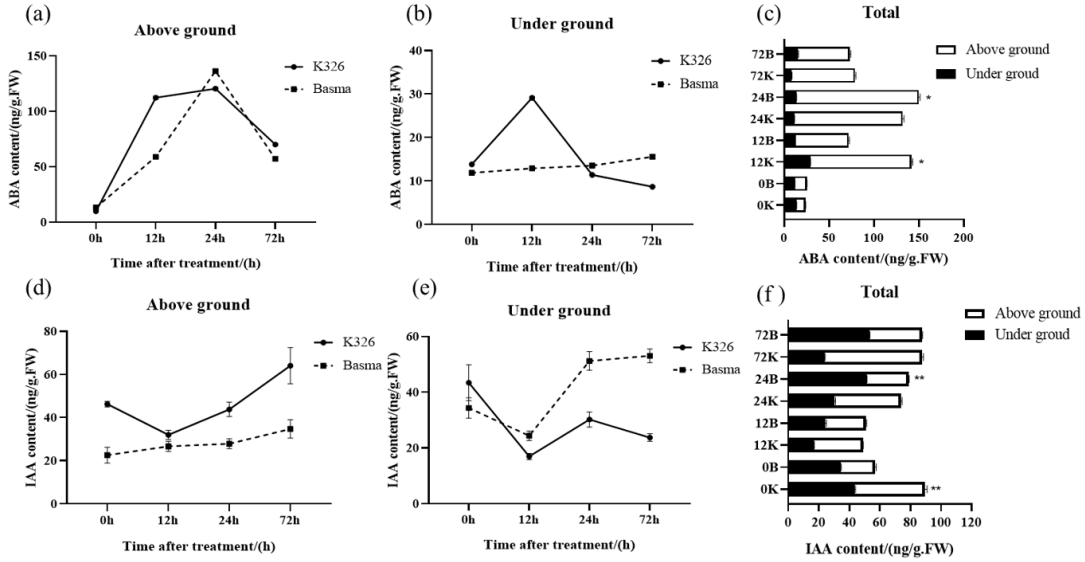


**Figure 6:** Comparison of hormone content of different salt-tolerant tobacco varieties at the seedings stage after salt stress treatment: ABA content of aboveground part (a) and underground part (b) after stress treatment of different varieties. ABA content statistics of whole plant (c) after stress treatment of different varieties. IAA content of aboveground part (d) and underground part (e) after stress treatment of different varieties. IAA content statistics of whole plant (f) after stress treatment of different varieties. * mean $P<0.05$, ** mean $P<0.01$.

## Relative Expression of *PIN* Family Genes and *YUCCA* Family Genes in Tobacco at the Seeding Stage

The relative expression of *PIN* family genes in tobacco varieties with different salt tolerance was determined during four periods of salt stress, as shown in Figure 7a 7b 7e 7f. Under salt stress, *PIN1-A* expression in Basma's aboveground part was significantly lower than K326's at 12 hours. However, at 24 h, the relative expression of *NtPIN1-A* gene in the aboveground part of Basma was 7-fold higher than that of K326. After salt stress, the *NtPIN4* gene expression of Basma in both aboveground and underground parts was always significantly higher than that of K326, which was 5.48-18.76-fold higher than that of K326.

The relative expression of *YUCCA* genes was measured by sampling aboveground and underground parts of two different tobacco varieties at four selected time periods (0h 6h 12h 24h) and the results are shown in Figure 7c 7d 7g 7h. The relative expression of *NtYUCCA6* increased in tobacco under salt stress. The relative expression of the *NtYUCCA6* gene in the underground part of the Basma variety was significantly higher than that of the K326 variety after salt stress, while the relative gene expression in the aboveground part of K326 was 4.67-fold higher than that of

Basma from 12 h to 24 h of treatment. Under salt stress, the underground gene expression of the two varieties had the same trend, which decreased significantly at 12 h and increased slightly at 24 h. However, the relative expression of *NtYUCCA8* gene in the aboveground part of Basma was significantly higher than that in the aboveground part of K326 and also significantly higher than that in the underground part of K326.

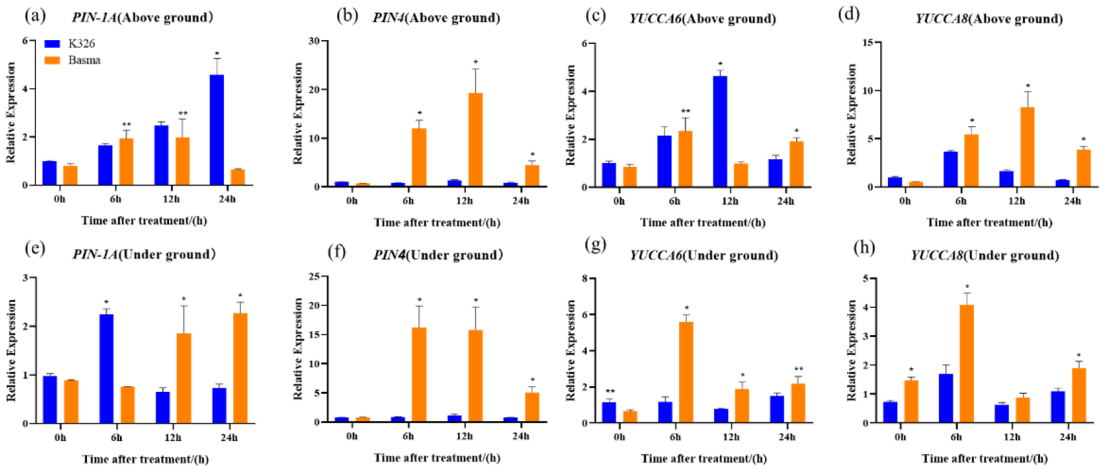


**Figure 7:** Comparison of genes in different varieties of seeding stage after salt stress treatment. Expression of *NtPIN1-A* (a), *NtPIN4* (b), *NtYUCCA6* (c), *NtYUCCA8* (d) in aboveground part after stress of different varieties. Expression of *NtPIN1-A* (e), *NtPIN4* (f), *NtYUCCA6* (g), *NtYUCCA8* (h) in underground part after stress of different varieties. * mean $P<0.05$, ** mean $P<0.01$.

## Discussion

### Differences in the Tobacco Growth

As the earliest organ to feel salt stress, plant roots will produce a series of physiological changes under salt stress, which will affect the growth of aboveground parts of plants. It was found that salt stress significantly decreased the root growth rate of Arabidopsis thaliana [22], so plant root length is a commonly used index in the study of salt stress. According to Alaya et al [23], different concentrations of salt in the growth environment will lead to different degrees of inhibition of cellulase and antioxidant enzymes and further inhibit plant growth. Especially with the increase of salt concentration, the inhibitory effect on root growth was enhanced. In this study, after 5 d of stress treatment, the root length growth of K326 tobacco under salt stress was only 60% of Basma. Therefore, from the point of view of root development after adding salt stress, Basma has higher salt tolerance than K326.

### Differences in ROS Content and Antioxidant Enzyme Activities

Under salt stress, ROS accumulate in plants, mainly in the form of $H_2O_2$ and $O_2^-$, and ROS can play a crucial role as signaling molecules in regulating various physiological processes in plants, inducing programmed cell death, regulating hormone signaling, photosynthesis, stomatal movement, and inducing cell signaling related to plant defense genes. However, the massive accumulation of ROS is also a common manifestation of the imbalance of metabolic mechanisms in organisms under adversity stress environment and is one of the important causes of oxidative damage to the plasma membrane. Along with the increase in ROS, plants scavenge excess ROS by increasing the activity of antioxidant enzymes such as SOD, CAT, and POD [24], and enhancing enzymatic and nonenzymatic radical scavenging capacity is an effective means to cope with oxidative stress. In rice KDML, RD6, and SKC1 genes mitigate the detrimental effects of reactive oxygen species (ROS), leading to enhanced seedling growth [25]. This improvement is attributed to elevated activities of antioxidant enzymes, which help counteract salt stress. Ren et al [26] used SNP (NO donor) solution, the activities of SOD and CAT increased, while the activity of MDA (malondialdehyde) de-

creased. Wang et al [27] enhanced the expression level of four antioxidant genes *ThGSTZ1*, *ThAPX*, *ThSOD*, and *ThPOD* by transgenic *Th2CysPrx*, thus increasing the activity of antioxidant enzymes and enhancing the scavenging ability of ROS, thus alleviating the damage to cells caused by salt stress. Differences in antioxidant enzyme activities also existed among different cultivars of the same crop [28]. The activities of SOD and POD in salt-tolerant rice varieties were significantly positively correlated with salt stress ability, and their ability to scavenge ROS was significantly higher than that of salt-sensitive varieties. Guan et al [29] showed that the relative expression of antioxidant system genes in salt--tolerant wild tomato was significantly higher than that in common tomato varieties. In this study, through the determination of antioxidant enzyme activity in seeding-stage tissues of different tobacco varieties, it was found that with the increase of salt stress treatment time, the antioxidant enzyme activity of Basma variety was always higher than that of K326 variety, indicating that the higher antioxidant enzyme activity is an important reason for the higher salt tolerance of Basma variety.

## Differences in Osmotic Substances

Under salt stress, plants regulate the intracellular osmotic potential by accumulating some compatible solutes, such as pro, soluble sugars, soluble proteins, and inorganic ions, to maintain water balance and thus protect the activity of many enzymes required for important intracellular metabolic activities [30]. By regulating the mass content of osmoregulators in the body, which is an important way for plants to resist osmotic stress brought about by salt stress, Pro, an important osmoregulatory substance, exists in plant cells in the free state, within the physiological pH range, with small molecular weight, high water solubility, and no net charge. Pro acts as an osmoprotectant to maintain plant growth under salt stress, protects the photosynthetic system, maintains ion homeostasis, and enhance antioxidant systems [31]. Soluble sugars mainly include glucose, sucrose and alginate, and their main functions are to stabilize the plasma membrane and protoplasts, protect soluble enzymes [32], and provide energy for the synthesis of organic matter. It was found the contents of osmotic regulating substances accumulated by different salt-tolerant flax varieties were different under salt stress, and the pro content in roots, stems and leaves of salt-tolerant flax varieties was significantly higher than that of sensitive materials [33]. Shadad et al [34] found that the accumulation of soluble protein and pro differed significantly in soybean with different salt tolerance under salt stress, with higher sugar and protein content in salt-tolerant varieties and lower in salt-sensitive varieties. In this study, two tobacco varieties showed higher accumulation of pro and soluble sugar after salt stress, but the accumulation rate of Basma was significantly higher than that of K326 after 12 h of stress, indicating that Basma had a faster regulation of osmotic accumulation and stronger resistance to salt stress than K326.

## Differences in the Accumulation of Na, K Elements, and *NtSOS1* Expression

Ionic toxicity is an important cause of plant damage due to salt stress. Under high concentrations of salt stress, excess $Na^+$ enters plant cells, inhibits cellular uptake of $K^+$, and disrupts the intracellular ionic balance [35]. In field production, the increase in salt concentration in soil also causes excessive $Na^+$ uptake by plants, which in turn disrupts the plant plasma membrane structure and affects the normal physiological metabolism of plants [36]. In addition to this, it has been shown that sodium-potassium ratio play a role in measuring the varietal salt tolerance of crops such as wheat, rice, and tomato [37-39], while Basma varieties have lower sodium-potassium ratio than K326. A study by Mekawy et al [33] also demonstrated that two crops with different salt tolerance flax, which had significant differences in sodium-potassium ratio of roots and stems under salt stress treatment. In this study, the accumulation of elemental Na was significantly higher in the K326 variety than in the Basma variety after salt stress treatment, and the accumulation in the underground part is greater than aboveground part. Some studies found that 0 salt mustard under salt stress accumulated more Na absorbed in the medium in the roots, thus maintaining a lower sodium-potassium ratio in the leaves and adapting to the saline environment. A similar phenomenon was observed in cotton under high salt stress [40, 41]. In order to maintain its own growth and normal photosynthesis, $Na^+$, which caused ion toxicity, was accumulated in stems and petioles, while $K^+$ was transferred to leaves to maintain the normal work of leaf photosynthesis. According to the results of accumulation and distribu-

tion of Na, K elements in two varieties after salt stress treatment, the Na content in the underground part of Basma varieties was significantly lower than that of K326. Therefore, we believe that one of the important reasons for the high salt tolerance of Basma species is its ability to regulate ion homeostasis.

SOS pathway is one of the most important signal transduction pathways under salt stress [42, 43]. The SOS pathway has been shown to play an important role in $Na^+$ efflux and includes SOS1, SOS2, and SOS3, which are conserved domains [44, 45]. SOS3 and SCaBP8 can sense salt-induced $Ca^{2+}$ signals and interact with SOS2 phosphorylates Ser1138 on SOS1, relieving the autoinhibition of SOS1 by the C-terminal inhibitory domain and activates SOS1, which transports $Na^+$ from the cytoplasm to the exosome and prevents $Na^+$ accumulation to toxic levels [46]. Guo and colleagues [47] have shown that the SOS1 overexpression mutant strain is more salt-tolerant than the wild type, with a significantly higher intracellular sodium-potassium ratio compared to the wild type. It is also believed that SOS1 indirectly regulates cellular potassium homeostasis and plays a crucial role in maintaining overall cellular ion balance. Therefore, we divides the treatment into aboveground and underground parts were selected for *NtSOS1* gene expression assays at four stages of salt stress treatment, 0 h, 6 h, 12 h, and 24 h. In this study, the *NtSOS1* expression in the roots of Basma variety was significantly higher compared to K326, especially the gene expression in the underground part at 12 h of treatment was 6.42 times higher than that of K326 variety, while the Na content in the underground part of Basma variety was much lower than that of K326 variety. This indicates that when exposed to salt stress, the high expression of *NtSOS1* in the roots of the Basma variety promoted the efflux of $Na^+$, which reduced the Na content in the underground part, decrease the sodium-potassium ratio, and effectively regulated the ion balance in the tissues. Therefore, the Basma variety has high salt tolerance.

## Differences in Phytohormones Content and Expression of Functional Genes Related to Growth Hormone

Plant cells respond to abiotic stress by inhibiting growth, such as the reduction in root and shoot elongation. In this process, plant hormones play a crucial role. When plants receive salt stress signals, stress-related hormones (ABA, salicylic acid, jasmonic acid, and ethylene) and growth and development-related hormones (growth hormone, gibberellin, cytokinin, and oleoresin sterols) play an important role in weighing growth and stress responses, with some hormones positively or negatively regulating plant salt tolerance. ABA, as one of the most important stress-response hormones, plays an important role in the defense against adversity stress, especially osmoregulation. Zhu et al, identified that the LRXs-RALFs-FER module in Arabidopsis cell walls regulates downstream signaling pathways under salt stress [48]. This regulation primarily modulates the increase in levels of abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA), which are crucial for maintaining cell wall integrity under abiotic stresses, as well as for growth regulation and plant survival under salt stress conditions. Salt and osmotic stresses lead to a rapid increase in endogenous ABA levels, which activate SnRK2 protein kinase, phosphorylate downstream effector proteins, and further regulate plant stomatal closure in response to osmotic stress [49]. In this study, the ABA level increased significantly from 12 to 24 h. Although the corresponding ABA hormone levels of the two varieties were different during salt stress, the difference of ABA levels between the two varieties decreased at 72 h in the later stage of salt stress. It is worth noting that after salt stress treatment, the distribution of IAA in the two tobacco varieties showed significant differences, and IAA also played a very important role in the regulation of plant adaptation to salt stress. The expression levels of most Aux/IAA genes were up-regulated in rice (Oryzasativa), tomato, pepper, and sorghum under different abiotic stress conditions [50-54]. In this study, Basma with high salt tolerance showed a significant increase in IAA content in its underground parts after 24 h of salt stress treatment compared to K326, a result that also corresponds to the characterization of Basma's root system that still grows and develops under salt stress, so we addressed this result in greater depth.

We focused our attention on the key genes involved in the synthesis and regulation of IAA. Mashiguchi K et al [55] found that two key enzymes are involved in the important synthetic pathway of growth hormone: tryptophan aminotransferase and flavin monooxygenase (YUCCA), the latter catalyzing the synthesis of the growth hor-

mone indole pyruvate from tryptophan, followed by direct conversion of *YUCCA* to IAA. Salt stress leads to redistribution of growth hormone and the localization of growth hormone transport proteins AUX1 and PIN1/2 is altered by salt stress and inhibits the activity of root meristematic tissue. Growth hormones can affect various physiological metabolic processes in plants, and YUCCA is the rate-limiting enzyme for plant growth hormone biosynthesis [56]. It has been shown that overexpression of the growth hormone synthesis key enzyme gene *AtYUCCA6* from Arabidopsis thaliana in poplar and potato [57, 58] produced transgenic varieties with excess growth hormone and all varieties also showed greater resistance to stress. PIN is considered to be the main export carrier of the phytohormone IAA runner protein and *PIN1*, *PIN2*, *PIN3*, *PIN4*, and *PIN7* genes have been isolated from Arabidopsis and other plants, and the role of these genes in the groundward and lightward responses of plant roots, root elongation growth, and organ development has been demonstrated. The role of these genes in the groundward and lightward responses of plant root systems, root elongation growth, and organogenesis and development was demonstrated. In a study of the Arabidopsis *PIN* gene family, *PIN1-4* and *PIN7* were found to be localized on the plasma membrane of specific cells to regulate growth hormone export [59]. *PIN4* is mainly involved in the transport process of root growth hormone to the resting center and its role is mainly to balance the overall distribution of other *PINs* in the root, thus ensuring proper root growth [60]. Salt stress causes dramatic changes in the expression of *PIN1c*, *PIN3*, and *PIN4*, which may lead to different distribution of root growth hormone and affect root growth. Overexpression of *AtPIN1* can promote the flow of growth hormone to the root tip and increase the number of lateral roots [61]. In this study, we observed a difference in root length between two tobacco varieties under salt stress. Our investigation revealed that *NtYUCCA6* and *NtYUCCA8* were significantly more expressed in Basma varieties than K326 under salt stress, resulting in higher IAA content and thus promoting root development in Basma varieties. This is also consistent with the results of root length measurements. The relative expression of the *PIN* family in the lower part of the ground was significantly higher in Basma varieties than in K326 varieties, especially the *PIN4* gene; this is also consistent with the results of root IAA content measurements, which were significantly higher in Basma varieties after salt stress treatment, indicating that Basma varieties can still synthesize IAA under salt stress and thus mitigate salt stress through IAA regulation effects. Furthermore, we noted an increase in antioxidant enzyme activity and overexpression of *NtSOS1*, which both contributed to maintaining $Na^+/K^+$ balance in Basma roots. Therefore, the high salt tolerance of Basma may be due to the IAA-regulated pathway. This needs to verified in a subsequent study using the IAA mutant of K326 (Figure 8).

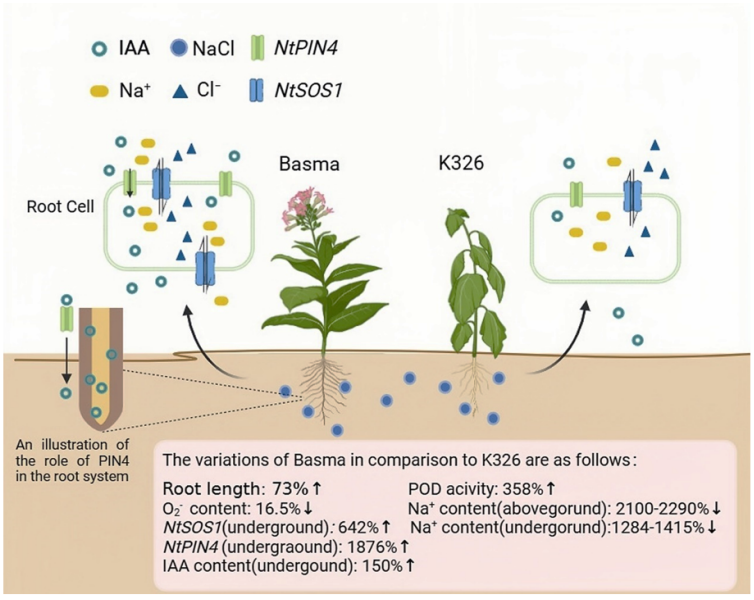


**Figure 8:** Mechanisms of differences in salt tolerance of different varieties of tobacco under salt stress

### Prospects for the Practical Application of the Findings

In this study, we clarified the differences in salt tolerance between the two tobacco varieties and preliminarily identified the reasons for Basma's high salt tolerance. These include the efficient scavenging of ROS, selective uptake of $Na^+$, and the uneven distribution of IAA. These factors collectively contribute to Basma's sustained growth and enhanced salt tolerance under salt stress conditions.

In actual production, we believe that improving the scavenging of reactive oxygen species and enhancing selective ion uptake, as discussed, can enhance crop salt tolerance. Fang et al have demonstrated that scavenging reactive oxygen species improves crop salt tolerance [62]. Specifically, Jun et al. synthesized a novel engineered nanomaterial (Cs-Se NMs) that, when applied at 300 mg/L to rice, significantly improved the activities of superoxide dismutase, catalase, and peroxidase in the aboveground parts of the plants, thereby enhancing salt tolerance. Additionally, the overexpression of the transgene *PagMYB73* in Jatropha curcas has been shown to enhance salt tolerance, primarily by regulating osmotic homeostasis and decreasing ROS levels through its promoter region [63]. Therefore, applying substances exogenously to reduce ROS content offers a viable method to mitigate salt damage in practical cultivation.

In terms of root morphology, it has been suggested that changes in root morphology can improve salt tolerance in plants. Julkowska M et al. showed that natural variation in root architecture among Arabidopsis thaliana lines was related to the response to salinity [64]. They found that varieties exhibiting high salt tolerance under salt stress had a reduced number of lateral roots and an elongated main root. This finding is not entirely consistent with our study, however, the root system remodeling by plants is believed to enhance their resistance to salt stress.

In terms of IAA content, it has been verified that foliar spraying of IAA can alleviate salt stress injury and improve the yield of crops such as maize [65], hemp [66], and tomato [67]. Additionally, the application of IAA to the root system can help mitigate the damage caused by salt stress. Feng et al [68], found that the exogenous application of 10 μmol/L IAA to the root system significantly increased the net photosynthetic rate and aboveground bioaccumulation of burley tobacco in a hydroponics experiment. Similar findings have been reported in cherry radish [69], where exogenous IAA application significantly increased both root and leaf fresh weight. In potatoes [70], IAA application to the culture medium was found to alleviate the effects of salt stress on the root system. Nevertheless, most of these studies were conducted in the laboratory, with fewer comprehensive field cultivation experiments. Therefore, we plan to apply IAA in tobacco field cultivation and study its practical effects on tobacco yield under salt stress conditions

As a final point, we will also consider applying biotechnological approaches to enhance the salt tolerance of tobacco, and further explore how the PIN gene family regulates tobacco's response to salt stress using mutant approaches in the laboratory.

## Conclusion

In this study, A comprehensive analysis of the salt tolerance mechanisms in the two tobacco varieties, Basma and K326, has unveiled significant disparities in their adaptive responses to saline stress. The conclusions drawn from this study are as follows: Firstly, enhanced antioxidant defense: The Basma variety exhibited a pronounced augmentation in the activities of antioxidant enzymes, such as SOD) POD, which were elevated by 1.16 to 3.58 times compared to the K326 variety. This increase in enzymatic activity was correlated with a diminished accumulation of ROS, particularly a 16.5% reduction in $O_2^-$ levels in Basma at the peak ROS accumulation relative to K326. Secondly, ion homeostasis regulation: the Basma variety effectively modulated ion homeostasis through the upregulation of *NtSOS1* gene expression, notably in the underground tissues, where the expression level was 6.42-fold higher than that of K326 at 12 hours post-saline stress imposition. This regulatory mechanism resulted in a marked reduction in sodium ion ($Na^+$) accumulation in the above-ground tissues (21.01 to 22.90 times lower than control conditions) and in the underground tissues (12.84 to 14.15 times lower), alongside a more balanced $Na^+/K^+$ ratio, indicative of superior ion compartmentalization capabilities. Finaly, hormonal balance modulation: Basma demonstrated a strategic modulation of

phytohormone levels, particularly the auxin indole-3-acetic acid (IAA). There was a 7-fold increase in *NtPIN1-A* expression in the above-ground tissues 24 hours post-saline stress, coupled with a consistent overexpression of *NtPIN4* in both above-ground and underground tissues. This hormonal adjustment supported root development and overall growth under stress, as evidenced by a 1.5-fold higher IAA content in the underground tissues of Basma compared to K326 after 72 hours of saline stress.

In summary, the superior salt tolerance of the Basma variety is attributed to its multifaceted adaptive strategies, which encompass the upregulation of antioxidant systems to counteract ROS accumulation, precise regulation of ion homeostasis through enhanced *NtSOS1* expression and management of the $Na^+/K^+$ ratio, and modulation of phytohormone balance, specifically IAA, to foster growth and stress adaptation. These findings provide key insights into the genetic and physiological basis for salt tolerance and suggest new research directions for how IAA might influence plant salt tolerance. Additionally, it is suggested that in practical cultivation, enhancing the salt tolerance of crops can be achieved by enhancing the scavenging of reactive oxygen species, improving root system structure, and applying indoleacetic acid, thus effectively enhancing the salt tolerance of crops.

## Declarations

## Ethics Approval and Consent to Participate

This study is in line with relevant institutions, national and international guidelines and factory ethics legislation. The plant seeds in this study were provided by Yuxi Zhongyan seed Co., Ltd and Baoshan aromatic Tobacco Co., Ltd. This lab has been granted permission.

## Consent for Publication

Not applicable

## Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Competing Interests

The authors declare that they have no competing interests.

## Funding

This research was funded by the Key Laboratory for Tobacco Cultivation of Tobacco Industry of China (No. 30800665).

## Authors' Contributions

XFY, YLW and LX conceived the research; YLW performed the experiments and conducted the data analysis; LX and YLW wrote the manuscript; HJZ revised the manuscript; XFY provided funding acquisition, supervision, and project administration. All authors have read and approved the manuscript.

## References


1. Singh A (2022) Soil salinity: A global threat to sustainable development. Soil Use and Management. 38: 39-67.

2. FAO (2023) Global Soil Partnership. Available online: https://www.fao.org/global-soil-partnership/resources/highlights/detail/en/c/1412475/

3. Qadir M, Quillérou E, Nangia V, Murtaza G, Singh M, Thomas RJ, et al. (2014) Economics of salt-induced land degradation and restoration. Nat. Resour. Forum. 38: 282-295.

4. El-Mageed T, Mekdad AA, Rady MO, Abdelbaky AS, Saudy HS, Shaaban A (2022) Physio-biochemical and agronomic changes of two sugar beetcultivars grown in saline soil as influenced by potassium fertilizer. J. Soil Sci. Plant Nutr. 22: 3636-54.

5. World Health Organization (2017) Shanghai declaration on promoting health in the 2030 Agenda for Sustainable Development. Health Promotion International. 32: 7-8.

6. Blumwald E (2000) Sodium transport and salt tolerance in plants. Current Opinion in Cell Biology. 12: 431-4.

7. Jones L, McQueen-Mason S (2004) A role for expansins in dehydration and rehydration of the resurrection plant Craterostigma plantagineum. FEBS Lett. 559: 1-3.

8. You J, Chan ZL (2015) ROS Regulation During Abiotic Stress Responses in Crop Plants. Frontiers in Plant Science. 6: 690-5.

9. Chen Y, Li H, Zhang S, Du S, Wang G, Zhang J, Jiang J (2022) Analysis of the Antioxidant Mechanism of Tamarix ramosissima Roots under NaCl Stress Based on Physiology, Transcriptomic and Metabolomic. Antioxidants. 11: 2362.

10. Assaha DVM, Ueda A, Saneoka H, Al-Yahyai R, Yaish MW (2017) The Role of Na+ and K+ Transporters in Salt Stress Adaptation in Glycophytes. Frontiers in Physiology. 8.

11. Qiu QS, Guo Y, Dietrich MA, Schumaker KS, Zhu JK (2002) Regulation of SOS1, a plasma membrane Na+/H+ exchanger in Arabidopsis thaliana, by SOS2 and SOS3. Proceedings of the National Academy of Sciences of the united states of America. 99: 8436-41.

12. Contreras-Cornejo HA, Macías-Rodriguez L, Cortés-Penagos C, López-Bucio J (2009) Trichoderma virens, a Plant Beneficial Fungus, Enhances Biomass Production and Promotes Lateral Root Growth through an Auxin-Dependent Mechanism in Arabidopsis. Plant Physiology. 149: 1579-92.

13. Saleem S, Iqbal A, Ahmed F, Ahmed M (2021) Phytobeneficial and salt stress mitigating efficacy of IAA producing Salt Tolerant strains in Gossypium hirsutum. Saudi Journal of Biological Sciences. 28: 5317-24.

14. Amirbakhtiar N, Ismaili A, Ghaffari MR, Nazarian Firouzabadi F, Shobbar ZS (2019) Transcriptome response of roots to salt stress in a salinity-tolerant bread wheat cultivar. PLoS ONE, 14: e0213305.

15. Zhaolong X, Qasim R, Ling X, et al. (2018) GmWRKY49, a Salt-Responsive Nuclear Protein, Improved Root Length and Governed Better Salinity Tolerance in TransgenicArabidopsis:J..Frontiers in Plant Science, 9: 809.

16. Xia KF, Wang R, Ou XJ, Fang ZM, Tian CG, Duan J, Wang YQ, Zhang MY (2012) OsTIR1 and OsAFB2 Downregulation via OsmiR393 Overexpression Leads to More Tillers, Early Flowering and Less Tolerance to Salt and Drought in Rice. PLoS ONE. 7: 364-73.

17. Geng G, Li R, Stevanato P, Lv C, Lu Z, Yu L, Wang Y (2020) Physiological and Transcriptome Analysis of Sugar Beet Reveals Different Mechanisms of Response to Neutral Salt and Alkaline Salt Stresses. Frontiers in Plant Science. 571864.

18. Ferreira LJ, Azevedo V, Maroco J, Margarida Oliveira M, Santos AP (2015) Salt Tolerant and Sensitive Rice Varieties Display Differential Methylome Flexibility under Salt Stress. PLOS one. 10: e0124060.

19. WANG Yuelin, YE Xiefeng, SONG Jiaqian, et al. (2020) Evaluation of salt tolerance of 34 tobacco varieties during germination based on artificial climate incubatorJ.. Tobacco Science and Technology, 53: 20-8.

20. Xu L, Song JQ, Wang YL, Liu XH, Li XL, Zhang B, et al. (2022) Thymol improves salinity tolerance of tobacco by

increasing the sodium efflux and enhancing the content of Nitric oxide and glutathione. BMC Plant Biol. 22: 31.

21. XF, Ling, TX, et al. (2016) Thymol Mitigates Cadmium Stress by Regulating Glutathione Levels and Reactive Oxygen Species Homeostasis in Tobacco Seedlings J. MOLECULES, 21.

22. Smolko A, Bauer N, Pavlović I, Pěnčík A, Novák O, Salopek-Sondi B (2021) Altered Root Growth, Auxin Metabolism and Distribution in Arabidopsis thaliana Exposed to Salt and Osmotic Stress. Int. J. Mol. Sci. 22: 7993

23. Alaya AB, Rabhi F, Djébali N, Hessini K (2021) Pyrenophora teres growth and severity of net blotch on barley under salt stress. European Journal of Plant Pathology. 161: 709-22.

24. Gebauer J, El-siddig K, Salih AA, Ebert G (2004) Tamarindus indica L. seedlings are moderately salt tolerant when exposed to NaCl-induced salinity. Scientia Horticulturae. 103: 1-8.

25. Pamuta D, Siangliw M, Sanitchon J, Pengrat J, Siangliw JL, Toojinda T, et al. (2022) Physio-biochemical traits in improved 'KDML105'jasmine rice lines containing drought and salt tolerance gene under drought and salt stress. Chil. J. Agric. Res. 82: 97-110.

26. Ren YF, Wang W, He JY, Zhang LY, Wei YJ, Yang M (2020) Nitric oxide alleviates salt stress in seed germination and early seedling growth of pakchoi (Brassica chinensis L.) by enhancing physiological and biochemical parameters. Ecotoxicology and Environmental Safety. 187: 109785.

27. Wang YY, Liu ZY, Wang PL, Jiang B, Lei XJ, Wu J, Dong WF, Gao CQ (2020) A 2-Cys peroxiredoxin gene from Tamarix hispida improved salt stress tolerance in plants. BMC Plant Biology. 20: 1-10.

28. Goswami K, Mittal D, Gautam B, Sopory SK, Sanan-Mishra N (2020) Mapping the salt stress-induced changes in the root miRNome in Pokkali rice. Biomolecules. 10: 498.

29. Guan QJ, Liao X, He ML, LI XF, Wang ZY, Ma HY, Yu S, Liu SK (2017) Tolerance analysis of chloroplast OsCu/Zn-SOD overexpressing rice under NaCl and NaHCO3 stress. PLoS One. 12: e0186052.

30. Zhang F, Wang Y, Liu C, Chen F, Ge H, Tian F, Yang T, Ma K, Zhang Y (2019) Trichoderma harzianum mitigates salt stress in cucumber via multiple responses. Ecotoxicology and Environmental Safety. 170: 436-45.

31. Teh CY, Shaharuddin NA, Ho CL, et al. (2016) Exogenous proline significantly affects the plant growth and nitrogen assimilation enzymes activities in rice (Oryza sativa) under salt stress. Acta Physiologiae Plantarum. 38: 151.

32. Guo R, Yang ZZ, Li F, Yan CR, Zhang XL, Liu Q, Xia L, Li HR, Zhao L (2015) Comparative metabolic responses and adaptive strategies of wheat (Triticum aestivum) to salt and alkali stress. BMC Plant Biology. 15: 170.

33. Mekawy AMM, Assaha DVM, Ueda A (2020) Differential salt sensitivity of two flax cultivars coincides with differential sodium accumulation, biosynthesis of osmolytes and antioxidant enzyme activities. Journal of Plant Growth Regulation. 39: 1119-26.

34. El-Samad HM, Shaddad MAK (1997) Salt tolerance of soybean cultivars. Biol Plant. 39: 263-9.

35. Yao Y, Sun Y, Feng Q, Zhang X, Gao Y, Ou Y, Yang F, Xie W, et al. (2021) Acclimation to nitrogen salt stress in Populus bolleana mediated by potassium/sodium balance. Industrial Crops and Products. 170: 113789.

36. Tufail A, Li H, Naeem A, Li TX (2017) Leaf cell membrane stability-based mechanisms of zinc nutrition in mitigating salinity stress in rice. Plant Biology. 20: 338-145.

37. Ashraf M, Khanum A (2010) Relationship Between Ion Accumulation and Growth in Two Spring Wheat Lines Differing in Salt Tolerance at Different Growth Stages. Journal of Agronomy & Crop Science. 178: 39-51.

38. Yan F, Wei H, Li W, Liu Z, Tang S, Chen L, et al. (2020) Melatonin improves K+ and Na+ homeostasis in rice under salt stress by mediated nitric oxide. Ecotoxicology and Environmental Safety. 206: 111358.

39. Manaa A, Ahmed HB, Valot B, Bouchet JP, Aschi-Smiti S, Causse M, Faurobert M (2011) Salt and genotype impact on plant physiology and root proteome variations in to-

mato. Journal of Experimental Botany, 62: 2797-813.

40. Wang Q, Guan C, Wang SM (2014) Coordination of AtHKT1;1 and AtSOS1 facilitates Na and K homeostasis in Arabidopsis thaliana under salt stress. Journal of Plant Biology. 57: 282-90.

41. Yu K, Ju E, Wang Z, Sun L, Huo Y, Zhu J, Pang J, et al. (2022) Potassium ameliorates cotton (Gossypium hirsutum L.) fiber length by regulating osmotic and K/Na homeostasis under salt stress. Physiologia Plantarum. 175: e13842.

42. Yang Y, Guo Y (2018) Unraveling salt stress signaling in plants. Journal of Integrative Plant Biology. 60: 796-804.

43. Yang Z, Wang C, Xue Y, Liu X, Chen S, Song C, Yang Y, Guo Y (2019) Calcium-activated 14-3-3 proteins as a molecular switch in salt stress tolerance. Nature Communications. 10: 1-12.

44. Liu JG, Han X, Yang T, Cui WH, Liu LJ (2019) Genome-wide transcriptional adaptation to salt stress in Populus. BMC Plant Biology. 19: 1-14.

45. Gong Z, Xiong L, Shi H, Yang S, Herrera-Estrella LR, Xu G, et al. (2020) Plant abiotic stress response and nutrient use efficiency. Science China Life Sciences. 63: 635-74.

46. Yang YQ, Wu YJ, Ma L, Yang ZJ, Dong QY, Li QP, et al. (2019) The Ca2+ sensor SCaBP3/CBL7 modulates plasma membrane H+-ATPase activity and promotes alkali tolerance in Arabidopsis. The Plant Cell. 31: 1367-84.

47. Guo Q, Meng L, Han J, Mao P, Tian XX, Zheng ML, Mur LAJ (2020) SOS1 is a key systemic regulator of salt secretion and K+/Na+ homeostasis in the recretohalophyte Karelinia caspia. Environmental and Experimental Botany. 177: 98-104.

48. Chunzhao Z, Wei J, Omar Z, et al. (2020) The LRXs-RALFs-FER module controls plant growth and salt stress responses by modulating multiple plant hormones J. National Science Review.

49. Takahashi Y, Zhang J, Hsu P K, et al. (2020) MAP3Kinase-dependent SnRK2-kinase activation is required for abscisic acid signal transduction and rapid osmotic stress responseJ..Nature Communications, 11: 12.

50. Jain M, Kaur N, Garg R, et al. (2006) Structure and expression analysis of early auxin-responsive Aux/IAA gene family in rice (Oryza sativa). Functional and Integrative Genomics. 6: 47-59.

51. Jain M, Khurana JP (2009) Transcript profiling reveals diverse roles of auxin-responsive genes during reproductive development and abiotic stress in rice. The FEBS Journal. 276: 3148-62.

52. Audran-Delalande C, Bassa C, Milla I, Regad F, et al. (2012) Genome-wide identification, functional analysis and expression profiling of the Aux/IAA gene family in tomato. Plant Cell Physiol. 53: 659-72.

53. Waseem M, Ahmad F, Habib S, Li Z (2018) Genome-wide identification of the auxin/indole-3-acetic acid (Aux/IAA) gene family in pepper, its characterisation, and comprehensive expression profiling under environmental and phytohormones stress. Scientific reports. 8: 12008.

54. Shen C, Bai YH, Wang SK, Zhang SN, Wu YR, Chen M, Jiang DA, Qi YH (2010) Expression profile of PIN, AUX/LAX and PGP auxin transporter gene families in Sorghum bicolor under phytohormone and abiotic stress. The FEBS Journal. 227: 2954-969.

55. Mashiguchi K, Tanaka K, Sakai T, Sugawara S, Kawaide H, Natsume M, et al. (2011) The main auxin biosynthesis pathway in Arabidopsis. Proceedings of the National Academy of Sciences. 108: 18512-7.

56. Won C, Shen X, Mashiguchi K, Zheng Z, Dai X, Cheng Y, et al. (2011) Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES OF ARABIDOPSIS and YUCCAs in Arabidopsis. Proceedings of the National Academy of Sciences. 108: 18518-23.

57. Ke QB, Wang Z, Ji CY, Jea CJ, Lee HS, Li HB, et al. (2015) Transgenic poplar expressing Arabidopsis YUCCA6 exhibits auxin-overproduction phenotypes and increased tolerance to abiotic stress. Plant Physiology and Biochemistry. 94: 19-27.

58. Kim JI, Baek D, Park HC, et al. (2013) Overexpression of Arabidopsis YUCCA6 in potato results in high-auxin

developmental phenotypes and enhanced resistance to water deficit. Mol Plant. 6: 337-49.

59. Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, et al. (2005) The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature. 433: 39-44.

60. Zhao J, Wang W, Zhou H, Wang R, Zhang P, Wang H, Pan X, Xu J (2017) Manganese Toxicity Inhibited Root Growth by Disrupting Auxin Biosynthesis and Transport in Arabidopsis. Front. Plant Sci. 8.

61. Omelyanchuk NA, Kovrizhnykh VV, Oshchepkova EA, Pasternak T, Palme K, Mironova VV (2016) A detailed expression map of the PIN1 auxin transporter in Arabidopsis thaliana root. BMC Plant Biology. 16.

62. Fang J, Peng Y, Zheng L, et al. (2023) Chitosan–Se Engineered Nanomaterial Mitigates Salt Stress in Plants by Scavenging Reactive Oxygen Species J. Journal of Agricultural and Food Chemistry, 72: 176-88.

63. Zhao K, Fan G, Yao W, et al. (2024) PagMYB73 enhances salt stress tolerance by regulating reactive oxygen species scavenging and osmotic maintenance in poplarJ.. Industrial Crops and Products, 208: 117893.

64. Julkowska M M, Koevoets I T, Mol S, et al. (2017) Genetic components of root architecture remodeling in response to salt stress J. The plant cell, 29: 3198-213.

65. Afridi G M, Ullah N, Ullah S, et al. (2023) Modulation of salt stress through application of citrate capped silver nanoparticles and indole acetic acid in maize J. Plant Physiology and Biochemistry, 2023, 201: 107914.

66. Yang Y, Liu S, Du G, et al. (2024) Exogenous Application of Phytohormones Alleviates Salt Injury to Growth, Physiological, and Biochemical Attributes of Hemp SeedlingsJ.. Journal of Plant Growth Regulation, 1-15.

67. Alam M, Khan M A, Imtiaz M, et al. (2020) Indole-3-acetic acid rescues plant growth and yield of salinity stressed tomato (Lycopersicon esculentum L.) J. Gesunde Pflanzen, 72: 87-95.

68. FENG Changchun, GAO Jun, XU Lifeng, et al. (2020) Effects of exogenous growth factors on the growth and development of roasted tobacco seedlings J. China Tobacco Science, 41: 27-31.

69. WU Mengdan, YE Xu, WEI Dayong, et al. (2023) Physiological effects of growth regulators in alleviating salt stress in cherry radish J. Jiangxi Journal of Agriculture, 35: 89-95.

70. Gull M, Sajid Z A, Aftab F (2023) Alleviation of salt stress in Solanum tuberosum L. by exogenous application of indoleacetic acid and l-tryptophanJ.. Journal of Plant Growth Regulation, 42: 3257-73.

71. Orozco-Cardenas M, Ryan CA (1999) Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. Proceedings of the National Academy of ences of the United States of America. 96: 6553-7.

72. Yamamoto Y, Kobayashi Y, Matsumoto H (2001) Lipid peroxidation is an early symptom triggered by Aluminum, but not the primary cause of elongation inhibition in Pea Roots. Plant Physiology. 125: 199-208.

73. Duan YF, Zhang WS, Li B, Wang Y, Li KX, Sodmergen, et al. (2010) An endoplasmic reticulum response pathway mediates programmed cell death of root tip induced by water stress in Arabidopsis. New Phytologist. 186: 681-95.