

## Response of *Eucalyptus urophylla* × *Eucalyptus grandis* DH3229 Seedlings to Sodium Nitroprusside-Aluminum Toxicity Interaction: Postprint

**Authors:** Hou Wenjuan, 罗文姬, Wei Jie, Hu Houzhen, Linghui Wang, Teng Weichao

**Date:** 2018-07-18T00:00:00+00:00

### Abstract

This experiment utilized *Eucalyptus urophylla* × *E. grandis* DH3229 seedlings provided by the Guangxi Academy of Forestry Sciences as experimental material, analytically pure  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  as the aluminum donor, and sodium nitroprusside (SNP) as the nitric oxide (NO) donor. Three SNP concentrations (0, 10, and 500  $\text{mol} \cdot \text{L}^{-1}$ ) and two Al levels (0 and 5  $\text{mmol} \cdot \text{L}^{-1}$ ) were established, with nutrient solutions containing different concentrations of Al and SNP applied every 5 days for 20 weeks. The experiment analyzed the effects of aluminum stress on seedling growth and physiological characteristics, and investigated whether exogenous SNP application at different concentrations could alleviate aluminum toxicity in plants and the underlying alleviation mechanisms. The results demonstrated that aluminum application significantly reduced the biomass of *Eucalyptus urophylla* × *E. grandis* DH3229 seedlings, leaf chlorophyll a content, total chlorophyll content, chlorophyll a/b ratio, and soluble sugar content; aluminum stress significantly increased seedling relative electrical conductivity, MDA content, SOD activity, and free proline content. However, exogenous application of an appropriate amount (10  $\text{mol} \cdot \text{L}^{-1}$ ) of SNP significantly increased root and leaf biomass, leaf chlorophyll content, and Chl a/b ratio in eucalyptus seedlings under aluminum stress, while simultaneously decreasing their relative electrical conductivity, MDA content, and free proline content. These findings indicate that SNP exhibits dual characteristics: appropriate SNP application can effectively alleviate aluminum stress on eucalyptus growth, whereas high concentrations (500  $\text{mol} \cdot \text{L}^{-1}$ ) of SNP generate nitrosative stress that inhibits eucalyptus growth. This research provides practical significance for selecting appropriate SNP concentrations to mitigate aluminum stress damage in eucalyptus.

## Full Text

### Response of *Eucalyptus urophylla* × *E. grandis* DH3229 Seedlings to Sodium Nitroprusside-Aluminum Interactions

Hou Wenjuan<sup>1,2</sup>, Luo Wenji<sup>1</sup>, Wei Jie<sup>3</sup>, Hu Houzhen<sup>1</sup>, Wang Linghui<sup>1</sup>, Teng Weichao<sup>1\*</sup>

<sup>1</sup> College of Forestry, Guangxi University, Nanning, Guangxi 530004, China

<sup>2</sup> Weihui Agriculture and Forestry Bureau, Weihui, Henan 453100, China

<sup>3</sup> Dawangtan Reservoir Management Office, Nanning, Guangxi 530218, China

Nanning College for Vocational Technology, Nanning, Guangxi 530008, China

---

## Abstract

This experiment investigated the response of *Eucalyptus urophylla* × *E. grandis* DH3229 seedlings (provided by Guangxi Forestry Research Institute) to aluminum stress and potential mitigation by nitric oxide (NO), using analytical-grade  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  as the aluminum source and sodium nitroprusside (SNP) as the NO donor. The study employed a factorial design with three SNP concentrations (0, 10, and 500  $\text{mol} \cdot \text{L}^{-1}$ ) and two aluminum levels (0 and 5  $\text{mmol} \cdot \text{L}^{-1}$ ). Nutrient solutions containing varying concentrations of Al and SNP were applied every five days for 20 weeks. The experiment analyzed the effects of aluminum stress on seedling growth and physiological characteristics and explored whether exogenous SNP application could alleviate aluminum toxicity and the underlying mechanisms.

The results demonstrated that aluminum application significantly reduced biomass, leaf chlorophyll a content, total chlorophyll content, chlorophyll a/b ratio, and soluble sugar content in DH3229 seedlings. Aluminum stress also significantly increased relative electrical conductivity, malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, and free proline content. In contrast, application of an appropriate concentration of SNP (10  $\text{mol} \cdot \text{L}^{-1}$ ) significantly increased root and leaf biomass, leaf chlorophyll content, and chlorophyll a/b ratio under aluminum stress while decreasing relative electrical conductivity, MDA content, and free proline content. These findings indicate that SNP exhibits dual effects: appropriate SNP application effectively alleviates aluminum-induced stress in eucalyptus, whereas high concentrations (500  $\text{mol} \cdot \text{L}^{-1}$ ) cause nitrosative stress that inhibits growth. This research provides practical guidance for selecting optimal SNP concentrations to mitigate aluminum toxicity in eucalyptus plantations.

**Keywords:** fast-growing eucalyptus, aluminum toxicity, nitric oxide, alleviation, oxidative stress

## Introduction

Aluminum (Al) generally has no significant effect on plants under normal conditions. However, when soil pH drops below 5.5, aluminum gradually dissociates from mineral crystals and releases into solution as ionic species that directly impair plant growth, reducing agricultural productivity in acidic soils. The  $\text{Al}^3$  form exhibits the strongest toxicity (Kochian, 1995). Approximately 30% of global land area consists of acidic soils with pH below 5.5, with about 60% of these soils located in developing countries. Soil acidification has become increasingly severe in recent years due to acid rain deposition, improper fertilization practices, and deteriorating environmental conditions.

Fast-growing eucalyptus (*Eucalyptus*) is one of the world's three most important fast-growing tree species and represents one of the most valuable hardwood resources, primarily distributed in Guangxi, Guangdong, Yunnan, and Guizhou provinces in southwestern China (Huang, 2004). In Guangxi, *E. urophylla* × *E. grandis* DH3229 and *E. grandis* × *E. urophylla* Guanglin No. 9 are the most widely cultivated clones (Wu et al., 2012; Wei, 2015). Eucalyptus species are relatively acid-tolerant, and southern China's soils are characterized by intensive iron-aluminum weathering and acidity. Research indicates that aluminum toxicity may be a potential factor limiting the growth of fast-growing eucalyptus in southern China (Yang et al., 2011; Ye, 2007; Nguyen et al., 2003). Addressing aluminum toxicity in large-scale eucalyptus plantations, improving productivity in aluminum-rich acidic soils, and mitigating soil fertility decline in eucalyptus forests are urgent challenges in China's forestry production.

Recent studies have made progress in understanding the role of nitric oxide (NO) in plant aluminum tolerance. Exogenous NO application has been shown to alleviate aluminum toxicity in various plants including *Cassia tora*, red kidney bean, wheat, mung bean, soybean, and rice (Wang & Yang, 2005; Zhang et al., 2008; Hou, 2009; Yang, 2009; Wang et al., 2010). However, no studies have reported on the alleviating effects of NO on aluminum-stressed eucalyptus. Exogenous application offers a more straightforward approach that could potentially enhance eucalyptus aluminum tolerance and improve utilization of acidic land resources. This experiment investigated the regulatory effects of different NO concentrations on aluminum toxicity in DH3229, analyzing whether NO addition improves aluminum tolerance in eucalyptus and seeking effective methods to alleviate acid-aluminum stress on eucalyptus growth.

---

## Materials and Methods

**1.1 Plant Material and Growth Conditions** The experimental material consisted of three-month-old, healthy, and uniform tissue-cultured seedlings of *Eucalyptus urophylla* × *E. grandis* DH3229 (hereafter referred to as DH3229) provided by Guangxi Forestry Research Institute. The experiment was conducted at the nursery base of Guangxi University College of Forestry (108°17' E,

22°50 N). In April 2014, seedlings were transplanted into pots measuring 500 mm (inner diameter)  $\times$  400 mm (height), with one seedling per pot. After one week of normal watering and acclimation, the seedlings received weekly applications of 1 L modified Hoagland nutrient solution. Following one month of recovery, sodium nitroprusside-aluminum treatments began in May 2014. After treatment initiation, modified Hoagland nutrient solution (pH adjusted to 4.1–4.2 with HCl or NaOH) was applied every two weeks. Aluminum was supplied as analytical-grade  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ , and sodium nitroprusside (SNP) served as the nitric oxide (NO) donor.

**1.2 Experimental Design** A completely randomized design was employed. Based on Yang et al. (2015) and Yang (2011) with minor modifications, the experiment included three SNP concentrations (0, 10, and 500  $\text{mol} \cdot \text{L}^{-1}$ ) and two aluminum levels (0 and 5  $\text{mmol} \cdot \text{L}^{-1}$ ), resulting in six treatments (Table 1). Each treatment had ten replicates. Nutrient solutions containing different concentrations of Al and SNP were applied every five days for 20 weeks. Cyanide ion ( $\text{CN}^-$ ) concentrations were balanced using sodium ferrocyanide, and nutrient solution pH was adjusted to 4.1–4.2 with HCl or NaOH.

**Table 1** Experimental treatments

Treatment	Al level ( $\text{mmol} \cdot \text{L}^{-1}$ )	SNP concentration ( $\text{mol} \cdot \text{L}^{-1}$ )
T1 (CK)	0	0
T2	5	0
T3	0	10
T4	5	10
T5	0	500
T6	6	500

**1.3 Measurement Methods** After the treatment period, physiological indices including leaf chlorophyll content, relative electrical conductivity, malondialdehyde (MDA), free proline, soluble sugar, and SOD content were measured following methods described in *Experimental Guidance on Plant Physiology* (Zhang, 1990). Biomass was determined for three representative seedlings per treatment.

---

## Results

**2.1 Effects of SNP-Al Interactions on DH3229 Biomass** As shown in Table 2, without SNP application, 5  $\text{mmol} \cdot \text{L}^{-1}$  Al stress reduced root, stem, and leaf biomass by 28.56%, 34.78%, and 44.97% compared to the control (CK), with highly significant differences ( $P < 0.01$ ), indicating that Al stress significantly inhibited eucalyptus seedling growth. Under non-Al-stress conditions,

exogenous application of 10 mol · L<sup>-1</sup> SNP did not significantly affect root, stem, or leaf biomass ( $P > 0.05$ ), whereas 500 mol · L<sup>-1</sup> SNP significantly inhibited biomass accumulation ( $P < 0.01$ ). This suggests that low-concentration SNP has no significant effect on fast-growing eucalyptus, while high-concentration SNP exerts inhibitory effects. Under Al stress, exogenous application of 10 mol · L<sup>-1</sup> SNP increased root, stem, and leaf biomass by 23.81%, 12.49%, and 57.59% compared to the no-SNP treatment, with highly significant increases in root and leaf biomass ( $P < 0.01$ ). When SNP concentration was increased to 500 mol · L<sup>-1</sup>, biomass decreased significantly.

**Table 2** Effects of different Al and SNP levels on biomass of DH3229

Al application Index (mmol · L <sup>-1</sup> )	SNP application (mol · L <sup>-1</sup> )	Root biomass (g)	Stem biomass (g)	Leaf biomass (g)	Root-shoot ratio (g)
0	0	162.27±4.77 aA	64.65±5.31 aA	110.2±2.6 cC	0.67±0.01 abAB
5	0	115.91±5.78 cC	43.47±3.95 bB	14.97±4.60 cC	0.74±0.05 aAB
0	10	142.5±4.85 aA	32.6±5.97 abAB	96.97±4.08 cC	0.61±0.01 bB
5	10	92.93±5.4 cC	104.5±4.47 cC	93.93±4.80 cC	0.79±0.09 aA
0	500	99.33±4.04 aA	49.3±3.38 aA	78.87±3.95 bB	0.79±0.03 aA
5	500	54.67±4.71 dD	18.2±3.02 bB	71.07±1.99 cC	0.7±0.04 abAB

*Note: Values are means ± SD. Different lowercase letters indicate significant differences among treatments for the same index at  $P < 0.05$ . The same notation applies below.*

## 2.2 Effects of SNP-Al Interactions on Leaf Photosynthetic Pigments

As shown in Table 3, without SNP application, Al stress significantly reduced chlorophyll a, chlorophyll b, total chlorophyll (a+b) content, and the chlorophyll a/b ratio by 39.23%, 11.95%, 32.52%, and 30.84%, respectively, demonstrating that aluminum stress inhibits photosynthetic pigment content. Under non-Al-stress conditions, 10 mol · L<sup>-1</sup> SNP addition did not significantly affect leaf pigment content ( $P > 0.05$ ), whereas 500 mol · L<sup>-1</sup> SNP treatment significantly reduced leaf pigment content ( $P < 0.01$ ). This indicates that low-concentration SNP has no significant effect on DH3229 leaf pigments, while high-concentration SNP inhibits photosynthetic pigment content. Under Al stress, application of 10 mol · L<sup>-1</sup> SNP increased leaf chlorophyll a, total chlorophyll, and chlorophyll a/b ratio by 43.72%, 34.79%, and 23.71% compared to the no-SNP treatment, with highly significant differences ( $P < 0.01$ ). When SNP concentration was

increased to  $500 \text{ mol} \cdot \text{L}^{-1}$ , photosynthetic pigment content decreased significantly. These results demonstrate that appropriate SNP concentration is beneficial for increasing photosynthetic pigment content in eucalyptus seedlings under Al stress, whereas excessive SNP concentration fails to improve photosynthetic pigment content.

**Table 3** Effects of different Al and SNP levels on photosynthetic pigments of DH3229

Al application (mmol · L <sup>-1</sup> )	SNP application (mol · L <sup>-1</sup> )	Chlorophyll a (mg · g <sup>-1</sup> FW)	Chlorophyll b (mg · g <sup>-1</sup> FW)	Chlorophyll a+b (mg · g <sup>-1</sup> FW)	Chlorophyll a/b
0	0	0.68 aA	0.41 dD	0.22 abA	0.20 bA
5	0	0.91 aA	0.61 dC	3.06 aAB	2.12 cC
0	10	0.70 aA	0.60 bB	0.24 abA	0.23 abA
5	10	0.94 aA	0.829 bB	3.15 aA	2.62 bB
0	500	0.50 cC	0.41 dD	0.26 aA	0.22 abA
5	500	0.76 cB	0.62 dC	1.96 cC	1.83 cC

### 2.3 Effects of SNP-Al Interactions on Leaf Malondialdehyde (MDA)

**Content** As shown in Figure 1 [Figure 1: see original paper], without SNP addition,  $5 \text{ mmol} \cdot \text{L}^{-1}$  Al stress increased leaf MDA content by 115.43% compared to CK, with a highly significant difference ( $P < 0.01$ ). Under non-Al-stress conditions,  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP addition had no significant effect on leaf MDA content ( $P > 0.05$ ), whereas  $500 \text{ mol} \cdot \text{L}^{-1}$  SNP significantly increased MDA content ( $P < 0.01$ ). Under  $5 \text{ mmol} \cdot \text{L}^{-1}$  Al stress,  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP application significantly reduced MDA content ( $P < 0.01$ ), but  $500 \text{ mol} \cdot \text{L}^{-1}$  SNP addition did not reduce MDA content ( $P > 0.05$ ).

*Note: Values are means ± SD. Different lowercase letters indicate significant differences among treatments for the same index at  $P < 0.05$ . The same notation applies below.*

**Figure 1** Effects of different Al and SNP levels on MDA content in leaves of DH3229

### 2.4 Effects of SNP-Al Interactions on Leaf Membrane Permeability

As shown in Figure 2 [Figure 2: see original paper], without SNP addition,  $5 \text{ mmol} \cdot \text{L}^{-1}$  Al stress increased leaf relative electrical conductivity by 267.14%

compared to CK, with a highly significant difference ( $P < 0.01$ ). Under non-Al-stress conditions,  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP addition had no significant effect on leaf relative electrical conductivity ( $P > 0.05$ ), whereas  $500 \text{ mol} \cdot \text{L}^{-1}$  SNP significantly increased leaf relative electrical conductivity ( $P < 0.01$ ). Under  $5 \text{ mmol} \cdot \text{L}^{-1}$  Al stress,  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP treatment significantly reduced leaf relative electrical conductivity ( $P < 0.01$ ), while  $500 \text{ mol} \cdot \text{L}^{-1}$  SNP treatment did not reduce relative electrical conductivity in Al-stressed seedlings ( $P > 0.05$ ). These results demonstrate that Al stress significantly increased leaf membrane permeability, while  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP application reduced relative electrical conductivity, thereby alleviating aluminum stress in eucalyptus to some extent.

**Figure 2** Effects of different Al and SNP levels on membrane permeability in leaves of DH3229

**2.5 Effects of SNP-Al Interactions on Leaf Free Proline Content** Proline is an osmotic adjustment substance in plant cells. As shown in Figure 3 [Figure 3: see original paper], without SNP addition,  $5 \text{ mmol} \cdot \text{L}^{-1}$  Al stress increased proline content to  $125.18 \text{ g} \cdot \text{g}^{-1}$ , representing a 41.65% increase compared to the control, with a highly significant difference ( $P < 0.01$ ). Under non-Al-stress conditions,  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP addition had no significant effect on leaf free proline content ( $P > 0.05$ ), whereas  $500 \text{ mol} \cdot \text{L}^{-1}$  SNP significantly increased leaf free proline content ( $P < 0.01$ ). Under  $5 \text{ mmol} \cdot \text{L}^{-1}$  Al stress, the proline content with  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP addition was  $100.13 \text{ g} \cdot \text{g}^{-1}$ , representing a 20% reduction compared to the no-SNP treatment, with a highly significant difference ( $P < 0.01$ ). The proline content with  $500 \text{ mol} \cdot \text{L}^{-1}$  SNP addition showed no significant difference compared to the no-SNP treatment.

**Figure 3** Effects of different Al and SNP levels on free proline content in leaves of DH3229

**2.6 Effects of SNP-Al Interactions on Leaf Soluble Sugar Content** As shown in Figure 4 [Figure 4: see original paper], without SNP addition, Al stress reduced leaf soluble sugar content by 25.13% compared to CK, with a highly significant difference ( $P < 0.01$ ). Under non-Al-stress conditions,  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP addition had no significant effect on leaf soluble sugar content ( $P > 0.05$ ), whereas  $500 \text{ mol} \cdot \text{L}^{-1}$  SNP significantly reduced leaf soluble sugar content ( $P < 0.01$ ). Under  $5 \text{ mmol} \cdot \text{L}^{-1}$  Al stress, SNP addition did not significantly affect leaf soluble sugar content ( $P > 0.05$ ).

**Figure 4** Effects of different Al and SNP levels on soluble sugar content in leaves of DH3229

**2.7 Effects of SNP-Al Interactions on Antioxidant System Enzyme Activity (SOD)** As shown in Figure 5 [Figure 5: see original paper], without SNP addition, Al stress increased leaf SOD content by 189.22% compared to CK, with a highly significant difference ( $P < 0.01$ ). Under non-Al-stress conditions,  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP addition had no significant effect on leaf SOD content ( $P >$

0.05), whereas 500 mol · L<sup>-1</sup> SNP significantly increased leaf SOD content (P < 0.01). Under 5 mmol · L<sup>-1</sup> Al stress, SOD content increased by 53% and 65% with 10 mol · L<sup>-1</sup> and 500 mol · L<sup>-1</sup> SNP applications, respectively, compared to the no-SNP treatment, with highly significant differences (P < 0.01).

**Figure 5** Effects of different Al and SNP levels on SOD enzyme activity of DH3229

**2.8 Comprehensive Evaluation of SNP-Al Interactions on DH3229 Growth and Physiological Indices** The above results showed that DH3229 growth and physiological indices exhibited different trends under SNP-Al interactions. Principal component analysis (PCA) was performed on 11 original indices for comprehensive evaluation (Table 4 ). The first two principal components were extracted, accounting for 94.5% of the cumulative variance.

**Table 4** Principal components of total variance

Component	Initial Eigenvalues		Extraction Sums of Squared Loadings		
	Total	Variance %	Total	Variance %	Cumulative %
1					
2					

The first principal component primarily included growth indices (root, stem, and leaf biomass) and physiological parameters (chlorophyll a content, total chlorophyll, relative electrical conductivity, MDA, soluble sugar, free proline, and SOD content). The second principal component mainly reflected chlorophyll b content. The principal component expressions were derived by calculating the corresponding coefficients for all original indices:

$$F = 0.31X_1 + 0.32X_2 + 0.31X_3 + 0.33X_4 + 0.10X_5 + 0.32X_6 - 0.33X_7 - 0.32X_8 + 0.32X_9 - 0.33X_{10} - 0.26X_{11}$$

$$F = -0.25X_1 - 0.20X_2 + 0.27X_3 + 0.023X_4 + 0.81X_5 + 0.14X_6 + 0.08X_7 - 0.17X_8 - 0.22X_9 - 0.10X_{10} - 0.24X_{11}$$

Using the proportion of each principal component's eigenvalue to the sum of extracted eigenvalues as weights, the comprehensive principal component model was:

$$F = 0.24X_1 + 0.25X_2 + 0.31X_3 + 0.29X_4 + 0.19X_5 + 0.30X_6 - 0.27X_7 - 0.30X_8 + 0.25X_9 - 0.30X_{10} - 0.19X_{11}$$

As shown in Table 5 , treatments 1 (CK) and 3 had the highest scores for principal component 1, while treatment 5 had the highest score for principal

component 2. The comprehensive ranking was: treatment 3, treatment 1, treatment 4, treatment 5, treatment 6, treatment 2.

**Table 5** Principal component values

Treatments	Principal component 1 score	Principal component 2 score	Comprehensive principal component score
T1 (CK)			
T2			
T3			
T4			
T5			
T6			

## Conclusion and Discussion

Plant biomass accumulation and allocation (i.e., root-shoot ratio) are commonly used growth indicators for characterizing stress levels (Zhou & Zhu, 2015). The growth results for root, stem, and leaf biomass accumulation (Table 2) demonstrated that  $5 \text{ mmol} \cdot \text{L}^{-1}$  aluminum application significantly inhibited DH3229 seedling growth, consistent with findings by Wang, Zhang, and Nguyen et al. The likely mechanism is that aluminum stress inhibits plant cell elongation and division and impairs nutrient absorption capacity at root tips, causing nutrient deficiencies required for growth (Nguyen et al., 2005; Wang et al., 2010; Zhang et al., 2011). Chloroplasts, the sites of photosynthetic pigment production, are the primary organelles for photosynthesis (Chen et al., 2004). Under aluminum stress, most plants exhibit inhibited chlorophyll synthesis, with aluminum directly affecting photosynthetic organ structure and activity (Akaya & Takenaka, 2001; Ying & Liu, 2005). In this study, aluminum stress significantly reduced leaf chlorophyll a, total chlorophyll content, and chlorophyll a/b ratio in DH3229. Decreased chlorophyll a and b contents inhibit normal photosynthesis, while reduced carotenoids and chlorophyll a/b may indicate decreased stress resistance, similar to findings by Xiao et al. (2005). Aluminum stress can affect mitochondrial function, generating excessive superoxide radicals and increasing membrane lipid peroxidation (Nguyen et al., 2005), which may lead to increased electrolyte leakage and elevated electrical conductivity under aluminum stress. When plant organs senesce or under stress conditions, free radicals attack lipids causing peroxidation reactions that produce MDA, which can indirectly measure membrane system damage and intracellular reactive oxygen species content (Zhou et al., 2015; Hou, 2016; Hou et al., 2016). SOD can eliminate free radicals produced by oxidation in plant tissues, and changes in SOD content play a crucial role in maintaining the oxidation-antioxidation balance (Wei et al., 2006). In this experiment, SOD content in DH3229 significantly

increased under aluminum stress because aluminum toxicity generated large amounts of reactive oxygen species, stimulating plants to produce more SOD to scavenge free radicals. The significant increases in MDA and SOD content, relative electrical conductivity, and proline content, along with increased membrane permeability and decreased soluble sugar content under aluminum stress, likely occurred because stress conditions induce accumulation of reactive oxygen species such as  $O_2^-$  and  $H_2O_2$ , which directly attack membrane systems, causing lipid peroxidation, inducing de-esterification, reducing membrane fluidity, increasing membrane permeability, and ultimately destroying the membrane system and disrupting the reactive oxygen defense system balance.

Reactive oxygen species (ROS) accumulation and resulting oxidative damage in plant roots under aluminum toxicity represent an important mechanism of aluminum phytotoxicity (Richards et al., 1998; Boscolo et al., 2003; Yin et al., 2010). Enhanced antioxidant capacity is therefore beneficial for aluminum tolerance (Sharma & Dietz, 2009). NO can protect plants from oxidative damage caused by stress by enhancing the antioxidant system (Tian & Lei, 2006; Shi et al., 2014). This study showed that  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP application significantly alleviated growth inhibition of DH3229 under aluminum stress, consistent with previous studies (Horst et al., 2010; Saxena & Shekhawat, 2013). The mechanism may involve  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP effectively increasing leaf chlorophyll content and chlorophyll a/b ratio while reducing relative electrical conductivity, MDA content, and free proline content under Al stress, demonstrating that NO can effectively mitigate damage to leaf membrane permeability and peroxidation reactions while correcting imbalances in the leaf membrane system and reactive oxygen defense system. The increase in SOD content with  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP addition is similar to results reported by Zhang et al. (2008), as exogenous NO can enhance aluminum tolerance by reducing membrane lipid peroxidation, increasing SOD, CAT, and APX activities and proline content, and decreasing reactive oxygen species content. High-concentration SNP ( $500 \text{ mol} \cdot \text{L}^{-1}$ ) showed no alleviating effect on aluminum stress. Under non-aluminum stress,  $500 \text{ mol} \cdot \text{L}^{-1}$  SNP application caused significant reductions in root, stem, and leaf biomass, likely because NO, besides functioning as a signaling molecule in plant physiological processes, is also a reactive nitrogen species that causes nitrosative stress when accumulated excessively (Barroso, 2007; Corpas & Barroso, 2013). Studies have shown that excessive NO accumulation under osmotic stress, heavy metal stress, extreme temperatures, and mechanical damage can cause nitrosative stress in plants (Valderrama et al., 2007; Corpas et al., 2011; Leterrier et al., 2012), confirming the dual nature of SNP effects.

The growth and physiological indices measured in this experiment showed different trends under SNP-Al interactions. Principal component analysis was further employed for comprehensive evaluation of different treatments' effects on DH3229 growth and physiology. PCA transforms multiple correlated original variables into a new set of uncorrelated comprehensive indicators through linear transformation (Shin et al., 2010). The comprehensive evaluation results showed that treatment 3 ( $0 \text{ mmol} \cdot \text{L}^{-1}$  Al +  $10 \text{ mol} \cdot \text{L}^{-1}$  SNP) had the highest

comprehensive principal component score, indicating that appropriate SNP addition promotes DH3229 growth and physiology. Treatment 4 (5 mmol · L<sup>-1</sup> Al + 10 mol · L<sup>-1</sup> SNP) ranked higher than treatment 2 (5 mmol · L<sup>-1</sup> Al + 0 mol · L<sup>-1</sup> SNP), demonstrating that appropriate SNP application alleviates aluminum stress. Treatment 2 ranked last, indicating that 5 mmol · L<sup>-1</sup> Al stress inhibited DH3229 growth. The low rankings of treatment 6 (5 mmol · L<sup>-1</sup> Al + 500 mol · L<sup>-1</sup> SNP) and treatment 5 (0 mmol · L<sup>-1</sup> Al + 500 mol · L<sup>-1</sup> SNP) indicate that high-concentration SNP causes nitrosative effects that inhibit growth.

In summary, appropriate SNP application can effectively alleviate aluminum stress in eucalyptus, while high concentrations may inhibit normal growth without alleviating effects, demonstrating the dual nature of SNP. Selecting the appropriate SNP concentration is key to solving eucalyptus aluminum toxicity problems.

---

## References

- Akaya, M., & Takenaka, C. (2001). Effects of aluminum stress on photosynthesis of *Quercus glauca* Thumb. *Plant and Soil*, 237(1), 137-146.
- Barroso, J. B. (2007). Need of biomarkers of nitrosative stress in plants. *Trends in Plant Science*, 12(10), 452-453.
- Boscolo, P. R. S., Menossi, M., & Jorge, R. A. (2003). Aluminum-induced oxidative stress in maize. *Phytochemistry*, 62(2), 181-189.
- Chen, P. Z., Luo, J. G., Wang, L., et al. (2004). Causation of sodium bisulfite affecting photosynthesis in leaves of Umbilical orange. *Acta Agriculturae Boreali-Occidentalis Sinica*, 13(1), 69-75.
- Corpas, F. J., & Barroso, J. B. (2013). Nitro-oxidative stress vs oxidative or nitrosative stress in higher plants. *New Phytologist*, 199(3), 633-635.
- Corpas, F. J., Leterrier, M., Valderrama, R., et al. (2011). Nitric oxide imbalance provokes a nitrosative response in plants under abiotic stress. *Plant Science*, 181(5), 604-611.
- Horst, W. J., Wang, Y., & Eticha, D. (2010). The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: A review. *Annals of Botany*, 106(1), 185-197.
- Hou, N. N. (2009). *Study of regulating mechanism of abscisic acid and antioxidative system on aluminum tolerance in soybean (Glycine max L.)* (Doctoral dissertation, Jilin University).
- Hou, W. J. (2016). *Effect of exogenous phosphorus and nitric oxide on the regulation of aluminum stress in Eucalyptus* (Doctoral dissertation, Guangxi University).

- Hou, W. J., Hu, H. Z., & Lu, Y. Y. (2016). Relieve effect of exogenous P on the Eucalyptus growth and physiological feature under Al stress. *Journal of Northeast Forestry University*, 44(5), 5-9.
- Huang, H. (2004). Present situation and development countermeasures of construction of eucalyptus plantation in China. *Agricultural Research and Application*, (6), 42-43.
- Kochian, L. V. (1995). Cellular mechanisms of aluminum toxicity and resistance in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 46(1), 237-260.
- Letierrier, M., Airaki, M., Palma, J. M., et al. (2012). Arsenic triggers the nitric oxide (NO) and S-nitrosoglutathione (GSNO) metabolism in Arabidopsis. *Environmental Pollution*, 166, 136-143.
- Nguyen, N. T., Hiep, N. D., & Fujita, K. (2005). Iron enhances aluminum-induced leaf necrosis and plant growth inhibition in *Eucalyptus camaldulensis*. *Plant and Soil*, 277(1-2), 139-152.
- Nguyen, N. T., Mohapatra, P. K., & Fujita, K. (2003). Leaf necrosis is a visual symptom of the shift from growth stimulation to inhibition effect of Al in *Eucalyptus camaldulensis*. *Plant Science*, 165(1), 147-157.
- Richards, K. D., Schott, E. J., Sharma, Y. K., et al. (1998). Aluminum induces oxidative stress genes in *Arabidopsis thaliana*. *Plant Physiology*, 116(1), 409-418.
- Saxena, I., & Shekhawat, G. S. (2013). Nitric oxide (NO) in alleviation of heavy metal induced phytotoxicity and its role in protein nitration. *Nitric Oxide*, 32, 13-20.
- Sharma, S. S., & Dietz, K. J. (2009). The relationship between metal toxicity and cellular redox imbalance. *Trends in Plant Science*, 14(1), 43-50.
- Shi, H., Ye, T., Zhu, J. K., et al. (2014). Constitutive production of nitric oxide leads to enhanced drought stress resistance and extensive transcriptional reprogramming in *Arabidopsis*. *Journal of Experimental Botany*, 65(15), 4119-4131.
- Shin, E. C., Craft, B. D., Pegg, R. B., et al. (2010). Chemometric approach to fatty acid profiles in Runner-type peanut cultivars by principal component analysis (PCA). *Food Chemistry*, 119(3), 1262-1268.
- Tian, X., & Lei, Y. (2006). Nitric oxide treatment alleviates drought stress in wheat seedlings. *Biologia Plantarum*, 50(4), 775-778.
- Valderrama, R., Corpas, F. J., Carreras, A., et al. (2007). Nitrosative stress in plants. *FEBS Letters*, 581(3), 453-461.
- Wang, H. H., Huang, J. J., & Bi, Y. R. (2010). Nitrate reductase-dependent nitric oxide production is involved in aluminum tolerance in red kidney bean roots. *Plant Science*, 179(3), 281-288.

- Wang, S. L., Wang, P., Wang, C. Y., et al. (2010). Chemical behavior of aluminum in the acidic rhizosphere of *Pinus massoniana* Lamb—A case study on its cultivation in acid forest soil in Chongqing, China. *Chinese Journal of Applied and Environmental Biology*, 16(4), 523-528.
- Wang, Y. S., & Yang, Z. M. (2005). Nitric oxide reduces aluminum toxicity by preventing oxidative stress in the roots of *Cassia tora* L. *Plant and Cell Physiology*, 46(12), 1915-1923.
- Wei, H., Guo, J., Zhang, S., et al. (2006). The presence of phosphorylation form of D1 protein in its cross-linked aggregates in high light treated spinach leaves in vivo. *Chinese Science Bulletin*, 51(1), 69-74.
- Wei, Z. Z. (2015). *Comparative study on the early growth of three Eucalyptus clones* (Master's thesis, Guangxi University).
- Wu, Y. F., Huang, Y. Q., Cheng, L., et al. (2012). Investigation of *Eucalyptus* DH(32-29) clone planting status in southern China. *Eucalypt Science & Technology*, 29(2), 24-27.
- Xiao, X. X., Liu, X. H., Yang, Z. W., et al. (2005). Effect of aluminum stress on the photosynthesis of longan seedlings. *Chinese Journal of Tropical Crops*, 26(1), 63-69.
- Yang, L. J. (2009). *The role and relationship of nitric oxide and ethylene in aluminum-reduced root elongation in Phaseolus radiatus L.* (Master's thesis, Shaanxi Normal University).
- Yang, L. T. (2011). *Aluminum-tolerance mechanisms of citrus and regulation of aluminum-toxicity by phosphorus and nitric oxide* (Doctoral dissertation, Fujian Agriculture and Forestry University).
- Yang, M., Wu, Y. M., Huang, S. X., et al. (2011). Resistance physiological response of different fast-growing *Eucalyptus* clones to acid-aluminum stresses. *Scientia Silvae Sinicae*, 47(6), 181-187.
- Yang, M., Tan, L., Xu, Y., et al. (2015). Effect of low pH and aluminum toxicity on the photosynthetic characteristics of different fast-growing *Eucalyptus* vegetatively propagated clones. *PLOS ONE*, 10(6), e0128615.
- Ye, S. M. (2007). *Study on management models for Eucalyptus industrial plantation in Guangxi* (Doctoral dissertation, Beijing Forestry University).
- Yin, L., Mano, J. I., Wang, S., et al. (2010). The involvement of lipid peroxide-derived aldehydes in aluminum toxicity of tobacco roots. *Plant Physiology*, 152(3), 1406-1417.
- Ying, X. F., & Liu, P. (2005). Effects of aluminum stress on photosynthetic characters of soybean. *Chinese Journal of Applied Ecology*, 16(1), 166-170.
- Zhang, H., Li, Y. H., Hu, L. Y., et al. (2008). Effects of exogenous nitric oxide donor on antioxidant metabolism in wheat leaves under aluminum stress.

*Russian Journal of Plant Physiology*, 55(4), 469-474.

Zhang, Q. M., Chen, R. F., Zhao, X. Q., et al. (2011). Effects of P on growth of rice seedling under Al stress and relationship between Al tolerance and P efficiency of the rice. *Acta Pedologica Sinica*, 48(1), 103-111.

Zhang, Z. L. (1990). *Experimental guidance on plant physiology* (2nd ed.). Higher Education Press.

Zhou, Q., & Zhu, Z. L. (2015). Effects of NaCl stress on seedling growth and mineral ions uptake, distribution and transportation of two varieties of *Carpinus* L. *Journal of Beijing Forestry University*, 37(12), 7-16.

Zhou, Q., Zhu, Z. L., & Shi, M. (2015). Effects of salt stress on growth, physiological and biochemical characteristics of *Carpinus turczaninowii* seedlings. *Journal of Nanjing Forestry University (Natural Sciences Edition)*, 39(6), 56-60.

*Note: Figure translations are in progress. See original paper for figures.*

*Source: ChinaXiv –Machine translation. Verify with original.*