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Effects of Exogenous Ascorbic Acid and Glutathione on Redox Balance and Nicotine in Tobacco After Topping (Postprint)

Authors: Luo Yinghong, Yiyang Zhang, Liu Xiaoying, Li Shuang, Kun Liu, Cui Chaogang, Tan Guanping, Jiheng Zhou

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Abstract

Elevated nicotine content is a prevailing issue in Chinese flue-cured tobacco. Effectively reducing leaf nicotine content and improving the industrial usability of flue-cured tobacco represents a significant challenge in tobacco production. Based on the phenomena that tobacco topping causes a sharp increase in nicotine content and mechanical damage triggers cellular oxidative burst, this study investigated the series of physiological changes induced by topping wounds. Two antioxidant treatments—ascorbic acid plus glutathione (AsA+GSH) and ascorbic acid (AsA) applied to the wound site after topping—were employed to suppress the elevation of reactive oxygen species (ROS). The study explored the relationship between jasmonic acid (JA)-stimulated nicotine accumulation and ROS levels, and compared the efficacy of the two methods in inhibiting ROS and nicotine increase. The results demonstrated that both AsA+GSH and AsA treatments effectively suppressed the increase in superoxide anion, hydrogen peroxide, and malondialdehyde in tobacco leaves. Hydrogen peroxide degraded more slowly than superoxide anion, leading to its accumulation in tobacco tissues. AsA+GSH and AsA treatments resulted in lower jasmonic acid content at 6 h post-topping compared with conventional topping, indicating an inhibitory effect on jasmonic acid production. After 96 h of treatment, leaf nicotine content in the AsA+GSH treatment was 21.5% lower than that in conventional topping, while the AsA treatment showed a 17.5% reduction. Furthermore, significant or highly significant correlations were observed among all measured parameters. Additionally, ROS levels in all treatments returned to control (non-topped) levels by 24 h post-topping. The experiment indicates that application of antioxidant substances (AsA+GSH) to topping wounds effectively suppresses the increase of reactive oxygen species, jasmonic acid, and nicotine content, revealing a close interrelationship among ROS, jasmonic acid,

and nicotine. AsA+GSH exhibits stronger antioxidant capacity than AsA alone and provides superior inhibition of post-topping nicotine accumulation.

Full Text

Influence of Exogenous Ascorbic Acid and Glutathione Applied to Wounds After Tobacco Topping on Redox Equilibrium and Nicotine Content

LUO Yinghong, ZHANG Yiyang, LIU Xiaoying, LI Shuang, LIU Kun, CUI Chaogang, TAN Guanping, ZHOU Jiheng

(Institute of Tobacco Research, Hunan Agricultural University, Changsha 410128, China)

Abstract

High nicotine content is a current problem in flue-cured tobacco in China, posing a significant challenge for improving tobacco quality and industrial usability. Based on the observations that tobacco topping causes a sharp increase in nicotine content and that mechanical damage triggers oxidative burst in plant cells, this study investigated the physiological changes induced by topping trauma. Two antioxidant treatments—ascorbic acid plus glutathione (AsA+GSH) and ascorbic acid (AsA) alone—were applied to topping wounds to suppress reactive oxygen species (ROS) accumulation and to explore the relationship between jasmonic acid-stimulated nicotine synthesis and ROS levels. The effects of these two methods on suppressing ROS and nicotine accumulation were compared.

The results showed that both AsA+GSH and AsA treatments inhibited the increase in superoxide anion, hydrogen peroxide, and malondialdehyde contents in tobacco leaves. Hydrogen peroxide degraded more slowly than superoxide anion and accumulated in tobacco tissues. At 6 hours after treatment, jasmonic acid content in the AsA+GSH and AsA treatments was lower than in conventional topping, demonstrating an inhibitory effect on jasmonic acid production. After 96 hours, leaf nicotine content in the AsA+GSH treatment was 21.5% lower than in conventional topping, while the AsA treatment showed a 17.5% reduction compared to conventional topping. Significant or extremely significant correlations were observed among all measured indicators. Additionally, ROS levels in all treatments returned to control (non-topped) levels by 24 hours after topping.

These findings demonstrate that applying antioxidant substances (AsA+GSH) to tobacco wounds after topping effectively suppresses the increase in ROS, jasmonic acid, and nicotine content, revealing a close relationship among these three factors. The AsA+GSH treatment exhibited stronger antioxidant activity than AsA alone and was more effective in inhibiting nicotine accumulation after topping.

Keywords: Ascorbic acid; Glutathione; Tobacco topping; Reactive oxygen species; Jasmonic acid; Nicotine

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Introduction

When plants are wounded, endogenous elicitors activate multiple signal transduction pathways, including those involving jasmonic acid, salicylic acid, and ethylene, ultimately triggering defense responses. However, the specific pathways connecting elicitors to these signaling cascades remain poorly understood. Nicotine, a secondary metabolite widely present in tobacco, is not only an important chemical component of tobacco leaves but also a key quality indicator in cigarettes. Low nicotine content results in weak flavor and flat taste, whereas excessive nicotine increases harshness and produces a pungent taste. In recent years, despite improvements in tobacco leaf appearance quality approaching international standards in some Chinese production regions, the average nicotine content in flue-cured tobacco has reached 3–4%, significantly higher than the normal level of below 3%. Therefore, controlling nicotine content within an appropriate range is crucial for ensuring high-quality tobacco production.

Topping is an essential agronomic practice in tobacco cultivation that simultaneously inflicts mechanical damage on the plant. Research has demonstrated that artificial mechanical damage can fully or partially simulate insect herbivory, activating direct defense response systems. Following mechanical injury, plant cells undergo hypersensitive reactions to avoid further damage, and cells surrounding the wound site may undergo programmed cell death (PCD). The most prominent chemical reaction during this PCD is the "oxidative burst," which disrupts the existing redox balance. Under normal conditions, reactive oxygen species (ROS) are decomposed by enzymatic systems including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX). Excess ROS can also be quenched by non-enzymatic compounds such as flavonoids, ascorbic acid, glutathione, -tocopherol, and carotenoids. However, during oxidative burst, ROS production dramatically exceeds the scavenging capacity of endogenous antioxidants, and the surplus ROS can react with virtually all biomolecules, including proteins, lipids, polysaccharides, and nucleic acids.

ROS also promote plant hormone synthesis and induce a series of resistance responses. ROS can attack linolenic, linoleic, and arachidonic acids in the plasma membrane, causing their peroxidation. Vick et al. proposed in the 1980s that jasmonic acid precursors originate from linolenic acid peroxidation products on chloroplast membranes, suggesting that ROS may induce jasmonic acid production. As a plant hormone, jasmonic acid can trigger nicotine synthesis as a defense response in tobacco, indicating a potential causal relationship among topping, ROS, jasmonic acid, and nicotine. While nicotine synthesis may not be regulated solely by jasmonic acid and likely involves multiple regulatory factors,

numerous studies have shown that ROS can induce salicylic acid, ethylene, and phytoalexin synthesis, all of which regulate plant stress responses. These regulatory pathways may interact synergistically; for example, jasmonic acid and ROS can co-regulate 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity during ethylene synthesis. Additionally, H O , as a relatively stable ROS, functions as a signaling molecule itself.

To address the problem of rapid nicotine increase after topping, researchers such as Cheng Xinsheng, Zong Na, and Huang Lan drew inspiration from oligophagous insects that feed on tobacco. They applied bionic signal molecules (BSM) derived from specific enzymes secreted by the labial glands of these insects to topping wounds and found that BSM could suppress the increase in jasmonic acid, lipoxygenase (LOX), and ornithine decarboxylase (ODC), ultimately inhibiting nicotine accumulation. Studies by Zhang Xinhua and Li Ying suggested that BSM inhibits oxidative stress, thereby suppressing nicotine increase after topping.

The process of nicotine synthesis induction in tobacco is extremely complex, with many aspects still unclear and controversial. Controlling one or a few enzymes in a specific pathway to reduce nicotine is impractical. However, extensive research indicates that various regulatory pathways are almost all linked to ROS. Therefore, controlling ROS levels after topping may weaken wound signals and ultimately achieve nicotine reduction. AsA, a non-enzymatic antioxidant, can effectively scavenge ROS and, together with APX, eliminate H O . GSH not only quenches free radicals and removes H O but also reduces dehydroascorbic acid (DHA), enabling the recycling of AsA for continuous ROS scavenging.

This study applied AsA and GSH to tobacco wounds after topping to eliminate the large amount of ROS produced, with the expectation of weakening wound signals and ultimately reducing nicotine content, providing a potential solution to the widespread problem of high nicotine content in Chinese tobacco leaves.

Materials and Methods

1.1 Experimental Materials The experiment was conducted from April to June 2014 on the rooftop of the Tobacco Research Institute at Hunan Agricultural University. The test variety was 'Yunyan 87' . Tobacco seedlings were grown in sand culture using plastic pots (bottom diameter 20 cm, top diameter 24 cm, height 24 cm) with small drainage holes, filled with sterilized river sand. Seedlings were transplanted at the 7-leaf stage and irrigated daily at 8:00 AM with Hoagland nutrient solution.

1.2 Experimental Reagents L-ascorbic acid (AsA), product number A2174, CAS 50-81-7, Sigma, plant cell culture tested; L-reduced glutathione (GSH), product number G6013, CAS 70-18-8, Sigma, cell culture tested, BioReagent, 98.0%, powder.

The 1% AsA solution was prepared following Jin Zhonghua’ s method: 0.1 g L-ascorbic acid was dissolved in deionized water, then 100 mg EDTA and 2 mL glacial acetic acid were added, and the volume was adjusted to 100 mL with deionized water. The solution was mixed well, stored in a brown reagent bottle, and refrigerated.

The 1% AsA + 1% GSH solution was prepared similarly: 0.1 g AsA and 0.1 g GSH were dissolved in deionized water, followed by addition of 100 mg EDTA and 2 mL glacial acetic acid, with the final volume adjusted to 100 mL. The solution was mixed well, stored in a brown reagent bottle, and refrigerated.

The buffer solution was prepared by adding 100 mg EDTA and 2 mL glacial acetic acid to a small amount of deionized water, then adjusting the volume to 100 mL. The solution was mixed well, stored in a brown reagent bottle, and refrigerated.

1.3 Experimental Treatments The experiment included five treatments: (1) T1: topping followed by application of 1% AsA; (2) T2: topping followed by application of 1% AsA + 1% GSH; (3) TS1: topping followed by application of buffer solvent; (4) TS2: conventional topping; and (5) CK: no topping. Topping was performed at the budding stage, and treatments were applied immediately afterward. For T1, T2, and TS1, 0.25 mL of the respective solution was applied to the wound using a wool brush immediately after topping.

Each treatment comprised 33 plants. At 3, 6, 9, 24, and 48 hours after treatment, three plants from each treatment were sampled to measure superoxide anion, malondialdehyde, and H O contents in all fresh leaves. Additionally, at 6 hours, fresh leaves and roots were sampled for jasmonic acid analysis. At 3, 6, 9, 24, 48, and 96 hours after treatment, three plants from each treatment were killed by heating to determine nicotine content in all leaves and roots.

1.4 Detection Methods Superoxide anion (· O) content was determined using the method of Wang Aiguo et al. Hydrogen peroxide (H O) content was measured using Ferguson’ s acetone extraction method combined with Liu Jun’ s extraction and decolorization method. The specific protocol followed Zhang Qian et al.

Malondialdehyde (MDA) analysis was performed according to literature methods with slight modifications. Fresh leaf samples (0.5 g) were de-veined and ground in a pre-cooled mortar with 2 mL of 0.05 mol · L ^{1} phosphate buffer (pH 7.8) and a small amount of quartz sand. The homogenate was adjusted to 10 mL in a graduated test tube. Five milliliters of the mixture were centrifuged at 16,000 r · min ^{1} for 15 minutes, and the supernatant was collected and stored at 5°C. For analysis, 1.5 mL of crude enzyme extract was mixed with 2.5 mL of 0.5% thiobarbituric acid in 5% trichloroacetic acid in a 5 mL test tube. The mixture was heated in a boiling water bath for 15 minutes, rapidly cooled, and centrifuged again. The supernatant was used to measure absorbance at 532 nm

and 600 nm (A and A). MDA concentration (C) was calculated using the formula: C = [(A - A) × A × V] / (0.155 × a × W), where A is the total reaction volume, V is the total extraction volume, a is the extraction volume in the reaction mixture, and W is the plant sample weight.

Jasmonic acid extraction and detection followed the methods of Baldwin et al. and Segarra et al. Nicotine content was determined using the UV spectrophotometry method of Jin Wenbo et al.

1.5 Data Processing **Change rate calculation**: TS2 (conventional topping) was used as the reference, with changes relative to CK representing the effect of topping. The change amount divided by time yielded the change rate. T1 and T2 (antioxidant treatments) were compared to TS2, with changes relative to TS2 representing the antioxidant effect, and the change amount divided by time representing the change rate.

We defined "TS2 change rate" as TS2, "T1 change rate" as TS2, and "T2 change rate" as TS2. ATS2 represents the content of the corresponding index at specific times after topping in TS2, AT1 represents the content in T1, AT2 represents the content in T2, and t represents the time after treatment.

Association of indicators with topping response: We defined the net response intensity (S) of each detection index to topping as follows: for superoxide anion, hydrogen peroxide, and malondialdehyde, S = ST - SCK; for jasmonic acid, S = T(6 h) - CK(6 h); for nicotine, S = T(96 h) - CK(96 h). ST represents the weighted value of each treatment from 0-48 h, where T(ih) is the content of the corresponding index at time i, and SCK represents the weighted value of CK from 0-48 h, where CK(ih) is the content in CK at time i.

Correlation analysis was performed to examine relationships among the response intensities of various indicators to topping. Data were processed using Microsoft Excel 2010 and SPSS 20.

Results

2.1 Effects of AsA and GSH Treatments on Nicotine Content After Topping Analysis of root nicotine content (Figure 1A) showed that CK (non-topped) maintained stable nicotine content throughout the 96-hour period. Topping caused a rapid increase in nicotine content, with all topped treatments (T1, T2, TS1, TS2) showing significantly higher nicotine content than CK at most time points, except for T2 at 3 hours and T1 and T2 at 9 hours. Application of AsA+GSH or AsA effectively inhibited nicotine increase, with T1 and T2 showing significantly lower nicotine content than TS1 and TS2. The difference between T1/T2 and TS1/TS2 was small at 3-24 hours but gradually increased at 48-96 hours. At 96 hours, T2 nicotine content was 21.5% lower

than TS2, while T1 was 17.5% lower than TS2, indicating that AsA+GSH was more effective than AsA alone in suppressing nicotine increase.

Analysis of leaf nicotine content (Figure 1B) revealed similar trends to those observed in roots. CK nicotine content remained stable for 48 hours, while all topping treatments showed varying degrees of increase. TS1 and TS2 increased most rapidly, remaining significantly higher than CK throughout 3–96 hours and also higher than T1 and T2. Notably, TS1 and TS2 showed no significant differences at any time point, indicating that buffer application alone did not affect nicotine content. Both T1 and T2 had significantly higher nicotine content than CK but significantly lower than TS1 and TS2, confirming that AsA+GSH or AsA application effectively suppressed nicotine increase. T2 showed significantly lower nicotine content than T1 at 9, 48, and 96 hours, further demonstrating the superior effect of AsA+GSH over AsA alone.

[Figure 1: see original paper]

2.2 Effects of AsA and GSH Treatments on Superoxide Anion Content After Topping As shown in Figure 2A, superoxide anion content in CK remained stable throughout the 48-hour period. Topping induced a dramatic increase in superoxide anion, with TS2 (conventional topping) showing much higher levels than CK at 3 hours, followed by rapid decline to near-CK levels by 24 hours, with no significant difference thereafter. TS1 (buffer application) showed no significant differences from TS2 during the 48-hour period. Application of AsA+GSH or AsA (T1 and T2) inhibited superoxide anion increase, with significantly lower content than TS1 and TS2 at 3 hours, though still significantly higher than CK. By 6 hours, T1 and T2 levels approached CK values and were significantly lower than TS1 and TS2, with no significant difference between T1 and T2.

Figure 2B illustrates the change rates of superoxide anion content. TS2 showed a high change rate at 3 hours after topping, which rapidly decreased to near zero by 24 hours. T1 and T2 also showed high degradation rates at 3 hours, which declined quickly thereafter, but with distinct patterns: T1 had a higher degradation rate than T2 at 3 hours, a lower rate at 6 hours, and similar rates at 9 hours. This suggests that AsA application was more effective at suppressing superoxide anion increase at 3 hours, while AsA+GSH was more effective at 6 hours. Both treatments converged to zero thereafter.

[Figure 2: see original paper]

2.3 Effects of AsA and GSH Treatments on H O Content After Topping Figure 3A shows that H O content in CK remained stable throughout the 48-hour period. Topping significantly increased H O content in all topped treatments (T1, T2, TS1, TS2). TS2 (conventional topping) exhibited an initial increase followed by a decrease, reaching maximum content at 6 hours and approaching CK levels by 48 hours with no significant difference. TS1 showed

significantly lower H O content than TS2 at 6, 9, and 24 hours. Application of AsA+GSH or AsA effectively suppressed H O increase, with T1 and T2 showing significantly lower content than TS1 and TS2 at 3 and 6 hours, significantly higher than CK, and significantly lower than TS2 at 9 and 24 hours. No significant differences were observed among treatments at 48 hours. T2 showed significantly lower H O content than T1 at 6 hours, with no significant differences at other time points.

Figure 3B shows that the change rates of TS2, T1, and T2 approached zero by 48 hours. TS2 had a markedly higher change rate than T1 and T2 before 24 hours. The degradation rates of T1 and T2 decreased gradually, with T2 showing higher rates than T1 at 0-9 hours, indicating that AsA+GSH was more effective than AsA alone in suppressing H O increase.

[Figure 3: see original paper]

2.4 Effects of AsA and GSH Treatments on Malondialdehyde Content After Topping Figure 4A shows that MDA content in CK remained essentially unchanged during the 48-hour period. Topping induced a dramatic increase in MDA, with TS2 reaching maximum content at 3 hours, decreasing rapidly between 3-6 hours, then declining slowly to near-CK levels by 48 hours with no significant difference. TS1 showed significantly lower MDA content than TS2 at 3 hours, with no significant differences thereafter. AsA+GSH or AsA application significantly inhibited MDA increase, with T1 and T2 showing an initial increase followed by decrease. Except for T1 at 6 hours (which showed no significant difference from TS1 and TS2), T1 and T2 had significantly lower MDA content than TS1 and TS2 at 3-24 hours, with no significant differences among treatments at 48 hours. T2 showed significantly lower MDA content than T1 at 3 and 6 hours, with no significant differences at other time points.

Figure 4B shows that the change rate curves for TS2, T1, and T2 followed an inverse function pattern, with rapid initial decline followed by gradual decrease. T2 had higher degradation rates than T1 at 3-9 hours, with similar rates thereafter, indicating that AsA+GSH was more effective than AsA in suppressing MDA increase.

[Figure 4: see original paper]

2.5 Effects of AsA and GSH Treatments on Jasmonic Acid Content After Topping According to Vick et al.' s octadecanoid pathway for jasmonic acid synthesis, jasmonic acid precursors derive from linolenic acid oxidation products in chloroplast membranes, which are associated with ROS. Therefore, jasmonic acid content should be influenced by ROS concentration.

Analysis of jasmonic acid in leaves (Figure 5A) and roots (Figure 5B) at 6 hours after treatment showed that CK had the lowest jasmonic acid content. Topping significantly increased jasmonic acid content, with TS2 showing several-fold

higher levels than CK. TS1 showed no significant difference from TS2, indicating that buffer application alone did not significantly affect jasmonic acid content. T1 and T2 were significantly lower than TS1 and TS2, demonstrating that AsA+GSH or AsA application effectively suppressed jasmonic acid increase. T2 showed significantly lower jasmonic acid content than T1, indicating that AsA+GSH was more effective than AsA alone.

Root jasmonic acid analysis showed that topping induced a greater increase in roots than in leaves, and the inhibitory effect of AsA+GSH or AsA was also more pronounced in roots. The relative differences among treatments were consistent with those observed in leaves.

[Figure 5: see original paper]

2.6 Response Intensities and Correlations Among Indicators After Topping The response intensities of detection indicators to topping are shown in Table 1. T1 and T2 showed markedly lower response intensities for all indicators compared to TS1 and TS2, with T2 showing weaker responses than T1. These results further confirm that AsA+GSH effectively suppressed the increase in all measured indicators, with stronger inhibitory effects than AsA alone.

Correlation analysis of response intensities among indicators is presented in Table 2. All detection indicators (superoxide anion, hydrogen peroxide, malondialdehyde, leaf jasmonic acid, root jasmonic acid, root nicotine, and leaf nicotine) showed significant positive correlations with each other, with some reaching extremely significant levels. This demonstrates that changes in these indicators after topping were not independent but rather interconnected and mutually influential.

Discussion

Topping, as a destructive agricultural practice, indeed induces changes in tobacco’ s internal chemical composition. The results showed that conventional topping caused a substantial increase in leaf superoxide anion content, which declined dramatically within 24 hours, indicating that tobacco possesses self-recovery capacity after damage, with the disrupted redox balance being reestablished over time. The initial superoxide anion content in the buffer treatment (TS1) was slightly lower than in conventional topping, possibly because the buffer solution temporarily isolated the wound from atmospheric oxygen, slowing the oxidative burst. AsA application effectively reduced superoxide anion concentration, while AsA+GSH showed no significant difference from AsA alone. At 3 hours after treatment, the superoxide anion degradation rate was faster with AsA than with AsA+GSH, but the opposite was observed at 6 hours. This may be explained by the glutathione-ascorbate cycle mechanism: AsA+GSH initially showed slightly weaker antioxidant capacity, but subsequently GSH could reduce oxidized AsA, enabling its recycling. Notably, although differences

among treatments were substantial initially, all superoxide anion concentrations converged to nearly identical levels after 24 hours, suggesting that tobacco requires approximately 24 hours to re-establish redox equilibrium after topping.

Topping also affected hydrogen peroxide content in tobacco. Except for the non-topped control, which showed stable H_2O_2 levels, all other treatments exhibited an initial increase followed by decrease, indicating that H_2O_2 is more stable than superoxide anion and accumulates during production, possibly serving as a stable ROS signaling molecule. This finding aligns with the research of Gechev et al. and Foyer et al. The results demonstrate that antioxidant application (AsA and GSH) effectively suppressed H_2O_2 increase, with AsA+GSH showing stronger effects than AsA alone—a trend distinct from that of superoxide anion, which can be explained by the glutathione-ascorbate cycle.

Malondialdehyde concentration reflects plasma membrane oxidation levels. In this study, MDA dynamics closely mirrored those of H_2O_2, which can be attributed to the chemical stability of MDA. The results indicate that AsA+GSH and AsA treatments effectively protected the membrane system by reducing ROS damage, with AsA+GSH showing superior effects.

These three indicators collectively represent ROS levels. The results demonstrate that topping effectively elevated ROS content and disrupted redox balance. Unlike previous studies by Zhang Xinhua and Li Ying that used BSM from oligophagous insect labial glands to inhibit ROS through unclear mechanisms, this study achieved similar effects through direct ROS quenching by AsA and GSH, with a clear mechanistic basis.

ROS can trigger multiple complex signaling pathways. Our analysis of jasmonic acid, the most representative signaling molecule, revealed that topping increased jasmonic acid content in both leaves and roots, while AsA+GSH and AsA treatments significantly reduced jasmonic acid levels compared to conventional topping. This suggests that AsA-GSH suppresses jasmonic acid increase by inhibiting ROS, a conclusion supported by Vick et al.' s research. Our jasmonic acid findings support the series of studies by Cheng Xinsheng et al.

As a plant hormone, jasmonic acid induces resistance responses, and nicotine, as a secondary metabolite, participates in tobacco defense. The experiment showed that antioxidant application effectively suppressed nicotine increase in both roots and leaves, with AsA+GSH showing stronger effects than AsA alone —a trend consistent with jasmonic acid dynamics. This suggests that nicotine changes are stimulated by jasmonic acid and are proportional to its content. Cheng Xinsheng et al. also sought to reduce nicotine by decreasing jasmonic acid, and our nicotine reduction effects were similar to their BSM results.

Although topping and antioxidant application independently affected ROS, jasmonic acid, and nicotine contents, these effects were not isolated but closely interrelated. Correlation analysis among the seven detection indicators (ROS components, jasmonic acid, and nicotine) revealed significant correlations, some

reaching extremely significant levels, confirming their interconnectedness and mutual influence.

This study demonstrates that applying antioxidants effectively suppressed the increase in superoxide anion, hydrogen peroxide, malondialdehyde, jasmonic acid, and nicotine after topping, with AsA+GSH showing superior antioxidant effects compared to AsA alone.

Antioxidant substances (AsA-GSH) applied to tobacco wounds after topping effectively reduced ROS, jasmonic acid, and nicotine contents, revealing close relationships among these factors. AsA+GSH exhibited stronger antioxidant capacity than AsA alone and better inhibited nicotine increase after topping.

While BSM indirectly inhibits oxidative stress through enzymatic action, AsA and GSH directly quench ROS, thereby suppressing jasmonic acid increase after topping. Using AsA and GSH instead of BSM offers cost reduction and improved efficiency, providing broader applicability and a practical solution to the problem of high nicotine content in Chinese flue-cured tobacco.

References

[1] Doke N, Miura Y. In vitro activation of NADPH-dependent O -generating system in a plasma membrane-rich fraction of potato tuber tissues by treatment with an elicitor from *Phytophthora infestans* or with digitonin[J]. *Physiological and Molecular Plant Pathology*, 1995, 46(1): 17–28.

[2] Wang G S, Chen W H, Xue C Q, et al. Discussion on factors of influencing nicotine level and measures for reducing nicotine level[J]. *Tobacco Science & Technology*, 2001(2): 38–42.

[3] Lyytikäinen-Saarenmaa P. The responses of scots pine, *Pinus sylvestris*, to natural and artificial defoliation stress[J]. *Ecological Applications*, 1999, 9(2): 469–474.

[4] Greenberg J T, Guo A, Klessig D F, et al. Programmed cell death in plants: A pathogen-triggered response activated coordinately with multiple defense functions[J]. *Cell*, 1994, 77(4): 551–563.

[5] Papadakis A K, Roubelakis-Angelakis K A. The generation of active oxygen species differs in tobacco and grapevine mesophyll protoplasts[J]. *Plant Physiology*, 1999, 121(1): 197–205.

[6] Fath A, Bethke P, Beligni V, et al. Active oxygen and cell death in cereal aleurone cells[J]. *Journal of Experimental Botany*, 2002, 53(372): 1273–1282.

[7] Apostol I, Heinstein P F, Low P S. Rapid stimulation of an oxidative burst during elicitation of cultured plant cells. Role in defense and signal transduction[J]. *Plant Physiology*, 1989, 90(1): 109–116.

[8] Chen H, Wu J L, Li X J. Generation and scavenging of reactive oxygen species in chloroplasts[J]. *Progress in Modern Biomedicine*, 2008, 8(10): 1979–1981.

[9] Wu S, Xiao L T. Metabolism and signaling conduction of the reactive oxygen species in plant[J]. *Journal of Hunan Agricultural University: Natural Sciences*, 2003, 29(5): 450–456.

[10] Gechev T, Gadjev I, Van Breusegem F, et al. Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes[J]. *Cellular and Molecular Life Sciences*, 2002, 59(4): 708–714.

[11] Trippi V S, Gidrol X, Pradet A. Effects of oxidative stress caused by oxygen and hydrogen peroxide on energy metabolism and senescence in oat leaves[J]. *Plant & Cell Physiology*, 1989, 30(2): 157–162.

[12] Vick B A, Zimmerman D C. Biosynthesis of jasmonic Acid by several plant species[J]. *Plant Physiology*, 1984, 75(2): 458–461.

[13] Shoji T, Yamada Y, Hashimoto T. Jasmonate induction of putrescine N-methyltransferase genes in the root of *Nicotiana sylvestris*[J]. *Plant & Cell Physiology*, 2000, 41(7): 831–839.

[14] Dat J F, Foyer C H, Scott I M. Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings[J]. *Plant Physiology*, 1998, 118(4): 1455–1461.

[15] Dat J F, Lopez-Delgado H, Foyer CH, et al. Parallel changes in H O and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings[J]. *Plant Physiology*, 1998, 116(4): 1351–1357.

[16] Van Camp W, Van Montagu M, Inzé D. H O and NO: Redox signals in disease resistance[J]. *Trends in Plant Science*, 1998, 3(9): 330–334.

[17] Ievinsh G, Tillberg E. Stress-induced ethylene biosynthesis in pine needles: A search for the putative 1-aminocyclopropane-l-carboxylic acid-independent pathway[J]. *Journal of Plant Physiology*, 1995, 145(3): 308–314.

[18] Schraudner M, Moeder W, Wiese C, et al. Ozone-induced oxidative burst in the ozone biomonitor plant, tobacco BelW3[J]. *The Plant Journal*, 1998, 16(2): 235–245.

[19] Overmyer K, Tuominen H, Kettunen R, et al. Ozone-sensitive arabidopsis rcd1 mutant reveals opposite roles for ethylene and jasmonate signaling pathways regulating superoxide-dependent cell death[J]. *The Plant Cell*, 2000, 12(10): 1849–1862.

[20] Dixon R A, Harrison M J. Activation, structure, and organization of genes involved in microbial defense in plants[J]. *Advances in Genetics*, 1990, 28: 165–234.

[21] Watanabe T, Seo S, Sakai S. Wound-induced expression of a gene for 1-aminocyclopropane-1-carboxylate synthase and ethylene production are regulated by both reactive oxygen species and jasmonic acid in *Cucurbita maxima*[J]. *Plant Physiology and Biochemistry*, 2001, 39(2): 121–127.

[22] Foyer C H, Noctor G. Tansley review No. 112. Oxygen processing in photosynthesis: Regulation and signalling[J]. *New Phytologist*, 2000, 146(3): 359–388.

[23] Cheng X S, Shen J, Cao L J. Effects of wounding signal regulations on the nicotine and other chemical composition in tobacco upper leaves[J]. *Chinese Journal of Tropical Crops*, 2008, 29(3): 333–337.

[24] Zong N, Wang C Z. Induction of nicotine in tobacco by herbivory and its relation to glucose oxidase activity in the labial gland of three noctuid caterpillars[J]. *Chinese Science Bulletin*, 2004, 49(14): 1380–1385.

[25] Huang L, Wang Z G, Shu J S, et al. Inhibitory effects of bionics signal molecule on principal alkaloids in tobacco[J]. *Acta Tabacaria Sinica*, 2010, 16(2): 1–5.

[26] Zhang X H, Yao Z D, Chen C W, et al. Reaction of tobacco to oxidation stress induced by topping and bionic signal molecules[J]. *Tobacco Science and Technology*, 2011(12): 61–64.

[27] Li Y, Wang C H, Zhou C Y, et al. Effects of topping and bionics signal molecule on oxidation stress of different flue-cured tobacco varieties[J]. *Chinese Tobacco Science*, 2013, 34(6): 20–23.

[28] Potters G, Horemans N, Bellone S, et al. Dehydroascorbate influences the plant cell cycle through a glutathione-independent reduction mechanism[J]. *Plant Physiology*, 2004, 134(4): 1479–1487.

[29] Jin Z H. Improvement about ascorbic acid solution make-up method[J]. *The Administration and Technique of Environmental Monitoring*, 2001, 13(6): 39–40.

[30] Wang A G, Luo G H. Quantitative relation between the reaction of hydroxylamine and superoxide anion radicals in plants[J]. *Plant Physiology Communications*, 1990(6): 55–57.

[31] Ferguson I B, Watkins C B, Harman J E. Inhibition by calcium of senescence of detached cucumber cotyledons: Effect on ethylene and hydroperoxide production[J]. *Plant Physiology*, 1983, 71(1): 182–186.

[32] Liu J, Lü B, Xu L L. An improved method for the determination of hydrogen peroxide in leaves[J]. *Progress in Biochemistry and Biophysics*, 2000, 27(5): 548–551.

[33] Zhang Q, Fu S Y, Li H L, et al. A rapid method for determination of hydrogen peroxide concentration[J]. *Spectroscopy and Spectral Analysis*, 2014, 34(3): 767–770.

[34] Zhang X Z, Chen F Y, Wang R F. *Plant Physiology Experiment Technology*[M]. Shenyang: Liaoning Science and Technology Press, 1994: 164-165.

[35] Baldwin I T, Zhang Z P, Diab N, et al. Quantification, correlations and manipulations of wound-induced changes in jasmonic acid and nicotine in *Nicotiana sylvestris*[J]. *Planta*, 1997, 201(4): 397-404.

[36] Segarra G, Jáuregui O, Casanvoa E, et al. Simultaneous quantitative LC-ESI-MS/MS analyses of salicylic acid and jasmonic acid in crude extracts of *Cucumis sativus* under biotic stress[J]. *Phytochemistry*, 2006, 67(4): 395-401.

[37] Jin B W, Dai Y, Yang J. *Tobacco Chemical Analysis and Flue Gas Analysis*[M]. Nanchang: Jiangxi Science and Technology Press, 1993: 123-125.

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