Thymol improves salinity tolerance of tobacco by increasing the Na^+ efflux and enhancing the content of NO and GSH

liang xu
Henan Agricultural University
Jia-Qian Song
Henan Agricultural University
yuelin wang
Henan Agricultural University
Xiao-Han Liu
Guangdong Shaoguan Tobacco Recuring Co., LTD
Xue-Li Li
China Tobacco Corporation Staff Training College
Bo Zhang
Henan Agricultural University
Ai-Jie Li
Henan University
Xie-Feng Ye (yexiefeng@163.com)
Henan Agricultural University
Jing Wang
Henan Agricultural University
Peng Wang
Wuhan Cigarette Factory of Hubei China Tobacco Industry Limited Liability Company

Research Article

Keywords: Thymol, Tobacco, NO, GSH, Na+ efflux

Posted Date: October 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-745750/v2

Abstract

Plants have evolved a lot of strategies to improve salt tolerance to cope with salt stress. Recent studies have suggested that thymol (a nature medicine) enhances the plant tolerance against abiotic stresses, but the mechanisms are rarely known. Here, we found that thymol played an important role in maintaining root growth under salt stress. Thymol rescued root growth from salt stress via ameliorating ROS (reactive oxygen species) accumulation, lipid peroxidation, and cell death. In addition, thymol enhanced the level of NO (nitric oxide) and GSH (glutathione) to repress ROS accumulation, further protecting the stability of cell membrane. Thymol-induced Na^+ efflux in roots and leaves under salt stress may depend on the upregulation of *SOS1*, *HKT1* and *NHX1*. Consequently, all of these evidences suggested that thymol improved tobacco salt tolerance via enhancing NO and GSH content as well as inducing Na^+ efflux.

1. Background

In recent years, soil salinization caused by natural and human factors has been becoming increasingly serious, and continuing to affect soil resources. It is one of the worldwide agroecological problems (Dong-xue, 2005; Jia et al., 2015). More than one-fifth of the world's arable land is currently under the threat of salt stress, arising a big challenge for the growth of plants (Zhang et al., 2012).

Salt stress impacts the plant growth (Xie et al., 2011). High salinity results in a decrease in germination rate of seeds, inhibition on the growth of primary root, decrease in the number of lateral roots, and withering and yellowing of leaves (Ischiropoulos and Al-Mehdi, 1995; Zhu, 2001). Salinity also accelerates chlorophyll decomposition and decreases photosynthesis. Salt stress has caused osmotic stress, ion-toxicity effects and oxidative stress in plants, which can damage the cellular components such as membrane lipids, proteins, and nucleic acids and cause metabolic dysfunction(Boudsocq and Lauriere, 2005; Munns and Tester, 2008; Suzuki et al., 2012; Zhu, 2016). As a result, the yield and the quality of crops always decrease upon salinity stress. Under salt stress, To cope with salinity stress, plants have evolved sophisticated mechanisms, including selective ion uptake/exclusion, compart-mentalization of toxic ions, synthesis of compatible products, adjustment of photosynthetic and energy metabolism, accumulation of antioxidative enzymes, regulation of hormones, and modification of cell structure (Zhang et al., 2012).

The most fundamental approach to solve salt stress problem is to prevent soil salinization and repair saline soil, but it has some limitations, such as difficult management, long period application, high cost, etc. Exogenous regulation of plant salt tolerance is a kind of potential alternative. Therefore, it is important to find cheap, safe, effective, and practical regulators to alleviate salt stress. At present, most of the exogenous substances are hormones, growth regulators, and signal substances. It is essential to develop novel regulators with potentiality in field application.

Thymol [5-methyl-2-(1-methylethyl) phenol] is a kind of plant essential oil; it is a monoterpene phenol that is easily soluble in organic solvents. Thymol is widely used in medical research because of the relatively low price and the least potential toxicity and risk. Thymol has good antibacterial and anti-inflammatory activity, and can protect mice liver by inhibiting lipid peroxidation (Alam et al., 1999). In food industry, thymol can be used as antioxidants and food additives to maintain the quality of fresh food (Castillo et al., 2014; González-Aguilar et al., 2013; Guarda et al., 2011). However, the application of thymol in plants is relatively rare. In this study, we investigated the effect of thymol on tobacco seedlings under salt stress and discussed its internal mechanisms.

2. Results

2.1. Thymol Mitigates Salt-Induced Inhibition of Tobacco Root Growth

To characterize the effect of salt on the growth of tobacco seedlings, the 6-day-old seedlings were treated with 0-200 mM NaCl for 72 h and the root length was measured. We found that 150 mM NaCl significantly inhibited root growth, resulting in a degree about 50% (Fig. 1a). Then the tobacco seedlings were allowed to grow in MS (Murashige & Skoog) medium containing 150 mM NaCl and different concentrations of thymol. Root length was slightly affected by thymol treatment alone (Fig. 1b). Compared to NaCl treatment alone, adding thymol at 50 and 100 mM remarkably increased root length (Fig. 1b). Using a time-course experiment, we cultured the seedlings with MS medium containing water (C), 150 mM NaCl (S)□50 μM thymol (T), or a mixture of them (S+T), respectively. The results indicated that roots treated with S+T showed higher root growth speed than that of S treatment (Fig. 1c and d), further supporting that thymol mitigated salt-induced inhibition of tobacco root growth.

2.2 Thymol suppresses ROS accumulation in Roots and Leaves under Salt Stress

Reactive oxygen species (ROS) accumulation, induced by salt stress, is widely recognized as an important cause of damage (Zhu, 2001). We tested whether the H_2O_2 content in tobacco seedlings was regulated by thymol. Treatment with 150 mM NaCl resulted in significant increase in H_2O_2 content; seedlings under S+T treatment had much lower H_2O_2 content, similar to the control group (Fig. S1 a). Then the endogenous H_2O_2 and levels were evaluated in situ in tobacco roots and leaves using DAB and NBT, respectively. The staining of seedlings grown on 150 mM NaCl was darker than control; seedlings treated with S+T showed slight staining (Fig. 2a, b, c and d). These results suggested that thymol decreased salt-induced ROS accumulation in tobacco seedlings.

In plants, ROS can act as signal to activate antioxidative system under stress conditions. Salt stress induced ROS accumulation, accompanying with the enhancement of three antioxidative enzymes, such as SOD, POD, and CAT (Fig. 2e, f and g). Adding thymol decreased the activity of these enzymes in salt-treated seedlings. This may due to the decrease in endogenous ROS level upon thymol application.

2.3 Thymol ameliorated salt-induced lipid peroxidation and cell death in roots and leaves

MDA is the main product of lipid peroxidation. The increased MDA content in tobacco seedlings showed a strong response to salt stress (Fig. S1 b), which was repressed by the application of thymol (Fig. S1 b). Shiff's Reagent was used to indicate lipid peroxidation *in situ*. Under salt stress, the roots and leaves were stained pink, while those treated with S+T were stained lighter (Fig. 3a and c). Trypan Blue was used to test cell death in tobacco roots and leaves. The results showed that root tips and leaves under salt stress showed extensive blue while other seedlings were stained slightly (Fig. 3b and d). These results suggested that thymol was able to attenuate lipid oxidation and cell death in tobacco seedlings upon salt stress.

2.4 Thymol increased the NO and GSH content in tobacco seedlings under salt stress

NO is an important defensive signaling molecule helping combat salt stress (Bai et al., 2011). The endogenous NO in tobacco roots was detected in situ by using specific fluorescent probe DAF-FM DA. Here we found that thymol enhanced endogenous NO level in roots under salt stress (Fig 4a). GSH is one of the major antioxidants in plants. The addition of thymol significantly increased GSH content in tobacco seedlings in the presence of NaCl or not (Fig 4b). These results suggested that NO and GSH may be involved in thymol-facilitated salt tolerance in tobacco seedlings.

2.5 Thymol mediated Na^+/K^+ transportation in tobacco seedlings under salt stress

Maintaining a balanced cytosolic Na^+/K^+ ratio has become a key salinity tolerance mechanism. Achieving this homeostatic balance requires the modulation of Na^+ and K^+ transporters and/or channels. The Na^+ and K^+ flux in root tip and leaves was measured by using Non-invasive Micro-test Technology (NMT). We found that the Na^+ influx in root tip was induced by salt while thymol caused Na^+ efflux (Fig. 5a), suggesting that the absorption of Na^+ was inhibited by thymol in root tip. Different with that in root tip, the Na^+ efflux was observed in leaves treated by salt or thymol (Fig. 5c). In addition, we measured Na^+ content in seedlings and expression levels of the known Na^+ transporter genes, including *NtSOS1*, *NtHKT1* and *NtNHX1*. Thymol decreased Na^+ content in seedlings under salt stress (Fig. 5e). And thymol enhanced the expression levels of *NtSOS1*, *NtHKT1* and *NtNHX1* as compared to that in salt treatment (Fig. 5g). These results suggested that thymol modulated Na^+ transporters to maintain K^+/Na^+ homeostasis in tobacco seedling under salt stress.

3. Discussion

A growing body of evidence suggests that thymol plays a role in the tolerance of plant stress resistance. In this study, we found that thymol enhanced plant tolerance via increasing NO and GSH content, and modulating Na^+/K^+ homeostasis in tobacco seedlings.

Increased ROS formation is observed in plants in response to both osmotic and ionic stresses associated with soil salinity, which further results in oxidative stress and cell damage(Flowers, 2004; Miller et al., 2010; Xie et al., 2011). ROS (e.g. H_2O_2 and superoxide anion), can damage most cellular macromolecules

directly and cause irreversible damage (Apel and Hirt, 2004; Kovtun et al., 2000). On the other hand, ROS have also been proposed to act as a signaling mediator of plant salinity tolerance. Plants scavenge excess ROS through antioxidant system in order to maintain cell homeostasis and reduce the harm of oxidative stress.

In the present study, we found that the growth of tobacco seedlings was strongly affected by salt stress. Salt stress significantly inhibited the root growth of tobacco seedlings, which was attenuated by thymol (Fig. 1). Under salt stress, H_2O_2 and both in leaves and roots were decreased by thymol, suggesting that thymol effectively prevented the over-accumulation of ROS in tobacco leaves and roots (Fig. 2). Thymol led to the decrease in the content of MDA and the lighter staining of shiff's reagent in tobacco seedlings under salt stress, suggesting that the lipid peroxidation and oxidative injury caused by salt stress were ameliorated by thymol (Fig. 3). ROS not only directly affect normal cellular functioning, but also lead to cell death (Van Breusegem and Dat, 2006). In our experiments, it could be suggested that the decrease in ROS accumulation caused by thymol resulted in the mitigation of lipid peroxidation and cell death in salt-treated tobacco seedlings.

NO, an essential messenger existing widely in plants, regulates multiple processes of plant growth and development. NO also modulates plant resistant responses against various abiotic stresses, including salt, drought, and heavy metals. NO helps to eliminate ROS likely through two kinds of pathway. First, NO can regulate ROS levels (Ischiropoulos and Al-Mehdi, 1995; Radi, 2004; Romero-Puertas and Sandalio, 2016). NO is not only able to regulate production but also H_2O_2 as it has been shown that the S-nitrosylation pattern of glycolate oxidase (GOX), one of the main H_2O_2 sources in the peroxisome, changes in response to Cd (Romero-Puertas and Sandalio, 2016). Second, GSNO (nitroso glutathione), generated by the reversibly reaction of NO and GSH, is an endogenous regulator of NO and GSH homeostasis (Noctor et al., 2012). GSH not only reacts directly with ROS species (Hernandez et al., 2010), but also participates in ASA-GSH cycle to scavenge ROS (Gill and Tuteja, 2010; Mittler, 2002). Here we found that thymol enhanced endogenous NO and GSH content in tobacco seedlings under salt stress. This may explain the decrease ROS content in salt-treated seedlings in the presence of thymol. However, further studies are need to find out whether and how thymol modulates GSNO to maintain the homeostasis of NO, GSH, and ROS upon salt stress.

Ionic stress, caused by excess Na^+ accumulation, is an important component of salt stress. To relieve Na^+ toxicity, some strategies have been developed decrease Na^+ content in the cytosol of plant cells, including suppressing the Na^+ uptake and enhancing the Na^+ compartmentalization (Tian et al., 2010). SOS1, plasmalemma Na^+/H^+ antiporter, transport Na^+ out of the cell; HKT, high affinity K^+ transporter, transports Na^+ from stems to xylem; NHX, sodium-hydrogen exchanger, transports Na^+ into the vacuole (Apse et al., 1999; Shi et al., 2002). In this study, Na^+ influx was observed in roots treated by salt, which was repressed by the addition of thymol. This may due to the activation of *NtSOS1* by thymol. Thymol also induced the expression of *NtHKT1* that is responsible for the tissue distribution of Na^+, which may also contribute to the decrease in the total Na^+ content in roots. K^+ in tobacco seedlings were not affected significantly.

Thus, thymol seems to maintain Na^+/ K^+ balance likely through the regulation of Na^+ transportation. In addition, *NtNHX1* was induced by thymol, which may help root cells compartmentalize Na^+ inside of vacuole to avoid Na^+ toxicity in cytosol. However, the subcellular distribution of Na^+ in thymol-treated tobacco seedlings needs further investigation.

4. Conclusion

In summary, application of thymol not only increased the NO and GSH content to scavenge the excessive accumulated ROS, but also decreased the Na^+ content to relieve ion toxicity in tobacco under salt stress. Finally, the action of thymol maintains the stability of tobacco seedling membrane system and reduced cell death to resist salt stress (Fig. 6). The detailed molecular mechanisms are still elusive, but these results provide a certain understanding of the physiological functions of thymol related to salt tolerance.

5. Materials And Methods

5.1. Plant culture, treatment, and chemicals

The tobacco seeds（K326）were washed for 10 times by using distilled water and sown on the surface of the culture medium in the petri dish after being disinfected with 0.2% potassium permanganate solution for 30 minutes. (The tobacco seeds were provided by Yuxi Zhongyan Limited liability company.) They were cultivated for 6-7 days in plant growth cabinet with a light intensity of 5000Lux, an air relative humidity of 50%, a photoperiod of 12 h, and the temperature at 26℃. Then the seedlings with a root length of 0.5 cm were selected and transplanted in the new petri dish with NaCl (0- 200 mM) and thymol (0- 200μM) alone or their combinations, for up to 72 h. The root length was measured every 12 h. After 72 h incubation, the plants were harvested for histochemical, physiological, and biochemical analysis.

5.2. Histochemical detection

The DAB, NBT, Shiff's reagent and trypan blue were used for the detection of H_2O_2, , lipid peroxidation and cell death in roots and leaves, according to our previous publication (Ye et al., 2016).

For the detection of indicators in roots, the 6-day-old seedlings treated for 72 h were transferred to different solutions at 25 ℃ for 20 minutes under light. Then the roots were washed with deionized water until apical discoloration was observed, and the root tip was photographed under stereoscopic microscope (SteREO Discovery.V8, ZEISS, Oberkochen, Germany).

The seedings were excised at the base of the stem and supplied DAB, NBT, trypan blue or Shiff's reagent for 6 h to indicated H_2O_2, content, lipid peroxidation and cell death in leaves. Then the leaves were washed and boiled with 95% alcohol for 20 minutes and photographed with stereomicroscope.

5.3. Determination of H_2O_2, MDA, GSH, NO content and enzymatic activity

The harvested plants were collected and ground to powder in a mortar containing liquid nitrogen by spectrophotometric method for the detection of H_2O_2 (hydrogen peroxide), MDA (malondialdehyde), reduced GSH (glutathione), POD (peroxidase), SOD (superoxide dismutase) and CAT (catalase). All kits were manufactured by the Suzhou Comin Biotechnology Co.Ltd, China. The subsequent measurement and data analysis were conducted following the operational manuals of the kits. The amount of NO was analyzed using DAF-FM DA staining and confocal laser scanning microscope and the kits were manufactured by the Beyotime Biotechnology Co.Ltd, China.

5.4 K^+ and Na^+ flux assays

The 6-day-old seedlings treated for 12 h were used for the K^+ and Na^+ determination. K^+ and Na^+ efflux in tobacco leaves and roots were detected using non-invasive micro-test technology (NMT; Younger LLC) as previously described (Chen et al., 2015).

5.5 Gene expression analysis

Total RNA extracted from root tips was used to analyze the relative gene expression level by using qRT-PCR (real-time quantitative reverse transcription polymerase chain reaction) according to our previous publication (Norling et al., 2017). Data were collected and analyzed by using ABI 7500 software (v. 2.0.6, Applied Biosystems) based on $2^{-\Delta\Delta CT}$ threshold cycle method. Primers for each gene in this study were presented in Table S1.

5.6 Statistical analysis

The mean ± standard deviation (SD) of ten replications was selected to present the data. After ANOVA, Student's t-test were used to identify significant differences ($P < 0.05$) among treatment means. LSD (least significant difference test) was used to test for significant difference ($P < 0.05$) among any different treatments in one experiment.

Abbreviations

ROS
Reactive oxygen species
NO
Nitric oxide
GSH
Glutathione
MS
Murashige & Skoog
DAB
Diaminobenzidine
NBT

Nitro blue tetrazolium chloride
H2O2
Hydrogen peroxide
MDA
Malondialdehyde
POD
Peroxidase
SOD
Superoxide dismutase
CAT
Catalase
DAF-FM DA
3-Amino,4-aminomethyl-2',7'-difluorescein, diacetate
GSNO
S-nitrosoglutathione
ASA
Ascorbic acid
GSH
Glutathione

Declarations

Availability of data and materials

All of the data and materials supporting our research findings are contained in the methods section of the manuscript. Details are provided in the attached Additional files.

Acknowledgements

We thank Jian Chen (Institute of Food Quality and Safety , Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China) for providing help in the experiments.

Authors' contributions

XFY, JW PW and LX conceived the research; JQS and YLW prepared the plant materials; XHL and XLL performed the experiments and data analysis; BZ and AJL conducted the data analysis. LX wrote the manuscript; XFY revised the manuscript. All authors have read and approved the manuscript.

Funding

This research was financially supported by the National Natural Science Foundation of China (41907076). The funding bodies played no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Declarations

Ethics approval and consent to participate.Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

Supplementary material

Primers used for qRT-PCR analysis (Table S1).

References

Alam, K., Nagi, M. N., Badary, O. A., Al-Shabanah, O. A., Al-Rikabi, A. C., Al-Bekairi, A. M., 1999. The protective action of thymol against carbon tetrachloride hepatotoxicity in mice. Pharmacol Res. 40, 159–63.

Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol. 55, 373–99.

Apse, M. P., Aharon, G. S., Snedden, W. A., Blumwald, E., 1999. Salt tolerance conferred by overexpression of a vacuolar Na+/H+ antiport in Arabidopsis. Science. 285, 1256–8.

Bai, X., Yang, L., Yang, Y., Ahmad, P., Yang, Y., Hu, X., 2011. Deciphering the protective role of nitric oxide against salt stress at the physiological and proteomic levels in maize. J Proteome Res. 10, 4349–64.

Boudsocq, M., Lauriere, C., 2005. Osmotic signaling in plants: multiple pathways mediated by emerging kinase families. Plant Physiol. 138, 1185–94.

Castillo, S., Pérez-Alfonso, C., Martínez-Romero, D., Guillén, F., Serrano, M., Valero, D., 2014. The essential oils thymol and carvacrol applied in the packing lines avoid lemon spoilage and maintain quality during storage. Food Control. 35, 132–136.

Chen, J., Wang, W. H., Wu, F. H., He, E. M., Liu, X., Shangguan, Z. P., Zheng, H. L., 2015. Hydrogen sulfide enhances salt tolerance through nitric oxide-mediated maintenance of ion homeostasis in barley seedling roots. Sci Rep. 5, 12516.

Dong-xue, L., 2005. World Resources of Saline Soil and Main Amelioration Measures. Research of Soil and Water Conservation.

Flowers, T. J., 2004. Improving crop salt tolerance. J Exp Bot. 55, 307–19.

Gill, S. S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 48, 909–30.

González-Aguilar, G., Ansorena, M. R., Viacava, G., Roura, S., Ayala-Zavala, J. F., Plant Essential Oils as Antifungal Treatments on the Postharvest of Fruit and Vegetables. 2013.

Guarda, A., Rubilar, J. F., Miltz, J., Galotto, M. J., 2011. The antimicrobial activity of microencapsulated thymol and carvacrol. Int J Food Microbiol. 146, 144–50.

Hernandez, M., Fernandez-Garcia, N., Diaz-Vivancos, P., Olmos, E., 2010. A different role for hydrogen peroxide and the antioxidative system under short and long salt stress in Brassica oleracea roots. J Exp Bot. 61, 521–35.

Ischiropoulos, H., Al-Mehdi, A., 1995. Peroxynitrit… mediated oxidative protein modifications. FEBS Letters. 364.

Jia, F., Wang, C., Huang, J., Yang, G., Wu, C., Zheng, C., 2015. SCF E3 ligase PP2-B11 plays a positive role in response to salt stress in Arabidopsis. J Exp Bot. 66, 4683–97.

Kovtun, Y., Chiu, W. L., Tena, G., Sheen, J., 2000. Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc Natl Acad Sci U S A. 97, 2940–5.

Miller, G., Suzuki, N., Ciftci-Yilmaz, S., Mittler, R., 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ. 33, 453–67.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–10.

Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 59, 651–81.

Noctor, G., Mhamdi, A., Chaouch, S., Han, Y., Neukermans, J., Marquez-Garcia, B., Queval, G., Foyer, C. H., 2012. Glutathione in plants: an integrated overview. Plant Cell Environ. 35, 454–84.

Norling, L. V., Ly, L., Dalli, J., 2017. Resolving inflammation by using nutrition therapy: roles for specialized proresolving mediators. Curr Opin Clin Nutr Metab Care. 20, 145–152.

Radi, R., 2004. Nitric oxide, oxidants, and protein tyrosine nitration. Proc Natl Acad Sci U S A. 101, 4003–8.

Romero-Puertas, M. C., Sandalio, L. M., 2016. Nitric Oxide Level Is Self-Regulating and Also Regulates Its ROS Partners. Front Plant Sci. 7, 316.

Shi, H., Quintero, F. J., Pardo, J. M., Zhu, J. K., 2002. The putative plasma membrane Na(+)/H(+) antiporter SOS1 controls long-distance Na(+) transport in plants. Plant Cell. 14, 465–77.

Suzuki, N., Koussevitzky, S., Mittler, R., Miller, G., 2012. ROS and redox signalling in the response of plants to abiotic stress. Plant Cell Environ. 35, 259–70.

Tian, H., Baxter, I. R., Lahner, B., Reinders, A., Salt, D. E., Ward, J. M., 2010. Arabidopsis NPCC6/NaKR1 is a phloem mobile metal binding protein necessary for phloem function and root meristem maintenance. Plant Cell. 22, 3963–79.

Van Breusegem, F., Dat, J. F., 2006. Reactive oxygen species in plant cell death. Plant Physiol. 141, 384–90.

Xie, Y. J., Xu, S., Han, B., Wu, M. Z., Yuan, X. X., Han, Y., Gu, Q., Xu, D. K., Yang, Q., Shen, W. B., 2011. Evidence of Arabidopsis salt acclimation induced by up-regulation of HY1 and the regulatory role of RbohD-derived reactive oxygen species synthesis. Plant J. 66, 280–92.

Ye, X., Tianxiao, L., Yanfeng, X., Cunfa, X., Wei, Z., Liangbin, H., Jian, C., Zhiqi, S., 2016. Thymol Mitigates Cadmium Stress by Regulating Glutathione Levels and Reactive Oxygen Species Homeostasis in Tobacco Seedlings. Molecules. 21.

Zhang, H., Han, B., Wang, T., Chen, S., Li, H., Zhang, Y., Dai, S., 2012. Mechanisms of plant salt response: insights from proteomics. J Proteome Res. 11, 49–67.

Zhu, J. K., 2001. Plant salt tolerance. Trends Plant Sci. 6, 66–71.

Zhu, J. K., 2016. Abiotic Stress Signaling and Responses in Plants. Cell. 167, 313–324.

Figures

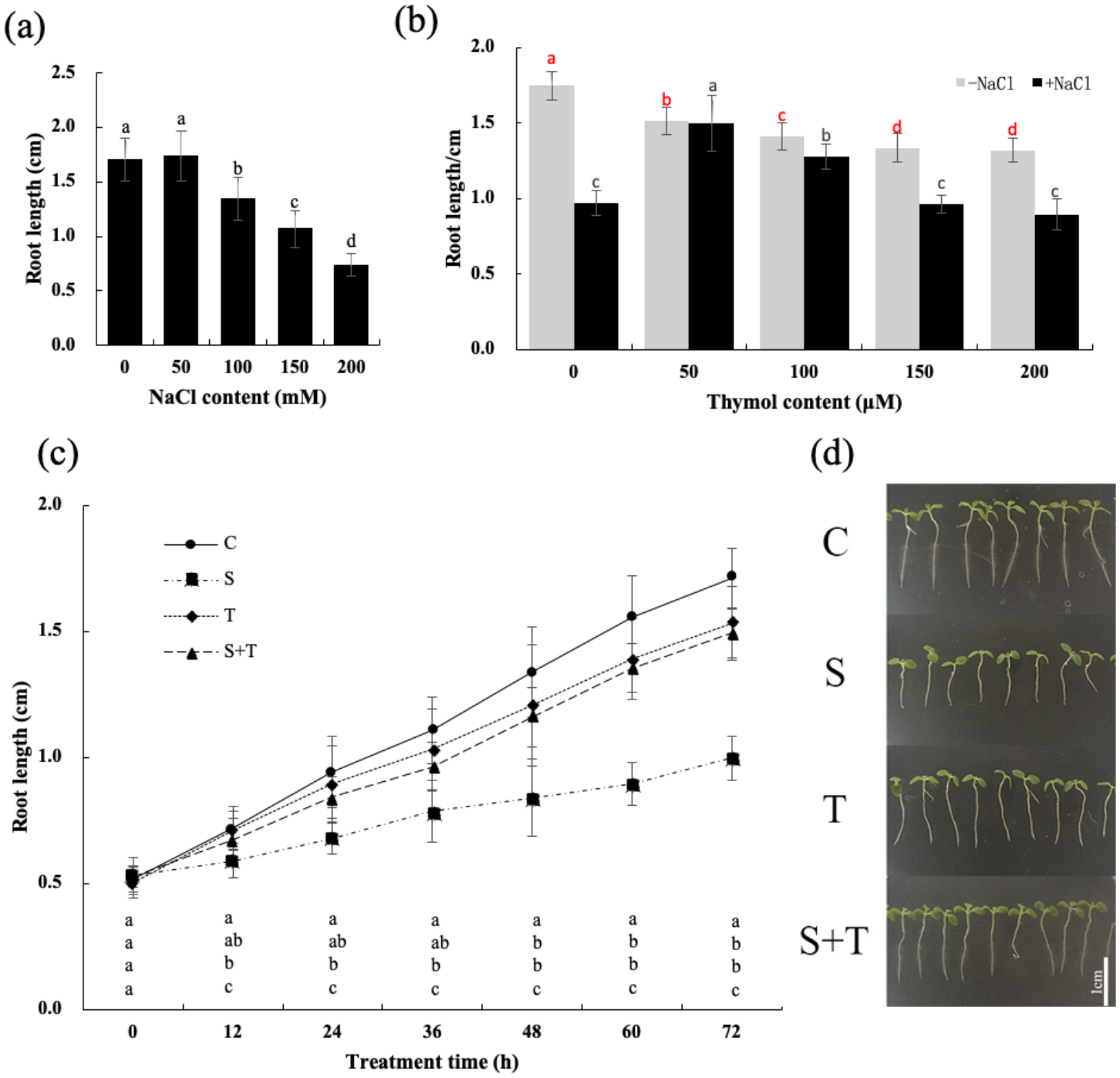

Figure 1

The effect of NaCl and thymol on the growth of tobacco seedings. (a) The 6-day-old tobacco seedings were transferred to plates containing 0-200 mM NaCl for the measurement of root length after 72 h. (b) The seedings were treated with 0-200 μM thymol and 150 mM NaCl for 72 h before the measurement of root length. (c) Tobacco seedings were treated with water (C), 150 mM NaCl (S), 50 μM thymol (T) or the mixture of them (S+T) separately for 72 h. The root length was measured each 12 h. (d) The seedings

were photographed after 72-h treatment. Different letters indicate the significantly different between the treatments ($p < 0.05$, ANOVA, LSD).

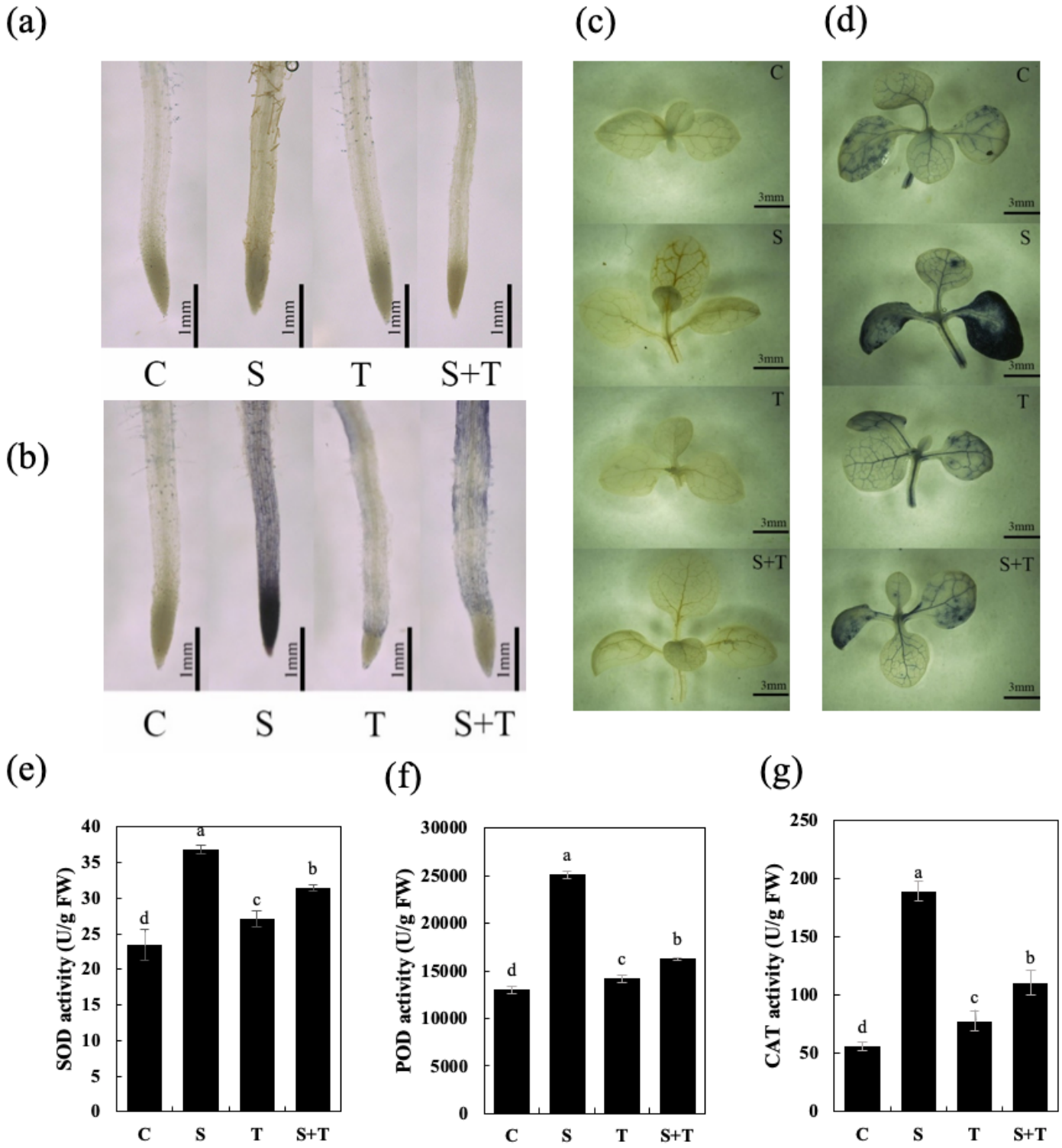

Figure 2

The effect of thymol on the accumulation of ROS in tobacco leaves and roots under salt stress. Tobacco seedings were treated with water (C), 150 mM NaCl (S), 50 μM thymol (T) or the mixture of them (S+T) separately for 72 h. The roots were stained with DAB (a) or NBT (b) for 20 minutes to indicated H2O2 and

O_2^(•-) content, respectively. The seedings were supplied DAB (c) or NBT (d) for 6 h to indicated H2O2 and O_2^(•-) content in leaves. The activity of (e) SOD, (f) POD and (g) CAT. Different lowercase letters in e-g, indicated that the mean values of three replicates were significantly different between the treatments (P< 0.05, ANOVA, LSD).

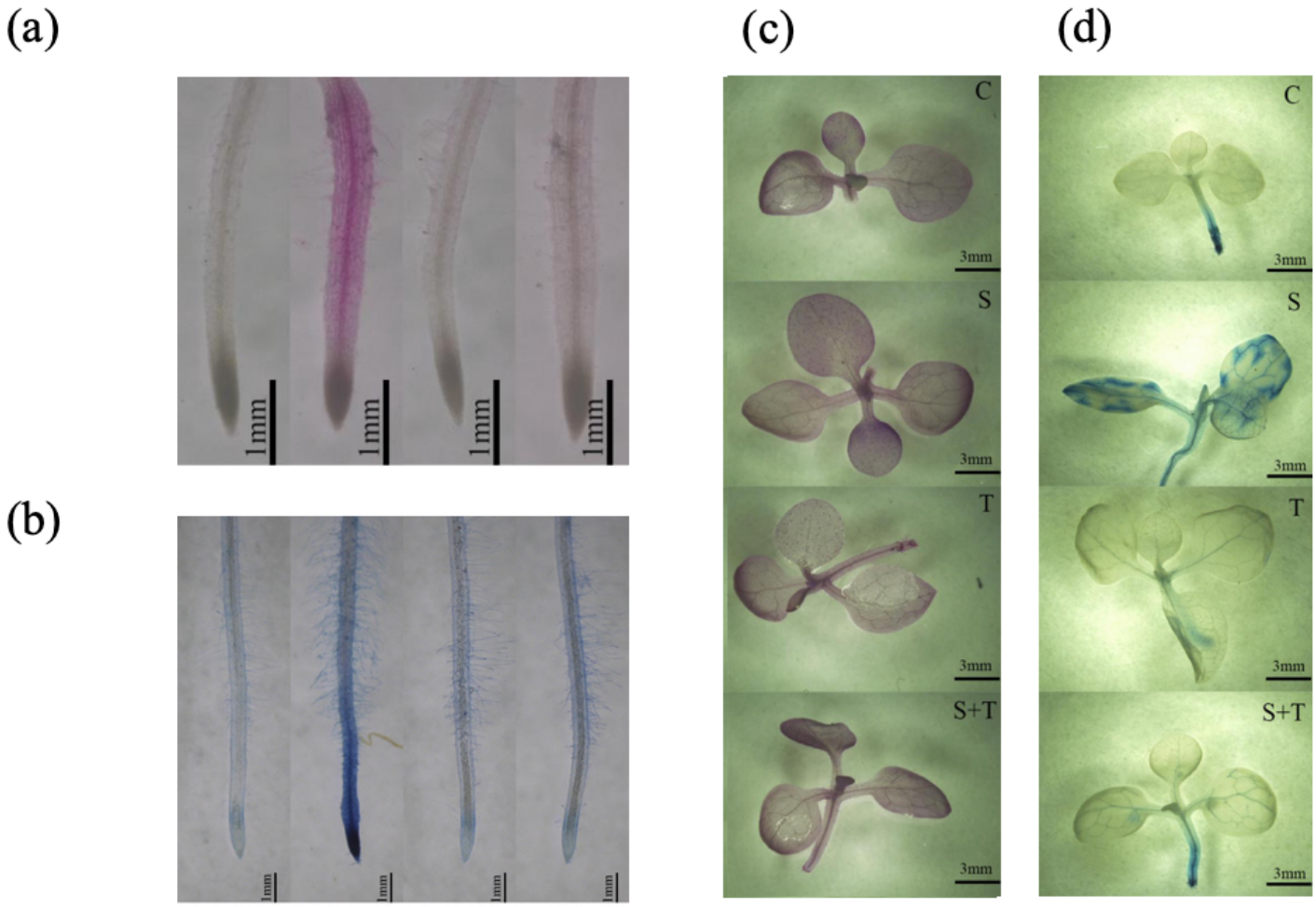

Figure 3

The effect of thymol on the lipid peroxidation and cell death in tobacco leaves and roots under salt stress. Tobacco seedings were treated with water (C), 150 mM NaCl (S), 50 μM thymol (T) or the mixture of them (S+T) separately for 72 h. The roots were stained with Shiff's Reagent (a) or Trypan Blue (b) for 20 minutes to indicated lipid peroxidation and cell death, respectively. The seedlings were supplied with Shiff's Reagent (c) or Trypan Blue (d) for 6 h to indicated lipid peroxidation and cell death in leaves.

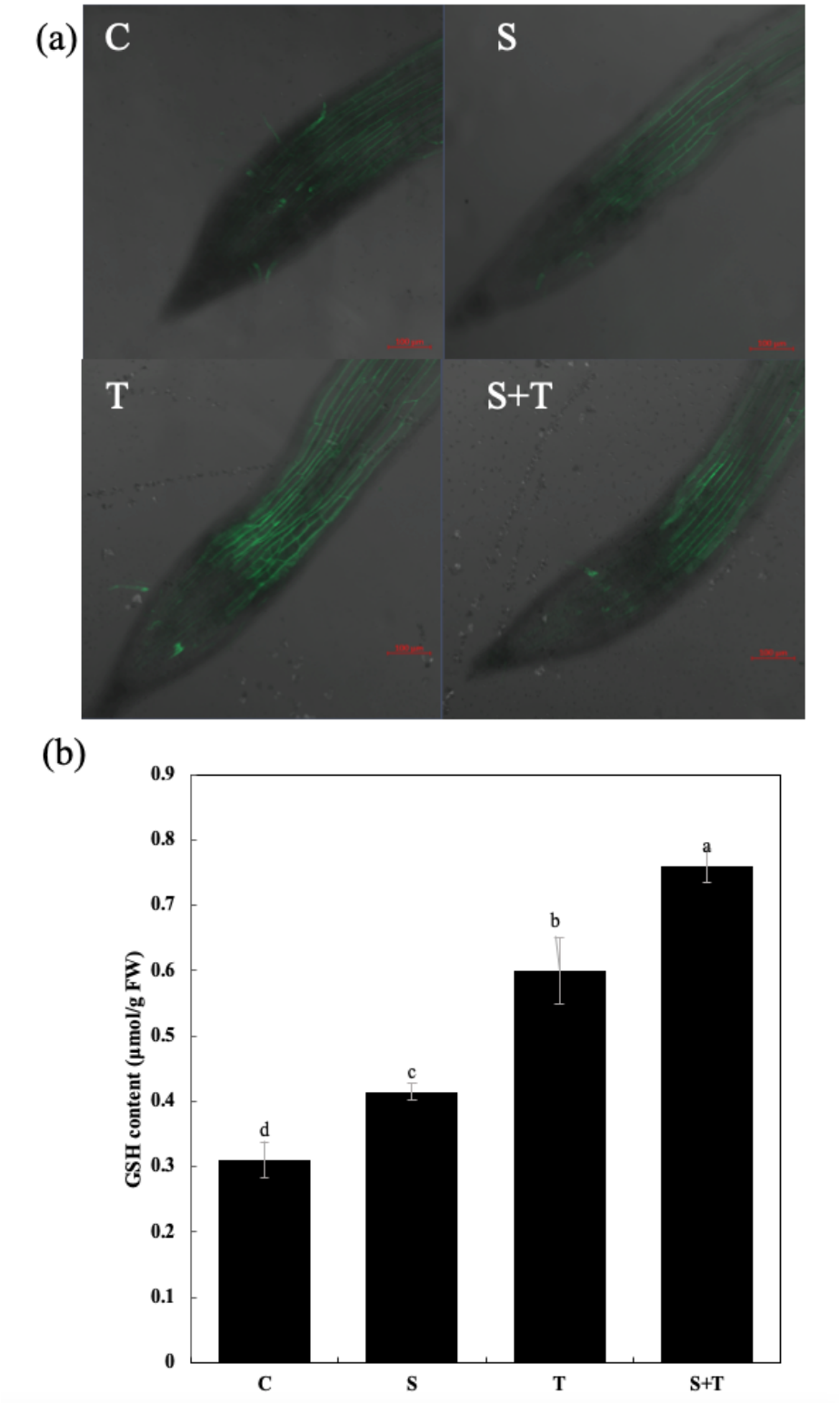

Figure 4

Effect of thymol on NO and antioxidant system in tobacco seedlings under salt stress. DAF-FM DA fluorescence indicating total NO level (a). (b) GSH content.. Different lowercase letters in b, indicated that the mean values of three replicates were significantly different between the treatments (P< 0.05, ANOVA, LSD).

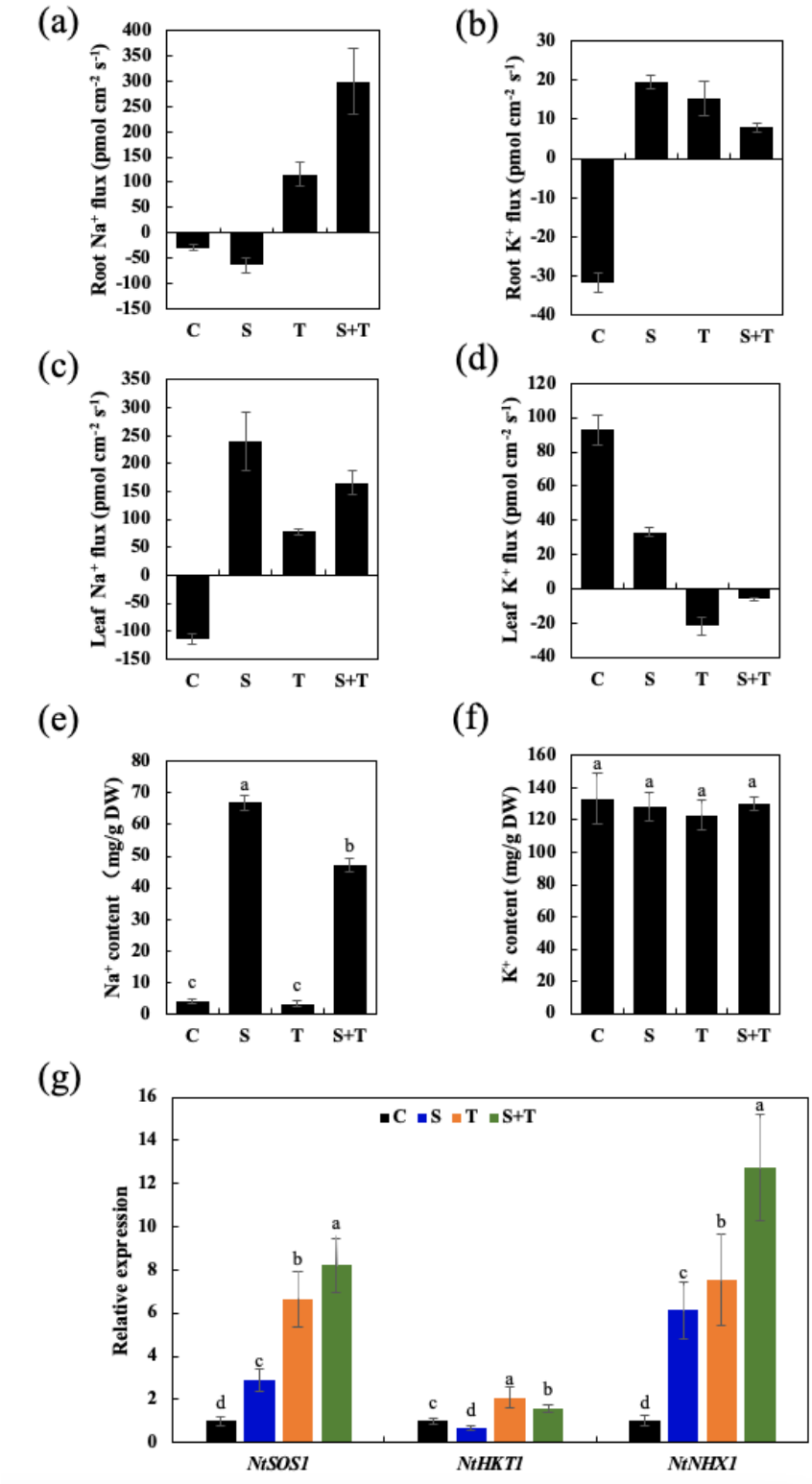

Figure 5

Effect of thymol on Na+ and K+ content and the expression of Na+ transporter genes in tobacco seedlings under salt stress. Na+ and K+ flux in tobacco roots and leaves (a-d). Na+ and K+ content in tobacco seedlings (e and f). Relative expression level of NtSOS1, NtHKT1 and NtNHX1 (g). Different lowercase letters indicated that the mean values of three replicates were significantly different between

the treatments (P< 0.05, ANOVA, LSD). The expression levels of NtSOS1, NtSOS1 and NtSOS1 in control were defined as “1”. Data are means ± SE (n=3).

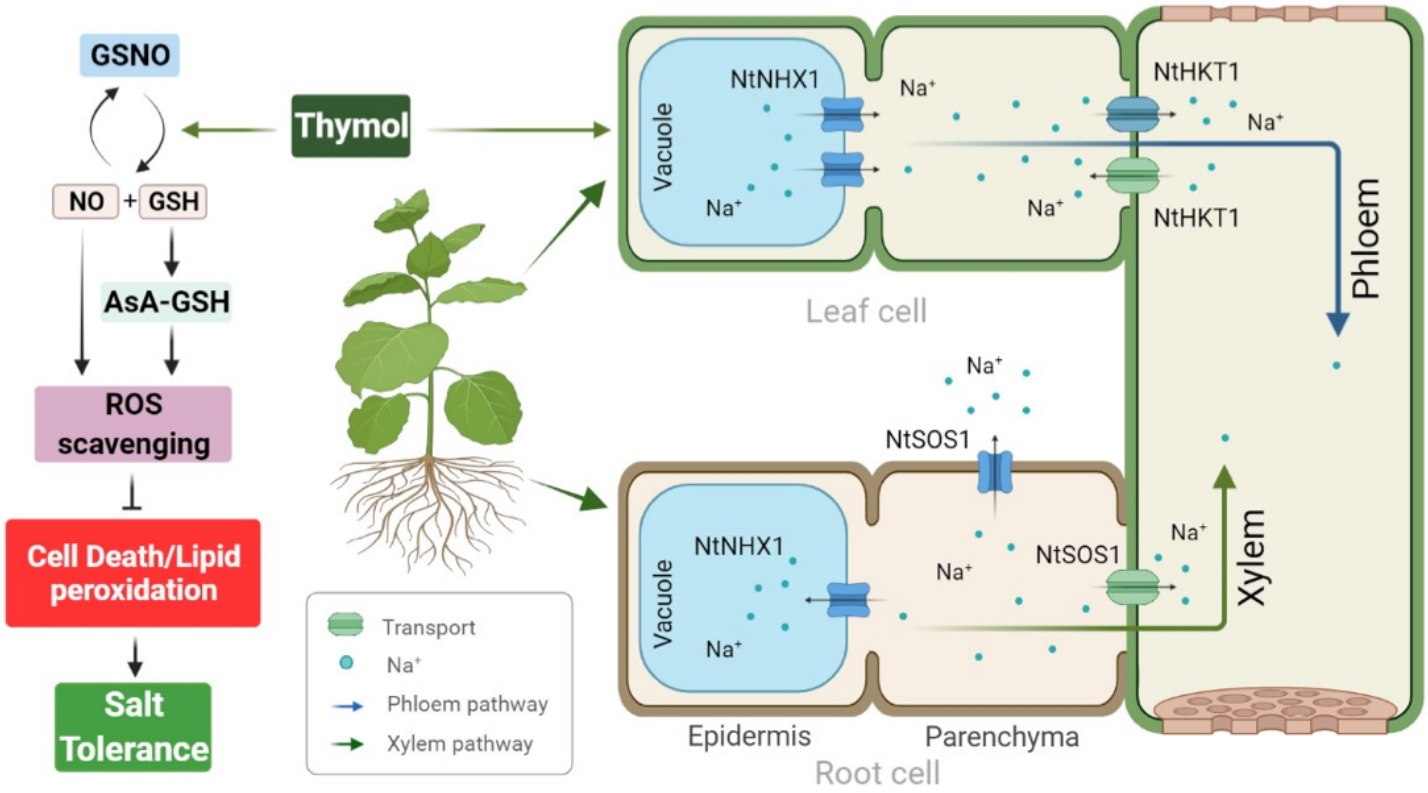

Figure 6

Schematic model for thymol-induced salt tolerance in tobacco seedlings. Basically, thymol mainly deploys two strategies to increase the salt tolerance of tobacco seedlings. First of all, thymol promoted the scavenging of ROS by promoting the decomposition of GSNO and increasing the content of NO and GSH. Secondly, activate NtNHX1 to stimulate vacuole retention of Na+, activate NtSOS1 to promote Na+ rejection, and activate NtHKT1 to promote the transfer of Na+ to xylem and phloem, thus helping to reduce the level of Na+ in leaves and roots. (Created with BioRender.com)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- S1qRTPCR.xlsx
- FigS1.png
- Totaldata.xlsx