Pak. J. Bot., 54(3): 911-916, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-3(4)

PHYTOSTEROL ALTERATIONS RESPONDING TO ROS INHIBITORS BY GC-MS IN *ARABIDOPSIS*

YUANYUAN WANG[1,2†], TIANLIN JIN[1,2†], XUE WANG[1,2] AND DACHENG LIANG[1,2*]

[1]*Engineering Research Center of Ecology and Agricultural Use of Wetland, Ministry of Education/Hubei Key Laboratory of Waterlogging Disaster and Wetland Agriculture, Jingzhou, Hubei 434025,China*
[2]*Hubei Collaborative Innovation Center for Grain Industry, Jingzhou, Hubei 434025, China*
[†]*These authors contributed equally to this work*
[*]*Corresponding author's email: dachengliang@yangtzeu.edu.cn; Tel: +86 13343510740; Fax: +86 716 8060813*

Abstract

Phytosterols, essential components of cellular membrane integrity, play an important role in plant growth, development, and responses to various types of stressors. Emerging studies have proposed that phytosterols are a result of adaptation to the aerobic environment during evolution. We reasoned that phytosterols could dramatically respond to changes in external reactive oxygen species (ROS) levels. The levels of five phytosterols were measured by gas chromatography-mass spectrometry (GC-MS) after *Arabidopsis* plants were treated with the ROS-altering drugs 2'-3'-dideoxycytidine (DDC), H_2O_2, Catalase (CAT), diphenyleneiodonium (DPI), and salicylhydroxamic acid (SHAM) and auxin transport inhibitors 2,3,5-triiodobenzoic acid (TIBA) and 1-N-naphthylphthalamic acid (NPA). We found that all redox-altering agents can dramatically reduce sterol levels, whereas CAT and NPA can partly increase them. Our results establish a link between redox balance and sterol level alteration.

Key words: ROS; ROS inhibitors; Sterol; Lipid rafts.

Introduction

Sterols, a type of lipid, are components of eukaryotic membranes, and have been suggested to maintain the domain structure of cell membranes by acting as membrane reinforcers (Dufourc, 2008). Together with sphingolipids (e.g., sphingomyelin and glycosphingolipids) and associated proteins, sterols are usually organized into liquid-ordered lipid domains, known as lipid rafts, which are thought to play an important role in fundamental biological processes, such as signal transduction, cellular trafficking, polarized secretion, membrane transport, and cytoskeleton reorganization (Simons & Ehehalt, 2002; Helms & Zurzolo, 2004; Bieberich, 2018). In terms of structure, cholesterol and ergosterol are the lipids enriched in animal and fungal cell membranes, respectively; however, their plant counterparts display a much greater structural variability. In addition to cholesterol, many cholesterol analogues in plants, such as campesterol, stigmasterol, and β-sitosterol, have been identified from detergent-resistant membranes, which are supposedly equivalent to lipid rafts (Mongrand *et al.*, 2004; Borner *et al.*, 2005). Plant species possess more than 250 different sterols (Akihisa *et al.*, 1991), but campesterol, stigmasterol, and β-sitosterol are the dominant constituents of plant sterol profiles, implying that fluctuation in the levels of these major sterols can affect the physical function of lipid rafts.

The specific functions of each sterol are largely unknown. From the viewpoint of evolution, the advent of sterols is teleologically related to the increasing concentrations of oxygen that occurred around 2.5 billion years ago (Galea & Brown, 2009). Therefore, sterols may be considered to have been part of a primitive cellular defense system against oxygen and ROS (ROS). Indeed, lipid rafts that are composed of phytosteorols work as an important redox signaling platform for the assembly of the NADPH oxidase complex and activation of downstream redox regulation of cell functions (Jin *et al.*, 2011). In plants, lipid rafts also contain NADPH oxidase (Mongrand *et al.*, 2004) and cytosolic NADH-ubiquinone oxidoreductases (Lefebvre *et al.*, 2007). The presence of these conserved components in lipid rafts strongly suggests that redox signaling is associated with the physical function of lipid rafts. The objective of the study was to examine the effects of several ROS-altering agents on the levels of five phytosterol compounds compared with the control samples.

Material and Methods

Plant materials and growth condition: *Arabidopsis thaliana* Col (Columbia wild type) was grown on a vertical plate containing 1X MS medium in a greenhouse for 7 days at 22°C under long days (15 h light and 9 h dark cycles) with white light illumination. Seven-day-old plants were transferred into a medium containing chemical compounds (DDC, H_2O_2, SHAM, DPI, CAT, TIBA, and NPA) for 7 days in a greenhouse under the same growing conditions. The dosages of these drugs are indicated in Table 1.

Phytosterol extraction and GC-MS analysis: Sterol extraction was performed according to the method described by Zhang et al. (2017) with some modifications. Freeze-dried samples (20 mg) were ground in liquid nitrogen and extracted with 4 mL of chloroform and methanol mixture (2:1, v/v), and then filtered through a rephiquik syringe filter (PVDF, 0.2 μm). The filtered mixture was further incubated at 70 °C for 1 h and dried using a vacuum freeze drier. 5α-cholestane (12.5 μL of 2 mg/mL) was added to the mixture as an internal standard. The samples were saponified with 2 mL 6% (w/v) KOH in methanol for 3 h at 90°C to release the sterol moiety of the steryl ester. Sterols were extracted three times with 2 mL of hexane and water (1:1) and dried using a vacuum freeze drier. The dried residues were derived with 100 μL BSTFA-TMCS (99:1) for 25 min at 75°C, and the volume

of the mixture was brought up to 500 µL with N-hexane. Sterol level was analyzed on DB-5 MS column (30 m × 0.25 mm × 0.25 µm, Agilent technologies) with helium as carrier gas. The temperature program was as follows: 100°C for 5 min., ramped to 280°C at 10°C/min., 300°C at 5°C/min., and held for 20 min (Zhang *et al.*, 2017).

Results

Seven-day-old *Arabidopsis thaliana* (Col) seedlings were treated with the ROS-altering drugs 2'-3'-dideoxycytidine (DDC), H_2O_2, CAT, diphenyleneiodonium (DPI), and salicylhydroxamic acid (SHAM), along with auxin transport inhibitors 2,3,5-triiodobenzoic acid (TIBA) and 1-N-naphthylphthalamic acid (Table 1), and the growth phenotype was usually obvious after 7 days (Fig. 1). We first tested the effect of H_2O_2 on sterol levels, given that it is a strong oxidant that is capable of oxidizing lipids and impacting membrane permeability (Floyd & Lewis, 1983; Bienert *et al.*, 2007; Ouchi *et al.*, 2019). We found that it significantly reduced campesterol and stigmasterol levels by 41% and 47%, respectively, relative to their levels in untreated plants, but slightly increased cholesterol level (Fig. 2A). These results prompted us to investigate whether the external reduction of H_2O_2 could increase sterol levels. A significant increase in cholesterol and campesterol levels when CAT was added to the growth medium (Fig. 2). Interestingly, the brassicasterol level was also greatly increased (more than three-fold) compared to that in untreated plants. Therefore, the presence of CAT, an antioxidant enzyme that scavenges H_2O_2 by dismutating it into molecular oxygen and water (Nicholls, 2012), can improve sterol levels.

We then sought to examine the effects of the superoxide dismutase inhibitor DDC (Maître *et al.*, 1993), a peroxidase inhibitor SHAM (Kukavica *et al.*, 2012), and a plasma membrane NADPH oxidase inhibitor, DPI (Bolwell *et al.*, 1998) on sterol level alterations. Plants treated with DDC had much less campesterol, stigmasterol, and β-sitosterol, but there was no obvious effect on cholesterol and brassicasterol levels (Fig. 3). SHAM strongly reduced the sterol content; in particular, cholesterol and brassicasterol were decreased to approximately 44% and 15% of basal levels, respectively. DPI also reduced all sterol levels except those of brassicasterol, with the greatest impact on cholesterol levels (decreased by 71%).

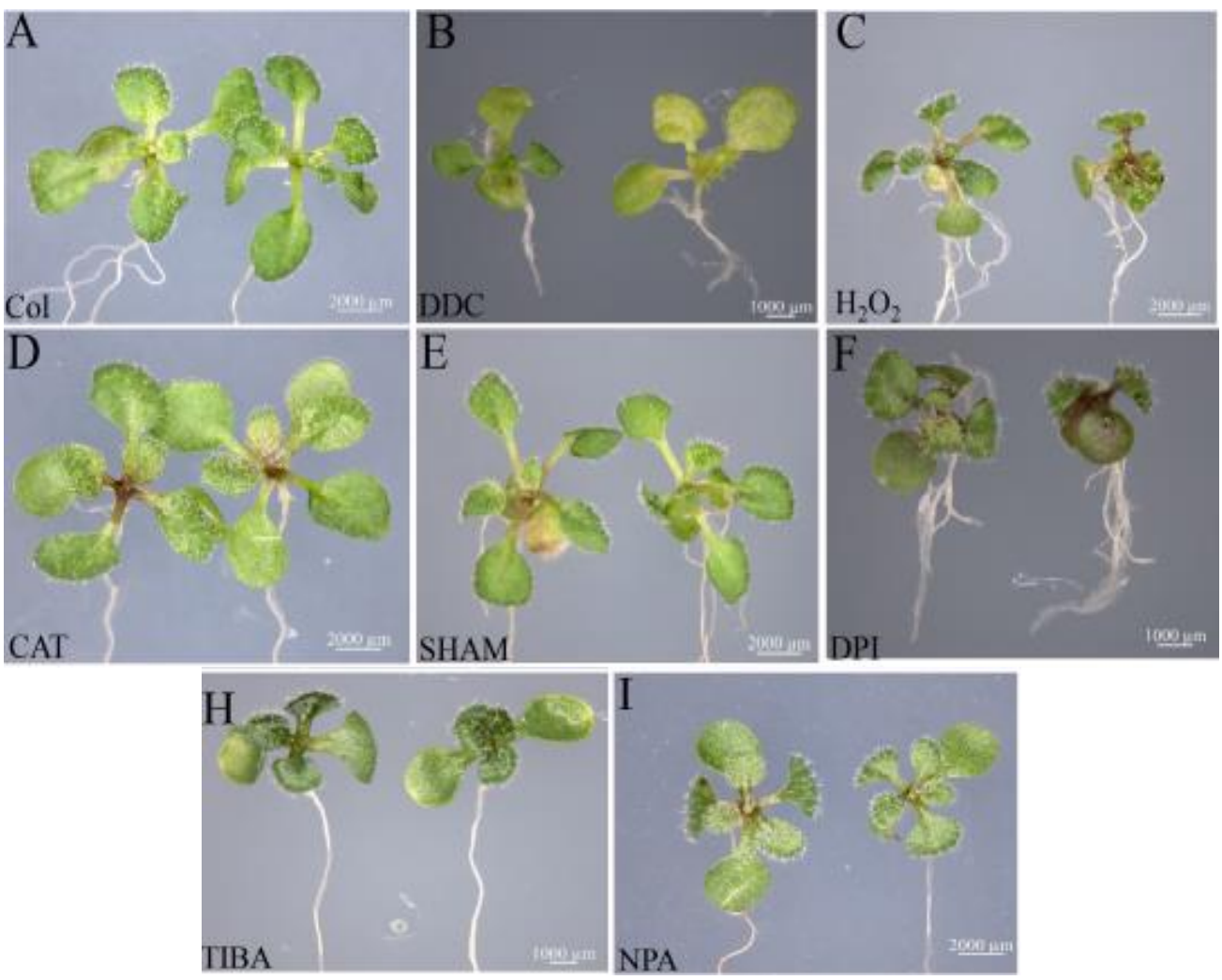

Fig. 1. The phenotypic changes in *Arabidopsis thaliana* (Col) treated with different drugs. A, *Arabidopsis* plants on drug-free medium. B, 14-day old *Arabidopsis* plants treated with DDC. C, 14-day old *Arabidopsis* plants treated with H_2O_2. D, 14-day old *Arabidopsis* plants treated with CAT. E, 14-day old *Arabidopsis* plants treated with SHAM. F, 14-day old *Arabidopsis* plants treated with DPI. G, 14-day old *Arabidopsis* plants treated with TIBA. H, 14-day old *Arabidopsis* plants treated with NPA.

Table 1. Various inhibitors used in this study.

Abbreviation name	Full name	Final concentration	Effect	reference
H_2O_2	Hydrogen peroxide	3 mM	Strong oxidant	(Floyd & Lewis, 1983; Bienert *et al.*, 2007; Ouchi *et al.*, 2019)
CAT	Catalase	100 μg/ml	Antioxidant enzyme to scavenge H_2O_2	(Nicholls, 2012)
DDC	Diethyldithiocarbamic acid	10 mM	Superoxide dismutase inhibitor	(Maître *et al.*, 1993)
SHAM	Salicylhydroxamic acid	100 μM	Peroxidase inhibitor	(Kukavica *et al.*, 2012)
DPI	Diphenyleneiodonium	1 μM	NADPH oxidase inhibitor	(Bolwell *et al.*, 1998)
NPA	N-l-naphthylphthalamic acid	100 μM	Polar auxin transport inhibitor	(Kliimbt, 1992)
TIBA	2,3,5-triiodobenzoic acid	50 μM	Polar auxin transport inhibitor	(Choi *et al.*, 1997)

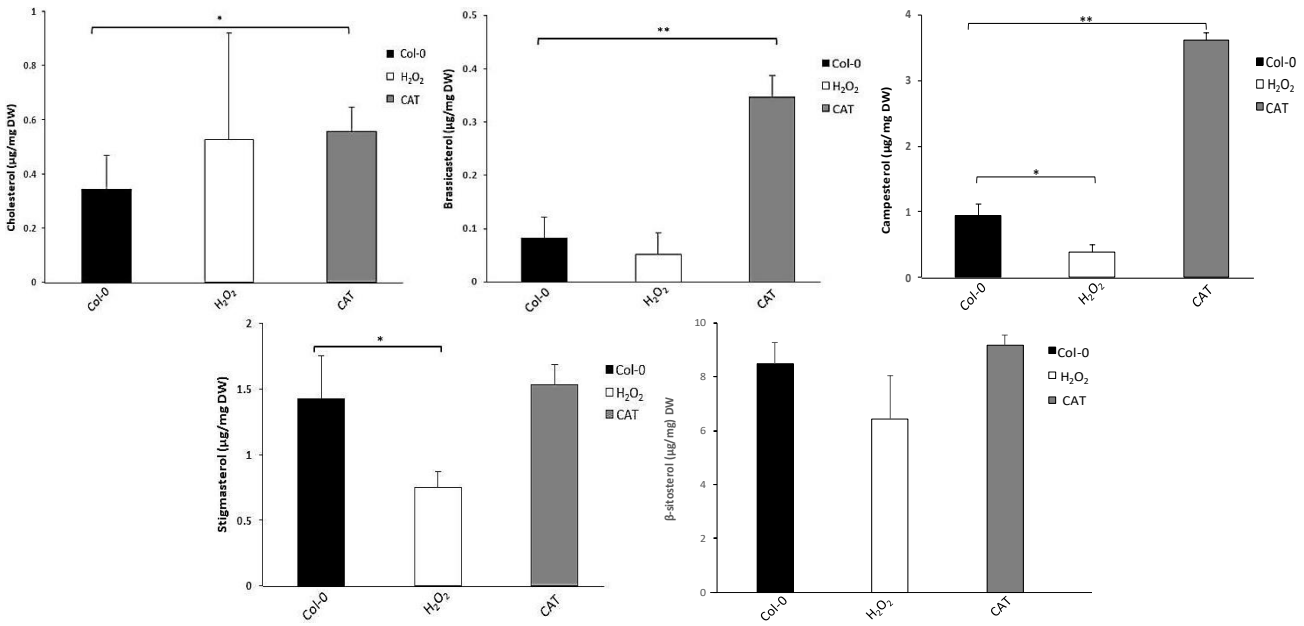

Fig. 2. Measurement of phytosterol levels after treatment with H_2O_2 and CAT. Col refers to the untreated control group. The content of cholesterol (A), brassicasterol (B), campesterol (C), stigmasterol (D), β-sitosterol (E) was measured under H_2O_2 and CAT treatment conditions. Error bars represent standard deviation over three independent experiments; the data were evaluated by Student's t-test to determine statistical significance. *: $p<0.05$; **: $p<0.01$.

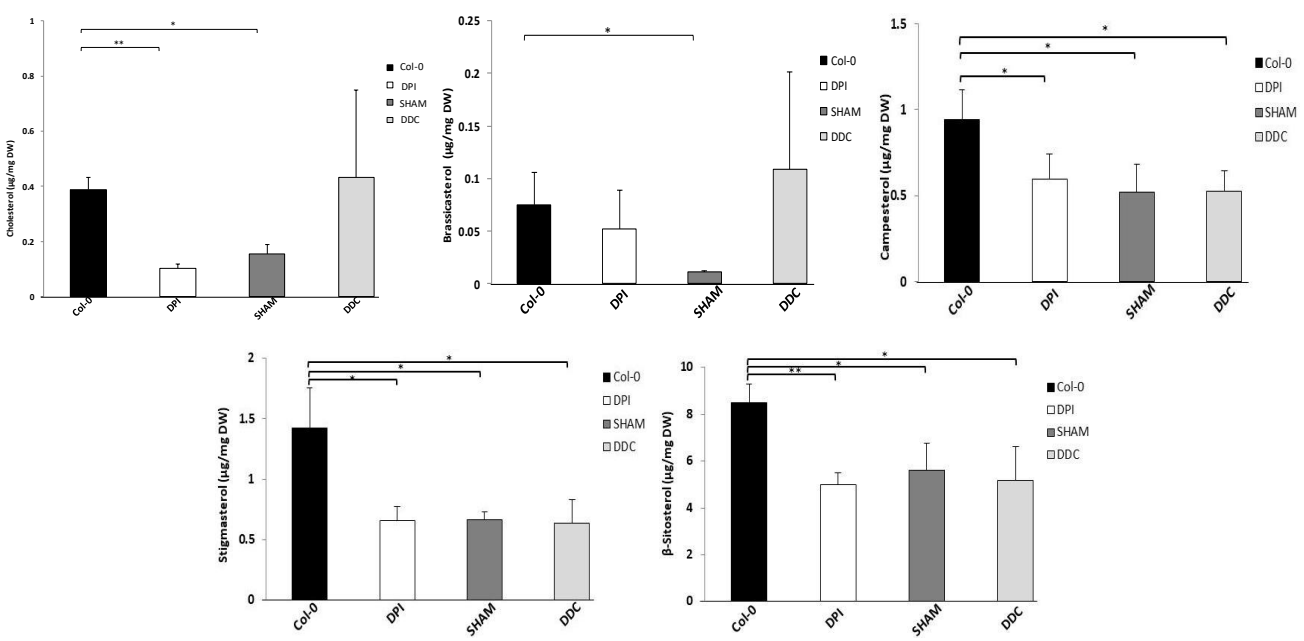

Fig. 3. Phytosterol level under DPI, SHAM, and DDC treatment. Col refers to the untreated control group. The contents of cholesterol (A), brassicasterol (B), campesterol (C), stigmasterol (D), and β-sitosterol (E) were measured under DPI, SHAM, and DDC treatment conditions. Error bars represent standard deviation over three independent experiments; the data were evaluated by Student's t-test to determine statistical significance. *: $p<0.05$; **: $p<0.01$.

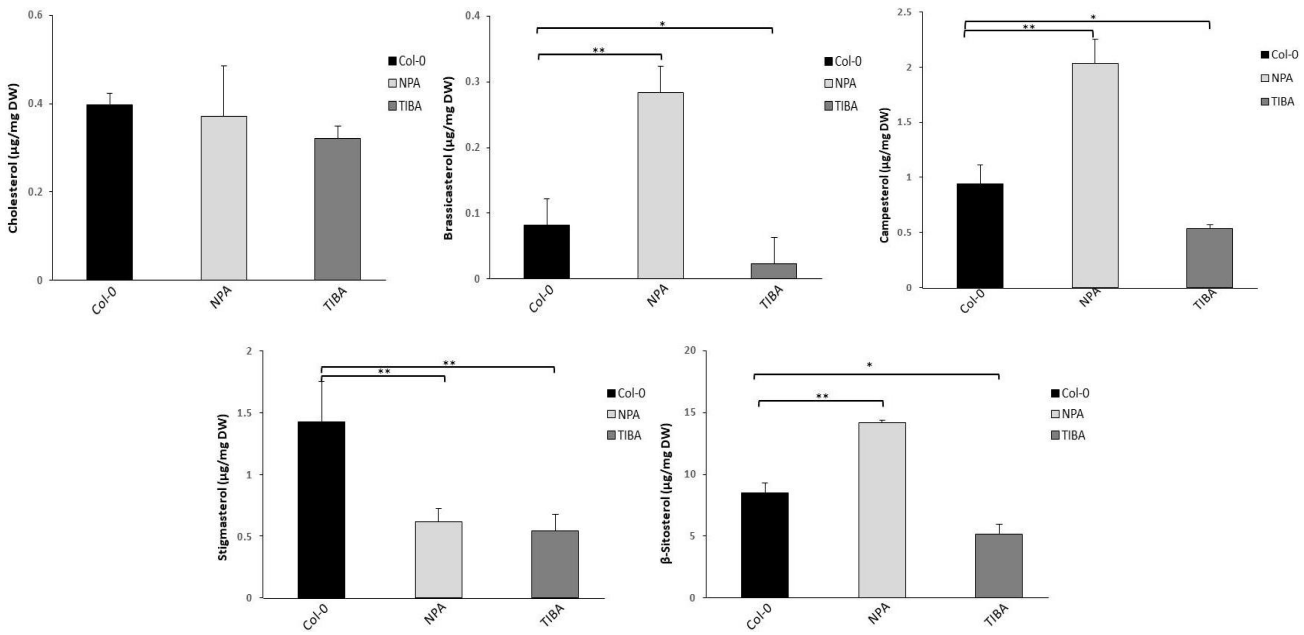

Fig. 4. Phytosterol measurement under TIBA and NPA treatment. The contents of cholesterol (A), brassicasterol (B), campesterol (C), stigmasterol (D), and β-sitosterol (E) were measured under NPA and TIBA treatment conditions. Error bars represent standard deviation over three independent experiments; the data was evaluated by Student's t-test to determine statistical significance. *: $p<0.05$; **: $p<0.01$.

Since sterols are essential components of lipid rafts and a previous study has shown that auxin efflux proteins are also associated with sterol- and sphingolipid-enriched lipid rafts (Yang *et al.*, 2013), we sought to test the effect of the auxin efflux inhibitors NPA (Kliimbt, 1992) and TIBA (Choi *et al.*, 1997) on sterol levels. As shown in Fig. 1, 100 μM NPA can strongly reduce plant size and inhibit lateral root formation after a 7-day treatment, suggesting an inhibitory effect. Interestingly, NPA treatment led to a significant increase in campesterol, brassicasterol, and β-sitosterol levels, but a decrease in stigmasterol. However, TIBA, reduced all sterol levels except those of cholesterol, suggesting that TIBA and NPA have different modes of regulation on sterol levels.

Discussion

Galea & Brown (2009) hypothesized the relationship between ROS and sterol formation more than 10 years ago. They reported that sterols might have evolved as an adaptive mechanism for protection against oxygen and/or ROS in eukaryotes. The synthesis of sterols, such as ergosterol in fungi, cholesterol in animals, and β-sitosterol in plants, is an oxygen-intensive process; 12, 11, and 11 molecules of O_2 are required for ergosterol, cholesterol, and β-sitosterol synthesis, respectively (Summons *et al.*, 2006). These sterols are susceptible to ROS oxidation, and the depletion of sterols increases susceptibility to cellular ROS attack (Iuliano, 2011). More specifically, the 5,6-double bond and the allylic C7 carbon in the B ring of cholesterol confer susceptibility to ROS and other radicals, such as peroxyl radicals (Murphy & Johnson, 2008). Since phytosterols contain the same ring skeleton as cholesterol, phytosterols would presumably be subjected to ROS oxidation in a manner similar to that of cholesterol in animals, resulting in oxidized derivatives, such as hydroxy-, keto-, epoxy-, and triol-phytosterol (Murphy & Johnson, 2008). Overall, our study documented an altered level of many sterols upon introduction of various ROS interfering agents, supporting the view that sterols work as a cornerstone for oxygen adaption and also a barrier to redox attack (Dotson *et al*., 2017; Zuniga-Hertz & Patel, 2019).

Because DPI can induce mitochondrial superoxide production by inhibiting NAD(P)H oxidase (Li *et al.*, 2003) and DDC can cause superoxide accumulation by inhibiting superoxide dismutase (Biagini *et al.*, 1995), sterol depletion could be associated with increased superoxide. However, caution is needed when interpreting these data. That is, the sterol decrease by DPI and DDC may not be directly attributed to the superoxide oxidizing attack on sterols, given that there was no reaction between superoxide and cholesterol in an *in vitro* experiment (Lee *et al.*, 1997). However, superoxide and H_2O_2 together led to the production of oxidized cholesterol molecules, such as keto-, hydroxy-, and hydro-cholesterol (Lee *et al.*, 1997), and the simultaneous production of superoxide and H_2O_2 could generate a strong inhibitory effect. Indeed, the application of SHAM, a peroxidase inhibitor that can promote ROS production (Brouwer *et al.*, 1986; Tsukagoshi *et al.*, 2010; Samuilov & Kiselevsky, 2016), led to a reduction in the contents of all measured sterols, suggesting that the strong inhibitory effect conferred by SHAM might be associated with the generation of multiple reactive species.

Compared with superoxide, H_2O_2 is much more stable and displays high oxidative activity, and an *In vitro* experiment showed that it did not oxidize cholesterol directly (Morzycki & Sobkowiak, 2015). In contrast, animal cells accumulated cholesterol after a short period of treatment with H_2O_2 (Ryu *et al.*, 2016), which is in agreement with previous findings showing that DHCR24 catalyzes the conversion of desmosterol to cholesterol by scavenging H_2O_2 (Waterham *et al.*, 2001; Lu *et al.*, 2008).

Similarly, the slightly eleveated level of cholesterol by H_2O_2 was also detected in *Arabidopsis* in this study, implying a conserved response in both animal and plant cell. Interestingly, the addition of CAT to the plant growth medium increased the contents of almost all sterols, strongly suggesting that the depletion of environmental H_2O_2 could boost endogenous sterol levels. Since H_2O_2 can function as a signaling molecule and is particularly involved in establishing intercellular tunnels (Liang, 2018), the current results do not clarify the target sterol of H_2O_2 oxidation, and further study is needed to elucidate the role of H_2O_2 signaling in sterol metabolism.

Lipid rafts play a significant role as signaling hubs (Simons & Toomre, 2000). Lipids rafts have been shown to be associated with one of the most important signaling pathways in plants, the auxin signaling pathway (Yang *et al.*, 2013). Our data showed that TIBA can lower the contents of all measured sterols, suggesting a link between auxin efflux and sterol-enriched lipid rafts. However, NPA, another auxin efflux inhibitor, exerted different effects on sterol content. In contrast to TIBA, NPA increased brassicasterol, campesterol, and β-sitosterol content. This difference could be due to the fact that TIBA and NPA belong to different classes of transporter inhibitors, and have the differing working mechanisms (Teale & Palme, 2017). Further studies are needed to clarify the role of these inhibitors in sterol level alteration.

Acknowledgement

This work was funded by National Natural Science Foundation of China (31671257).

References

Akihisa, T., W.C.M.C. Kokke and T. Tamura. 1991. Naturally occurring sterols and related compounds from plants. In: (Eds.): Patterson, G. and W. Ness. Physiology and Biochemistry of Sterols, 172-228. American Oil Chemists' Society, Champaign, Illinois.

Biagini, G., D. Sala and I. Zini. 1995. Diethyldithiocarbamate, a superoxide dismutase inhibitor, counteracts the maturation of ischemic-like lesions caused by endothelin-1 intrastriatal injection. *Neurosci. Lett.*, 190(3): 212-216.

Bieberich, E. 2018. Sphingolipids and lipid rafts: Novel concepts and methods of analysis. *Chem. Phys. Lipids*, 216: 114-131.

Bienert, G.P., A.L. Møller, K.A. Kristiansen, A. Schulz, I.M. Møller, J.K. Schjoerring and T.P. Jahn. 2007. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *J. Biol. Chem.*, 282(2): 1183-1192.

Bolwell, G.P., D.R. Davies, C. Gerrish, C.-K. Auh and T.M. Murphy. 1998. Comparative Biochemistry of the oxidative burst produced by rose and french bean cells reveals two distinct mechanisms. *Plant Physiol.*, 116(4): 1379-1385.

Borner, G.H.H., D.J. Sherrier, T. Weimar, L.V. Michaelson, N.D. Hawkins, A. MacAskill and J.A. Napier. 2005. Analysis of detergent-resistant membranes in arabidopsis. Evidence for plasma membrane lipid rafts. *Plant Physiol.*, 137(1): 104-116.

Brouwer, K.S., T. van Valen, D.A. Day and H. Lambers. 1986. Hydroxamate-Stimulated O_2 uptake in roots of *Pisum sativum* and *Zea mays*, mediated by a peroxidase: Its consequences for respiration measurements. *Plant Physiol.*, 82(1): 236-240.

Choi, Y.E., H.S. Kim, W.Y. Soh and D.C. Yang. 1997. Developmental and structural aspects of somatic embryos formed on medium containing 2,3,5-triiodobenzoic acid. *Plant Cell Rep.*, 16(11): 738-744.

Dotson, R.J., C.R. Smith, K. Bueche, G. Angles and S.C. Pias. 2017. Influence of Cholesterol on the Oxygen Permeability of Membranes: Insight from Atomistic Simulations. *Biophysical Journal*, 112(11): 2336-2347.

Dufourc, E.J. 2008. Sterols and membrane dynamics. *J. Chem. Biol.*, 1(1-4): 63-77.

Floyd, R.A. and C.A. Lewis. 1983. Hydroxyl free radical formation from hydrogen peroxide by ferrous iron-nucleotide complexes. *Biochemistry*, 22(11): 2645-2649.

Galea, A.M. and A.J. Brown. 2009. Special relationship between sterols and oxygen: were sterols an adaptation to aerobic life? *Free Radic. Biol. Med.*, 47(6): 880-889.

Helms, J.B. and C. Zurzolo. 2004. Lipids as targeting signals: Lipid rafts and intracellular trafficking. *Traffic*, 5(4): 247-254.

Iuliano, L. 2011. Pathways of cholesterol oxidation via non-enzymatic mechanisms. *Chem. Physiol. Lipids*, 164(6): 457-468.

Jin, S., F. Zhou, F. Katirai and P.L. Li. 2011. Lipid raft redox signaling: molecular mechanisms in health and disease. *Antiox. & Redox Signal.*, 15(4): 1043-1083.

Kliimbt, D. 1992. NPA inhibits secretion of amylases by barley aleurone cells and auxins can overcome this inhibition. *Bot. Acta*, 106(1993): 52-57.

Kukavica, B.M., S.D. Veljovicc-Jovanovicc, L. Menckhoff and S. Lüthje. 2012. Cell wall-bound cationic and anionic class III isoperoxidases of pea root: biochemical characterization and function in root growth. *J. Exp. Bot.*, 63(12): 4631-4645.

Lee, J.H., D.W. Shoeman, S.S. Kim and A.S. Csallany. 1997. The effect of superoxide anion in the production of seven major cholesterol oxidation products in aptoric and protic conditions. *Int. J. Food Sci. Nutr.*, 48(2): 151-159.

Lefebvre, B., F. Furt, M.A. Hartmann, L.V. Michaelson, J.P. Carde, F. Sargueil-Boiron and M. Rossignol. 2007. Characterization of lipid rafts from Medicago truncatula root plasma membranes: a proteomic study reveals the presence of a raft-associated redox system. *Plant Physiol.*, 144(1): 402-418.

Li, N., K. Ragheb, G. Lawler, J. Sturgis, B. Rajwa, J.A. Melendez and J.P. Robinson. 2003. DPI induces mitochondrial superoxide-mediated apoptosis. *Free Radic. Biol. Med.*, 34(4): 465-477.

Liang, D. 2018. A salutary role of reactive oxygen species in intercellular tunnel-mediated communication. *Front. Cell Dev. Biol.*, 6: 2.

Lu, X., F. Kambe, X. Cao, Y. Kozaki, T. Kaji, T. Ishii and H. Seo. 2008. 3β-Hydroxysteroid-Δ24 reductase is a hydrogen peroxide scavenger, protecting cells from oxidative stress-induced apoptosis. *Endocrinol.* 149(7): 3267-3273.

Maître, B., L. Jornot and A.F. Junod. 1993. Effects of inhibition of catalase and superoxide dismutase activity on antioxidant enzyme mRNA levels. *Amer. J. Physiol.*, 265(6 Pt 1): L636-643.

Mongrand, S., J. Morel, J. Laroche, S. Claverol, J.P. Carde, M.A. Hartmann and M. Bonneu. 2004. Lipid rafts in higher plant cells: purification and characterization of Triton X-100-insoluble microdomains from tobacco plasma membrane. *J. Biol. Chem.*, 279(35): 36277-36286.

Morzycki, J.W. and A. Sobkowiak. 2015. Electrochemical oxidation of cholesterol. *Beilstein J. Org. Chem.*, 11: 392-402.

Murphy, R.C. and K.M. Johnson. 2008. Cholesterol, reactive oxygen species, and the formation of biologically active mediators. *The J. Biol. Chem.*, 283(23): 15521-15525.

Nicholls, P. 2012. Classical catalase: ancient and modern. *Arch Biochem. Biophys*., 525(2): 95-101.

Ouchi, Y., K. Unoura and H. Nabika. 2019. Role of oxidized lipids in permeation of H_2O_2 through a lipid membrane: molecular mechanism of an inhibitor to promoter switch. *Sci. Rep*., 9(1): 12497.

Ryu, H.M., Y.J. Kim, E.J. Oh, S.H. Oh, J.Y. Choi, J.H. Cho and C.D. Kim. 2016. Hypoxanthine induces cholesterol accumulation and incites atherosclerosis in apolipoprotein E-deficient mice and cells. *J. Cell. & Mol. Med.*, 20(11): 2160-2172.

Samuilov, V.D. and D.B. Kiselevsky. 2016. Salicylhydroxamic acid enhances the NADH-oxidase activity of peroxidase in pea mitochondrial and chloroplast suspensions. *Moscow Uni. Biol. Sci. Bull.*, 71(1): 19-23.

Simons, K. and D. Toomre. 2000. Lipid rafts and signal transduction. *Nature Rev. Mol. Cell Biol.*, 1(1): 31-39.

Simons, K. and R. Ehehalt. 2002. Cholesterol, lipid rafts, and disease. *J. Clin. Invest.*, 110(5): 597-603.

Summons, R.E., A.S. Bradley, L.L. Jahnke and J.R. Waldbauer. 2006. Steroids, triterpenoids and molecular oxygen. *Philosophical transactions of the Royal Society of London. Series B, Biol. Sci.*, 361(1470): 951-968.

Teale, W. and K. Palme. 2017. Naphthylphthalamic acid and the mechanism of polar auxin transport. *J. Exp. Bot.*, 69(2): 303-312.

Tsukagoshi, H., W. Busch and P.N. Benfey. 2010. Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. *Cell*, 143(4): 606-616.

Waterham, H.R., J. Koster, G.J. Romeijn, R.C. Hennekam, P. Vreken, H.C. Andersson and D.R. FitzPatrick. 2001. Mutations in the 3beta-hydroxysterol Delta24-reductase gene cause desmosterolosis, an autosomal recessive disorder of cholesterol biosynthesis. *Amer. J. Human Gen.*, 69(4): 685-694.

Yang, H., G.L. Richter, X. Wang, E. Młodzińska, N. Carraro, G. Ma and M. Jenness. 2013. Sterols and sphingolipids differentially function in trafficking of the Arabidopsis ABCB19 auxin transporter. *The Plant J.*, 74(1): 37-47.

Zhang, Z., Y.L. Ruan, N. Zhou, F. Wang, X. Guan, L. Fang and X. Shang. 2017. Suppressing a putative sterol carrier gene reduces plasmodesmal permeability and activates sucrose transporter genes during cotton fiber elongation. *Plant Cell*., 29(8): 2027-2046.

Zuniga-Hertz, J.P. and H.H. Patel. 2019. The Evolution of Cholesterol-Rich Membrane in Oxygen Adaption: The Respiratory System as a Model. *Frontiers in Physiology*, 10: 1340.

(Received for publication 14 October 2020)