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STABILIZATION OF DISTURBANCES IN MEMBRANE PHOTOCHEMICAL REACTIONS IN WHEAT SEEDLINGS UNDER COLD STRESS BY NATURAL EXOGENOUS SAPONINS

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Abstract

The chloroplast membrane is under the constant effect of external factors (temperature, light, nutrition, etc.) resulting in certain structural changes. Low temperature is known to contribute to a decrease in the fluidity of the lipid phase of the membrane. Metabolites of cell lipid metabolism, in turn, can facilitate the mobility of protein components. Modifying these conditions, we used the incubation of *Triticum aestivum* L. seedlings at a low temperature (4°C) for various periods as a hardening effect of membrane lipids *In vivo*. Adding a fraction of saponins – natural detergents, isolated from the medicinal plant *Glycyrrhiza glabra* into the nutrient solution provoked a change in the state of the thylakoid membrane. Under these conditions, the behavior of the change in the delayed chlorophyll *a* fluorescence and pigments activity in the light-harvesting antenna were studied *In vivo* using the absorption spectra. It was shown that in wheat seedlings in the absence of saponins, after 4 hours of cold stress, the activity of the electron transport chain in the PS II decreases, which is associated with the blocking of electron transfer in the quinone acceptor site. Longer cold stress contributed to the restoration of these changes. The maximum change in the compositions of photosynthetic pigments occurred after 48 hours of cold stress. The introduction of saponins into the nutrient solution prevented changes in the reactions of the electron transport chain of PS II, which was correlated with the restoration of the pigment pool in the light-harvesting antenna. The latter is probably associated with an adaptive increase in membrane stability. The corrective effect of the saponin fraction is possible due to the restoration of lipid components of the thylakoid membrane, and the transition of the membrane from a quasicrystalline to a liquid state. Restoration of membrane fluidity stimulates the function of membrane proteins – electron carriers in the PSII electron transport chain and stabilizes the light-harvesting pigment complex.

Key words: Photosystem II activity, Delayed Chl *a* fluorescence, Photosynthetic pigments, Antioxidant.

Introduction

Adverse environmental factors cause the accumulation of reactive oxygen species in the plant cell and chloroplasts (superoxide O^{*-} anion radical, hydroxyl OH*, H_2O_2, singlet oxygen ($^1O_2^*$), electrophilic alkoxy RO^+, organic peroxy radicals ROO•, triplet excited chlorophyll $^3Chl^*$) causing oxidative stress, which leads to a significant disruption of biological processes (Ahmad *et al.*, 2008; Rantala *et al.*, 2020). One of the reasons leading to such disorders is damage to the membrane system of chloroplasts. The thylakoid membranes of the chloroplasts of higher plants contain three glycolipids, namely, monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG), and one phospholipid, phosphatidylglycerol (PG). The lipids of chloroplast membranes contain polyunsaturated fatty acids at unusually high relative levels (Reszczyńska & Hanaka, 2020). Low temperatures cause hardening of the lipid components and a change in the ratio of unsaturated and saturated fatty acids, which suppresses the barrier function of the membrane system. As a result of these damages, the activity of ion transport across membranes, the disintegration of the electron transport chains (ETC) of mitochondria and chloroplasts are disrupted, which disturbs energy metabolism and photosynthesis (Zhang *et al.*, 2019; Ashrostaghi *et al.*, 2022). Photosystem II (PS II) and the formation of antenna complexes are known to be most seriously damaged at low temperatures. Accumulation of reactive molecules in chloroplasts leads to a decline in PSII activity (Foyer, 2018). To protect against reactive molecules, their rapid detoxification is necessary, otherwise, the generation of reactive molecules increases and leads to a rapid light-dependent turnover of the D_1 protein in PSII (Järvi *et al.*, 2015). Plants have various effective defense systems that resist the development of oxidative stress. Low-molecular compounds, carotenoids, stress proteins, antioxidant enzymes, and other protective compounds are synthesized or activated, which prevent the damaging effect of reactive oxygen species. They provide flexibility in regulating the redox potential, increasing the resistance of photosynthesis to the action of negative factors (Scandalios, 2005; Ganiyeva *et al.*, 2021; Swapnil *et al.*, 2021). The increased interest in medicinal plants motivates more in-depth research to identify and obtain new medicines (Habibah *et al.*, 2023; Mammedova *et al.*,

2018). The antioxidant properties of plants are determined by the high content of compounds capable of quenching free radicals (Jafarova *et al.*, 2019). The medicinal plant Liquorice (*Glycyrrhiza glabra*) is of great value in pharmacological research. Phenolic compounds in their phytochemical composition play an important role in bioprocesses as correctors of redox reactions. Thus, in the roots of licorice (*Radix glycyrrhizae*), the most valuable compound is saponin – glycyrrhizin, which has a membranotropic property. In the presented paper, the role of saponin, as a membrane-active compound, in the protection of the thylakoid membrane against low-temperature stress has been revealed.

Material and Methods

The object of the research was seven-day-old wheat seedlings (*Triticum aestivum* L.) grown under controlled conditions in a water medium. Plants incubated in an aqueous solution containing the sum of saponins (saponin+) (at a concentration of 500 μg/100 ml) and in the absence of saponin (saponin –) were exposed to low-temperature stress in a cold chamber (4°C) for 4, 24, and 48 hours. Saponins were obtained from the roots of *Glycyrrhiza glabra* grown in the Republic of Azerbaijan by the method of triple extraction with water-alcohol mixtures in a water bath (50g of raw material + 350ml). Elution was carried out with 95% ethylene alcohol. The resulting eluate was evaporated to a volume equal to half the mass of the raw material with the addition of 25% acetone (pH 7.8). The filtered eluate was washed with acetone and chloroform (Weng *et al.*, 2009). Antioxidant (AO) activity of extract *Glycyrrhiza glabra* was determined on the stable radical model DPPH by the method using the computer program Perella Scientific Inc. Amherst, USA (Molyneux, 2004). The study of the composition of native forms of chlorophyll (Chl *a*, Chl *b*) and carotenoids (Car) was carried out at room temperature on a Furye Cary 50 Scan Varian spectrophotometer. The kinetics of ms DF was measured using a phosphoroscope as described previously (Gasanov *et al.*, 2015). A sample in silica SUPRASIL cuvette was irradiated with continuous white light (250 W m^{-2} s^{-1}) passing through 2 cm of a $CuSO_4$ solution. The light passed through holes on the rotating wheel of the phosphoroscope (three holes, 120° apart) in such a way that 0.3 ms of excitation was followed by 1.25 ms of dark and 0.3 ms of registration of the delayed light emission (three cycles of excitation/dark/registration per one full turnover of the phosphoroscope wheel). The delayed light was measured with a photomultiplier varied glass cut-off KC9 filter (>680 nm). The signal passing through an amplifier was recorded by an electrical potential recorder (Rubin *et al.*, 1985; Goltsev *et al.*, 2009). The studies were carried out *In vivo*. Excell program was used for statistical data processing.

Results

Saponins are known to have a wide range of biological and physiological properties. Participating in biochemical processes and being membrane-active compounds, they contribute to the restoration of the lipid membrane. The saponin fraction was obtained from *Radix glycyrrhizae* and antioxidant activity (AO) was determined from the stable radical model by the DPPH method (Table 1).

Table 1. DPPH reaction of *Glycyrrhiza glabra* root extract.

Samples	IC 50(μg/ml)*
Trolox	17.1 ± 0.1
Glycyrrhiza glabra extract	11.9 ± 0.1

Compared to the action of Trolox, the reaction with DPPH for *Glycyrrhiza glabra* was 11.9 mg/ml, confirming the high AO activity of the medicinal plant used. In order to diagnose the state of the ETC of PS II under the effect of low positive temperatures on *Triticum aestivum* L. seedlings, we studied the induction transitions of the light curves of delayed Chl *a* fluorescence (ms DF Chl *a*). In an intact system, this reaction is closely related to metabolic processes and is regulated by the cell following its energy needs (Gasanov *et al.,* 2007). The observed decline in fluorescence characteristics under stress, both in the fast phase (fast phase/stationary phase) and in the slow phase (slow phase/stationary phase) of fluorescence, indicated the blocking of the electron transfer reaction in the Q_B region. These changes depended on the duration of the low-temperature stress effect on seedlings (Figs. 1 and 2).

After 4 hours of low-temperature exposure of leaves (saponin –), indicators of f.p./st.p. and sl.p./st.p. fluorescence was decreased by 2 times. Due to the effect of saponins on wheat seedlings under cold conditions, the fluorescent characteristics of leaves (saponin+) were increased by 5 and 4.5 times. Compared to 4 hours, during 24 hours of low-temperature stress, the fluorescence indicators of both f.p./st.p. and sl.p./st.p. in the leaves (saponin –) was increased and almost corresponded to the indicators of the control (24°C).

The action of saponins led to an effective increase in these parameters. In this time period, the amplitude of both f.p/st.p and sl.p/st.p fluorescence during the action of saponins on seedlings was increased 3 times in leaves (saponin+) relative to the untreated variant (saponin –) and was slightly decreased after 48 hours of cold exposure (Figs. 1 and 2).

Analysis of the absorption spectrum showed changes in the Chl a_{680}/Chl b_{645} ratio in leaves at low temperatures (Fig. 3).

In the leaves (saponin –), a decrease in the Chl *a*/Chl *b* ratio was observed as exposure time increased under cold stress. By 48 hours, this ratio was decreased by 1.6 times compared to 4 hours of exposure, suggesting a lower tolerance of Chl *a* to cold. The presence of saponin under these conditions leads to an increase in the ratio of Chl *a*/ Chl *b* after 4 hours by 20%, after 24 hours by 30%, and after 48 hours by 28% (Fig. 3). Besides, the highest indicator of saponin action occurs after 24 hours of cold stress, which correlates with the restoration of saponin activity of PSII ETC.

As seen in (Table 2), the content of carotenoids in untreated seedlings under the action of a low positive temperature for 48 hours remained almost at the same level.

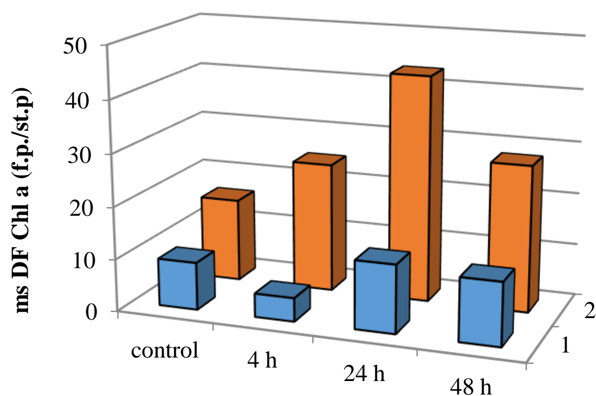

Fig. 1. Changes in the ratio of fast phase fluorescence to stationary fluorescence (f.p./st.p.) in ms of delayed Chl *a* fluorescence of leaves (1-saponin – and 2-saponin +) of wheat (*Triticum aestivum* L.) seedlings exposed to a low positive temperature (4°C) for 4, 24, and 48 hours.

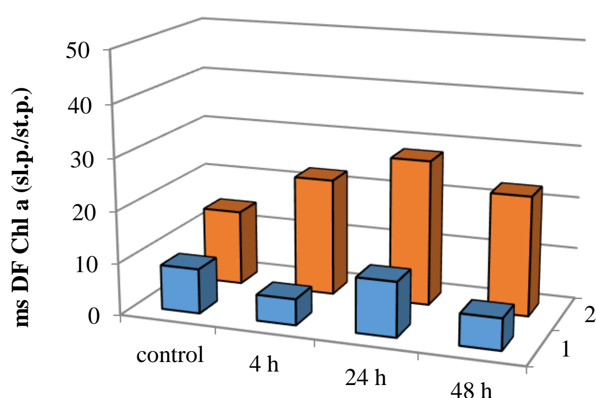

Fig. 2. Changes in the ratio of slow phase fluorescence to stationary fluorescence (sl.p./st.p.) in ms of delayed Chl *a* fluorescence of leaves (1-saponin – and 2-saponin +) of wheat (*Triticum aestivum* L.) seedlings exposed to a low positive temperature (4°C) for 4, 24, and 48 hours.

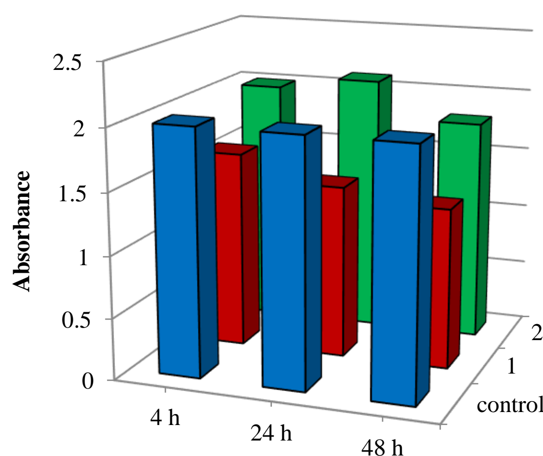

Fig. 3. Changes in the ratio of Chl a_{680}/Chl b_{645} under the influence of a low positive temperature (4°C) in leaves (1-saponin – and 2-saponin +) of *Triticum aestivum* L. after 4, 24, and 48 hours.

Discussion

It is known that under physiological conditions, the membrane is in a liquid crystal state and a selective barrier for cellular elements. Under conditions of low-temperature stress, membrane permeability is damaged as a result of the inhomogeneous solidification of lipid components and disruption of its fluidity (Bychkov *et al*., 2020; Liu *et al*., 2018; Iqbal *et al*., 2023). Besides, the action of low temperature can cause dehydration in the cell, which also leads to disruption of the functions of biomacromolecules and membrane components (Banerjee & Roychoudhury, 2019; Muzi *et al*., 2016). As shown (Figs. 1 and 2), the effect of low temperature on wheat seedlings resulted in a significant decrease in electron transfer on both donor and acceptor sides of PSII ETC. Disturbance of the barrier function of the thylakoid membrane leads to changes in the accumulation of photosynthetic pigments, which resulted in a change in the Chl *a*/Chl *b* ratio and the content of carotenoids. Low temperature causes monomerization of light-harvesting forms of chlorophyll *a* and less stability of the pigment-protein and protein-protein interactions (Elfman *et al*., 1984; Banerjee & Roychoudhury, 2016; Rochaix & Bassi, 2019) (Fig. 3, Table 2). Our studies showed the blocking of electron transfer reactions on both donor and acceptor sides, resulting in an increase in stationary fluorescence, explaining the decline in the f.p./st.p. and sl.p./st.p. ratios (Table 3).

Partial restoration of fluorescent characteristics in seedlings deprived of saponins by 48 hours of cold stress is attributed to adaptive reactions (Yeshi *et al*., 2022). The low-molecular and enzymatic antioxidant system is activated under stress conditions and provides protection for chloroplasts and photosynthesis, which are the most sensitive to low positive temperatures (Paredes & Quiles, 2015). Triterpene saponins have a high protective effect. Their antioxidant effects on homeostasis and apoptosis of plant and animal cells have been studied (Cui *et al*., 2020; Klimov, 1997; Faizal & Geelen, 2013). The membrane-active properties of saponins are reflected in the restoration of reactions occurring in ETC of PS II, in the content of chlorophyll and carotenoids, which ensure the stability of the light-harvesting pigment complex (Zhao *et. al.*, 2020; Gill & Tuteja, 2010). It is also possible that saponins, being low molecular weight antioxidants, are able to quench reactive oxygen species (lifespan 2 ms) around PSII. This restores the interaction between the PSII reaction center, the proton gradient, and the stability of electron transfer in the ETC.

The results obtained will expand current ideas on the use of semi-synthetic (hybrid) structures based on various intact photosynthetic systems – cells, plant thylakoid membranes.

Conclusion

Saponins obtained from the medicinal plant *Glycyrrhiza glabra* have a membranotropic effect. Restoring the thylakoid membrane from cold damage activates the work of protein electron carriers in the PSII electron transport chain and stabilizes the light-harvesting pigment complex.

Table 2. Changes in the carotenoid content of wheat (*Triticum aestivum* L.) leaves (saponin – and saponin +) under a low positive temperature (4°C).

λ, nm	Control 24°C	4 hours		24 hours		48 hours	
		Saponin –	Saponin +	Saponin –	Saponin +	Saponin –	Saponin+
425	1.9 ± 1.0	1.5 ± 0.3	3.3 ± 1.4	2.5 ± 1.6	3.9 ± 0.3	1.7 ± 0.2	3.8 ± 0.4
445	1.5 ± 0.6	1.2 ± 0.2	2.7 ± 0.4	1.6 ± 0.3	3.7 ± 0.2	1.4 ± 0.1	3.6 ± 0.3
470	1.4 ± 0.3	1.1 ± 0.1	2.0 ± 0.3	1.5 ± 0.2	2.8 ± 0.2	1.1 ± 0.1	2.7 ± 0.2

Under the influence of saponins for 24 hours, an increase in the content of carotenoids occurs, which also corresponds to the time of restoration by saponins of both the Chl *a*/Chl *b* ratio and ETC activity in PSII

Table 3. Changes in stationary fluorescence of wheat (*Triticum aestivum* L.) leaves (saponin – and saponin +) under the influence of a low positive temperature (4°C) for 4 hours.

Saponin – 24°C	Saponin – 4°C	Saponin + 24°C	Saponin + 4°C
1.63 ± 0.04	2.66 ± 0.20	0.78 ± 0.24	0.61 ± 0.13

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References

Ahmad, P., M. Sarwat and S. Sharma. 2008. Reactive oxygen species, antioxidants and signaling in plants. *J. Plant Biol.*, 51(3): 167-173. doi:10.1007/bf03030694

Ashrostaghi, T., S. Aliniaeifard, A. Shomali, S. Azizinia, J.A. Koohpalekani, M. Moosavi-Nezhad and N.S. Gruda. 2022. Light intensity: the role player in cucumber response to cold stress. *Agronomy*, 12(1): 1-19. https://doi.org/10.3390/agronomy12010201

Banerjee, A. and A. Roychoudhury. 2016. Plant responses to light stress: oxidative damages, photoprotection and role of phytohormones. In: (Eds.): Ahammed, G. and J.Q. Yu. *Plant Hormones Under Challenging Environmental Factors*. Springer, Science+Business Media, Dordrecht, 181-213. doi: 10.1007/978-94-017-7758-2_8

Banerjee, A. and A. Roychoudhury. 2019. Cold Stress and Photosynthesis. In: (Eds.): Ahmad, P., M.A. Ahanger, M.N. Alyemeni and P. Alam. *Photosynthesis, Productivity, and Environmental Stress*. John Wiley & Sons Ltd., UK, 27-37. doi:10.1002/9781119501800.ch2

Bychkov, I.A., N.V. Kudryakova,V. Kuznetsov and V.V. Kusnetsov. 2020. Cold stress activates the expression of genes of the chloroplast transcription apparatus in *Arabidopsis thaliana* plants. Biochem. and Biophy., 494(1): 235-239. doi: 10.1134/S160767292005004X

Cui, Y., B. Liu, X. Sun, Z. Li, Y. Chen, Zh. Guo, H. Liu, D. Li, Ch. Wang, X. Zhu and Y. Shi. 2020. Protective effects of alfalfa saponins on oxidative stress-induced apoptotic cells. *Food & Func*, 11(9): 8133-8140. doi: 10.1039/d0fo01797c

Elfman, B., N.P.A. Huner, M. Griffith, M. Krol, W.G. Hopkins and D.B. Hayden. 1984. Growth and development at cold-hardening temperatures. Chlorophyll–protein complexes and thylakoid membrane polypeptides. *Can. J. Bot.*, 62(1): 61-67. doi.org/10.1139/b84-010

Faizal, A. and D. Geelen. 2013. Saponins and their role in biological processes in plants. *Phytochem. Rev.*, 12(4): 877-893. doi: 10.1007/s11101-013-9322-4.

Foyer, C.H. 2018. Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. *Environ. Exp. Bot.*, 154: 134-142. doi.org/10.1016/j.envexpbot.2018.05.003.

Ganiyeva, R., S. Dadashova, J. Jafarova and R. Gasanov. 2021. Protection with a bioactive composition based on plumbagin (*Ceratostigma plumbaginoides* Bunge) and Na-ascorbate pigments and PS II in oxidative stress. *Eurasian Uni. Sci.*, 6(87): 8-13. https://doi.org/10.31618/ESU.2413-9335.2021.1.87.1388.

Gasanov, R., S. Aliyeva, S. Arao, A. Ismailova, N. Katsuta, H. Kitade, Sh. Yamada, A. Kawamori and F. Mamedov. 2007. Comporative study of the water oxidizing reactions and the millisecond delayed chlorophyll fluorescence in photosystem II at diferent pH. *J. of Photochem. and Photobiol.*, 86(2): 160-164.

Gasanov, R., S. Aliyeva and F. Mamedov. 2015. Delayed fluorescence in a millisecond range – a probe for donor side-induced photoinhibition in photosystem II. In: (Eds.): Itoh, S., P. Mohanty and K.N. Guruprasad. *Photosynthesis: Basics to applications*. I.K. International Publishing House Pvt. Ltd., New Delhi, India, 101-107.

Gill, S.S. and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.*, 48(12): 909-930. doi: 10.1016/j.plaphy.2010.08.016.

Goltsev, V., I. Zaharieva, P. Chernev and R.J. Strasser. 2009. Delayed chlorophyll fluorescence as a monitor for physiological state of photosynthetic apparatus. *Biotech. Equip.*, 23(1): 452-457. doi:10.1080/13102818.2009.10818461.

Habibah, N.A., N. Nugrahaningsih, Y.U. Anggraito, S. Safitri, F. Musafa and N. Wijawati. 2023. Influence of plant growth regulators on the phenolic composition of *Elaeocarpus grandiflorus* J.E. Smith (Elaeocarpaceae) cell culture. *Pak. J. Bot.*, 55(5): 1623-1631. http://dx.doi.org/10.30848/PJB2023-5(17).

Iqbal, N., A. Masood, H. Mubeen, F. Deeba, R. Waheed, I. Noreen, A. Zafar and P. Khanum. 2023. ATP synthase subunits: structure and role in plants under stress conditions. *Pak. J. Bot.*, 55(5): 1661-1668. http://dx.doi.org/10.30848/PJB2023-5(11).

Jafarova, J., R. Ganiyeva, S. Bayramova and R. Gasanov. 2019. The nature of PS II reactions stability under oxidative stress. *Bangladesh J. Bot.*, 48(4): 1029-1035. https://doi.org/10.3329/bjb.v48i4.49051.

Järvi, S., M. Suorsa and E.M. Aro. 2015. Photosystem II repair in plant chloroplasts–Regulation, assisting proteins and shared components with photosystem II biogenesis. *Biochem. Biophys. Acta*, 1847(9): 900-909.

Klimov, S.V. 1997. Bioenergy concept of plant adaptation to low temperatures. *Adv. Mod. Biol.*, 117(2): 133-154.

Liu, X., Y. Zhou, J. Xiao and F. Bao. 2018. Effects of chilling on the structure, function and development of chloroplasts. *Front. Plant Sci.*, 9: 1715.

Mammadova, H.G., S.V. Serkerov and A.N. Alasgarova. 2018. Investigation of the biologically active substances obtained from *Johrenia paucijuga* (DC) of the species Bornm. *J. Plant Biochem. Physiol.*, 6(1):1000208.

Molyneux, P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin. J. Sci. Technol.*, 26(2): 211-219.

Muzi, C., L. Camoni, S. Visconti and P. Aducci. 2016. Cold stress affects H^+-ATPase and phospholipase D activity in Arabidopsis. *Plant Physiol. Biochem.*, 108: 328-336.

Paredes, M. and M.J. Quiles. 2015. The effects of cold stress on photosynthesis in *Hibiscus* plants. *Plos One*, 10(9): e0137472.

Rantala, S., S. Järvi and E.M. Aro. 2020. Photosystem II: Assembly and turnover of the reaction center D1 protein in plant chloroplasts. In: (Ed.): Roitberg, B.D. *Reference Module in Life Sciences*. Elsevier, 1-8. doi:10.1016/B978-0-12-809633-8.21404-0.

Reszczyńska, E. and A. Hanaka. 2020. Lipids composition in plant membranes. *Cell Biochem. Biophys.*, 78: 401-414.

Rochaix, J.D. and R. Bassi. 2019. LHC-like proteins involved in stress responses and biogenesis/repair of the photosynthetic apparatus. *Biochem. J.,* 476(3): 581-593.

Rubin, A.B., T.E. Krendeleva, P.S. Venediktov and D.N. Matorin. 1985. Initial photosynthetic processes and photosynthetic productivity. Sel'skokhozyaĭstvennaya. *Biologiya*, 6: 81-92.

Scandalios, J.G. 2005. Oxidative stress: Molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz. J. Med. Biol. Res.*, 38(7): 995-1014.

Swapnil, P., M. Meena, S.K. Singh, U.P. Dhuldhaj, Harish and A. Marwal. 2021. Vital roles of carotenoids in plants and humans to deteriorate stress with its structure, biosynthesis, metabolic engineering and functional aspects. *Curr. Plant Biol.*, 26: 100203.

Weng, A., D. Bachran, C. Görick, Ch. Bachran, H. Fuchs and M.F. Melzig. 2009. A simple method for isolation of Gypsophila saponins for the combined application of targeted toxins and saponins in tumor therapy. *Planta Med.*, 75(13): 1421-1422.

Yeshi, K., D. Crayn, E. Ritmejerytė and P.H. Wangchuk. 2022. Plant secondary metabolites produced in response to abiotic stresses has potential application in pharmaceutical product development. *Molecules*, 27(1): 313. https://doi.org/10.3390/molecules27010313.

Zhang, H., J. Dong, X. Zhao, Y. Zhang, J. Ren, L. Xing, Ch. Jiang, X. Wang, J. Wang, Sh. Zhao and H. Yu. 2019. Research progress in membrane lipid metabolism and molecular mechanism in peanut cold tolerance. *Front. Plant Sci.*, 10: 838. doi:10.3389/fpls.2019.00838.

Zhao, Y., Q. Han, Ch. Ding, Y. Huang, J. Liao, T. Chen, S. Feng, L. Zhou, Zh. Zhang, Y. Chen, Sh. Yuan and M. Yuan. 2020. Effect of low temperature on chlorophyll biosynthesis and chloroplast biogenesis of rice seedlings during greening. *Int. J. Mol. Sci.*, 21(4): 1390.

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