Proceedings of the 5th Balkan Scientific Conference on Biology

Kostadinova, S., Mollov, I., Dzhambazov, B., Naimov, S., Vassilev, K. & Georgiev, B. (Eds.)
Plovdiv, Bulgaria ● 15-16 April 2021 ● pp. 89-96

BalkanBio

Functional and Structural Feature of Photosynthetic Apparatus of Some Halophytic and Glycophytic Representatives from Genus Lactuca (Asteraceae)

Violeta N. Peeva[1*], Viktoria A. Hristova[2], Svetlana M. Momchilova[3], Dimitrina P. Koleva[2], Albena P. Ivanova[1], Albena B. Momchilova[4], Liliana T. Maslenkova[4]

1 - Bulgarian Academy of Sciences, Institute of Plant Physiology and Genetics,
"Acad. G. Bonchev" Str. bl. 21, 1113 Sofia, BULGARIA
2 - Sofia University "St. Kliment Ohridski", Faculty of Biology, 8 "Dragan Tsankov" Str., 1164 Sofia, BULGARIA
3 - Bulgarian Academy of Sciences, Institute of Organic Chemistry with Centre of Phytochemistry,
"Acad. G. Bonchev" Str. bl. 9, 1113 Sofia, BULGARIA
4 - Bulgarian Academy of Sciences, Institute of Biophysics and Biomedical Engineering,
"Acad. G. Bonchev" Str. bl. 21, 1113 Sofia, BULGARIA
*Corresponding author: lili.maslenkova@gmail.com

Abstract. In the present study, chloroplast ultrastructure, PSII functionality and lipid and fatty acids pattern of isolated chloroplasts have been used in order to characterize structural and functional peculiarities of photosynthetic apparatus in some halophytic and glycophytic *Lactuca* species. The comparative studies of *Lactuca tatarica* (L.) C.A. Mey, *L. serriola* L. and *L. quercina* L. have shown distinctive features of thylakoid membrane system, chlorophyll thermoluminescence emission and kinetic parameters of PSII oxygen-evolving reactions. The analysis of lipid classes and fatty acids composition of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyl diacylglycerol (SQDG) and phosphatidyldiacylglycerol (PG) show the existence of qualitative and quantitative differences that can contribute in this regard. The results show specific characteristics of photosynthetic membranes in halophytic and glycophytic *Lactuca* species, reflecting different adaptive strategies of the studied species to environmental conditions in their natural habitats.

Key words: chloroplasts, fatty acids, lipid composition, *Lactuca quercina, Lactuca serriola*, *Lactuca tatarica*, oxygen evolution, photosynthetic activity, thermoluminescence.

Introduction

In natural habitats, the environmental factors (temperature, water, light intensity, salinization, etc.) have a complex influence on the plants. Fluctuations in environmental factors as well as various unfavorable situations induce physiological adaptation by influencing the activity of primary metabolic reactions such as photosynthesis, which is a major physiological process that determines plant growth and productivity (Lichtenthaler, 1998). The properties of photosynthetic apparatus may contribute to a great extent to plant habitat separation and to adaptation to environment factors governing mechanisms for effective light energy utilization. The physiological properties of chloroplast membrane such as permeability, selectivity, etc., varied in dependence of environment conditions, resulting in changes in the physical orientation of membrane lipids and functional activity of thylakoids. The response of the cell in such cases is a series of quantitative and qualitative alterations in the lipid composition in order to restore the initial orientation and the physical properties of the membrane.

The genus *Lactuca* L. (Asteraceae) comprises about 100 wild species occurring in Europe, Asia, Africa and North America. Since ancient times, some *Lactuca* species have been well known as dietary and medicinal plants. Plants of the genus *Lactuca* have been shown to produce sesquiterpene lactones (SL) (Wang et al., 2010) as their characteristic secondary metabolites. Sesquiterpene lactones may play a highly significant role in human health, as pharmaceutical agents, due to their potential for the treatment of cardiovascular disease and cancer (Chadwick et

al., 2013). Wild *Lactuca* species e.g., *L. virosa* L. and *L. saligna* L. are being used in breeding programs for the introduction of virus resistance into commercial lettuce (Tamaki et al., 1995)

In Bulgaria, seven *Lactuca* species can be found: *Lactuca tatarica* (L.) C.A. Mey, *L. serriola* L., *L. quercina* L., *L. viminea* (L.) J.Presl & C.Presl, *L. saligna* L., *L. perennis* L., *L. aurea* (Vis. & Pančić) Stebbin (Stoyanova et al., 2015). In the present study were selected three species, a halophyte *L. tatarica* and two glycophytic species *Lactuca serriola* and *L. quercina*.

The aim of this study was to investigate the structural-functional peculiarities of chloroplast membranes influenced by the specific environmental conditions in different natural habitats of some halophytic and glycophytic *Lactuca* species.

Materials and Methods

Plant material and habitats. Sampling of leaves from selected *Lactuca* species was made in flowering stage from different natural habitats during the period July - August 2019. The glycophyte *Lactuca serriola* is a drought-resistant species mainly found in solar habitats, urban places, along railways, landfills, etc. (D'Andrea et al., 2009). The plants were collected around the city of Sofia from agricultural field near Lozen village (42.6017° N, 23.4827° E, altitude 650 m). The glycophyte *Lactuca quercina* inhabits mainly shadow and a semi-shade oak, beech forests and shrubland communities. The plants were collected in Rila Mountain in beech forest on the territory of Rila Monastery Nature Park (42.1334° N, 23.3401° E, altitude 1200 m). In nature, halophyte *Lactuca tatarica* grows in meadows, in steppes and semi-deserts, as well as in salty soils, for example, along sea coasts. The samples were collected from the Bulgarian Black sea coast near the village of Shabla (43.5379° N, 28.5352° E, altitude 47 m).

Isolation of broken chloroplasts (thylakoids). Averaged samples of leaves of 3-4 plants collected were homogenised in 50 mM Na-tricine (pH 7.8), containing 3 mM Na-ascorbate, 10 mM NaCl, 5 mM MgCl2, 0.4 M sucrose and 5% PEG-6000. The resulting slurries were passed through 8 layers of cheesecloth and the broken chloroplasts (thylakoids) were collected by centrifugation at 1000 x g for 10 min. The pellets were washed twice in 10 mM Na-tricine (pH 7.8) containing 0.4 M sucrose, 10 mM NaCl and 5 mM MgCl and then resuspended to concentration of 1 mg Chl/ml in 50 mM Na-Mes (pH 6.5) instead of Tricine buffer and stored on ice for 1 h in the dark before measurements. The pigment content was determined spectrophotometrically (Lichtenthaler, 1987).

Oxygen-evolving reactions. Oxygen-evolving reactions were measured using polarographic oxygen rate electrode (Joliot-type) and thylakoid membranes (100 µl sample volume, 300 µgChl/ml) without any artificial electron acceptors, as described in (Zeinalov, 2002). The initial oxygen burst was recorded after irradiation with continuous white light (450 µmol photons m^{-2} s^{-1}). Deconvolution of the oxygen burst decay was performed by fitting of the function with two exponential components: $A_1e(tk_1) + A_2e(tk_2)$, where A_1 and A_2, and k_1 and k_2 were the rate constants of the fast and slow components of the oxygen burst decay, respectively.

Thermoluminescence. Thermoluminescence (TL) measurements were carried out in darkness using computerized equipment, described in detail in (Zeinalov & Maslenkova, 1996 a). The samples were kept in the dark for 2 h before measurements. Samples of isolated thylakoid membranes ware illuminated at 2-5°C to generate charge pairs within the PSII reaction centres and then rapidly cooled down in liquid nitrogen to trap those charge-separated states. Subsequent warming of the samples reveals thermoluminescence emission with characteristic peaks (Sane & Rutherford, 1986). Decomposition an analysis of TL glow curves was carried out using Origin Pro 8.

TEM analysis. For TEM analysis small segments (1-2 mm^2) from the middle part of fully expanded leaves were taken and fixed in 3% (m/v) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 12 h at 4°C. The leaf segments were post fixed in 1% (m/v) $KMnO_4$ in the same

buffer for 2 h at room temperature. After dehydration by increasing concentrations of ethyl alcohol (from 25 to 100%), the samples were embedded in Durcupan (Fluka, Buchs, Switzerland) and cross-sectioned with Reichert-Jung (Wien, Austria) ultramicrotome. Observation and documentation were performed by JEOL 1200 EX (Tokyo, Japan) electron microscope.

Lipid extraction. The lipids were extracted with chloroform/methanol/water as described by Bligh & Dyer (1959). The fresh aerial part of plant (25-30 g) was homogenized with 20 ml methanol and refluxed for 5 minutes in order to inactivate the lipases. An equal volume of chloroform was added and after 24h the mixture was filtered, and an equal amount of water was added. The lower layer (total lipophilic extract) was evaporated under vacuum and kept at –30°C. The amounts of lipophilic components were determined gravimetrically.

Lipid and fatty acid analysis. For lipid classes and fatty acids analyses part of the total lipophilic extract (50 mg) was applied on 20×20 cm silica gel G (Merck) plate (layer thickness 0.5 mm) and then the plate was developed with chloroform-methanol-acetone-acetic acid (70:14:24:0.4 v/v/v/v) as a mobile phase. The spots of the main lipid classes were visualized under UV-light, scrapped off with the silica gel layer and transferred in small vials with Teflon screw caps. Five ml of 15% acetyl chloride in absolute methanol were added and the vials were heated for 4 hours at 55°C (Christie, 1989). After cooling, the samples were diluted with water and the obtained fatty acids methyl esters (FAME) were extracted twice with hexane (2×5 ml). The FAME in combined hexane extracts were purified by preparative thin–layer chromatography (TLC) on 20×20 cm silica gel G (Merck) plates (layer thickness 0.5 mm) developed with hexane–acetone (95:5 v/v). The spots of the FAME were visualized under UV light, scrapped off with the silica-gel layer and eluted with diethyl ether. The amount of each sample was determined gravimetrically.

Fatty acid analysis was performed using Gas Chromatograph with Flame Ionization Detector Agilent 7890B, equipped with Agilent7693 Autosampler with 10 μl syringe and with capillary column SGE BPX70 (60 m x 0.25 mm x 0.25 μm). Nitrogen was the carrier gas at flow rate of 1.2 ml/min. The column temperature was programmed from 80°C (hold for 1 min) to 130°C by step of 8°C/min and then to 250°C by step of 5°C/min. The injector and detector temperatures were 245°C and 255°C, respectively; split 15:1. Instrument control, data acquisition and data processing were performed by GC software Clarity v.8.0.0.125. The fatty acids were identified by comparison of the retention times with that of reference mixture F.A.M.E. Mix C8-C24 (Sigma-Aldrich). The relative amounts of the fatty acids were determined from peak areas of the respective methyl esters.

The fluidity of the membrane lipids was expressed by the level of unsaturation, calculated as double bond index (DBI = 18:1 x 1 + 18:2 x 2 + 18:3 x 3).

Results and Discussion

Due to their immobile lifestyle, plant organisms are able to survive only by their ability to build rapid and highly adaptive responses to ever-changing environments. Under field conditions the situation is frequently much more complicated, since various interferences between numerous factors co-occur. As a means of overcoming abiotic and biotic limitations, plants have different adaptive and protective strategies.

As model plants to study structural-functional peculiarities of chloroplast membranes influenced by the specific environmental conditions some glycophytic and halophytic *Lactuca* species that inhabits different areas are chosen. *L. serriola* is a glycophytic drought-resistant species mainly found in solar habitats whether glycophytic *L. quercina* generally inhabits humid areas preferably in shadow or in partial shade, and is considered drought intolerant. *L. tatarica* is an extreme halophyte plant from Bulgarian Black sea coast.

The influence of environmental factors is manifested in alterations in structure of thylakoid membranes and the photochemical efficiency of photosystems, especially photosystem II (PSII) as main stress sensitive site in plants. The possibility of fast and reliable monitoring of the effectiveness of the operation of PSII oxygen-evolving enzyme complex of thylakoid membranes is the first prerequisite to solve the site and mechanisms of injury and adaptation to specific environmental conditions in different natural habitats.

TL glow curve parameters were used to access the functional features of PSII. TL proved to be a very sensitive and reliable biophysical method for investigation of the functioning of both PSII donor and acceptor side components (Sane, 2004). TL signals have been assigned to result from the thermal-activated recombination of the trapped electrons and stabilized positive equivalents on the reduced quinone acceptors (QA or QB) and on the S_2 (or S_3) oxidation state of the water-splitting complex, respectively. The illumination of dark-adapted chloroplast suspensions isolated from fully hydrated *Lactuca* leaves by continuous white light revealed a glow curve with a B band temperature maximum positioned at around 28.5–31 ◦C in *L. tatarica* (Table 1). The respective maximum in *L. seriola* and *L. quercina* chloroplast membranes appeared at lower temperature of 25-26°C and 23-24°C, respectively. The overall intensities of TL B-band were maximal in *L. seriola* samples and reaching minimal value in *L. tatarica* chloroplast membranes.

It is generally accepted that the amplitude of TL B-band is proportional to the number of centers having $S_{2(3)}$ Q_B^- charge pairs after flash illumination, while the maximal emission temperature of this band is a measure for redox span between the separate charges (Sane & Rutherford, 1986). It is reasonable to suggest that the dynamics in the relative number and stability of PSII reaction centers of the investigated *Lactuca* species can reflect some specific adaptive characteristics of the photosynthetic system of halophytic plants and glycophytic species with different drought tolerance to the environmental conditions in their natural habitats.

Table 1. Changes in the kinetic parameters of thermoluminescence (TL) and oxygen-evolving reactions of isolated *Lactuca* thylakoids. T_{max} (ºC) and B-band (%) are the emission temperature and the amplitude of the main TL B-band, recorded after one turn-over flash; A is the oxygen burst under continuous irradiation; A_1 and A_2, represent amplitudes of fast and slow components of initial oxygen burst.

Species	**T_{max} (°C)**	**B-band (%)**	**A (%)**	**A_1/A_2**
Lactuca seriola	25-26	100.0	100.0	2.46
Lactuca tatarica	28.5-31	70.7	72.3	2.22
Lactuca quercina	23-24	87.8	86.5	2.84

Another reliable approach used to study the properties of PSII complex in *Lactuca* thylakoids was to compare the kinetics of oxygen-evolving reactions (Table 1). The amplitude (A) of the initial oxygen burst and the area under the curve (which is a measure of the oxygen volume evolved) are proportional to all functionally active oxygen-evolving centers (i.e., both PSIIα in the grana and PSIIβ in the stroma domains). The decay kinetics after the oxygen burst are fitted with two exponential decay functions and the ratio A_1/A_2 of the obtained amplitudes for the fast (A_1) and the slow (A_2) components corresponds to the ratio of functionally active PSIIα to PSIIβ centers. The results suggest some decrease in the proportion of functionally active PSIIα centers in thylakoids in halophytic and drought tolerant *Lactuca* species which could be attributed to the reduced grana formation and dominant operation of the cooperative mechanism of oxygen evolution in stroma situated PSIIβ centers. (Maslenkova et al., 1993). It is supposed that the cooperative mechanism is realized by diffusion of oxygen precursors mainly within PSIIβ centers and is

characterized by a time constant lower than that of the non-cooperative Kok's mechanism, realized by PSIIα centers (Zeinalov & Maslenkova, 1996 b). The cycling of the rest operating PSIIα was typical for higher plants, thus suggesting no peculiarities in this parameter of investigated *Lactuca* species (data not shown).

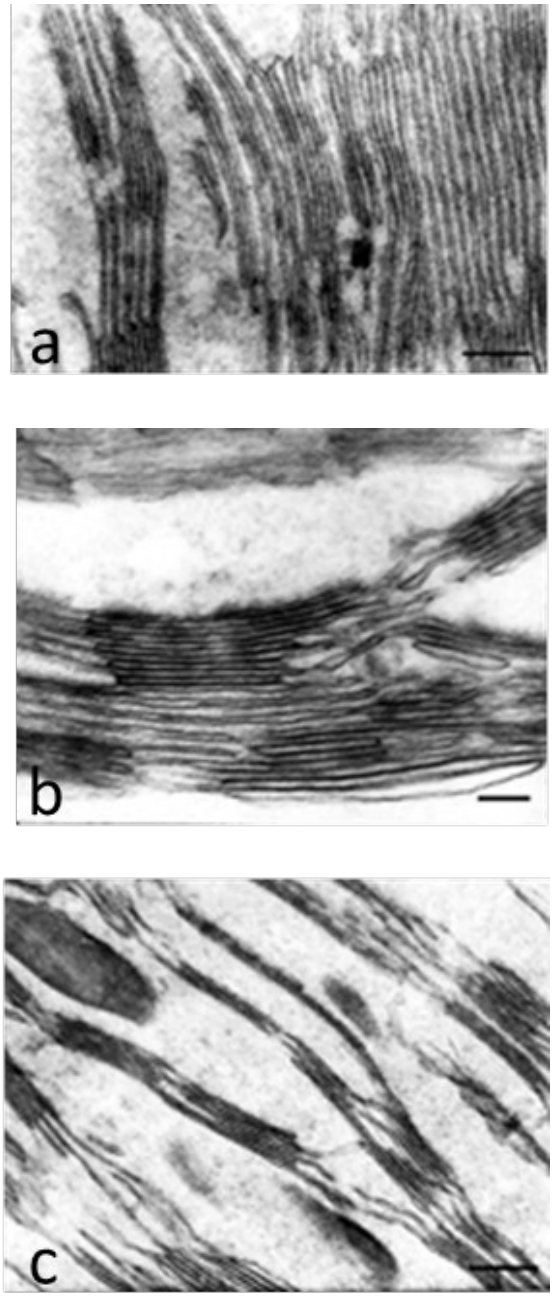

Fig. 1. Chloroplast ultrastructure in different *Lactuca* species:
a - *L. seriola,* **b** – *L. quercina,* **c –** *L. tatarica* (scale bar 200 nm).

The microscopic investigations obtained by TEM-analysis revealed that the structure of the chloroplasts in *L. seriola* were characterized by an elliptical shape and well-developed inner membrane system (Fig. 1-a). The grana represented different height. The number of thylakoids in them varies from 8-10 to 35, connected by evenly spaced stromal thylakoids of different lengths.

Chloroplasts in *L. quercina* are characterized by a well-developed inner membrane system composed of broad grana areas, most of them of low (5-8 thylakoids) and medium hight with about 15 thylakoids (Fig. 1-b). Most stromal thylakoids are short and fragmented between some faces.

Chloroplasts in halophyte *L. tatarica* species are characterized by a visibly smaller volume of the inner membrane system (Fig. 1-c). The grana are low (2 to 5-6 thylakoids) evenly distributed in the stroma and associated with long stromal thylakoids.

It is well known that lipids play an essential role in maintaining the integrity and functional activity of chloroplast membrane (Lichtenthaler, 1987). Differences in lipid and fatty acid composition may be species-specific or due to adaptation to varied environmental conditions. The lipid restructuring in the membranes as well as changes in the unsaturation of fatty acids play an important role in the acclimation of the photosynthetic machinery to changes in various forms of environmental stress (Allakhverdiev et al., 2010). In this respect the elucidation of the specific difference in the lipid classes and fatty acid composition of *Lactuca* membranes could bring important information in the interpretation of the obtained results of their functional activity (Table 2).

Table 2. Lipid classes in thylakoid membranes isolated from *Lactuca* leaves. The values obtained are means ± s.e. from three parallel measurements.

Species	Lipid classes (% of total)				
	MGDG	DGDG	SQDG	PG	DGDG/MGDG
L. tatarica	36.67±0.3	27.77±0.4	17.77±0.3	17.77±0.2	0.76
L. seriola	35.84±0.1	26.41±0.1	22.64±0.1	15.09±0.7	0.74
L. quercina	40.91±0.1	30.68±0.5	12.50±0.5	15.91±0.1	0.75

Photosynthetic membranes of plants are characterized by a high content of glycolipids dominated by galactolipids MGDG and DGDG. The fractions of MGDG and DGDG were higher in drought intolerant *L. quercina*, but the DGDG/MGDG ratio is nearly equal in all samples (Table 2). The fractions of the minor lipid constituents of thylakoid membrane show a gradual decrease of the amount of SQDG from *L. seriola* (22.64%) to *L. tatarica* (17.77%) and *L. quercina* (12.5%) while the difference in the amount of PG between investigated species was smaller.

Six common fatty acids were detected in the lipid classes of the respective thylakoid preparations, including palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) and the species variations in their relative content are presented in Table 3. The most abundant fatty acid in all the lipid classes was linolenic acid followed by palmitic and linoleic acids. The high level of unsaturation (Table 3), calculated as double bond index (DBI = 18:1 x 1 + 18:2 x 2 + 18:3 x 3) was most obvious for MGDG reaching slightly lower values in *L. quercina*. The content of polyunsaturated fatty acids in the lipid matrix is one of the major factors determining membrane fluidity. The high level of lipids unsaturation provides the mobility of lateral separated pigment-protein complexes and electron carriers in the electron transport chain thus affecting the effectiveness of photosynthetic machinery. It has been proven that polyunsaturated fatty acids in thylakoid lipids play an important role in the stability of oxygen evolving machinery and increases in unsaturated fatty acids in membrane lipids protects PSII against photoinhibition (Sui & Han, 2014).

Table 3. Species variations in relative fatty acids content of lipid classes. The values obtained are means ± s.e. from three parallel measurements; double bond index (DBI = 18:1 x 1 + 18:2 x 2 + 18:3 x 3); n.d. – not detected.

Lipid classes	Species	16:0	16:1	18:0	18:1	18:2	18:3	DBI
MGDG	*L. tatarica*	6.0±0.1	n.d.	1.6±0.2	0.5±0.7	2.6±0.2	86.5±0.1	265.2
	L. seriola	4.1±0.3	0.2±0.1	0.8±0.1	0.5±0.4	0.9±0.1	85.5±0.3	258.8
	L. quercina	10.5±0.3	n.d.	2.1±0.1	0.9±0.1	3.7±0.2	71.1±0.2	221.6
DGDG	*L. tatarica*	44.7±0.2	0.54±0.1	5.7±0.3	1.5±0.1	2.1±0.1	21.1±0.5	47.9
	L. seriola	23.9±0.5	1.0±0.2	2.8±0.1	0.8±0.1	1.5±0.1	24.7±0.3	77.8
	L. quercina	30.6±1.1	n.d.	5.6±0.1	1.5±0.4	3.2±0.1	45.0±0.3	97.9
SQDG	*L. tatarica*	40.2±1.3	8.9±0.2	5.2±0.2	2.2±0.3	8.6±1.8	25.6±0.1	96.2
	L. seriola	13.3±0.6	0.6±0.1	3.5±0.1	8.5±1.0	9.0±0.8	26.3±0.7	105.4
	L. quercina	33.0±0.1	7.0±0.3	5.1±0.1	2.0±0.1	9.3±0.2	33.2±1.1	120.2
PG	*L. tatarica*	32.1±1.1	10.5±0.5	5.9±0.1	2.4±0.1	11.2±0.1	25.8±0.3	102.2
	L. seriola	19.1±0.2	5.6±0.1	5.7±0.2	4.1±0.1	4.2±0.1	27.3±0.1	94.4
	L. quercina	32.9±0.4	10.2±0.2	3.5±0.2	1.8±0.2	11.8±0.1	24.8±0.1	99.8

Conclusion

During our studies of halophytic *L. tatarica* and glycophytic species *L. seriola* and *L. quercina*, using a highly sensitive TL and polarographic techniques were demonstrated high capability of chloroplast membranes to maintain efficient PSII function. In the same time, we observed some peculiarities of PSII redox reactions and mechanisms of oxygen evolution that can reflect specific adaptive strategies of the photosynthetic system, under environmental conditions in their natural habitat. Salt and drought tolerant species demonstrated some stabilization of PSII charge pairs, evidenced by higher temperature maximum of the main TL B peak and a decrease in the proportion of functionally active PSIIα centers which could be attributed to the reduced grana formation and dominant operation of the cooperative mechanism of oxygen evolution in stroma situated PSIIβ centers. The analysis of lipid classes and their fatty acids composition show the existence of qualitative and quantitative differences that can contribute in this regard. The results of our experiments show specific characteristics of photosynthetic membranes in halophytic and glycophytic *Lactuca* species, reflecting different adaptive strategies to environmental conditions in their natural habitats.

Acknowledgements

This work was supported partially by grant КП-06-Н21/12/2018 of National Science Fund, Bulgaria and by Contract DO1-154/28/08/2018/ „Scientific Infrastructure on Cell Technologies in Biomedicine (SICTB)".

References

Allakhverdiev, S., Los D. & Murata, N. (2010). Regulatory roles in photosynthesis of unsaturated fatty acids in membrane lipids. In H. Wada, N. Murata (Eds). *Lipids in Photosynthesis, AIPH, 30*, 373–388. doi:10.1007/978-90-481-2863-1_17.

Bligh, E. & Dyer, W. (1959). A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol., 37*, 911-917. doi: 10.1139/o59-099.

Chadwick, M., Trewin, H., Gawthrop, F. & Wagstaff, C. (2013). Sesquiterpenoids Lactones: Benefits to Plants and People. *Int. J. Mol. Sci., 14*, 12780–12805. doi: 10.3390/ijms140612780.

Christie, W. (1989). HPLC & GC-Mass Spectrometry in the Analysis of Fatty Acids. In R.C. Cambie (Ed.). *Fats for the Future.* (pp. 335–344). Chichester, UK: Ellis Horwood.

D'Andrea, L., Broennimann, O., Kozlowski, G., Guisan, A., Morin, X., Keller−Senften, J. & Felber, F. (2009). Climate change, anthropogenic disturbance and the northward range expansion of *Lactuca serriola* (Asteraceae). *Journal of Biogeography*, *36(8)*, 573-1587. doi: 10.1111/j.1365-2699.2008.02060.x.

Lichtenthaler, H. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology, 148*, 350–382. doi: 10.1016/0076-6879(87)48036-1.

Lichtenthaler, H. (1998). The stress concept in plants: an introduction. *Ann N. Y. Acad. Sci. Jun*., 30, 851, 187-98. doi: 10.1111/j.1749-6632.1998.tb08993.x.

Maslenkova, L., Zanev, Y. & Popova, L. (1993). Adaptation to salinity as monitored by PSII oxygen evolving reactions in barley thylakoids. *J. Plant Physiol., 142*, 629–634.

Sane, P. (2004). Thermoluminescence. A technique for probing photosystem II. Methods in Molecular Biology. In R. Carpentier (Ed.)*, Photosynthesis Research Protocols.* (pp. 229–248). Totowa, NJ: Humana Press Inc. Doi: 10.1385/1-59259-799-8:229.

Sane, P. & Rutherford, A. (1986). Thermoluminescence from photosynthetic membranes. In Govindjee, J. Amesz, J. & D.C. Fork (Eds.). *Light Emission by Plants and Bacteria*. (pp. 329–361). Academic Press, Elsevier Inc.

Stoyanova, S., Stoyanov, K., Raycheva,Tz., Desheva, G., Uzundzhalieva, K., Valchinova, E. (2015). Wild relatives of cultivated plants - inventorization and summarization of information for dissemination, use, conservation and proposals for future politics. Online database. Retrieved from http://www.botanica.gallery.

Sui, N. & Han, G. (2014). Increases of unsaturated fatty acids in membrane lipids protects photosystem II from photoinhibition under salinity in different halophytes. *J. Agricult. Sci., 6(12)*, 251–264.

Tamaki H., Robinson R., Anderson J. & Stoewsand G. (1995). Sesquiterpene Lactones in Virus-Resistant Lettuce. *J. Agric. Food Chem. 43, 1*, 6–8.

Wang X-X., Gao X. & Jia Z-J. (2010). Sesquiterpenoids from Lactuca tatarica. *Fitoterapia, 81*, 42 – 44. doi: 10.1016/j.fitote.2009.07.001.

Zeinalov, Y. & Maslenkova, L. (1996a). A computerized equipment for thermoluminescence investigations. *Bulg. J. Plant Physiol., 22*, 88–94.

Zeinalov, Y. & Maslenkova, L. (1996b). *Mechanisms of photosynthetic oxygen evolution*. *Handbook of Photosynthesis.* In M. Pessarakli (Ed.). (pp. 129–150). New York, USA: Marcel Dekker.