Photoinhibition of primary photosynthetic processes in hydrated *Polytrichum commune*: Analysis of non-photochemical quenching affecting species resistance

Gabriella Nora Maria Giudici

University of Naples Federico II, Department of Agriculture, 80055 Portici, Italy

Abstract

A moss from the alpine environment of the Jeseníky mountains, *Polytrichum commune*, was studied under lab-induced light stress to analyse photoinhibition (PI) stress response; three PI doses were used: PAR 1500 µmol m^{-2} s^{-1} for 60 min., 1200 µmol m^{-2} s^{-1} for 60 min. and 1200 µmol m^{-2} s^{-1} for 30 min.; in the last one the added component of slight desiccation stress was added. Chlorophyll fluorescence parameters were plotted as time series, immediately before and after the PI treatments, then every 20 minutes for three hours (recovery period). F_V/F_M, Φ_{PSII} and NPQ parameters and quenching components were analysed. Decreasing courses and final values of F_V/F_M and Φ_{PSII} parameters along with increased values of NPQ clearly indicated PI stress response, although not very severe. Quenching parameters analysis showed a dominant role played by xanthophyll pigments along with changes in PS II in the non-photochemical energy quenching. Dehydration contributed additional value to NPQ. All these factors are consistent with the adaptation of the species to harsh conditions of alpine environments.

Key words: NPQ quenching, *Polytrichum*, chlorophyll fluorescence, photoinhibition, lamellae, alpine environment

DOI: 10.5817/CPR2019-2-14

Introduction

Photoinhibition (PI) is a decrease of photosynthetic performance due to high light exposure. High light can cause closure of photosynthetic reaction centres, therefore inducing an excess of adsorbed energy which in turn cannot be utilized through photosynthetic pathways and could be dangerous to structures and complexes of the whole chloroplastic apparatus. Mosses and lichens in alpine environments are usually high light tolerant species as a result of evolutionary adaptation to bright days which can occur through the year. Since the pioneering study of Murray et al. (1993), PI as well as photoprotective mechanisms activated during PI has been in focus in a great variety of mosses. Majority of them were found PI tolerant or resistant. However, some sensitive species have been identified as well (*e.g. Pleuro-*

Received October 25, 2019, *accepted* December 18, 2019.
*Corresponding author: G. N. M. Giudici <gabriellanoramaria.giudici@unina.it>
Acknowledgements: The author wishes to thank the ECOPOLARIS project (CZ.02.1.01/0.0/0.0/16_013/0001708) for the infrastructure, Czech Ministry of Education for the facilities and the staff of the EEL Laboratory (CzechPolar2 infrastructure LM2015078).

zium schreberi from shade habitats - Hájek et al. 2009). Only few studies have dealt with mechanistic aspects of photoinhibition and photoprotection of desiccation-tolerant photoautotrophs (Heber et al. 2007, Veerman et al. 2007, Heber 2008, Heber et al. 2009). Apart from the studies focused on PI in mosses in wet state, there have been several others dealing with photoprotective mechanisms in desiccating mosses (*e.g.* Csintalan et al. 1999, Nabe et al. 2007). High light resistance is the result of biochemical, physical and structural traits and protective mechanisms. Maximum and effective quantum yield (F_V/F_M, Φ_{PSII}) of photosystem II along with slow Kautsky kinetics are widely used to measure plant response to stress inducing factors, together with non-photochemical quenching (NPQ) values. Stress factors cause general lowering of F_V/F_M and Φ_{PSII} transient and flattening of shapes (*i.e.* general lowering of their peaks), see *e.g.* Jägerbrand and Kudo (2014). At the same time they induce increase in NPQ time course, whose components are indicative of the different activated mechanisms. In mosses, non-photochemical quenching has been studied as dependent on desiccation and photoinhibition Heber et al. (2006). It is generally accepted that there are three different components of non-photochemical quenching (reviewed by *e.g.* Yamakawa et al. 2012). The first mechanism, is active in hydrated mosses and controlled by the protonation of a thylakoid protein. Other two mechanisms are activated in desiccating thalli. One of them permits exciton migration towards the light-harvesting complexes, antenna pigments in particular, where fast thermal dissipation takes place. The third mechanism is based on the reversible photo-accumulation of a radical that acts as a quencher of excitation energy in reaction centres of photosystem II. The drought-induced quenching brings the acceleration of the chlorophyll fluorescence decay rate and rapid dissipation of excitation energy into heat (Yamakawa and Itoh 2013). Although many studies dealt with PI and desiccation, little has been written about photoinhibition in fully wet state. In the study we analysed chlorophyll fluorescence induction curve supplemented with analysis of quenching mechanisms in fully wet *Polytrichum commune*.

Material and Methods

General characteristics of Polytrichum commune

The species within the genus *Polytrichum* have several adaptations helping them to keep water in the thallus and maintain photosynthesis in leaves. They comprise of 1) the ability of water transport from the base of the plant thanks to water-conducting cells (hydromes, in central strand of the stem, and hydroids, in the costa of leaves) and 2) special structures on the upper leaf surface (lamellae). The lamellae are ridges-like structures that run parallel to each other over the length of the leaf. They are several cell layers long and tall and a single cell wide (8 μm in our sample) (Fig. 1). The lamellae cells are rich in chloroplasts and increase the effective area for photosynthesis (Thomas et al. 1996). They are effective in keeping water in photosynthesizing cells even during initial phase of desiccation. Moreover, moist air remains between the lamellae protecting the leaves from fast dehydration. Lamellar cells may undergo structural and functional changes in some periods of growing season, more pronounced in winter time (Ljubešic et al. 2005). *Polytrichum commune* is easily identified because of the lamella apical cell which is indented or cup-like shaped (Fig. 1).

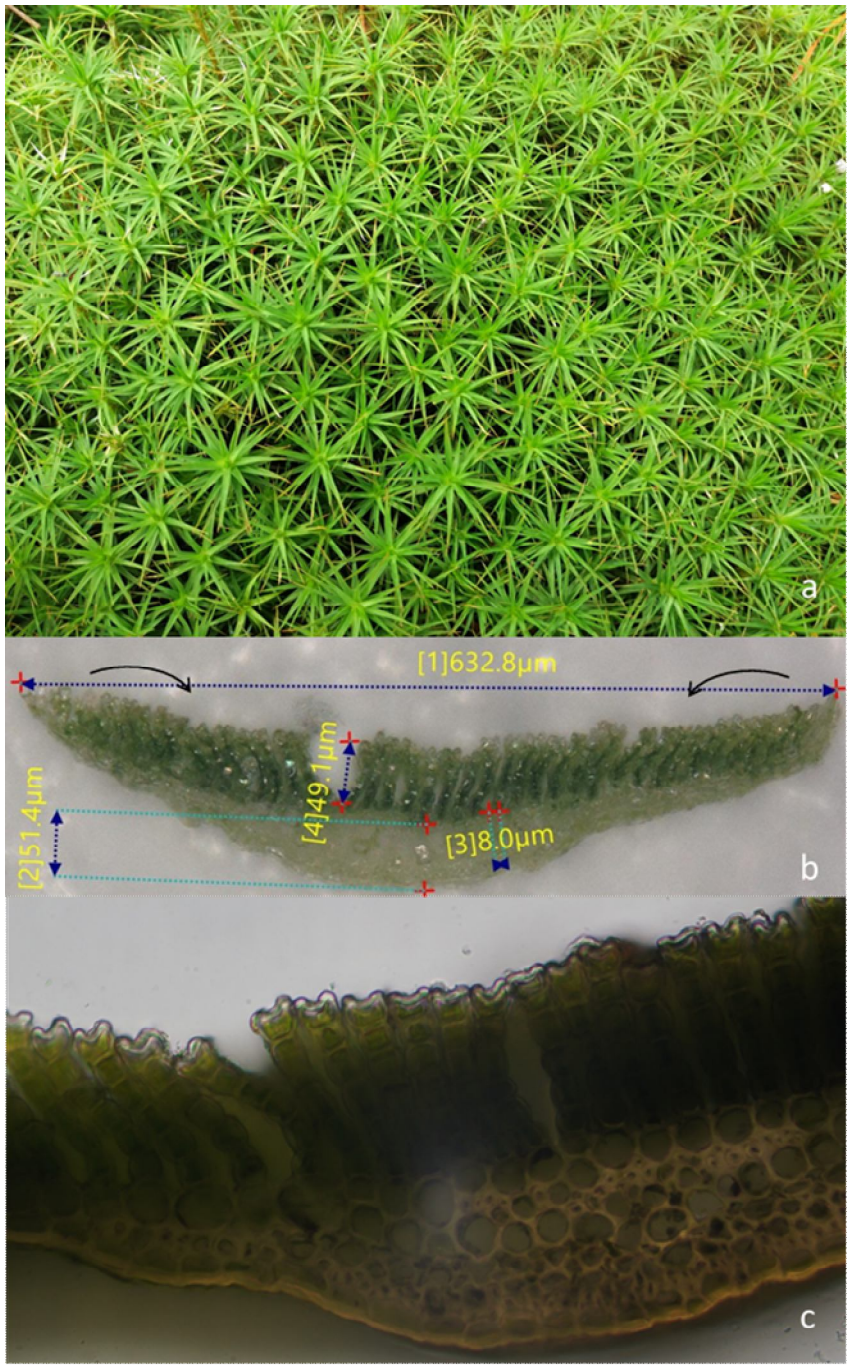

Fig. 1. *Polytrichum commune* leaves (a) and microscopy section of a single leaf showing lamellae (b). The arrows show the direction of the movement when the leaf folds inside to protect from dehydration. Apical cells (c) with the typical indented shape; chloroplasts are visible through the cells.

Experimental plants collection and handling

Shoots of *Polytrichum commune* were collected in Tabulové skály rocks, Jeseníky Mts. (1415 m a.s.l.), and stored in wet condition (regularly sprayed) under dim light (20 µmol m^{-2} s^{-1}) at 5°C. Experiments were run soon after the collecting. Shoots were put in small containers with distilled water and kept fully wet by constantly spraying them with distilled water during the treatments and the measuring periods; ice was put around, but not in the containers, to maintain low temperature (below 10°C). Three light treatments were induced (LED source PSI SL3500-498, Photon Systems Instruments, Czech Republic) at PAR 1500 µmol m^{-2} s^{-1} for 60 min. (n°1), 1200 µmol m^{-2} s^{-1} for 60 min. (n°2) and 1200 µmol m^{-2} s^{-1} for 30 min. (n°3), in which we added the desiccation components, *i.e.* we used a set of shoots which were fully hydrated (100% RWC) along with another set which had 85-90% RWC at the beginning of the PI; during the photoinhibition, distilled water was sprayed around the samples and above them, enough to prevent rapid desiccation, but not increasing the actual RWC. During the three hours measurements, water was sprayed around the samples but not directly on them. Chlorophyll fluorescence parameters were plotted as time series, immediately before and after the PI treatments, then every twenty minutes for three hours (recovery period) after the end of the PI treatments. F_V/F_M, Φ_{PSII} and NPQ, were recorded after 5 min. pre-darkening with a PSI Handy Fluorcam HFC-010 (Photon Systems Instruments, Czech Republic). Other parameters were calculated with the following equations:

$$NPQ = (F_M^*-F_M')/F_M' \quad \textit{Eqn.}\ 1$$

$$qN = (F_M- F_0)^*– (F_M'- F_0')/(F_M- F_0)^* \quad \textit{Eqn.}\ 2$$

$$qE = (F_M^*-F_M'')/(F_M- F_0)^* \quad \textit{Eqn.}\ 3$$

$$qI = (F_M- F_0)^*– (F_M''- F_0'')/(F_M- F_0)^* \quad \textit{Eqn.}\ 4$$

$$F_V/F_M =(F_M- F_0)/F_M \quad \textit{Eqn.}\ 5$$

F_M^* was always referred to control (before PI) values
$(F_M - F_0)^*$ F_M and F_0 were always referred to control (before PI) values

Results

Time courses analysis of F_V/F_M and Φ_{PSII}

In our experiment, both parameters F_V/F_M and Φ_{PSII} were indirectly correlated with the severity of light treatments, their values decreasing with increasing doses (*i.e.* time and intensity). The time courses in both parameters showed: first a clearly defined drop immediately after the end of PI, then a quick but smaller rise within the following 15 min. (fast phase recovery), finally a slower increasing recovery phase toward control levels (before PI), (slow phase recovery) (Fig. 2).

In Φ_{PSII}, only the second treatment (1200 µmol m^{-2} s^{-1} for 60 min.) showed a marked difference between the fast and slow phase of recovery, the other two treatments having a slight and average constant rate of the rising parameter. In all cases, the above-mentioned check points of the curves occurred always at the same time, in both parameters in all treatments. Majority of recovery (*i.e.* above 75% of initial values) was reached within 15 min. after the end of PI in both parameters.

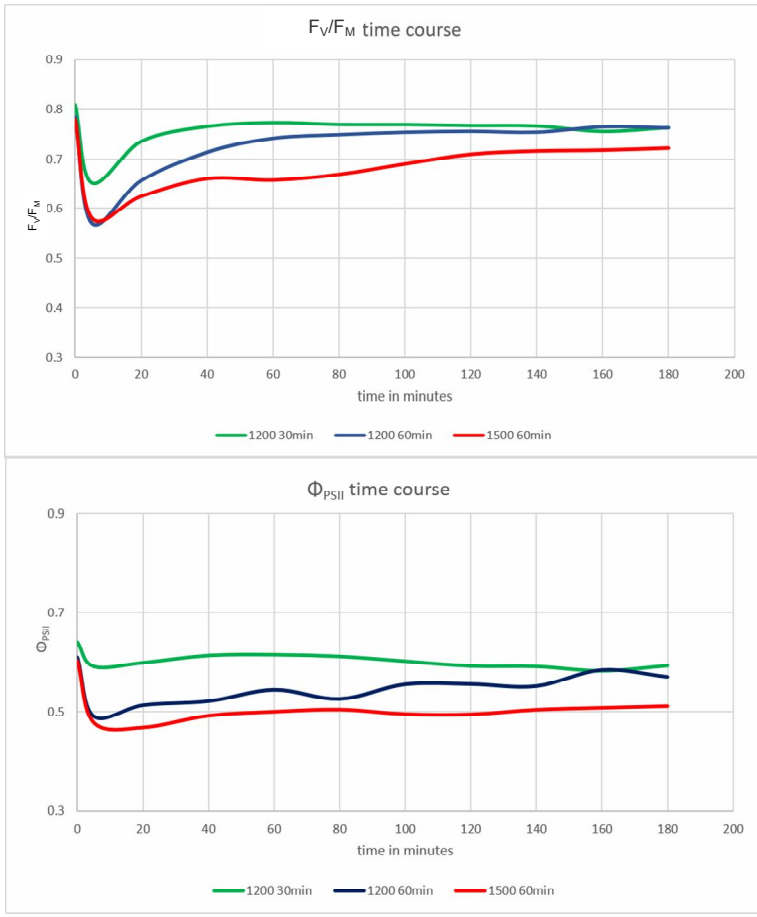

Fig. 2. F_V/F_M and Φ_{PSII} average time courses, before (control time = 0) and during three hours after the treatment (max value of standard deviation 0.11) .

Control values, before PI, were never reached after three hours of recovery period in any of the treatments for both parameters.

NPQ showed a rise peak in the first measurements after the end of PI, followed by a two-phase decline, first quick then slow. However, the least severe treatment (1200 µmol m^{-2} s^{-1} for 30 min.) showed constant rate of decrease during the recovery period (Fig. 3).

In contrast with F_V/F_M and Φ_{PSII} courses, there was no other synchronism check points on the curves related to different PI doses than the rising peaks.

Values of NPQ were found directly dose-dependent. Steepness of NPQ curve before reaching the peak was in a direct relationship with the PI dose, in contrast with time course of recovery phase (Fig. 4). Once again final NPQ values did not reach control values in any treatment.

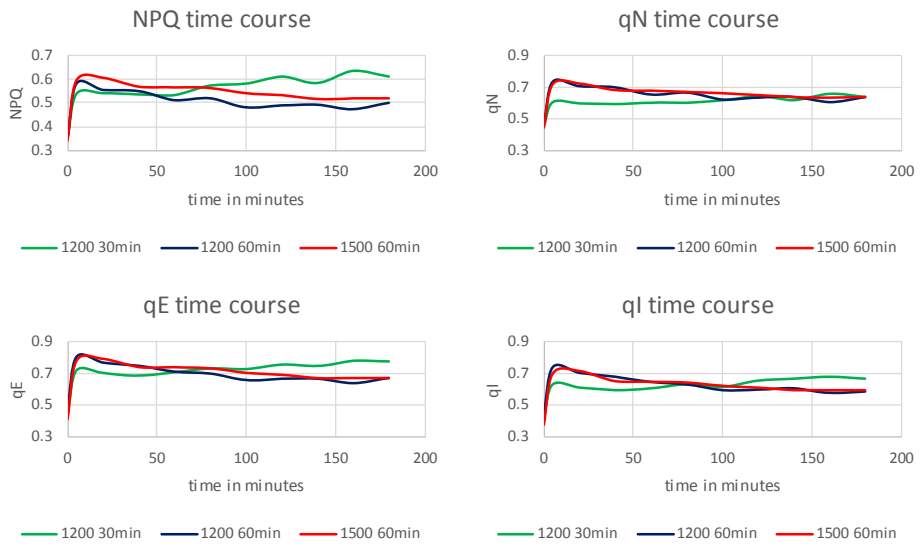

Fig. 3. NPQ, qN, qE, qI average time courses (max of average standard deviation 0.36).

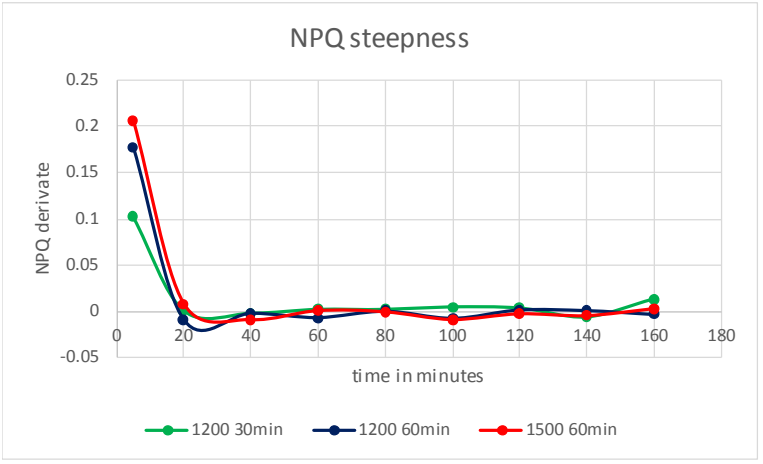

Fig. 4. NPQ steepness time course.

Quenching components

qN, qI and qE were considered. All showed comparable time courses, the general trend being comparable to that of NPQ, with more pronounced peaks and higher similarities among the curves. In all treatments, the fast phase of recovery was more prolonged in the highest dose.

Discussion

All treatments led to photoinhibition in the moss; it is clearly indicated by the lowering of both F_V/F_M and Φ_{PSII} time courses proportional to the PI dose, by the drop of F_V/F_M and Φ_{PSII} values immediately after the PI and by F_V/F_M and Φ_{PSII} final values (after three hours of recovery) lower than control (before PI). Moreover, photoprotecting mechanisms were activated as shown by the quick increase of NPQ immediately after the PI, once again proportional to PI dose. Light dose-dependent increase in NPQ was well documented in *P. formosum* (Marschall and Proctor 2004).

Non-photochemical quenching composing parameters showed a slightly higher proportion of the quick reversing component (qE), suggesting that a dominant role was played by the xanthophyll pigments cycle, as well as conformation and functional changes in PS II (related to the qI component). It must be noticed that in our data the addition of qE + qI is not below 1 because in the equation we used to calculate the parameters we kept constant F_M and F_0 values referred to control (*see* Equations 3 and 4).

It could be said that the doses we used induced a mild PI because of the following reasons:

1. After three hours of recovery, even though control values were not reached, the decrease of final values was less than 8% of initial values in all treatments (15% only in Φ_{PSII} final values of the most severe treatment, 1500 μmol m^{-2} s^{-1} for 60 min.)
2. The lowering of F_V/F_M and Φ_{PSII} curves was not substantial (less than 20%)
3. Majority of recovery during the recovery period was achieved quickly (70% in the first 10 min.).

Lowest treatment

The NPQ values in the lowest treatment (1200 μmol m^{-2} s^{-1} for 30 min.) showed a time course different from the others because of the additional effect of desiccation during the measuring time: the values that should decline quickly and continuously after the peak of the first measurement, decreased instead slowly, to rise again 40 min. after the end of the PI (dehydration effect). By the end of the recovery period, we observed NPQ values higher than the peak (Fig. 5); which could be explained by the additional effect of desiccation as was reported for *Polytrichum formosum* (Proctor et al. 2007) in which decreasing RWC caused increasing values of NPQ between 80 and 50% of RWC.

Confirmation is added by the decreasing course of Fs (Fig. 5), which is reported to be correlated with desiccating conditions. In desiccating mosses, Fs decreases thanks to desiccation-induced quenching as reported by *e.g.* Heber et al. (2007) for *Rhytidium rugosum*.

Capacity of protective mechanisms forming NPQ is species-specific in mosses. Some mosses have orange carotenoid pigments which may help prevent photosystem damage. For *Ceratodon purpureus,* photoprotective carotenoids including violaxanthin are reported (Post 1990). Proportion of underlying biophysical, biochemical and molecular responses activated in photoinhibited mosses and forming NPQ remains unclear because of the complex manner of PI response of chloroplastic apparatus. Mosses share many of photoprotective mechanisms with the vascular plants, however, there are some key differences in the photoprotection available (Robinson and Waterman 2014).

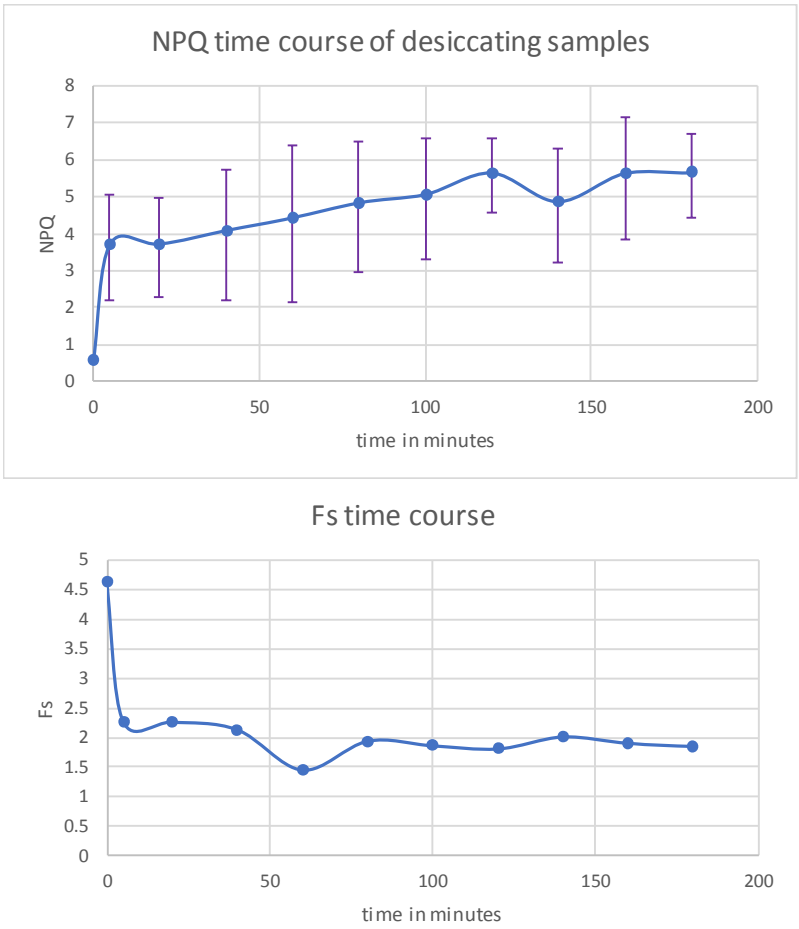

Fig. 5. NPQ and Fs average time course in the samples exposed to desiccation (*i.e.* samples which were not kept in fully wet state by constant spraying) in treatment n°3 (1200 µmol m^{-2} s^{-1} for 30 min.).

In mosses, fastest component of non-photochemical quenching occurs within PS II antenna system by the action of two essential light-harvesting complex (LHC)-like proteins, photosystem II subunit S (PSBS) in plants and light-harvesting complex stress-related (LHCSR) (Pinnola et al. 2015). Recent studies (*e.g.* Stella 2016, Dikaios et al. 2019) confirmed the role of LHCSR proteins in NPQ, particularly in thermal dissipation in photoinhibited mosses. Some studies, however, suggest that LHCSR proteins may be active in energy quenching (qE) as well (Alboresi et al. 2010, Gerotto et al. 2012).

Apparently, lichens possess a dominant mechanism of photoprotection which does not require zeaxanthin or a protonation reaction (Heber 2011).

Conclusions

According to our data, *Polytrichum commune* could be considered medium resistant to photoinhibition relating to the PI doses we used; moderate water loss could increase protective mechanisms activation (non-photochemical quenching in particular) but does not influence the overall photosynthetic performance. Protective mechanisms are activated in case of intense light and moderate water deficiency, which are both occurring in the mountains, after the snow cover is melted down and water availability is limited. Further studies should point to the extreme conditions of both light and desiccation levels which could be tolerated by this moss in the field. Since mosses are poikilohydric autotrophs active during winter period, low temperature photoinhibition of thalli in wet state should be taken into account when evaluating species-specific differences in resistance to photoinhibition (Lovelock et al. 1995a, b).

References

ALBORESI, A., GEROTTO, C., GIACOMETTI, G. M., BASSI, R. and MOROSINOTTO, T. (2010): *Physcomitrella patens* mutants afected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization. *Proceedings of National Academy of Science* USA, 107: 11128-11133.

CSINTALAN, Z., PROCTOR, M. C. F. and TUBA, Z. (1999): Chlorophyll fluorescence during drying and rehydration in the mosses *Rhytidiadelphus loreus* (Hedw.) Warnst., *Anomodon viticulosus* (Hedw.) Hook. & Tayl. and *Grimmia pulvinata* (Hedw.) Sm. *Annals of Botany*, 84: 235-244.

DIKAIOS, I., SCHIPHORST, CH., DALL'OSTO, L., ALBORESI, A., BASSI, R. and PINNOLA, A. (2019): Functional analysis of LHCSR1, a protein catalyzing NPQ in mosses, by heterologous expression in *Arabidopsis thaliana*. *Photosynthesis Research*, 142: 249-264.

GEROTTO, C., ALBORESI, A., GIACOMETTI, G. M., BASSI, R. and MOROSINOTTO, T. (2012): Coexistence of plant and algal energy dissipation mechanisms in the moss *Physcomitrella patens*. *New Phytologist*, 196: 763-773.

HÁJEK, T., TUITTILA, E.-S., ILOMETS, M. and LAIHO, R. (2009): Light responses of mire mosses: a key to survival after water-level drawdown? *Oikos*, 118: 240-250.

HEBER, U. (2008): Photoprotection of green plants: A mechanism of ultra-fast thermal energy dissipation in desiccated lichens. *Planta*, 228: 641-650.

HEBER, U. (2011): Lichens and Bryophytes: Light Stress and Photoinhibition in Desiccation/ Rehydration Cycles – Mechanisms of Photoprotection. *In*: U. Lüttge, E. Beck, D. Bartels (eds.): Plant Desiccation Tolerance. Springer, 386 p.

HEBER, U., BILGER, W. and SHUVALOV, V. A. (2006): Thermal energy dissipation in reaction centres and in the antenna of photosystem II protects desiccated poikilohydric mosses against photo-oxidation. *Journal of Experimental Botany*, 57(12): 2993-3006. doi: 10.1093/jxb/erl058

HEBER, U., AZARKOVICH, M. and SHUVALOV, V. A. (2007): Activation of mechanisms of photoprotection by desiccation and by light: Poikilohydric photoautotrophs. *Journal of Experimental Botany*, 58: 2745-2759.

HEBER, U., BILGER, W., TÜRK, R. and LANGE, O. L. (2009) Photoprotection of reaction centres in photosynthetic organisms: mechanisms of thermal energy dissipation in desiccated thalli of the lichen *Lobaria pulmonaria*. *New Phytologist*, doi: 10.1111/j.1469-8137.2009.03064.x

JÄGERBRAND, A. K., KUDO, G. (2014): Short-term responses in maximum quantum yield of PSII (Fv/Fm) to ex situ temperature treatment of populations of bryophytes originating from different sites in Hokkaido, Northern Japan. *Plants* (Basel). 2016 Jun; 5(2): 22. doi: 10.3390/ plants5020022.

LJUBEŠIC, N., WRISCHER, M., PREBEG, T. and DEVIDÉ, Z. (2005): Structural changes of lamellar cells in leaves of the moss *Polytrichum formosum* Hedw. during winter freezing and thawing processes. *Acta Botanica Croatica*, 64: 219-226.

LOVELOCK, C. E., OSMOND, C. and SEPPELT, R. D. (1995a): Photoinhibition in the Antarctic moss *Grimmia antarctici* card when exposed to cycles of freezing and thawing. *Plant Cell and Environment*, 18: 1395-1402.

LOVELOCK, C. E., JACKSON, A. E., MELICK, D. R. and SEPPELT, R. D. (1995b): Reversible photoinhibition in Antarctic moss during freezing and thawing. *Plant Physiology*, 109: 955-961.

MARSCHALL, M., PROCTOR, M. C. F. (2004): Are bryophytes shade plants? Photosynthetic light responses and proportions of chlorophyll *a*, chlorophyll *b* and total carotenoids. *Annals of Botany*, 94: 593-603.

MURRAY, K. J., TENHUNEN, J. D. and Nowak, R. S. (1993): Photoinhibition as a control on photosynthesis and production of *Sphagnum* mosses. *Oecologia*, 96: 200-207.

NABE, H., FUNABIKI, R., KASHINO, Y., KOIKE, H. and SATOH, K. (2007): Responses to desiccation stress in bryophytes and an important role of dithiothreitol-insensitive non-photochemical quenching against photoinhibition in dehydrated states. *Plant and Cell Physiology*, 48(11): 1548-1557.

POST, A. (1990): Photoprotective pigment as an adaptive strategy in the Antarctic moss *Ceratodon purpureus*. *Polar Biology*, 10: 241-245.

PINNOLA, A., GHIN, L., GECCHELE, E., MERLIN, M., ALBORESI, A., AVESANI, L., PEZZOTTI, M., CAPALDI, S., CAZZANIGA, S. and BASSI, R. (2015): Heterologous expression of moss Light-harvesting Complex Stress-related 1 (LHCSR1), the chlorophyll a-xanthophyll pigment-protein complex catalyzing non-photochemical quenching, in *Nicotiana* sp. *The Journal of Biological Chemistry*, 290: 24340-24354.

PROCTOR, M. C. F., LIGRONE, R. and DUCKETT, J. G. (2007): Desiccation tolerance in the moss *Polytrichum formosum*: Physiological and fine structural changes during desiccation and recovery. *Annals of Botany*, 99: 75-93.

ROBINSON, S., WATERMAN, M. (2014): Sunsafe bryophytes: Photoprotection from excess and damaging solar radiation. *In*: D. T. Hanson, S. K. Rice: Photosynthesis in bryophytes and early land plants. Springer, pp. 113-130.

STELLA, G. R. (2016): Light stress and photoprotection in green algae, mosses and diatoms. Ph.D. thesis, University of Verona and University Pierre et Marie Curie, 144 p.

THOMAS, R. J., RYDER, S. H., GARDNER, M. I., SHEETTZ, J. P. and NICHIPOR, S. D. (1996): Photosynthetic functions of leaf lamellae in *Polytrichum commune*. *The Bryologist*, 99: 11.

VEERMAN, J., VASILEV, S., PATON, G. D., RAMANAUSKAS, J. and Bruce, D. (2007): Photoprotection in the lichen *Parmelia sulcata*: The origins of desiccation induced fluorescence quenching. *Plant Physiology*, 145: 997-1005.

YAMAKAWA, H., FUKUSHIMA, Y., ITOH, S. and HEBER, U. (2012): Three different mechanisms of energy dissipation of a desiccation-tolerant moss serve one common purpose: To protect reaction centres against photo-oxidation. *Journal of Experimental Botany*, 63: 3765-3775.

YAMAKAWA, H., ITOH, S. (2013): Dissipation of excess excitation energy by drought-induced nonphotochemical quenching in two species of drought-tolerant moss: desiccation-induced acceleration of photosystem II fluorescence decay. *Biochemistry*, 52: 4451-4459.