HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

DOCTORAL SCHOOL OF BIOLOGICAL SCIENCES

The Thesis of the PhD Dissertation

SMALL-SPATIAL SCALE ECO PHYSIOLOGICAL AND STRESS-INDUCED (SALICYLIC ACID) BIOCHEMICAL INVESTIGATIONS ON DESICCATION-TOLERANT BRYOPHYTE *SYNTRICHIA RURALIS*

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Title: Small-spatial scale ecophysiological and stress-induced (salicylic acid) biochemical investigations on desiccation-tolerant bryophyte *Syntrichia ruralis*

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1. BACKGROUND & OBJECTIVES

1.1 Introduction

According to a special report by (Masson-Delmotte *et al.,* 2018) the global warming rate is currently estimated to be 0.2 °C per decade; therefore, it is possibility that the average global temperature may reach 1.5 °C higher in between 2032 and 2050. Climate change is now one of the most important threats to biodiversity and consider as a major factor for the degradation of ecosystem (Hooper *et al.,* 2012). Research studies have been done and identified the transformation of climate from temperate to dry hot semi-deserts in southern Europe (Spinoni *et al.,* 2018). Cryptogamic species (such as algae, fungi, lichens, and bryophytes) collectively formed biological soil crusts which perform important ecological function e.g., production in dry grasslands (Bartholy, Gelybó and Pongrácz, 2007). Biological soil crusts (BSCs) comprising of communities are the major component of the soil surface in arid areas (Evans and Johansen, 1999).

The present scenario suggests that the alternation in environmental factors such as high temperature, increasing CO_2 levels, pattern changes in the distribution of rainfall, UV radiation causes oxidative stress due to global warming. These changes can also affect the habitats and microhabitats conditions at different ecosystem levels. Bryophytes are facing challenges due to climate change as their photosynthetic and biochemical activities primarily depend on their external environmental conditions (Proctor and Pence, 2002). Desiccation tolerant mosses such as *Syntrichia ruralis* (Hedw.) were reported and found abundant between the scattered tufts of dominant grasses *Festucetum vaginatae danubiale* association (Csintalan *et al.,* 2000).

Chlorophyll *a* fluorescence techniques were used to study the photosynthetic performance of moss cushions on different exposition (slopes).

Another study of this thesis based on biochemical aspects of dehydration and rehydration cycles. The objective of this study was to measure the changes in the antioxidant enzymatic analysis, namely catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (POD) during dehydration (slow drying) and rehydration in *S. ruralis* to understand the antioxidant defense response in different seasons. Furthermore, the research focused on the examinations of seasonal variation to study the functioning of photosynthetic apparatus associated photoprotective mechanism in bryophytes caused due to salicylic acid (SA) pre-treatment.

1.2 Objectives

This thesis was conducted on the following experiments to achieve the main objectives and questions:

1. How can affect the small-spatial expositions (NE and SW slope) on physiological activity of bryophyte *S. ruralis* moss cushions seasonally?
2. Are there any differences and consequences in antioxidant enzymatic activities (APX, POD, CAT) of *S. ruralis* cushions during desiccation and rehydration either seasonally or small-spatial scale levels?
3. What are the effects on protein content and lipid peroxidation (MDA content) collected from slope in *S. ruralis* moss cushions during desiccated and rehydrated state either seasonally or small-spatial scale levels?
4. What are the effects of Salicylic acid (SA) pre-treatment after long-term of desiccation on physiological activity measured by chlorophyll *a* fluorescence method and antioxidant enzymatic activities?

2. MATERIALS AND METHODS

2.1 Site description & plant material

The semi-arid sandy grassland site at Bócsa village in the Southern Great Plain region of southern Hungary. Co-ordinates are (central Hungary 46°53′29″ N, 19°26′35.6″ E). These sandy grasslands are part of the Kiskunság National Park, Bugac in the Hungarian Great Plain. *S. ruralis* (Hedw.) Weber & Mohr (synonymous: *Tortula ruralis*) belong to family Pottiaceae and known as sandhill screw moss. They are found in the form of extensive mats on open exposed areas of sandy dunes in semi-arid sandy grassland which plays an important role in binding sand particles (Csintalan *et al.,* 2000). Moss cushions were collected from sandy dunes of semi-arid sandy grassland in air-dried conditions during late winter season (March 2018), spring (May 2018), summer (July 2018) and autumn (October 2018) from two different microhabitats north-east (NE) and south-west (SW) slopes based on the orientation of sandy dunes and dominant wind direction.

2.2 Experimental set-up

Dense and intact cushions of *S. ruralis* were collected in air-dried conditions and kept inside paper bags (16×13.5 cm) and at room temperature for 48 h in the open paper envelopes. These air-dried samples were cleaned and separated from the sand particles before conducting the experiment. After cleaning, these moss cushions were transferred on a wet filter paper in a one-fourth water-filled plastic box container (21.5×14×7 cm) in six replicates for rehydrated treatment. Three moss cushions samples were placed in each plastic box container. For rehydration treatment, they were sprayed in the morning with distilled water to maintain hydration for 72h and if necessary, under room temperature conditions. For desiccated treatment, shoots were weighed and placed in petri dishes for slow dehydration for 48h. A similar experiment set-up was followed in different seasons.

Fresh weight, dry weight and water content percentage has been measured for each rehydrated and dehydrated period.

2.3 Chlorophyll *a* fluorescence measurement

Chlorophyll *a* fluorescence measurement was carried out on the moss cushions with a modulated chlorophyll fluorometer Hansatech Ltd. (King 's Lynn, UK) FMS II (Gödöllő). Calculation and definitions for chlorophyll fluorescence parameters (Fv/Fm, ΦPSII, qP) were followed as per (Roháček, Soukupová and Barták, 2002) and (NPQ, qN) as per (Proctor, 2003), respectively. The samples were maintained at a fully hydrated condition for 48 h at room temperature and placed it nearby the window. Prior to Fv/Fm measurements, the samples were kept in dark conditions for 30 mins. This parameter has been widely used to measure the physiological condition of a plant in stress and estimates the maximum quantum efficiency of Photosystem II. The values Fv/Fm for fully saturated, healthy, and unstressed material are around in the range between 0.76 to 0.83 (Proctor, 2003). Measurements of 6 replicates were taken from each slope: NE and SW in four different seasons at room temperature. The samples were placed in the fluorometer and Fo (minimum fluorescence yield), Fm (maximum fluorescence yield) were recorded. The light intensity of the modulated measuring beam (1.6 kHz) was 100-150 nmol photons m^{-2} s^{-1}, actinic light (650 nm, 370 µmol photons m^{-2} s^{-1}) was used to assess steady-state fluorescence and the maximum fluorescence level was measured with saturating white light pulses of 3000 µmol photons m^{-2} s^{-1}. The effective PSII quantum yield (ΦPSII), the potential quantum yield of PSII (Fv/Fm) the photochemical fluorescence quenching (qP) and non-photochemical quenching (NPQ) was observed from dark-adapted samples using chlorophyll fluorometry method described by (Genty, Briantais and Baker, 1989).

The protocol of measurement of chlorophyll fluorescence quenching were calculated by equations reported in (Roháček, 2002; Proctor, 2003). A pulse-modulated chlorophyll fluorometer (FMS 2) were used for conducting chlorophyll fluorescence measurements. The system is operated through a serial connection with window PC and data is presented as a real-time chart recorder emulation & Parameters (easy identification format of key experimental events). Data was recorded in FMS 2 can be saved in windows software for full analysis in the laboratory (with MODFLUOR & PREVIEW software) and converted to excel files.

2.5 Spectrophotometric antioxidant enzymatic analysis

2.5.1 Extraction of plant material

About 0.3 g moss shoots (rehydrated and desiccated) were used to determine the antioxidant enzymatic activity, protein content and 0.2 g used for the lipid peroxidation. These shoots were ground to a fine powder in liquid nitrogen and homogenized in 2 mL of potassium phosphate extraction buffer (125 mM, pH = 7.8) using a pre-chilled mortar and pestle under cold condition. The extract was centrifuged at 4 °C for 10 min at 15,000 rpm in a cooling centrifuge (HERMLE Z216 MK). The supernatant was used to determine the activity of ascorbate peroxidase (APX; EC 1.11.1.11), catalase (CAT; EC 1.11.1.6), and guaiacol peroxidase (POD; EC 1.11.1.7) according to (Dazy, Masfaraud, and Férard, 2009) with some modifications. Molar extinction coefficient (ε) was used to calculate the enzymatic activities and expressed in terms of $mmolmin^{-1}mg^{-1}$ protein content or Units/mg protein content. The formula for all enzymatic activity was calculated from the equation given below:

$$\frac{(\Delta Abs\ *\ Vassay\ *\ Ve.extraction)}{(\varepsilon\ *1*\ Vext\ for\ assay*\ protein\ content)}$$

Where, ΔAbs = Ratio of absorbance per unit time

Vassay = 1mL (Total volume of reaction)

Ve. extraction = 2 mL (Total Volume of extraction)

Vext for assay = 0.1 mL (Volume of plant extract)

ε = Molar Extinction coefficient

2.6 Spectrophotometric assays for antioxidant enzymes

2.6.1 Assay of Ascorbate peroxidase (APX)

APX reaction mixture consisted of 125 mM potassium phosphate buffer (pH = 7.0), 5 mM Na-ascorbate, 1 mM Na_2-EDTA, 100 mM H_2O_2 and 0.1 mL plant enzyme extract was completed to a final volume 1mL. The decrease in oxidation of ascorbate in a reaction mixture were measured for 100 sec, 25 °C at 290 nm and extinction coefficient ($\varepsilon = 2.8\ mM^{-1}cm^{-1}$).

2.6.2 Assay of Catalase (CAT)

Catalase activity was determined by measuring the decrease in the H_2O_2 concentration at absorbance 240 nm. The CAT reaction mixture (1 mL) contained 125 mM potassium phosphate buffer (pH = 7.0), 100 mM H_2O_2 and 0.1 mL plant enzyme extract were added to initiate the reaction. The decrease in the H_2O_2 concentration in a reaction mixture were measured for 340 sec, 25 °C and extinction coefficient ($\varepsilon = 36.6\ mM^{-1}cm^{-1}$)

2.6.3 Assay of Guaiacol Peroxidase (POD)

POD reaction mixture (1 mL) contained 125 mM potassium phosphate buffer (pH = 7.0), 34 mM guaiacol, 100 mM H_2O_2, 0.1 mL plant enzyme extract and completed to 1 mL final volume. The increase in Tetra guaiacol concentration in a reaction mixture was measured at 470 nm for 150 sec, 25 °C and extinction coefficient ($\varepsilon = 36.6\ mM^{-1}cm^{-1}$)

2.6.4 Protein determination

The concentration of protein was determined according to (Bradford, 1976) with modification. Bovine serum albumin (BSA) was used to prepare the standard curve.

Protein content was measured based on the reaction of the Coomassie Blue G-250 dye-binding assay with extinction coefficient at 595 nm (ε = 43000 $M^{-1}cm^{-1}$). Enzyme extracts of samples from both microhabitats were measured spectrophotometrically (SHIMADZU UV-1061 UV-visible spectrophotometer) at 595nm wavelength.

2.6.5 Lipid peroxidation

Lipid peroxidation was measured as the amount of MDA content determined by thiobarbituric acid (TBA) reaction according to (Heath and Packer, 1968) with some modification. 0.2 g of moss shoots were homogenized in 2 mL of 0.1% TCA extraction buffer under cold conditions. The suspension was centrifuged at 15,000 rpm for 10 min at 4°C and supernatant was collected. Replicates consisted of 200 µL of the supernatant, 1800 µL of TCA (20%) −TBA (0.5%) buffer was added. The assay mixture was heated at 95 ºC for 30 min. The content was cooled to end the reaction for 5-10min on ice and re-centrifuged at 10,000 rpm for 10 min at 4 °C. The absorbance was recorded at 532 nm and corrected for 600 nm. MDA content expressed in nmol/g dw by using the extinction coefficient of 155 $mM^{-1}cm^{-1}$.

2.7 Salicylic acid (SA) pre-treatment

2.7.1 Preparation of SA solution for the treatment

In the pilot study, chlorophyll fluorescence was detected under different SA concentrations: 10 µM (low), 0.001 M, (medium), 0.01 M (high). Medium concentration was selected and prepared by dissolving 0.1381g in 1000mL distilled water and transferred the solution in a spray bottle.

2.7.2 Experimental set-up

Plant material was collected from the flat areas of semi-arid sandy grassland in air-dried form during spring, summer, autumn seasons. In the laboratory, they were cleaned and transferred to petri dishes.

Samples were divided into 6 petri-dishes included 3 for control (distilled water treatment) and 3 for SA treatment. Samples were rehydrated by placing them in petri dishes under SA treatment for 72 h. After 6 hours of rehydration, the fresh weight and dry weight after one week were measured for all the samples. Water content (WC%) was calculated by $WC = [(FW-DW)/DW] \times 100$ (Péli *et al.*, 2011), where FW is the fresh weight while DW is the oven-dried weight of the sample.

2.7.3 Measurements of chlorophyll *a* fluorescence parameter

In the beginning, samples were sprayed with distilled water for control petri dishes and SA solution (0.001 M) for SA Petri-dishes. After 6 h of rehydration, chlorophyll fluorescence was measured daily on all the samples from different seasons until 3 days and then on the 10th day. Hansatech pulse modulated chlorophyll fluorometer (FMS 2) was used to measure the chlorophyll fluorescence parameters Fv/Fm, ΦPSII and NPQ. The similar protocol to measure the chlorophyll fluorescence was according to section 2.3. After the measurement, data saved in FMS 2 and converted to excel files for data analysis.

2.7.4 Enzyme extraction and Spectrophotometric antioxidant enzymatic assays

0.3 g moss shoots (control and SA-treated) were ground to a fine powder in liquid nitrogen and homogenized in 2 mL of potassium phosphate extraction buffer (125 mM, pH = 7.8) using a pre-chilled mortar and pestle. The extract was centrifuged at 4 °C for 10 min at 15,000 rpm in a cooling centrifuge (HERMLE Z216 MK). Antioxidant enzymatic activities were evaluated, and protein content was calculated after 72 h of treatment. Antioxidant enzymatic analysis has been done on control and SA treatment samples. APX, CAT and POD enzymatic analysis were performed and followed accordingly mentioned previously.

2.8 Statistical analysis

Statistical analyses were performed using the statistical software R programming language version 3.5.3 for Windows (R development Core Team, Auckland, New Zealand). All the experimental data were tested for normality and homogeneity tests using the Shapiro–Wilk's test and Levene's test, respectively. An independent sample t-test was performed to compare the mean values between the NE and SW slopes. A one-way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) parametric test was performed. ANOVA post-hoc (Tukey's test) was performed at 95% confidence level to determine the significant differences between each pair of seasons with different parameters.

3. RESULTS & DISCUSSIONS

3.1 Measurements of chlorophyll *a* fluorescence parameter

3.1.1 Effects of slopes on chlorophyll *a* fluorescence parameter

Chlorophyll fluorescence measurements were detected in *S.ruralis* showed recovery of Fv/Fm within three days in the rehydrated state between NE and SW slope, respectively. An independent sample T-test was performed to calculate the mean and significant values of two slopes with respect to fluorescence parameters. There was no significant difference in Fv/Fm. In *S. caninervis* desert moss, Fv/Fm found relatively steady at around 0.7 (Zhang, Zhao and Wang, 2017) and within the range 0.76 to 0.85 also reported in (Csintalan, Proctor and Tuba, 1999). However, ΦPS II and qP differed significantly between the NE and SW slopes. One of the possible hypothesis may be the adjustments and alteration of their optimum conditions (Hamerlynck *et al.,* 2002) by exposing to high amount of incoming solar radiation on SW facing sides on the sandy dunes during daytime. NPQ and qN between the slope (p-value ≤ 0.05) respectively. Fv/Fm, ΦPSII and qP has positive correlation with qN, NPQ (Maxwell and Johnson, 2000). Thus, a change in NPQ and qN values may be estimated by change in the ΦPSII and qP values.

3.1.2 Effect of seasons on chlorophyll *a* fluorescence parameter

One-way analysis of variance (ANOVA) was performed to determine the significant values of fluorescence parameters with respect to different seasons. ΦPS II has no significant difference whereas Fv/Fm, qN, NPQ values were found to be statistically significant within each pair of seasons. In the results, Fv/Fm values showed a significant difference in summer and spring. The mean value of Fv/Fm was found low in the spring season which might be due to minimum temperature value (below 0 °C) in March 2018.

In this period, sampling sites were covered with snow as the effect of a late winter and as a result delayed the recovery of photosynthetic activity of moss cushions that might have been due to cold temperature stress. Seasonal variations were observed in all the fluorescence parameters values indicated different level of stress in *S. ruralis*. Fv/Fm values were observed higher in both slopes in the summer period which may indicate the effect of environmental stress conditions. In too hot and dry conditions, mosses become dormant because they have a short active period in the morning hours to activate photosynthetically when moisture is available (Tuba, Proctor and Csintalan, 1998; Csintalan *et al.,* 2000). Mosses experienced high irradiance and high temperature, as a result, they became inactive in the desiccated state (Kalapos and Mázsa, 2001). In the autumn and winter season, Fv/Fm values were found similar with each other. During colder conditions, mosses assimilate effectively and can continue their growth in a hydrated state under moderate temperature and irradiance except for snow cover period (Kalapos and Mázsa, 2001). The photochemical quantum yield of photosystem II (ΦPSII) showed maximum value in the winter, followed by autumn, the spring, and the minimum values in the summer season. Another similar parameter is qP with ΦPSII that gives an indication of the proportion of open PSII centres and relates to altered efficiency (Maxwell and Johnson, 2000). Similarly, when light is excessive, NPQ values become higher and indicate the protection of photosystem apparatus from excess excitation energy (Demmig-Adams and Adams, 2006). Therefore, qN and NPQ values were shown to be higher during the summer season.

3.1.3 Effect of slopes and seasons interaction on chlorophyll a fluorescence parameter

Multi-variate analysis of variance (MANOVA) results was shown there were no significant differences in photochemical quenching parameters.

However, non-photochemical quenching parameter has a significant difference with respect to slopes within seasons. Chlorophyll fluorescence parameters measured during different seasons showed a $p \leq 0.05$ expect for ΦPS II which may indicate a seasonal variation.

3.2 Measurements of antioxidant enzymatic activities

3.2.1 Determination of Water Content (WC%) under rehydration treatment for antioxidant activities

Water content (WC%) were measured and calculated by using the fresh (FW) and oven-dried (DW) weight of the samples after small intervals of rehydration (2h, 6h, 12h, 24 h, 72 h) which shows changes in water content during rehydration-dehydration cycle. Water content was expressed as a percentage.

3.2.2 Effect of antioxidant enzymatic activities during rehydrated and desiccated states

The activities of APX, CAT and POD tended to be higher in material from the NE compared to the SW facing slopes in all seasons except opposite trend was seen in summer season. Activities tended to be higher in desiccated states than in rehydrated material for both slopes. In both rehydrated and desiccated states, all activities were higher in summer and winter season and lower in spring and autumn. For material from the NE and SW facing slopes CAT activities did not vary much throughout the year in both states. It seems likely that the differences in the enzyme activities in the mosses growing on the two slopes are a consequence of the more stressful conditions on the NE facing slopes. Conditions on the SW slope are better (e.g., favourable light conditions, better availability of water) for moss growth. This is suggested by a recent study on the photosynthetic efficiencies of mosses sampled from the two slopes (Ruchika, Csintalan and Péli, 2020).

Similarly, higher activities of antioxidative enzymes suggests than mosses growing on the NE slope might be experiencing greater stress**.** In summer and winter season, qN and NPQ values were reported higher that may indicate the stressful environmental conditions (high light exposure and temperature variations or differences in water condition) in these two seasons.

3.2.3 Variation in protein determination (protein content) between the slopes (NE and SW) in seasons in the rehydrated and desiccated states

On rehydration, the protein content was observed increased and desiccation resulted in a decrease level of the protein synthesis in all seasons in both NE and SW slopes. Overall, in spring and autumn season, protein content was found increased whereas in summer and winter season it become decreased. Based on slope-wise, protein content was not significantly different in rehydrated states as well as in desiccated states. Based on season-wise, protein content was significantly different ($p \leq 0.05$). Cruz de Carvalho *et al.*, (2014) reported that there is a down-regulation of the synthesis of proteins during drying conditions. Similarly, in this present study, results observed lower protein values during desiccation.

3.2.4 Variation in lipid peroxidation (MDA content) between the slopes (NE and SW) in seasons in the rehydrated and desiccated states

MDA content differed significantly between each season in rehydrated states and desiccated states. It was not significantly different between the slopes. The concentration of the oxidized lipid MDA tended to be higher in in desiccated material than rehydrated material. The lower level of lipid peroxidation in moss shoots suggests that this moss might be better protected from oxidative damage during rehydration. However, in contrast, Zhang, Zhao and Wang (2017) reported that in species *Bryum argenteum* and *Barbula fallax*, MDA content increased first within 24 h and then declined at 48 h and 72 h later stages of desiccation stress.

It seems likely that measuring MDA alone may give a rather poor indicator of oxidative stress in tissues and as suggested by De Dios Alché (2019), other molecules such as 4-hydroxy-nonenal (HNE) may be a more sensitive indicator of oxidative stress.

3.3 Measurements of SA pre-treatment on antioxidant enzymatic activities

3.3.1 Determination of water content (WC%) under rehydration

Water content showed changes in control vs SA treated samples in different three seasons during rehydration period. Water content was expressed as a percentage. In all the seasons, the water content (%) in the SA treatment was found to be higher (but not significantly in all cases) as compared to their respective controls at 0 h. In the case of SA treated, specifically, the trend of the water content increased at 12 h and steadily decreased as the time progressed, while in the case of control samples the trend was variable.

3.3.2 Effect of SA and seasonal variation on chlorophyll a fluorescence parameters

Chlorophyll *a* fluorescence parameter values of SA-treated moss cushions were compared with control samples in different seasons. Fv/Fm values were found to be significantly different ($p \leq 0.05$) compared to the mean values with days of treatment in each season except for the autumn season on day 10. In the spring season, ΦPS II values were shown significantly in all three days except on day 10 whereas in summer season day 1 and day 10 were found not significant. In the autumn season, day 1 showed significant differences, but other days were not significantly different NPQ values were significantly different on day 2 in spring season and day 2, 3, 10 in the summer season whereas in autumn season on day 1, 2.

Fv/Fm values are found in the range of 0.79 to 0.83 approximate optimal values in most plant species and lowered values indicating the condition of plant stress (Maxwell and Johnson, 2000). Similar results were reported in (Beckett et al. 2000) where NPQ values were shown higher ABA hormone pre-treatment in the moss *Atrichum undulatum* and *A. androgyne* (Marschall and Beckett, 2005).

3.3.3 Protein Determination

Mean values were calculated, and ANOVA post-hoc Tukey's test was performed to find out the difference in the protein content between the control and SA treatment. There was no significant difference season wise and within same season. ROS causes inhibition of protein synthesis or protein denaturation (Scheibe and Beck, 2011). However, there is a down-regulation of the synthesis of proteins during drying conditions (Cruz de Carvalho *et al.,* 2014).

3.3.4 Effect of SA Treatment on antioxidant enzymatic activity

Antioxidant enzymatic activity results were represented in two different ways i.e., treatment-wise (control and SA treated) within the same season and season-wise. Based on treatment wise for the APX activity, there was a significant difference between the control and SA-treated for spring, summer, and autumn seasons whereas based on seasons, the APX activity in the case of control and SA in spring was significantly different to summer and autumn seasons, respectively. No significant difference was observed between summer and autumn. The CAT activity in SA-treated samples was very slightly increased as compared to their respective control values in spring, autumn and summer. A season-wise comparison showed CAT activity in control is significantly different to CAT activity in summer, while the CAT activity in SA-treated in autumn was significantly different to that in summer.

The POD activity in SA-treated in spring was significantly different to that in SA-treated in summer. Based on treatment-wise, no significant difference was observed between control and SA-treated in spring season, respectively except summer and autumn season.

According to (Sattler, Calsou and Boiteux, 2007), low temperature and water stress leads to overproduction of ROS that causes the oxidative damage to the cells. Moss cushions might be induced these enzymes to scavenge ROS and enhancing their tolerance during the different seasons (Thakur and Kapila, 2017). Hydrogen peroxide is produced during oxidative stress caused by the overproduction of ROS is decomposed by peroxidase enzymes (Reddy, Kumar and Jyothsnakumari, 2005). An earlier study has been reported that several bryophytes showed significant antioxidant activity and possessed with efficient antioxidant enzyme systems. Antioxidant peroxidase was characterized in the liverwort *Marchantia polymorpha* L. that found different from other known peroxidases in vascular plants (Hirata, Ashida and Mori, 2002). Similarly, the role of ascorbate peroxidase was found in the removal of hydrogen peroxide in a moss *Brachythecium velutinum* and *M. polymorpha* (Paciolla and Tommasi, 2003).

4. NEW SCIENTIFIC RESULTS

1. For photochemical parameters, qP and ΦPSII differed significantly in small-spatial scale on *S. ruralis* moss cushions collected from the north-east (NE) and south-west (SW) slopes in rehydrated state.
2. For non-photochemical parameters, qN and NPQ were significant between the two slopes.
3. Seasonal variations in photochemical parameters as Fv/Fm, qP, and non-photochemical parameters, as qN and NPQ were significantly different between each pair of seasons. Fv/Fm values were higher in summer and lower in spring season. qP parameter was observed higher in spring and lower in autumn season whereas, ΦPSII was maximum in winter and minimum values in summer season. For qN and NPQ, both showed higher values in summer and lower in autumn season.
4. The applicant with her supervisors prepared protocols to calculate the enzymatic activity for CAT, APX and POD enzymes.
5. The enzymatic activities of APX, CAT, POD and MDA contents were showed variations significantly differed within each seasons but not significant between the slopes.
6. In Salicylic Acid (SA) treated mosses, Fv/Fm values were increased in spring and autumn season and ΦPSII was reduced significantly during spring and summer season. NPQ values were found significantly differed in few days of treatment and showed inclined pattern in SA-treated mosses during spring and autumn season than control values and opposite trend in summer season.
7. Antioxidant enzymatic activities of APX and CAT in SA-treated mosses were increased except POD activity than control values.

5. CONCLUSION AND RECOMMENDATIONS

The study showed significant seasonal variations in chlorophyll *a* fluorescence parameter of samples collected from NE and SW slopes. The presence of these differences in photosynthetic properties of *S. ruralis* in such a small-spatial scale of the microhabitat refers to the high adaptation ability and sensitivity level of mosses to the smallest changes in environmental factors. The main finding of this work is the contrasting behaviour of all the enzymatic activities in the green shoot apex during rehydrated and desiccated states in different seasons and along with SA-pre-treatment. The role of some antioxidant enzyme in desiccation tolerance may be different, basically depending on the actual metabolic balance of mosses. Their enzymatic activity might be not influenced only by water conditions but also by other environmental factors such as temperature, light, and soil conditions which need to be further investigated in the future. Effects of changing the climate for the ecosystem especially for vegetation is more traceable by small spatial-scale investigation of such adaptable plants as mosses and can serve for prediction in the future. Furthermore, more research will be needed to study the impact of climate change on desiccation-tolerant cryptogamic specie

6. LIST OF PUBLICATIONS

1. Ruchika, Csintalan, Z., Péli, E.R. (2020a) 'Seasonality and small spatial-scale variation of chlorophyll a fluorescence in bryophyte *S. ruralis* (Hedw.) in semi-arid sandy grassland, Hungary, *Plants*, 9(1), pp. 92. https://doi.org/10.3390/plants9010092.
2. Ruchika, Csintalan, Z., Péli, E.R. (2020b) 'Effect of salicylic acid pre-treatment after long-term desiccation in the moss *S. ruralis* (Hedw.) Web. And Mohr., *Plants*, 9(9), pp. 1097. https://doi.org/10.3390/plants9091097.
3. Ruchika, Csintalan, Z., Veres K., Péli, E.R. (2021) 'Seasonal variation of antioxidant enzymatic responses in the desiccation-tolerant bryophyte *S. ruralis* (Hedw.) Web. & Mohr., *Columella Journal of Agricultural and Environmental Sciences,* 8(1), pp. 37-50. https://doi.org/10.18380/SZIE.COLUM.2021.8.1.37.

Conferences (Posters) and Workshop

1. Preliminary study of chlorophyll *a* fluorescence measurement on *Syntrichia ruralis* (Hedw.) from different microhabitats.
   Ruchika, Zsolt Csintalan, Evelin Péli Ramóna
   In: Book of Abstracts XXII Symposium of Cryptogamic Botany, Conference: Lisbon, Lisboa, Portugal, (2019.07.24. - 2019.07.26), p. 54.
2. Antioxidant enzymatic analysis on bryophyte *Syntrichia ruralis* (Hedw.) in semi-arid sandy grassland, Hungary.
   Ruchika, Zsolt Csintalan, Evelin Péli Ramóna
   In: Coudert Yoan *et al.* (eds.) Bryology2019-abstracts-posters, Conference: Madrid, Spain (2019.07.09. - 2019.07.12) p. 72.
3. Participated in the workshop entitled 'Eagle Hill Natural Science Fall Workshop in Bryophytes: Mosses and Liverworts in Maine, USA.

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