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

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I dedicate this thesis to my honourable supervisor

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ABBREVIATIONS

µL	microliter
µmol	micromole
^{0} C	degree Celsius
$^{1}O_2$	singlet oxygen
ANOVA	analysis of variance
ABA	abscisic acid
APX	ascorbate peroxidase
Asc	ascorbate
C_3	3- carbon atoms
CAT	catalase
Chl	chlorophyll
cm	centimetre
CO_2	carbon dioxide
Cu	copper
df	degree of freedom
DNA	deoxyribonucleic acid
Fe	Iron
Fm	chl fluorescence at a saturating radiation pulse in the dark-adapted state
Fo	ground fluorescence in the dark-adapted state
Fv/Fm	maximum photochemical quantum yield of PSII
FW	fresh weight
g	gram
GR	glutathione reductase
GSH	glutathione
h	hour
H_2O	water
H_2O_2	hydrogen peroxide
KDa	kiloDalton
LHCP	light harvesting chlorophyll proteins
$m^{-2}s$-1	square meter per second
MANOVA	multivariate analysis of variance
MDA	malondialdehyde

mg	milligram
mL	milliliter
Mm	millimeter
mM	millimole
$mM^{-1}cm^{-1}$	millimolar per centimeter
Mn	manganese
MPa	mega pascal
Na_2EDTA	disodium Ethylenediaminetetraacetic acid
Na-ascorbate	sodium ascorbate
NADPH	nicotinamide adenine dinucleotide phosphate
NE	north-east
Nm	nanometer
nmol/g dw	nanomole per gram dry weight
$NO^{\cdot}$	nitric oxide
NPQ	non-photochemical quenching
O_2	oxygen
O_2^{-}	superoxide radicals
$OH^{\cdot}$	hydroxyl radicals
PEP	phosphoenolpyruvate
POD	guaiacol peroxidase
PSI	photosystem I
PSII	photosystem II
qN	non-photochemical fluorescence coefficient
qP	photochemical fluorescence coefficient
RH	relative humidity
RNA	ribonucleic acid
ROS	reactive oxygen species
Rpm	revolutions per minute
RUBISCO	ribulose-1,5-bisphosphate carboxylase-oxygenase
SA	salicylic acid
SD	standard deviation
Se	selenium
SE	standard error of mean
SW	south-west

TBA	thiobarbituric acid
TCA	trichloroacetic acid
UV	ultra-violet
WC	water content
Zn	zinc
ε	molar Extinction coefficient
ΦPSII	effective photochemical quantum yield of PSII

1 INTRODUCTION

1.1 Foreword

Climate change is the major component of global environmental change caused by anthropogenic activities which become the biggest problem in this century. Masson-Delmotte *et al.,* (2018) reported that 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. Global warming has the major influence on the patterns of rainfall and its alternations may directly impact on the primary productivity (Radu and Duval, 2018). The present scenario suggests that the alternation in environmental factors such as rise of temperature, increasing atmospheric CO_2 levels, changes in the precipitation, UV radiation causes oxidative stress due to global warming. These changes can also affect the habitats and microhabitats conditions at different ecosystem levels which become one of the important topic for intensive research especially in arid and semiarid grasslands. Spinoni *et al.* (2018), reported that the transformation of climate from temperate to dry hot semi-deserts in southern Europe.

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). A great challenges has been facing by the scientists to understand how plant species respond to climate change for the conservation of biodiversity. How the rapid climate change will affect on the morphology, physiology and on their metabolism of many different species of bryophytes and it still has been poorly studied which are also ecologically important groups of plants. Bryophytes are of small size, poikilohydric and they are highly dependent on external environment for water and nutrients for their survival and reproduction (Proctor, 2000). Many species of bryophytes are sensitive to relatively high temperatures as a result tropical lowlands are now become small habitats for bryophytes (Zotz and Bader, 2009). Furthermore, negative impacts has been suggested on photosynthetic activities and growth rates of moss species in sub-arctic heathlands (Bjerke *et al.,* 2011). Several European bryophyte species are predicted to decline their distributions, while other species will expand their habitats as they approach at northern limits (Bergamini, Ungricht and Hofmann, 2009; Hodd, Bourke and Skeffington, 2014).

Therefore, in this present study more focus has been highlighted to the experiments which deals with physiological activity, biochemical basis to understand the consequences of climate change on bryophytes and their adaptations to survive in terrestrial environment.

Researchers have been drawing significant attention to the further exploration of bryophyte ecophysiology for climate change will provide new knowledge and helpful in the conservation of bryophytes. In the Hungarian Great Plain, is one of the most important vegetation type of the Carpathian basin type. They are facing an environmental problem of desertification caused by human-induced global environmental change. It has a major impact on grasslands and forests, altering species richness (Kertész *et al.*, 2017). Cryptogamic species (such as algae, fungi, lichens, and bryophytes) collectively formed biological soil crusts which perform important ecological function such as 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).

A more than decade research has been investigated the mechanism of desiccation tolerance on vascular plants and non-vascular plants. Earlier many works of literature were reviewed (Bewley, 1979; Bewley and Krochko, 1982; Proctor, 1990; Oliver and Bewley, 1997) on desiccation tolerance in bryophytes. Earlier studies on the desiccation tolerance were undertaken on mosses and ferns and especially on the moss *Syntrichia ruralis* (Hedw.) (Bewley, 1979; Bewley and Krochko, 1982). In mosses, it was reported that *Tortula ruralis* (synonymous with *Syntrichia ruralis*) was found as the first desiccation-tolerant plant which has some specialized repair mechanism (Farrant and Moore, 2011). This moss has been most studied in all bryophytes with respect to its physiological, biochemical, cellular and desiccation responses (Smirnoff, 1993; Oliver and Bewley, 1997). *S. ruralis* serves as an experimental model plant system in bryophytes to understand desiccation tolerance. There has been less information in literature about the biochemical mechanism of desiccation tolerance, researchers are more interested in the studies mainly focus on the impact of climate change with desiccation-tolerant (DT) bryophytes in recent years. Global climate change increases the demand for research conduct in monitoring and studying photosynthetic activity in the field of plant physiology and ecophysiology.

Previously few experiments were conducted to study the photosynthetic behaviour of *S. ruralis* moss after re-moistening in time (Tuba, Csintalan and Proctor, 1996). As it is the steppe-grassland and sand-dune moss, showed significant early-morning photosynthesis due to heavy dewfall which provide sufficient water supply. In the dry state, they remained unchanged but after rehydration, they regained fully and rapidly to their photosynthetically active state. These plants can stay in the desiccated state for months or years without dying then upon rehydration are able to recover to their full metabolic activity within minutes, hours or a few days (Tuba, Proctor and Csintalan, 1998; Péli *et al.*, 2005). There is lack of knowledge of special adaptation strategy in small-spatial scale referring chlorophyll fluorescence response to desiccation tolerance.

Therefore, chlorophyll fluorescence measurements were examined as an indicator to understand how mosses respond to environmental changes. Thus, it becomes an important area to study in terms of current climate change scenarios where temperature and precipitation are anticipated to change on a worldwide scale.

Investigation in small-scale seasonal variation of metabolic activity of *Syntrichia spp*. can serve forward information concerning the effects of future climatic changes e.g., the desertification aspect. Another study of the thesis based on biochemical aspects of desiccation and rehydration cycle and measured the changes in the antioxidant enzymatic analysis, namely catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (POD) during desiccation (slow drying) and rehydration in *S. ruralis* to understand the antioxidant defense responses in different seasons (spring, summer, winter, autumn). 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. The main objective of this thesis was to study the possible impact of climate change in a Hungarian grassland with the combination of eco-physiological and biochemical aspects on a desiccation-tolerant bryophyte from a different slope in semi-arid sandy grassland.

1.2 Objectives

This thesis 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?

The hypothesis of the study formulated as follows:

Hypothesis 1. Effect of exposition (slopes) has some influence on chlorophyll fluorescence parameters either seasonally or at small-spatial scale.

Hypothesis 2. Differences of the antioxidant enzymatic activities can be detected in chlorophyll fluorescence parameters either seasonally or at small-spatial scale during desiccation and rehydration.

Hypothesis 3. Protein content and lipid peroxidation (MDA content) show some variation seasonally or at small-spatial scale during desiccation and rehydration.

Hypothesis 4. The effect of Salicylic acid (SA) pre-treatment is expressed in chlorophyll fluorescence parameters and antioxidant enzymatic activities seasonally during desiccation and rehydration.

2 LITERATURE REVIEW

2.1 What are bryophytes?

Bryophytes are commonly referred to as mosses which represented as the first dwellers of the land plant history. The occurrence of bryophytes can find easily on most habitats from the dry surfaces of deserts to the sweat water as a submerged plants, from the poles to the equator such as wet, humid, or boggy areas such as damp land area, damp rocks, forests and tree trunks. The term 'bryophytes' has its origin in the Greek language, which refers to plants that swell upon hydration (Vanderpoorten and Goffinet, 2009). They occur mostly in terrestrial biomes and well-known boreal regions, forests, extreme habitats such as rocks and temperate grasslands around the world (Müller *et al.,* 2012). Bryophytes are small plants and have a dominant gametophyte which support the sporophyte for sexual reproduction. They are divided into three divisions: liverworts (Marchantiophyta), mosses (Bryophyta) and hornworts (Anthocerotophyta). All three groups of bryophytes belongs to the earliest green plants that move to the land where mosses were among the first plants making a step forward from a life of water to a life on land and they successfully colonized to terrestrial habitats. Bryophytes are differ from tracheophytes in having a dominant gametophyte with dependent sporophyte. They lack meristematic tissue, lignin, tracheids (but hydroids has similar role like tracheids to transport water and minerals) and sieve cells (but leptoids has similar role like sieve cells to transport sucrose). Hydroids and leptoids are present in some mosses such as species of Polytrichaceae (Ligrone and Duckett, 1994). Thus, they also term as non-tracheophytes or non-vascular plants (Glime, 2017a).

Mosses are plants composed of simple stem bearing leaflets, typically arranged in spiral rows. They can absorb water and nutrients through their whole gametophyte body. They differ from vascular plants in lacking water-bearing xylem tracheids or vessels (Vanderpoorten and Goffinet, 2009). Hornworts have thalloid vegetative gametophyte which may be dissected but never bears leaves. These species lack water-conducting cells in both generations of the life cycle (Gunathilaka, 2019). Bryophytes may reproduce both asexually and sexually. Asexual reproduction occurs when sporophyte releases spores from capsule and sexual reproduction occurs when gametes fused to produce zygote. The sporophyte remains attached to the gametophyte which supplies nutrients (Vanderpoorten and Goffinet, 2009). Bryophytes are the most successful group of plants than higher plants based on several species, topographical distribution on all landmass and their natural surrounding diversification. They are unique among land plants and haploid green gametophyte is their dominant stage and has short-lived of the diploid sporophyte (Slack, 2011). They are very specific for microhabitats and have diversified ecologically during their evolution.

Some specialized structures are found in mosses that have physiological functions such as leaf axillary hairs which secrete mucilage for juvenile leaves to prevent from dehydration, small leaf-like structures paraphyllia on pleurocarpous moss stems which provide photosynthetic surface area and lamellae on the upper surface of the leaflets in Polytrichaceae and some Pottiaceae (Buck and Goffinet, 2000).

2.2 Description and characteristics of *Syntrichia ruralis* (Hedw.)

S. ruralis (synonym: *Tortula ruralis*) is commonly known as twisted moss which belongs to family Pottiaceae (Matthews, 1993). It is a short, erect moss that forms loose to dense tufts. Shoots are up to several centimetres long but often partly buried in the soil. Leaves are bright green in wet conditions and twisted golden-brown in dry conditions (Crumm and Anderson, 1981). It is generally a xerophytic, highly drought and desiccation-tolerant moss (Mishler and Oliver, 1991). It is a dessert moss and known as star moss due to its morphological structure. In North America, it is known as star moss and in Europe as the hairy screw moss and derives its Latin name from its twisted peristome and leaves around the central stalk in a tortuous movement as its dries (Oliver, Velten and Wood, 2000a). It is also known as sand-hill screw moss that is found abundantly in the form of extensive mats in open exposed areas of sandy dunes in semi-arid grassland in Hungary (Csintalan *et al.,* 2000) and the Hungarian name is "háztető moha" (rooftop moss) that refers to its preferred habitat as shown in **Figure 1.**

Figure 1. A moss cushion of *S. ruralis* (Hedw.) in dried state from semi-arid sandy grassland, Hungary (Source: own photo).

Taxonomic classification of *S. ruralis* was done by Weber and Mohr (1803) as follows:

Kingdom: Plantae

Phylum: Bryophyta

Class: Bryopsida

Order: Pottiales

Family: Pottiaceae

Genus: *Syntrichia*

Species: *ruralis*

2.3 Role of bryophytes in semi-arid grasslands and in a changing environment

In Europe, grasslands are one of the widely distributed vegetation types, especially in the Hungarian Great Plain. These grasslands are facing a major environmental problem i.e., continues drifting towards desertification. In nitrogen (N) and carbon (C) cycles, grassland ecosystems play important role between the land surface and the atmosphere (Machon *et al.,* 2011). Some studies were carried out on the sites of Hungarian grasslands on net ecosystem exchange (NEE) of CO_2, respiration processes (Balogh *et al.,* 2005; Balogh, Biro and Pinter, 2008) and on the greenhouse gas (GHG) flux (Nagy *et al.,* 2005, 2007). Due to the large territorial coverage of the *S. ruralis* in Hungarian grasslands, they can contribute considerably to the C-balance of this sandy vegetation in certain seasons (Juhász *et al.,* 2005). Approximately 11% of Hungary are covered by grasslands and they are an important sink for CO_2 (Nagy *et al.,* 2011). Semi-natural grasslands in central Europe are created either by grazing and mowing depending on the management at the coastline or in the high mountains where the forest growth is inhibited by harsh environmental conditions.

Succession and forest re-growth replace grassland vegetation in the long run (Ellenberg and Leuschner, 2010). The productivity of semi-natural grasslands is highly depended on resource supply. Hence, except the floodplains which has natural nutrient influx, semi-natural grasslands are mostly nutrient-deficient due to constant removal of biomass for example in hay meadows (Dieterich and Beinlich, 2009). Central European grasslands have become an extremely species-rich habitat for both flora and fauna even under such low-productive conditions over the centuries. Moreover, diversity of herbivorous species and species of higher trophic levels are promoted by the richness of plant species (Balvanera *et al.,* 2006). In the last decades, increased land use and landscape scale eutrophication caused severe losses in bryophytes of grasslands (Müller *et al.,* 2012). The nutrient status of the grassland is of great importance for the dominance of vascular plants and for bryophyte vegetation. Consequently, species richness and diversity of bryophytes are considerably differing among different grassland types (Klaus and Müller, 2014).

Dry grasslands are characterized by nutrient-poor conditions and periodical drought. Bryophytes along with lichens can dominate the vegetation of these low productive dry grasslands e.g., 63 species in Estonian calcareous dry grassland (Ingerpuu, Kull and Vellak, 1998), while 153 species in Sweden calcareous dry grassland (Löbel, Dengler and Hobohm, 2006). A key for the bryophyte dominance is the low-nutrient availability of the system along with high desiccation tolerance of some bryophyte species withstanding long phases of drought but recovering within a few hours after rewetting (Oliver, Velten and Mishler, 2005).

In temperate zones, different factors can cause drought conditions: microclimate, extreme soil conditions, deep groundwater tables and topographic exposure on steep slopes with quick run-off and high radiation. Consequently, several different types of dry grasslands can be found, differing in soil substrate, exposition, altitude, species pool, site history and management. These dry grasslands can be distinguished: siliceous dry grasslands, grasslands on rock substrate along with distinct inclination and on sandy substrate such as xeric grasslands on dunes (Ellenberg and Leuschner, 2010). In Hungary, the open sand steppes as a prominent representatives of open dry grassland type, typically with *Festucetum vaginatae* cöenotaxons prevalence (Borhidi, 1996). Bryophytes make a significant contribution to grassland diversity and facilitate fundamental ecosystem functioning such as carbon storage in nutrient-poor environments, increase water retention capacity and interacting with vascular plants by seedling establishment (Lindo and Gonzalez, 2010). Furthermore, bryophytes in grasslands could be used as indicators to track nutrient fluxes, pollutants, or climate change in grasslands (Müller *et al.*, 2012).

The geographical distribution of microclimate is a key important for the selection of habitat for many species (Rich and Weiss, 1991; Mantilla-Contreras, Schirmel and Zerbe, 2012). Climate change appears to have an impact on species occurrences by changing the precipitation and increasing the frequency of extreme weather events (Easterling *et al.*, 2000; Parmesan, Root, and Willig, 2000). Therefore, more investigation of the habitats and on affected species become crucial important and offer a new insights for understanding how species are respond to changes in microclimate and also studying for conservation biology. Furthermore, they must emphasis on the distinguish between (semi) natural habitat and artificially created habitats (e.g., agricultural fields, grasslands) because environmental conditions (e.g., water supply, availability of nutrients, microclimate) and ecological processes are different in both types of habitats (Pinke *et al.*, 2012). Microclimate is an important factor in establishing the sand dune slack communities of the Kiskunság sand ridge which plays a role in preserving plant species such as *Carex flacca* Schreb., *Carex humilis* Leyss., *Chrysopogon gryllus* (L.) Trin., *Molinia caerulea* (L.) Moench, *Salix rosmarinifolia*, *Scirpoides holoschoenus*, and *Thalictrum simplex* L. (Bátori *et al.*, 2014).

Several factors have been contribute to the changes in the species composition of dune vegetation and the most important are the climate change and human-induced drought (Thomas, Knight and Wiggs, 2005; Margóczi, Szanyi and Aradi, 2007; Yizhaq, Ashkenazy and Tsoar, 2009). Apart from the climate change-induced drought, local human activities (e.g., afforestation, groundwater extraction, and intensive farming) also have had a strong negative influence on the groundwater table which reported decline in water table (Tölgyesi and Körmöczi, 2012). Due to the changes in the vegetation of the sandy dune, climate change predict a significant increase in temperature and decrease in precipitation in summer (Bartholy, Gelybó and Pongrácz, 2007) and Kiskunság Sand Ridge area become more vulnerable to drought and fire (Blanka, Mezősi and Meyer, 2013).

Bryophytes are mostly found in terrestrial biomes, forests and extreme habitats such as rocks and they are important component of temperate grasslands which contribute to grassland diversity and influence ecosystem functions (Zechmeister *et al.*, 2002; Müller *et al.*, 2012). Lindo and Gonzales (2010) was investigated the interaction of bryophytes with vascular plants where bryophytes provide habitats for numerous microbes and other animal species. Due to environmental changes, there must be detailed study related to the role of bryophytes in the functioning of ecosystem seems crucial. Like vascular plants, bryophytes maintain gene flow between populations and colonize to other habitats by dispersal vectors (Pharo and Zartman, 2007). Hence, they have various dispersal strategies such as sexual via spores and asexual via vegetative through specialized propagules (gemmae) and gametophyte fragments where later on these gametophyte produce protonema and then a new bryophyte plant (Frey and Kürschner, 2011). Spores are very tiny in structure and can be easily transported by wind and disperse to long distances and they have high longevity (Vanderpoorten and Goffinet, 2009).

Growth forms of bryophytes occur in grasslands can be categorized into four different forms; the first category includes the dominant tufts of branching moss species which makes extensive mats when moisture and nutrient are available such as pleurocarpous species (*Rhytidiadelphus squarrosus, Climacium dendroides, Rhytidium rugosum*) and peat mosses (*Sphagnum* species).

The second category includes creeping growth forms with long branches and stems which tolerate high nutrients levels, overgrowing by vascular plants (*Brachythecium spp., Eurhynchium spp., Amblystegium spp*.). The third category comprises of short erect mosses which is small or less dense turfs with short life cycles such as acrocarpous species (*Acaulon sp., Bryum sp., Phascum spp., Pleuridium spp*.). Furthermore, the fourth category consists of thallose or rosettes moss species such as liverworts and hornworts (*Riccia and Anthoceros*) (Klaus and Müller, 2014).

Bryophytes also has competition with vascular plants for water, nutrients and light and both groups of plants have different strategies for nutrient uptake but direct competition for nutrients is very weak whereas for light, competition is much stronger (Rydin, 2009).

Ingerpuu and Kupper (2007) reported that the cover of vascular plants found reduced by an extensive moss cover, which was favoured in years with high precipitation on dry calcareous grasslands. They have ability to hold twenty-times their own weight in water and used their complete external surface to absorb the nutrients from the surrounding (Klaus and Müller, 2014). They also survived and grow in harsh and nutrient-limited environment e.g., *Sphagnum* mosses in bogs and they shift their growth period by tolerating the extremely cold conditions to avoid competition with vascular plants (Ingerpuu and Kupper, 2007). Thus, it shows important characteristics feature of bryophytes for tolerating dryness and cold and maintains coexistence with strongly competitive vascular vegetation. In earlier reports, several author highlighted the importance of protective role of a moist moss layer which subjective to a summer drought or different seasonal wet-dry cycles (During and Van Tooren, 1990; Ryser, 1993; Bissels, Hölzel and Otte, 2004).

Bryophytes are the most important primary producer which contributes fixation of carbon in peatlands. The most important contribution of bryophyte vegetation in grasslands is ecosystem functioning of grasslands such as retention of nutrients and water (Klaus and Müller, 2014). Special tissue of bryophyte structure has a very high holding capacity of up to 1400% of their dry mass (Proctor, 2008). Thus, bryophytes serve role in storage of water and slowly re-delivering into the surrounding and it considered as an important trait of bryophyte communities (Michel *et al.*, 2013). It also contribute to nutrient cycling by storing nutrients and releasing from the decomposition of bryophyte litter and later on increase the nitrogen availability for vascular plants (Startsev and Lieffers, 2006). Therefore, furthermore more research should also be done on bryophytes to find contributions for studying their roles and effects in a changing environment.

2.4 Desiccation tolerance in Bryophytes

Desiccation tolerance is a characteristic feature of bryophytes (Oliver and Bewley, 1997; Alpert and Oliver, 2002; Proctor and Pence, 2002; Wood, 2007) but not universal. It is considered as a key component in the evolution of plants to colonize successfully on the land (Oliver, Velten and Wood, 2000a). Land plants have been evolved and developed a more efficient water transportation mechanism because of desiccation tolerance lost from vegetative tissues and retained with reproductive structures (Proctor and Tuba, 2002).

According to phylogenetic studies on land plants suggest desiccation tolerance was a requirement for a transition of land (Oliver, Tuba and Mishler, 2000b). Due to diversification, it is further hypothesized that vegetative desiccation tolerance independently revolved several times, giving rise to the resurrection plants observed today (Oliver, Velten and Wood, 2000a). This can be defined as going up to the desiccated state and returning to normal function upon rehydration, if the ability of an organism to dry to equilibrium with dry air (50 % RH and 20 °C, corresponding water potential of -94 MPa) and to resume normal metabolic function on rehydration (Bewley, 1979; Proctor *et al.,* 2007a).

Vegetative desiccation-tolerant plants are categorized into two groups based on their rate of drying: (1) Fully desiccation-tolerant plants (include the algae, lichens, and mosses) as they can tolerate the total loss of free protoplasmic water, withstand rapid drying, and possess constitutive tolerance. (2) Modified desiccation-tolerant plants (include higher plants) as they can withstand slow drying and possess inducible cellular protection mechanisms (Oliver, Wood and Mahony 1998; Oliver, Velten and Wood, 2000a). Those bryophytes which are more desiccation-tolerant are termed as fully desiccation-tolerant bryophytes whereas desiccation-tolerant vascular plants termed as modified desiccation-tolerant plants (Oliver and Bewley, 1997). Vegetative tissues of plants can tolerate more water deficit, as a result, they play an important role in the development of drought-tolerant crops. Among higher plants, if vegetative tissues are desiccation-tolerant (DT) then these being termed as resurrection plants (Farrant *et al.,* 2004). They are a small but diverse group of land plants characterized by their tolerance to extreme drought or desiccation. They have the unique ability to survive months to years without water and those species which are desiccation sensitive (DS) are termed as recalcitrant. Susceptibility is another term that is the inability of the plants which do not survive under abiotic stresses whereas resistance is the ability of the plants which survive under abiotic stresses. Plants developed mainly two strategies for survival against water deficit: (1) enable the cells to lose basically all water equilibrating with the atmosphere (poikilohydric organisms) and (2) prevent water loss to the atmosphere, by waxy layers, internal water conduction through specialized conducting vessels or pores (homoiohydric organisms) which is belongs to drought avoidance.

Poikilohydric means a lack of ability to regulate water content and organisms directly depend on the environment for its water supply whereas homoiohydric means the capacity of plants to regulate water content from the environment through a system of roots (Proctor and Tuba, 2002). It appears that both poikilohydric and homoiohydric possess a common ancestral that developed desiccation tolerance (Oliver *et al.,* 2000b).

This common ancestral showed desiccation tolerance to involve in the intertidal space, optimizing the carbon absorption and light capture for photosynthesis. Some species evolved through lines that kept these mechanisms functional while others lost the capacity to tolerate desiccation in the vegetative state. However, these plants keep the desiccation tolerance potential present in their genome, mainly through seeds (Tweddle *et al.*, 2003), pollen and spores (Hoekstra, 2002).

The desiccation-tolerant (DT) plants are poikilohydric and capable of surviving the loss of 90-95% of their cell water content. Based on the retention of photosynthetic apparatus, DT plants may be subdivided into (1) homoiochlorophyllous (HDT) and (2) poikilochlorophyllous (PDT) types (Tuba, Proctor and Csintalan, 1998). The HDTs retain their chlorophyll on desiccation, whereas in PDTs on desiccation results in the loss of chlorophyll, which must be resynthesized following remoistening (Tuba, 2008). Poikilohydric DT mosses can survive during dry conditions and recover themselves fully on rehydration (Alpert and Oliver, 2002). In previous literature, poikilohydric strategy has been studied in moss *S. ruralis* (Willis, 1964; Schonbeck and Bewley, 1981a,b; Tuba, 1985; Oliver 1991; Tuba, Csintalan and Proctor, 1996) belongs to the homoiochlorophyllous (HDT) groups and cannot maintain constant internal water content by regulating water loss (Proctor, Ligrone and Duckett, 2007b).

Cryptogams occupy almost all-natural surroundings from the tropics to cold and hot deserts. Bryophytes are the earliest group of first land plants which faced extremely dry conditions during movement onto the terrestrial habitat (Benicci 2008). Bryophytes are the components of the ecosystem, it may significantly affect the environment through successive drying up and rewetting cycles (Lakatos, 2011). Bryophytes implement desiccation tolerance as a common and successful alternative life strategy. Some previous reviews were focused on the comparison between poikilohydry and homoihydry (Proctor and Tuba, 2002), on physiological and ecological aspects of bryophytes (Turetsky, 2003; Proctor, Ligrone and Duckett, 2007b) and on anthropogenic aspects such as agriculture, medicine, and global change (Alpert, 2005; Zotz and Bader, 2009). The field of study is turning out to be more attractive to a broad variety of researchers for the understanding of desiccation tolerance in the context of global warming and land-use change. In response to drought stress, plants adapted three main strategies i.e., drought escape, drought avoidance and desiccation tolerance (Levitt, 1980). The ability to escape from periods of drought commonly known as drought escape in which plant dies during drought periods whereas spores and vegetative propagules remains viable in the ground. This strategy is adapted by annual mosses of family Pottiaceae. When plants withstand in a period of drought by maintaining internal water balance, refer as drought avoidance.

This strategy is rarely adapted in bryophytes which is linked through morphological variations to store and transport water, for example presence of hyaline cells in *Sphagnum* species (Vanderpoorten and Goffinet, 2009). Another alternative strategy is desiccation tolerance where bryophytes can recover a normal metabolism upon rehydration after losing their cell water content (Proctor, 2000). Desiccation tolerance is widespread in the plant kingdom including ferns, mosses and their spores, pollen and seeds of higher plants, vegetative tissue of resurrection plants but not gymnosperm plants (Oliver, 1996). In orthodox seeds, studies found that cellular protection mechanisms are involved in desiccation tolerance (Alpert and Oliver, 2002). Bryophytes and vascular plants show contrasting adaptive strategies to drought. Higher plants developed the conduction system where underground roots provides the availability of water to the leaves whereas bryophyte adapted the alternative strategy of photosynthesizing and growing when water is available (Vanderpoorten and Goffinet, 2009).

Effect of ABA hardening treatments on aspects of desiccation tolerance has been studied in the liverwort *Dumortiera hirsuta* and the moss *Atrichum androgynum* and results found increase desiccation tolerance in partial dehydration and in ABA treatments. *D. hirsuta* was more responsive to ABA hardening than *Atrichum* and thus, mechanism of hardening induced appear quite different in two species (Marschall and Beckett, 2005). Among various group of mosses, *Sphagnum* mosses are the most important carbon fixers in peatland habitats. Some physiological properties has been studied in *Sphagnum recurvum* under different environmental conditions. Total chl concentration was approx. 2.0 mg g^{-1} (dw.) found similar as in other open exposed habitats mosses. Other feature, electron flow does not saturate and continue to rise with increasing irradiance, and it shows high levels of NPQ. Sucrose and fructan are major soluble carbohydrates found in *S. recurvum* (Marschall and Laufer, 2002). Another study on photosynthetic responses has been reported on *Sphagnum angustifolium* to study the effect of exogenous ABA on aspects of desiccation tolerance.

Chl fluorescence parameters were monitored during slow or rapid desiccation and upon rehydration. Results indicated recovery of PSII activity in ABA hardening indicated an effective protection against light stress (Marschall and Borbély, 2011). Nitrate reductase activity has been detected in the desiccation-tolerant moss *S. ruralis* and the liverwort *Porella platyphylla*. During first hour of rehydration, *S. ruralis* showed a rapid fall in NR activity during light and modest decrease in dark whereas *P. platyphylla* showed little change in the light and increase in the dark (Marschall, 1998).

2.4.1 Desiccation tolerance in *Syntrichia ruralis*

S. ruralis serves as an experimental model plant system in bryophytes for stress tolerance mechanism which will become useful for understanding how plants respond to the changes of environment. Most initial works were done on *S.ruralis* and few experiments have shown that the desert moss *S. caninervis* can survive rapid desiccation (within 30 minutes) to approximately -540 MPa for up to six years, returning to normal metabolic activity upon rehydration (Oliver *et al.,* 2009). Desiccation tolerance is the ability of the plants to survive drying below the absolute water content of 0.1 g H_2O g^{-1} dry mass (g g^{-1}) (Farrant, 2010). Previous work reported that in the moss *S. ruralis*, they indicated a level of constitutive cellular protection associated with induced recovery system during rehydration for tolerance against desiccation (Oliver, 1996; Oliver and Bewley, 1997). In *S. ruralis*, cell membrane and plasma membrane remain intact in the process of drying (Oliver *et al.,* 2009). During desiccation, many bryophytes have mechanisms for cellular protection, but this varies even within closely related species in one genus. An ultrastructural study using freeze-fracture preparations showed that organelles and membranes in *S. ruralis* and the resurrection plant *Selaginella lepidophylla* were not disrupted in the desiccated state (Brighigna *et al.,* 2002).

Proteins required for cellular protection against desiccation are already present to maintain membrane integrity in *S. ruralis* (Platt, Oliver and Thomson, 1994). There has been controversy about the constitutive or non-constitutive mechanism in such mosses as *S. ruralis*, latter processes variously described as a repair-based mechanism (Mayaba and Beckett, 2001). Previously, the idea of the repair-based mechanism was reported by (Bewley, 1979; Bewley and Krochko, 1982) related to the recovery of normal function by cell membrane following rehydration. Later, fine-structural changes were studied through electron micrographs of freshly rehydrated bryophytes reviewed by Oliver and Bewley (1997) and Bewley and Oliver (1992). Physiological work was done in bryophytes by Alpert and Oliver (2002); Oliver, Velten and Mishler (2005) discussed a substantial role for constitutive mechanism in desiccation tolerance.

Infrared gas analysis and CO_2 uptake experiments showed instant reactivation of respiration and return to normal rates of net photosynthesis within 30-60 min in such species *S. ruralis*, *Grimmia pulvinata* and *Andreaea rothii* (Tuba, Csintalan and Proctor, 1996; Proctor and Pence, 2002) also showed that photosynthesis begins within a few minutes after rehydration (Proctor and Smirnoff, 2000). Earlier work has been reported on *S. ruralis* to study the limits of desiccation tolerance by desiccating the samples either rapidly or slowly to different tissue water contents.

Results showed slow drying caused temporary increased in dark respiration and electrolyte leakage and a slight inhibition in growth while rapid drying caused visible injury, reduced chlorophyll ration and enhanced electrolyte efflux with severely inhibited gross photosynthesis and linear growth (Schonbeck and Bewley, 1981a). Previously, fewer studies have been done on a physiological and molecular level to understand the mechanism behind the desiccation tolerance in bryophytes reported that *S. ruralis* are adapted and abundant in the semi-arid habitats and can tolerate high irradiation and temperature fluctuations (Oliver, Velten and Mishler, 2005). 'Blackspots' of desiccated moss carpet of *S. ruralis* is visible in summer in semi-arid sandy grassland, Hungary. In addition, it contributes 18-20 % to the total cover found between the scattered tufts of dominant grasses (*Festucetum vaginatae danubiale)* association and plays important role in the function of this community (Csintalan *et al.*, 2000).

2.5 General overview of the photosynthesis in bryophytes

In photosynthesis, two light reactions occur simultaneously at the two photosystems PSI and PSII reaction centers. The main function of light harvesting antenna (LHC) pigments is to absorb the light energy and distribute or transfer it to the photosystems reaction centers which used to oxidize water to oxygen, reduce $NADP^+$ and produce ATP (Misra, Misra and Singh, 2012). At room temperature, most of the chlorophyll a fluorescence emission at 685 nm originates in the antenna complexes of PSII (Govindjee, 2004). Bryophytes are evolving in terrestrial and in aquatic environments from long time and adjusting to different climatic changes by surviving through different environmental variations (Hanson and Rice, 2014). Photorespiration is first discovered in bryophytes that contributes to the loss of CO_2 in light compared to dark. Its rate is greater that dark respiration in C_3 plants (Dilks, 1976). Bryophytes leaves are one-cell thick with no epidermis and no stomata. Therefore, these cells are exposed directly to light and have access to atmospheric gases directly. Due to simpler structure of their leaves, it makes possible to make immediate advantage of photosynthetic possibilities for bryophytes (Glime, 2017b). Some studies were elaborated the structural modifications adapted by bryophytes for their survival. In *Polytrichum commune,* leaf lamellae increase the surface areas (Thomas *et al.*, 1996).

2.5.1 Photosynthetic apparatus: Chloroplast

The structure of chloroplasts in mosses can changes with different wavelengths of light. These changes were reported by Zurzycki (1974) in *Funaria hygrometrica* and in *Marchantia polymorpha* (Fredericq and Greef, 1968). Bryophytes have chlorophylls *a* and *b* and organized within a chloroplast like green algae and vascular plants.

The light- harvesting chlorophyll protein (LHCP) complexes and fatty acids are different from vascular plants (Glime, 2017b). Alpha (α) and beta (β)-carotene, zeaxanthin, neoxanthin, lutein and violaxanthin all are the most common antenna pigments. As in vascular plants, the chlorophyll antenna system traps energy from different wavelengths and transfer to chl *a* (Boston *et al.*, 1991).

Several studies have been done to locate a pathway among bryophytes. The ratio of RUBISCO carboxylase activity was found more than PEP carboxylase activity (Farmer, Maberly and Bowes, 1986; Keeley, Deniro and Sternberg, 1986). Finally, Raven, Macfarlane and Griffiths (1987) evaluated that bryophytes have C_3 photosynthetic pathway that is based on the CO_2 compensation point. Although some aquatic bryophyte such as *Fontinalis antipyretica* suggesting CO_2 concentrating mechanism (Raven *et al.,* 1998). Further, evidence was supported this mechanism in members of anthocerophyta (hornworts), they can update CO_2 uptake through proteinaceous bodies known as pyrenoids. Plants with this kind of CO_2-concentrating mechanism were reported higher affinity for external CO_2 than C_3 plants (Hanson, Andrews and Badger, 2002). Still these mechanism does not clearly understand how it works.

Photosynthetic products can be stored in different forms in mosses and liverworts. Sucrose is soluble sugar in mosses (Suire, 1975) and sugar alcohols in liverworts (Suleiman *et al.*, 1979). In some habitats, where photosynthesis is limited by light, the plants may assist by sugars to maintain carbon balance (Graham *et al.,* 2010). Photoinhibition is a phenomenon of light induced damage to PSII activity and decreases the photosynthetic capacity of plants (Murata *et al.* 2007). Adamson *et al.* (1988) reported the reduction in photosynthetic efficiency in *Schistidium antarctici* during moderate light intensity or low temperature that indicated temperature has a major role in photoinhibition. On the other hand, the desiccation-tolerant moss *S. ruralis* var. *arenicola* suffered little damage to the chlorophyll as compared to desiccation intolerant mosses *Dicranella palustris* (Seel, Hendry and Lee, 1992a). Photo-protective quenching mechanism is the ability of the plants that redistribute the energy in a way which avoids the damage from high light intensities. Accessory pigments can help in filtering the light such as zeaxanthin stabilizes the energy level (Glime, 2017c).

In the moss *Rhytidiadelephus squarrous,* the light quenching of chlorophyll fluorescence originated in the antenna system. However, in vascular plants such as *Arabidopsis thaliana* happened in the reaction center (Bukhov *et al.*, 2001). Chlorophyll a:b ratios and chl concentrations are depended on changes in light conditions (Martin and Churchill, 1982). Marschall and Proctor (2004), reported that chl a:b ratios, chl: carotenoid ratios and chl concentration were correlated with photosynthetic photon flux density (PPFD) at 95 % saturation level in bryophytes.

The total chlorophyll on dry weight basis was reported in late summer and winter than in early summer in the desiccation-tolerant *S. ruralis* (Mishler and Oliver, 1991). Photosynthesis is limited by light, temperature, CO_2 availability and availability of water (Glime, 2017d). Water availability has a major role in the distributions of bryophyte. However, they are first colonized the land from water (Mishler and Churchill, 1985). Thylakoid membranes of green plants consist of photosynthetic antenna molecules which absorbed light energy by chlorophyll, carotenoids and other pigment molecules (Maxwell and Johnson, 2000; Strasser *et al.*, 2000; Govindjee, 2004). When light energy is absorbed, photosynthetic system can undergo with three outcomes a) photochemistry (energy used for photosynthesis b) dissipation in the form of heat c) re-emitted as fluorescence. Any increase in the efficiency of one process would lead to a reduction in the yield of the other two processes (Misra, Misra and Singh, 2012). Therefore, the yield of chlorophyll fluorescence can provide details about the changes in the efficiency of photochemistry and heat dissipation.

2.5.2 Limiting factors

There are the main controlling conditions which can limit the rate of photosynthesis and they are refers as limiting factors such as light intensity, temperature, CO_2 concentration and another important availability of water (Glime, 2017d). Gerdol *et al.,* (1998) reported that the productivity is affected by night-time temperatures, nutrients at optimum temperatures on *Sphagnum capillifolium* and also suggested that different enzymes become active at different temperatures and different pH levels and enzymatic reactions found limited at unfavourable temperatures. Bryophytes has one-cell thick leaves, and they are susceptible to damage caused by high light intensity (UV-light). Hamerlynck *et al.,* (2002) found that in desiccation-tolerant species such as *S. ruralis* had lower biomass, and lower tissue N, C (% dry mass) and chlorophyll concentrations than shade plant of the species. They transported them to shade and these desiccation-tolerant species able to adjust to the lower light conditions by increasing their PSII yields and *vice versa* results were observed with shade plants. Cleavitt (2002) demonstrated that high light intensities can the damage chlorophyll and they can cause photoinhibition in *Mnium spinulosum* restricted to deep shade whereas *Bryum pseudotriquetrum* limited by moisture reflects lack of desiccation tolerance. Structural adaptations or protective pigments plays important role in adaptation from high light intensities whereas in vascular plants they have protective epidermal layers and palisade layers in most groups which serves to absorb light before reaches the photosynthetic tissue of the spongy mesophyll (Glime, 2017d). However, bryophytes do not have these structures, but they are more involved in reducing the damage effects of high light at the level of cellular protection (Robinson and Waterman, 2014).

In Hawaiian mosses, structural traits (cell size, cell wall thickness, leaf area, canopy density) were found aligned with microhabitat irradiance (Waite and Sack, 2010). Bryophytes have filters that might help to filter the light e.g., lamellae is found in *Polytrichum juniperinum,* filaments in *Crossidium abe*rrans, hyaline cells in *Bryum argenteum, Hedwigia ciliate,* epiphytes like *Octoblepharum albidum, Leucobryum glaucum* and awns found in *Tortula, Syntrichia* species (Glime, 2017d). A unique spectral feature was found in species from boreal wetlands. Three group of mosses e.g., *Sphagnum,* feather mosses and brown mosses has different cellular characteristics. They suggested these mosses respond to various spectrum visible, near infrared and short-wave infrared (Bubier, Rock and Crill, 1997). Coloured pigments can also filter the light and help in reducing the light energy, thus protecting the chlorophyll and DNA. Ethylene is responsible for the production of red pigments which are particularly common at low temperatures and in bright light and inhibits the synthesis of carotenoids and chlorophyll (Kang and Burg, 1972). Violaxanthin is known to increase in response to high light. Zeaxanthin enhances light quenching in an atmospheric 20% CO_2 which appear distinct feature as compared to vascular plants *Arabidopsis* and spinach (Bukhov *et al.,* 2001) and thus reducing the energy reaching the chlorophyll *a.*

Mosses that live in desiccation conditions are also suffer light damage during hot and dry periods. *S. ruralis* retains all its pigments with recovering its photosynthetic functions upon rehydration as it dehydrates in the morning and its recovery depends on light conditions and drying speed. However, in vascular plants they rapidly loose capability of their photosystem II under desiccation condition. (Hamerlynck *et al.,* 2002). In desiccation-tolerant bryophytes protein protonation is associated with high levels of zeaxanthin which indicate capable of dissipating excess light energy (Heber, 2001).

Some mosses are avoiding from intense radiation by hiding under the rocks and avoiding the damage to pigments. In Californian Desert, *S. inermis* using the rock as a filter and surviving under beneath quartz pebbles (Werger and During, 1989). In inselbergs of Bosmansland, South Africa, some bryophyte communities found beneath stones e.g., *Bryum argenteum, Riccia spp*., other pottiaceae species *Chamaebryum, Gigaspermum* buried to a depth of a few centimeters. In Antartica, bryophytes occur beneath rocks and sand (Smith, 2000) such as *Hennediella heimii* found lives under the quartz rocks. In Mojave Desert, conditions are very intense and hot but *S. caninervis* has found under translucent quartz rocks as rocks helps to maintain a longer hydration period (Glime, 2017d).

Distribution of bryophytes and growth rate is determined by the temperature of the microclimate and highlighted the importance of temperature in Azorean forest (Glime, 2017d). Bryophytes may experience dependent on temperatures and also reflect as one of the limiting factors. Mosses have different optimum and lethal temperature. In aquatic mosses, several *Fontinalis* species perform well at 20 ^{o}C for a period of time then they lose their growth rate (Glime and Acton, 1979). In arctic mosses, the net photosynthetic rate in *Sanionia uncinate* was found nearly constant at near-saturating light levels across the range of 7 to 20 ^{o}C and these species experienced a large increase in the gross photosynthesis with temperature (Uchida *et al.*, 2002).

In coastal British Columbia, Canada, peatland (*Sphagnum* spp.) had found lower temperature thresholds than in *Pleurozium schreberi* and *Racomitrium lanuginosum* which indicate a threshold effect on bryophyte productivity (Asada, Warner and Banner, 2003). Bryophytes alter their optimum temperature and acclimate to temperature and adjusts to new conditions for photosynthesis. For example, in *Leucodon sciuroides*, low temperature induced the dissipation of absorbed light energy and this ability protect the photosynthetic apparatus and upon return to temperatures above freezing point permits bryophyte to survive high light intensity at lower temperature limits. This moss has become acclimated to the new temperature (Deltoro *et al.*, 1999). The desiccation-tolerant *S. ruralis*, experienced increases in Fv/Fm, NPQ and ΦPSII in sun plants transplanted to shade which may indicated the short-term adjustments to changing light levels permits the species to take advantage of new shade habitat (Hamerlynck *et al.*, 2002).

CO_2 concentration often become limits the plant productivity. CO_2 is a limiting resource therefore some plant species such as in green algae (Chlorophyta) have specialized proteinaceous structure associated with chloroplasts, namely as pyrenoids which has ability to concentrate CO_2 and permits them to store inorganic carbon for later (Smith and Griffiths, 1996). Bryophytes take advantage of CO_2 emitted through soil respiration. In New Zealand temperate rainforest, bryophytes used only about 10% of the CO_2 emitted from the soil microbes and contributed to carbon fixation in the boreal forest (Delucia *et al.*, 2003). Water availability is also as important limiting factor. Uchida *et al.* (2002) reported that *Sanionia uncinata* has found higher photosynthetic activities only on rainy days which may indicated the water limits the productivity in Svalbard, Norway. Similarly, importance of water for the growth of *Sphagnum* species has been reported (Asada, Warner and Banner, 2003). In dry habitats, various bryophytes had significantly different responses to water content, light, desiccation (Alpert and Oechel, 1987). Such studies give illustrations on the adaptability of bryophytes with distinct conditions.

The two rock-dwelling mosses *Grimmia pulvinate* and *Anomodon viticulosus* found a sharp peak of non-photosynthetic quenching during remoistening and recover slowly in less desiccation-tolerant *Rhytidiadelphus loreus* (Csintalan, Proctor and Tuba, 1999). Species also respond different to humidity. In *Plagiomnium acutum*, photosyhthetic rates were found higher on cloudy and rainy days as compared to *Herpetineuron toccoae* and lower rates for sunny days (Li et al., 1999). In the desert moss *S.ruralis*, water was absorbed gradually throughout much of the night, and it provided sufficient water for the moss for net photosynthesis immediately after dawn and suggested this short time period was sufficient for repairing following long-term desiccation damage (Csintalan *et al.*, 2000).

2.6 Chlorophyll fluorescence in bryophytes and Fluorescence Monitoring System (FMS2)

Chlorophyll fluorescence is one of the most popular techniques which provides information directly related to photosynthetic performance in plants. It is a non-invasive measurement of photosystem II (PSII) activity (Murchie and Lawson, 2013). It provides detailed information about a plant status in a short period of time which improves the research study in the field of crop ecology and plant ecophysiology. In recent decades, the use of this technique become increased due to research in the improvement of crop plants especially for the screening of desirable plant traits (Baker and Rosenqvist, 2004).

Chlorophyll fluorescence is a measure of re-emitted light from the chlorophyll molecules from an excited state to a non-excited state. Light absorbed by chlorophyll molecules can be categorized into two processes: photochemical and non-photochemical processes in photosynthetic systems. In the photochemical process, absorbed energy can drive photosynthesis (photochemistry) whereas in the non-photochemical process, absorbed energy re-emitted in the form of heat and light (fluorescence).

This chlorophyll fluorescence emission provides valuable information about the quantum efficiency of photochemistry and heat dissipation (Murchie and Lawson, 2013). A chlorophyll fluorometer is designed to measure the chlorophyll fluorescence emission from plant samples. Hansatech Instruments manufacture chlorophyll fluorometers based on two different measurement techniques. First one is Continuous Excitation chlorophyll fluorometer: It is designed to measure the Kautsky Induction or Fast chlorophyll fluorescence Induction. It used high light intensity from red LEDs to induce a fast response from a dark-adapted sample. It is a multi-purpose tool that prevents ambient light leakage into the highly sensitive photodiode to detect chlorophyll fluorescence. Second one is Pulse-modulated chlorophyll fluorometer: It is designed to separate chlorophyll fluorescence from ambient light.

It used rapid pulsing excitation light to induce pulsed fluorescence emission. The system uses a highly sensitive photodiode to record the pulsed signal and neglect any non-pulsed signal (**Figure 2).**

Figure 2. FMS 2 system (Hansatech Instrument) during the chl fluorescence measurement in the laboratory of the Department of Plant Physiology and Plant Ecology, Institute of Agronomy Gödöllő.

In this thesis, Pulse Amplitude Modulation (PAM) fluorometry (Schreiber, Schliwa and Bilger, 1986; Schreiber, 2004) used for chlorophyll fluorescence measurements. PAM is widely used chlorophyll fluorescence technique which also called as quenching analysis of modulated fluorescence along with saturation pulse-method. A high intensity of light is used in this technique which would be switched on and off at high frequency and detector is used to measure the fluorescence emission only thereby providing us a more efficient system to measure chlorophyll fluorescence (Schreiber, 2004).

Photochemical quenching parameters such as the maximum quantum efficiency of PSII photochemistry (Fv/Fm), the effective PSII quantum yield (ΦPSII), the photochemical fluorescence quenching (qP) and non-photochemical quenching parameters such as non-photochemical fluorescence quenching (qN) and non-photochemical quenching (NPQ) has been determined through this technique in this present study. Modulated chlorophyll-fluorescence techniques showed how fast recovery of the photosystems can be during rehydration. Species such as *S. ruralis* of dry sunny habitat show high level of NPQ referring to its photoprotection role.

When sunlight much exceeds the quantum requirements for photochemistry in photosynthesis under natural conditions, it referred as photoinhibition. Under these conditions, the ability to restore the damaged PSII reaction centre becomes suboptimal and an irreversible inhibition of PSII can be detected as a decrease in the chlorophyll fluorescence ratio (Fv/Fm) (Misra, Srivastava and Strasser, 2001a,b; Misra, Srivastava and Strasser, 2007). Therefore, this parameter is useful to measure the scope of photoinhibition in photosynthesis. Non-photochemical quenching (NPQ) of chlorophyll fluorescence is used as an indicator to study the level of energy dissipation in the LHC II of PSII which provides protection from photodamage (Misra, Misra and Singh, 2012).

Previously, chlorophyll *a* fluorescence measurement has been used for study desiccation tolerance in bryophytes (Proctor and Smirnoff, 2000; Robinson *et al.* 2000; Cleavitt, 2002; Proctor, 2003; Lüttge, Meirelles and De Mattos, 2008; Cruz de Carvalho, Branquinho and Marques Da Silva, 2011) and for studying photoprotection during rehydration (Beckett, Marschall and Laufer, 2005). Recently chlorophyll fluorescence techniques has been used to understand the photosynthetic response with light and moisture (Cui *et al.,* 2009; Hájek *et al.* 2009). Mostly, fluorescence emission were measured by pulse-amplitude modulated method by means of the saturation pulse which allows quenching of absorbed light energy (Misra, Misra and Singh, 2012). Later on, Liepina and Ievinsh, (2013) reported that chlorophyll *a* fluorescence measurements could be rapid and efficient tool for eco-physiological studies which allows to study the differences in the photochemistry of photosynthesis for bryophytes species growing in different microhabitats.

Chlorophyll fluorescence measurements has been used in wide range application in the field of forestry, crop productivity and stress adaptation in plants (Baker and Rosenqvist, 2004; Strasser, Tsimilli–Michael and Srivastava, 2004; Roháček, Soukupová and Barták, 2008). Besides this fast chlorophyll fluorescence can be used as a sensitive device for detection of salt sensitivity and other environmental stress factors (Misra, Srivastava and Strasser, 2001a; Misra, Srivastava and Strasser, 2007). This method has keep on continuously used by researchers that has a great advantage in the field of bryophyte ecophysiology.

In this thesis study, we also used chlorophyll fluorescence technique to study the photosynthetic responses during different environmental stress conditions.

2.7 Antioxidant enzymatic system in plants

Antioxidants are those compounds that inhibit the oxidation due to the chain reactions of free radicals. They are broadly classified into three groups (Sindhi *et al.,* 2013).

1. The first group of antioxidants are the enzymes which include catalase, superoxide dismutase, peroxidases, and glutathione reductase along with the minerals like Se, Cu, Zn, Fe, Mn, etc. that act as cofactors of these enzymes.
2. The second group of antioxidants includes glutathione, vitamin E (tocopherols), vitamin C, lipoic acid, albumin, carotenoids (vitamin A), phenolics and flavonoids.
3. The third group of antioxidants includes a complex group of enzymes like DNA repair enzymes, transferases, lipases, proteases, methionine sulfoxide reductase, etc. which are used for repair of damaged DNA, damaged proteins, oxidized lipids and peroxides (Irshad and Chaudhuri, 2002).

In recent years, most of the studies has been done on ROS as it plays an important role in signalling, controlling processes such as growth, development, and their response to biotic and abiotic environmental factors. ROS family include free radicals which are highly reactive atoms or molecules with an unpaired electron such as singlet oxygen ($^1O_2$), superoxide (O_2^-), hydroxyl radical (OH) and nitric oxide (NO) and non-radical members like H_2O_2 and O_2. Major source of ROS production in plants are those organelles that has a highly oxidizing metabolic activity or with an intense rate of electron flow, such as mitochondrion, peroxisome and chloroplast.

Peroxidases are present in the cell walls along with these organelles (Foyer and Shigeoka, 2011). Plant NADPH oxidases are also enzymatic source of ROS, and they act as key signalling nodes in the network of ROS regulation in plants with integrating various signal transduction pathways with ROS signalling and mediating multiple biological processes including cell growth and plant development, biotic and abiotic stress responses (Marino *et al.,* 2012). During stress conditions, ROS production become increased and acts as a signal for the activation of stress response antioxidant pathways; enzymatic and non-enzymatic (Baxter, Mittler and Suzuki, 2014). Plants produce alternative oxidases as potion to this antioxidant system that have ability to prevent the excess generation of ROS in the electron transport chains of mitochondria. Other mechanisms such as movement and curling of leaf or rearranging of photosynthetic apparatus may also represent to avoid the over-reduction of ROS by balancing the amount of energy absorbed by the plant with the CO_2 availability (Mittler, 2002).

Oxidative stress is caused due to various environmental stresses, but drought and heat are two main stress factors and limiting the survival of moss biocrusts in arid areas (Chongfeng *et al*., 2017). Desiccation is one of the stress which causes increase in oxidative damage (Smirnoff, 1993) whereas decreases in the cellular water content and organelles with high rates of electron flow such as chloroplasts, mitochondria, peroxisomes upregulates the production of ROS and hydrogen peroxide (H_2O_2) (Mittler, 2002; Scheibe and Back, 2011).

These stresses increased reactive oxygen species (ROS) production inside the plant cell. These reactive oxygen species (ROS) react with proteins, lipids, and nucleic acids, thereby causing damage to enzymes (Wolff *et al.,* 1986; Halliwell, 1999), membranes (Senaratna and McKersie, 1983; Leprince *et al.,* 2000) and chromosomes (Dizdaroglu, 1994). ROS also called reactive oxygen species which are the products of partial reduction of atmospheric O_2. There are well known four forms such as superoxide radical (O_2^-), hydroxyl radicals ($OH^·$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), each have a half-life and an oxidizing potential. They can oxidize in cellular components like lipids, proteins, DNA and RNA which may cause cell death (Mittler, 2002; Cruz de Carvalho, 2008). In all plants, they have an antioxidant system for the detoxification of excess ROS and maintain the balance and control the overproduction of ROS species with antioxidant enzymatic and non-enzymatic activities.

In resurrection plants, proteomic studies has been carried out and results showed an increase in ROS-scavenging enzymes (Ingle et *al.,* 2007; Jiang *et al.,* 2007). On the other hand, during desiccation photosynthetic system is blocked then there is gradually a decrease in proteins related to photosynthetic activity to avoid the formulation of ROS has been reported in Oliver *et al.* (2010). In terrestrial mosses, ROS production in response to desiccation and rehydration has been determined (Minibayeva and Beckett, 2001; Mayaba *et al.,* 2002; Beckett *et al.,* 2004). Bryophytes are among the pioneer colonizers that are found on extreme conditions, and they are well adapted to oxidative stress (Nagae, Nakata and Takahashi, 2008). During desiccation, antioxidant enzymes play a role in tolerance for long survival in the desiccated state (Kranner and Birtic, 2005). In Seel *et al.,* (1992b) has reported the correlation between the desiccation tolerance and various activated antioxidant enzymes in the mosses *Tortula ruraliformis* and *Dicranella palustris*. In moss *Atrichum androgynum,* CAT and SOD were not found responsible for desiccation tolerance induced by hardening treatment (Mayaba and Beckett, 2003). In liverwort *Monoclea forsteri,* MDA content has been found increase in the gametophytes which was indicated by oxidative damage during dehydration (Hooijmaijers, 2008).

Antioxidant defense system responses has been investigated in some mosses growing on rocks *Barbula fallax, Erythrodontium julaceum* and *Bryum argenteum* (epilithic mosses) in different terrestrial habitats in Guizhou, Southwest China (Zhang, Zhao and Wang, 2017). Among these species, *E. julaceum* demonstrated the strongest resistance followed by *B. fallax* and *B. argenteum*. One of the major antioxidant systems, the ascorbate system has been studied in two bryophytes *Brachythecium velutinum* (moss) and *Marchantia polymorpha* (liverwort) which found that ascorbate content was maintained in the moss after drought stress while it declines in the liverwort (Paciolla and Tommasi, 2003).

In this thesis, the focus was on the ROS scavenging mechanism mediated by enzymatic enzymes highlighting the role of ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (POD) in stress tolerance. These antioxidant enzymes are in different sites of plant cells where ROS produced under both normal and stressful conditions such as cell wall, plasma membranes, chloroplasts, mitochondria, peroxisomes, and endoplasmic reticulum (ER) where they work together to detoxify the ROS (Noctor and Mhamdi, 2017).

2.7.1 Ascorbate peroxidase (APX, EC 1.11.1.11)

It is a hydrogen peroxide scavenging enzyme found in prokaryotes, fungi, plants, and mammals. It catalyses the oxidation of cellular components by either hydrogen peroxide or organic hydroperoxides. In plants, the peroxidases are referred to as guaiacol peroxidases because guaiacol is used as an electron donor which helps in biosynthesis of lignin and ethylene and in the degradation of indole-3-acetic acid (Asada, 1992).

It is found in the moss gametophytes both in the reduced and oxidized form which utilize ascorbate in removing H_2O_2 by ascorbate peroxidase and reconvert to ascorbate its oxidation products by means of dehydroascorbate reductase and monodehydroascorbate reductase.

In the cytosolic fraction, ascorbate oxidase activity was measured suggesting localization of the enzyme different from more evolved organisms (Paciolla and Tommasi, 2003). It is one of the major antioxidant species and synthesized in plant cells. It is metabolized by the two enzymes: ascorbate peroxidase and ascorbate oxidase found in different cell localization. It is present in chloroplasts, cytosol, and vacuole and apoplastic space of leaf cell in high concentrations. It catalyses the reduction of H_2O_2 using the substrate dehydroascorbate as following reaction:

$$\text{Ascorbate} + H_2O_2 \xrightarrow{\text{APX}} \text{Dehydroascorbate} + 2H_2O$$

One mole of H_2O_2 oxidizes one mole of ascorbate to produce one mole of dehydroascorbate (Nakano and Asada, 1981).

2.7.2 Catalase (CAT EC 1.11.1.6)

Catalases are the tetrameric heme-containing enzymes which is responsible for the degradation of the reactive oxygen species (ROS). This enzyme is the unique antioxidant enzyme that does not require any reducing agent in contrast with APX enzyme used ascorbic acid and POD used guaiacol as a substrate.

It catalyses the decomposition of H_2O_2 to give H_2O and O_2 (Aebi, 1984) as following reaction:

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O_2 + O_2$$

Catalase is an oxidoreductase enzyme which decomposes H_2O_2 to water and molecular oxygen (Baek, Kwon and Park, 2000). Peroxidase activities play protective roles in enhancing their tolerance under unfavourable conditions (Ghorbanli, Tehran and Niyakan, 2012). Bryophytes are sensitive plants that produce secondary metabolites that strengthen them with strong antioxidative machinery to cope with biotic and abiotic stress (Xie and Lou, 2009). The antioxidant defence response protects the cell organelles and cell membranes against oxidative damage. Overproduction of ROS disrupting the structure of the cell and reacts with lipids and proteins leads to cell damage under unfavourable conditions (Aslanbaba *et al*., 2017).

2.7.3 Guaiacol peroxidase (POD, EC 1.11.1.7)

POD is a heme-containing enzyme composed of 40-50 kDa monomers that eliminates excess H_2O_2 both during normal metabolism and stressful conditions. It also plays a pivotal role in the biosynthesis of lignin and participate in defense against biotic stress by degrading indole acetic acid (IAA) and utilizing H_2O_2 in the process (Corban *et al.,* 2011).

$$H_2O_2 + \text{Guaiacol} \xrightarrow{\text{POD}} \text{Tetra guaiacol} + H_2O_2$$

2.7.4 General characteristics of salicylic acid (SA)

In the entire plant kingdom, salicylic acid is worldwide distributed and its history back to 1878, when it was Germany's highest selling drug in the world. The word salicylic acid came from the Latin word "Salix" meaning willow tree, and the name was given by Rafacle Piria as salicylic acid in 1938 (Hayat, Ali and Ahmad, 2007; Hayat *et al.,* 2010).

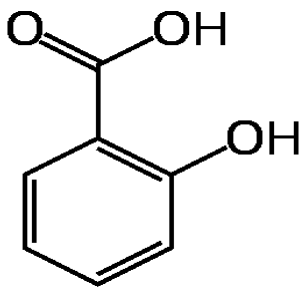


Figure 3. Chemical structure of salicylic acid.

It is a phenolic-type endogenous plant growth regulator (**Figure 3)** that has an aromatic ring with a hydroxyl group or its functional derivative.

SA is found in a free state in a crystalline powder state with a melting point of 157-159 ^{o}C and a pH of 2.4 (Raskin, 1992a). It has been found that salicylic acid plays a key role in controlling plant development, growth, interaction with other organisms and reacting to environmental stresses (Raskin, 1992a,b; Senaratna *et al.,* 2000). In addition, its role is obvious in the germination of seeds, fruit yield, glycolysis, flowering in thermogenic plants, ion uptake and transport, photosynthetic rate, stomatal conductance, and transpiration (Hayat *et al.,* 2010). Salicylic acid and other salicylates are known to affect the various physiological and biological activities of plants and may play a key role in regulating their growth and productivity (Arberg, 1981).

2.7.5 Interaction of SA with antioxidant system

Stressful conditions trigger the production of ROS in plants thereby causes oxidative stress (Panda, Chaudhury and Khan, 2003). These stresses may lead to damage the biomolecules such as lipids, proteins, and nucleic acids (Smirnoff, 1993). In vascular plants, SA was found to enhance when applied exogenously at suitable concentrations (Knörzer *et al.,* 1999). Salicylic acid treatment resulted in temporary reduction of catalase activity and increased H_2O_2 level (Janda *et al*., 2003) and tolerance against the oxidative stress in vascular plants.

In other report, it was found that SA enhanced the activities of antioxidant enzymes, CAT, POD and SOD while spraying exogenously to the drought stressed tomato plants (Hayat *et al.,* 2008). Based on previous research, it may be concluded that SA acts a potent plant growth regulator that modulate the various plant growth responses. It enhances the plant growth and productivity, induces systematic acquired resistance (SAR) in plants thereby provide a considerable protection against biotic stress, it also alleviated the toxic effects generated in plants due to the exposure of various abiotic stresses such as temperature, desiccation, irradiance, heavy metals and salinity stress (Hayat *et al.,* 2010). Previous literature cited clearly mentioned the important roles of SA in plants and potentially reduces the disastrous effects generated by various biotic and abiotic stresses. However, still demands of a lot of work to be carried more information related to the biosynthesis pathways, mechanism of action and other regulatory roles of SA. In future, more research is also needed in the field of exogenous application of SA in non-vascular plants. They are the experimental model organisms that contribute to study of evolution of plant hormones. Salicylic acid (SA) can inhibit the later stages of bud formation in *Funaria hygrometrica* in a dose-dependent manner. Results indicated that these mosses might use SA as developmental signals (Christianson and Duffy, 2002). However, further research is needed to clarify and understand their distribution and to study the mechanism behind signal transduction in bryophytes (Sabovljević, Vujičić and Sabovljević, 2014).

The environmental stresses lead to the production and accumulation of reactive oxygen species (ROS) in various cell organelles (Poór, 2020). In plants, catalase and peroxidases help in scavenging ROS and provide protection against oxidative damage which causes inactivation of cell functions (Singh, Eapen and D'souza, 2006).

In this thesis, the level of plant stress was examined in desiccation-tolerant bryophyte; *S. ruralis*. It is found abundantly in the form of extensive mats in open exposed areas of sandy dunes in a semi-arid grassland (Csintalan *et al.*, 2000). Chlorophyll *a* fluorescence parameter was measured and examined its seasonal variation to study the functioning of photosynthetic apparatus and associated photoprotective mechanism in *S. ruralis* (Ruchika, Csintalan and Péli, 2020a). The impacts of exogenous SA pre-treatment on the activities of antioxidant enzymes: Ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxidase (POD) in *S. ruralis* during the different three seasons (spring, summer, autumn, winter) were also investigated (Ruchika, Csintalan and Péli, 2020b).

3 MATERIALS AND METHODS

3.1 Site description

The investigated semi-arid sandy grassland site can be found at Bócsa village in the Southern Great Plain region of southern Hungary. It is located in the higher part of the Danube-Tisza Rivers and the middle of Bács-Kiskun county. Co-ordinates are (central Hungary 46°53′29″ N, 19°26′35.6″ E) is shown in **(Figure 4).** These sandy grasslands are part of the Kiskunság National Park, Bugac in the Hungarian Great Plain. It is known for one of the most characteristics features of grassland vegetation types in Hungary. In these sandy grasslands, sand hills are created by strong winds from several years which cover a large area of square kilometres of the region resulting in a unique landscape around the village. The vegetation is semi-arid sandy grassland dominated by *Carex stenophylla* (Wahlbg.), *Cynodon dactylon* (L.), *Festuca pseudovina* (Hack. Ex Wiesb.) and *Salvia pratensis* (L.) Pers. The grassland has been under management from the last 20 years and part of the Kiskunság National Park (Nagy *et al.*, 2007).

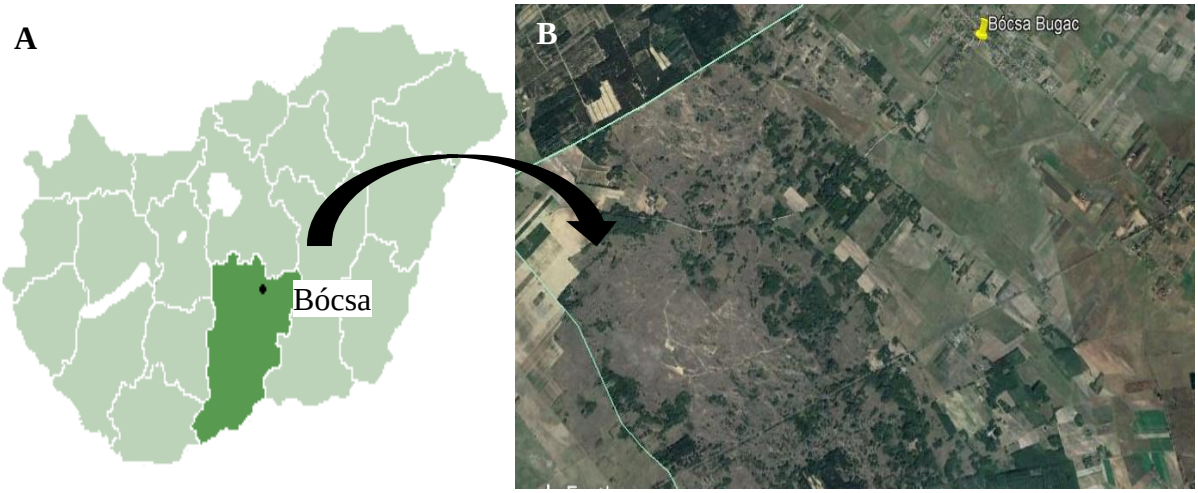


Figure 4 A. Hungarian map showing Bócsa village in the Southern Great Plain, available at (https://www.pngwing.com/en/search?q=B%C3%A1cs-Kiskun+ **B.** Satellite view from google earth showing plant material collecting site.

3.2 Meteorological Data

The climate of the site is semi-arid temperate continental; the average yearly precipitation is 562 mm (Nagy *et al.*, 2007). In the investigated year (2018), changes in the monthly average meteorological parameters (temperature, photosynthetically active radiation, precipitation, and relative humidity) at the Bugacpuszta site (46.69° N, 19.60° E; 111 m above sea level) showed in **Figure 5 A&B.**

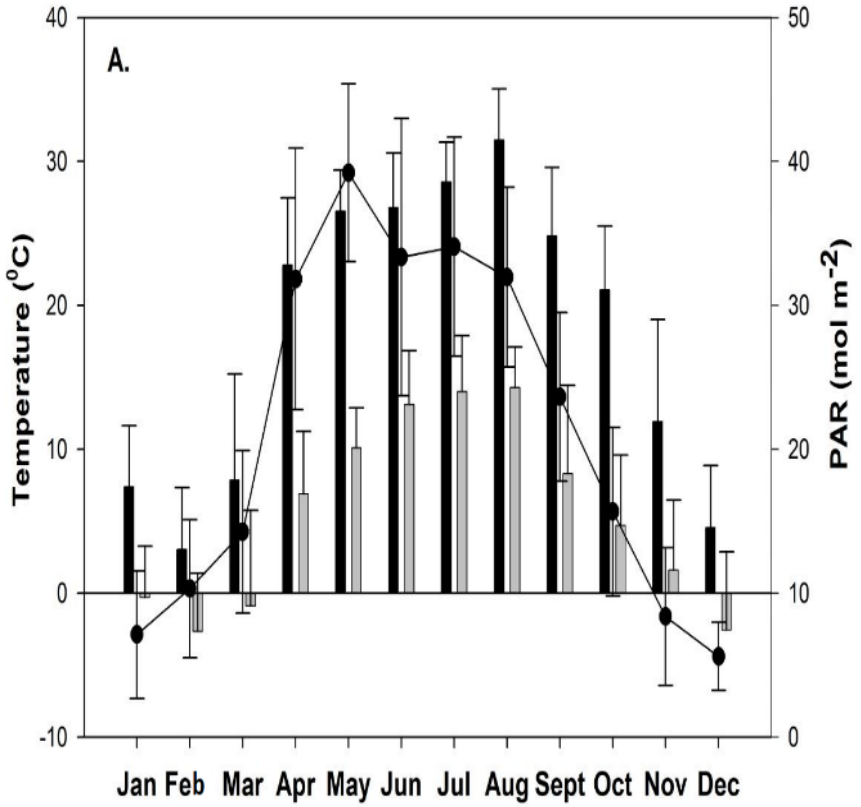


Figure 5 A. Monthly average of the maximum values of air temperature (0 ^{o}C, T max, black bars, and T min, grey bars) and the photosynthetically active radiation (PAR, in mol m^{-2}) values.

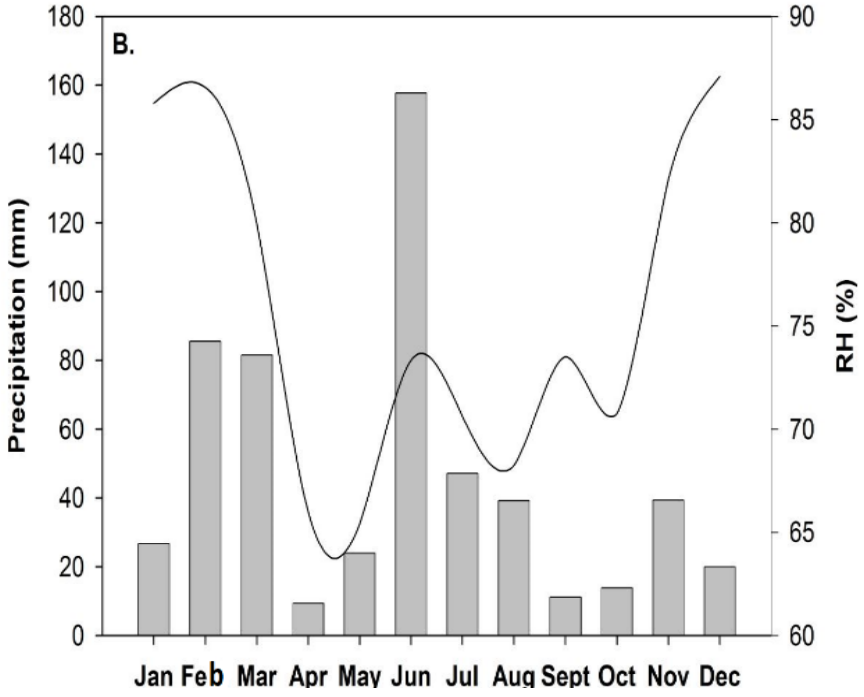


Figure 5B. Monthly sum of precipitation (mm) and average relative humidity (%) during the study period in 2018.

3.3 Sample collection and experimental set-up

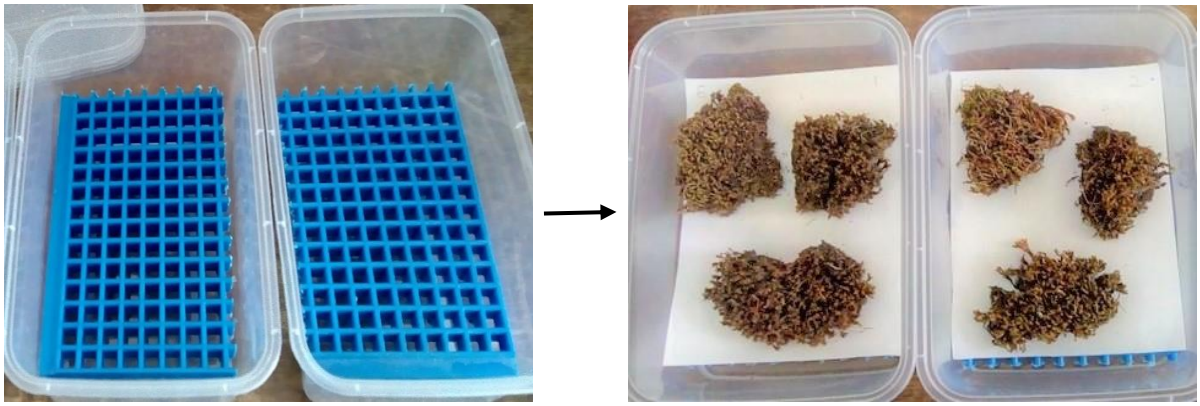
Moss cushions were collected from sandy dunes of semi-arid sandy grassland in air-dried conditions for winter samples (see Figure 5A) during early spring season (March 2018), late spring season (May 2018), summer (July 2018) and autumn (October 2018) from two different microhabitats north-east (NE) and south-west (SW) slopes on the basis of the orientation of sandy dunes and dominant wind direction.

Figure 6. Moss cushions of *S. ruralis* (air-dried) in semi-arid sandy grassland (Source: own photo)

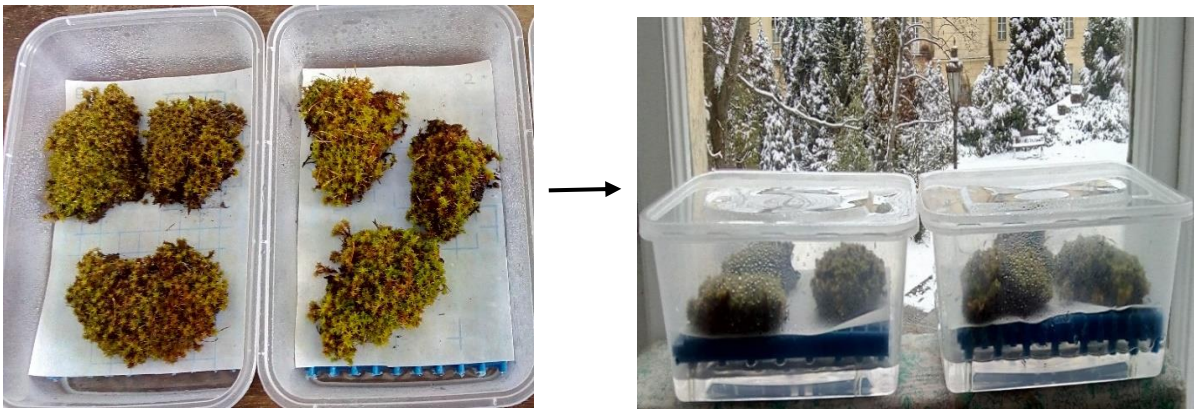
The main aspect of the collection of mosses was the exposition. The collection date was after a drier period for each season but in typical periods of a certain season when the cushions were relatively dry. It is very general and typical in this area because of the climatic condition and the sandy soil coverage which has a fast water loss.

3.3.1. 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.



A. Plastic box containers B. Field samples (air-dried)



C. Samples under rehydrated state D. Samples were covered by lid

Figure 7. Flow-chart showing the experimental set-up of plant samples performed in the laboratory of the Department of Plant Physiology and Plant Ecology, Institute of Agronomy, Gödöllő.

After the experimental set-up, measurement of fresh weight and dry weight of moss cushions were recorded, and shoots of samples were distributed after weighing according to different treatments. 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) and drying out at 80 ^{o}C, respectively; WC = [(FW - DW) / DW] ×100 (Péli *et al.*, 2011).

3.4 Chlorophyll *a* fluorescence measurement for the physiological study

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, 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 and 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), non-photochemical fluorescence quenching (qN) 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, the calculation of fluorescence parameters and the standards is based on the following equations (Roháček, 2002; Proctor, 2003).

Equation 1. Potential quantum yield of PSII

$$\frac{\mathrm{Fv}}{\mathrm{Fm}} = \frac{\mathrm{Fm} - \mathrm{Fo}}{\mathrm{Fm}}$$

Equation 2. Effective PSII quantum yield

$$\Phi\mathrm{PSII} = \frac{\mathrm{Fm'} - \mathrm{Fs}}{\mathrm{Fm'}}$$

Equation 3. Photochemical fluorescence quenching

$$\mathrm{qP} = \frac{(\mathrm{Fm'} - \mathrm{Fs})}{(\mathrm{Fm'} - \mathrm{Fo'})}$$

Equation 4. Non-photochemical fluorescence quenching

$$\mathrm{qN} = \frac{(\mathrm{Fm} - \mathrm{Fm'})}{(\mathrm{Fm} - \mathrm{Fo'})}$$

Equation 5. Non-photochemical quenching

$$\mathrm{NPQ} = \frac{\mathrm{Fm}}{\mathrm{Fm'}} - 1$$

A pulse-modulated chlorophyll fluorometer (FMS 2) were used for conducting chlorophyll fluorescence measurements. It is a tool versatile pulse-modulated instrument and powerful tool for photosynthesis research. This tool is designed to measure chlorophyll fluorescence emission from samples engaged in photosynthesis. It consists of a control unit, a fibre optic, leaf clips, a multi-battery charger, and the main adapter. The system is operated through a serial connection with window PC and data is presented as a real-time chart recorder emulation and 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.

3.5 Spectrophotometric antioxidant enzymatic analysis

3.5.1 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 ^{o}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. For rehydration treatment, rehydrated shoots were weighed and stored in liquid nitrogen to perform the antioxidant enzymatic assay. 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. Rehydrated, desiccated shoot tips and the main shoot with green shoot apex of *S. ruralis* are shown in (**Figure 8. A, B and C respectively).**

Molar extinction coefficient (ε) was used to calculate the enzymatic activities and expressed in terms of mmolmin-1mg-1 protein content or Units/mg protein content. The formula for all enzymatic activity was calculated from the equation given below:

Equation 6. Enzymatic activity

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

Figure 8A. Rehydrated; **B**. desiccated shoot tips; **C**. main shoot with green shoot apex of *S. ruralis*

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

3.5.2 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 (ε = 2.8 $mM^{-1}cm^{-1}$).

Calculations:

The enzyme activity was calculated from the equation given below:

Equation 6. Enzyme activity of APX

Enzyme activity of APX = $\frac{(\Delta Abs \times 1 * 0.1000\ L \times 2mL)}{(2.8 \times 1 \times 0.1mL \times protein\ content)}$ ($mmol.min^{-1}.mg^{-1}$. protein content or Units/mg protein content)

3.5.3 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, 25^0C and Extinction coefficient (36.6 $mM^{-1}cm^{-1}$).

Calculations:

The enzyme activity was calculated from the equation given below:

Equation 7. Enzyme activity of CAT

Enzyme activity of CAT = $\frac{(\Delta Abs \times 1 * 0.1000\ L \times 2mL)}{(0.0366 \times 1 \times 0.1mL \times \text{protein content})}$ (mmol.min^{-1}.mg^{-1}. protein content or Units/mg protein content)

3.5.4 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 1mL final volume. The increase in Tetra guaiacol concentration in a reaction mixture was measured at 470 nm for 150 sec, 25 ^{O}C and Extinction coefficient (ε = 26.6 mM^{-1}cm^{-1})

Calculations:

The enzyme activity was calculated from the equation given below:

Equation 8. Enzyme activity of POD

Enzyme activity of POD = $\frac{(\Delta Abs \times 1 * 0.1000\ L \times 2mL)}{(26.6 \times 1 \times 0.1mL \times \text{protein content})}$ (mmol.min^{-1}.mg^{-1}. protein content or Units/mg protein content)

3.5.5 Protein determination

Protein determination was performed to calculate the enzymatic activity based on protein content. 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}). This dye was used for the quantification of soluble protein content. Enzyme extracts of samples from both microhabitats were measured spectrophotometrically (SHIMADZU UV-1061 UV-visible spectrophotometer) at 595nm wavelength.

3.5.6 Lipid peroxidation (MDA content)

Lipid peroxidation was measured as the amount of MDA (malondialdehyde) 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 ^{o}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 ^{o}C 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 ^{o}C. The absorbance was recorded at 532 nm and corrected for 600 nm. The MDA content expressed in nmol/g dw by using the extinction coefficient of 155 mM^{-1}cm^{-1}.

3.6 Salicylic acid (SA) pre-treatment

3.6.1 Preparation of SA solution for the treatment

In the pilot study, we examined chlorophyll fluorescence under different SA concentrations: 10 µM (low), 0.001 M (medium), 0.01 M (high). Medium concentration was selected to conduct the final experiment. Salicylic acid solution with concentration of 10^{-3} mM was prepared by dissolving 0.1381g in 1000 mL distilled water and transferred the solution in a spray bottle. After 6 hours of rehydration, the fresh weight of all the samples from 6 petri dishes at the beginning of the experiment and dry weight was measured after one week at the end of the experiment.

Plant material was collected from the flat areas of semi-arid sandy grassland in air-dried form during spring, summer and autumn seasons, respectively. In the laboratory, they were cleaned and transferred to large size Petri-dishes nearby to the window. Samples were divided into 6 Petri-dishes included 3 for control (distilled water treatment) and 3 for SA treatment **(Figure 9)**. Samples were rehydrated by placing them in Petri dishes under SA treatment for 72 h.

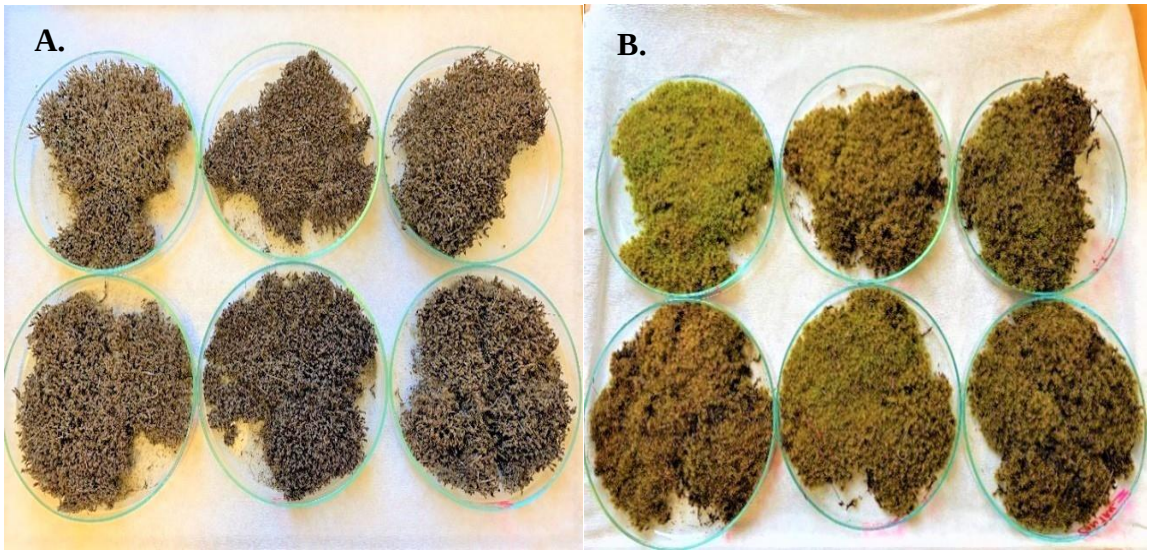


Figure 9. Petri-dishes showing *S.ruralis* samples **A**. Before the salicylic acid (SA) treatment (in the air-dried form) **B**. After the salicylic acid (SA) treatment (6 hrs rehydration). Above one row of petri-dishes represent as SA treated and below one row as control samples.

After performing various experiments on chlorophyll fluorescence measurements and antioxidant enzymatic studies, only few important parameters (Fv/Fm, ΦPSII and NPQ) has been detected for SA pre-treatment. As samples were collected from open exposed areas in semi-arid grassland therefore, effects of slopes does not considered in this experiment.

3.6.2 Measurements of chlorophyll *a* fluorescence parameters

At the beginning of the experiment, 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 parameters were measured daily on all the samples from different seasons until 3 days and then on the 10^{th} day. Hansatech pulse modulated chlorophyll fluorometer (FMS 2) was used to measure the chlorophyll fluorescence parameters Fv/Fm, ΦPSII, NPQ. The similar protocol to measure the chlorophyll fluorescence was according to section 3.4. After the measurement, data saved in FMS 2 and converted to excel files for data analysis.

3.6.3 Enzyme extraction and spectrophotometric antioxidant enzymatic assays

To determine the activity of antioxidant enzymes, 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). The supernatant was used to determine the activity of all the enzymes (section: extraction of plant material). 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 in section 3.5.2, 3.5.3, 3.5.4, respectively.

3.7 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) parametric test and multivariate analysis of variance (MANOVA) parametric test was done to study the interaction within slope and seasons. 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.

4 RESULTS & DISCUSSIONS

4.1 Measurements of chlorophyll *a* fluorescence parameter

4.1.1 Effects of slopes (NE and SW) on chlorophyll *a* fluorescence parameter

Chlorophyll fluorescence measurements were taken on desiccation-tolerant bryophyte *S. ruralis* showed recovery of Fv/Fm (ratio of variable to maximum fluorescence) 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 **(Table 1).** In, photochemical fluorescence quenching parameters (Fv/Fm, ΦPSII, qP), there was no significant difference in Fv/Fm between the slopes whereas ΦPSII and qP parameter differed significantly (p-value ≤ 0.05) between NE and SW slopes. In, non-photochemical fluorescence quenching parameters, qN and NPQ also showed significant differences between the slopes (p-value ≤ 0.05), respectively.

Table 1. Mean of Chlorophyll *a* fluorescence parameters (Fv/Fm, ΦPSII, qP, qN, NPQ) for *S.ruralis* between the slopes NE and SW.

Microhabitat\Chl parameter	**Fv/Fm**	**ΦPS II**	**qP**	**qN**	**NPQ**
Northeast (NE)	0.750± 0.006	0.260± 0.008	0.582± 0.012	0.905± 0.005	4.504± 0.217
Southwest (SW)	0.752± 0.006	0.305±0.009***	0.659±0.013***	0.879± 0.008*	3.724±0.229*
t-test	-0.259	-3.845	-4.198	2.548	2.466
df	45.981	45.125	45.523	38.764	45.873

Data are expressed as mean± SE of six replicates each parameter. p-values are expressed along with (*) between the slopes indicated the different levels of statistical significance where (*, ** and *** represent $p \leq 0.01$, 0.001 and 0.0001, respectively)

Dark-adapted Fv/Fm values reflect the potential quantum efficiency of PSII and used as a sensitive indicator for studying plant photosynthetic performance (Maxwell and Johnson 2000). The parameters Fv /Fm represent electron transport efficiency and ΦPSII represent photochemical efficiency in the PSII reaction center (Zhang, 1999). Fv/Fm provides an estimate of the maximal quantum efficiency of PSII in healthy or unstressed material which is generally around 0.76 to 0.83 (Proctor, 2003). In *S. caninervis* desert moss, Fv/Fm values were found relatively steady at around 0.7 (Zhang, Zhao and Wang, 2017). Similarly. in the results Fv/Fm values has similar mean values and represented the recovery after remoistening the air-dried samples.

However, their values was found within the similar range 0.76 to 0.85 reported in (Csintalan, Proctor and Tuba, 1999). Samples from both slopes are represented as same species therefore, there is no significant difference was observed.

ΦPSII is the most useful parameter in photochemical quenching processes which measures the efficiency PSII photochemistry and as an indicator of overall photosynthesis and relates to achieved efficiency (Genty, Briantais and Baker, 1989). ΦPSII is the proportion of absorbed energy which is used in photochemistry and there is strong linear relationship between ΦPSII and efficiency of carbon fixation, under laboratory conditions whereas qP provides an indication of the proportion of PSII open reaction centres (Maxwell and Johnson, 2000). ΦPSII and qP can be interrelated by a third parameter Fv/Fm (Genty, Briantais, and Baker, 1989; Maxwell and Johnson, 2000). In our result, Fv/Fm values were slightly higher in in the samples collected from SW slope **(Table 1)**. Therefore, ΦPSII and qP values could be estimated higher and indicated better efficiency of PSII photochemistry. 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 of chlorophyll fluorescence is often taken as an indicator of a mechanisms for preventing over-excitation of reaction centres (Ivanov and Edwards, 2000). qN is an important non-photochemical quenching parameter, which protects the photosynthetic mechanism from damage by heat dissipation when light energy absorption exceeds absorption of light (Müller, Li and Niyogi, 2001). Photochemical parameters (Fv/Fm, ΦPSII and qP) has positive correlation with non-photochemical parameters (qN, NPQ) (Maxwell and Johnson, 2000). Thus, a change in qN and NPQ values may be estimated by change in the ΦPSII and qP values. Therefore, in this present study, higher values of qN and NPQ were detected in the samples from the NE slope that could be indicated the photoinhibition and photoprotection (Proctor and Bates, 2018). The study revealed that ΦPSII, qP, qN, NPQ as chlorophyll *a* fluorescence parameters showed small-spatial scale variations that were statistically significant between both slopes.

4.1.2 Effects 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. ΦPSII has no significant difference whereas Fv/Fm, qN, NPQ values were found to be statistically significant within each pair of seasons as shown in **(Table 2)**.

Table 2. Analysis of variance (ANOVA) results of chlorophyll *a* fluorescence parameter values (Fv/Fm, ΦPSII, qP, qN, NPQ) between slopes (NE and SW) with respect to the different seasons (spring, summer, autumn, winter) in *S. ruralis*.

Season	Fv/Fm	ΦPSII	qP	qN	NPQ
Spring	0.714±0.006 a	0.280±0.013 a	0.670±0.021 b	0.904±0.005 bc	4.086±0.022 a
Summer	0.780±0.002 c	0.273±0.013 a	0.613±0.021 ab	0.920±0.003 c	5.212±0.187 b
Autumn	0.754±0.005 b	0.285±0.011 a	0.590±0.018 a	0.860±0.010 a	3.164±0.243 a
Winter	0.756±0.005 b	0.290±0.016 a	0.609±0.017 ab	0.884±0.012 ab	3.994±0.370 a

Data are expressed as mean ± SE of six replicates from the both the slopes. Different alphabets are represented a significant difference among all four seasons at p≤0.05.

In the results, Fv/Fm values showed a significant difference in summer and spring compared to winter and autumn **(Table 2)**. 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 **(Figure 5A)**. 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* is shown in (**Figure 10).** The highest value of maximum photochemical efficiency of photosystem II (Fv/Fm) was observed during hot and dry summer season (July) followed by late winter (March) then autumn season (October) and the lowest value was observed in the spring season (May). 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 to be 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.

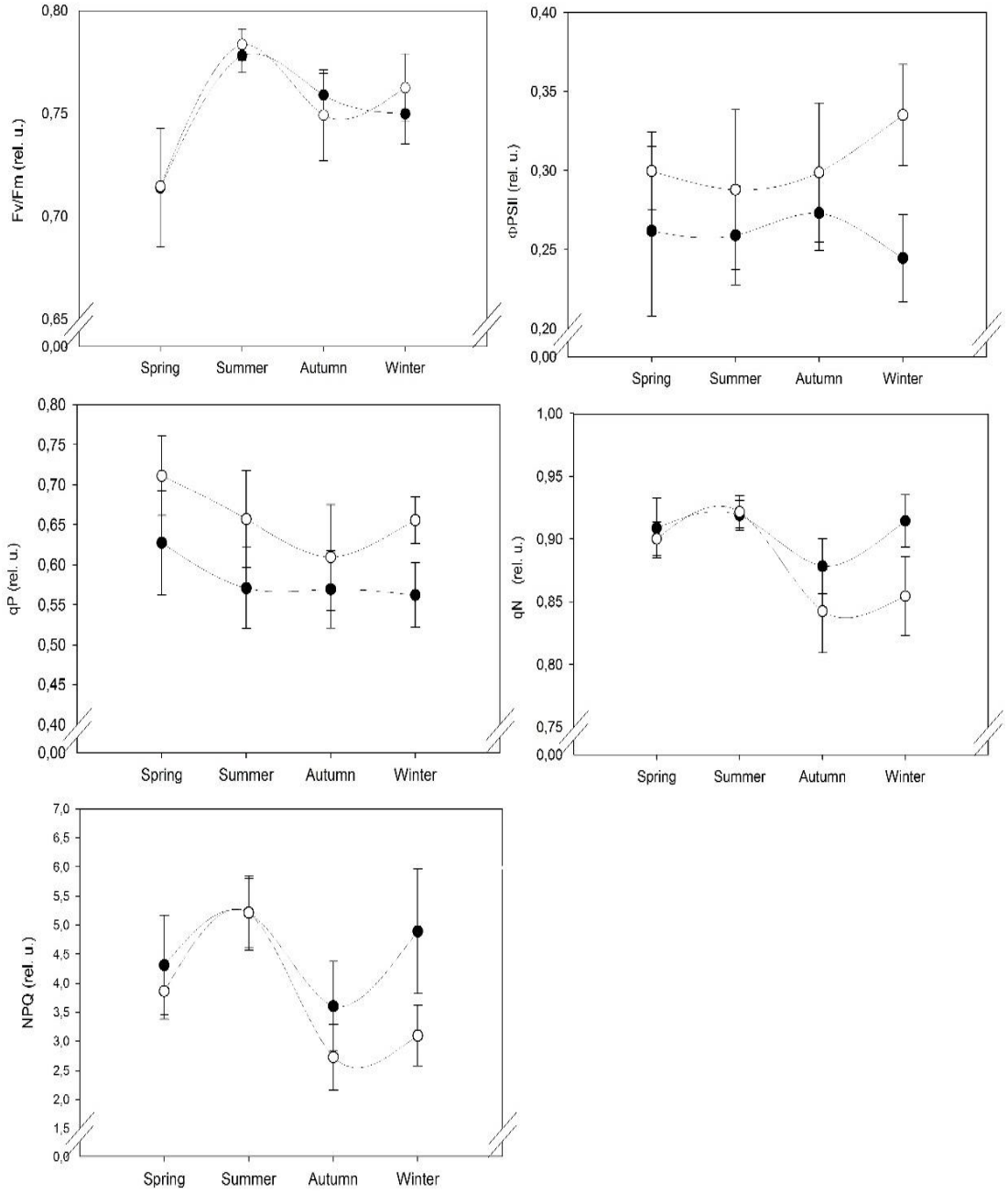


Figure 10. Mean values of chlorophyll a fluorescence parameters (Fv/Fm, ΦPSII, qP, qN, NPQ) for *S.ruralis* were plotted against four seasons (spring, summer, autumn, winter) in two different microhabitats NE: north-east (●), SW: south-west (○) slopes. Error bars represent standard error of mean.

The coefficient of photochemical fluorescence quenching (qP) is observed to be higher in the spring season followed by summer than winter and the lowest values are in the autumn season. Although, ΦPSII was observed to be highest in winter, it was not found to be significant across all the seasons and followed with little variation in autumn and spring seasons. Lower values were found in the hot and dry summer and reflected the least photochemical efficiency (Kalapos and Mázsa, 2001).

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). In **(Figure 10),** qP values are shown to be maximum in the spring season. In spring and winter season, we observed higher values for qP and ΦPSII parameters, respectively in the SW slope which indicated the higher photosynthetic efficiency in the SW slope as compared to NE slope. It could be due to better conditions such as longer favourable light conditions, higher temperature and availability of water which might allow a longer active period for moss cushions in the SW slope on the dunes of semi-arid sandy grassland.

Coefficient of non-photochemical fluorescence quenching (qN) and non-photochemical quenching (NPQ) showed similar trends, and both have maximum values in the summer season followed by spring and winter season and minimum values in the autumn season. Non-photochemical processes for energy dispersion in the PSII act as mechanisms to reduce photoinhibition during a period of high light intensity (Maxwell and Johnson, 2000). On comparing the seasons, higher values of these parameters were recorded in the summertime **(Figure 10)**. *S. ruralis* belongs to sun-exposed habitats that exhibit photoprotection at higher irradiance (Proctor and Bates, 2018). 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.

In spring, there were little variations in qN and NPQ values which might be due to the transition from colder to warmer conditions. Also, in this season, temperatures increase rapidly, and mosses dry out in only a few hours after sunrise and become metabolically inactive due to high light exposure that causes photoinhibition (Marschall, 2017). Previous studies reported that rapid recovery of the photosynthetic system from dehydration allows it to react rapidly for short wet periods. This reaction due to the low water requirement of *S. ruralis* species enables it to survive during the hot and dry summer (Csintalan *et al.*, 2000). In an open sun-exposed habitat, high values of NPQ indicated water stress and high-light protection mechanisms (Marschall and Proctor, 2004). In another moss species, *Atrichum androgyne* similar results were reported that NPQ were increased during rehydration due to photoprotection and showed a high level of desiccation tolerance (Marschall and Beckett, 2005). In the winter season, higher values were observed in qN and NPQ parameters in NE slope (**Figure 10)**, which seemed to have resulted from a below-zero temperature which leads to cold temperature stress. In this temperate climatic zone, spring, autumn, and winter generally have a favourable water supply but the summer season is strongly water limited.

The main growing season in the open sandy grassland is the late spring in Central and Eastern Europe (Csintalan *et al.,* 2000). The small spatial scale microclimatic conditions may also modify the seasonal differences. The variations in chlorophyll fluorescence parameters observed were due to different environmental natural conditions in semi-arid sandy grassland in various seasons. Comparing the expositions, higher values were observed in NE slope for qN, NPQ except for Fv/Fm, ΦPSII, qP parameters and this might be due to fluctuations in some abiotic factors for example., temperature, humidity, and light irradiance, soil parameters, precipitation.

4.1.3 Effects of slopes and seasons on chlorophyll *a* fluorescence parameters

Multi-variate analysis of variance (MANOVA) results were shown there were no significant differences in photochemical quenching parameters (Fv/Fm, ΦPSII, qP) values. However, non-photochemical quenching parameters (qN and NPQ) has a significant difference with respect to slopes within seasons (**Table 3).** These seasonal variations might be due to different environmental conditions such as irregular precipitation patterns, temporal distribution in xeric habitats (Margóczi, Aradi and Busa-Fekete 2007; Yizhaq, Ashkenazy and Tsoar, 2009).

Climate change causes a significant increase in temperature and a decrease in precipitation in summer for the Kiskunság sand ridge (Bartholy, Gelybó and Pongrácz, 2007) and makes this area vulnerable to drought (Blanka, Mezősi and Meyer, 2013). Chlorophyll fluorescence parameters measured during different seasons showed a $p \leq 0.05$ expect for ΦPSII which may indicate a seasonal variation.

Table 3. Multivariate Analysis of Variance (MANOVA) results of chlorophyll *a* fluorescence parameter values (Fv/Fm, ΦPSII, qP, qN, NPQ) between the slopes NE and SW with respect to different seasons (spring, summer, autumn, winter) in *S. ruralis.*

Variables	Groups	Sum of squares	df	F-value	P-value
Fv/Fm	Slopes	6.07×10^{-5}	1	0.18	0.67
	Seasons	0.27	3	27.08	9.8×10^{-10}***
	Slopes: Seasons	8.02×10^{-4}	3	0.79	0.5036
ΦPSII	Slopes	0.25	1	14.77	4.23×10^{-4}***
	Seasons	1.83×10^{-3}	3	0.36	0.78
	Slopes: Seasons	8.34×10^{-3}	3	1.64	0.19
	Slopes	0.69	1	20.82	4.70×10^{-5}***

qP	Seasons	4.23×10^{-3}	3	4.23	0.01
	Slopes: Seasons	5.4×10^{-3}	3	0.54	0.65
qN	Slopes	7.85×10^{-5}	1	12.81	9.21×10^{-4}***
	Seasons	0.02	3	13.07	4.28×10^{-6}***
	Slopes: Seasons	7.07×10^{-3}	3	3.84	0.016*
NPQ	Slopes	7.30	1	11.93	1.32×10^{-3}**
	Seasons	25.49	3	13.88	2.37×10^{-6}***
	Slopes: Seasons	5.23	3	2.84	0.049*

p-values are expressed along with (*) indicated the different levels of statistical significance where ($p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$).*

4.2 Measurements of antioxidant enzymatic activities

4.2.1 Determination of Water Content (WC%) under rehydration for antioxidant activities experiments

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 **(Figure 11).**

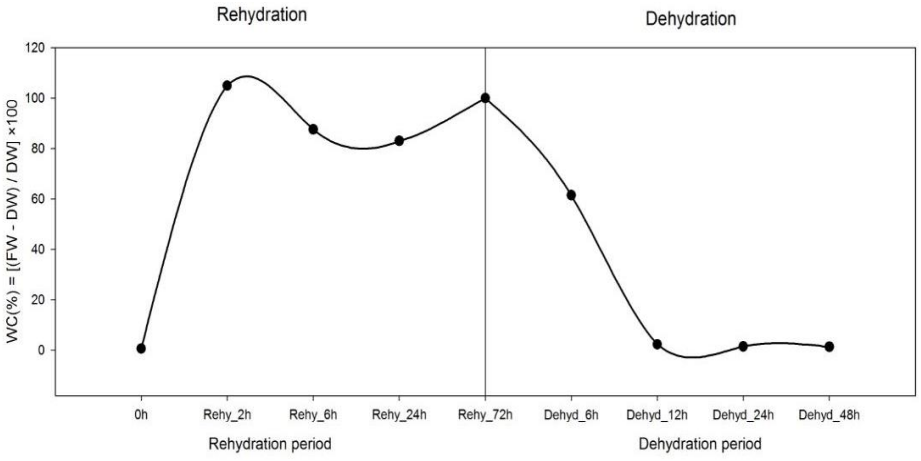


Figure 11. Graphical representation of water content percentage (WC%) during rehydration period (left) and dehydration period (right).

4.2.2 Effect of antioxidant activities (APX, CAT, POD) in mosses collected from the NE and SW slopes in the rehydrated and desiccated states

Antioxidant enzymatic activity results were represented in two different states, i.e., rehydrated (Rehy) and desiccated (Desic) between north-east (NE) and south-west (SW) slopes with respect to different period of collection **(Figure 12)**. Our results showed that mosses growing on the NE slope have higher enzymatic activities (APX, CAT and POD) in both the rehydrated and desiccated states as compared to the SW slope in all seasons except opposite trend was observed in summer season. 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, 2020a). 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. In this study, it also showed higher activities in summer and winter seasons. All the activities were followed similar trend upon rehydration and desiccation, these activities increased first from spring to summer season and then declined in autumn season. Again, it was increased in the colder winter season. 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 which could be enhancing the activities of antioxidant activities indicating stress conditions.

In both rehydrated and desiccated states, all activities were higher in summer and winter season and lower in spring and autumn. APX **(Figure 12A-B)** and POD **(Figure 12E-F)** activities were showed variations throughout the year in both rehydrated and desiccated states between both NE and SW slopes. CAT activities did not vary much throughout the year in both rehydrated and desiccated states for the material from the NE and SW facing slopes **(Figure 12C-D)**.

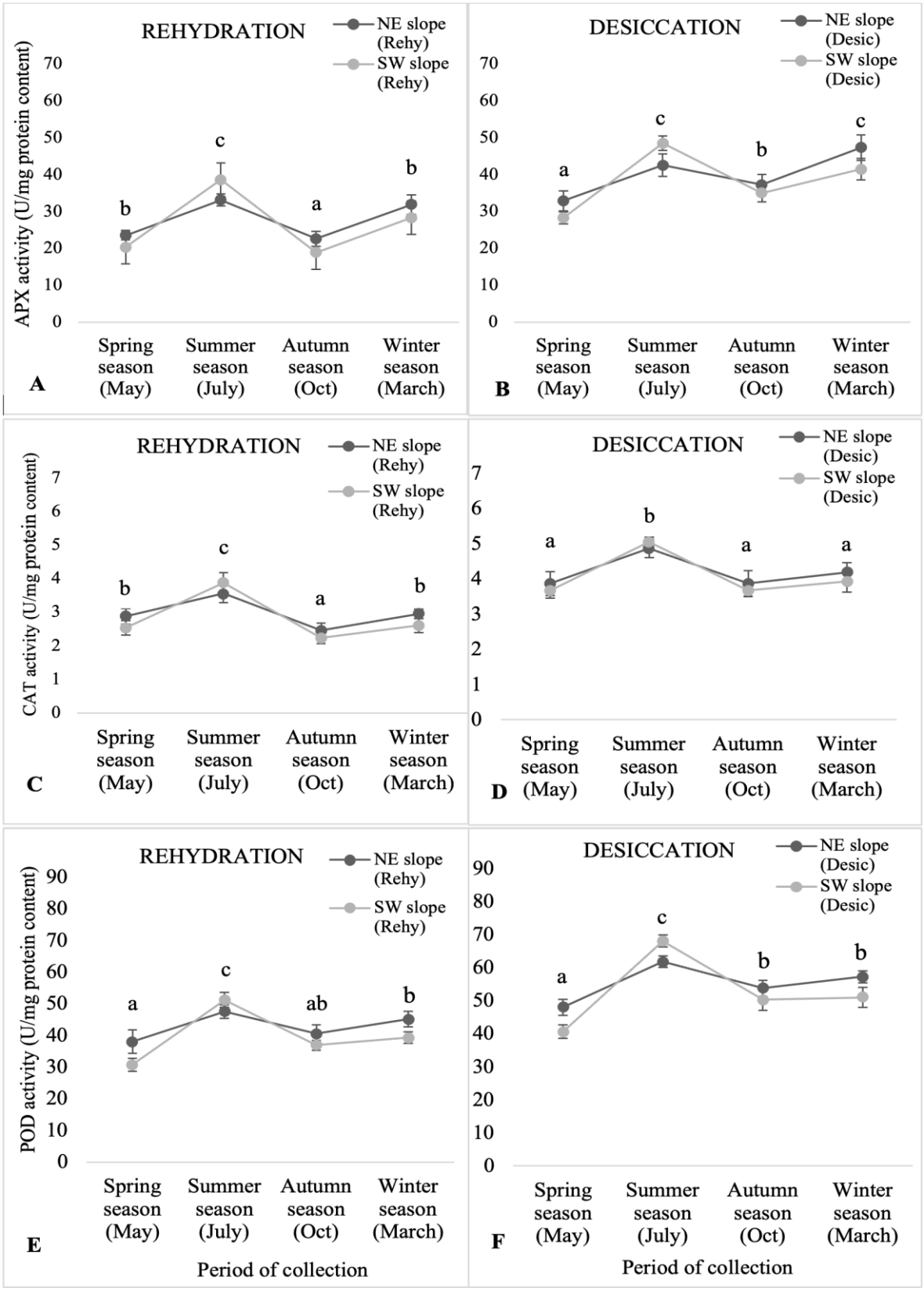


Figure 12. Effect of activity of antioxidant enzymes in *S. ruralis* : (**A-B**) APX ; (**C-D**) CAT; (**E-F**) POD in rehydrated (Rehy) and desiccated(Desic) states between north-east (NE) and south-west (SW) slopes with respect to different period of collection (Spring, Summer, Autumn, Winter season). The mean values (n = 5) ± SD with different alphabetical letters is significantly different at $p \leq 0.05$ using ANOVA post-hoc (Tukey's test).

Ascorbate peroxidase (APX) enzyme plays a key role in eliminating H_2O_2 and therefore it is an important component of the antioxidant system (Najami *et al.,* 2008). APX activity was lower following rehydration **(Figure 12A)**, presumably because of the reduction in oxidative stress. Bansal and Srivastava (2017) also reported a reduction in APX activities during rehydration in the moss *Brachythecium procumbens.*

Catalase enzyme (CAT) is an important antioxidant enzyme that breaks down H_2O_2 to form water and oxygen (Zhang, Zhao and Wang, 2017). In rehydrated mosses, CAT activities were observed similar in NE and SW slope **(Figure 12C)**. Results are generally consistent with studies on other mosses that have found that CAT activity does not vary greatly during wetting /drying cycles, suggesting that CAT is probably a largely constitutive defence against oxidative stress (Mayaba and Beckett, 2003).

Guaiacol peroxidase (POD) activity, which will also remove H_2O_2, increased during slow desiccation in all moss samples as compared to rehydrated states **(Figure 12E)**. Similar increases in POD activity have been observed during desiccation in *Brachythecium velutinum* (Paciolla and Tommasi, 2003), *B. procumbens* (Bansal and Srivastava, 2017), *Octoblepharum albidum* (Lubaina, Meenu and Murugan, 2013) and *Dicranum scoparium* (Onele *et al.,* 2018).

All the activities tended to be higher in desiccated states than in rehydrated material for both slopes. In plants, the production of antioxidant enzymes is one of the strategies to defend themselves from ROS injury during desiccation (Seel *et al.,* 1991; Oliver and Bewley, 1997). These mosses were collected from exposed areas in semi-arid sandy grassland that showed increased antioxidant enzymatic activities. However, similar results were also reported in the moss *S. caninervis* Mitt. collected from exposed areas that showed the highest antioxidant enzyme activity (Yin and Zang, 2016). During dehydration, plants deal with the water-deficit condition which causes lower water potential and declines the primary metabolism in bryophytes (Dinakar, Djilianov and Bartels, 2012). In the desiccated state, accumulation of ROS increases the damage to proteins and lipids in the chloroplast also in mitochondria, peroxisomes, and plasma membrane (Scheibe and Beck, 2011).

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

Protein content were represented in two different states, i.e., rehydrated (Rehy) and desiccated (Desic) between north-east (NE) and south-west (SW) slopes with respect to different period of collection **(Figure 13A-B).**

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 desiccated states. Based on season-wise, protein content was significantly different ($p \leq 0.05$).

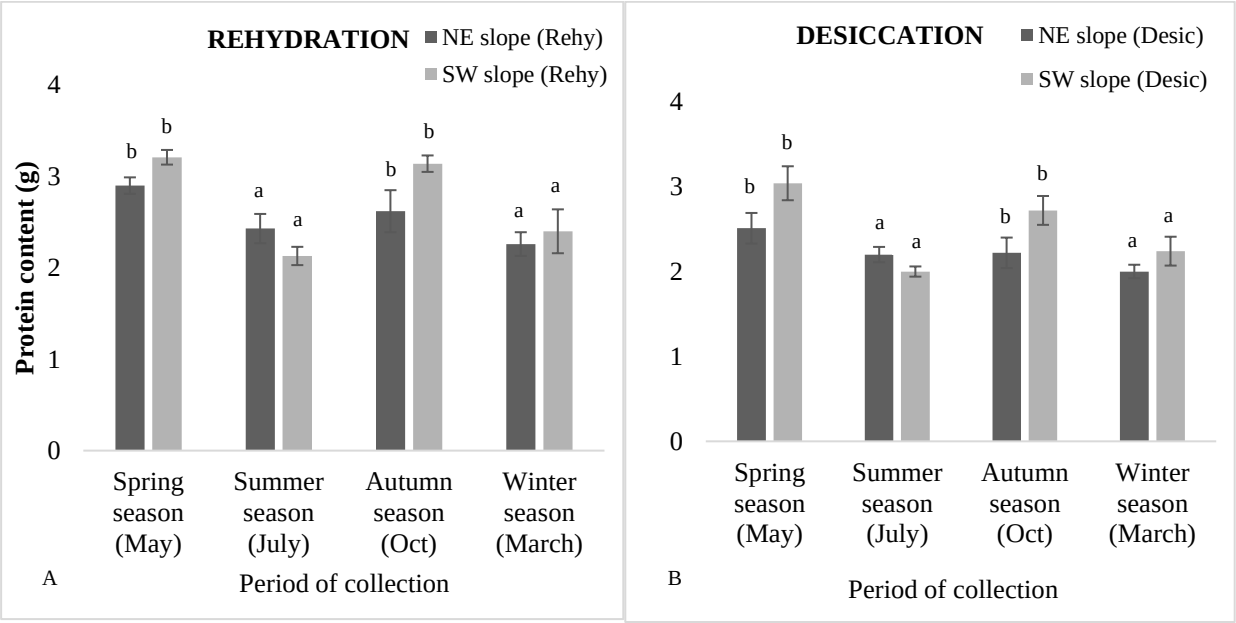


Figure 13. Protein content in *S. ruralis* (**A-B**) in rehydrated (Rehy) and desiccated (desic) states between north-east (NE) and south-west (SW) slope with respect to different period of collection (Spring, Summer, Autumn, Winter season). The mean values (n = 5) ± SD with different alphabetical letters is significantly different at $p \leq 0.05$ using ANOVA post-hoc (Tukey's test).

Results showed higher protein content during the summer season the mosses of NE slope than the SW slope's one, while during another seasons the SW's one was higher the NE slope's one. 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 **(Figure 13)** which may indicate the damage of proteins. Higher values in antioxidant enzymatic activities might be indicated higher water deficit condition and imbalance of ROS production in NE slope. In the previous report, protein synthesis induced during rehydration (Oliver *et al.,* 2004). Similarly, it may indicate the higher protein content values in the rehydrated state in both NE and SW slopes.

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

MDA content differed significantly ($p \leq 0.05$) 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 desiccated material than rehydrated material (**Figure 14A-B**). In all seasons, MDA content was found higher in NE slope except summer season as compared to SW slope.

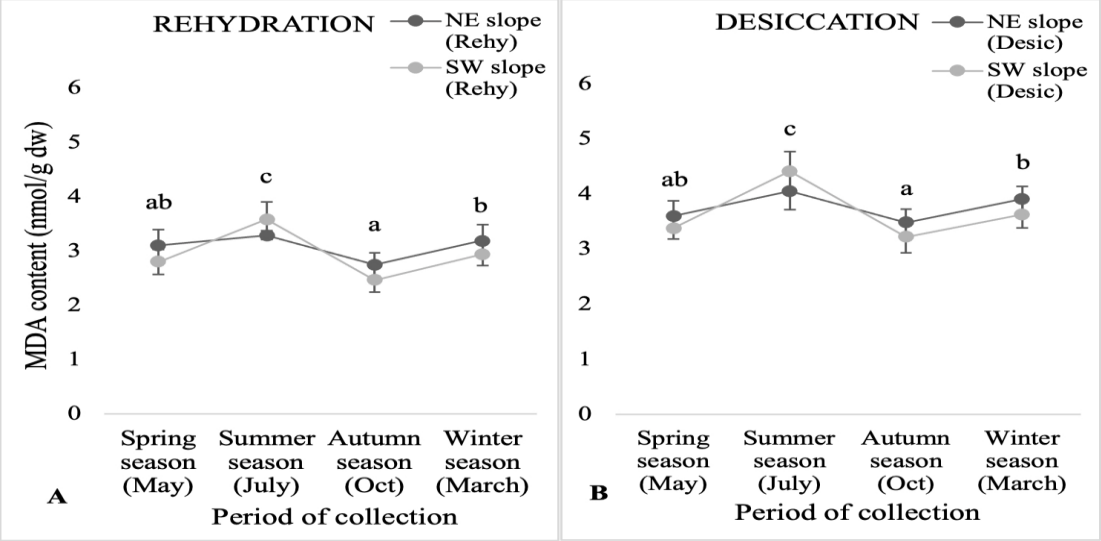


Figure 14. MDA content in *S. ruralis* (**A-B**) in rehydrated (Rehy) and desiccated (desic) states between north-east (NE) and south-west (SW) slope with respect to different period of collection (Spring, Summer, Autumn, Winter season). The mean values (n = 5) ± SD with different alphabetical letters is significantly different at $p \leq 0.05$ using ANOVA post-hoc (Tukey's test).

Lipid peroxidation (MDA content) is used as an indicator to measure the degree of oxidative damage in plants (Liu *et al.,* 2013). Increased stress is probably the reason for the higher MDA levels in mosses growing on the NE slope compared with those growing on the SW slope. Similar results were observed that MDA content increased during desiccation while comparing with rehydration **(Figure 14).** 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.

Future studies on desiccation-induced changes in lipids in mosses should probably use indicator molecules other than MDA. In the present study, the activities for all enzymes (APX, CAT, POD) tended to be lower in the rehydrated compared to the desiccated state. Previous studies in *S. ruralis* (Oliver, 1991; Oliver and Bewley, 1997; Oliver, Wood and O' Mahony, 1998), and *Anomodon viticulosus* and *Racomitrium lanuginosum* (Proctor and Smirnoff, 2000) indicated the importance of constitutive protection with an induced repair mechanism upon rehydration. It appears that the H_2O_2 scavenging antioxidant enzymes form part of the inducible mechanism. During rehydration, processes such as photosynthesis, respiration and protein synthesis return to normal and suggesting recovery from stress (Oliver, 1991; Cruz de Carvalho, Branquinho and Marques Da Silva, 2011; Cruz De Carvalho *et al.*, 2014).

Although more frequent sampling occasions would have been desirable, the results also suggested that the antioxidant enzymatic activities might affected by increasing temperatures from spring to summer season (April to July 2018) and by declining temperatures from autumn to winter season (October to late March 2018). In summer and winter season, enzymatic activities differed greatly between collections and treatments, which indicated that anti-oxidative systems may be performed an important role in balancing the production of free radicals and adjust level of protective enzymes to provide protection in extreme environment. In the present study, collections were made on representative days of each of the four seasons, in an attempt to obtain an overview of how the activities of the enzymes vary throughout the year.

In this present study, we investigated the activities of the antioxidant enzymes APX, CAT, POD with protein determination and MDA content in the desiccation-tolerant moss *S. ruralis*, comparing material collected from the NE and SW slopes in different seasons. Our results showed significant seasonal variations in antioxidant enzymatic activities in the rehydrated and desiccated states for the slopes. In general, higher activities of the antioxidant enzymatic activities were found in mosses collected from the NE slope. In both states, the highest activities occurred in mosses collected during summer and winter season and the lowest activities were found during the spring and autumn season. Besides seasonal differences in the activities of the antioxidant enzymes, the small spatial-scale exposures i.e., the NE and SW slope orientation also can modify the expression of these enzymes. The role of some antioxidant enzyme in desiccation tolerance may be different, basically depending on the actual metabolic balance of mosses. Their enzymatic activity is not only influenced by water conditions but also by other environmental factors such as temperatures, light, and soil conditions) which need to be further investigated in the future.

4.3 Measurements of SA pre-treatment on antioxidant enzymatic activities

4.3.1 Determination of Water Content (WC%) under rehydration in SA treatment

Water content showed changes in control vs SA treated samples in different three seasons during rehydration period **(Figure 15).** 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.

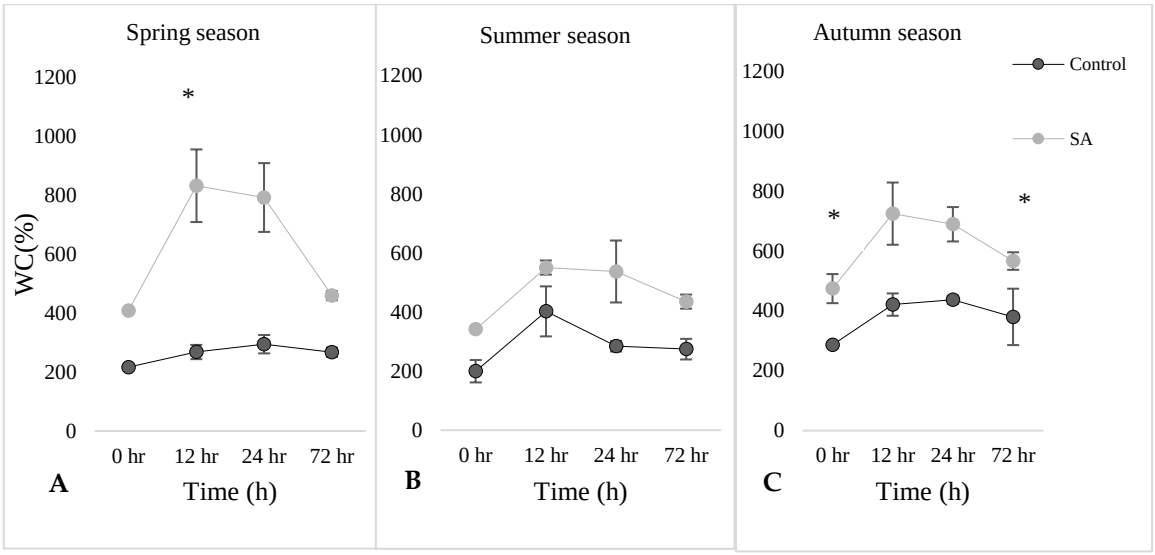


Figure 15. The water content percentage (WC%) in the leaves of *S. ruralis* after SA treatment during rehydration period under SA treatment in different seasons (**A** - spring, **B** - summer, **C** - autumn). The mean values (n = 3) ± SD marked with an asterisk (*) are significantly different at $p \leq 0.05$ using ANOVA post-hoc Tukey's test.

4.3.2 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 **(Figure 16A-I)**. 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, ΦPSII 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 parameter values of SA-treated samples were found lower on day 1 in each season as compared to the control values with other days of treatment **(Figure 16A-C)**. Spring and autumn season were slightly varied to each other but in the summer season, higher variation was found. In the spring season, the lowest Fv/Fm values in SA-treated samples were found on day 1 (0.157 ± 0.02) and the highest on day 10 (0.810 ± 0.01). On day 1, SA-treated samples for spring and autumn season were lower than the summer season.

However, from day 2 to day 10 observed values were lower in the summer season as compared with the control values. In the summer season, lowest values were observed on day 2 (0.173 ± 0.05) and highest on day 10 (0.614 ± 0.07). In the autumn season, the lowest was on day 1 (0.112 ± 0.03) and highest on day 10 (0.780 ± 0.01). For ΦPSII parameter, SA-treated values were found lower on day 1 and then increased until day 10 in the spring season **(Figure 16D-F)**. The lowest value was recorded on day 1 (0.030 ± 0.007) and highest on day 10 (0.274 ± 0.05). Initially, values recorded in the summer season was decreased till day 2 and then showed inclined pattern. SA-treated samples were found lowest on day 2 (0.045 ± 0.037) and highest on day 10 (0.227 ± 0.07). In the autumn season, the lowest values were observed on day 1 (0.024 ± 0.01) while the highest on day 3 (0.332 ± 0.02) for SA-treated samples. From day 1, ΦPSII values increased continuously till day 10.

NPQ values were slightly varied to each other during spring and autumn season whereas higher variation was seen in the summer season **(Figure 16G-I)**. Lowest NPQ values were found on day 1 for SA-treated samples in each season: 0.726 ± 0.11 for the spring season, 0.415 ± 0.19 for the summer season and 0.337 ± 0.07 for the autumn season. Highest values for SA-treated samples were recorded on day 10 in each season: 2.171 ± 0.28 for the spring season, 1.019 ± 0.22 for the summer season and 2.185 ± 0.25 for the autumn season.

In this study, we examined how the effects of SA pre-treatment relates to photosynthetic efficiency using chlorophyll *a* fluorescence method and determined the antioxidant enzymatic activities. Seasonal variation was observed and studied in moss cushions collected from three different seasons. Moss cushions were monitored for three consecutive days and till day 10 under control and SA-treated conditions. The ratio of variable and maximum fluorescence (Fv/Fm) in a dark-adapted state is used as an important and sensitive indicator of plant photosynthetic performance in chlorophyll fluorescence measurement. It indicates the maximum efficiency of PSII when all PSII centres are open. 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).

The results showed an inclined trend from day 1 till day 10 in SA-treated samples while comparing with control samples in spring and autumn season in **(Figure 16)**. These increased fluctuations indicated the recovery of moss cushions after a long term of desiccation within each season in a different way.

However, in summer season, declined pattern was observed in the Fv/Fm values in **(Figure 16B)** for SA-treated samples. In spring and summer season, ΦPSII values showed a declined pattern for SA-treated samples as compared to control **(Figure 16D,E)**. However, opposite trend was observed in autumn season **(Figure 16F)**. It might indicate some protective mechanism towards photoinhibitory damage in response to stress could be due to combined effect of SA treatment and high temperature during (July 2018) period of collection which might reduce the photosynthetic efficiency. In general, fewer reaction centres were opened during that time which lowered the Fv/Fm ratio, and the plant may be experienced greater stress (Baker and Rosenquist, 2004).

For non-photochemical quenching parameters **(Figure 16 G, H, I)**, SA-treated samples showed the higher values of NPQ during the spring and autumn season but lower in the summer season. 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). NPQ reflects the level of energy dissipation in the form of heat energy. During rehydration, its increase provides benefit to the moss cushions by the heat dissipation in excess or photoprotection indicates the higher level of desiccation tolerance in *S. ruralis* moss cushions. But it is still not clear how NPQ is related to SA tolerance levels.

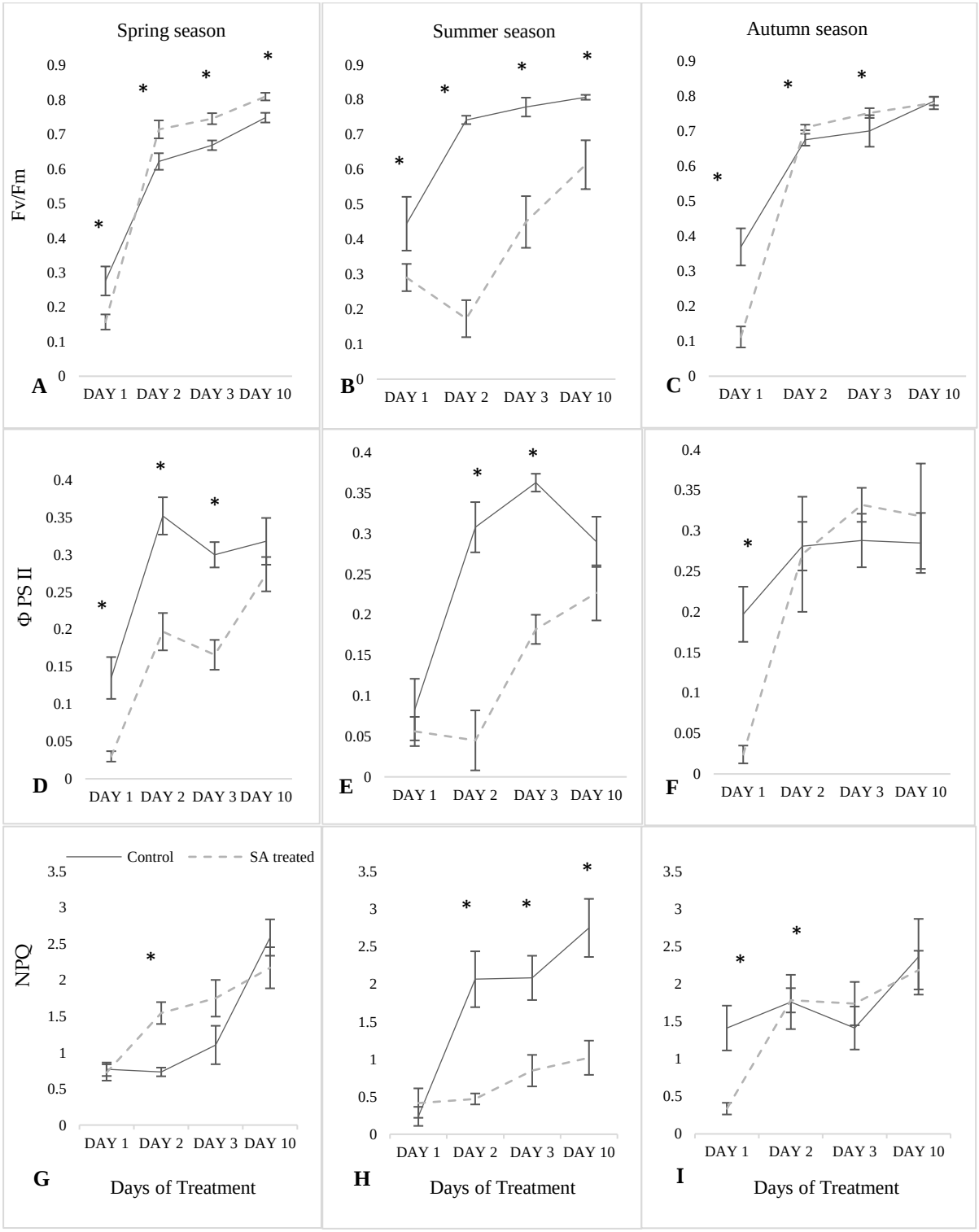


Figure 16. Changes of kinetic of chlorophyll a fluorescence Fv/Fm: (**A–C**); ΦPSII: (D–F); NPQ: (**G–I**) in *S. ruralis* after SA treatment respect to different seasons (spring, summer, autumn). The mean values (n = 3) ± SD marked with an asterisk (*) are significantly different at $p \leq 0.05$ using ANOVA post-hoc Tukey's test.

In **(Table 4,5.6)** showed ANOVA results for chlorophyll *a* fluorescence parameter values.

Table 4. ANOVA results of chlorophyll a fluorescence parameter values (Fv/Fm, ΦPSII, NPQ) between days of treatment for the spring season in *S. ruralis* between SA treated and control samples.

Chl *a* fluorescence parameter	Days of treatment	df	Mean sq	F-value	P-value
Fv/Fm	Day 1	1	3.54×10^{-1}	30.7	***
	Day 2	1	2.17×10^{-2}	33.62	***
	Day 3	1	1.4746×10^{-2}	60.49	***
	Day 10	1	9.364×10^{-3}	52.65	***
ΦPSII	Day 1	1	2.7716×10^{-2}	64.28	***
	Day 2	1	6.078×10^{-2}	32.67	***
	Day 3	1	4.46×10^{-2}	122.5	***
	Day 10	1	4.797×10^{-3}	2.112	0.184
NPQ	Day 1	1	4.694×10^{-3}	0.433	0.529
	Day 2	1	1.652	12.52	**
	Day 3	1	1.0379	3.949	0.0821
	Day 10	1	4.355×10^{-1}	2.149	0.181

p-values are expressed along with (*) indicated the different levels of statistical significance where (, ** and *** represent $p \leq 0.01$, 0.001 and 0.0001, respectively)*

Table 5. ANOVA results of chlorophyll a fluorescence parameter values (Fv/Fm, ΦPSII, NPQ) between days of treatment for the summer season in *S. ruralis* between SA treated and control samples.

Chl *a* fluorescence parameter	Days of treatment	df	Mean sq.	F-value	P-value
Fv/Fm	Day 1	1	5.96×10^{-2}	7.732	*
	Day 2	1	8.094×10^{-1}	529.8	***
	Day 3	1	2.702×10^{-1}	34.99	***
	Day 10	1	9.312×10^{-2}	28.76	***
ΦPSII	Day 1	1	1.746×10^{-3}	1.909	0.204
	Day 2	1	1.7254×10^{-1}	66.19	***
	Day 3	1	8.152×10^{-2}	341.9	***
	Day 10	1	9.872×10^{-3}	1.204	0.304
NPQ	Day 1	1	7.87×10^{-2}	2.868	0.129
	Day 2	1	6.356	21.13	**
	Day 3	1	3.819	12.41	**
	Day 10	1	7.482	22.82	**

p-values are expressed along with (*) indicated the different levels of statistical significance where (, ** and *** represent $p \leq 0.01$, 0.001 and 0.0001, respectively)*

Table 6. ANOVA results of chlorophyll a fluorescence parameter values (Fv/Fm, ΦPSII, NPQ) between days of treatment for the autumn season in *S. ruralis* between SA treated and control samples.

Chl *a* fluorescence parameter	Days of treatment	df	Mean sq	F-value	P-value
Fv/Fm	Day 1	1	1.6589×10^{-1}	87.79	***
	Day 2	1	3.133×10^{-3}	15.85	**
	Day 3	1	6.401×10^{-3}	5.566	*
	Day 10	1	4.84E-05	0.195	0.67
ΦPSII	Day 1	1	7.513×10^{-2}	26.55	***
	Day 2	1	2.64×10^{-4}	0.087	0.776
	Day 3	1	4.8×10^{-3}	2.559	0.148
	Day 10	1	2.614×10^{-3}	0.91	0.368
NPQ	Day 1	1	2.8856	60.34	***
	Day 2	1	1.21×10^{-3}	0.015	***
	Day 3	1	2.661×10^{-1}	1.657	0.234
	Day 10	1	7.916×10^{-2}	0.492	0.503

p-values are expressed along with (*) indicated the different levels of statistical significance where (, ** and *** represent $p \leq 0.01$, 0.001 and 0.0001, respectively)*

4.3.3 Protein determination

ANOVA was performed to calculate the mean values and statistical differences of the protein content between control and SA treated values with respect to different seasons by Tukey's pairwise comparisons in (**Figure 17)**. These results showed that the highest protein content (2.8±0.66) $mg.g^{-1}$.FW in the summer season and lowest protein content (2.3±0.18) and (2.4±0.36) $mg.g^{-1.}$FW in the spring and autumn season respectively were found in SA treated samples. There was no significant difference season-wise and within same season.

Protein content was observed decreased in SA-treated as compared to control **(Figure 17)**. It may indicate the protein denaturation due to the inhibitory response of SA in different seasonal conditions. In the desiccated state, accumulation of ROS increases the damage to proteins and lipids in the chloroplast also in mitochondria, peroxisomes, and plasma membrane. 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).

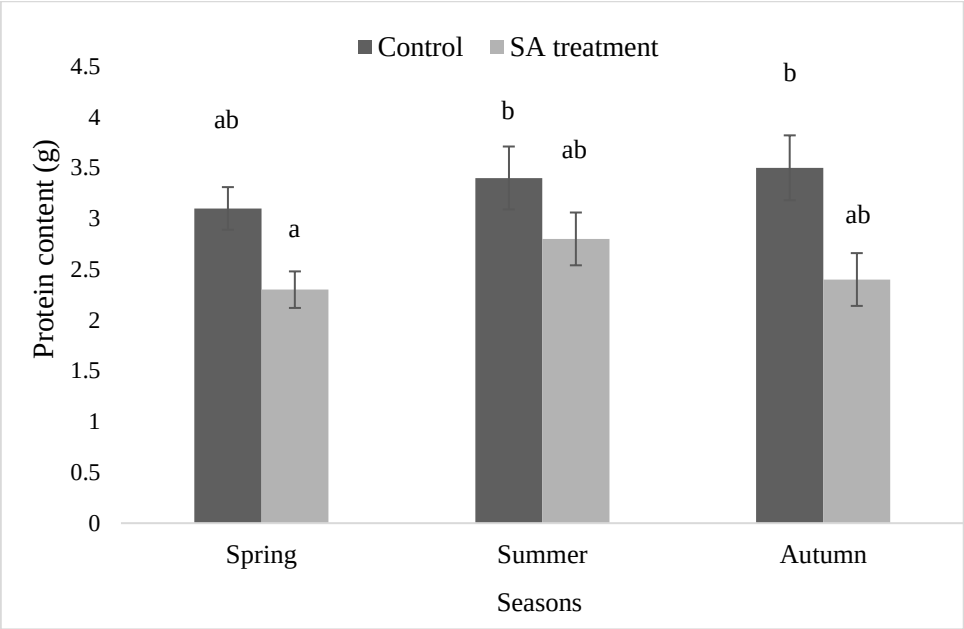


Figure 17. Protein content in *S. ruralis* with respect to different seasons (spring, summer, autumn). The mean values (n = 5) ± SD with different alphabetical letters are significantly different at $p \leq 0.05$ using ANOVA post-hoc (Tukey's test).

4.3.4 Effect of SA pre-treatment on antioxidant enzymatic activities

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 **(Figure 18A,B,C)**. 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. The increased APX and CAT activity in SA pre-treated samples was reported as compared to the control values in different seasons (autumn, spring, summer) expect POD activity which showed an opposite trend. The highest values for APX activity were reported in the spring season (98.82±10.23) and lowest values (77.59±17.98) during the autumn season. On the basis of 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 **(Figure 18A)**.

The CAT activity in SA-treated samples was very slightly increased as compared to their respective control values in spring, autumn and summer **(Figure 18B)**. Lowest CAT values were found in the summer season (3.57±0.64) and highest in autumn season (5.46±1.13). 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.

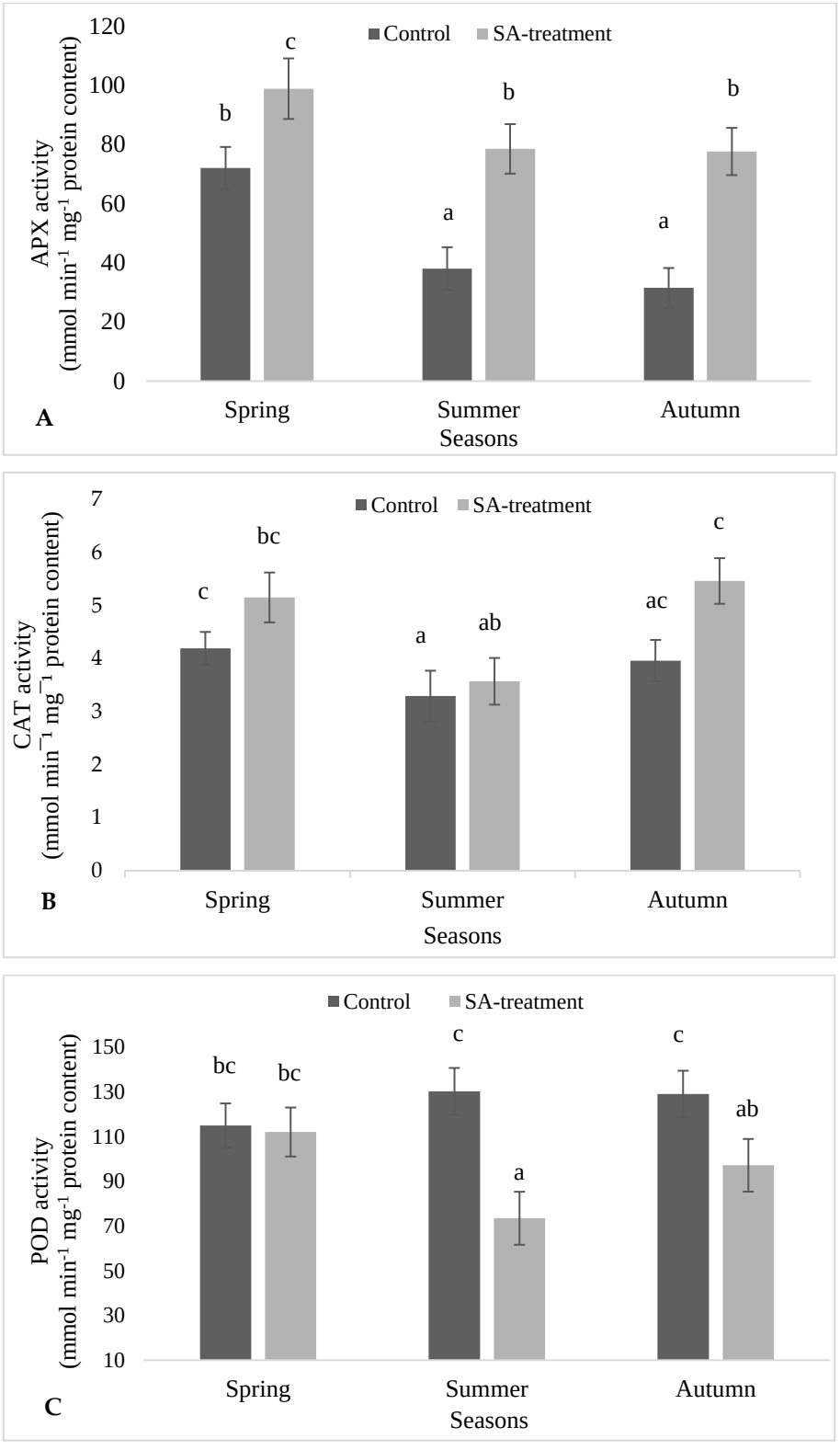


Figure 18. The effect of SA treatment on the activity of antioxidant enzymes in *S. ruralis*: (**A**) APX; (**B**) CAT; (**C**) POD with respect to different seasons (spring, summer, autumn) Mean values (n = 5) ± SD marked with different alphabetical letter are significantly different at $p \leq 0.05$ using ANOVA post-hoc (Tukey's test).

The POD activity in SA-treated samples in spring was significantly different to that in SA-treated ones in summer.

Lowest POD values were found in the summer season (73.44±0.64) and highest in the spring season (111.98±10.96) **(Figure 18C)**. Based on treatment-wise, no significant difference was observed between control and SA-treated in spring season, respectively except summer and autumn seasons. Enzymatic activities (APX, CAT and POD) within control and SA treated values were found significant (representing by different letters) in **(Figure 18).**

Catalase activity showed slightly variation in all seasons but significantly not different between SA-treatment and control **(Figure 18B)**. Ascorbate peroxidase under SA-treatment were found to be increased which caused a decrease in oxidative stress during each season **(Figure 18A)**. This might be related to the protective mechanism against the stressful conditions caused due to pre-treatment of SA compared with control values. Another unfavourable conditions occurred may be due to temperature and water stress depending on different seasons. 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).

5 NEW SCIENTIFIC RESULTS

1. For photochemical parameters, qP and ΦPSII differed significantly in small-spatial scale on *Syntrichia 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.

6 CONCLUSION & RECOMMENDATIONS

The main objective of the thesis was to the study the effect of antioxidant enzymatic activities between the two slopes located within a small distance from each other in desiccation-tolerant *S. ruralis* moss cushions collected from semi-arid sandy grassland in Hungary. Furthermore, this study showed clear and significant seasonal variations in chlorophyll *a* fluorescence parameter samples collected from NE and SW slopes. The results referring different photosynthetic activity and stress tolerance of dominant cryptogam species (*S. ruralis*) depending on the exposition in small-spatial scale and the role and significance of exposition in the grassland carbon budget.

The results indicated better photosynthetic performance in the south-west slope (SW) in contrast to the north-east slope (NE) in all seasons. The presence of differences in photosynthetic properties 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. *Syntrichia ruralis* is one of the key components of the cryptobiotic crust in semi-arid sandy grassland which are the main producers and relevant participants in the biological (mainly the carbon cycle) circulation of this ecosystem. Maximal utilization of better environmental factors (e.g., light) was shown in the higher activation of the photochemistry of SW slope species opposite to NE ones where the non-photochemical energy dissipation is more expressed. The cost of the more effective light absorption also attends to the need for greater water supply which was also limited in this habitat. Therefore, maintenance of the balance in photosynthesis (light reaction and carbon cycle) basically determined the spreading and production of mosses. 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.

The activities of the antioxidant enzymes APX, CAT, POD and MDA content in the desiccation-tolerant moss *S. ruralis* were also investigated by comparing the material collected from the NE and SW slopes in different seasons. The results clearly showed significant seasonal variations in antioxidant enzymatic activities in the rehydrated and desiccated states. In general, higher activities of the antioxidant enzymatic activities were found in mosses collected from the NE slope. In both states, the highest activities occurred in mosses collected during summer and winter season and the lowest activities were found during the spring and autumn season. Besides seasonal differences in the activities of the antioxidant enzymes, the small spatial-scale exposures i.e., the NE and SW slope orientation also can modify the expression of these enzymes. The role of some antioxidant enzyme in desiccation tolerance may be different, basically depending on the actual metabolic balance of mosses.

Their enzymatic activity is not only influenced 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.

In SA pre-treatment, a significant effect on photosystem II in the moss *S.ruralis* was observed. This would be the first study of SA pre-treatment on *S. ruralis* to explore the mechanism behind the defence system in bryophytes. It contributes to a better understanding of abiotic interaction in non-vascular plants. Increased activity of antioxidant enzymes has been suggested as an adaptive protective mechanism against oxidative damage due to pre-treatment of SA. It can be concluded that seasonal variation has been observed in chlorophyll fluorescence and antioxidant system after long term of desiccation in *S. ruralis* species that could be because of SA and might be due to fluctuations in conditions of their habitat, duration of light intensity, temperature and precipitation. The present work can be useful in finding the role of the SA hormone in bryophytes. In conclusion, 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. Another important finding was the application of exogenous SA pre-treatment first time on desiccation-tolerant bryophyte. Furthermore, more research will be needed to the study the impact of climate change on desiccation-tolerant cryptogamic species.

7 SUMMARY

In semi-dry areas or grasslands, cryptogamic species such as algae, fungi, lichens, and bryophytes, all are collectively contributing to form biological soil crusts (BSCs) or cryptobiotic crusts (CBCs). They are the major component and unique feature in arid habitats. They play important role in the biodiversity of the ecosystem, soil stability, nitrogen fixation and biomass production. Recently, researchers are focusing in these dry areas sectors due to global climate change which causes alternation in the environmental factors such as temperature, increasing CO_2 levels, pattern changes in the distribution of rainfall, UV radiation. These alternations can disturb the habitats and microhabitats conditions in dry areas. Bryophytes are facing challenges especially in these areas as their photosynthetic and biochemical activities depends on their external environmental conditions.

In this thesis, a desiccation-tolerant bryophyte *Syntrichia ruralis* was collected from NE and SW slope in sandy semi-arid grassland of Hungary. These sandy grasslands are part of the Kiskunság National Park, Bugac in the Hungarian Great Plain. Chlorophyll *a* fluorescence measurements were detected on both samples and photochemical (Fv/Fm, qP, ΦPSII) and non-photochemical (qN, NPQ) parameters were studied in rehydrated states. Moreover, antioxidant enzymatic activities (APX, CAT, POD) has been demonstrated during rehydration and desiccation states along with protein and MDA content with respect to seasons and slope wise. Application of SA pre-treatment was also investigated, and measurements were conducted on chlorophyll a fluorescence parameter (Fv/Fm, ΦPSII, NPQ) along with antioxidant enzymatic activities.

The results clearly showed significant seasonal variations in the chlorophyll *a* fluorescence parameters and also in antioxidant enzymatic activities samples collected from north-east (NE) and south-west (SW). Similar result has been found in protein and MDA content also have seasonal variations. In both states, the highest enzymatic activities occurred in mosses collected during summer and winter season and the lowest activities were found during the spring and autumn season. Besides seasonal differences in the activities of the antioxidant enzymes, the small spatial-scale exposures i.e., the NE and SW slope orientation also can modify the expression of these enzymes. The role of some antioxidant enzyme in desiccation tolerance may be different, basically depending on the actual metabolic balance of mosses. In future perspectives, there is need to do more findings on biochemical aspects and more enzymatic activities will be investigated that will later contribute more to understanding the mechanism behind the desiccation tolerance in non-vascular plants. More investigations on application of exogenous SA pre-treatment will have to study on desiccation-tolerant bryophyte in future.

8 ÖSSZEFOGLALÁS

A félszáraz területeken, gyepekben, a kriptogám fajok, úgymint algák, gombák, zuzmók és mohák közösségei alkotják a biológiai talaj kéregrétegét (BSCs) vagy más néven a kriptobiotikus kérget (CBCs). Ezen közösségek meghatározói és egyedi sajátosságai a száraz élőhelyeknek. Fontos szerpük van az ökoszisztéma biodiverzitásában, a talaj stabilitásában, a nitrogén fixálásban és a biomassza produkcióban. Manapság, ezen száraz területek a globális klímaváltozás jelenségének köszönhetően kerülnek a kutatások középpontjába, mely folyamat a környezeti faktorok változásait eredményezi, úgymint a hőmérséklet, az emelkedő CO_2 koncentráció, a csapadékeloszlás és UV sugárzás mintázatának változásai. A mohák jó indikátorai e változásoknak, különösen a száraz területeken, mivel fotoszintetikus és biokémiai aktivitásuk a külső környezeti tényezők függvényében változik.

A dolgozatban, a vizsgálatokhoz a kiszáradástűrő *Syntrichia ruralis* mohafaj gyűjtése magyarországi, félszáraz homokpusztagyep buckáinak ÉK és DNY kitettségű oldalairól történt. Ezen nyílt homokpusztagyepek a magyar Alföld területén a Kiskunsági Nemzeti Park részei. A területről begyűjtött minták újranedvesítését követő, klorofill a fluoreszcencia (fotokémiai: Fv/Fm, qP, ΦPSII és nemfotokémiai: qN, NPQ) paramétereinek mérése történt. A vizsgálatok részét képezték továbbá az antioxidáns enzimaktivitás (APX, CAT, POD) mérések, fehérje és MDA tartalom meghatározások, újranedvesítés és kiszáradás során, szezonálisan és a kitettség függvényében. Klorofill a fluoreszcencia mérések (Fv/Fm, ΦPSII, NPQ) és antioxidáns enzimaktivitás mérések a minták SA hatásának vizsgálata során is történtek.

Eredményeink szignifikáns változásokat mutatnak mind a fluoreszcencia paraméterek mind az antioxidáns enzimaktivitás tekintetében a különböző kitettségű mikroélőhelyek között szezonálisan. Hasonló eredményeket kaptunk a fehérje és MDA tartalomra vonatkozólag is. A legmagasabb aktivitásokat mindkét kitettség esetén a nyári és a téli időszakban gyűjtött mohák vizsgálatainál kaptuk, míg a legalacsonyabbakat a tavaszi és őszi időszakokban. A szezonális különbségek mellett, a különböző antioxidáns enzimaktivitások a kitettségek között is mutattak eltérést. Az különböző antioxidáns enzimek kiszáradástűrésben betöltött szerepe eltérő lehet, alapvetően a mohák aktuális metabolikus aktivitásának függvényében. A jövőben, további biokémiai vonalon alapuló, feltáró és enzimaktivitás vizsgálat szükséges a nem edényes növényekben zajló kiszáradástűrő mechanizmusok hátterének megértéséhez. A SA exogén alkalmazásának hatásai a stresszélettani vizsgálatokban szintén jövőbeli feladataink egyike.

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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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11 APPENDICES

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