Final report of the project PD 109445 entitled „Different physiological ways of morphological and adaptation diversities of plant desiccation tolerance”

Principal investigator: Evelin Ramóna Péli

Period: 1 September 2013 – 31 August 2018

Our previous results indicated the different level of desiccation tolerance in many living system such as cyanobacteria, peat mosses and vascular plant species which are a represent contrasting strategies to solve the same ecological problem at drought conditions. Different strategies contrast greatly in the timescale of rehydration and desiccation events to which they are adapted of course, HDT (homoiochlorophyllous) and PDT (poikilochlorophyllous) categories overlap in their ecological adaptation.

Aim of the project

The main aim of this work was to obtain a better understanding of the responses of plants to desiccation as a stress factor in their different ecophysiological aspects. Furthermore, we would like to reveal some histo-anatomical characters which can play a special role in the mechanism of desiccation tolerance and the differences among species of the same genus or among members of different families.

Unscheduled project events and changes during the project

The project would be suspended for 2 years from 22.02.2014. My 2nd son was born in 22.02.2014. so I turned my request to Prof. Dr. Anna Erdei, the chairwoman of Life Sciences of OTKA to suspended my research project in order to upbringing my sons. The permission of the break of the research project was approved (EIK-7394/2012.10.17.), the date of the project would be modified, the period of the research postponed for next two years.
1 January 2017 there was the changing institution (from Hungarian Academy of Sciences (Plant Ecology Research Group) to University of Veterinary Medicine). Performance of the technical tasks of that research time was hardly blocked by the long-continued administration conversion. The financial administration of HAS found it difficult to transfer the project to UVM, the project was closed during seven months and the situation only normalized by August 2017.

Results

The project contains two main tasks:

1) tasks related to the ecophysiological properties of desiccation tolerant plants

2) tasks related to desiccation tolerance investigations of some Hungarian (from Bereg Plain) peat mosses (*Sphagnum sp.*)

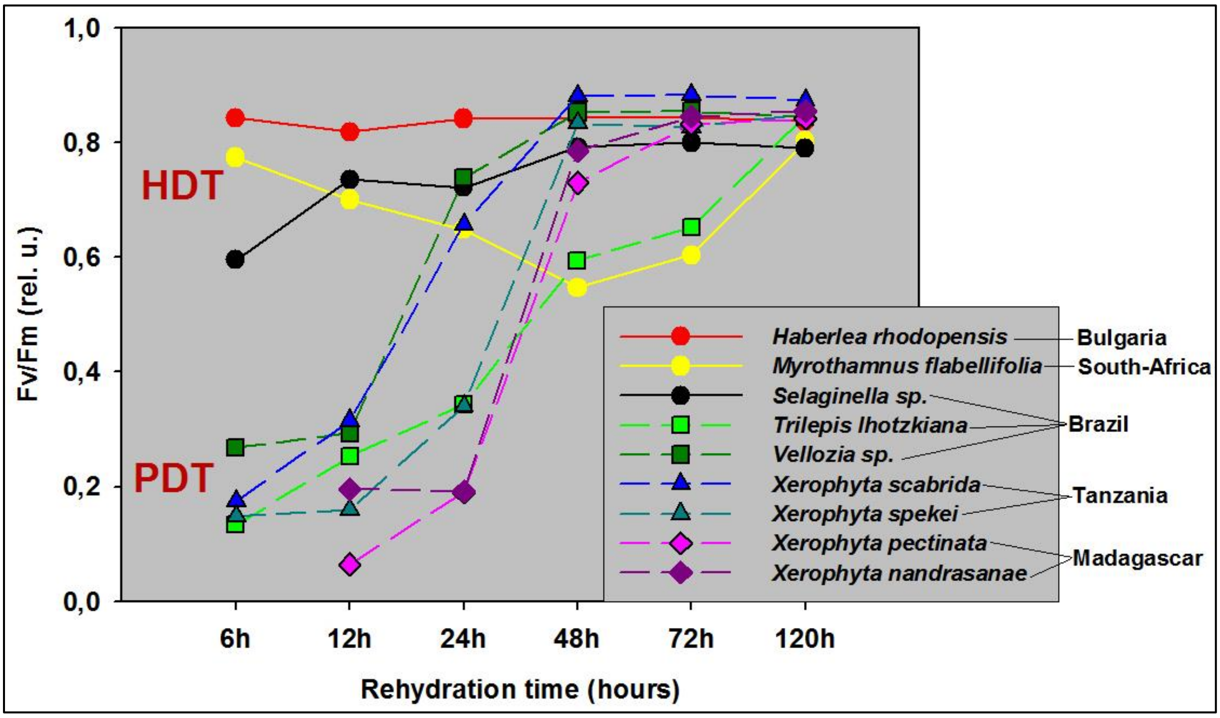
1) tasks related to the ecophysiological properties of desiccation tolerant plants

Water uptake and water use efficiency of PDT plants considerably differ from other vascular plants due to their special ecological adaptation strategies. Vascular DT plant leaves in general require external liquid water for rehydration. In the case if the leaves are to remain turgid for more than a few hours after rain, the continuity of water column within the xylem vessels must be restored as the tissues rehydrate.
Histological traits of the living leaves of desiccation-tolerant *Xerophyta scabrida* (Pax) Th., Dur. et Schinz. were investigated after safranin staining to examine the dynamics of rehydration and the process of water transport by safranin impregnation differences. Staining that appeared on the leaf's surface epidermal glands after 30 minutes of remoistening in living *X. scabrida* leaves suggested that glandular complexes could take part in the water uptake from the beginning of the rehydration process, when the xylem might not have been filled up, yet. Sclerenchyma staining started from the xylem while dead *X. scabrida* leaves were not able to rehydrate fully. The dynamics of rehydration by the orientation of immersing leaves was also studied. Leaves immersed into the solution with their base downward became fully impregnated earlier than the leaves sank into the dye with their apex downward. Faster water supply in the former case might have been related to the earlier recovery of xylem integrity and the resultant spatially continuous water supply. The results indicate that the living leaves of desiccation-tolerant *X. scabrida* were able to uptake significant water amount not only (and not primarily) through their vascular tissues but by external water conduction. It is supposed that the water movement outside the xylem namely extraxylar water transport plays a dominant role in the leaf hydraulics of desiccation-tolerant plants by refilling the cavitated xylem vessels during rehydration. Due to the specific binding of safranin to lignifying cell walls and its fast spread from the xylem to the sclerenchymatous bundle sheaths also confirm the potential role of sclerenchyma in the water movement of leaf tissues.

The objective of our other approach is to demonstrate some special and unique histological characters and their variability of the DT plants in *Xerophyta* genus that belong to the same genus living in different ecological environment which can take part in the mechanism of their desiccation tolerance ability. We hypothesize that these differences reflect diverse levels of their survival chance depending on the timescale of dehydration. These features context to their basic physiological parameters, on the one hand, can help distinguishing the desiccation tolerant plant species from drought tolerant ones. On the other hand, they can also contribute to more extensive knowledge about the ecological aspects of desiccation tolerance which is based on the isolated microclimatic adaptation under similar environmental conditions.

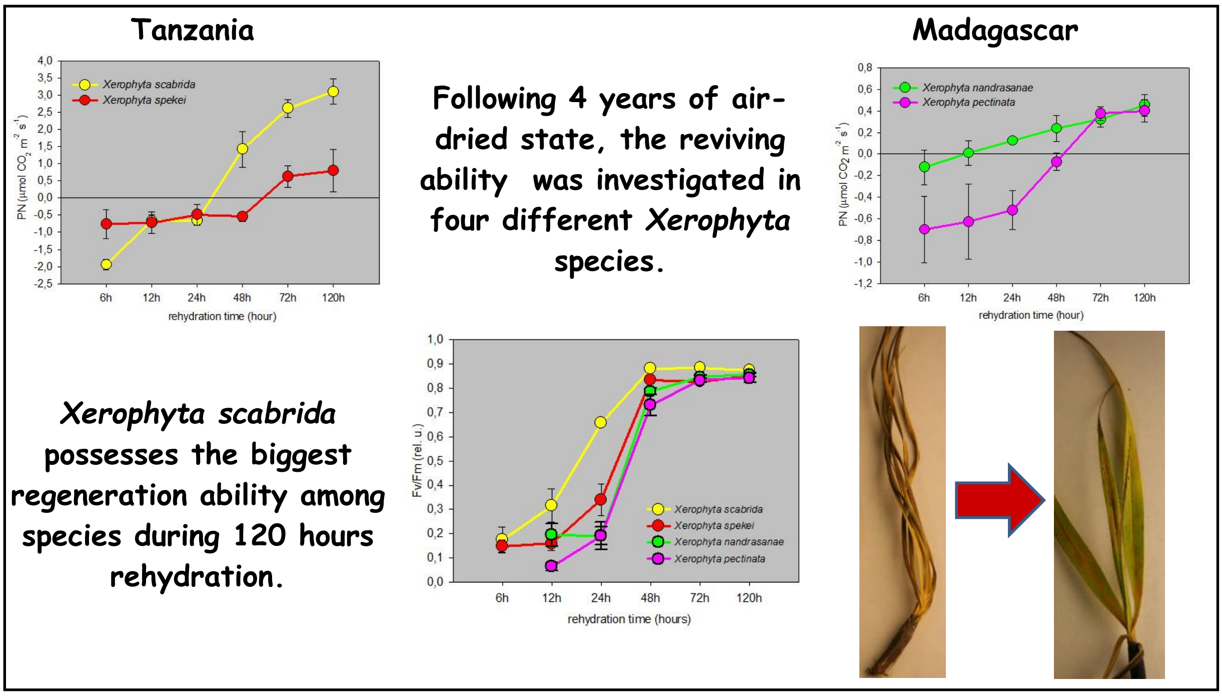
The investigated Xerophyta species had some characteristics which are typical for drought tolerant plants. Their leaf surface was ribbed. On the leaf surface of *X. scabrida* and *X. villosa* can not be found any nonglandular trichomes. They have entire leaf margins with spikes. The leaf margins of *X. villosa* were specifically thickened. Leaves of the other three species (*X. pectinata*, *X. nandrasanae*, *X. spekei*) were covered by trichomes, which seemed denser in the margins of the leaf and along the midrib. On the abaxial leaf side of *X. pectinata* and *X. spekei* dense indumentum can be found. *X. nandrasanae* had fewer pubescent leaves. On the leaf margins of the latter species multicellular, branched hairs can be found. Glandular trichomes occurred along the midrib on the abaxial leaf surface of *X. pectinata*. The base of these trichomes showed intense autofluorescence similarly to the base of the the abaxial trichomes in *X. nandrasanae*. Besides nonsecretory hairs, shorter glandular trichomes also appeared the leaf blade of *X. spekei*. Glands can be found on the surface of the leaf in *X. scabrida*. Large glands were observed on the abaxial side of the leaf, while such glands could only be seen on the margins on the other side. Glands form regulated lines on the leaf surface, which was well noticed along the midrib on the abaxial leaf side. The blade of *X. villosa* had a V-shaped furrow along the adaxial midrib with characteristic papillae. Leaf glands and papillae may play a role in desiccation tolerance. A lot of sclerenchyma, which can be observed in the mesophyll of the investigated species, is also a xeromorphic feature. In some plants sclerenchyma elements or layers may play a role in the reduction of absorbed radiation. Besides sclerenchymatous bundle sheath, longitudinal sclerenchyma girders also ran in the leaves. These were present mainly in the adaxial side. The adaxial sclerenchyma girders of *X. pectinata* and *X. nandrasanae* were round shape in the cross sections. Frequently, large-celled, parenchymatous tissue, serving as water storage can also be observed in the leaves of xerophytic plants. Groups of isodiametric cells can be found between the vascular bundles of *X. pectinata*, may also take part in the water storage. The shape of the sclerenchyma fibres, occurred above the bundle sheaths, were plate-like, but the others, taking place between the vascular bundles, were round shape under the adaxial epidermis of *X. scabrida*. Sclerenchyma sheets occur under both epidermis of *X. spekei* and *X. villosa*. Three investigated species (*X. nandrasanae*, *X. scabrida*, *X. spekei*) with dorsiventral leaves had a lot of palisade mesophyll cells which is also characteristic for xeromorph plants. The palisade mesophyll cells of *X. spekei* showed a characteristic arrangement, which suggest that they may play a role in the folding of the leaves during desiccation. Such arrangement could also be seen around the vascular bundles of *X. scabrida*. Water transport between the epidermis and the vascular bundles is much higher through this palisade tissue. Vascular bundles associated with not only parenchymatous, but also sclerenchymatous bundle sheath. During desiccation when parenchyma cells undergo massive cell wall folding and leaves shrink, these sclerenchyma tissues may play an important role in the maintenance of leaf consistency.

Physiological recovery scale and degree of these plants is useful for understanding plant interactions like colonization benefits under severe growth limitations and also plant-environment relationships which determine their production in their harsh ecosystems. Significance of recovery and spending time in desiccated period is the basis of a successful surviving strategy because the carbon assimilation during hydrated periods must be higher than carbon costs with carbon losses during desiccated periods. Periods and effects can probably be changing due to global climate change.



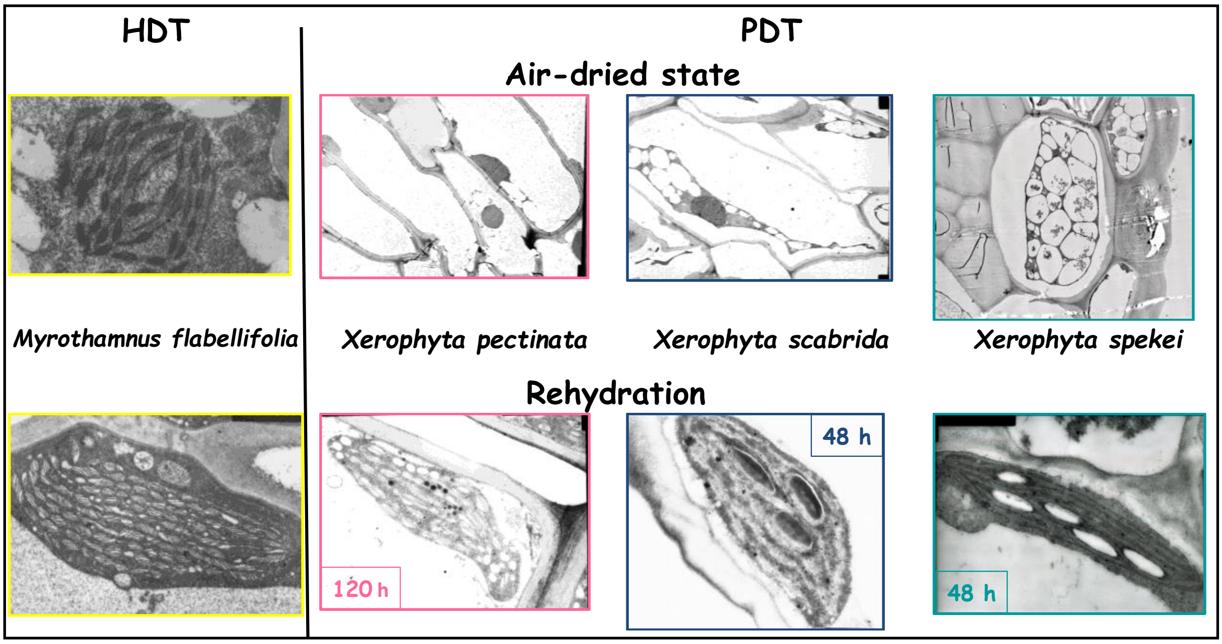
Changes in the maximal quantum yield of PSII (Fv/Fm) of different HDT and PDT plant species deriving from different habitats during rehydration. Species of HDT strategy well reflects the early regeneration ability after rehydration due to remaining their chlorophyll contents on dehydrating states. PDT plants need more time for activation because they must rebuild their dismantled chloroplasts structure. (unpublished)

We try to compare the regeneration ability of different HDT and PDT plant species deriving from different habitats based on their physiological aspects after being in 4 or more years of air-dried state. For 5 days of rehydration we were following the changes of photosynthetically features (through measuring PN the net photosynthesis and Fv/Fm -the maximal quantum yield of PSII) and their morpho-anatomical characters. Differences e.g. in the quality of the regeneration of different *Xerophyta* species (PDT) are also observed in respiration-photosynthesis transition and the level of CO_2 assimilation values. In comparison with different habitats and periods in desiccation states, the whole recovery based on Fv/Fm values followed 48 h and 72 h rehydration dependent on the species.



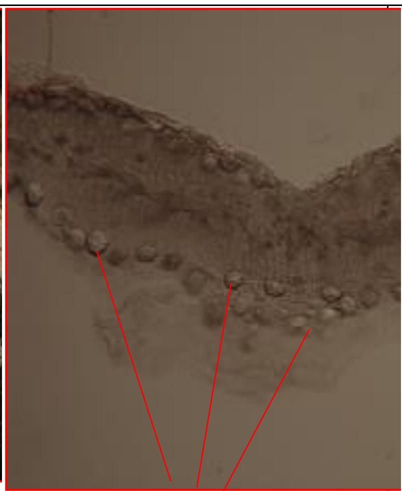
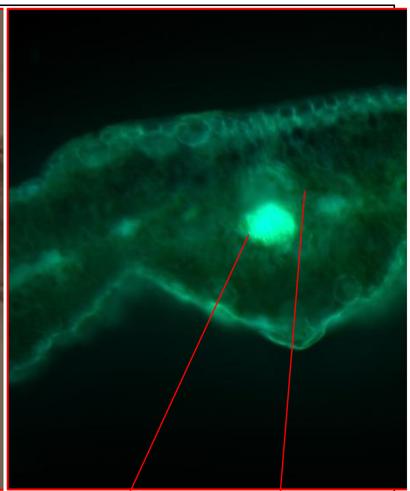
(unpublished).

We can also see the differences in time according to chloroplast reorganization levels. Not only spending time in air-dried state but also local, climatic environmental differences may play an important role e.g. in the same genus belonging to the same DT strategy.



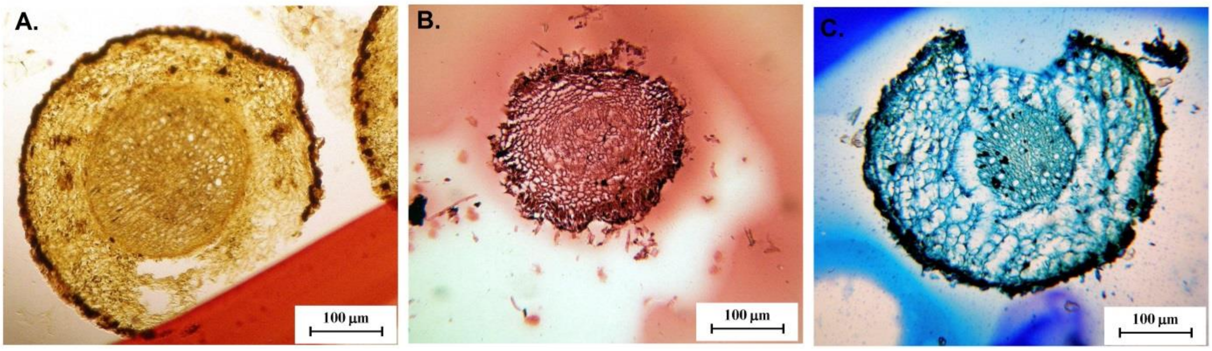
The cell inside changes following rehydration in different PDT and HDT plants. In mesophyll cells of the Xerophyta sp. and M. flabellifolia, the large central vacuole present in hydrated tissues is replaced by a number of smaller vacuoles, which serve to fill the cytoplasm, minimising organelle compaction and membrane appression and preventing plasmalemma withdrawal. It can be seen the differences in the time according to chloroplast reorganization levels. In the case of two Tanzanian species (X.scabrida and X. spekei) the chloroplast rebuilding processes is faster resulted in developed chloroplast form following 48 h *rehydration than Madagascan one (X. pectinata) where it is expressed by 120 h rehydration. (unpublished).*

Studied HDT plants are: *Haberlea rhodopensis* from Southern Europe and *Myrothamnus flabellifolia* from South African region. Epidermis of *Haberlea rhodopensis* consists of only one row with glands. Multicellular, unbranched (non-glandular) and glandular trichromes are also covered on the leaf surface. During dehydration-rehydration cycle significant changes were observed in the leaf. For the period of dehydration epidermis became thinner, but its cells remained closely to each other so it can help to retain the consistency of the leaf tissues. It is also necessary because the mesophyll turned into highly disintegrated. During rehydration mesophyll was restructured. Palisade parenchyma constituted compact and well-distinguished layer and in the spongy parenchyma regular intercellular spaces were formed again which can participate in leaf shrinkage.
The other HDT plant studied is *Myrothamnus flabellifolia*. The leaf surface is ribbed. Stomata are covered by protective secretion in both dehydrated and rehydrated states. The shape of the epidermis cells is irregular and polygon. Stoma is amaryllis type. Stomata are sunk a bit or they are on the same level with the epidermis. This resurrection plant possesses fanlike leaves composed of sclerenchyma-rich ribs that remain unfolded whereas the inter-rib vegetative tissue shrinks during dehydration. The unique arrangement of alternating ridges and furrows allows the leaves to fold parallel to the leaf face upon dehydration. This probably facilitates a rapid return to the hydrated stage.

		
***Myrothamnus flabellifolia* in dry season**	**liposomes**	**vascular bundle sheat** **spongy parenchyma**

The leaf shape is longitudinal in cross-section. Under the epidermis vacuoles containing condensated phenolics (polyphenols like tannin) can be found which play role in desiccation tolerance. It is proposed that photooxidative stress is also reduced by the presence of waxes which may function to reflect light away from the leaf surface. Calcium oxalate druse crystals also can be found in the mesophyll. (unpublished).

Histological changes of the roots and leaves of *Haberlea rhodopensis* were investigated during dehydration and rehydration. Vegetative organs of *Haberlea rhodopensis* have large flexibility, which contributes to its ability to tolerate and survive the extreme drought stress. Width and cell size of their tissues decreased at least with 1/5 part of the control during desiccation. However, some of them could undergo more than 50 % reduction to the end of the desiccation period. Considering roots, it is established that the vascular cylinder lost the water quicker and supplied it slower than the primary cortex. In contrast, during rehydration the water supply of the primary cortex proved to be quicker. Structural changes of the leaves, i.e. disintegrated parenchyma tissues, can restructure in the rehydrated plants, which indicated considerable adaptation of the plants to dry habitat. Adaxial epidermis and spongy parenchyma were more sensitive to water status changes than the other leaf tissues during dehydration and rehydration, as well. Root tissues, primarily due to the vascular cylinder, seemed to react more sensitively to water deficiency than leaves. However, parenchyma tissues of the leaves changed more quickly during the dehydration-rehydration cycle than the primary cortex of the roots indicating that the plasticity and fast regeneration of the leaves were mainly due to these kinds of plant tissues. Simultaneous investigation of the main vegetative organs in the whole plant gives possibility to better knowing the basic background of desiccation tolerance. In the mesophyll layer of the leaves, the spongy parenchyma was responsible for the effective water uptake and transport while the palisade layer could help the reversible and fast regeneration of the photosynthesis with their larger amount of chloroplast. This "division of labour" could also be observed between the root system tissues where the crucial function of the primary cortex could be the fast water uptake. These differences in the regeneration abilities of the main vegetative organ layers can make HDT strategy successful. HDT plants preferred habitats where the desiccated periods are relatively short, and plants can react immediately and quickly to the alternating and periodic dry and wet conditions on a day-to-day basis.



Micrographs of Haberlea rhodopensis roots in different time of dehydration and rehydration cycle. ***A***. *Root cross section of control (hydrated) root without staining.* ***B.*** *Root cross section after 72 h dehydration staining with safranin solution.* ***C.*** *Root cross section after 96 h rehydration staining with methylene blue. (Bar =100 µm). (unpublished)*

2) tasks related to desiccation tolerance investigations of some Hungarian (from Bereg Plain) peat mosses (*Sphagnum sp.*)

Our study is to provide a seasonal overview of functional physiological mechanisms of a unique and isolated peat moss dominated area in Hungary. Our study shows that functional differences can also exist within relatively small mire not only seasonally but also depending on microsites or types of plant communities. These results contribute to our understanding of dynamic changes of peatlands, mire ecosystem functioning at the edge of their distribution and predict ecosystem responses to climate change, which can be potential factors both on global carbon cycle and global change. Our findings showed that the decomposition rates were more dependent on vegetation type than C/N ratio and this relationship was also revealed at a small spatial scale. Measurements had been conducted throughout a year by placing litterbags filled with *Sphagnum* biomass in three vegetation types (open peat bog, poor fen, alder carr*)* of a mire ecosystem in Hungary. Peat decomposition rates differed to a great extent; the slowest decomposition rate (39.1±9.52%) was in the alder carr, indicating that slower decomposition could be characteristic for this kind of vegetation type of mire. Between *Sphagnum* dominated microhabitats, open peat bog showed medium (65.57±4.05) while poor fen the fastest (68.61±5.5) rates in decomposition. The C/N ratio of the *Sphagnum* litter showed significant decrease ($P<0.005$) in all studied micro-environments. Slower N release was observed from litter of *Alnus* dominated association (31.3±6.9%) compared to *Sphagnum* dominated ones (56.5±8.3%). The microclimatic effects on peatland vegetation basically influence their plant composition and their metabolisms which reflected a shift in decomposition even at a relatively small separated area.

The other aim of our study is to provide a seasonal overview of functional physiological mechanisms of a unique and isolated peat moss dominated area in Hungary. Net ecosystem exchange (NEE) of carbon dioxide, Normalized Difference Vegetation Index (NDVI values), chlorophyll fluorescence parameter (Fv/Fm) of dominant *Sphagnum* and vascular plant species were measured seasonally in two Hungarian *Sphagnum* dominated mires under *in situ* field conditions. NEE ranged from -1.08 to -2.89 μmol m^{-2} s^{-1} in the spring and autumn but fell to 0.68 to –2.52 μmol m^{-2} s^{-1} (a negative value indicates ecosystem uptake) under higher light flux density (PPFD of 1100 μ mol m^{-2} s^{-1}) during the summer period. NDVI values showed the highest rates in summer (between 0.756-0.882) and the lowest rates were measured in spring (between 0.426-0.612) in all investigated microhabitats. The maximal photochemical activity (Fv/Fm) of the dominated species reflected the seasonal and microclimatic adaptation; showed lower values in spring and autumn (0.505-0.847) while these parameters are characterized by higher values (0.8-0.857) in all dominated species in summer. Our study shows that functional differences can also exist within relatively small mire not only seasonally but also depending on microsites or types of plant communities.

Peat mosses of different ecosystems can possess significant production biological value depending on their abundance and dominance in a certain association. Vertical position of certain peat mosses (*Sphagnum spp.)* in the hollow-hummock situation showed characteristic and consistent pattern. Especially typical this kind o formation in open and treeless area of tundra and taiga zones, where the hollow-hummock formations are more compact and complex with conspicuous diversification of *Sphagnum* species. *Sphagnum fuscum, S. capillifolium* és *S. rubellum* species are dominant in the apical zone of the hummock. They are lay under enhanced environmental stress in flat fen to be got sort close to each other avoiding the intensified desiccation. In case of Hungarian peat bogs (in Bereg plain), the structure of the hummock is more loose, typical apical species – have better tolerance against desiccation – are: *Sphagnum palustre, S. magellanicum.* The members of *Sphagnum recurvum* group *(S. angustifolium, S. fallax, S. flexuosum*) have smaller head size and slender habitus furthermore, rather can be found in the hollow region. Hummock species showed smaller variability in the ability of the water storage of peat moss head than hollow species but latter can retain less water because of their morphology (smaller head size). Its also important fact, that the certain *Sphagnum* vegetation flecks - especially in hummock case - almost never consist of only one peat moss species. Collective presence of different peat mosses refer to better water holding capacity and performance of more effective strategy which show higher diversification. In spite of these facts, the peat moss associations are extremely sensitive for environmental changes showed e.g. *S. magellanicum* have almost absolutely disappeared from the hummock region of Bereg plain (Hungary) of late years, which can be one of the effect of global climate change.

Publication activity

The work plan of the present is represented in many publications, the principal investigator is the first author in all of them. Taking into account the novel nature of the work, we expect a high number of citations in the coming years. In the last period of the project, the scientific community was informed about the progress in the project, about preliminary results on posters presented on international and domestic conferences (see the publication list in mtmt data base). Further publications can be expected before long are under preparation from the above results and it is planned to submit in the near future.

Manuscript under review (submitted 21.08.2018 for Flora):

Evelin Ramóna Péli, Katya Georgieva, Helga Nagy-Déri (2018): Desiccation-rehydration effects on leaves and roots histology of *Haberlea rhodopensis*.