PEG-MEDIATED OSMOTIC STRESS RESPONSES OF WHEAT-BARLEY ADDITION LINES

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Abstract: Photosynthetic responses of three wheat-barley addition (add) lines exposed to PEG-induced drought stress and under rewatering period were investigated in order to improve wheat drought tolerance by the help of barley chromosomes. The wheat-barley disomic addition lines (2H, 3H, 4H) the wheat line (*Triticum aestivum* L. cv. 'Mv9kr1') were found to have better responses to osmotic stress relative to the parental barley cultivar (*Hordeum vulgare* L. cv. 'Igri'). Addition lines with 2H and 4H chromosome from barley used similar strategy of acclimation to osmotic stress. These lines were able to avoid drastic water loss as well as exhibiting only a slight decrease in stomatal conductance (g_s) in contrast to barley. At the same time, photosynthetic processes in 4H addition seemed to be more sensitive to the decreased relative water content (RWC) of leaves caused by 21% PEG resulting reduction in stomatal to non-stomatal limitation ratio and impaired recovery ability. 3H addition line could be characterized as the most dehydration tolerant among the examined lines on the basis of water wasting responses shown by high gs, decreased intrinsic water use efficiency and more successfully sustained shoot biomass production in contrast to root. Changes in Y(II) parameters were moderate in the addition lines indicating that the electron transport processes were not damaged by osmotic stress. Our results suggest that wheat line also avoided being dehydrated similar to 2H and 4H add but the relatively high RWC under severe water deficit was primarily due to the pronounced stomatal closure. Changes in shoot-root ratio and net CO_2 assimilation rate (P_N) was also similar to those in 4H add. Although the maintained root growth and strong decreased g_s may be the indicators of drought avoidance in barley, in spite of these traits low RWC was observed which contributed to the significantly impaired P_N primarily limited by the non-stomatal processes. Considering to drought sensibility, we concluded that barley genotype Igri is not the most suitable gene source for improving water stress tolerance of wheat but 2H addition line seemed to be more resistant to osmotic treatments than wheat and could be used in wheat breeding programs in the future.

Keywords: barley, biomass production, drought avoidance, drought tolerance, leaf gas exchange, wheat

INTRODUCTION

Water shortage, increased requirement for food production and/or less and less arable land are thought to be the main problems of the annual agricultural which demand to develop crop plants with an acceptable productivity (Tardieu 2012). It is estimated that wheat (*Triticum aestivum* L.) global grain yield must be doubled (Rajaram 2001) even under unfavourable environmental effects such as limited water availability. Improvement for adaptation to drought can be achieved by transposition of genomic regions controlling the survival of plants under water deficit. Several quantitative trait loci (QTLs) associated with drought-related traits have been determined in barley therefore it could be a potential gene source for development of drought tolerance in wheat. For example, QTLs affecting osmotic adjustment, relative water content and water soluble carbohydrates were mapped on 2H, 3H and 4H barley chromosomes (Teulat *et al.* 2002, Diab *et al.* 2004). In malting barley, QTLs for photochemical activity of PSII are also located on the 2H, 3H and 4H barley chromosomes (Wójcik-Jagła *et al.* 2013). Selecting plants which have efficient water saving strategy may be a way to minimise the water consumption of agriculture (Condon *et al.* 2004). Genomic regions controlling the rate of water-use-efficiency (WUE) have been also determined in the barley genome (Chen *et al.* 2012).

The first reaction of a plant stressed by drought may be a decline in water loss through the decrease of stomatal aperture (Henson *et al.* 1989). Stomatal conductance (g_s) makes the determination of an important factor associated with drought tolerance, called intrinsic water use efficiency (WUE_i) possible (Ehdaie and Waines 1993, Molnár *et al.* 2007). This value is controlled by photosynthetic CO_2 assimilation processes and stomatal resistance to water loss (Sinclair *et al.* 1984); therefore it can be determined as the ratio of P_N and g_s, as described by Martin and Ruiz-Torres (1992). It has been reported that closed stomata result increased WUE through the inhibition of transpiration more than CO_2 diffusion into the chloroplasts at the initial stage of water deficit (Chaves *et al.* 2009). Not only the WUEi but the biomass productivity of crop cultivars may be important criteria for drought tolerance (Blum 1993), but WUEi is not always positively correlated to crop yield (Tuberosa 2012). Through decreased g_s

which may occur quickly as a short-term adaptation mechanism, plants are able to moderate water loss under water scarcity (Chaves 1991, Cornic 1994, Molnár *et al.* 2004), thereby relative water content (RWC) goes down very little. Despite of this fact stomata also could play role as a limiting factor against the diffusion of CO_2 into the chloroplasts of mesophyll cells which is termed as stomatal limitation (L_s), followed by parallel declining in the net photosynthetic rate (P_N) under water stress (Cornic 2000, Lawlor and Cornic 2002, Medrano *et al.* 2002). Not only the value of P_N but also many other photosynthetic parameters such as carboxylation efficiency or electron transport rate may show a strong correlation with g_s even more than water status itself (Medrano *et al.* 2002). Some studies describe g_s as an important determining factor in the change of P_N even at severe water deficit and the role of metabolic factors may be unconsidered (*e.g.* Cornic and Fresneau 2002), while g_s and metabolic factors (RuBP and ATP) could limit together the assimilation even under moderate drought according to others (Tezara *et al.* 1999, Medrano *et al.* 2002). Brodribb (1996) described a biphasic model according to the changes in the intercellular CO_2 concentration (C_i) under increasing drought. In the first phase, a substantial reduction in C_i was noticed as g_s decreased. In the second non-stomatal phase, increment in C_i and irreversible photoinhibition was detected while g_s reached a minimum level.

Plant biomass is a crucial parameter but not necessarily correlated with grain yield in wheat under drought stress (Paul *et al.* 2016). At the same time the asymmetric growth of root and shoot makes it possible to compensate negative effects of unfavourable water supply. More intense root dry biomass production compared to shoot dry matter may contribute to higher relative water content (RWC) in plants which avoid being water stressed (Morgan 1984). The higher root biomass could also result an increased drought tolerance (Hoffmann and Burucs 2005) since low RWC affects particular processes of photosynthesis negatively (Chaves 1991, Cornic 1994). Therefore, preservation of water status may become significant to maintenance assimilation capacity and growth (Akram 2011).

Under water deficit photochemistry declines and energy dissipation shows higher value (Guo *et al.* 2013) resulting imbalance between energy capture and metabolism (Lawlor and

Tezara 2009). Under drought conditions when the CO_2 assimilation is impaired it is important to protect chloroplast against extra harmful reduction force by terminal dissipation of excess light energy from the PSII reaction centre (Ruban and Horton 1995, Horton *et al*. 2005). This rapidly activated regulatory mechanism could be detected as non-photochemical quenching (NPQ) (Horton *et al.* 2005). When lack of water becomes scarce, the quantum yield of non-photochemical quenching (YNPQ) and the amounts of the products of xanthophyll cycle (zeaxanthin and antheraxanthin) increased significantly (Tambussi *et al.* 2002) thereby may be minimize the damage of PSII. If plants are high sensitive to water deficit, damages may occur in PSII reaction centres (Murata *et al.* 2007) resulting intensification in the quantum yield of nonregulated energy dissipation (Y(NO)).

   The goal of this study was to compare photosynthetic responses to the polyethylene-glycol (PEG) induced water deficit in three wheat-barley addition lines in relation to the wheat and barley parental genotypes. The measured parameters were employed as a selection system for sorting wheat-barley introgression lines that have better drought tolerance and/or recovery capacity than the wheat genotype. The basis of our selection is the values of relative water content, biomass production, gas exchange and fluorescence induction parameters characterizing the ability of drought resistance suggested by literature data.

MATERIALS AND METHODS

Plant materials
Triticum aestivum L. cv. 'Mv9kr1'wheat line, *Hordeum vulgare* L. cv. 'Igri' barley cultivar and Mv9kr1-Igri wheat-barley disomic addition lines 2H, 3H and 4H (2H add, 3H add and 4H add, respectively) (developed by crossing Mv9kr1 wheat with Igri barley) were investigated. Genotypes were produced in the Agricultural Research Institute of Hungarian Academy of Sciences (Martonvásár). The disomic addition lines carry the full genome of wheat and one extra homologous chromosome pair of barley genotype 'Igri' 2H, 3H or 4H (Molnár-Láng *et al* 2000).

Culture condition and induction of osmotic stress

Seedlings were grown in 1500 cm³ pots containing half-strength modified Hoagland nutrient solution (Nagy and Galiba 1995) in growth chambers with normal CO_2 concentration at 20/25ºC. The light intensity for growth was 200 µmol (photon) m^{-2} s^{-1} and circadian illumination 12 h dark/12 h light was applied. Water deficit was induced in 4-week old plants by increasing the osmotic pressure of the hydroculture medium through the addition of polyethylene glycol (PEG 8000, Sigma, St. Louis, MO). Measurements were made after the 7-day treatment with 15% and 21% PEG and after 2 and 7 days of rewatering. The applied PEG concentrations resulted in osmotic potentials of –0.7 MPa and –1.75 MPa. All the experiments were performed on intact leaves or leaf segments of wheat cultivars and the hybrid lines.

Determination of RWC and dry matter production

Drought response of plants was monitored through determination of relative water content (RWC) and dry matter production of roots and shoots. The RWC was determined as RWC = (FW – DW) x 100 / (SW – DW), where FW is the fresh weight, SW is the water-saturated weight and DW is the oven dry weight for 12 h at 105ºC. Dry matter productions were estimated by harvest method. The shoot and root dry mass (g/plant) was determined on 7-week old plants at the end of whole experimental period and data were compared with the values for control plants of same age, grown in Hoagland solution without PEG.

CO_2 gas exchange and chlorophyll fluorescence measurements

The CO_2 assimilation of intact leaves was measured with an infrared gas analyser (Analytical Development Co. Ltd., United Kingdom) with 6.25 cm² assimilation surface. The net CO_2 assimilation rate (P_N), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were calculated in the light-saturated state of photosynthesis using the equations of von Caemmerer and Farquhar (1981) at 360 ppm CO_2 levels under 1,000 µmol(photon) m^{-2} s^{-1} light intensity. L_s and L_{ns} parameters were obtained from P_N versus C_i curves as described by Lawlor (2002), between 0–1,000 ppm CO_2 at light saturated state of photosynthesis using a gas

diluter. The intrinsic water use efficiency (WUE$_i$) will be calculated as P_N/g_s as described by Martin and Ruiz-Tores (1992).

Changes in fluorescence efficiency were measured with a pulsed-amplitude modulation fluorometry (PAM 101–103, Heinz Walz Effeltrich, Germany). The minimal fluorescence yield of the dark-adapted state (F$_0$) was detected after 15 min dark adaptation. The maximal fluorescence yield of the dark-adapted state (F$_m$) and maximal fluorescence yield of the light-adapted state (F$_m$') were determined by applying saturating flashes (8,000 µmol m^{-2} s^{-1}) lasting 0,8 s. Photosynthesis was induced by continuous illumination (actinic light) of leaves at 1,000 µmol m^{-2} s^{-1} light for 15 min. The fluorescence parameters were calculated as described by van Kooten and Snel (1990) and Klughammer and Schreiber (2008a) on the basis of the following equations: effective quantum yield of PS II, YII = (Fm'–F)/Fm'=ΔF/Fm'; quantum yield of regulated energy dissipation, Y(NPQ) = (F/F$_m$') – (F/Fm), quantum yield of non-regulated energy dissipation, Y(NO) = F/Fm.

Statistics

The results are the means ± LSD5% of five measurements on different plants per treatment for CO_2 gas exchange, chlorophyll fluorescence and RWC parameters and of eight measurements per treatment for the biomass parameters.

RESULTS AND DISCUSSION

Impact of PEG-induced water stress on relative water content, dry matter production and gas-exchange parameters

The alterations of some processes in photosynthesis could be partly attributed to the changes in RWC under different water conditions (Chaves 1991, Cornic 1994) hence RWC can be considered as a sensitive indicator of drought stress (Clavel *et al.* 2005). The reduction in RWC was much more pronounced in Igri (barley) compared to the other lines when the 15% PEG was applied (*Figure 1A*). Under more severe drought (21% PEG), 3H add also reacted remarkable drop while 2H add, 4H add and Mv9kr1 (wheat) showed RWC of approximately 90%. At the same time, RWC values of barley and 3H add recovered rapidly after the second day of re-watering. The high RWC in wheat could be attributed to significant closure of stomata at 21% PEG contrast to 2H and 4H add lines (*Figure 1B*) which have retained hydration of their tissues even with a higher stomatal conductance (g_{as}) in contrast with 3H add line. 3H add also did not closed its stomata under the treatments but this reaction reflected in lower RWC. When the reduction in g_s is more intensive, than that in assimilation rate (P_N) results improved intrinsic water use efficiency (WUE_i). The stomatal control led to an increase in WUE_i in wheat and barley under 21% PEG in relation to moderate stress (*Figure 1C*). In the case of wheat, the better WUE_i was primarily attributed to the significant drop in g_s and less to the changes in P_N (*Figure 2A*). 3H and 4H add showed the lowest WUE_i value at severe water deficiency, primarily caused by higher g_s and not by a large reduction in P_N. Treatment with 15% PEG significantly reduced P_N in all genotypes but the decrease was the most prominent in barley. Raising the PEG concentration (21% PEG) similar changes in P_N were represented by wheat and 4H add, it means approx. 34% and 37% loss of control value while the impairing was milder (approx. 23% loss) in 2H and 3H add line. Usually, drought tolerant barley cultivars show successfully maintained RWC of leaves when water availability is limited (Matin *et al.* 1989). In our research P_N was strongly correlated with RWC in wheat ($R^2=0,99$) and in barley ($R^2=0,97$) under osmotic treatments so the assimilation associated processes are more sensible to the loss of water content in parental lines but less in the

addition lines, especially in 3H add. Inhibition of P_N was the most pronounced in the case of barley with 67% loss compared to its own control but responded fast with only 26% loss to the recovered RWC under the re-watering period. None of the examined lines showed full recovery of P_N but the most prominent result was detected in 3H add with less than 8% loss of control. 15% PEG induced significantly higher intercellular CO_2 concentration (C_i) in the case of all genotypes excepting 2H add (*Figure 2B*). Further substantial increment was observed only in barley. If Ci shows increase at low g_s not only stomatal resistance but also impaired metabolic processes may be responsible for the inhibited photosynthesis (Brodribb 1996, Zhou *et al.* 2007). Consequently, metabolic limitation became significant in barley at the maximum water deficit indicated by higher C_i and strong stomatal closure. Although 21% PEG also significantly enhanced C_i in 3H and 4H add relation to their control level but it was noticed under substantially less decrease of stomatal aperture than in the barley line.

It is also essential whether the stomatal or non-stomatal limitation is dominated in the impaired CO_2 assimilation. At mild and moderate water stress stomata closure is the primal inhibitor of photosynthesis and less affected by biochemical processes (Bota *et al.* 2004), but at stronger water deficit the role of impaired metabolic processes may intensify at low g_s (Flexas and Medrano 2002), the latter is termed as non-stomatal limitation of photosynthesis (L_{ns}). L_{ns} reached higher values compared to stomatal limitation (L_s) in the examined lines even under slight osmotic stress (*Figure 2C, D*). When water deficit intensified, further decreased in the L_s/L_{ns} ratio was noticed in 4H add and barley with different stomatal response. In contrast to this observation, 2H add was the only line in which L_s exceeded L_{ns} value under 21% PEG indicating better drought tolerance. In the beginning of regeneration period L_s became the dominant factor in relation to L_{ns} in wheat contrast with Igri, 4H add and 3H add. Although L_{ns} was not abolished by 7-day rewatering in the case of any lines at the same time barley showed the most substantial reduction in relation to its value under 21% PEG indicating prominent ability to recover after water deficit.

The root and shoot dry weight of wheat and 4H add were equally depressed by osmotic treatments contributing to the

unchanged shoot/root ratio (*Table 1*) The root dry mass production of 2H add and 3H add was also negatively affected by deficient water availability (approx. 32% loss) more than shoot growth resulting raised shoot/root ratio especially in case of 3H add. Although barley maintained root growth more successfully than the other lines the shoot production was the most limited by water deficit resulting significantly reduced shoot/root ratio. More intense root growth compared to shoot could result higher RWC (Morgan 1984) through maximizing water uptake. Not only the asymmetric growth but the closure of stomata is the main feature of plant following drought avoidance strategy. Although the root growth of barley and the stomatal responses are indicative of drought avoidance in spite of these features barley was not able to sustain its water status indicating water stress sensitivity. Moreover, the decrased shoot/root ratio under drought conditions may contribute to yield loss due to the reduced assimilating area (Hoffmann *et al.* 2009).

Impact of PEG-induced water stress on chlorophyll a fluorescence parameters
Chlorophyll fluorescence measurement can be applied to discriminate between drought tolerant and sensitive wheat (Sayar *et al.* 2008). The effective quantum efficiency of PSII photochemistry (Y(II)) is directly related to the assimilation rate (Edwards and Baker 1993) therefore YII was utilised to examine perturbation of photosynthesis performance. Disturbance of CO_2 fixation and/or the damaged PSII contribute to the reduced Y(II). The latter may be caused only by stronger stress thereby PSII damages structurally (Tambussi *et al.* 2005). Y(II) began to decrease significantly in 4H add and in the parental lines even at 15% PEG concentration but no further noticeable reduction was observed when drought became more pronounced (*Figure 3A*). In the case of 2H add drought events did not caused significant depression on Y(II) moreover it was noticed no considerable changes in chlorophyll a fluorescence parameters were measured under the whole experiment reflected by negligible L_{ns} too. In the end of re-watering period addition lines could recover successfully their Y(II) while this parameter did not reach the value that of control in barley and wheat.

Under drought conditions when CO_2 availability is limited by depressed g_s, absorbed light energy can exceed that energy which is needed for Calvin cycle thus may result increment of the photoprotective processes (Chaves *et al.* 2009) such as NPQ and down regulation in Y(II) (Demmig-Adams *et al.* 1996). Y(NPQ) intensified substantially in parental lines when 15% PEG was applied parallel with the downregulation of Y(II) but further changes were not measured under severe water deficit. (*Figure 3A, B*). In the case of 3H add and 4H add, 15% PEG resulted higher Y(NPQ) but it was diminished to the control level by 21% PEG in 3H add and remained constant in 4H add. Induction of Y(NPQ) was not necessary in 2H add during the limited water availability indicating a positive effect of maintained g_s against the over-reduction of photosynthetic electron transport rate. The 7-day rewatering caused a reduction in Y(NPQ) for the most genotypes especially for barley in relation to value under 21% PEG.

If plants are highly sensitive to water deficit, damages could be detected in PSII reaction centres (Murata *et al.* 2007) as it reflects by the increment in the quantum yield of nonregulated energy dissipation (Y(NO)) in barley already at 15% PEG level (*Figure 3C*). At the same time only slight alterations were detected in Y(NO) for the addition lines and wheat under stress conditions. Moreover, this difference in Y(NO) values between the barley and the other lines showed further increment at maximum water deficit. High Y(NO) in barley could contribute to the insufficient recovery of Y(II) under favourable water supply while it was attributed to Y(NPQ) rather than PSII damages in the case of wheat. These results suggest that drought treatments had no noticeable effect on the capacity of primary charge separation in wheat and addition lines. These plants were able to compensate the effects of excess light mostly through the photoprotective regulated dissipation mechanisms while the intensified Y(NPQ) seems to be not enough efficient to avoid PSII damages in barley.

CONCLUSIONS

We compare photosynthetic and physiological responses to two different degrees of water deficit and rewatering in wheat-barely derivatives with the parental lines. On the basis of parameters, it could be determined which hybrid could be suitable to increase the tolerance of wheat against drought stress.

Drought tolerance is a complicated trait involving several physiological, morphological and biochemical processes. Stomatal closure is the most efficient way to reduce water loss allowing impaired WUE (Chaves *et al.* 2009) but keeping high g_s even under drought conditions has been considered a mode of drought resistance trait (Johnson *et al.* 1987). 2H add and 4H add not only kept open their stomata but also were able to retain their water status more successfully in contrast to barley and 3H add. Moreover, 2H add and 4H add maintained their RWC at a level similar to that of wheat without intense stomatal closure. This suggests that an active osmoregulation mechanism may exist in these lines contributing to efficient water uptake. In 2H add the CO_2 assimilation and the photosynthetic electron transport processes were slightly influenced by osmotic stress and shoot/root ratio showed increment but the biomass production was more depressed than that of wheat (*Table 1*). Although 2H and 4H add responded similar to osmotic treatments according to their g_s 4H add seems to be more sensitive the loss in RWC. This sensitivity was shown by the decreased L_s/L_{ns} ratio at stronger water deficit and the impaired ability to restore its P_N value largely due to L_{ns}. 3H add could be characterized as the more dehydration tolerant genotype among the examined lines on the basis of water wasting responses shown by opened stomata, low WUEi and increased shoot/root ratio. This line showed the most promising shoot biomass production under the water deficit. Despite of significant decreased RWC 3H line seems to be less sensitive to water loss than barley according to their P_N and L_{ns}, Y(II) and Y(NO). Although treatment with 15% PEG was accompanied by significant drop in P_N in the case of all genotypes under stronger osmotic treatment wheat line was able to produce a satisfactory assimilation level in spite of low g_s and after all its WUE$_i$ value elevated to the control level. It seems that wheat avoids being dehydrated by the lower g_s at more severe osmotic effect. This response might be essential to

preserve water content of leaves which were strongly correlated with P_N under the PEG treatments. The less limited CO_2 fixation and increased Y(NPQ) may contribute to protect PSII from damages but recovery of Y(II) and P_N were not fully. At the same time, among the examined lines root and shoot biomass production of wheat was the most pronounced. From our results, barley seems to be the most drought sensitive genotype, as regards RWC, P_N, L_{ns} and Y(NO) parameters in spite of the maintained WUEi and root growth. The change of Y(II) in barley was similar to its P_N indicating a close correlation (R^2= 0,89) under the whole experiments. Consequently, the decreased Y(II) may be attributed to the significant disturbance of CO_2 assimilation indicated by elevated L_{ns} and suggests low tolerance to water deficit in Igri. In spite of the unfavourable responses of barley, its regeneration ability is prominent as far as RWC and P_N are concerned. It may the consequence of the less retention of root growth under water deficit.

Finally, the results suggest that, photosynthetic responses of 2H add were less sensitive to water stress than the parental wheat genotype and could be useful genetic material in wheat breeding programmes. At the same time wheat also showed significantly better drought tolerance than barley therefore the examined barley genotype is not the most suitable gene donor for improving drought stress tolerance of bread wheat.
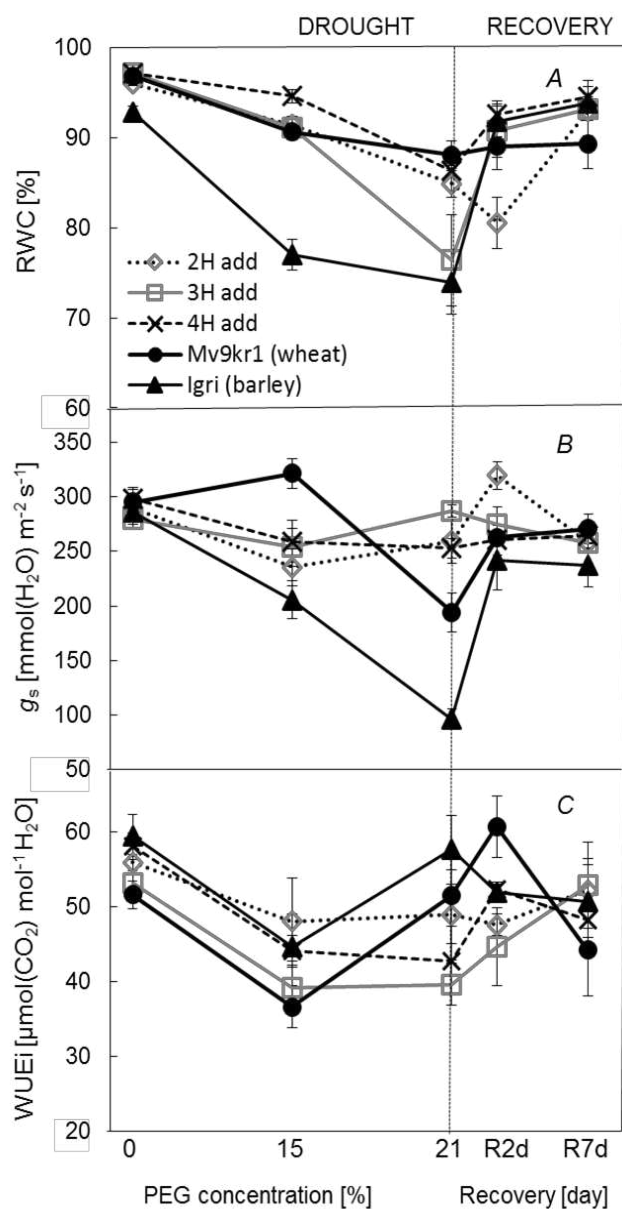
Figure 1. Effects of increasing osmotic stress followed by 7 days of regeneration on relative water content (RWC) (A), stomatal conductance (g_s) (B), intrinsic water-use-efficiency (WUE$_i$) (C) under 1,000 μmol (photon) m^{-2} s^{-1} light intensity. Vertical bars represent ± SD.
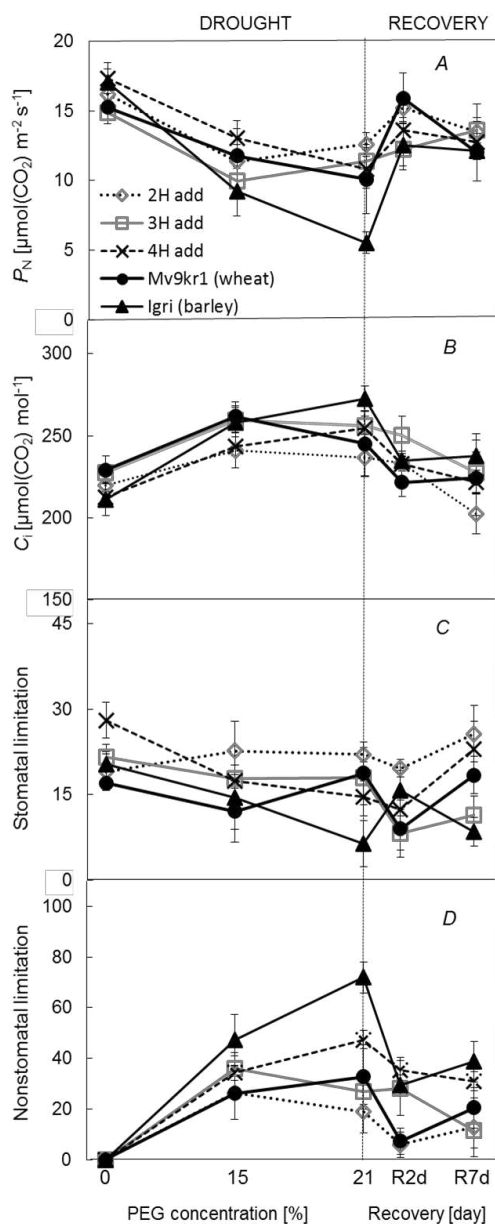
Figure 2. Effects of increasing osmotic stress followed by 7 days of regeneration on net photosynthetic rate (P_N) (A), intercellular CO_2 concentration (C_i) (B), stomatal limitation (L_s) (C), nonstomatal limitation (L_{ns}) (D) under 1,000 μmol (photon) m^{-2} s^{-1} light. Vertical bars represent ± SD.
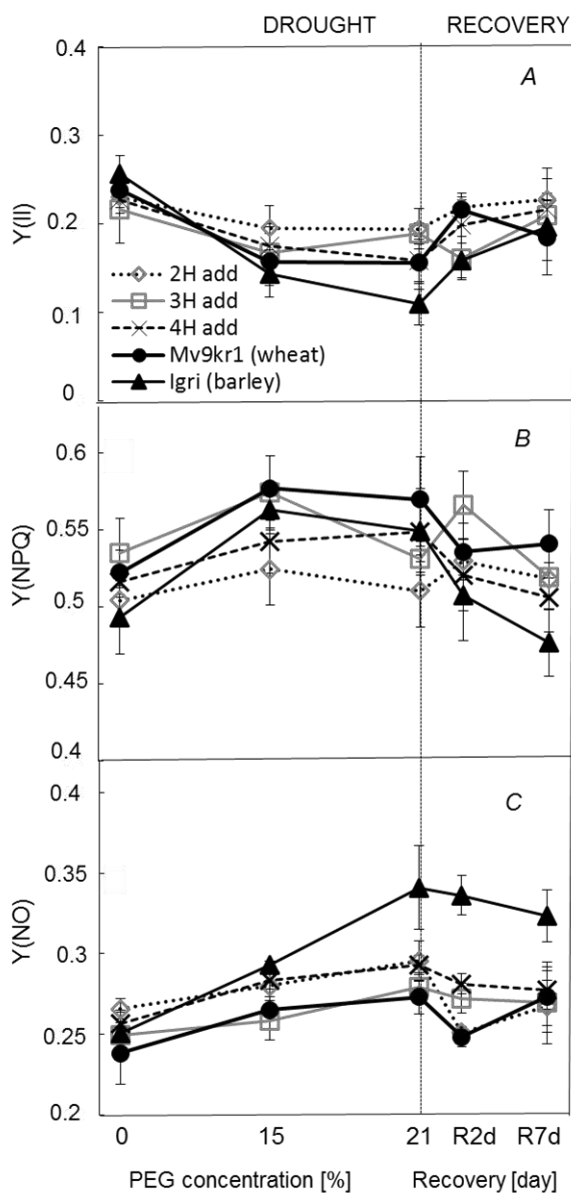
Figure 3. Effects of increasing osmotic stress followed by 7 days of regeneration on effective quantum yield of PS II photochemistry (Y(II)) (A), quantum yield of regulated energy dissipation (Y(NPQ)) (B), quantum yield of non-regulated energy dissipation (Y(NO)) (C) under 1,000 μmol (photon) m^{-2} s^{-1} light intensity. Vertical bars represent ± SD.

Table 1. The shoot/root ratio and biomass production of root and shoot (g/plant) expressed in terms of dry matter for 21% PEG-treated (stress) and control plants of similar age grown in nutrition solution without PEG (control).

Genotypes	Root dry weight (g)		Shoot dry weight (g)		Shoot/root ratio	
	Control	Stress	Control	Stress	Control	Stress
2H, Mv9kr1-Igri addition	0,047±0,031	0,032±0,014 (68,08%)	0,13±0,020	0,102±0,031 (78,46%)	2,766	3,187
3H, Mv9kr1-Igri addition	0,034±0,022	0,023±0,009 (67,64%)	0,074±0,017	0,069±0,019 (93,24%)	2,176	3,0
4H, Mv9kr1-Igri addition	0,026±0,011	0,021±0,013 (80,77%)	0,078±0,021	0,062±0,015 (79,49%)	3,0	2,952
Mv9kr1 (wheat)	0,069±0,04	0,0609±0,029 (88,40%)	0,294±0,108	0,258±0,079 (87,75%)	4,261	4,233
Igri (barley)	0,034±0,018	0,032±0,010 (94,11%)	0,170±0,083	0,120±0,033 (70,59%)	5,0	3,750

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UNDERSTORY FOLIAGE COVER IN AN HUNGARIAN OAK FOREST IN 2012

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Abstract: The serious oak decline was reported for the 1979–80 period and 63.0% of adult oaks died in a mixed oak forest in the Síkfőkút site, in the north-eastern part of Hungary. The data were used to obtain (1) quantitative information on shrub canopy, including foliage cover percentage of the shrub layer, mean cover of shrub species in 2012; (2) structural information on shrub foliage arrangement and (3) true description from the present ecological condition of the subcanopy layer. In the monitoring plot, the foliage cover condition of specimens only above 8.0 m was observed in 2012. The data relating to the projected foliage areas of high shrubs have been evaluated and foliage map waas digitized in ArcGis. It was measured a very low value of the foliage cover. The shrub layer cover was 61.5%, the duplex- and multiplex was 13.1% and only 76.8% total foliage cover in the understorey. The mean cover of the shrub species changed between 0.5 m^2 and 6.4 m^2 in the high shrub layer. The maximum mean cover values were recorded by the dominant woody species (*Acer campestre*, *Acer tataricum* and *Cornus mas*) in the shrub community. The GIS analysis confirmed that total foliage cover of the subcanopy layer and of the higher dominant woody individuals was 522.4 m^2. Our results suggest that the foliage cover size of shrub layer was a very low in 2012 and was measured similar low mean cover values of shrub species.

Keywords: ArcGis, shrub layer, *Acer campestre*, mean cover, shrub foliage cover, georeferencing

INTRODUCTION

Numerous studies used different structural variables, biotic and abiotic indicators to long-term detect and monitor the forests health and dynamics (Jukola-Sulonen *et al.* 1990; Strand 1995; Wicklum and Davies 1995). Several biotic and abiotic factors have been considered as important in forest health studies, such as

extreme weather conditions, drought, storms, heat (Drobyshev *et al.* 2008; Bolte *et al.* 2010), and insect fluctuations (Moraal and Hilszczanski 2000), disease outbreaks (Mistretta 2002) or human induced influences such as climate change, air pollution and fires (Signell *et al.* 2005; Kabrick *et al.* 2008). These factors may modify the functioning of the whole forest ecosystem and may lead to tree decline events.

The tree decline has heavily affected oak species and especially *Quercus petraea* Matt. L. (sessile oak) trees in European countries and naturally in Central Europe (Freer-Smith and Read 1995; Führer 1998; Thomas and Büttner 1998). An increasing decline of oak forests has been observed in many regions of Hungary since 1978. The oak decline in Slovakia began in 1976, in former Yugoslavia in 1979 and finally reaching the eastern regions of Austria in 1984 (Hämmerli and Stadler 1989). The stand dynamics of oak forests in Europe has been a topic of interest and concern to resource managers and scientists in the last period. Many studies have examined the effects of tree decline on stand (especially on canopy layer) development but relatively few research papers deal with shrub community and shrub layer dynamics in the process of tree decline (Alaback and Herman 1988; Gracia *et al.* 2007; Gazol and Ibáñez 2009).

Different structural variables can be used as indicators of disturbance regime and management practices in forests (Zumeta and Ellefson 2000; Larsson and Danell 2001). These variables are among others cover. Open areas and tree species diversity had a positive relationship to shrub cover because a diverse overstorey generally created more canopy gaps (Gazol and Ibáñez 2009). Canopy layer stand structure strongly influences understorey cover by resources, altering microsites and environmental conditions by light availability (Alaback 1982; Oliver and Larsson 1996). Shrub cover is linked to habitat quality and a number of interconnected ecological processes (Carey 1995; Hagar *et al.* 1996).

The species composition of the canopy layer was stable until 1979 and the healthy density of *Q. petraea* and of *Quercus cerris* L. (Turkey oak) also remained constant in the study site. The massive dieback of oaks started in 1979–80 and 68.4% of *Q. petraea* trunks died until 2007. The oak decline of the sample site resulted in an opening of the canopy. The overall species composition of the canopy has changed little, only some trees of *Tilia cordata* Mill. and

Carpinus betulus L. lived as new species in the site. The results of Jakucs (1988) suggested that the soil acidification induced by disappearance of mycorrhiza fungi and the air pollutants that promote water and nutrient absorption have been considered and identified as primary causes of deciduous forest decline.

Most papers only used changes in structural conditions in the tree layer to monitor the ecological process in the forest community after tree decline (Bussotti and Ferretti 1998; Brown and Allen-Diaz 2009). The studies of shrub species performed have mostly focused on the static population structure (age and size structures) (Tappeiner *et al.* 1991; Stalter *et al.* 1997). Other studies have focused on the cover and diversity of shrubs (Kerns and Ohmann 2004; Gracia *et al.* 2007). The decreased oak tree density led to numerous significant structural changes in the understorey such as herbaceous layer and shrub layer. The objectives of this study were to (1) give quantitative information on shrub canopy, including foliage cover percentage of the shrub layer, mean cover of shrub species in 2012; (2) describe structural information on shrub foliage arrangement and finally (3) give description from the present ecological condition of the subcanopy layer.

MATERIAL AND METHODS

Study site
The 27 ha reserve research site is located in the Bükk Mountains of northeast Hungary (47°55′ N, 20°46′ E) at a distance of 6 km from the city of Eger and at an altitude of 320–340 m a.s.l. The site was established in 1972 by Jakucs (1985) and has been used for the long-term study of forest ecosystems. The mean annual temperature is 9.9°C and mean annual precipitation typically ranges from 500 to 600 mm. The mean annual temperature and precipitation are based on measurements at the meteorological recording tower of the site. Descriptions of the geographic, climatic, and soil conditions, and vegetation of the forest were reported in detail by Jakucs (1985, 1988). Paper of Koncz *et al.* (2011) showed the seed bank and herbaceous vegetation condition of the forest. The most common forest association in this region is sessile oak and Turkey-oak forest community (*Quercetum petraeae-cerris*) with a dominant canopy of *Q. petraea* (sessile oak) and *Q. cerris*

(Turkey oak) deciduous tree species. Both oak species are important dominant native deciduous tree species of the Hungarian natural woodlands. Other codominant tree species in the site included *C. betulus*, *Prunus avium* L. and *T. cordata*. The plots under study were made up of evenly aged temperate deciduous forest trees that were at least 100 years old and had not been thinned for more than 50 years.

Sampling and statistical analysis
The necessary data of shrub layer condition has been collected at a definite period on a 48 m × 48 m monitoring plot. The permanent subplots (4 m × 4 m) were established in 1972. The data were obtained from a 24ha study site at regular intervals within the site. The investigations were performed during the growing season in 2012.

   Canopy trees were classified as sessile oak and Turkey-oak tree species > 13.0 m in height and ≥10.0 cm in diameter at 1.3 meters above ground (dbh). Shoot height and shoot diameter of the shrub individuals were the two main variables recorded. Specimens which were higher than 1.0 m were categorized as high shrub. The lower specimens were categorized as low shrub. Stems < 50.0 cm height of oak species were inventoried and identified to oak seedlings. The term "dominant woody" is used to refer to *A. campestre*, *A. tataricum* and *C. mas* species that play a key role in an understorey based on the high densities and largest mean sizes for the shrub community.

   The following measurements were carried out in each subplot: was detected foliage cover percentage and mean cover condition of high shrub individuals. In 2012 were sampled 486 high shrub individuals and then subjected to the mean cover analysis of shrub species. Box plot method used for graphically depicting groups of shrub's foliage cover data through their quartiles. Foliage cover, duplex- and multiplex cover (shrub canopy overlapped other shrub species) of the high shrub layer and total cover of the all high shrub individuals were also recorded. Location and cover of all high shrub specimens were mapped in each subplot. A projected foliage area cartogram of high shrubs was prepared using a method described in several studies (Jakucs 1985; Misik *et al.* 2007). Since 2012, data relating to the projected foliage areas of high shrubs have been evaluated and foliage map waas digitized in ArcGis. ArcGIS is a

geographic information system (GIS) for working with maps and geographic information in our study site. After the scanning, in the sample site was surveyed 24 pieces hand drawn segments and these segments were georeferencing on the basis of the cardinal gps coordinates to the EOV coordination system. On the basis of the delineated foliage margin on the georeferenced segments we vektorized all living individuals of the shrub species in the monitoring plot. Territorial and circumferential data belonged to the all shrub individuals. The total cover of the understorey was recorded on the basis of the contraction of the all polygon. The foliage cover overlapes of the shrub layer was determined by means of the "Intersect" ArcGIS tool. The distributed georeferring, digitalization and the actions of the geographical information system was making with the ArcMap 10.4 geographical information software. Based on the digitized map we estimated the foliage area of shrub species with the GIS environment.

RESULTS

In 2012, 11 native woody species were identified across the entire study area in the high shrub layer; *Acer campestre* L. (field maple), *Acer tataricum* L. (Tatar maple), *Cerasus avium* L. (wild cherry), *Cornus mas* L. (European cornel), *Cornus sanguinea* L. (common dogwood), *Crataegus monogyna* Jacq. (common hawthorn), *Euonymus europaeus* L. (common spindle), *Euonymus verrucosus* Scop. (warted spindle), *Ligustrum vulgare* L. (common privet), *Lonicera xylosteum* L. (fly honeysuckle) and *Tilia cordata* Mill. (small-leaved linden) were present as high shrub species in the sample site. It was determined the foliage cover sizes of these species.

In 2012 was measured a very low size of the foliage condition. The shrub layer cover was 61.48%, the duplex- and multiplex cover was 13.11% and was recorded only 76.83% total foliage cover in the understorey. This dataset was the lowest from the starting of the measurements (*Table 1*).

Table 1. Foliage cover condition in the understorey shrub layer in 2012 and between 1972-2007 period in Síkfőkút.

year	foliage cover (%)	duplex and multiplex cover (%)	total cover (%)
2012	61.48	13.11	76.83
1972-2007	77.57	41.47	129.53
mean	69.53	27.29	103.18

The mean cover of the shrub species individuals changed between 0.54 m² and 6.39 m² in the high shrub layer. It was measured 4.29-6.39 m² mean foliage cover by the dominant woody species of the shrub community. The highest cover values were detected for a single *C. avium* and single *T. cordata* tree species with 12.98 m² and with 11.12 m² under the oak canopy layer (*Table 2*).

Table 2. Mean foliage cover (±S.D.) of the species in the understorey shrub layer in 2012.

species	measured individuals number	cover (m²)	S.D.
Acer campestre	133	6.12	6.10
Acer tataricum	22	4.29	3.24
Cerasus avium	1	12.98	0.00
Cornus mas	85	6.39	4.52
Cornus sanguinea	29	0.54	0.65
Crataegus monogyna	47	2.19	2.19
Euonymus europaeus	10	1.42	1.62
Euonymus verrucosus	142	1.06	1.15
Ligustrum vulgare	14	0.73	1.72
Lonicera xylosteum	2	0.87	0.68
Tilia cordata	1	11.12	0.00

Box plot showed the cover distribution of high shrub species individuals in 2012. The median cover value of *C. mas* was the maximum; the lowest values were recorded by *C. sanguinea, E. verrucosus* and *L. vulgare*. The maximum standard deviation of cover values were detected by *A. campestre* individuals. Many field maples reached the tree size; the cover dataset was confirmed this statement (*Figure 1*).
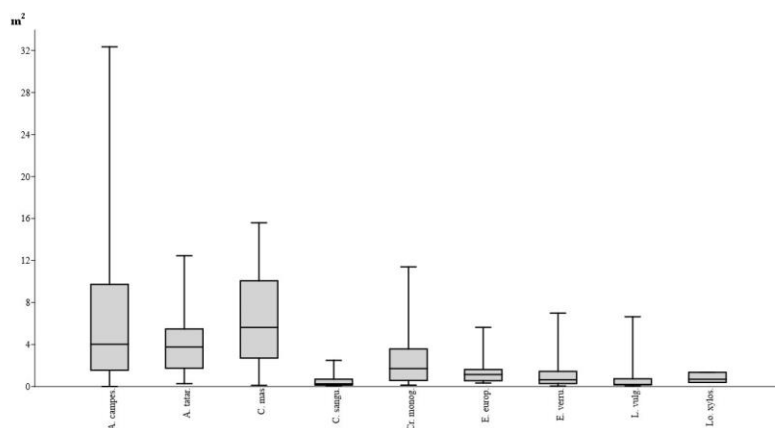
Figure 1. Statistical summary of cover sizes distribution of high shrub species on the monitoring plot in 2012. Boxes shown are the 25–75% percentile, median, minimum and maximum values (N = 486). (Notation: A. campes. = *Acer campestre*; A. tatar. = *Acer tataricum*; C. mas = *Cornus mas*; C. sangu. = *Cornus sanguinea*; Cr. monog. = *Crataegus monogyna*; E. europ. = *Euonymus europaeus*; E. verru. = *Euonymus verrucosus*; L. vulg. = *Ligustrum vulgare*; Lo. xylos. = *Lonicera xylosteum*)

In the subcanopy layer (between 8.0-13.0 m), and above this layer 3 dominant woody species were identified across the entire study area: *A. campestre*, *A. tataricum* and *C. mas*. The field maple was the dominant species with 53 individuals in the subcanopy and tree layer of the study site. The GIS analysis confirmed that total foliage cover of the subcanopy layer and of the higher dominant woody individuals was 522.38 m². Mean cover of *A. campestre* individuals was 8.26 m² in the subcanopy and tree layer; it was measured 3.47 m² and 11.61 m² mean foliage cover values by *A. tataricum* and *C. mas* individuals. The *C. mas* individuals has got the biggest mean foliage cover in the subcanopy layer. Above the 8.0 m height limit, the field maple is the dominant species, because nearly 84.0% percent of the total cover has got own individuals. It was found single *C. avium* and single *T. cordata* tree individuals in the subcanopy (*Table 3*).

Table 3. Mean (±S.D.) and total foliage cover of the woody and tree species individuals in the subcanopy layer in 2012.

species	mean cover (m^2)	S.D.	total cover (m^2)	density (ind. ha^{-1})
Acer campestre	8.26	6.22	437.97	230
Acer tataricum	3.47	2.21	13.89	17
Cerasus avium	12.98	0.00	12.98	4
Cornus mas	11.61	5.59	46.42	17
Tilia cordata	11.12	0.00	11.12	4
sum	-	**-**	**522.38**	**272**

DISCUSSION

Nowińska (2010) examined the ecological impact of canopy and canopy gaps on the development of lower forest layers in five protected phytocoenoses of oak-hornbeam habitats (natural and regeneration stands) and oak-pine habitats in the Wielkopolska National Park of Western Poland. Foliage gaps do not affect bryophyte richness and cover. The impact of gaps on the herb layer is not uniform for the investigated forests. Gaps often, though not in all cases, cause a significant increase in the total herb layer cover, vascular antrophophyte cover, vascular species richness and a greater Shannon diversity. In our site In the study of Gracia *et al.* (2007) the relationship between shrubs cover and basal area of overstory was only significant for two species, and in both two cases, cover decreased with basal area. Other studies have shown negative correlations of understory cover with canopy trees basal area (Gilliam and Turrill 1993) presumably because of differential light availability.

Tree species influenced shrub, herb and moss cover size (Klinka *et al.* 1996). Results from Kerns and Ohmann (2004) suggest that forest community structure, stand development, site disturbance history and environment all interact to influence shrub layer cover in Oregon landscape. Shrub cover was negatively correlated with *Tsuga heterophylla* basal area and density of shade tolerant tree individuals. Total shrub cover increased significantly ($P < 0.001$) with time since canopy gaps. Furthermore, the increase in total species foliage cover was significantly more pronounced in mature than old-growth stands of Canada (De Grandpré *et al.* 2011). Total

cover of the understory shrub layer increased slightly, but nonsignificantly in a mature oak forest of USA, from 45.0% in 1950 to 51.0% in 1969 and 1979.

Misik *et al.* (2013) reported that in the studied oak forest the shrub layer foliage cover increased remarkably from 1972 to 1988; since 1993 onwards there has not been a clear tendency in the change of the shrub layer cover. No significant differences were revealed between oak tree density and duplex- and multiplex cover in the shrub community (P > 0.05). *A. campestre* individuals showed a significant mean percent cover increase (P ≤ 0.05) after serious oak decline. Besides than higher foliage cover was recorded by *C. mas* and *A. tataricum* species 6.4 and 4.5 m^2 mean cover. In our present study the cover size in the understory showed an important decreasing in the last five years. The most important reason of the foliage decrease was the weather condition, because the summer was very hot and extreme dry in 2012 (Sippel and Otto 2014).

CONCLUSIONS

In 2012 was measured a very low size of the foliage condition. The shrub layer's cover size dataset was the lowest from the starting of the measurements, from 1972. The mean cover of the shrub species individuals changed between 0.54 m^2 and 6.39 m^2 in the high shrub layer. The dominant woody species of the shrub community have got the maximum mean foliage cover with 4.29-6.39 m^2 values. The median cover value of *C. mas* was the maximum; the lowest values were recorded by *C. sanguinea*, *E. verrucosus* and *L. vulgare* species individuals. The maximum standard deviation of cover values were detected by *A. campestre* individuals and many field maples reached the tree size. The GIS analysis confirmed that total foliage cover of the subcanopy layer and of the higher dominant woody individuals was 522.38 m^2. The cover dataset in the shrub community was a very low in 2012 and showed considerably decreasing compared with the last three decades cover values.

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BRYOFLORISTICAL DATA FROM THE APUSENI MOUNTAINS (ROMANIAN WESTERN CARPATHIANS, TRANSILVANIA)

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Abstract: The main aim of this study was to explore the bryophyte diversity and distribution patterns in the Apuseni Mountains. From our collections hitherto 108 bryophyte species were identified. The 31 Marchantiophyta and 77 Bryophyta species belong to 74 genera of 40 families. Among them the endangered *Campylium protensum, Didymodon cordatus* and the critically endangered *Ditrichum gracile* and *Schistidium robustum* are to be mentioned.

**Keywords:** bryoflora, rare species, Apuseni Mts., Romania

INTRODUCTION

The bryological research of the climatologically and geologically very variable and biologically very rich Apuseni Mountains started at the beginning of XXth century but till now its investigation is far from complete (Győrffy 1903; Péterfi 1908, 1910; Boros 1942a, 1942b, 1951; Páll 1960, 1962, 1963; Colectiv 1966; Boros and Vajda 1967, 1974; Ştefureac 1975, 1977; Plămadă and Goia 1994). Investigations have continued intensively in the past twenty years (Goia 2001, 2005; Goia and Mătase 2001; Jakab 1999, 2000; Goia and Schumaker 2000, 2002, 2003a, 2003b, 2004; Goia and Ştefănuţ 2004; Plămadă *et al.* 2000; Lüth 2002, and others). The enumeration below shows, that many places are still unexplored and still there are just a few data from the Codru-Moma Mountains (Mohan 1996, Ardelean 2006) and Pădurea Craiului (Raţiu and Boşcaiu 1967). Csűrös (1981) gave a wide overview of the natural conditions and of Apuseni Mountains and its flora and fauna. Touristic travelers find a professional guide in Mátyás (1988). We started our intensive bryological exploration in 2002 and published a series of papers from this area, some of them as result of the

fruitful cooperation with Irina Goia, bryologist at the Babeş-Bolyai University (Orbán and Sass-Gyarmati 2003, Pócs 2005, Pócs *et al.* 2002, Sass-Gyarmati *et al.* 2005a, 2005b, Sass-Gyarmati *et al.* 2008a, 2008b).

Study area
The Bihor Mountains occupy a central position within the Apuseni mountains range (*Plate I. photo 1*). The characteristic karstic topography is widely developed, parallel to places with other sedimentary and volcanic bedrocks. The Bihar Massif is the highest part of the Apuseni Mountains, part of the western Carphatians. It is roughly 25 km long from northwest to southeast and 14 km wide. The summit is gently sloping, broken by a few peaks and carved out by two glacial valleys. Cucurbăta Mare (1849m) is the highest point. Gârda Seacă valley is a narrow canyon with up to 250m high walls cut into the limestone bedrock. Coteţul Dobreştilor spring is the main outlet of the Ocoale-Coteţul Dobreştilor aquifer in the Gârda Seacă valley. The Gârda-Ocoale area almost entirely consists of sedimentary deposits ascribed to the Bihor Unit (Orăşeanu *et al.* 2007). The Pădurea Craiului group are in the northwestern part, while the Codru-Moma massif are the southwestern part of the Apuseni Mountains (*Figure 1*).
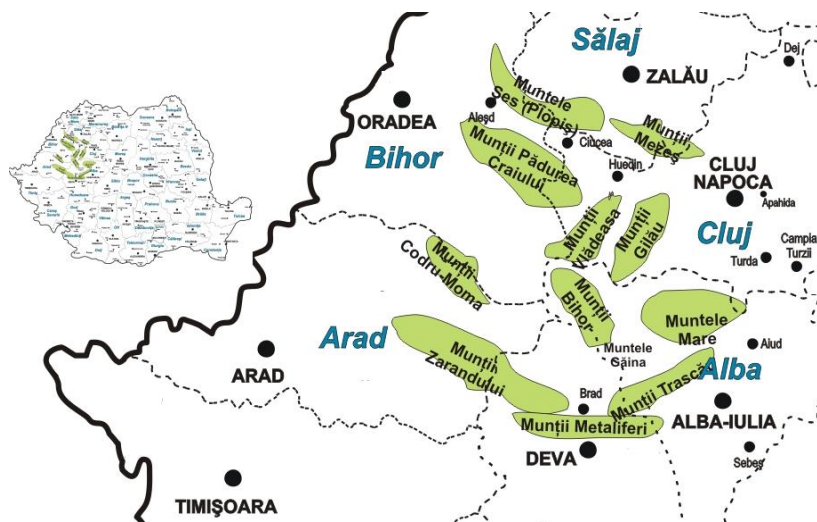
Figure. 1. Detailed map of the investigated area

MATERIAL AND METHODS

Authors visited the Apuseni Mountains between 22-27 June 2006. The collection was made in the various vegetation types: bogs, meadows, beech and spruce forests and subalpine belts. The specimens were collected by Tamás and Sarolta Pócs, Andrea Sass-Gyarmati, András Vojtkó and identified by Tamás Pócs and Andrea Sass-Gyarmati. The Romanian distribution of mosses was established from Plămadă (1998) and Mohan (1998), while that of the liverworts from Ştefănuţ (2008). The nomenclature of liverworts follows Ştefănuţ (2008), modified by Söderström *et al.* (2016), nomenclature of mosses follows Hill *et al.* (2006). The classification of liverworts (Marchantiophyta) follows Söderström *et al.* (2016), while the classification of mosses (Bryophyta) follows Goffinet and Shaw (2009). The species in each family are arranged in alphabetical order. Species names are followed by the collecting site number, and by the substrate on which they were grown. The collected specimens are deposited in the Herbarium of Eger (EGR). The list of collecting sites are listed in the Appendix.

RESULTS

List of species
During the field study, 108 bryophyte species were found in the investigated area. The 31 Marchantiophyta and 77 Bryophyta species belong to 74 genera of 40 families.

Marchantiophyta
Pelliaceae
Pellia endiviifolia (Dicks.) Dumort. – 1, 6: on soil

Conocephalaceae
Conocephalum conicum (L.) Dumort. – 2: on irrigated rocks

Anastrophyllaceae
Barbilophozia lycopodioides (Wallr.) Loeske – 10: on granitic
    rocks

Barbilophozia sudetica (Nees ex Huebener) L.Söderstr. – 10: on rocks

Mesoptychia bantriensis (Hook.) L.Söderstr. et Váňa – 5: on limestone rocks

Sphenolobus minutus (Schreb. ex D.Crantz) Berggr. – 10: on granitic rocks

Lepidoziaceae
Bazzania trilobata (L.) Gray. – 21: on soil

Calypogeiaceae
Calypogeia muelleriana (Schiffn.) Mül. Frib. – 11: on soil

Cephaloziaceae
Cephalozia bicuspidata (L.) Dumort. – 11: on transitional bog
Cephalozia pleniceps (Austin) Lindb. – 18: on vertical cliff

Scapaniaceae

Diplophyllum albicans (L.) Dumort. – 1, 2: on rocks
Diplophyllum taxifolium (Wahlenb.) Dumort. – 10: on granitic rock
Scapania aequiloba (Schwägr.) Dumort. – 5: on rocks
Scapania aspera H. Bernet et M. Bernet – 5: on rocks
Scapania undulata (L.) Dumort. –1, 2, 11: on irrigated rocks

Jubulaceae
Frullania dilatata (L.) Dumort. – 1, 3, 16, 18: on bark

Jungermanniaceae
Jungermannia atrovirens Dumort. – 1: on rocks
Jungermannia hyalina Lyell – 1: on soil
Syzygiella autumnalis (DC.) K.Feldberg, Váňa, Hentschel et Heinrichs – 1: on rocks
Tritomaria exsectiformis (Breidl.) Loeske – 1: on rocks
Tritomaria exsecta (Schmidel) Schiffn. ex Loeske – 2: on irrigated rocks
Leiocolea badensis Jörg. – 4: on limestone rocks

Lophocoleaceae

Lophocolea heterophylla (Schrad.) Dumort. – 3: on decaying log

Plagiochilaceae
Pedinophyllum interruptum (Nees) Kaal. – 1, 4: on rocks
Plagiochila porelloides (Torr. ex Nees) Lindenb. – 2: on irrigated rocks; 5: on soil

Lejeuneaceae
Cololejeunea calcarea (Lib.) Schiffn. – 18: on rocks
Lejeunea cavifolia (Ehrh.) Lindb. – 1, 2: on irrigated rocks; 10: on rocks; 16: bark of *Carpinus*
Radulaceae
Radula complanata (L.) Dumort. – 1, 3: bark of *Acer* sp.; 7: bark of *Viburnum lantana;* 17: bark of *Fagus*; 16, 18: on bark

Metzgeriaceae
Metzgeria conjugata Lindb. – 2: on irrigated rocks; 18: on bark
Metzgeria furcata (L.) Dumort. – 16: bark of *Carpinus*; 21: bark of *Fagus*
Metzgeria violacea (Ach.) Dumort. – 7: bark of *Viburnum*; 18: bark of *Acer campestre*

Bryophyta
Sphagnaceae
Sphagnum capillifolium (Ehrh.) Hedw. – 11: on turf
Sphagnum centrale C.E.O. Jensen – 11: on turf
Sphagnum magellanicum Brid. – 11: on turf
Sphagum russowii Warnst. – 10, 11: on turf

Andreaeaceae
Andreaea alpestris (Thed.) Schimp. – 10: on rocks; 11: on irrigated rocks
Andreaea rupestris Hedw. var. *rupestris* – 10: on rocks

Polytrichaceae
Pogonatum urnigerum (Hedw.) P. Beauv. – 9: on earth covered rocks
Polytrichastrum alpinum (Hedw.) G. L. Sm. – 10, 11: on rocks
Polytrichastrum longisetum (Sw. ex Brid.) G. L. Sm. – 1: on soil

Polytrichum piliferum Hedw. – 9: on earth covered rocks; 10: on soil

Encalyptaceae

Encalypta streptocarpa Hedw. – 4: on limestone rock
Encalypta rhaptocarpa Schwaegr. – 13: on soil
Encalypta vulgaris Hedw. – 3: on rocks

Grimmiaceae

Racomitrium sudeticum (Funck) Bruch. & Schimp. – 9, 11: on rocks
Schistidium apocarpum (Hedw.) Bruch & Schimp. – 4: on rocks
Schistidium robustum (Nees & Hornsch.) H. H. Blom – 1: on stonebridge

Seligeriaceae

Seligeria acutifolia Lindb. – 18: on vertical cliff
Seligeria patula (Lindb.) I. Hagen – 7: on vertical cliff
Seligeria pusilla (Hedw.) Bruch & Schimp. – 13: on earth covered rocks

Fissidentaceae

Fissidens adianthoides Hedw. – 17: on rocks
Fissidens crispus Mont. – 13: on rocks
Fissidens dubius P. Beauv. – 4, 5: on limestone rocks

Dicranaceae

Dicranella heteromalla (Hedw.) Schimp. – 1: lignicolous; 9: on earth covered soil
Dicranum scoparium Hedw. – 1: on bark; 6, 17: on soil
Dicranum montanum Hedw. – 1: on bark
Paraleucobryum longifolium (Hedw.) Loeske – 1: on soil

Ditrichaceae

Ceratodon purpureus (Hedw.) Brid. – 9: on soil
Ditrichum gracile (Mitt.) Kuntze – 4: on rocks; 5: on soil

Rhabdoweisiaceae

Dichodontium palustre (Dicks.) M. Stech. – 8, 12: on irrigated rocks
Rhabdoweisia fugax (Hedw.) Bruch & Schimp. – 10: on rocks

Pottiaceae
Barbula convoluta Hedw. – 5, 13: on earth covered soil
Didymodon cordatus Jur. – 3: on vertical cliff
Didymodon tophaceus (Brid.) Lisa – 4: on rocks
Tortula muralis Hedw. – 20: on rocks
Tortella tortuosa (Hedw.) Limpr. – 3, 5, 17: on rocks
Weissia sp. Hedw. – 1: on rocks (without capsules)

Cinclidotaceae
Cinclidotus aquaticus (Hedw.) Bruch & Schimp. – 7: on submerged
    limestone rock
Cinclidotus fontinaloides (Hedw.) P. Beauv. – 7, 14: on irrigated
    limestone rock

Orthotrichaceae
Orthotrichum diaphanum Schrad. ex Brid. – 3: bark of *Salix*
Orthotrichum lyellii Hook. & Taylor – 3: bark of *Salix*
Orthotrichum obtusifolium Brid. – 3: bark of *Acer pseudoplatanus*
Orthotrichum speciosum Nees –19: bark of *Salix*
Ulota crispa (Hedw.) Brid. – 16: on bark; 19: bark of *Salix caprea*;
    21: bark of *Fagus*

Bartramiaceae
Bartramia halleriana Hedw. – 1, 11: on rocks
Bartramia pomiformis Hedw. – 4, 5: on rocks
Philonotis fontana (Hedw.) Brid. – 6: on marshy meadow
Plagiopus oederianus (Sw.) H. A. Crum & L. E. Anderson – 5: on
    rocks

Bryaceae
Bryum capillare Hedw. var. **capillare** – 13: on limestone rocks
Bryum elegans Nees – 13: on rocks
Bryum pseudotriquetrum (Hedw.) P. Gaertn. – 1: on irrigated rocks
Bryum rubens Mitt. – 5: on rocks
Mniaceae
Mnium lycopodioides Schwaegr. – 1, 4: on limestone rocks
Plagiomnium undulatum (Hedw.) T. J. Kop. – 1, 6: on earth covered
    rocks
Pohlia nutans (Hedw.) Lindb. – 9, 10: on earth covered rocks

Pohlia wahlenbergii (F. Weber & D. Mohr.) A. L. Andrews – 2, 10: on rocks

Rhizomnium punctatum (Hedw.) T. J. Kop. – 1, 2: on irrigated rocks

Amlystegiaceae
Campylium protensum (Brid.) Kindb. – 6: on marshy meadow

Hylocomiaceae
Hylocomium splendens (Hedw.) Schimp. – 1, 2: on rocks
Pleurozium schreberi (Willd. ex Brid.) Mitt. – 11: on soil

Leskeaceae
Pseudoleskeella catenulata (Brid. ex Schrad.) Kindb. – 7, 13: on rocks

Thuidiaceae
Abietinella abietina (Hedw.) M. Fleisch. – 16: on soil
Thuidium delicatulum (Hedw.) Schimp. – 4, 13: on rocks
Thuidium tamariscinum (Hedw.) Schimp. – 2: on rocks

Brachytheciaceae
Eurhynchium striatum (Hedw.) Schimp. – 6: on marshy meadow
Homalothecium sericeum (Hedw.) Schimp. – 13: on limestone rock

Plagiotheciaceae
Plagiothecium denticulatum (Hedw.) Schimp. – 5: on rocks
Climaciaceae
Climacium dendroides (Hedw.) F. Weber & D. Mohr – 6: on soil

Hypnaceae
Campylophyllum halleri (Hedw.) M. Fleisch. – 5: on vertical cliff
Ctenidium molluscum (Hedw.) Mitt. – 4: on rocks
Hypnum cupressiforme Hedw. – 20: on rocks
Hygrohypnum luridum (Hedw.) Jenn. – 5: on irrigated rocks
Orthothecium rufescens (Dicks. ex Brid.) Schimp. – 5: on rocks (*Plate I, photo 3*)
Ptilium crista-castrensis (Hedw.) De Not. – 5, 17: on rocks

Neckeraceae
Neckera crispa Hedw. – 4: on limestone rock

Neckera complanata (Hedw.) Hüb. – 1: on bark; 5: bark of *Acer*; 19: on rocks

Thamnobryum alopecurum (Hedw.) Gangulee – 15: on rocks

Anomodontaceae

Anomodon viticulosus (Hedw.) Hook & Taylor – 13: on limestone rock

DISCUSSION

The results of this study suggest that the bryophyte diversity is quite high in the Apuseni Mountains. The main reason for that can be the several habitat types that can be found in this area.

Metzgeria violacea (Ach.) Dumort. – (*Plate I, photo 5*) – is an oceanic („atlantic") geographical element – vulnerable (Ştefănuţ and Goia 2012) in Romania. It is known from Cobleş Valley, Arieşeni (Goia 2001, Goia and Ştefănuţ 2004, Goia and Schumaker 2004). We reported it from several localities from the Bihor and Pădurea Craiului Mountains as result of our collection trip. (Pócs 2006 as *M. temperata*, Sass-Gyarmati *et al.* 2007, 2008a, 2008b). The species was known earlier from Romania in the Cindrel Mts. (Boros and Vajda 1967 as *M. fruticulosa*, Gündisch 1977, Ştefureac 1986, Drăgulescu 2003).

Cephalozia pleniceps (Austin) Lindb. – rare liverwort from Bihor Mts. known only from Galbena valley and Padiş (Jakab 2000). According to Ştefănuţ (2008) it is distributed both in the Eastern and Southern Carpathians.

Leiocolea badensis Jörg. – known only from two localities from Bihor Mountains: Vadu Crişului, Casa Zmăului (Boros 1942a, b; Boros 1951) and Şuncuiuş (Boros 1942b, 1951). Rare species distributed just in a few localities in Romania: Harghita County – Topliţa; Bucegi Massif – Bucşoiu Peak, Retezat Mountains, toward Custura Peak and Argeş County - Great Gorges of Dâmboviţa River (Ştefănuţ 2008).

Lophozia sudetica (Nees ex Huebener) Grolle – it is known only from two localities – Galbena Valley (Goia 2001) and Arieşul Mare Basin (Goia and Schumacker 2002). We have collected it on the rocks from the Cucurbăta Mare peak. Rare species distributed in Rodna, Căliman, Ciucaş, Făgăraş Mountains, Bucegi Massif, Cozia Mountain and Sibiu County: Cisnădioara and Sadu Valley (Ştefănuţ 2008).

Cinclidous aquaticus (Hedw.) Bruch & Schimp. **–** has been indicated as vulnerable species – we have found it on permanently irrigated rocks at Izvorul Coteţul Dobreştilor. *C. fontinaloides* (Hedw.) P. Beauv. is a relatively rare species in Bihor Mountains, occurring on temporary irrigated rocks. (*Plate I, photo 4*). Both species have scattered distribution in the Romanian Carpathians.

Seligera acutifolia (Lindb.) Broth. (*Plate I, photo 2*) – is a mainly sub-Mediterranean and montane taxon (Düll 1984). It is scattered throughout Europe. Besides on Sicily it has also been found on the Italian mainland, in Belgium, (former) Czechoslovakia, France, Germany, Great Britain and Greece, in Ireland, Norway, Poland, Romania, Sweden and former Yugoslavia (Düll 1985). It occurs in limestone rock crevices, data deficient (DD) in Romania (Ştefănuţ and Goia 2012). We have found it on a shaded limestone rocks at Poarta lui Ionel Cave in the Ordâncuşa Valley.

Seligeria patula (Lindb.) Broth. – is an Alpine-Carpathian species, it was observed at several other places in the Bihor Mts., as in Ordâncuşa Valley, Galbena gorge, at Barsa ice cave, and from Boghii Valley, near the Oşelu waterfall (Pócs 2006). *Seligeria patula*, similarly to *S. tristicha*, is always embedded in a thin or thick layer of cryptobiotic crust dominated by cyanobacteria.

Ditrichum gracile (Mitt.) Kuntze (syn.: *D. crispatissimum* C. Müll. Hall.) Paris **–** a critically endangered species which requires constant humidity, *D. flexicaule* is more drought resistant (Casas *et al.* 1990). As it was not distinguished for long time from *D. flexicaule*, therefore its Romanian distribution should be investigated.

Mnium lycopodioides Schwaegr. – vulnerable species, so far it was known only from a single locality in the Bihor Mountains – Stâna de Vale (Péterfi 1908). Based on Mohan (1998) it is distributed in a few localities in the Retezat and Bucegi Mountains, Strunzinu in Maramureş and Codrul secular Slătioara in Jud. Suceava.

Didymodon cordatus Jur. – endangered species, a member of the southern-temperate European element, it is widespread in southern Europe, extending N to Germany and Belgium and E to the Caucasus and Turkey. According to Mohan it occurs only in Vidra in the Munţii Apuseni, furthermore on Cetate Deva and at Lapuş in Maramureş.

Campylium protensum (Brid.) Kindb. – is an endangered species in Romania, many bryfloristical works did not separated it from its very close relative and more frequent *C. stellatum*. The high red list category of *C. protensum* should be reevaluated after taxonomical revision of herbarium specimens and future fieldworks, to provide the reliable distribution.

Schistidium robustum (Nees & Hornsch.) Blom – critically endangered species in Romania (Ştefănuţ and Goia 2012), which is communicated in the country only from two localities: Mt. Rarău in the Eastern and Dâmbovicioara (Piatra Craiului Mts.) in the Southern Carpathians (Blom 1996). It is a calciphilous element known from the higher mountains and boreal parts of Europe and North America, from the Yukon area to the Caucasus Mountains.

These records well reflect the multilateral character of the flora of Apuseni Mountains (see also Ştefureac 1975). Csergő (2002) has written an interesting study on the possible places of preglacial, interglacial and glacial relic populations of calciphilous flora in Apuseni Mountains. She emphasized the importance of peatlands, sheltered valleys, gorges, rockslides, rock faces and screes, as refugium areas, where relic populations could survive. We definitely should add to these the caves and cave entrances, with their special microclimate (very steady temperature, continuous high air moisture and limited light conditions). The steady temperature seems to be very cool in summertime, approaching the annual mean temperature, but this has great significance in wintertime, when in many caves frost does not occur. This seems to

ensure the life possibilities of Atlantic and Mediterranean elements, both plants and animals. (It is well known, that the caves in Apuseni Mountains have an invertebrate fauna very rich in endemics). These findings should enhance the knowledge of bryoflora, our results emphasizes the importance of further research in this highly valuable area.
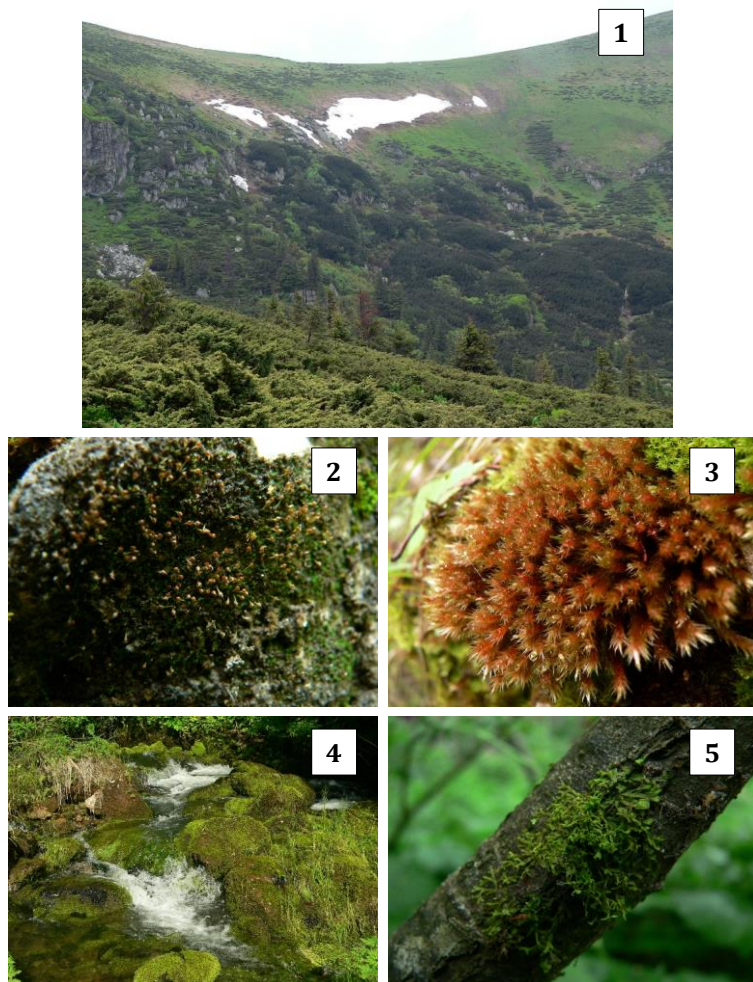
Plate I. Photo 1. View from the Cucurbăta Mare summit**. Photo 2.** *Seligeria acutifolia* (Lindb.) Broth. **Photo 3.** *Orthothecium rufescens* (Brid.) B.S.G. **Photo 4**. *Cinclidotus aquaticus* (Hedw.) Bruch & Schimp. and *C. fontinaloides* (Hedw.) P. Beauv. **Photo 5.** *Metzgeria violacea* (Ach.) Dumort.

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APPENDIX

List of collecting sites from the Apuseni Mts. (Nyugati Szigethegység)

1. Munţii Bihorului (Bihar Hegység), Headwaters of Crişul Negru (Fekete Körös) River on the NW slope of Vf. Cucurbăta Mare (Nagy Bihar csúcs), at 1050 m alt. N 46°26'53.2", E 22°40'06.8" Hab.: *Abieti-Fagetum* on schist bedrock. Coll.: S. & T. Pócs, A. S.-Gyarmati. Date: 22.06.2006. No. 06025

2. Munţii Bihorului (Bihar-hegység), Headwaters of Crişul Negru (Fekete-Körös) river on the NW slope of Vf. Cucurbăta Mare (Nagy Bihar csúcs), below the Amazon Falls, at 946 m alt. N 46°27'08.9", E 22°39'54.1" Hab.: Shady gorge with *Alnus viridis* bushes. Coll.: S. & T. Pócs, A. S.-Gyarmati. Date: 22.06.2006. No. 06026

3. Munţii Bihorului (Bihar-hegység), 1.5 km NW of Gârda de Sus village near Dobreşti, in the Gârda Seacă Valley at 765 m alt. N 46°28'36.1", E 22°48'43.8". Hab.: Riverine *Salix* bush and calcareous cliffs. Coll.: S. & T. Pócs, A. S.-Gyarmati. Date: 23.06.2006. No. 06027

4. Munţii Bihorului (Bihar-hegység), Gârda Seacă Valley, 6 km NW of Gârda de Sus village, at 816-825 m alt. N 46°29'42.6", E 22°47'12.9". Hab.: E facing limestone cliff in the river gorge. Coll.: S. & T. Pócs, A. S.-Gyarmati. Date: 23.06.2006. No. 06028

5. Munţii Bihorului (Bihar-hegység), Gârda Seacă Valley, 7 km NW of Gârda de Sus village, at 840 m alt. on the E foot of Dosul Hodobanii. N 46°29'50.8", E 22°47'03.4". Hab.: E facing limestone cliff in the river gorge, covered partly by *Seslerietum rigidae* sward. Coll.: S. & T. Pócs, A. S.-Gyarmati. Date: 23.06.2006. No. 06029

6. Munţii Bihorului (Bihar-hegység), Gârda Seacă Valley, E of Hodobani village and of the Piatra Tăuzului summit, at 920 m alt. N 46°30'50", E 22°47'10.1". Hab.: Meadows, spring bog and *Abieti-Fagetum*. Coll.: S. & T. Pócs, A. S.-Gyarmati. Date: 23.06.2006. No. 06030

7. Munţii Bihorului (Bihar-hegység), Izbuc Coteţul Dobreştilor, 3 km NW of Gârda de Sus and 1 km from Dobreşti, in the Gârda Seacă Valley at 770 m alt. N 46°28'40.9", E 22°48'32.8". Hab.: In shady cliffs along streamlet. Coll.: S. & T. Pócs, A. S.-Gyarmati Date: 23.06.2006. No. 06031

8. Munţii Bihorului (Bihar-hegység), in the saddle between Vf. Cucurbăta Mică and Vf. Cucurbăta Mare (Kis és Nagy Bihar csúcsok), at 1690 m alt. N 46°26'07, E 22°41'. Hab.: In a snow valley, at the edge of melting snow, on dripping granitic rocks. Coll.: S. & T. Pócs, A. S.-Gyarmati Date: 24.06.2006. No. 06032

9. Munţii Bihorului (Bihar-hegység), on the summit ridge of Vf. Cucurbăta Mare (Nagy Bihar csúcs), at 1840 m alt. N 46°26'20", E 22°41'24.8". Hab.: Subalpine grassland and granitic scree. Coll.: S. & T. Pócs, A. S.-Gyarmati. Date: 24.06.2006. No. 06033

10. Munţii Bihorului (Bihar-hegység), on the NNE slope of the summit ridge of Vf. Cucurbăta Mare (Nagy Bihar csúcs), at 1830 m alt. N 46°26'20", E 22°41'24.8". Hab.: On granitic cliffs. Date: 24.06.2006. Coll.: T. Pócs, A. S.-Gyarmati and A. Vojtkó. No. 06034

11. Munţii Bihorului (Bihar-hegység), in the NE facing glacial valley (V. Cepilor) below Vf. Cucurbăta Mare (Nagy Bihar csúcs), at 1554 m alt. N 46°26'39.1", E

22°41′45.3″. Hab.: Subalpine dwarf pine (*Pinus mugo*) bush interwoven by streamlets and transitional bogs. Date: 24.06.2006. Coll.: T. Pócs, A. S.-Gyarmati and A. Vojtkó. No. 06035

12. Munţii Bihorului (Bihar-hegység), in Valea Cepilor 3 km NE of Vf. Cucurbăta Mare (Nagy-Bihar csúcs), at 1150 m. N 46°26′59.2″, E 22°42′33.03″. Hab.: On the irrigated stones of rivulet at the lower limit of spruce forest belt. Coll.: T. Pócs. Date: 24.06.2006. No. 06036

13. Pădurea Craiului (Király-erdő). Cheile Albioarei gorge in the valley of Roşia streamlet N of Ţarina village, 5-6 km WNW of Roşia, at 380m alt. N 46°50′26.3″, E 22°22′16.8″. Hab.: *Asplenio-Seslerietum rigidae* swards on limestone cliffs, with many pine trees and *Ceterach officinarum*. Coll.: S. & T. Pócs, A. S.-Gyarmati. Date: 25.06.2006. No. 06038

14. Pădurea Craiului (Király-erdő). Cheile Albioarei gorge near Peştera Jofi (Zsófi bg.), in the Roşia valley N of Ţarina village, 6 km WNW of Roşia, at 400 m alt. N 46°50′54.3″, E 22°22′09.4″. Hab.: Calcicolous swards surrounded by mesic forest. Coll.: T. Pócs Date: 25.06.2006. No. 06039

15. Pădurea Craiului (Király-erdő). Sohodol Valley (Aszóvölgy) near Albioara Forest House, at 420 m alt. N 46°50′59″, E 22°22′12″. Hab.: *Phyllitidi-Fagetum.* Coll.: S. & T. Pócs, A. S.-Gyarmati. Date: 25.06.2006 No. 06040

16. Pădurea Craiului (Király-erdő). V. Topliţa (Tapolca) E of Lacul Vida above Luncasprie village, at 250-270 m alt. N 46°51′47″, E 22°19′05″ Hab.: *Carpino-Fagetum* with scattered *Abies alba*, along streamlet, on red sandstone. Coll.: S. & T. Pócs, A. S.-Gyarmati. Date: 25.06.2006. No. 06041

17. Munţii Bihorului (Bihar-hegység), on the carstic plateau, at the top of E edge of Cheile Ordâncuşii gorge, 1 km NNW of Scărişoara (Aranyosfő), at 1090-1125 m altitude, N 46°28′25.6″, E 22°51′36.4″. Hab.: Montane beach forest (*Abieti-Fagetum*) on calcareous, rocky soil. Coll.: T. Pócs, A. S.-Gyarmati Date: 27.06.2006. No. 06043

18. Munţii Bihorului (Bihar-hegység), in limestone gorge of Cheile Ordâncuşii NE of Gârda de Sus, near the Poarta lui Ionel Cave, at 775 m alt. N 46°27′59.05″, E 22°50′17.55″. Hab.: *Asplenio-Poëtum nemoralis* and *Seslerietum rigidae transsilvanicum* on the half shady, vertical cliffs. Coll.: T. Pócs, A. S.-Gyarmati. Date: 27.06.2006. No. 06044

19. Munţii Bihorului (Bihar-hegység), near the upper end of Cheile Ordâncuşii gorge NE of Gârda de Sus, at 862 m alt. N 46°28′42.09″, E 22°50′36.07″. Hab.: *Asplenio-Poëtum nemoralis* and *Seslerietum rigidae transsilvanicum* on the half shady, vertical cliffs. Coll.: T. Pócs, A. S.-Gyarmati. Date: 27.06.2006. No. 06045

20. Munţii Codru-Moma (Béli-hegység). At the entrance of Câmpineasca Cave (Jókai bg.) at N end of Izbuc village, at 410 m alt. N 46°27′09.2″, E 22°27′44.8″. Hab.: Dry limestone rocks with xerothermic vegetation. Coll.: T. Pócs, A. S.-Gyarmati. Date: 27.06.2006. No. 06046

21. Munţii Codru-Moma (Béli-hegység). Dosul Laurului Reserve 8 km NNW of Zimbru village, 15 km N of Gurahonţ town, jud. Arad, at 550-600 m alt. N 46°23′52″, E 22°22′50″. Hab.: *Carpino-Fagetum*, only locality of *Ilex aquifolium* in Romania. Coll.: T. Pócs, A. S.-Gyarmati. Date: 27.06.2006. No. 06047

SHORT-TERM CHANGES IN THE BRYOPHYTE FLORA IN THE BOTANICAL GARDEN OF ESZTERHÁZY KÁROLY UNIVERSITY (EGER, NE HUNGARY)

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Abstract: Two research surveys were made to check the bryophyte species in the botanical garden: one investigation was started before the reconstruction took place and one after the works ended.
Altogether 46 taxa (2 liverworts and 44 mosses) were recorded. Changes of the bryophyte species diversity (10 mosses disappeared and 5 new mosses appeared) were evidently connected with the garden reconstruction and consequently with changes of the habitats, respectively their destruction and creation of the new ones.

Keywords: mosses, liverworts, life strategy, disturbance

INTRODUCTION

Examples of bryophyte floristical research within some European botanical gardens or arboreta could be mentioned (e. g. Vajda 1954, Ştefureac and Lungu 1961, Menzel 1984, Stech 1996, Fukarek 2006, Eckstein and Burghardt 2008, Teutsch 2011, Szűcs 2017), however botanical garden research related to short- or long-term changes of the bryophyte species diversity has not yet been accomplished. At present, the comparison of species distributions before and after certain time intervals has been carried out only in case of cities (e. g. Oxfordshire (Jones 1991), London (Duckett and Pressel 2009) or Katowice City (Stebel & Fojcik 2016)).

The objectives of our work are to examine the changes in bryophyte flora of the botanical garden of Eszterházy Károly University in reaction to the full reconstruction of the area.

MATERIAL AND METHODS

Two research efforts were made to check the bryophyte species in the botanical garden: one investigation was started before the reconstruction took place (September of 2014) and one after the works ended (June of 2017).

The evaluation of the life strategies of bryophytes comparing the most represented strategy types in two collecting times was carried out according to During (1979) and Orbán (1984). Nomenclature follows the classification of Király (2009) for vascular plants, Papp *et al.* (2010) for liverworts and Hill *et al.* (2006) for mosses. Specimens have been deposited in bryophyte collections of the Eszterházy Károly University in Eger (EGR).

Study area
The botanical garden (BG) of Eszterházy Károly University is situated in North-East Hungary, at the eastern border of Eger town. The management of the garden is provided by the Department of Botany. Its territory is 1 ha and is under local protection of the Town of Eger. The BG was founded in 1967, but the complete reconstruction of the garden took place between 2011-2015: the change of its whole structure has included new habitats and sidewalks network, the introduction of a new irrigation system, and planting many new herbaceous plants and some trees. The garden has been divided according to the vegetational regions of Hungary (*Figure 1*) and the living collection of plants is devoted mainly to the flora of the Carpathian Basin. The collection consists of about 100 species of trees and shrubs and about 600 species of herbaceous plants. The BG is a member of Hungarian Association of Arboreta and Botanic Gardens (HAABG).

The geographical position of the BG is N47° 54' 23.9", E 20° 23' 23.6", at an altitude of 230 m a.s.l. The climate of the garden is mild with a shift to the sub-continental features, with hot summer, moderately cold winter and quite low precipitation values: according to climate diagrams generated from 30-years global history with hourly weather data (Meteoblue 2017) and the data from Climate-data.org (2017), the average annual temperature is 9,9 °C with July and August as the warmest months and with the coldest temperatures in January. The frost-free period usually starts in the middle of May and lasts till the end of September. In

average, there are only max. 4,3 snow days per month in winter (from October to March). The average annual precipitation reaches 543 mm with maximums in May and July. The strongest and at the same time the most frequent winds blow from West and North directions.

According to Geological map of Hungary, 1:200 000, the area of the BG belongs to Kiscelli Clay geological unit: open-marine clay, clay marly silt, clay mar. The soil type is slightly acidic brown forest soil with higher percentage of clay in the soil texture (Budai and Gyalog 2010).
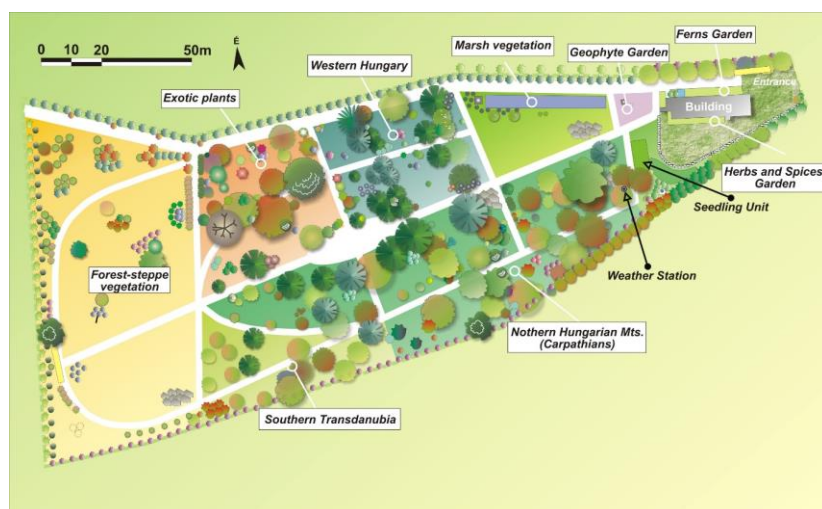
Figure 1. The map of the botanical garden of Eszterházy Károly University.

RESULTS AND DISCUSSION

The changes of bryophyte species diversity are tightly connected with the garden reconstruction and consequently with changes of the habitats, respectively their destruction and creation of new ones. The most striking habitat alterations concern for example the removal of an old shadowy limestone rock-garden or the establishment of a small artifitial wetland on an area of former shrub and weedy vegetation. Through the maintenance of the wetland, occasionally plots of open surface could be found there, as

a habitat suitable for colonization by new bryophyte species (e.g. *Marchantia polymorpha*, *Physcomitrium pyriforme*).

As an example for a regeneration process, *Climacium dendroides* can be mentioned from among the moss species. The distribution area of this species decreased during the works, but the moss survived and shows increasing tendency.

The central part of the garden forms a grove of high trees, most of them are over 50 years old. The dense canopy of trees has been partly opened up by thinning of weak, sick and dangerous individuals. There was no change in the species composition of epiphytes, because the old trees of the garden have not been disturbed.

The weedy herb layer under the trees has been removed and replaced by new plantations, only smaller patches at the northern edge of the garden have remained untouched.

The cause of changes in species composition of the garden was evidently the reconstruction process. *Table 1* shows the disappeared and the new species after the reconstruction works.

Table 1. The disappeared and the new species after the reconstruction works.

Disappeared species	Disappeared habitat	New species	New habitat
Abietinella abietina	mown lawn	*Marchantia polymorpha*	marsh vegetation
Homalothecium lutescens	mown lawn	*Brachythecium rivulare*	marsh vegetation
Homalothecium philippeanum	limestone rock-garden	*Funaria hygrometrica*	marsh vegetation
Homomallium incurvatum	limestone rock-garden	*Leptodictyium riparium*	marsh vegetation
Hylocomium splendens	mown lawn	*Physcomitrium pyriforme*	marsh vegetation
Rhytidium rugosum	mown lawn		
Schistidium crassipilum	limestone rock-garden		
Thuidium assimile	mown lawn		
Orthotrichum anomalum	limestone rock-garden		
Orthotrichum cupulatum	limestone rock-garden		

The bryophytes found in the studied area are listed below indicating species names and the substrates on which they were found. Species marked with a cross are identified as disappeared species and species with star are identified as a new species to the botanical garden. The Life strategy considered for each taxon is added.

List of taxa
In total, 46 species were found, out of these 44 were mosses and 2 liverworts.

Marchantiophyta

Frullania dilatata (L.) Dumort. (long-lived shuttle) – bark of *Morus alba*
*****Marchantia polymorpha*** L. (colonist) – on wet soil

Bryophyta

†***Abietinella abietina*** (Hedw.) M.Fleisch. (perennial) – on exposed soil
Amblystegium serpens (Hedw.) Schimp. (perennial) – bark of *Morus alba*
Anomodon attenuatus (Hedw.) Huebener (perennial) – bark of tree
Barbula unguiculata Hedw. (colonist) – on bare soil
Brachytheciastrum velutinum (Hedw.) Ignatov & Huttunen (perennial) – on soil and bark of trees
*****Brachythecium rivulare*** Schimp*.* (competitive perennial) – on wet soil
Brachythecium rutabulum (Hedw.) Schimp*.* (competitive perennial) – on soil
Brachythecium salebrosum (Hoffm. ex F.Weber & D.Mohr) Schimp. (competitive perennial) – bark of *Morus alba*
Bryum argenteum Hedw. (colonist) – on soil
Bryum caespiticium Hedw. (colonist) – on soil
Calliergonella cuspidata (Hedw.) Loeske (competitive perennial) – on wet soil

Climacium dendroides (Hedw.) F.Weber & D.Mohr (competitive perennial) – on shaded and wet soil

Ceratodon purpureus (Hedw.) Brid. (colonist) – on soil

Fissidens taxifolius Hedw. (colonist) – on shaded soil

*****Funaria hygrometrica** Hedw. (fugitive) – on disturbed and shaded soil

†**Homalothecium lutescens** (Hedw.) H.Rob. (perennial) – on exposed soil

†**Homalothecium philippeanum** (Spruce) Schimp. (perennial) – on limestone

†**Homomallium incurvatum** (Schrad. ex Brid.) Loeske (colonist) – on limestone

†**Hylocomium splendens** (Hedw.) Schimp. (competitive perennial) – on shaded and wet soil

Hypnum cupressiforme Hedw. (stress tolerant perennial) – on soil, bark of trees and stones

*****Leptodictyium riparium** (Hedw.) Warnst. (perennial)– on wet soil

Orthotrichum affine Schrad. ex Brid. (colonist) – bark of *Acer pseudoplatanus*

†**Orthotrichum anomalum** Hedw. (colonist)– on limestone

†**Orthotrichum cupulatum** Hoffm. ex Brid. (colonist) – on limestone

Orthotrichum diaphanum Schrad. ex Brid. (colonist) – bark of *Morus alba*, *Pyrus pyraster* and *Acer pseudoplatanus*

Orthotrichum pallens Bruch ex Brid. (pioneer colonist) – bark of *Morus alba*

Orthotrichum pumilum Sw. ex anon. (colonist) – bark of *Morus alba* and *Acer pseudoplatanus*

Orthotrichum speciosum Nees (short-lived shuttle) – bark of *Morus alba*

Orthotrichum stramineum Hornsch. ex Brid. (colonist) – bark of *Acer pseudoplatanus*

Orthotrichum striatum Hedw. (short-lived shuttle) – bark of *Acer pseudoplatanus*

Oxyrrhynchium hians (Hedw.) Loeske (pioneer colonist) – on soil

*****Physcomitrium pyriforme** (Hedw.) Bruch & Schimp. (annual shuttle) – on wet and bare soil

Plagiomnium cuspidatum (Hedw.) T.J.Kop. (competitive perennial) – on shaded soil

Plagiomnium undulatum (Hedw.) T.J.Kop. (competitive perennial) – on soil

Platygyrium repens (Brid.) Schimp. (stress tolerant perennial) – bark of *Morus alba*

Pseudoscleropodium purum (Hedw.) M.Fleisch. (perennial) – on soil

Pylaisia polyantha (Hedw.) Schimp. (stress tolerant perennial) – bark of *Morus alba*

†***Rhytidium rugosum*** (Hedw.) Kindb. (competitive perennial) – on soil

Rhytidiadelphus squarrosus (Hedw.) Warnst. (competitive perennial) – on wet soil

†***Schistidium crassipilum*** H.H.Blom (colonist) – on limestone

Sciuro-hypnum populeum (Hedw.) Ignatov & Huttunen (perennial) – bark of *Morus alba*

†***Thuidium assimile*** (Mitt.) A.Jaeger (perennial) – on soil

Tortula lanceola R.H.Zander (annual shuttle) – on bare soil

***Tortula muralis*** Hedw. (colonist) – on stones and concrete

Life strategies

There is a slight difference between 2014 (before reconstruction works) and 2017 (after reconstruction works) status concerning the number of species according to the life strategy categories (*Figure 2*).

The disappeared species belong to the colonist (*Homomallium incurvatum, Orthotrichum anomalum*, *Orthotrichum cupulatum, Schistidium crassipilum*), the competitive perennial (*Hylocomium splendens*, *Rhytidium rugosum*) and the perennial (*Abietinella abietina*, *Homalothecium lutescens*, *Homalothecium philippeanum*, *Thuidium assimile*) categories.

The new taxons belong to the annual shuttle (*Physcomitrium pyriforme*), fugitive (*Funaria hygrometrica*), colonist (*Marchantia polymorpha*), competivive perennial (*Brachythecium rivulare*) and perennial (*Leptodictyium riparium*) life strategy categories.
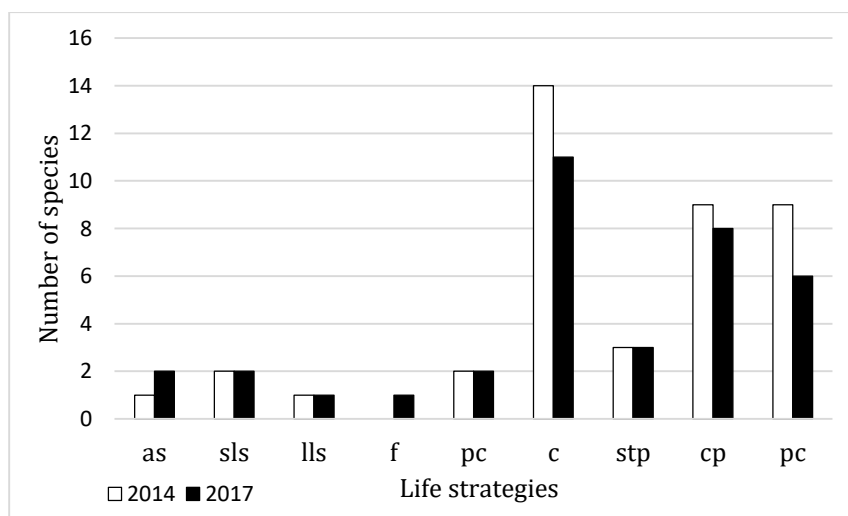
Figure 2. Life strategies of detected bryophyte species growing in the botanical garden before (2014) and after reconstruction work (2017).
(Notation: as = annual shuttle; sls = short-lived shuttle; lls = long-lived shuttle; f = fugitive; pc = pioneer colonist; c = colonist; stp = stress tolerant perennial; cp = competitive perennial and p = perennial).

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ECOPHYSIOLOGY OF BRYOPHYTES IN A CHANGING ENVIRONMENT

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Abstract: Studies of the impact of climate change on plants are generally based on vascular plants. Bryophytes basically differ from tracheophytes in having a smaller size and a poikilohydric strategy for water and nutrients. Their survival and growth are highly dependent on their external environment. Bryophytes are able to lose most of their cell water without dying, and resume normal metabolism after rehydration, gaining positive carbon balance over wet-dry cycles. This sort of adaptation is called desiccation tolerance. Desiccation tolerance is a common phenomenon among bryophytes: cells of bryophytes in exposed sunny sites can switch from full turgor to air dryness quickly, while the species of moist habitats dry more slowly, and can suffer stress by even moderate drying. Bryophytes can maintain efficient photosynthesis under low light conditions, have low chl a/b ratios, and their optimum growth is possible within a limited temperature range. When certain bryophytes' CO_2 assimilation is suppressed, photorespiration activity increases and becomes the main electron sink. Bryophytes, as sensitive components of various vegetation types, are capable of effective light absorption during their desiccation, rehydration, freezing and melting, with the help of coexisting alga and vascular plant energy dissipation mechanisms. They have a relatively low optimal temperature for growth (with narrow T range for net photosynthetic gain), with only minor differences between the optimum temperatures for net photosynthesis in polar, temperate and tropical populations of the same species. Bryophytes have a low acclimatization potential for high temperatures, taking into account that they are generally drought avoiders. Temperature acclimatization potential is of high importance for the physiological basis of altitudinal distribution and the likely responses of bryophytes to climate change. Bryophytes with their small and resistant spores are able to disperse over long distances by wind, which might help their survival in a changing environment. Dominant vascular vegetation might change as temperature will increase; however, suitable microhabitats for bryophytes might still persist. The abundance and species composition of bryophytes in plant communities is predicted to be altered, just as the function of the whole ecosystem. Based on recent literature and own data, the author makes an attempt to summarize the physiological mechanisms, morphological features and alternative strategies that make bryophytes successful in a constantly changing terrestrial environment. These plants represent a sophisticated solution to the challenges of life at their

scale. Further exploration of bryophyte ecophysiology in the changing and stressful environment will provide new information that will assist bryophyte conservation.

INTRODUCTION

The changing environment means the same altering environmental factors for bryophytes as for vascular plants. During global climate change drought, extreme values in the distribution of rainfall or other types of precipitation, more frequent high temperatures, rising CO_2 levels, and UV-A, UV-B radiation rise often or continuously occur and can cause abiotic stress. As a result of all of these changes, habitats and microhabitats may change, the desertification of certain areas can start, a change of ecosystem structure and function appears, and the mineral cycle and the local and global carbon balance also change.

Based on recent literature and own data, the author aims to summarize the physiological mechanisms, responses, morphological and other adaptations, and alternative strategies that make bryophytes successful in a constantly changing terrestrial environment.

Responses to water stress

Bryophytes are ancient land plants. Their desiccation tolerance is general, but not universal. Due to their smaller size and poikilohydric strategy they cannot be independent of the water content of their environment. Their cellular responses to water stress do not differ from those of vascular plants. Therefore bryophytes are ideal model plants to study the physiological basis of desiccation tolerance. The physiology of bryophytes differs in major respects from that of vascular plants by virtue of their smaller size. Unlike vascular plants, the leafy shoots of bryophytes equilibrate rapidly with the water potential in their surroundings and tend to be either fully hydrated or desiccated and metabolically inactive (Proctor *et al.* 2007b). In the course of drying out and rehydrating they must pass through the levels of water stress

experienced by drought-tolerant vascular plants. They only transiently face the problem of metabolizing under water stress. It is similar to a "drought avoidance" strategy in vascular plants (Marschall 2010). The time required to recover from desiccation increases and degree of recovery decreases with length of desiccation; both also depend on temperature and intensity of desiccation. Recovery of respiration, photosynthesis and protein synthesis takes place within minutes or an hour or two; recovery of the cell cycle, food transport and the cytoskeleton may take 20 hours or more. Positive carbon balance is essential to surviving repeated cycles of drying and wetting; significant growth requires continuously wet periods of a few days or more. The mechanisms of desiccation-tolerance in bryophytes, including expression of LEA proteins, high content of non-reducing sugars (Marschall *et al.* 1998) and effective antioxidant and photo-protection, are at least partly constitutive, allowing survival of rapid drying, and employ an active rehydration-induced repair and recovery mechanism. During their recovery phase the changes in gene expression resulting from mRNA sequestration and alterations in translational controls elicited upon rehydration are also important to repair processes following rewetting (Proctor *et al.* 2007b). Desiccation-tolerant angiosperms do not survive if they are dehydrated in less than 12 hours, because of the existence of time-consuming inductive cell protection mechanisms (Oliver *et al.* 2000, Marschall 2010). However, desiccation-tolerant bryophytes can survive desiccation even if it occurs in less than 12 hours, due to their constitutive protection and an active rehydration-induced repair and regeneration mechanism. To understand the differing physiological processes and stress responses of bryophytes compared with higher plants' reactions it is essential to know the actual water status of the bryophyte tissue. Cell water relations in bryophytes essentially are the same as those of other plant cells and can be described by the 'Höfler diagram'. Bryophytes' pressure (P-V) curves and Höfler diagrams are different from those typical for vascular plants in only a single but essential respect: substantial external capillary water is generally present, and is physiologically important (Proctor 2000). Surface water amount can vary widely without affecting cell water status, which can result in difficulties in expressing precise actual water content (WC). Knowledge of full-turgor WC is principal to calculate RWC and can be determined

only from experimental P-V measurements. The different adaptive types of bryophytes and higher plant cells respond similarly to water deficit. Bryophytes are much more tolerant of high (or very low) temperatures dry than wet. Species of constant moist and shade are the most sensitive to desiccation.

Adaptation in the photosynthesis of bryophytes, light protection mechanisms

Differing and also frequently changing habitat light conditions, several types of bryophyte morphology for the operation of CO_2 diffusion, different degrees of desiccation-tolerance in the different bryophyte species, as various factors, necessitate the existence of different forms of photosynthesis adaptation. Bryophyte species, although living in a wide range of light conditions, still show uniform features as shade plants (based on evidence including fine structural features of the chloroplasts, the typically low chl a/b ratios in the range of shade-adapted vascular plants, and the saturation of their photosynthesis at 20% of full sunlight), therefore tradition has regarded them as shade plants (Marschall and Proctor 2004). Bryophytes include but are not inherently shade plants. It is intuitively reasonable that poikilohydric photosynthetic organisms should be adapted to function at relatively low light levels. During periods of bright, dry sunny weather bryophytes will be dry and metabolically inactive. Most of their photosynthesis takes place in rainy or cloudy weather (<20% of full sunlight). Best adapted to photosynthesis under high light are presumably the species of mires, springs and other wet habitats, which remain constantly moist in full sun. There is great variation in the light responses of bryophytes: shade-loving species saturate at a PPFD of 100-300 μmol m^{-2} s^{-1} (responses are similar to those of shade-loving vascular plants), sun-exposed species at a PPFD of 1000 μmol m^{-2} s^{-1}. In this species REFR rises almost linearly (does not saturate) with increasing irradiance, and they show extraordinary high levels of NPQ, which can be suppressed by DTT. This suggests high levels of xanthophyll-cycle-mediated photoprotection, similar to that of higher plants, extra excitation energy dissipating as heat. 1-qP generally stabilises at around 0.3 to 0.4. Responses of this kind are found in a taxonomically and ecologically diverse range of bryophytes. PPFD response patterns

in bryophytes having complex ventilated photosynthetic systems are similar to those of vascular plants and very different from sun-exposed responses (Marschall *et al.* 2000). Photosynthetic responses of a range of *Polytrichaceae* vary in lamella development (Proctor *et al.* 2007a). In sun-exposed bryophytes O_2 and CO_2 are largely interchangeable as electron sinks and CO_2-uptake accounts for ~60% of the low PPFD saturation value. Shade-adapted species appear less able to use O_2 as electron sink, or to generate high NPQ at high irradiance. Light-saturation levels for species of open sun-exposed habitats are lower than for vascular sun plants and are probably limited by CO_2 diffusion into unistratose leaves; this limit can only be exceeded by bryophytes with ventilated photosynthetic tissues, which provide an increased area for CO_2 uptake (Marschall *et al.* 2000). Contrary to bryophyte species of wet and constantly moist areas, species of open sun-exposed habitats can reach air-dry state from full turgor relatively quickly.

The retaining of photosynthetic capacity in bryophytes after 7-d-darkness (photosynthetic activity immediately recovered after the subsequent illumination) is in contrast with the loss of photosynthetic capacity and degreening in higher plants exposed to prolonged dark. These responses of photosynthesis (and also of respiration) presumably contribute to conservation of resources when photosynthesis is prevented (by, for example, temporary burial under dead leaves) but allows rapid resumption of photosynthesis when the plant is illuminated (Marschall 2010).

The simultaneous presence of excitation energy and molecular oxygen, as occurs in the membranes of plants, may lead to the formation of reactive oxygen species and thus to photoinduced damage (Croce 2015). In desiccation-tolerant and open sun-exposed habitat bryophytes thermal energy dissipation with extremely high NPQ values is the most important element of water stress and high-light protection mechanisms. The safest and most flexible way of dissipating extra excitation energy absorbed by chlorophylls is the release of heat in the photosynthetic apparatus. NPQ, also known as non-photochemical quenching, consists of heat dissipation of chlorophyll (1Chl *) in excited singlet states. Kinetically, NPQ is a complex process, its biochemical, biophysical, physiological and ecological characterization has intensified over the last 20 years. Basically, 3 components are involved in NPQ regulation: (1) the proton gradient (ΔpH) generated between the

thylakoid membrane lumen and the side of the stroma generated by the photosynthetic proton pump; (2) violaxanthin cycle activity and the formation of zeaxanthin; (3) the PsbS protein (García-Plazaola *et al.* 2012). Although many of the light protection mechanisms of bryophytes are common with higher plants, there are also fundamental differences. In other respects, the mechanisms found in the algae ancestors that vascular plants have lost during their evolution have been retained by bryophytes (eg. LHCSR protein related thermal energy dissipation). Many bryophyte species during their desiccation, rehydration, freezing and melting are capable of absorbing light, without suffering photooxidative damage, due to their effective light protection mechanisms of energy dissipation. In vascular plants, NPQ (non-photochemical quenching) is based on the activity of PSBS protein, while in *Chlamydomonas reinhardtii* green algae it requires LHCSR, another protein (LHC-like polypeptide). For the time being, *Physcomitrella patens* is the only known / described bryophyte in which both of these proteins are present and active in the induction of NPQ. Marschall and co-workers' (2017) investigations on desiccation-tolerant *Porella platyphylla* (Marschall and Proctor 1999) and desiccation-sensitive *Sphagnum angustifolium* were directed at detecting photosynthetic activity, light protection and other regeneration mechanisms during varying degrees and durations of desiccation and rehydration with the use of violaxanthin cycle inhibitor, plastis and nuclear-encoded protein synthesis inhibitors. They showed that during the regeneration of thylakoid function related photosynthetic processes in the light xanthophyll cycle has great importance: higher zeaxanthin-dependent and lower ratio of DTT-insensitive NPQs were confirmed. Desiccation-tolerant bryophytes are characterized by the fact that they do not suffer from photooxidative damage due to the coexistence of both algal and higher plants zeaxanthin-dependent NPQ mechanisms and also a desiccation-induced thermal energy dissipation.

Alternative e-sinks: FLV-, photorespiration-dependent e-sinks
At least two large electron sinks, FLV-dependent and photorespiration-dependent electron sinks, were found to operate in photosynthetic organisms. Both electron sinks guide excess photon-energy to O_2 (Ilik *et al.* 2017). Photosynthetic organisms in

aquatic environments prefer the FLV-dependent electron sink before photorespiration, while higher plants use photorespiration as an electron sink (Shimakawa *et al.* 2017a). Using land plants (liverworts, ferns, gymnosperms, and angiosperms), Hanawaa and co-workers (2017) compared photorespiration activities and estimated the electron flux driven by photorespiration to evaluate its electron-sink capacity at CO_2-compensation point. Liverworts showed photorespiration activity in which electron flux occupied more than 70% of the photosynthetic linear electron flux under conditions when CO_2 assimilation was suppressed. Starting from liverworts to gymnosperms, photorespiration activity increased and became the main alternative electron flux. Their results show that the first land plants, liverworts, started to use photorespiration as an electron sink. When certain bryophytes' CO_2 assimilation is suppressed, photorespiration activity increases and becomes the main electron sink (Hanawaa *et al.* 2017). Gerotto and co-workers (2017) showed that in *Physcomitrella patens*, Flavodiiron (FLV) proteins act as an electron sink to avoid photosynthetic electron transport chain over-reduction after any increase in illumination and are fundamental for protection under fluctuating light conditions.

Physiological adaptations, responses to temperature in bryophytes

Bryophytes have a relatively low optimal temperature for growth (with narrow T range for net photosynthetic gain), including only minor differences between the optimum temperatures for net photosynthesis in polar, temperate and tropical populations of the same species. Bryophytes have a low acclimatization potential for high temperatures, taking into account that they are generally drought avoiders (He *et al.* 2016). Temperature acclimatization potential is of high importance for the physiological basis of altitudinal distribution and the likely responses of bryophytes to climate change. The dominant vascular vegetation presumably changes as T rises; however, a microhabitat suitable for bryophytes can be further preserved. For example, the appearance and occurrence of desert mosses in certain microclimatic areas of desert habitats is unique and a real milestone.

Other bryophyte ecophysiology related thoughts
In a globally changing environment certain bryophyte species can become extinct, contributing to a decline in biodiversity, and invasive species may spread, displacing their less competitive taxonomic relatives. It is important to acquire knowledge about the reproductive and ecophysiological properties of invasive bryophytes and also to further develop *in vitro* bryophyte culture techniques and to take further steps to utilize them for conservation biological purposes. Bryophytes with their small and resistant spores are able to disperse over long distances by wind and other carriers, which might help their survival in a changing environment. The effects of UV-A, B radiation on bryophytes have usually been studied in laboratory experiments. Less data is available from natural habitats. Growth responses to seasonal changes in UV radiation have been observed, mainly in circumpolar heights.

CONCLUSION

Bryophytes share most of their physiology with other land plants (Proctor 2000). Modern bryophytes are the end-product of the same 400+ million year period of separate evolution as modern vascular plants. They are not primitive precursors of vascular plants, but diverse and highly evolved representatives of an alternative adaptation strategy. Due to their successful strategy they are prominent in oceanic temperate forests, tropical cloud forests, bogs and fens, polar and alpine fellfields and tundras. The dominant vascular vegetation presumably changes as T rises; however, a microhabitat suitable for bryophytes can be further preserved. Further exploration of bryophyte ecophysiology in a changing and stressful environment will provide new information that will assist bryophyte conservation.

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