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IN SILICO IDENTIFICATION OF PUTATIVE BARLEY PHYTOCHROME INTERACTING FACTORS (PIFs)

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Abstract

Phytochrome Interacting Factors (PIFs) are plant transcription factors; members of the basic helix-loop-helix (bHLH) protein family. PIFs interact with the light sensitive phytochrome photoreceptors, thus they play pivotal role in light signaling, influencing a vast number of physiological processes. In spite of their importance, only a few studies focus on the identification of PIFs in different plant species until now. In this *in silico* study, we identified barley (*Hordeum vulgare* L.) bHLH proteins, and divided them into subfamilies by phylogenetic analysis. A total of 9 barley bHLH sequences were classified as VII (a+b) subfamily members. Since this group contains also those sequences, which were applied as reference PIF proteins (isolated from other plant species), we consider these 9 proteins as putative barley PIFs. These results provide a useful dataset for the forthcoming verification, analysis and functional analysis of barley PIF proteins.

Keywords: barley, *in silico* analysis, phylogenetic tree, bHLH protein family, PIF

Összefoglalás

A PIF (Phytochrome Interacting Factor) transzkripciós faktorok a bHLH (bázikus hélix-hurok-hélix) domént tartalmazó fehérjecsaládba tartoznak. Fontos szerepet játszanak a fény által aktivált jelátviteli útvonalakban, mivel képesek kapcsolatot kialakítani a fitokróm fotoreceptorokkal, amelyek a spektrum vörös és távoli-vörös tartományában rendelkeznek elnyelési maximummal. Mindezidáig csak kevés publikáció ismert, amelyek különböző növények PIF génjeinek azonosításáról szól. Ebben a tanulmányban *in silico* módszerekkel azonosítottuk az árpa (*Hordeum vulgare* L.) bHLH fehérjéit, majd filogenetikai módszerekkel alcsoportokba osztottuk őket. Összesen 9 egyedi árpa fehérjét azonosítottunk a VII (a+b) alcsoportból, amely más növény fajokban a PIF szekvenciákat is tartalmazta, tehát feltételezésünk szerint sikerült árpa PIF fehérjéket azonosítanunk. Eredményeink alapul szolgálhatnak a jövőben az árpa PIF fehérjék összehasonlító analíziséhez, majd azok funkcionális vizsgálataihoz.

Kulcsszavak: árpa, *in silico* analízis, filogenetikus fa, bHLH fehérje család, PIF

Introduction

For higher plants, light is one of the most important environmental factors that affect almost every process during the whole life cycle. The well characterized photoreceptors are responsible for detecting and absorbing light, each in a specific wavelength-range. Phytochromes (Phys) are red (R; λ_{max} ~660 nm) and far-red (FR; λ_{max} ~730 nm) light absorbents, while cryptochromes, phototropins, the Zeitlupe family and UVR8 photoreceptors are blue light and/or UV radiation sensitives (reviewed by Demarsy et al., 2018). In *Arabidopsis thaliana* (*At*) the Phy family is one of the most well characterized photosensors, its genome encodes 5 members of them, designated PhyA to PhyE (Clack et al., 1994). The monocot species, such as *Oryza sativa* (*Os*) or *Hordeum vulgare* (*Hv*) have 3 members of the Phy family, namely PhyA, PhyB and PhyC (Mathews and Sharrock, 1996). They exist in two interconvertible forms, the red light (λ_{max} ~660 nm) absorbing Pr form (biologically inactive) and the far-red light (λ_{max} ~730 nm) absorbing Pfr form (biologically active) in response to light signals. The Pr form is photoconverted to Pfr form under R light, which is reversible by exposure to FR light or independently from light (dark revision), but it is a slower conversion (Rockwell et al., 2006). The bioactive Pfr form is involved in two signaling pathways, one involves Constitutive Photomorphogenic 1 (COP1) and Elongated Hypocotil 5 (HY5) proteins, the other involves the Phytochrome Interacting Factors, hereafter PIFs (Ni et al., 1998, 1999; Lu et al., 2015).

PIFs are basic helix-loop-helix (bHLH) transcription factors, they play crucial roles in regulation of the expression level of numerous target genes, controlling hormonal signaling, abiotic (high temperature, light, circadian) and biotic (defense responses) pathways (reviewed by Paik et al., 2017). The bHLH structural motif is specific for a superfamily of dimerizing transcription factors that is characterized by two helical sequences connected with a loop (Toledo-Ortiz et al., 2003). Most of these proteins have an extra basic region in the N-terminal part of the HLH (reviewed by Jones, 2004). PIFs have been characterized mostly in the dicot model organism *Arabidopsis*, and almost nothing has been studied in the economically and agronomically important monocot species. The *Arabidopsis* genome encodes at least 7 PIF proteins (reviewed by Pham et al., 2018) and the rice genome encodes almost the same number (six) of PIF-like sequences (Nakamura et al., 2007), but no studies have been published about PIFs in barley or in bread wheat yet.

The aim of this study was to identify the bHLH proteins in barley and classify them into phylogenetic subfamilies, then to select those subgroup(s) that contains putative PIFs, based on reference sequence comparison.

Materials and Methods

bHLH domain sequences

The latest version (release-40) of the barley proteome (ftp://ftp.ensemblgenomes.org/pub/release-40/plants/fasta/hordeum_vulgare/pep/) was obtained from the Ensembl Genomes (Kersey et al., 2018) site for *in silico* analysis. Hidden Markov Model (HMM) based search with the Pfam (Finn et al., 2016) HLH domain profile (PF00010) was performed to identify putative bHLH protein sequences using the HMMER v3.0 software package (Eddy, 2009). The predicted bHLH protein sequences were manually curated for increasing redundancy. The sequence logos as graphical representation of protein alignments were generated by WebLogo v2.8.2 tool (Crooks et al., 2004).

Sequence alignment, phylogenetic analysis and motif search

For the identification of barley bHLH protein subfamilies and especially the barley PIFs, representative bHLH protein sequences from each subfamily from *At* and *Os* were collected and analyzed. AtPIF, AtPIL, OsPIF and *Glycine max* (Gm) PIF sequences belonging to the VII (a+b) subfamily (Pires and Dolan, 2010) were also added to the alignment (Table 1). The Clustal Omega (EMBL-EBI) web tool (Sievers et al., 2011) and the MEGA X software package (Kumar et al., 2018) were used for the multiple alignment of the bHLH protein sequences and Simple Phylogeny (EMBL-EBI) web tool (Larkin et al., 2007) was applied to generate phylogenetic tree data by Neighbor-Joining method as well. The phylogenetic tree was edited and visualized using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). The phylogenetic tree was represented in a radial tree layout and cladogram transformed branched.

Table 1 Representative members of bHLH proteins from each subfamily from At and Os; AtPIF, AtPIL, OsPIF and GmPIF sequences, used in multiple sequence alignment along with the barley bHLH protein sequences. At sequences were obtained from the literature (Pires and Dolan, 2010), *Os and Gm sequences were downloaded from the NCBI database (OsPILlike13: XP_015618074.1, OsPILlike15: XP_015619034.1, GmPIF3: NP_001340167.1).*

Subfamily	**bHLH protein sequences**
Ia	AtbHLH094 AtbHLH099 OsbHLH044
Ib (1)	AtbHLH095 OsbHLH146
Ib (2)	AtbHLH118 AtbHLH162 OsbHLH168
II	AtbHLH089 AtbHLH091 OsbHLH142
III (a+c)	AtbHLH027 AtbHLH029 OsbHLH156
IIIb	AtbHLH061 AtbHLH116 OsbHLH004
III (d+e)	AtbHLH004 AtbHLH005 OsbHLH009
IIIf	AtbHLH002 AtbHLH012 OsbHLH016
IVa	AtbHLH018 AtbHLH025 OsbHLH018
IVb	AtbHLH011 AtbHLH047 OsbHLH061
IVc	AtbHLH105 AtbHLH115 OsbHLH059
IVd	AtbHLH041 AtbHLH092 OsbHLH026
Va	AtbHLH046 AtbHLH141 OsbHLH031
Vb	AtbHLH030 AtbHLH032 OsbHLH042
VII (a+b)	OsbHLH152 AtPIL1 AtPIL2 AtPIF3 AtPIF4 AtPIL5 AtPIL6 AtPIF7 OsPILlike13 OsPILlike15 GmPIF3
VIIIa	AtbHLH052 AtbHLH053 OsbHLH178
VIIIb	AtbHLH040 AtbHLH140 OsbHLH123
VIIIc (1)	AtbHLH083 AtbHLH086 OsbHLH127
VIIIc (2)	AtbHLH054 AtbHLH085 OsbHLH128
IX	AtbHLH122 AtbHLH128 OsbHLH111
X	AtbHLH110 AtbHLH112 OsbHLH073
XI	AtbHLH069 AtbHLH082 OsbHLH114
XII	AtbHLH060 AtbHLH062 OsbHLH095
XIII	AtbHLH156 AtbHLH157 OsbHLH149
XIV	AtbHLH143 AtbHLH145 OsbHLH138
XV	AtbHLH134 AtbHLH136 OsbHLH154

Results

The barley proteome contains at least 163 bHLH domains

To identify the putative HvPIF sequences, the latest version of the whole barley proteome was used for a Hidden Markov Model based search (*hmmsearch*) applying the HLH profile. 1036 bHLH domains were obtained, then this protein records were manually curated for increasing the redundancy. This reduction revealed that the originally obtained 1036 bHLH domains mean 163 unique bHLH sequences in barley. Even so 183 records were used in the phylogenetic analysis, because in some cases we could not select only one gene variant.

Key amino acids in the barley bHLH domain

To get the most correct (without repetitions that distort the result) barley bHLH consensus sequence database, multiple sequence alignment was performed with the 163 unique bHLH domains, and a graphical representation as sequence logos of this alignment were generated (Figure 1). We found that the consensus amino acid residues of barley bHLH domain (with the minimal 50% identity) in the basic region are: E_4, R_5, R_8, R_9; while in the Helix 1 region: E_{10}, I_{12}, N_{13}, L_{19}, L_{22}, V_{23}, P_{24}; in the Loop region: D_{54}; and finally in the Helix 2 region: A_{56}, L_{59}, A_{62}, I_{63}, Y_{65} and L_{70}.

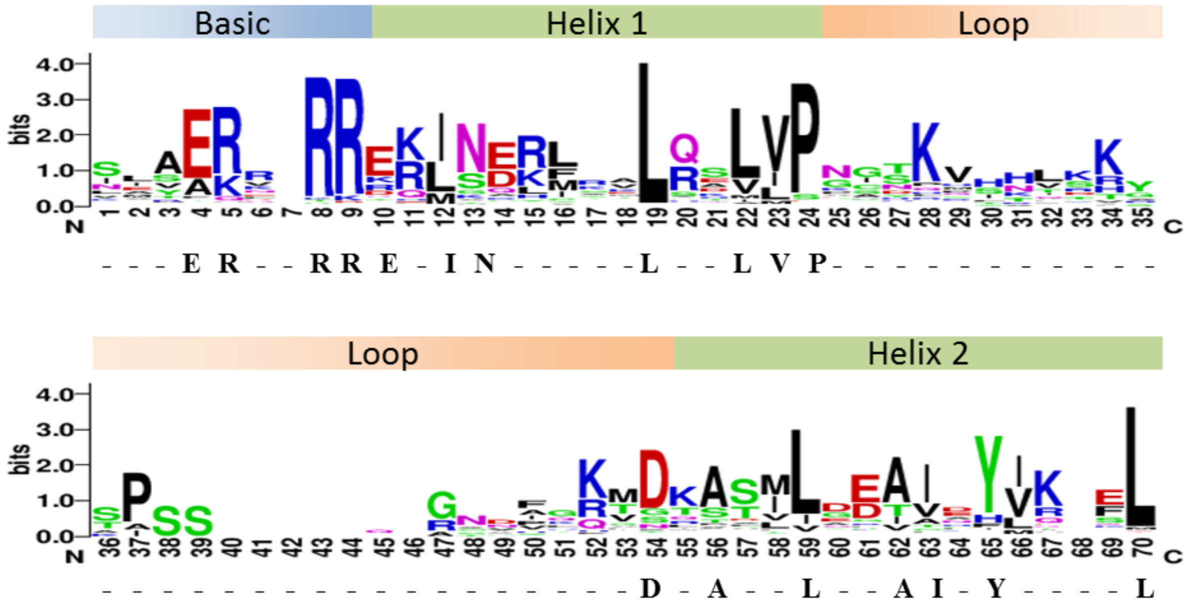

Figure 1 Graphical representation of barley bHLH sequence alignments. The labels at top represent the basic, helix 1, loop and helix 2 regions of the barley bHLH domain. The total height of the letters means the sequence conservation in that position in bits, while the proportions of amino acids in one position are shown as relative heights of individual letters. Coloring scheme was applied according to chemical properties. The bold letters under logos represent the consensus sequences of barley bHLH sequence with 50% identity. The numbering starts from a hypothetical site at the end of basic region.

Phylogenetic subfamilies of the barley bHLH proteins

To categorize the barley bHLH proteins into subfamilies, phylogenetic analyses were performed with the 183 amino acid sequences. To label the identified subfamilies and find the putative HvPIF sequences, we included several known sequences from each subfamily and also several known PIF amino acid sequences from other plant species (listed in Table 1). The phylogenetic analyses revealed that the barley bHLH domains can be separated into 25 subfamilies (Figure 2). The labels are numbered according to the system used for At and Os by Pires and Dolan (2010).

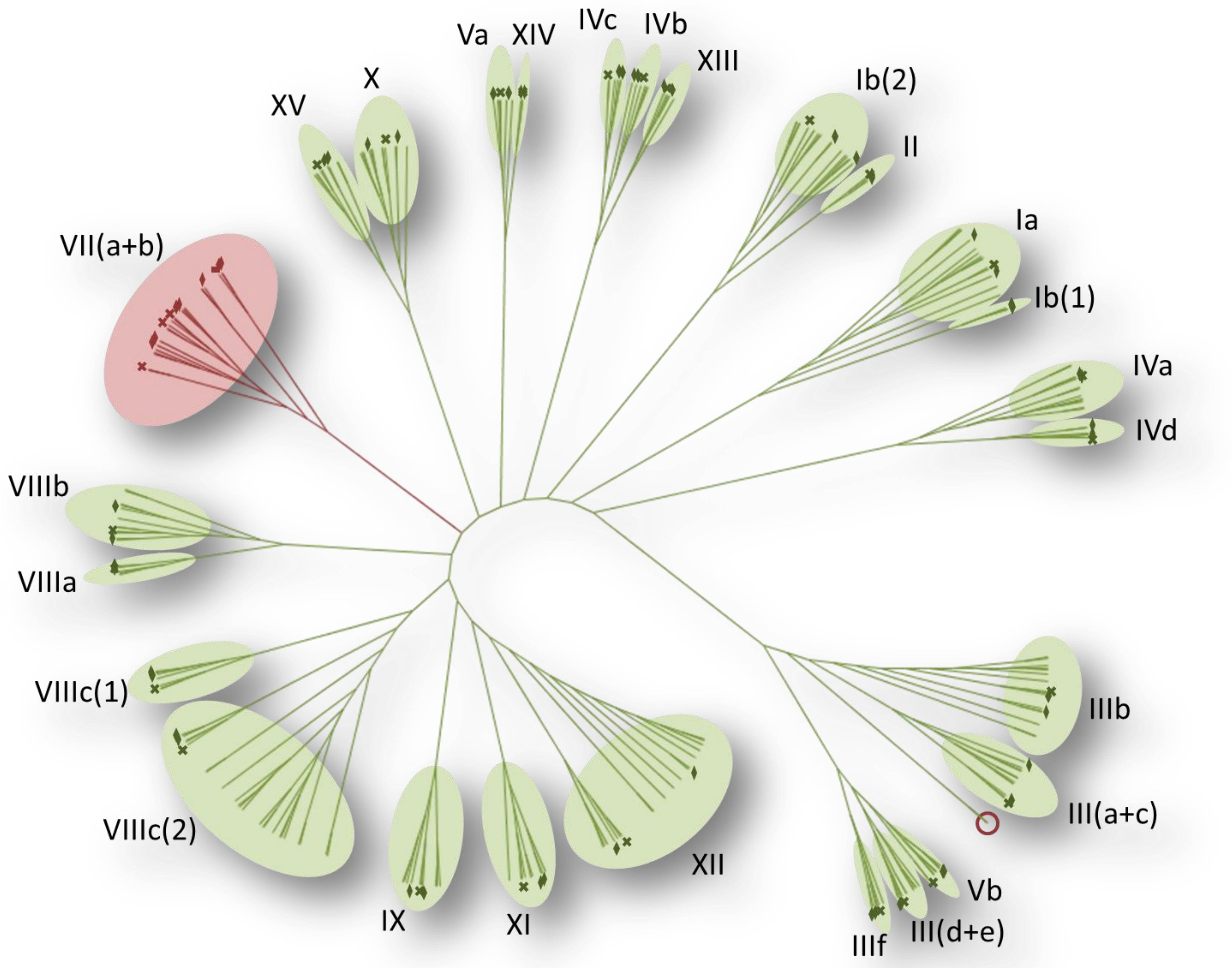

Figure 2 Phylogenetic tree of the barley bHLH proteins, shown as an unrooted cladogram. Representative members from At, Os and Gm were added to the analyses and marked at the tip of branches with diamond, cross and rectangle, respectively. The colored subgroups represent the barley bHLH subfamilies. The red bubble serves the VII (a+b) subfamily that probably contains the putative HvPIFs. Red circle marks the orphan members.

It turned out that two barley bHLH domain sequences cannot be grouped into any subfamilies. They showed a high level of divergence from other bHLH proteins, therefore these sequences (namely HORVU1Hr1G020370.2 and HORVU4Hr1G049550.3) were classified as ‘orphans’ (in the Figure 2 marked with red circle). On the other hand, we found that the XIV subfamily does not contain any barley bHLH sequences, suggesting that this subfamily is not highly conserved between plant species.

VII (a+b) subfamily contains at least 9 barley sequences

The phylogenetic analyses show that the VII (a+b) subfamily contains at least 11 sequences out of the 183 bHLH hits. Three (namely HORVU1Hr1G054260.3, HORVU1Hr1G054260.4 and HORVU1Hr1G054260.9) of the 11 sequences were not considered as unique hits, because these hits are from those ones that we could not classify as unique bHLH proteins. Thorough examination of these 3 sequences revealed that only one gene variants (namely HORVU1Hr1G054260.3) contains bHLH domain in the N-terminal region, so the other two variants were not further used in this study. Overall, we found that a total of 9 unique members of the VII (a+b) subfamily contain putative PIF sequences among the 163 identified barley bHLH proteins.

Discussion

In order to find the putative bHLH sequences in barley, we performed a Hidden Markov Model based search (*hmmsearch*) using the whole barley proteome and the Pfam (Finn et al., 2016) HLH profile. This search identified 163 unique bHLH sequences being encoded in the barley genome. Using *hmmsearch*, another study revealed that the number of these sequences are just the same in the *Arabidopsis* proteome (158) or in *Oryza sativa*, where 173 bHLH sequences were reported (Pires and Dolan, 2010). Multiple sequence alignment was performed to find the consensus sequence of these 163 bHLH proteins. The consensus amino acid (AA) residues of barley bHLH domain showed high similarities with the bHLH domains from *Arabidopsis* (Toledo-Ortiz et al., 2003) and *Zea mays* (Kumar et al., 2016) as well. In the *Arabidopsis* bHLH (*At* bHLH in Figure 3) sequence 16 AAs were found with at least 50% identity, whereas 26 AAs were reported from *Zea mays* (*Zm* bHLH in Figure 3) with the same similarity. In this current study, 18 residues were found (with at least 50% identity) in the barley bHLH sequences (Figure 3).

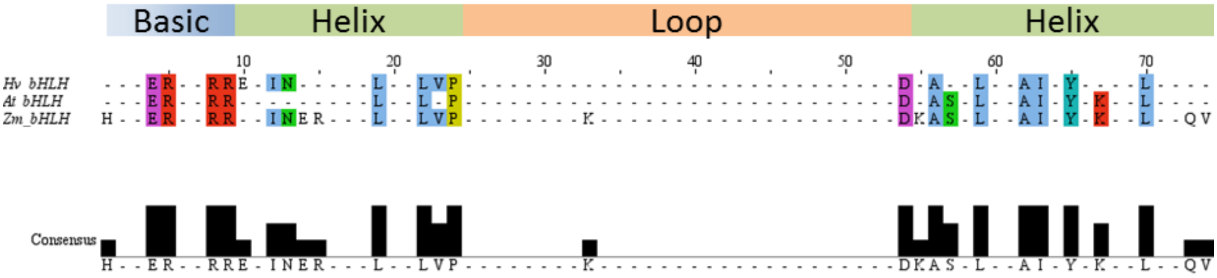

Figure 3 Multiple sequence alignment of the barley bHLH, the Arabidopsis bHLH (Toledo-Ortiz et al., 2003) *and the Zea mays bHLH* (Kumar et al., 2016) *domains with 50% identity . The labels at top represent the basic, helix 1, loop and helix 2 regions of bHLH domain. The total height of the black columns represents the sequence conservation in that position. Coloring scheme was applied for visualizing the residues. The numbering starts from a hypothetical site at the end of basic region.*

Comparing the three species, we assume that the common, i.e. consensus AAs represent pivotal residues with essential functions, therefore they are unchanged, conserved elements of the bHLH domain in Angiosperms. Thorough examination of those AAs, which are the same in *Arabidopsis* and *Zea mays* (S_{57} and K_{67}), but differ in barley reveals that they are just the same in barley, just the level of their identity is slightly less than the applied 50% threshold (S_{57}: 45.6% and K_{67}: 49.4%), therefore, these two AAs were also identified as elements of the consensus sequence. Based on the similarities of these elements, we assume that the functions of the bHLH proteins are conserved in *Arabidopsis*, maize and barley (and probably in Angiosperms). There was only one position (namely E_{10}) that was conserved neither in *Arabidopsis* nor in *Zea mays*, but indeed, it was in barley. Whether this AA has any special effect on the barley bHLH protein functions or not is still not known.

To find those barley bHLH sequences, which can be considered as putative PIFs, first we studied the 183 bHLH amino acid sequences compared to the ‘reference’ sequences (listed in Table 1) to compute a phylogenetic tree, and then, based on the topology of the tree, we defined 25 subfamilies of barley bHLH proteins. These subfamily separations were highly consistent with the previously published phylogenetic analyses carried out on *Arabidopsis* and *Oryza sativa* (Pires and Dolan, 2010), as well as on *Zea mays* sequences (Kumar et al., 2016). Of the 268 proteins analyzed, only two (HORVU1Hr1G020370.2 and HORVU4Hr1G049550.3) were not clearly classified into any of the 25 subfamilies, thus they were marked as ‘orphans’. These two protein sequences show high degree of sequence divergence from the other bHLH domains, therefore they may be considered as pseudogenes or they may represent a special subfamily, which is not present in the *Arabidopsis* bHLH tree. It is also possible that these two hits are members of the III (a+c) subfamily, which is closely located to them.

Subfamily XIV does not contain any of the studied barley sequences. Only one study, Imai et al. (2006) has been published in which a member of this minor subfamily was functionally characterized.

To identify the most relevant bHLH hits as putative PIF sequences from the 183 proteins, known PIFs, as reference sequences, were collected and added to the analysis from other plant species (*At*, *Os*, *Gm*). The phylogenetic analyses revealed that all of the studied PIFs were classified into the VII (a+b) subfamily, as it could be predicted from the literature (Pires and Dolan, 2010), thus confirming the accuracy of this novel phylogenetic tree. Overall, 9 unique barley members of the VII (a+b) subfamily were identified which probably contain the barley PIF sequences (Table 2). To find out which one of these are actually members of the HvPIF transcription factor family, *in vivo* function analyses are needed as further studies.

Table 2 Characteristics of the barley VII (a+b) subfamily from the bHLH proteins.

Transcript ID	Chr	Genomic DNA size (bp)	Number of Amino Acids
HORVU1Hr1G017900.1	1H	3463	493
HORVU1Hr1G054260.3	1H	1736	464
HORVU2Hr1G060680.1	2H	2239	362
HORVU2Hr1G104040.6	2H	567	188
HORVU5Hr1G011780.1	5H	4871	341
HORVU5Hr1G093310.11	5H	2104	547
HORVU5Hr1G102240.5	5H	3033	396
HORVU6Hr1G088020.4	6H	1685	296
HORVU7Hr1G026560.2	7H	1876	338

Conclusion

In this study, *in silico* analyses have provided information about the barley bHLH protein family and the putative PIF transcription factors in barley. Phylogenetic analysis identified the subfamilies of the barley bHLH domain sequences and revealed 9 unique sequences that show high level of similarity to PIFs from other plant species. This result will serve as valuable resources for future research in plant molecular biology, especially in light signaling studies.

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A NEW TREND IN EDUCATION AND RESEARCH AT BUDAPEST BUSINESS SCHOOL: GREEN CATERING

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Abstract

Budapest Business School, as an institute of higher education of the 21th century, is committed to the goals of sustainable development. These goals of 17 fields where adopted by 193 countries at the United Nations Conference at New York. Among these 17 fields several ones are linked to our institute. The goal most linked is the aim of sustainable consumption and prevention of climate change through modification of consumption patterns. In this sense the Faculty of Commerce, Catering and Tourism has chosen sustainable catering and hospitality as the research field of its Research Center.

Detection and calculation of the environmental impacts can be given by several indices. Methodology and content of these indices are continuously developing and their relevancy can be from local to global scale. Determination of the processes and the impacts gives heavy tasks because of modification and number of the different methods.

Among the several indices carbon-footprint is the most suitable to the goals of our Research Center. Introduction of the methodology of carbon-footprint is of high importance: this indicator provides opportunity to measure the environmental impact and sustainability of the catering sector, provides the opportunity of making comparisons, and can help to influence the decision making of the consumers and of the contractors.

A case study is summarizing the difficulties of the calculation of carbon-footprint in the field of catering, its advantages and limiting factors beside the detailed introduction of the carbon-footprint literature.

Keywords: sustainable development, Budapest Business School, product carbon-footprint

Összefoglalás

A Budapesti Gazdasági Egyetem, mint XXI. századi felsőoktatási intézmény elkötelezett a fenntartható fejlődési célok iránt. Ezeket 2015. szeptemberében, a New Yorkban megtartott ENSZ-csúcstalálkozón 193 ország által egyhangúlag elfogadott határozat fogalmazta meg. A 17 terület közül több is szorosan kötődik intézményünkhöz. Karunk profiljához és lehetőségeinkhez leginkább a felelős fogyasztás és termelés, valamint az ehhez kapcsolódó fellépés az éghajlatváltozás ellen célja köthető.

Ennek szellemében a Kereskedelmi-, Vendéglátóipari és Idegenforgalmi Kar Kiválósági Központja egyik fő kutatási területeként a vendéglátás környezetterhelésének tanulmányozását határozta meg. Ennek során többek között azt vizsgálja, hogy a vendéglátás mennyire felel meg a fenntarthatósági szempontoknak, milyen módon és mértékben javítható a fenntartható jellege.

Introduction

Environmental issues have impacts on hospitality industry in a similar way to those arising in other sectors (Taylor et al., 2017). One third of tourists' costs is to be spend for food consumption in the hosting country (Torres, 2000). Food service provision imposes significant environmental impacts (Filimonau et al., 2017). Filimonau and colleagues (2017) suggest that public knowledge on the climate significance of restaurant food choice should be reinforced. Carbon-footprint calculation and carbon labelling of the served dishes should be a suitable solution. According to Brunner et al. (2018) changes in human diets has a significant potential for greenhouse gas emission mitigation. The 29% of global emissions of GHGs are from agriculture and food production (Schmidt Rivera et al., 2014, Vermeulen et al., 2012). Espinosa-Orias and Azapagic (2018) estimated the carbon footprint of commercial and home-made sandwiches and found that the impact from the home-made is two times lower than for the ready-made one. In general, the results of Schmidt Rivera and colleagues (2014) suggest that the impacts of the home-made meal are lower than for the equivalent ready-made meal. Tourists usually consume ready-made meal during the stay at a destination.

Budapest Business School, as a higher educational institute is committed to sustainable development, founded its Sustainable Catering Research Center in the autumn of 2017. This Research Center supports financially several scientifically important goals in the field of Catering and Tourism. Research proposals are of 3 years cycle. One of these research aims is to make a temptation to calculate the carbon-footprint of some products of served food in restaurants. As a first step of the project the researchers summarized the literature of carbon-footprint calculation methods, carbon-footprint of the agricultural cultivation specified for food industry and food products. Carbon-footprint of some food materials used for catering is calculated. Why carbon-footprint was chosen as research field among others of the Research Center? Several indices are used to describe the environmental impact human activities, for example of food industry, catering and tourism.

The World Business Council for Sustainable Development (WBCSD) has settled nine crucial targets with indicators in its document Action2020, and the Hungarian adaptation incorporated five of them by Business Council for Sustainable Development in Hungary:

1. food and feed,
2. sustainable lifestyles,
3. employment,
4. climate change,
5. water.

Detailed goals for these main aims are summarized in *Figure 1*.

Among the fields and targets of Acton2020 Budapest Business School Sustainable Catering Research Center is linked to Food and Feed, Sustainable lifestyles by its research proposals. The realization of the targets can be detected by indicators. In *Figure 2.* the indicators most relevant in the research of the current project are signed in bold and italic.

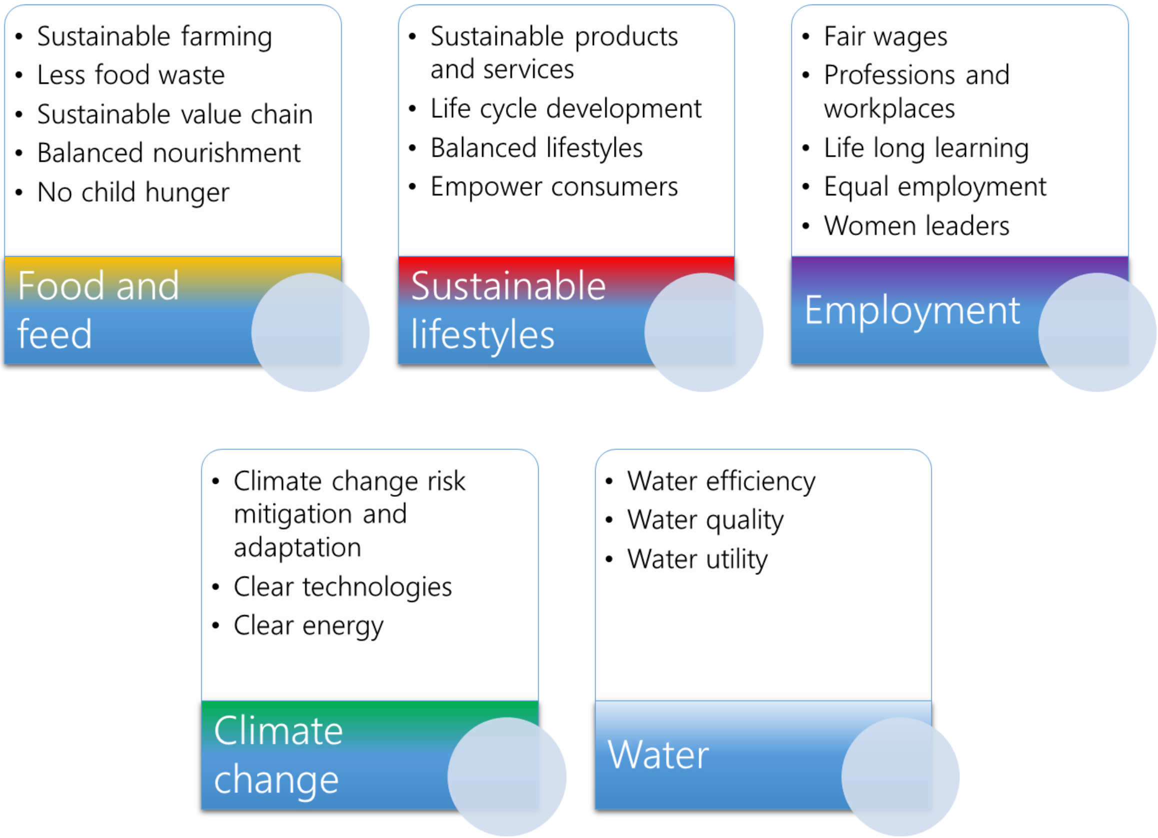

Figure 1. Main and detailed targets of Action2020 (https://action2020.hu/en/celok/)

Figure 2. Targets and indicators of Action2020 most relevant in the current project

Definition of carbon footprint was given by several authors (Mujica et al., 2016, Rebolledo-Leiva et al., 2017). For example according to Wiedmann and Minx (2008) carbon footprint is a part of the ecological footprint, that measures the total amount of carbon-dioxide emitted directly or indirectly to the atmosphere by a certain activity or person or surface, etc. and the surface unit that is capable to neutralize it. Carbon-footprint can be considered as a part of ecological footprint, and Mancini et al. (2016) developed a refining method for carbon-footprint calculation in this sense.

In general carbon-footprint summarizes the greenhouse gas emission equal to carbon-dioxide amount of the production or manufacturing of a product or service. The specific carbon-footprint of Hungary is favorable in comparison to other European countries (*Table 1.*). Greenhouse gas emission is important as an aspect of contamination of the environment, and main accelerative force of global climate change. Nowadays contribution of food production and food industry to the greenhouse gas emission and climate change gets more and more highlight among its environmental impacts.

Table 1. Greenhouse gas emission per capita of the member countries of European Union (given in carbon dioxide equivalent) (European Environmental Agency)

Country	1990	1995	2000	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Austria	10.4	10.2	10.3	11.5	11.1	10.8	10.7	9.8	10.4	10.1	9.7	9.7	9.2	9.4	9.4
Belgium	15	15.6	15.1	14.2	13.9	13.5	13.4	12.1	12.6	11.5	11.1	11.1	10.5	10.8	10.8
Bulgaria	12	9	7.3	8.4	8.6	9.1	9	7.8	8.3	9	8.4	7.7	8.2	8.7	8.4
Czech Republic	19.4	15.4	14.7	14.6	14.7	14.8	14.2	13.3	13.5	13.3	12.9	12.4	12.2	12.3	12.4
Denmark	14	15.3	13.7	12.7	14.1	13.2	12.5	11.8	11.9	10.9	10	10.3	9.5	9	9.3
United Kingdom	14.2	13.3	12.6	12.1	11.9	11.6	11.1	10.1	10.3	9.4	9.6	9.3	8.6	8.3	7.9

EU (28 countries)	**12**	**11.2**	**10.8**	**10.8**	**10.7**	**10.6**	**10.3**	**9.6**	**9.7**	**9.4**	**9.3**	**9.1**	**8.7**	**8.8**	**8.7**
Finland	14.5	14.3	13.8	13.5	15.7	15.3	13.8	13	14.4	12.9	11.9	12	11.1	10.5	11.1
France	:	9.3	9.3	9	8.8	8.6	8.5	8	8.1	7.7	7.7	7.6	7.1	7.1	7.1
Greece	10.4	10.6	11.9	12.6	12.3	12.5	12.2	11.5	10.9	10.6	10.4	9.6	9.4	9.1	8.8
Netherlands	15.1	15.5	14.4	13.8	13.5	13.4	13.3	12.8	13.5	12.6	12.2	12.2	11.7	12.2	12.2
Croatia	6.8	5	5.8	7	7.1	7.4	7.1	6.7	6.6	6.5	6.1	5.9	5.7	5.8	5.9
Ireland	16.1	16.7	18.5	17.3	16.8	16.2	15.6	14.1	13.9	12.9	12.9	12.9	12.8	13.2	13.5
Poland	12.3	11.4	10.2	10.4	10.8	10.9	10.7	10.2	10.7	10.7	10.5	10.4	10.1	10.2	10.5
Latvia	10	5.2	4.5	5.2	5.5	5.8	5.6	5.4	6.1	5.8	5.8	5.8	5.8	5.9	6
Lithuania	13.1	6.2	5.6	6.9	7.1	7.9	7.7	6.3	6.7	7.1	7.2	6.8	6.9	7	7.1
Luxemburg	34.5	26.1	24.3	30.7	29.7	28.2	27.5	25.8	26.5	25.5	24.2	22.7	21.5	20.4	19.8
Hungary	**9.1**	**7.4**	**7.3**	**7.6**	**7.5**	**7.3**	**7.2**	**6.6**	**6.6**	**6.5**	**6.1**	**5.8**	**5.9**	**6.3**	**6.3**
Germany	15.9	13.9	12.9	12.3	12.4	12.1	12.2	11.4	11.8	11.8	11.8	12	11.4	11.4	11.4
Norway	12.3	11.9	12.4	12.1	12	12.3	11.8	11.1	11.5	11.2	11	10.8	10.7	10.7	10.5
Italy	9.2	9.5	9.9	10.2	10	9.8	9.5	8.5	8.7	8.4	8.1	7.5	7.1	7.3	7.2
Portugal	6.2	7.2	8.3	8.5	8	7.8	7.6	7.2	6.9	6.8	6.6	6.5	6.6	7	6.9
Romania	10.7	8	6.3	7	7.1	7.3	7.2	6.3	6.1	6.4	6.2	5.8	5.8	5.9	5.8
Spain	7.5	8.4	9.7	10.3	10	10.1	9.2	8.2	7.9	7.9	7.7	7.2	7.3	7.5	7.3
Switzerland	8.4	8	7.9	7.8	7.8	7.5	7.6	7.3	7.5	6.9	7	7.1	6.5	6.4	6.4
Sweden	8.5	8.5	8	7.6	7.6	7.4	7.1	6.5	7.1	6.6	6.2	6	5.8	5.7	5.6
Slovakia	14	10.1	9.2	9.5	9.5	9.2	9.3	8.4	8.6	8.4	8	7.9	7.5	7.6	7.6
Slovenia	9.3	9.4	9.6	10.3	10.4	10.4	10.7	9.7	9.6	9.6	9.3	9	8.1	8.2	8.6

Materials and methods

The carbon-footprint is a sustainability indicator that gives numerically the amount of emitted greenhouse gases during the life cycle of the product. Life cycle assessment (LCA) is a proper tool to calculate potential environmental impacts of products or systems (Civancik-Uslu et al., 2018). LCA is a methodology used to evaluate the environmental impacts of products and services by taking into account all the production and consumption stages, from the production of raw materials to the end of life, including all intermediate steps (Bicalho et al., 2017), therefore it has high data inquiry. The uncertainty of product information is a critical question to estimate product carbon footprint for product life cycle (He et al., 2018). The methodologies used for the calculation of the carbon-footprint are not uniformed. Even in 2017 the standardization of the latest indicators has not been succeeded and a unique internationally accepted standard has not been developed (Lombardi et al., 2017). Crop production is strongly linked to food production and while different methods are available to account for GHG emissions in life cycle assessments (LCA) of crop production, there are no standard procedures (Goglio et al., 2018) even in this field. Mostly PAS 2070 guidelines can be followed or ISO 14067 standard can be used. ISO 14067 (Greenhouse gases — Carbon footprint of products — Requirements and guidelines for quantification and communication) standard is based on the standards of lifecycle analyses and assessment, eco-labelling and environmental reporting (ISO 14040. 14044. 14020. 14024. 14025) (www.iso.org). The principle of the standards of the Intentional Organization of Standardization is the PDCA cycle or named also as Deming-cycle (*Figure 3.*).

The basis of the carbon-footprint methodology is lifecycle assessment. The process begins the settle of goals and establish of the system boarders. In case limits are well defined the following step is life cycle inventory analyses, and then lifecycle impact analysis. Finally lifecycle assessment summarizes all the environmental impacts of the process and gives a report for further innovations or planning of improvement (*Figure 4.*)

Schaltegger and Csutora (2012) give a holistic view of carbon inventory analysis methods and carbon-footprint calculation methods that is rapidly developing field of environmental management. Stechemesser and Guenther (2012) provide a literature summery about the methodology of carbon inventory and carbon account.

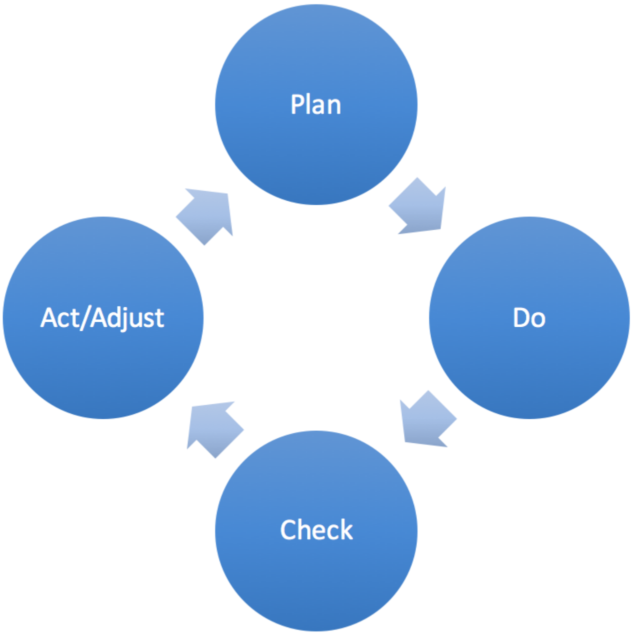

Figure 3. PDCA cycle /Deming-cycle/ (https://iinnovatemag.com/blog/techniques/what-is-deming-cycle-shewhart-cycle-pdca/)

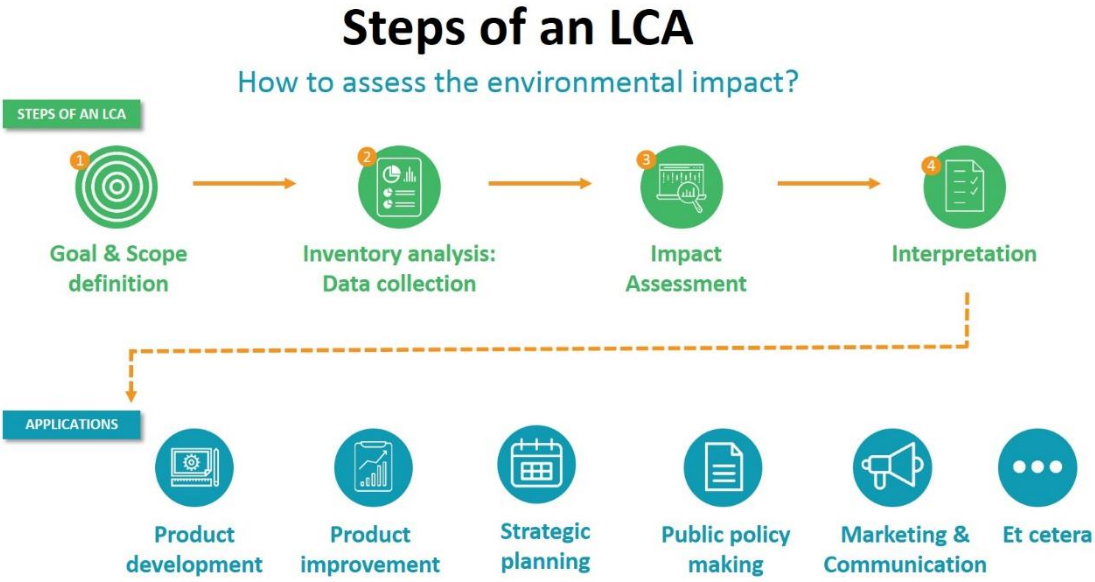

Figure 4. Steps of a lifecycle assessment (http://www.blonkconsultants.nl/what-is-life-cycle-assessment/?lang=en)

Results

Frozen sweet maize product of Mirelite Mirsa PLC was chosen as test material and carbon-footprint was calculated with bottom-up method. Mirelite Mirsa PLC has environmental protection, sustainability and support of local producers at principal position in its business strategy. Hungary is one of the main producers of sweet maize products in Europe.

Dr. Viktor Losó, expert of the company was on our help and provided several useful information about the production. A special thank should be expressed in this form to him by the researchers. The interview led with him gave the basic information to compose the flowchart of the steps of the production system (*Figure 5.*). This was the first stage towards calculating the carbon-footprint of frozen sweet maize per ton.

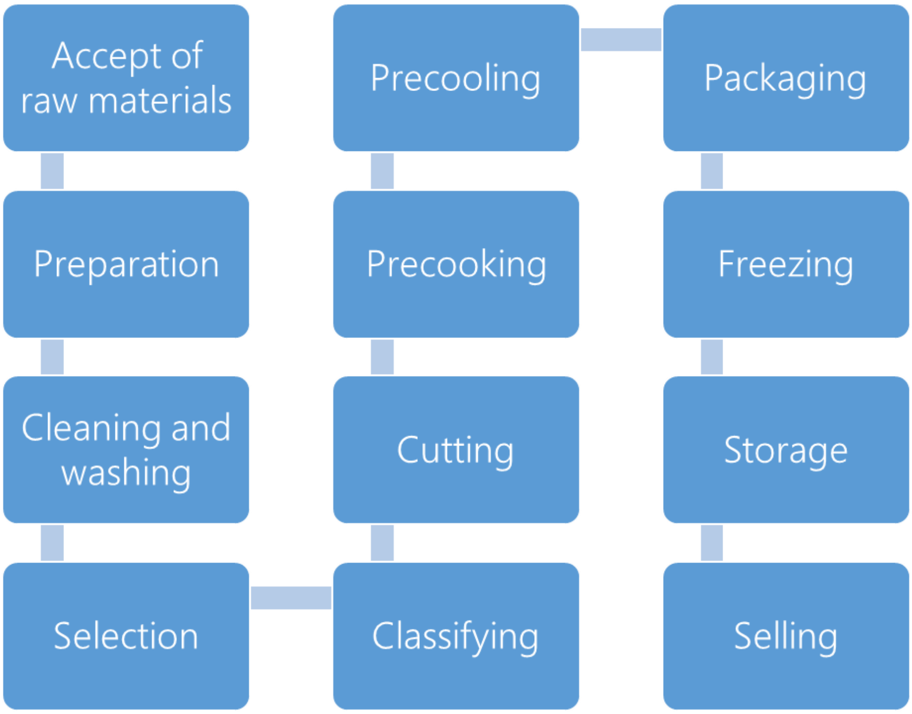

Figure 5. Flowchart of the frozen food industry products

Data at the stages shown in *Figure 5.*, for calculation of carbon-dioxide emission are provided by the interview with the expert, nominal performance of the machines, technical descriptions, transport information, waste management, international databases. *Table 2.* summarizes the emissions of the life cycle of 1 ton frozen sweet maize.

Table 2. Parts of carbon-footprint of 1 ton frozen sweet maize

	Transport	Production	Storage	Waste management	Total
Maize	66.46	4.56	124.97	0.09	196.08
Packaging	52.62	0	0	0.812	53.432
Total	119.08	4.56	124.97	0.902	**249.512**

The carbon footprint of packaging in the phase of production and storage is not part of the carbon-footprint of frozen sweet maize product, but the emission of the transport of the packaging material used in the production is calculated in the CF of the product because Mirelite Mirsa PLC solves the transportation.

This value of the carbon-footprint of frozen sweet maize is calculated just for a selected part of the production. For the scarcity of information the system borders was chosen according to the data available from the Mirelite Mirsa PLC. The most serious limitation of the method is that unfortunately the willingness of providing data suitable for such calculation is poor in the stakeholders of the whole production chain. In this case only the data of the production steps of frozen maize has been provided, but information about the cultivation's carbon-footprint cannot be taken into account, nor the production of the packaging material. As the borders of the system can be freely chosen during the calculation and the limiting factor is the information, the results are not comparable to other values. The calculation method is building bottom-up the value of the carbon footprint for the selected part of the production and follows the guidelines of the life cycle assessment, but has no strict rules. This is the other limitation of such calculations and makes hard to compare to other result. The information and data used in these type of evaluations also vary by the system borders, by technology used, data available and by geographical area.

Discussion

We have calculated the carbon footprint of sweet maize on the basis of an in-depth interview and manual data collection. With the in-depth interview, we have measured the steps of the manufacturing process, while in the course of the manual data collection we have developed a database of CO_2 emissions for the specific performance of the machines. For such a “simple” (less processed) product as frozen sweet maize, only at Mirelite Mirsa PLC we had to take into account the performance of 48 machines. These are closely and directly related to the manufacturing process. In accordance with the LCA approach, when calculating the carbon footprint, CO_2 emission associated with the stages of transport, storage and waste management of the75-step process should also be specified. Experiencing the difficulties of data collecting, we proposed that the University support the research center by obtaining a software and database for the carbon footprint calculation. This process is taking place just now. With this program, we would like to research how the carbon footprint of a product changes with each step of a particular workflow. In parallel with the methodology study, we plan to evaluate the practical utilization of the carbon footprint. We are looking for partners to accomplish this research.

Acknowledgement

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AIR TEMPERATURE AND PRECIPITATION EVOLUTION IN THE AREA OF KESZTHELY

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Abstract

In this brief study some local signs of global climate modification are presented based on the statistical analysis of long-term air temperature and precipitation data for Keszthely (Hungary). In the Agrometeorological Research Station in Keszthely continuous meteorological measurements have been recorded since 1871, therefore an adequate length of data is available for analysis. Based on our results, a significant warming tendency is dominant, an increasing trend in air temperature can be established especially from the 1980s. Over the 116-year study period, statistically significant increase of 0.1°C per decade was found with 9.94±0.77°C annual average. Regarding to rainfall a downward trend of 47 mm per 100 years was observed.

Keywords: air temperature, rainfall, climate change, time-series analysis, Keszthely (Hungary)

Összefoglalás

Tanulmányom célja a globális klímaváltozás helyi megnyilvánulásainak bemutatása a keszthelyi hosszú idősoros léghőmérséklet és csapadék adatok éghajlati statisztikai elemzése alapján. A keszthelyi Agrometeorológiai Kutatállomáson már 1871 óta végeznek folyamatos meteorológiai méréseket, így kellő hosszúságú adatsor áll rendelkezésünkre. Az eredmények alapján elmondható, hogy jelentős felmelegedési tendencia figyelhető meg a térségben, főképpen az 1980-as évektől kezdődően. A 116 éves vizsgált időszakban évtizedenként 0.1°C-os, statisztikailag szignifikáns hőmérséklet-emelkedés mutatható ki, az átlaghőmérséklet 9.94±0.77°C. A csapadékösszegek tekintetében 47 mm/100 év csökkenő tendencia tapasztalható.

Kulcsszavak: léghőmérséklet, csapadék, éghajlatváltozás, idősorelemzés, Keszthely (Magyarország)

Introduction

Global climate change has accompanied the history of Earth. Recent developments are fundamentally different from changes due to natural causes (Kertész, 2001). Human emissions of greenhouse gases have changed the composition of the atmosphere over the last two centuries and caused global climate modification on Earth. Climate change is the best reflected by the evolution of air temperature. During the 20th century, warming occurred in two phases. The first was from the 1910s to the 1940s and 1950s followed by a mild cooling until the 1970s. From the beginning of the last quarter of the century a rapid, intense warming has begun (Folland et al., 2018). Warming is not gradual, it varies by different locations and seasons (IPCC, 2007). The national average is also reflecting to the global changes, but shows slightly higher warming tendencies (Szalai et al., 2005). In Hungary, spatial change is not uniform, vary by region, distribution is mainly determined by the zonality and the relief. In most parts of the country the average annual temperature is between 10 and 11°C. Relief is well reflected in the annual mean temperature. The lowest values usually appear in higher areas, in the regions of Bakony and Alps, or in the North Hungarian Mountains, where the average temperature is usually below 8°C. Values higher than 11°C are located on the south-southwest slopes and south of the country. The spatial distribution of the average temperature from southwest to northeast is decreasing due to the effect of the Mediterranean Sea and the Siberian anticyclone. Air temperature characteristics are well suited to global trends, but due to Hungary is a small country, its variability is higher.

The extent and distribution of precipitation in Hungary has also changed. This process is most likely due to higher temperatures. Hydrological cycle may become more intense, with an increasing proportion of precipitation in the form of heavy rainfall. Hungary's water balance shows a deficit. According to Domonkos (2003) a slow precipitation decrease was observed during the 20th century. Regarding to the Carpathian Basin Bartholy and Pongrácz (2007, 2010) found an increased frequency and intensified tendency in the extreme values and decreasing precipitation amounts in the second half of the 20th century. The most extreme events occurred during the summer months (Bartholy et al., 2005). Lakatos and Bihari (2011) revealed stronger decrease in precipitation sums in the Transdanubian region than in other parts of the country. The largest precipitation decline occurred in springs, nearly 20% of the total between 1901-2009. Despite of the small area, Hungary also has a significant difference in precipitation by region. The western and south-western parts of the country are more humid, while as well as the higher-lying areas where the precipitation in some small spatial spots exceeds the 800 mm. The most arid region is located in the Great Plain on a long-term average of less than 500 mm. The annual rainfall decreases from southwest to northeast. Most precipitation falls between May and July. In a significant part of the country, especially in the southern part of Transdanubia, on the south-eastern slopes of the Transdanubian Mountains and the Dunazug Mountains, a secondary rainfall may occur in Autumn (October-November). According to Szalai et al., (2005) there were more humid years in the first half of the century, and over the 1901-2004 period an 11% of decrease in precipitation was reached.

The regional impact of global warming appears in the manifestation and enhancement of extreme climatic events. In addition to this, a considerable change in the intensity of temperature or precipitation extremities can be observed as well. The decrease in the number of frosty days and the increase of hot days clearly show an accelerated warming process. Extreme weather phenomena may become more common and according to IPCC (2011), it is very likely that the number of hot extremities and heat waves will increase. The frequency, intensity and duration of hot waves are expected to increase throughout Europe, while the occurrence of winter extremes of cold and frosty days is expected to decrease. A considerably increased number of summer days (T_{max}>25°C) and tropical nights (T_{min}≥20°C) were observed in Hungary, in recent decades (Lakatos et al., 2007). According to Spinoni et al. (2015a) in the period of 1961-2010 a general tendency of a more frequent, longer, more severe and intense heat wave events was found in the Carpathian region in every season. The tendency of cold waves was generally less frequent, shorter, less severe and less intense. Drought variables show a moderate increase in Carpathian regions in the period 1950-2012. The frequency, duration, and severity of drought increased in the past decades, in particular from 1990 onwards. This region is one of the highest drought risk areas (Spinoni et al., 2013, 2015b). Briffa et al. (2009) also found a significant clustering of dry summers in the most recent decades. Analyzing in a very long-term context, trend displays towards wetter summer conditions from the end of the 17th century until the beginning of the 19th century, followed by a continuous trend towards drier conditions. Drying summers are found obvious in the latter part of the 20th century, especially in central Europe.

Materials and methods

Keszthely (Hungary, N 46°44’, E 17°14’) is located in Transdanubia, near Keszthely Bay, in the catchment area of Lake Balaton surrounded by hills from the north. Its weather is influenced by the unique microclimate of the lake, as well as the wind changing influence of Keszthely Hills. These hills are located in the western part of the Bakony, which is in the way of the Mediterranean, Atlantic and Continental air masses. This area is very sensitive for climate change. The southern slopes of the mountains are particularly affected by the Mediterranean effect, which is enhanced by the climate change of the water mass of Lake Balaton. It also has an effect on the air humidity, and its surface plays an important role in the reflection of solar radiation (Antal, 1974).

The aim of this study was to analyze the long-term data series of the meteorological measurements of air temperature and precipitation at Keszthely from the point of view of climate modification and statistics. Meteorological observations at Keszthely have a long detailed historical background, measurements of the meteorological elements are continuous from 1871. For the air temperature analysis monthly homogenized mean data from 1901 to 2016 were used. Homogenization of air temperature data was done using MASH software (Szentimrey, 1999). After homogenization, the data were analyzed on an annual, seasonal and monthly scale. Development of precipitation was examined based on a monthly precipitation sums from the beginning of the observation until 2016 and on a daily scale from 1971.

Results

Changes in air temperature

The results derived from the observed data showed a significant rising trend in the air temperature for Keszthely. The annual average was found 9.94±0.77°C during the period of 1901-2016 (*Fig. 1*). This value was corresponded to the mean value in Hungary. For this entire period a temperature rise of 0.1°C per decade was resulted. Calculating from the most intense warming phase from the 1980s, the average temperature in the 1981-2010 period was already 10.31°C, with an intense rise of 0.36°C. Extended this interval to 2016, the average was 10.46°C with a rise of 0.43°C per 10 year.

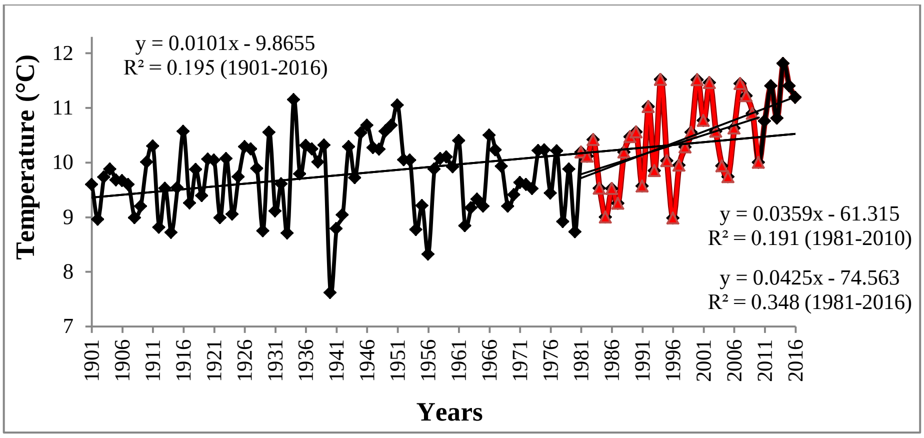

Fig. 1 Tendency of annual mean air temperatures between 1901-2016 (p<0.001), 1981-2010 (p=0.016) and 1981-2016 (p<0.001), in Keszthely

The mean annual temperature range is 21°C. 1940 proved to be the coldest year in Keszthely with an average annual temperature of 7.6°C, the hottest one was 2014 with 11.8°C. The absolute minimum monthly average was -9.96°C, which occurred in February 1929, the maximum average in 1992 August with 23.89°C. In *Fig. 2* the past changes and the increasing intensity of warming were shown. Annual mean temperature in the entire period were compared to the climate norm of the 1981-2010. The anomaly is clearly showing the recent intensive warming process.

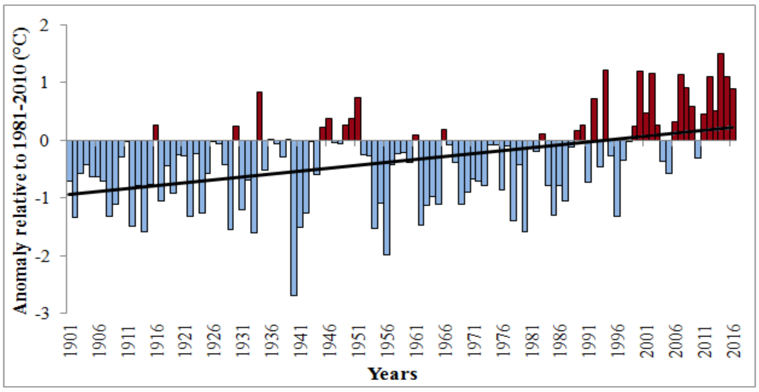

Fig. 2 Anomalies of the annual average temperatures of Keszthely (from 1901 to 2016) compared to 1981-2010 climate norm

Changes in ten-year averages of homogenized annual mean temperatures was presented in *Fig. 3*. After the warming period of 1901-1950, three decade long cooling period turned into a steadily warming phase especially from the 1980s. Based on the characteristics of the temperature evolution, Keszthely fits well with the global tendencies.

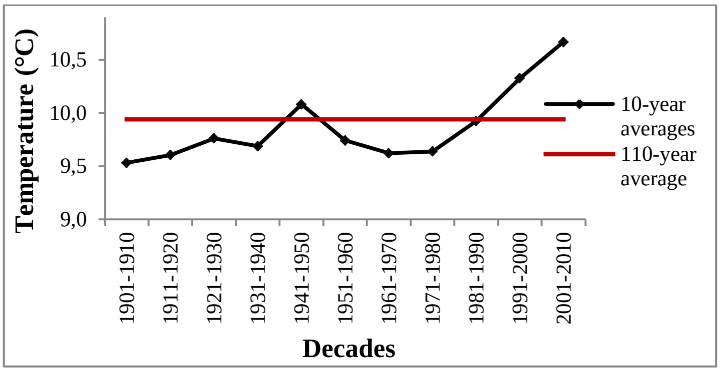

Fig. 3 The relationship of ten-year averages of homogenized annual mean air temperatures to the average of 110 years

Applying 10-year-moving averages was highlighted the upward trend in the air temperature in the last decades. (*Fig. 4*).

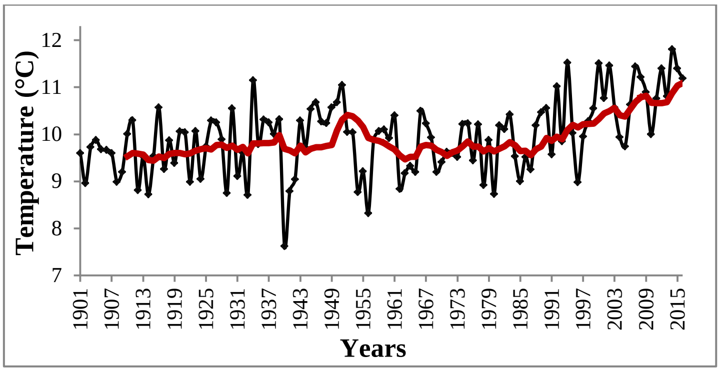

Fig. 4 Annual air temperature tendency (1901–2016) with moving average (k=10)

Regarding to the most recent climate from 2001 with the exception of the years 2004 and 2005, the average temperature of each year was exceeded the normal value (9.96°C) of 1971-2000 (*Fig. 5*). The average temperature of 2001-2016 period was 10.9 which was 0.9°C higher than the 30-year average of the last century.

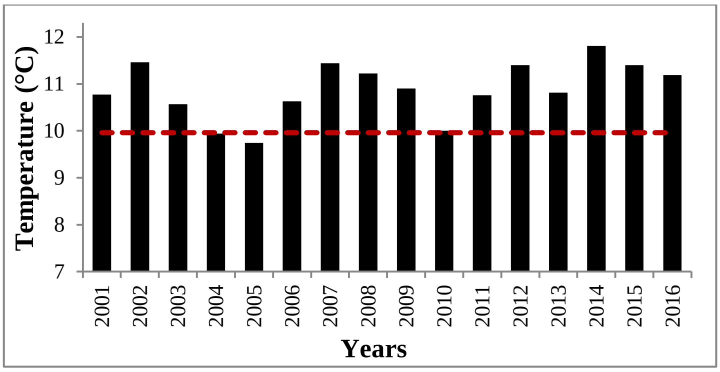

Fig. 5 The trend of changes in most recent annual temperatures (2001-2016) in comparison to the mean value of 1971-2000

A trend alignment for seasonal analysis was also prepared. The seasonal air temperature was investigated for the entire data set and from the beginning of the intensively warming period of 1981 (*Table 1*). Spring was proved to be the most warming season for the whole period, the changes were proved to be significant with the exception of autumn and winter of the last climate normal. The average temperature of each season has increased in the 1981-2010 period. Springs and summers represented the highest temperature rises for both intervals.

Table 1 Summary of seasonal mean temperatures and temperature rises

Season	**1901-2016**		**1981-2010**	
	Mean temp. (°C)	**Temp. rise (°C)**	**Mean temp. (°C)**	**Temp. rise (°C)**
Spring	10.24	1.38	10.76	1.35
Summer	19.37	1.26	19.82	1.51
Autumn	10.08	1.01	10.20	0.68
Winter	1.47	0.87	1.74	0.88

The ten-year average of the homogenized seasonal average temperatures was compared to the average of 110 years. Changes in 10-year averages was reflected to the modifications which occurred in the last decades and also showed clearly that spring and summer are the most warming seasons.

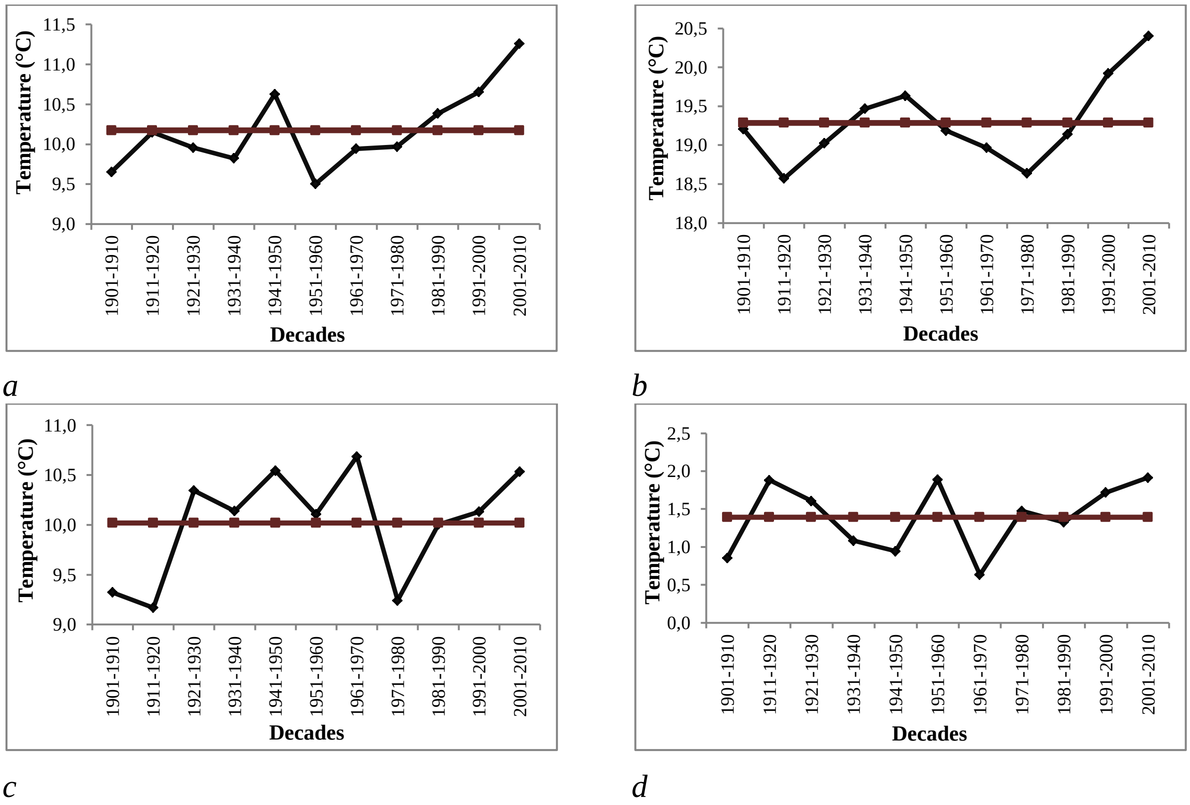

Fig. 6 The relationship between the ten-year averages of the mean temperatures and its 110 years average (a springs; b summers, c autumns, d winters)

On a monthly scale the hottest period of the year was the end of July and the beginning of August, while the coldest average was in January. The monthly temperature distribution is illustrated in *Fig. 7*.

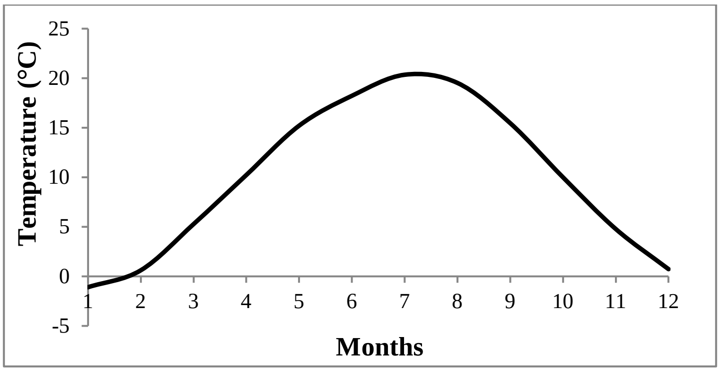

Fig. 7 The average monthly temperatures in Keszthely between 1901 and 2016

The air temperature was more balanced in the warmer months, the annual variability in summer was usually lower than in the colder seasons. (*Fig. 8*).

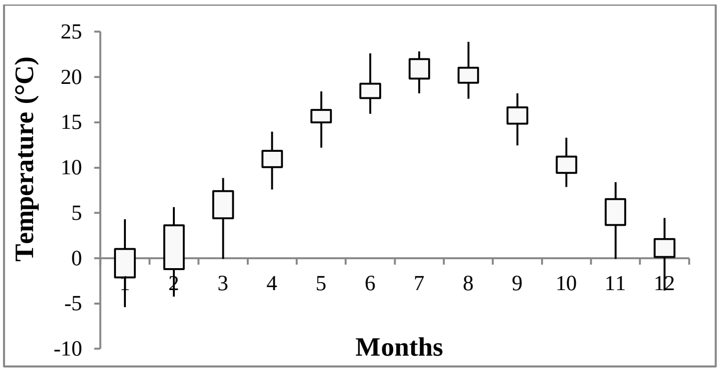

Fig. 8 The average monthly values of lower, upper quartile and extreme temperatures of Keszthely in the period of 1981-2010

Changes in precipitation

The 146-year annual average rainfall of Keszthely was 673±137 mm. Based on the entire dataset the precipitation decreases by 0.47 mm per year which resulted a total of 47 mm less rainfall in 100 years (*Fig. 9*). The range of data was 755 mm and the average absolute difference was 108 mm. According to Szász (1994), the risk of less precipitation is threatening Hungary.

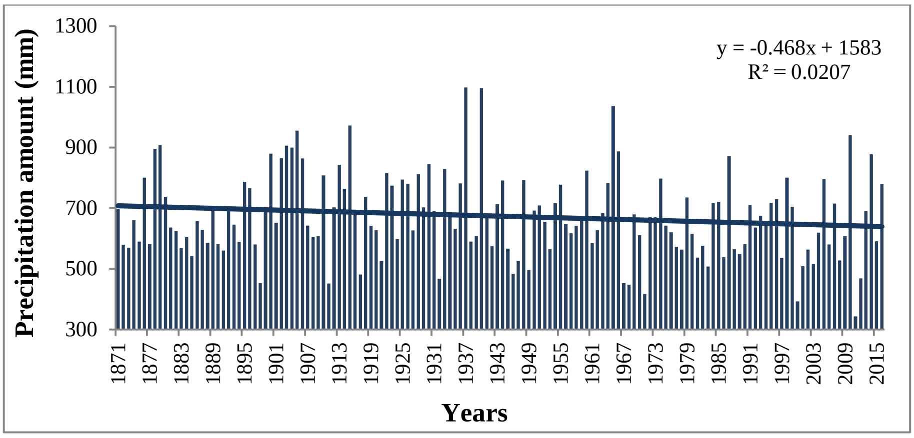

Fig. 9 The development of annual precipitation sums in Keszthely (1871-2016), p=0.083

Based on the linear trend, a significant downward tendency was fitted to the data. The decrease was also apparent from the average annual rainfall in the period from 1981 to 2010 which was resulted only 637 mm. Examined the annual precipitation amounts, these values was fluctuated on a wide scale, with a relatively large variation. Over the 146-year study period there were 16 years when the annual rainfalls were over 850 mm, moreover in three cases they were higher than 1000 mm (1098 mm in 1937, 1096 mm in 1940 and 1036 mm in 1965). There were 12 years, when the annual amounts remained below 500 mm, from which 2 years were extremely dry (393 mm in 2000 and 343 mm in 2011). The difference was more than twice between the lowest and the highest measured rainfall. Thirty-year standard climatic normal values were generated for ten-year slid periods presented in *Fig. 10*. From the beginning of the second third of the 20th century a significant decrease was observed.

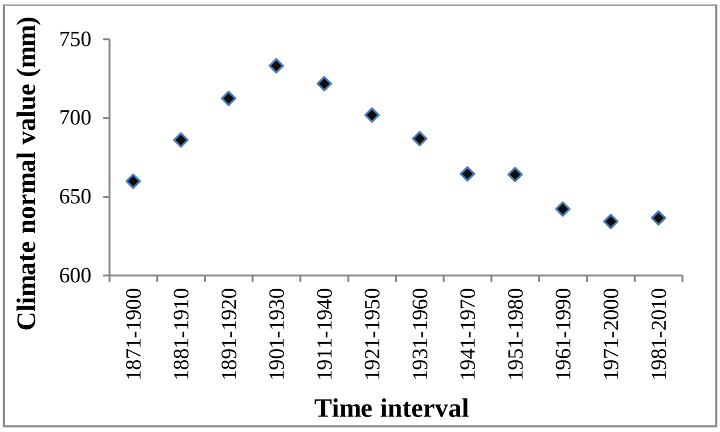

Fig. 10 Ten years slid climate normal values between1871-2010

The driest period was January-March, and in the rest of the year the precipitation was relatively evenly distributed (*Fig. 11*). For the whole period the rainiest month was June and the driest one was January. From March, the rainfall gradually increased until June. Among the monthly sums, the precipitation amounts of April (-15.9 mm per 100 years) and October (-18.9 mm per 100 years) showed statistically decreasing trends. These tendencies are unfavourable for the agricultural cultivation in this region. With the exception of August, September and December, a downward trend in precipitation was observed in comparison to the entire period.

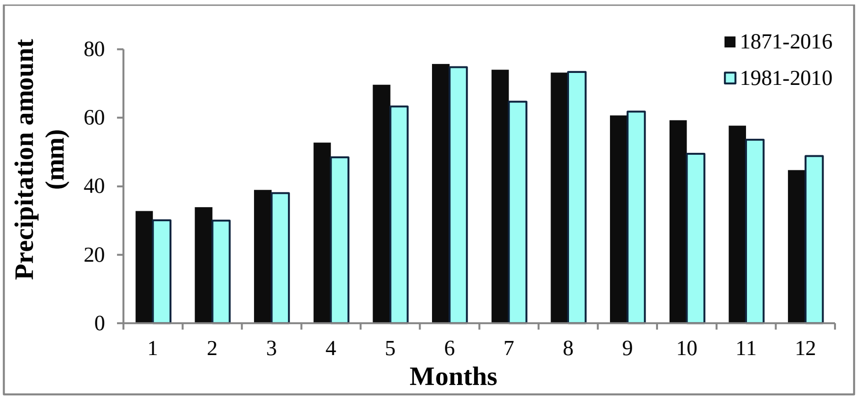

Fig. 11 The monthly average rainfalls between 1871-2016 and 1981-2010

The incidence and change in the occurrence of events and periods with more abundant precipitation or persistent drought also characterize the climate change. Days with precipitation of more than 20 mm showed a slight increasing tendency (*Fig. 12*). The upward trend in average daily precipitations suggests that precipitation was increasingly falling in the form of short-term, intense showers and thunderstorms.

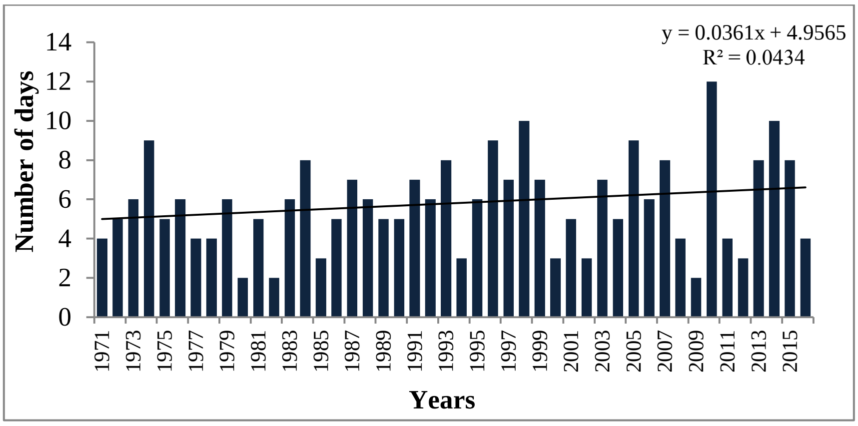

Fig. 12 The number of days with ≥20 mm precipitation between 1971 and 2016

Most arable is hard to tolerate rainless periods of 5-10 or more days and suffer irreversible damage (Szász, 1994). *Fig. 13* shows the length of dry periods in two different time intervals: from 10 to 14 days and over 15 days without precipitation. Between 1971 and 2016, on average there were two 15-day and three 10-14-day periods without precipitation yearly. In Keszthely, less rainy day were observed as we approached to the present. The longest period when the daily rainfall was less than 1 mm, had increased in the last decades.

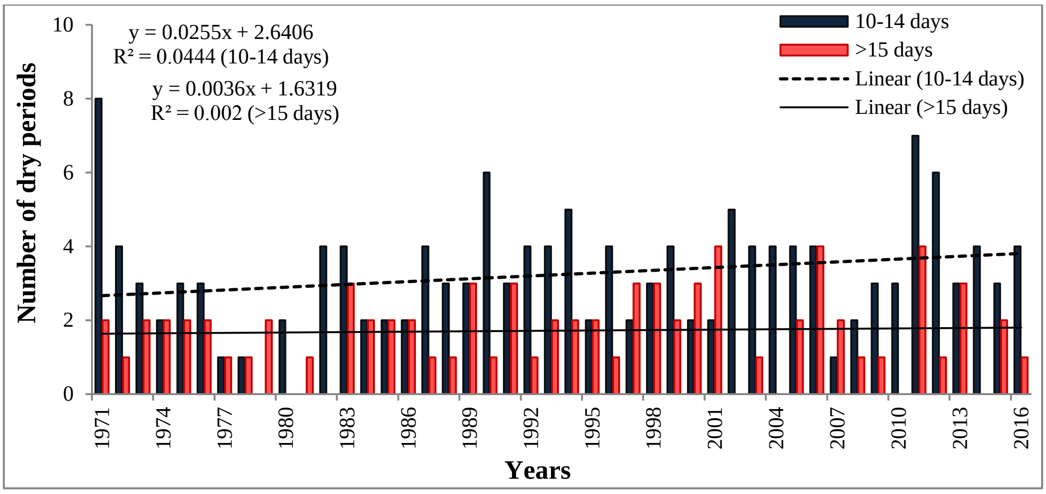

Fig. 13 The number of dry periods between 1981-2016

Discussion

Local scale study on the air temperature and precipitation trends showed the modification of the climate in Keszthely region. The results obtained a significant warming and downward precipitation tendency. The climate analysis highlights that information from development of these variables for short to long term planning in different sectors of economy is indispensable. Water management, agriculture, food production, biodiversity, forestry, tourism and human health can be the most affected sectors in the future. Increasing number of hot days during summers will have impacts towards higher energy consumption, as well. Due to warming and decreasing precipitation the rate of evaporation will be higher than the incoming amounts, which can affect on the water mass of Lake Balaton and its catchment area. If this trend continued in a long-term, the water level would decline. As a short-term positive effect, the water mass heats up sooner (in spring) and cools down later (in autumn), which could have beneficial on tourism and beaching by extending the holiday season. In accordance with other studies, for adjacent territories the trend of increasing risk of summer draughts needs to be taken into account. From the point of view of risk assessment it is very important to mapping this very vulnerable region and contributes to prioritization of measures for mitigation and adaptation of negative impacts. Climate warming has resulted in a significant upward shift in species optimum elevation and location, besides natural migration is proved slower than the climate spatial shifts. Therefore, projecting the climate change may likely drive major part of Hungarian agriculture to irreversible transformation. Shift of varieties is expected and variety selection is required. For planning of agro-technical measures are needed for a short and long term planning in struggle with the draught and high temperatures damage, and consequently risks from diseases and pests. Because of the drought and rising temperatures, the risk from forest fires may also grow. As a positive effect, the prolongation of the growth season is expected.

Acknowledgement

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DECOMPOSITION DYNAMICS OF *PHRAGMITES AUSTRALIS* LEAVES, STALKS AND RHIZOMES IN THE AREA OF LAKE BALATON AND KIS-BALATON WETLAND

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Abstract

The decomposition of plant litter is an important mechanism in regard to energy and nutrient dynamics of ecosystems. The decomposition dynamics of three plant parts of *Phragmites australis* (leaves, stalks and rhizomes) and the changes of total nitrogen and phosphorous concentrations were examined in Lake Balaton and Kis-Balaton Wetland for 230 days. The commonly applied litter bag technique was used, with two mesh sizes (litter bag mesh sizes ø = 3 mm; and plankton net bag mesh sizes ø = 900 µm). Leaf litter mass loss generally did not differ between the two mesh sizes and the study sites. The highest decomposition rates were observed at rhizomes (k=0.0051) and the slowest at stalks (k=0.0004). At the end of the investigation period, the remaining nutrient concentration was different in the three plant parts of *P. australis*. Nitrogen and phosphorous at the stalks in Lake Balaton was higher compared to the initial concentration. In the case of the leaves and rhizomes a decrease was observed.

Key Words: *Phragmites australis,* Lake Balaton, Kis-Balaton Wetland, leaf litter decomposition

Összefoglalás

A növényi anyag bomlása fontos mechanizmusa az ökoszisztémák energia- és tápanyagdinamikájának. 230 napos kísérletben vizsgáltuk a közönséges nád (*Phragmites australis*) három növényi részének (levél, szár és rizóma) lebontási ütemét és a visszamaradt teljes nitrogén és foszfor mennyiségét a Balaton (tó) és a Kis-Balaton (wetland) területén. A kísérlet során avarzsákos módszert alkalmaztunk, két lyukbőséggel (avarzsák lyukátmérő ø = 3 mm és planktonháló zsák lyukátmérő ø = 900 µm). A nád növényi részeinek tömegvesztése általában nem különbözött a két lyukbőségű zsák és a kísérleti területek között. A bomlási sebesség a leggyorsabb a rizóma esetében volt (k = 0,0051), míg a szárnál figyeltük meg a legalacsonyabb értékeket (k = 0,0004). A vizsgálati időszak végén a nád három növényrészében mért visszamaradt tápanyag-koncentráció eltérő volt. A balatoni nád szár esetében a nitrogén és a foszfor magasabb volt, mint a kezdeti koncentráció. A levél és a rizóma esetében csökkenés volt megfigyelhető.

Kulcsszavak: *Phragmites australis,* Balaton, Kis-Balaton, avarlebontás

Introduction

Common reed (*Phragmites australis* Cav. (Trin.) is one of the most abundant wetland plants world-wide (Schaller et al, 2016). *P. australis* substantially improves the total nitrogen and total phosphorous removal efficiency in wetland ecosystems, due to its high growth rate and great capacity for nutrient accumulation in stalks, roots, and rhizomes (Vymazal, 2005). Litter decomposition rates and nutrient dynamics also depend on a large extent on chemical properties (e.g., C, N and P concentrations) of the plant detritus material (Enríquez et al., 1993; Lee and Bukaveckas, 2002) and physical-chemical conditions of the water (Faye et al., 2006, Pozo, 1993). To better protect and manage lake and wetland ecosystems, it is important to understand the interacting forces that supports their functioning (Raposeiro et al, 2017).

In this study, the decomposition rate and the remaining nutrient concentrations were investigated in the Keszthely Bay of Lake Balaton and Fenéki Pond of Kis-Balaton Wetland using three plant parts of *P. australis* litter (leaves, rhizomes and stalks) in a 230 days long experiment.

Materials and Methods

Study Sites

The study was conducted the Lake Balaton and Kis-Balaton Wetland, Hungary. Lake Balaton is the largest shallow lake in Central Europe (Crossetti et al., 2013), connected to the Kis-Balaton Wetland, which serves as a filter for the lake (Anda et al., 2017). Lake Balaton lies in the western part of Hungary at an altitude of 104 m; its area is 589 km^2 and its average depth is 3 m (Dill, 1990). The westernmost of the four bays of Lake Balaton is Keszthely Bay, where an experiment was set up (17° 14' 46.3" E and 46° 43' 32.1" N, Fig. 1.). In order to protect the water quality of Lake Balaton, a wetland reconstruction of Kis-Balaton (Hídvégi and Fenéki Pond) was completed in 2015. Second part in our experiment was conducted in the Ingói Bay of Fenéki Pond (17° 11' 46.4" E and 46° 38' 37.4" N, Fig. 1.). Unlike Keszthely Bay, this area is typically a wetland.

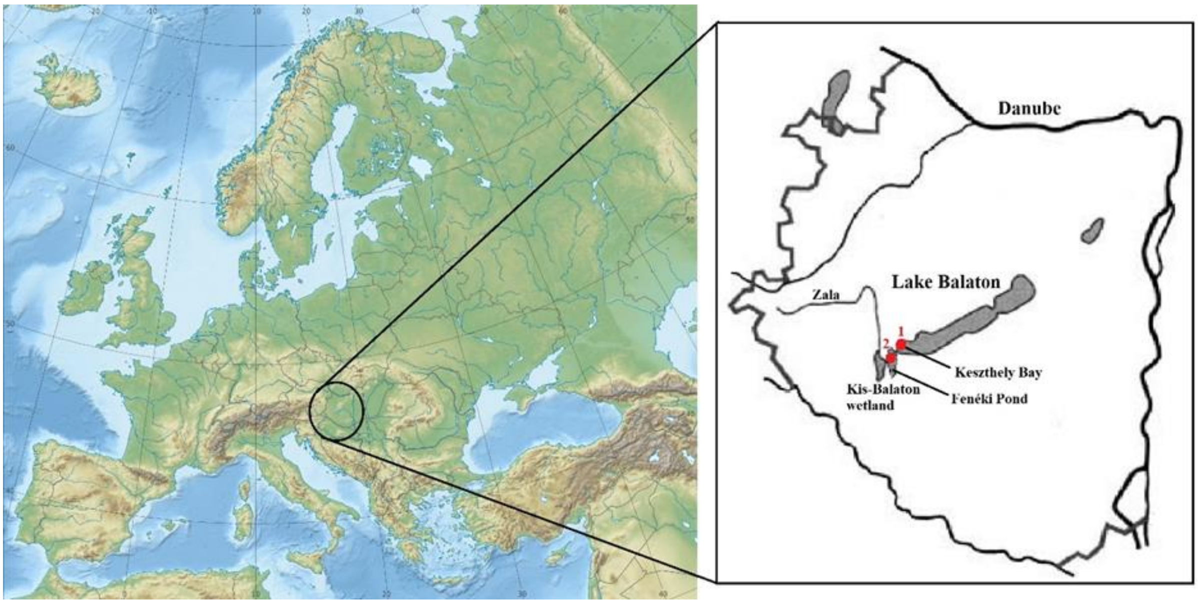

Figure 1. The sampling sites of Lake Balaton (1) and Kis-Balaton Wetland (b) (hu.wikipedia.org)

Plant Litter Decomposition

The decomposition of the dominant emergent macrophyte, *Phragmites australis* was analysed with the litter bag technique (Bärlocher et al., 2005). Standing dead leaves, stems and rhizomes were collected in October 2017 from the area of Lake Balaton and Kis-Balaton Wetland. Samples were air dried at room temperature to a constant weight. Whole parts of *P. australis* were fragmented to produce a coarse litter containing natural proportions. 10-10 g sample of plant material was transferred into polyethylene bags with two mesh sizes (litter bag mesh size ø = 3 mm; and plankton net bag mesh size ø = 900 µm). A total of 360 litter bags were positioned on 16 November 2017 at the water–sediment interface of the experimental area. Three replicates of the sample bags were collected 14, 32, 48, 60, 74, 123, 144, 158, 197 and 230 days after the start of the experiment. On each sampling occasion water samples were taken, from which pH, conductivity, PO_4^-, NH_4^+, NO_3^-, SO_4^{2-}, Cl^- were determined in the laboratory. Plant material was transported to the laboratory and washed with water. Samples were air dried for approximately 10 days to a constant weight. The dried litter was weighed to determine weight loss. The concentrations of phosphorous and nitrogen of the litters were determined using a spectrophotometer method at the end of experiment.

Data analysis

Litter decomposition rates were calculated of the simple first-order model, which assumes that litter decomposes at a constant rate over time (Jenny et al., 1949; Olson, 1963):

$$W_t = W_0 e^{-kt}$$

where t is the time (d), W_t the litter dry matter remaining at time t relative (g), W_0 the initial litter dry matter at time 0 (g), e the base of natural logarithm and k is the decomposition rate coefficient (d^{-1}). Differences in dry mass remaining, litter nutrient concentrations among plant parts and the two study sites were examined by least-significant difference approach of t-test using the Microsoft Office Excel 2016.

Results and Discussion

Water quality

Regarding the physical and chemical variables of the water samples from the two study sites (Table 1), there was a significant difference between the pH values of the two sites ($p<0.001$). The pH was higher in Lake Balaton, than in Kis-Balaton Wetland, which can be explained by the presence of organic acids in water (Gaudet and Muthuri, 1981). There was no significant difference ($p=0.3695$) in conductivity between the two sampling points, but Kis-Balaton Wetland has a higher conductivity in average (758.2±164.16 $\mu S\ cm^{-1}$). Dahrouga et al. (2016) made the statement, that denser bacterial biomass causes higher conductivity . Esteves (1988) concluded, that conductivity values also related to the trophic level of water.

Table 1 Changes in the main physical and chemical parameters of water during the decomposition period in Lake Balaton and Kis-Balaton Wetland

	Lake Balaton	**Kis-Balaton Wetland**
pH	8.3 ± 0.29	7.5 ± 0.32
Conductivity ($\mu S\ cm^{-1}$)	677.8 ± 134.0	758.2 ± 164.16
NO_3^- ($mg\ l^{-1}$)	u.r.	u.r.
NH_4^+ ($mg\ l^{-1}$)	0.77 ± 0.35	1.08 ± 1.17
SO_4^{2-} ($mg\ l^{-1}$)	187.0 ± 92.80	172.5 ± 112.55
PO_4^{3-} ($mg\ l^{-1}$)	0.31 ± 0.20	0.61 ± 0.35
Cl^- ($mg\ l^{-1}$)	43.2 ± 16.45	43.0 ± 21.76

The presence of microorganisms and ammonia-volatility (Reddy and Sacco, 1981) caused low concentration of NO_3^-.. Between the two habitats, there were no significant differences regarding SO_4^{2-} ($p=0.6247$) and Cl^- ($p=0.7242$) concentrations. . Kis-Balaton

Wetland had higher NH_4^+ concentrations (1.08±1.17 mg l^{-1}), than Lake Balaton (0.77±0.35 mg l^{-1}), the difference is not significant ($p=0.1705$). The PO_4^{3-} concentrations of the two study sites were different on a large-scale ($p=0.0307$), twice as high in Kis-Balaton Wetland, than in Lake Balaton. Excessive concentrations of N and P are the most common causes of eutrophication in freshwater lakes and reservoirs (Correll, 1998). Eutrophication - the excessive enrichment of mineral nutrients in receiving waters - results an excessive production of autotrophs, especially algae and cyanobacteria, which could be observed in the study sites.

Emergent plant decomposition

Respect of *P. australis* leaves, 54% - 59% of the initial dry mass remained in the litter bags after 230 days of incubation in Lake Balaton and Kis-Balaton Wetland (Figure 2a and b). The rate was higher in the litterbags, than plankton net bags. There was no significant correlation between leaf litter mass losses in the large and small mesh size bags in Lake Balaton ($p=0.5231$) and Kis-Balaton Wetland ($p=0.2814$). The amount of the remaining *P. australis* stalks (Figure 2c and d) were 76% in the litter bags (Lake Balaton), whereas 80 % retained in the plankton net bags (Kis-Balaton Wetland) of the original weight. The reduction in the dry weight of *P. australis* rhizomes (Figure 2e and f) in two water bodies was 50 - 53%. No significant difference was found between the litter bags and the plankton net bags either in Lake Balaton (stalks $p=0.1247$; rhizomes $p=0.0945$) or in Kis-Balaton Wetland (stalks $p=0.7256$; rhizomes $p=0.1036$).

There were significant correlations in the litter bags between leaves and stalks (Lake Balaton $p<0.001$; Kis-Balaton Wetland $p<0.001$), leaves and rhizomes (Lake Balaton and Kis-Balaton Wetland $p<0.001$) and stalks and rhizomes (Lake Balaton and Kis-Balaton Wetland

$p<0.001$). There were no significant correlations between Lake Balaton and Kis-Balaton Wetland regarding leaves ($p=0.1122$), stalks ($p=0.3158$) and rhizomes ($p=0.1274$) in the mass loss. A similar tendency was observed for plankton net bags.

Duke et al. (2015) described 49% weight loss was in *Phragmites* litter in Lake Erie. (144 days). At the same place, Rothman and Bouchard (2007) detected 86% weight loss during the 208 days of the investigation period. Findlay et al. (2002) examined the decomposition dynamics of *P. australis* in the Tivoli North Bay of the Hudson River and found that during the 3 years study period 28.5% of the original weight remained. The conflicting results among other studies comparing decomposition of *Phragmites* litter can be explained by the importance of environmental conditions.

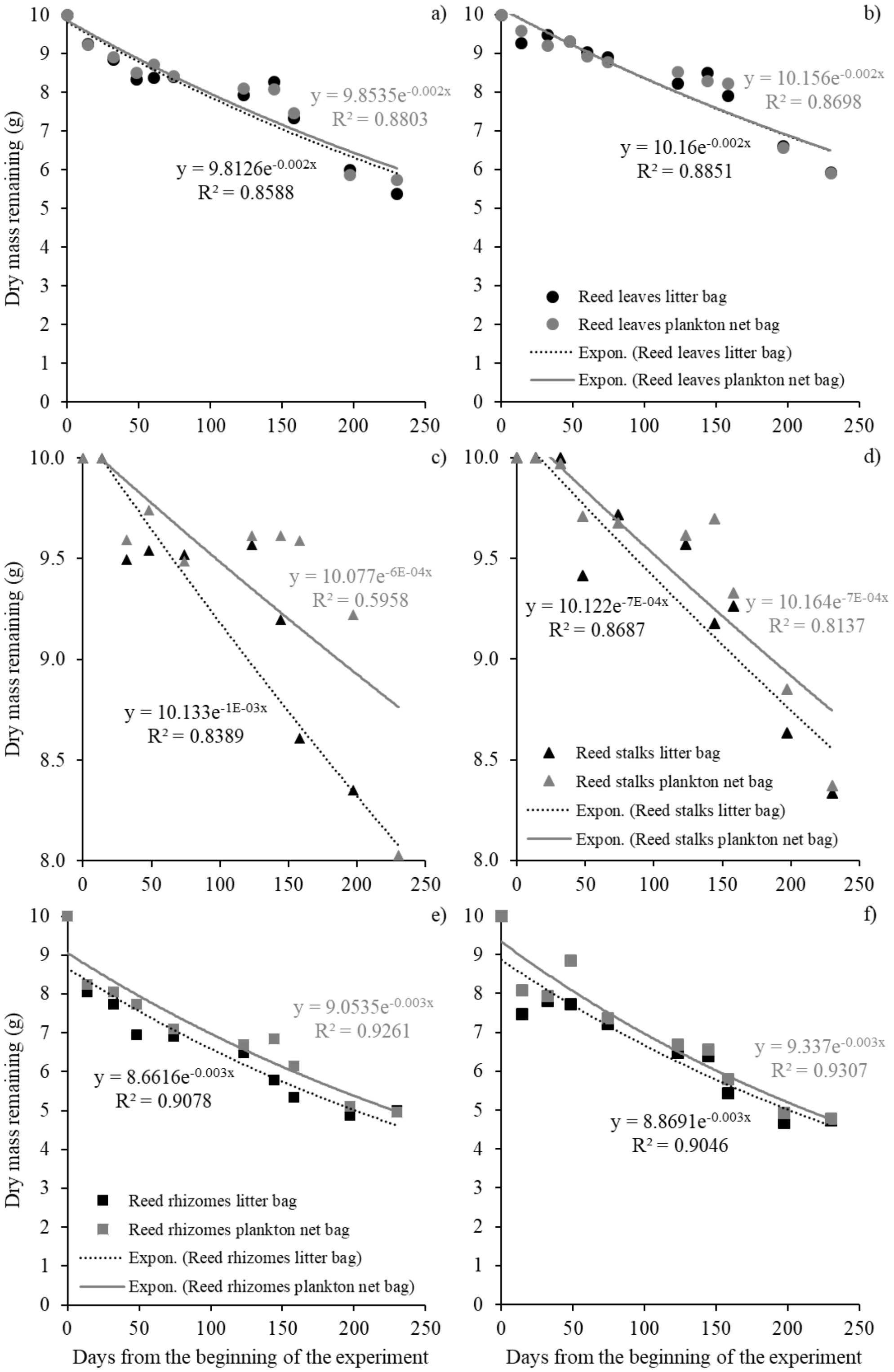

Figure 2. The remaining dry mass for P. australis leaves (a and b), stalks (c and d) and rhizomes (e and f) during the 230 days long experiment in the area of Lake Balaton (a, c and e) and Kis-Balaton Wetland (b, d and f)

The exponential decay coefficients of *P. australis* leaves, stalks and rhizomes are presented in Figure 3. The k-values of rhizomes were high in Lake Balaton in the litterbags (k=0.0051), while k of stalks were low in Kis-Balaton Wetland in the plankton net bag (k=0.0004). The exponential decay coefficients were high in Lake Balaton in case of leaves and stalks and, in Kis-Balaton Wetland (both types of bags) in case of rhizomes. The rhizomes in the litterbags in Lake Balaton and Kis-Balaton Wetland were classified to the medium, all other samples to the slow category. Zhang et al. (2014) investigated the decomposition of *P. australis* leaves and stalks in area of the Shanyutan wetland in 2010 (90 and 210 days experimental period). They observed the k values of 0.0018-0.0053 for the leaves and 0.00096-0.00275 for the stalk. Their experimental results were close to ours.

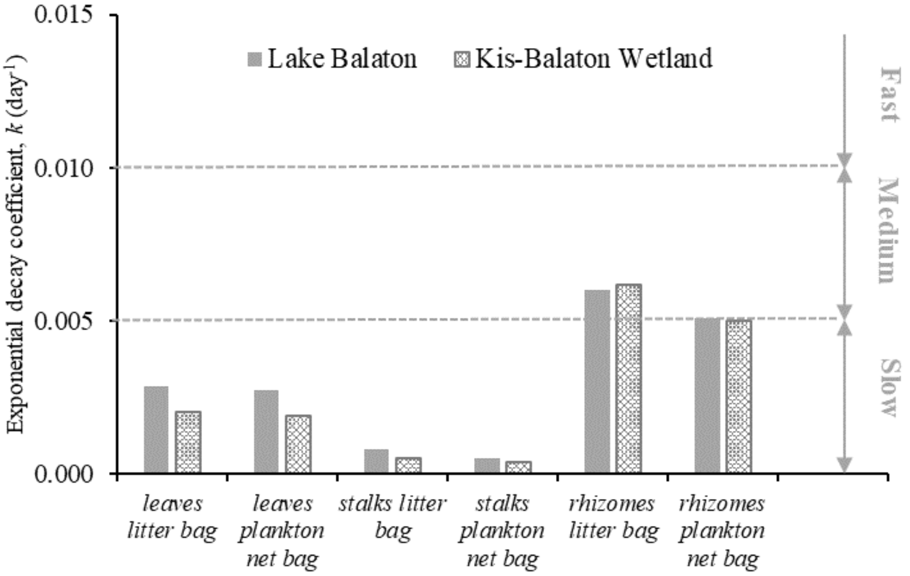

Figure 3. Decomposition coefficients (k) of P. australis leaves, stalks and rhizomes in Lake Balaton and Kis-Balaton Wetland

The remaining N and P concentrations in the decaying litter

Changes in the total nitrogen and total phosphorus contents in *P. australis* leaves, stalks and rhizomes at the beginning and end of the investigation period are presented in Figure 4. The initial phosphorous concentration was high in the rhizomes in Lake Balaton (0.104%) and Kis-Balaton Wetland (0.106%), while low in the stalks in Lake Balaton (0.012%) and Kis-Balaton Wetland (0.014%). At the end of the investigation period phosphorous concentration was reduced to near zero in the leaves and rhizomes, but in the stalks its amount increased.

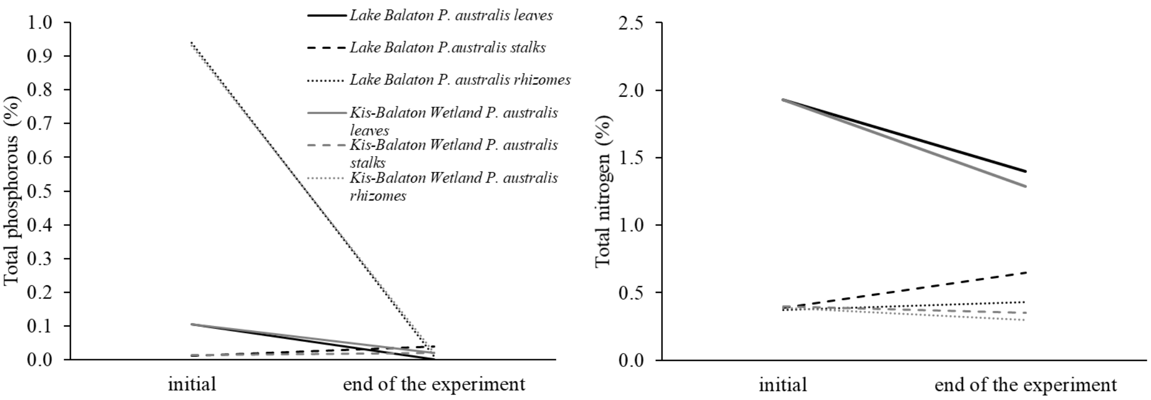

Figure 4. The remaining total nitrogen and total phosphorus contents in the P. australis leaves, stalks and rhizomes in the area of Lake Balaton and Kis-Balaton Wetland

The initial nitrogen concentration was high in the leaves (Lake Balaton 1.931%, Kis-Balaton Wetland 1.933%) and low nitrogen contents were detected in rhizomes (0.390% and 0.399%) and stalks (0.371% and 0.389%). The amount of phosphorus increased in the stalks in Lake Balaton, in all other samples the nitrogen content did not change (in Lake Balaton in rhizomes). The reason for the increase of nutrients was likely a biofilm formation, which could not be removed completely during the washing of the samples.

Zhang et al. (2014) observed, that by the end of their experiment, the litter nitrogen concentration in the leaves, stalks and flowers increased by 12% and 99% of the initial value of the sediment-surface phase for *P. australis*. The high phosphorus leaching from macrophytes may be related to the large inorganic fraction of phosphorus in tissues (Twilley et al., 1986). The increased P concentration during decomposition is usually caused by decomposer microbes associated with the plant tissue immobilizing P nutrient for their own growth (Pagioro and Thomaz, 1999; Ozalp et al., 2007). Köchy and Wilson (1997) suggested that nutrient loss depended on both litter quality (C, N, P) and nutrient availability in the surrounding environments.

Acknowledgement

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RELATIONSHIP BETWEEN WATER SUPPLY AND CROP YIELD COMPONENTS ON TWO SOYBEAN VARIETIES

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Abstract

Water stress is one of the most significant abiotic stress factors that leads to the loss of soybean production worldwide. The objective of this study was to quantify the water stress effects on two indeterminate type soybean varieties, *Sinara* (*Sn*) and *Sigalia* (*Sg*). Two water treatments were used: unlimited watering and 50% water withdrawal compared to optimal one. The experiment was set up in a modified evapotranspirometer. Seed yield, total dry matter, number of pods and seeds were measured during the harvest. Our results showed that water depletion significantly reduced the examined examined crop yield components. However, the varieties did not always react the same way to the lack of water: *Sn* produced higher yield with optimal water supply, while *Sg* produced higher number of pods and seeds.

Key Words: soybean, *Glycine max* L., water stress, unlimited watering, evapotranspirometer

Összefoglalás

A vízstressz az egyik legjelentősebb abiotikus stressz tényező, amely a szója termeléskiesését eredményezi világszerte. A vizsgálat célja volt, hogy meghatározzuk két indeterminált szójafajta, a *Sinara* (*Sn*) és a *Sigalia* (*Sg*) vízstresszre adott válaszát. Két vízellátást alkalmaztunk: korlátlan vízellátást és 50% -os vízmegvonást az optimálishoz képest. A kísérletet átalakított evapotranspirométerekben állítottuk be. A termésmennyiséget, a teljes szárazanyagot, a hüvelyek és magok számát a betakarítás során mértük. Eredményeink azt mutatták, hogy a vízmegvonás jelentősen csökkentette a vizsgált elemeket. A fajták azonban nem mindig reagáltak ugyanolyan módon a vízhiányra: az *Sn* az optimális vízellátással magasabb terméshozamot produkált, míg az *Sg* több hüvelyt és magot nevelt.

Kulcsszavak: szója, *Glycine max* L., vízstressz, korlátlan vízellátás, evapotranspirométer

Introduction

Drought is one of the most important environmental stresses in agriculture (Pardo et al., 2015). Soybean is the most widely grown oil and protein crop in the world. Extremely hot weather and water stress – as the impacts of global climate change - negatively affect the soybean production (Ergo et al., 2018). Furthermore, soybean yield losses due to unpredictable variability of precipitation and limited ground water reservoirs continue to exist (Le et al., 2012). Many efforts have therefore been made to improve crop productivity under water-limited conditions.

One of our options for preventing soybean yield loss is to increase irrigated areas (Heatherly, 1983), while the other one is the growing of drought-tolerant genotypes (Chapman 2008). In the future, the combination of the two previously mentioned possibilities is likely to achieve optimum yield and its high quality. It is crucial to develop strategies for coping with the effects of biotic stress to assist in stabilizing yield under stress conditions (Ries et al., 2012). To realize the expected yield production, it is necessary to know more about the specific soybean water stress response in more details.

The objective of this work was to determine the amount of crop yield, numbers of pods and seeds and total dry matter in two drought tolerant soybean varieties in evapotranspirometers (*Sinara, Sn* and *Sigalia, Sg*) exposed to water deficit during the growing season in 2018 and compared to that obtained under unlimited watering.

Materials and Methods

The experiment was conducted during the growing season of 2018 at the Agrometeorological Research Station of University of Pannonia, Georgikon Faculty, located in Keszthely (Hungary). Two soybean varieties from Karintia Ltd. *Sn* and *Sg* (of maturity group 0 and indeterminate growth habit) were sowed on April 26th. These varieties were chosen because of their wide use by Hungarian farmers, the similar length of their reproductive phases and their good drought tolerance. The seeds were hand-planted in evapotranspirometers with a stand density of 60 plants m^{-2}.

The evapotranspirometers are 2x2 m^2 surface area and 1 m deep open tanks, sunken under the ground, which are filled with soil (Ramann-type brown forest soil) and covered with seeded or planted vegetation (Figure 1). A water table is maintained in the soil at a given depth to secure optimal water supply. In our experiment, four of the eight evapotranspirometers were used for water withdrawal, so that only half of the optimal water supply was given to the plants, while unlimited watering was given to the other four tanks (Anda et al., 2018).

Figure 1. The evapotranspirometers with soybean

In each water supply level and variety, 5-5 plants were selected and harvested randomly for determining the average yielding characteristics. Plants were cut by hand, all pods of these plants were counted. Pods were opened by hand, retrieved seeds were dried until constant weight.

Differences in seed yield, total dry matter, the number of seed yield and pods in the two water supplies and varieties were examined by the least-significant difference approach of t-test and regression analysis using Microsoft Office Excel 2016.

Results and Discussion

The relationship between seed yield/plant and total dry matter/plant was positive and highly significant in the two varieties and two water treatments (Figure 3). The optimal water supply resulted in a proportional increase in seed yield and total dry matter. This relationship was linear ($R^2 = 0.75$; $p < 0.001$). Compared to optimal level, seed yield and total dry biomass in half-water supply decreased significantly in both varieties. In the case of *Sn*, the dry matter decreased by 27.6% ($p < 0.001$) and seed yield by 47.8% ($p < 0.001$). Reductions of dry matter (18.2%, $p < 0.001$) and seed yield (41.4%, $p = 0.001$) in *Sg* were lower than in *Sn*, under water shortage. No difference was found in dry matter between the two varieties for unlimited watering ($p = 0.095$) and water withdrawal ($p = 0.176$). There was no difference between the two varieties in the seed yield of the water deprivation ($p = 0.452$), but besides the unlimited water supply there was a significant difference ($p < 0.001$) for the benefit of *Sn*. This means that *Sn* under unlimited water supply had higher seed yield compared to *Sg*, but did not respond worse to water withdrawal than *Sg*.

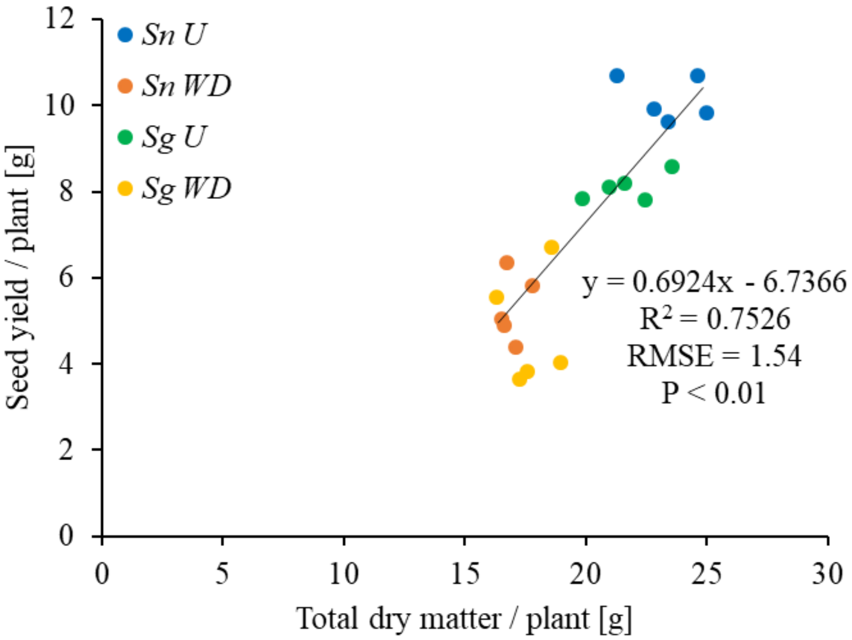

Figure 2. The relationship between seed yield and total dry matter for each water supply level (U – unlimited watering; WD – water deprivation) and variety (Sn – Sinara, Sg – Sigalia)

A linear relationship (Figure 3) was also found ($R^2 = 0.82$; $p < 0.001$) between the number of seeds and the number of pods tested in the two water supplies and varieties. Water withdrawal proportionally decreased the number of pods (*Sn* $p = 0.003$; *Sg* $p < 0.001$) and seeds (*Sn* p = 0.041; *Sg* p < 0.001). In addition in optimal water supply, *Sg* had 17.3% (p = 0.006) more pods and 17.7% (p = 0.003) more seeds, than *Sn*. In water deprivation there was a small difference between the two varieties in the number of pods (0.8%, p = 0.915), but there was a significant difference in the number of seeds: *Sn* produced 24% more seeds than *Sg* (p = 0.007). It can be concluded, that *Sg* developed more seeds and pods in the optimum water supply as compared to *Sn*, but the number of seeds was very sensitive due to water withdrawal.

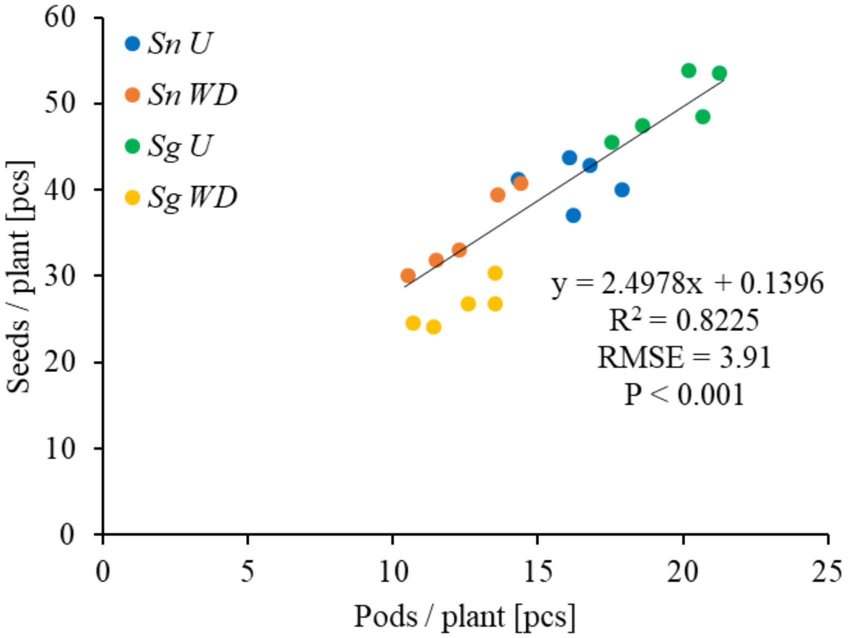

Figure 3. The effect of the number of seed yield and pods for two varieties (Sn – Sinara, Sg – Sigalia) and two water supplies (U – unlimited watering; WD – water deprivation)

In the experiment of Ergo et al. (2018), yield decreased by 43% under water stress (the number of seeds by 24% and their weight by 25%) in comparison to controls (non-heat-stressed and non-water-stressed plots). However, no difference was found between the two soybean genotypes (Syngenta Company SPS4×4 RR and SPS4×99 RR) in the yield, weight of grains and number of grains they used in different water availabilities. Dornbos and Mullen

(1991) had similar results when plants were grown under severe drought conditions. Karam et al. (2005) studied soybean using three deficit irrigation treatments (R2, R5 and R7 phenological stages), besides, a control was designed to receive a full irrigation at 100% of field capacity with no water restriction. According to their results, the water loss in the R5 phenophase resulted in a high deficit against to other water treatments. Compared to the control seed yield (28.1%), total dry matter (6.9%), number of pods (17.8%) and seeds (19.0%) decreased

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COMPARISON OF LEAF AREA OF SOYBEAN UNDER UNLIMITED WATERING AND RAIN-FED CONDITION

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Abstract

Different methods have been used to measure leaf area of soybean (*Glycine max* L.). In this study, high resolution photos of soybean leaves were processed using a histogram-based threshold method. Leaf area was measured for soybean in the growing season of 2018 at the Agrometeorological Research Station, Keszthely, Hungary, in evapotranspirometers (unlimited watering) and on the field with natural water supply (rain-fed plots). The experiment was carried out with two varieties (*Sinara* and *Sigalia*). During the investigation period leaf area was measured weekly. The results showed that there was no significant difference in leaf area between the two varieties. Optimum water supply for *Sigalia* resulted in significantly higher leaf area compared to natural water supply.

Key Words: soybean, *Glycine max* L., leaf area, unlimited watering, natural water supply

Összefoglalás

A szója (*Glycine max* L.) levélterületének mérésére különböző módszereket alkalmaznak. Vizsgálatunkban a szója leveleket fotóztuk, és hisztogram alapú szegmentálással dolgoztuk fel. A szója levélterületét 2018 tenyészidőszakában mértük a Keszthelyi Agrometeorológiai Kutatóállomáson, evapotranspirométerekben (korlátlan öntözés) és a természetes vízellátású szántóföldi körülmények között. A kísérletet két szójafajta bevonásával (*Sinara* és *Sigalia*) végeztük. A vizsgálati időszak alatt a levélterületet hetente mértük. Az eredmények azt mutatták, hogy a két fajta levélterülete között nem volt szignifikáns különbség. A *Sigalia* optimális vízellátása szignifikánsan magasabb levélterületet eredményezett a természetes vízellátású parcellán mért levélterületekhez képest.

Kulcsszavak: szója, *Glycine max* L., levélterület, korlátlan vízellátás, természetes vízellátás

Introduction

Soybean yield is highly dependent on weather conditions (Sentelhas et al., 2015). Most of the Hungarian soybean crops are grown under rain-fed conditions. Based on previous observations, the local effects of climate change are increasingly detectable. Additionally, worldwide crop productivity under rain-fed conditions will need to be enhanced to meet increasingly growing demand for food (Bhatia et al., 2008).

Gas exchange processes (mainly CO_2 and water vapour) between the atmosphere and the canopy occur through the leaves. From the size of the leaf area the crop biomass and final yield of the plant can be deduced (Kross et al., 2015). However, there are many environmental factors affecting the leaf area and yield, especially the available water and air temperature.

Climate change gives a high importance of local leaf area measurements for monitoring crop growth conditions on the fields (Canisius et al., 2010; Liu et al., 2010a). The leaf area can be directly or indirectly measured by several methods (Gower et al. 1999; White et al. 2000, Asner et al. 2000), using either a leaf area meter or a specific relationship of dimension to area via a shape coefficient (Grace, 1987; Barclay, 1998; Sellin, 2000). Using high-resolution digital cameras and image processing software are a relatively new method of determining leaf area.

In this study, the temporal dynamics of leaf area during the growing season of 2018 were measured in two soybean varieties (*Sinara* and *Sigalia*) of two different water supply levels (unlimited watering and rain-fed plots).

Materials and Methods

Experiments were conducted at the Agrometeorological Research Station, Keszthely, Hungary in the growing season of 2018. The study was carried out in evapotranspirometers (unlimited watering plots) and under field conditions (rain-fed plots). Prior to planting, 150 kg NPK ha^{-1} was applied during early spring. The two varieties of indeterminate soybeans (*Sinara* and *Sigalia*) were planted with 24 cm row spacing on 26 April 2018. Leaf area was weekly estimated from leaves taken from 10-10 plants in the two water supplies and two varieties.

Direct methods can provide reasonable estimates of leaf area. Digital photography is a popular, affordable and easy to use tool, which can be used to obtain field information. Proving the efficiency of digital photo analysis, Liu and Pattey (2010a) determined leaf area indexes (*LAI*) with a Li-Cor LAI-2000 Plant Canopy Analyser and vertical gap fractions derived from digital photographs, and they found a strong logarithmic relationship between the two methods ($R^2 = 0.84$).

In the study a digital camera (Canon EOS 7D) was used. Colour photos were weekly taken *in situ* on the study site from above the soybean leaves looking downward vertically. The leaves were placed in front of a red cardboard, then photographed, from a distance of approximately 0.2 m above the leaves, using automatic exposure and maximum resolution (Figure 1).

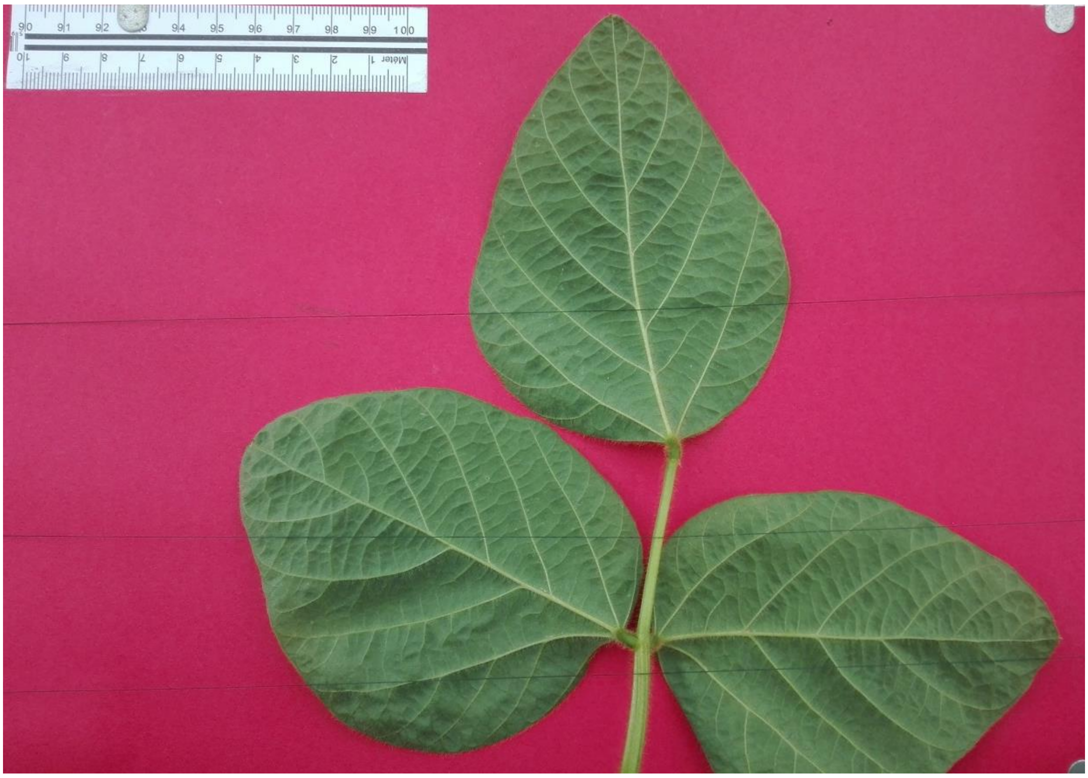

Figure 1. A soybean trifoliate on a red cardboard

The colour images were recorded in .jpeg formats. Each pixel of a photo consists of three digital numbers which are light intensity quantized in the red, green and blue bands. The photos were processed using a histogram-based threshold method to separate the green leaves from the red cardboard, using the SGDIP 0.1 program (Soós, 2010). The great advantage of the method is that the digital camera is an affordable way to acquire field information, furthermore the recorded images can be stored for later reviews (Liu and Pattey, 2010b).

Results and Discussion

Weekly mean leaf area in the growing season of 2018 (Figure 2) ranged from 168.2 ± 33.2 cm^2 (June 1, *Sigalia*) to 1955.35 ± 152.3 cm^2 (July 20, *Sinara*) in evapotranspirometers. *Sinara* has a higher leaf area like a tendency, although there was no significant difference between the two varieties (p=0.804).

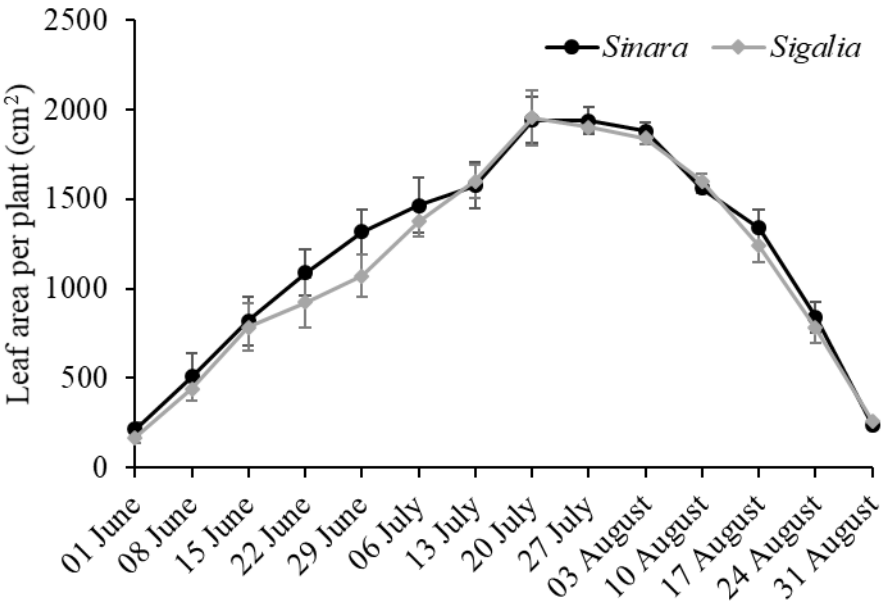

Figure 2. The weekly variation in leaf area of varieties Sinara and Sigalia using unlimited watering plots in the growing season of 2018

The weekly leaf area averages were also similar to that of obtained results for rain-fed plots (Figure 3). In the beginning, the two varieties of leaf area did not differ, but after June 29th the leaf area measured at *Sigalia* were lower. Overall, there was no difference between the leaf area developments of *Sinara* and *Sigalia* during the growing season (p=0.590).

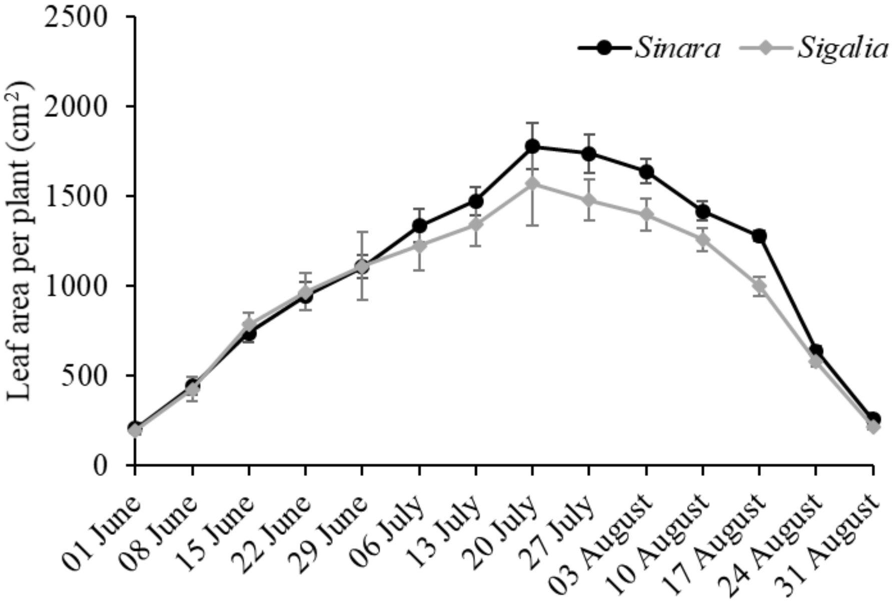

Figure 3. The weekly variation in leaf area of varieties Sinara and Sigalia using rain-fed plots in the growing season of 2018

When comparing the water supplies, it can be observed that for both varieties, there were larger leaf areas for unlimited watering plots than for rainfed crops. Higher maximums of leaf area, by 8.4% (p=0.572) for *Sinara* and by 19.6% (p=0.001) for *Sigalia* were measured, but the difference was only significant for *Sigalia*. In conclusion *Sigalia* - compared to *Sinara* - responds better to the changes in water supply.

Many studies deal with the exact exploration of the plant-water relationship, so there are many different results in the literature regarding soybean. Viña et al. (2011) measured 45.5% higher maximum leaf area in irrigated soybean than in natural water supply plants. In the experiment of Suker and Verma (2012) peak LAI varied from 4.4 to 5.6 m^2 m^{-2} in irrigated soybean, and for rain-fed soybean from 3.2 to 4.6 m^2 m^{-2}. The results of Karam et al. (2005) showed, that irrigation during R5 and R7 stages of plant growth had no effect on the leaf area patterns. The low crop growth rate at the previously mentioned stages can be caused by the high carbon and nitrogen translocation rates from the leaves to the seeds (Zeiher et al., 1982, De Souza et al., 1997).

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MODEL STUDY TO INVESTIGATE THE TOXIC INTERACTION BETWEEN KYLEO HERBICIDE AND LEAD ACETATE ON CHICKEN EMBRYOS

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Abstract

The aim of this study was to determine the individual and combined toxic effects of Kyleo herbicide (160 g/l 2,4-D; 240 g/l glyphosate) and lead acetate on the development of chicken embryos. The chicken eggs were dipped in the solution or emulsion of the test materials for 30 minutes before starting of incubation. The applied concentration of lead acetate was 0.01% and of herbicide Kyleo was 1%. The treatments were performed on day 0 of incubation, and the embryos were examined on day 19 by the followings: rate of embryo mortality, body weight, type of developmental anomalies by macroscopic examination. The body weight was evaluated statistically by one-way ANOVA with Tukey and Dunnett post-test, the mortality of embryo and the developmental anomalies were analysed by Fisher test. Lead acetate alone and in combination with herbicide significantly reduced the body weight of the embryos but statistically increased the mortality of embryo. Single and concomitant administration of lead acetate increased the rate of embryo mortality. Developmental abnormalities were observed sporadically due to the single and simultaneous administration of the test items. Based on the results there is a possibly additive toxic interaction between the lead acetate and Kyleo that can highly reduce the viability of the embryos or can lead to extinction of wild birds.

Keywords: chicken embryo, 2,4-D, glyphosate, lead acetate, toxic interaction, embryotoxicity

Összefoglalás

Napjainkban az emberiség egyik legnagyobb megoldásra váró problémája a természeti környezet növekvő mértékű elszennyeződése. A különböző vegyi anyagok egyidejűleg komoly kémiai terhelést jelenthetnek az egész élővilág számára, és mivel az interakciókra vonatkozóan csak kevés adat áll a rendelkezésünkre, ezért a legveszélyesebb nehézfémek körébe sorolt ólom, illetve egy széles körben alkalmazott herbicid, a glifozát és 2,4-D kombinált hatóanyagú Kyleo együttes méreghatásának tanulmányozását végeztük el. Arra kerestük a választ, hogy a környezetszennyezésből adódó állandó nehézfém-expozíció, kiegészítve egy gyakorlati permetlé töménységben alkalmazott Kyleo terheléssel; milyen változásokat indukál a tesztszervezetként választott házityúk-embrió fejlődésében. A kezeléseket a keltetést megelőző 0. napon végeztük el. Az embriókat vizsgálati anyagokból készült különböző koncentrációjú 38°C-os oldatba vagy emulzióba merítettük 30 perc kontakt időre. A kísérlet során az ólom-acetátot 0,01%-os, a herbicidet 1%-os koncentrációban alkalmaztuk. Az eredmények értékelése a keltetés 19. napján történt. A makroszkópos vizsgálat keretében lemértük az élő embriók testtömegét, feljegyeztük az elhullásokat és a fejlődési rendellenességeket és értékeltük azok gyakoriságát. A vizsgálati anyagokkal elvégzett egyedi és együttes kezelések eredményeként a gyomirtóval egyedileg kezelt csoporton kívül a kezelt csoportokban az embriók testtömeg értékei szignifikánsan kisebbek voltak a kontroll csoporthoz viszonyítva. Az egyedi és együttes kezelések következtében jelentkező elhullások száma - a herbiciddel egyedileg kezelt csoport kivételével - szignifikánsan emelkedett a kontroll csoporthoz képest. A fejlődési rendellenességek sporadikusan fordultak elő a kezelt csoportokban, teratogén hatás nem volt igazolható.

Kísérletünkben felhasznált 0,01%-os ólom-acetát oldat és Kyleo herbicid 1%-os emulziójának egyedi méreghatása embriótoxikus volt a tojásban fejlődő házityúk-embriókra. A kísérleti anyagok együttes alkalmazása során az embriótoxikus dózisú ólom-acetát mellett a növényvédelmi gyakorlatban felhasznált Kyleo gyomirtó szeres kezelés fokozta az embriótoxicitást, a toxikus interakció additív jellegű volt.

Introduction

The chemical plant protecting process is one of the most important polluting activities in the agricultural production. The ecosystem of a given habitat can be contaminated simultaneously by sprayed pesticides and other xenobiotics, e.g. heavy metals due to the agricultural activities during the plant protecting processes. Therefore, the chemical load can be occurred as a complex problem, so the combined toxic effect, i.e. toxic interaction of at least two substances can expected and the components can modify the effect of each other. Recently, the examination of the combination of heavy metals and other chemicals gained significant ground in both avian (Fejes et al., 2001; Kertész, 2001) and mammalian (Institóris et al., 2001; Pecze et al., 2001) toxicology research studies. Furthermore, the interaction effects are examined not only in the field of ecotoxicology, but also in all other areas that deal with health care and chemical safety issues (Oskarsson, 1983; Danielsson et al., 1984; Speijers and Speijers, 2004).

The different agricultural areas offer sources of food, shelter and breeding places to wild birds, therefore the sprayed pesticide and other chemical substances can contaminate not only the adults, but the embryos developing in egg, as well. The eggs of the wild birds may be exposed to different chemicals on the cultivated lands at the same time and their toxic effects may appear in embryo mortality and developmental anomalies.

Teratological tests carried out on avian embryos provide useful data for environmental protection and facilitate the development of environmental-friendly chemical plant protection techniques (Várnagy et al., 1996).

The chicken embryo test is quick and accurate and allows the chemical impact on the embryo to be investigated. The further advantage of this method is its low cost, the sensitivity against various agents, as well as its high degree of similarity to the morphological development of mammals (Korhonen et. al. 1981, 1982). Chicken embryos is a proper model animal. Testing with it is providing the protection of wild bird population.

The aim of our study was to examine the toxic effect and interaction of lead acetate and glyphosate and 2,4-D containing herbicide (Kyleo) on chicken embryos after single and simultaneous administration of the test items by immersion technique.

Materials and Methods

Fertile chicken eggs of Farm breeds (Goldavis Ltd., Sármellék, Hungary) were used in the study. The eggs based on their size and weight, were divided into four homologue groups (40 eggs/group). The experimental design is presented in Table 1. They were incubated in a Ragus type table incubator (Wien, Austria, in 2018) assuring the required temperature (37-38°C), the relative humidity (50-65%) and the daily twice rotation of them to prevent the adhesion of the embryo to the egg-shell (Bogenfürst, 2004).

Table 1. Experimental Design

Group	**No of eggs**	**Treatment**	
		Lead acetate	**Kyleo**
I (control)	40	-	-
II	40	0.01%	-
III	40	-	1%
IV	40	0.01%	1%

Before starting of incubation the eggs were immersed for 30 minutes into a solution or emulsion with a temperature of 38ºC, that were prepared from the test items or from their combination. Then they were placed onto a filter paper after treatment to soak the unnecessary liquid.

During the single and simultaneous administration lead acetate (Reanal-Ker Ltd., Budapest) was applied with a concentration of 0.01%. The dose was selected based on previous studies to produce toxic effect alone but not to all treated eggs (Fejes, 2005). The 2,4-D and glyphosate containing herbicide, Kyleo (Nufarm Hungary Ltd., 160 g/l; 360 g/l) was used as 1% solution corresponding to that used in plant protection practice. The control group was treated with avian physiological saline solution (NaCl, 0.75 w/v%). The details of the experimental design are presented in *Table 1*. All eggs and embryos were examined and processed on day 19 of incubation. During the processing rate of embryo mortality, body weight of embryos and type of developmental anomalies were registered.

The distribution of body weight of the live embryos was controlled by Comparison-Quantile Plot and was analysed statistically by one-way ANOVA. Data of groups were compared by

Tukey and Dunnett tests. The statistical analysis of the results of embryo mortality and developmental abnormalities were performed by Fisher's exact test (Baráth et al., 1996).

Results

The average body weight of the embryos was 20.27±1.27 g in Group II that was significantly lower as compared to the control group (21.35±1.27 g; p=0.05). Due to Kyleo treatment the body weight was reduced to 20.72±1.57 g without statistical difference compared to the control.

The simultaneous administration of lead acetate and Kyleo resulted in significant decrease of average body weight (Group IV: 20.21±1.59 g; p=0.05) as compared to the control (p=0.05) (*Figure 1*).

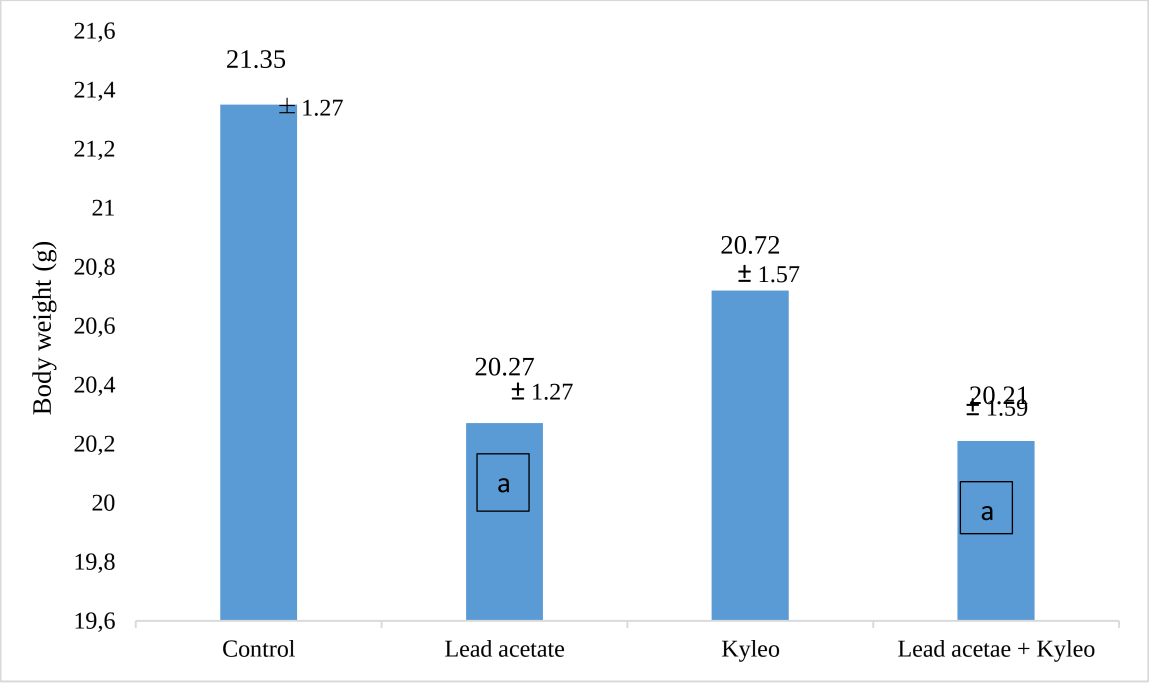

Figure 1. Body weight of the chicken embryos on day 19 of incubation from teratogenicity test on Kyleo and lead acetate after single and simultaneous administration (g)

a: Significant decrease as compared to the control(p=0.05)

The results of the embryo mortality and the developmental abnormalities are presented in *Table 2.*

There was no died embryos in the control group. The single administration of lead acetate increased the mortality up to 17.9% in Group II. The changes were statistically different ($p=0.05$) as compared to the Group I (control). The application of 1% Kyleo caused 5.1% of mortality in the treated embryos of Group III that was not significant as compared to the control. Due to the simultaneous administration of 0.01% lead acetate and 1% Kyleo (Group IV) induced significant increase ($p=0.5$) of embryo mortality (20.5%). Due to the simultaneous application of the test items the mortality rate of the embryos was increased up to 1.15 times higher compared to the Group II and 4 times higher compared to Group III. This increase is closely addition effect compared to the single treatments.

Table 2. Embryonic death and developmental anomalies from teratogenicity test of lead acetate and Kyleo in chicken embryos after single and combined administration

Group	Treatment	No of embryos showing abnormality/No of live embryos	Death No/ No fertile eggs	Rate of developmental anomalies (%)	Mortality (%)
I	Control	0/39	0/39	0	0
II	Lead acetate	2/39	7/39[a]	5.1	17.9
III	Kyleo	3/39	2/39	7.7	5.1
IV	Lead acetate + Kyleo	3/39	8/39[a]	7.7	20.5

a: Significant difference as compared to the control ($p=0.05$)

Developmental abnormalities were not recorded in the control group. The 0.01% lead acetate induced leg deformation and open abdomen (Group II: 2) without statistical difference as compared to Group I. The applied concentration of Kyleo (1%) and lead acetate (0.01%) induced leg deformation, growth retardation and beak malformation.

Discussions

The single treatment of lead acetate with 0.01% concentration induced embryotoxic effect in chicken embryo that manifested in significant decrease of body weight and elevated rate of embryo mortality.

The results of the individual teratogenicity studies on lead acetate in chicken are in accordance with results of toxicity studies in other species. Depending on the dose, lead has embryotoxic potential and may cause developmental anomalies (Ferm and Carpenter, 1967; Várnagy and Budai, 1995). Similar results were found in chicken embryos treated with 0.01% lead acetate (lower body weight, higher rate of embryo mortality) but the developmental anomalies were not significant versus the control group (Juhász, 2009).

The glyphosate and 2,4-D containing Kyleo plant protection product with herbicidal action was also embryotoxic on chicken embryos and resulted in non-significant reduce of body weight and increase of mortality.

Glyphosate containing Roundup herbicide was examined by other researchers in Wistar rats. Dams were treated orally with 500, 750 and 1000 mg/kg glyphosate via drinking water. The results showed 50% mortality rate of dams treated with 1000 mg/kg glyphosate. Skeletal alterations were observed in foetuses of the dams in groups treated with 500, 750 or 1000 mg/kg. Based on these data can be concluded that the glyphosate containing RoundUp is toxic to dams and induces developmental retardation of the foetal skeleton (Dallegrave et al., 2003).

Other studies reveal that acute treatment of male rats with 2,4-D caused reproductive system toxicity mainly with dose of 200 mg/kg (Marouani et al., 2016).

Due to the simultaneous application of lead acetate and Kyleo the embryo mortality was statistically higher than the individual effect.

Generally, the simultaneous application of heavy metals and pesticides may cause significant increase of their toxic effect in comparison with the individual toxicity of the applied components (Juhász, 2006).

According to the published literature the toxicity of many pesticide combinations is at least additive. In some cases pesticide mixtures, if they particularly contain insecticide component, have been shown to be synergistic effects, with reported increase in toxicity up to 100-fold. However, these effects are species, time and dose dependent, therefore difficult to predict it routinely (Thompson, 1996).

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CLIMATE CHANGE AND AGRICULTURE SECTOR IN EGYPT: EFFECTS AND ADAPTATION

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Abstract

The review aims to present an overview of the effects of climate change and the adaptation strategies to mitigate its impacts on the agriculture sector in Egypt. The main changes in the Egyptian climate would be an increase in temperature, evapotranspiration and sea level. Simulation studies show that reduce production, increase water demands of crops and lose agricultural lands are likely to be the main impacts of climate change on the Egyptian agriculture sector. The effects of climate change on aquaculture can be on natural resources that are necessary for aquaculture productions such as water availability, land, feed, seed, and energy inputs. However, different adaptation options involve that improving the technical water application efficiency and water conservation, as well as selection and breeding tolerant crops to heat, salinity and water use efficiency. Changing the time of sowing and management practices, encouraging farmers to adopt crops that high return with less water use and developing new crop models can consider other agricultural options for adapting climate change. The government should take extreme efforts to enhance the productivity of the agriculture sector and mitigate the risks of climate change impacts.

Keywords: climate change, Egyptian agriculture, Nile delta, vulnerability

Introduction

Egypt occupies the northeastern corner of Africa from 24° to 36° East Longitude and 22° to 31° North latitude. It is bordered in the east by the Red Sea and in the north by the Mediterranean Sea (Figure 1). The total land area of Egypt is 997,688 km^2, which can divide to three major geographical regions: the Nile Valley (Upper Egypt, Lower Egypt and the Nile Delta), the Eastern Desert, and the Western Desert (El-Ramady et al., 2013). Egypt has an arid climate with hot dry summers (May to October) and moderate winters (November to April). The average annual temperature is 14°C in winter and around 30°C in summer (El-Ramady et al., 2013). Rainfall in Egypt is minimal except in a narrow band along the northern coastal areas with 100–200 mm. The precipitation in the Delta is 40–60 mm, and in Middle Egypt less than 20 mm, while, in the south and the desert area there is no rain at all (Elmenoufy et al., 2017).

Most agricultural activities are located in Nile Valley (from the High Aswan Dam in the South to the Nile Delta in the North). The Nile Delta region is regarded as an important region because it contains over 60 % of the population and 63 % of the agricultural lands (Hereher, 2009). Agriculture is an essential sector in Egypt because it accounted for about 12 % of the gross domestic product and employed 29 % of the labour force (Tellioglu and Konandreas, 2017). Egypt has a unique agricultural system and all agricultural lands are irrigated with Nile River water which is stored in Lake Nasser behind the High Aswan Dam (Attia, 2009). Agriculture lands that used are around three million hectares (Table 1) (Kniivilä et al., 2013).

Table 1. Agricultural land in Egypt

Agro-climatic zone	Area (10^3 feddans)*	Percentage (%)
Western Desert	74.626	0.91
Along Suez Canal	157.819	1.92
Nile Valley	2795.001	33.97
Nile Delta	5200.330	63.20
Total	8227.776	100.00

**A feddan is the Egyptian unit. It is equal to (1.1 acres or 0.48 hectares), (Hereher, 2009)*

Agricultural year in Egypt has three seasons to grow plants. The first season is winter, which starts from October to December and plants will be harvested from April to June. Usually, this season includes the main crops such as wheat, barley, berseem (an Egyptian clover used for fodder), lentils, winter onions and winter vegetables. The second season is summer, which starts from March to June and plants will be harvested from August to November. This season includes crops cotton, rice, maize, sorghum, sesame, groundnuts, summer onions and summer vegetables. The third growing season is called ‘Nili’ and it is a delayed summer season. This season includes crops rice, sorghum, berseem and some vegetables (Kniivilä et al., 2013). Besides, there are perennial crops such as sugarcane in Upper and Middle Egypt and grapes, citrus, bananas, mangoes, olives and dates (Kniivilä et al., 2013).

Figure 1. Map of Egypt

Despite all controversies and debates, climate change is a reality with a broad range of adverse and dire implications on the earth. These implications will have direct or indirect effects on food production systems and global biodiversity. Egypt is regarded as one of the expected countries to be vulnerable to climate change impacts, and any attempt to assess the future of Egyptian agriculture must consider the complex interactions of climate change impacts and the growth of population (Figure 2). The task is made all the more difficult by the possibility of a significant changing trend expected to result from the climate change effects. The purpose of this review is to provide general information about the impacts of predicted climate change on the agriculture sector in Egypt. Also, a brief discussion about agricultural adaptation options may contribute to significant mitigation to the impacts of climate change.

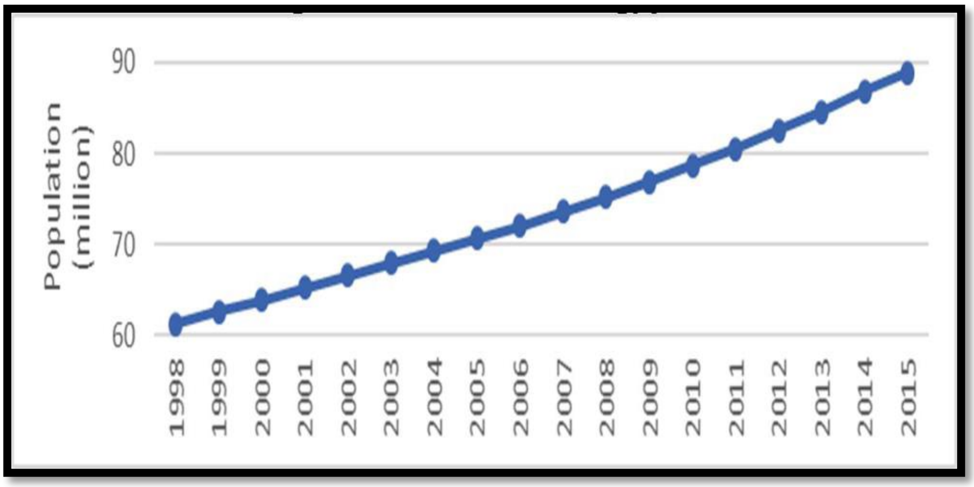

Figure 2. Total population in Egypt, (CAPMAS, 2016)

Historical and Predicted Changes in Egyptian Climate

Historical Changes in Egyptian Climate

Data collected by Cairo stations indicated that there is a general trend towards warming of the air temperature (Figure 3). These trends are in agreement with trends in the global mean surface temperature since the late 20th century. The most probable cause of the recent observed warming is a combination of internally and externally forced natural variability and sources (Hussein and Mohamed, 2016).

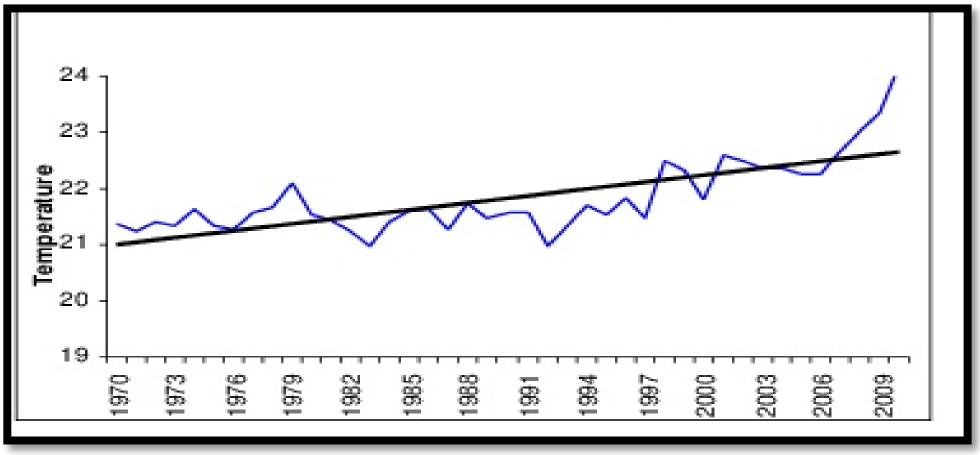

Figure 3. Mean annual temperature trends for Cairo/Egypt, (Hussein and Mohamed, 2016)

The historical rainfall data also showed an increase in the rainfall amount over whole Egypt during the period of 1980 – 2015, with the intensity of about 70mm (Figure 4). However, the rainfall is not considered a valuable water resource in Egypt because Egypt receives more than 95% of its various freshwater resources from outside its international borders. According to the historical and observed data, there is a decrease in the availability of total renewable freshwater per capita in Egypt (Figure 5). An ever-increasing population, climate change and upstream Nile projects are expected to intensify water scarcity in Egypt (Tellioglu and Konandreas, 2017).

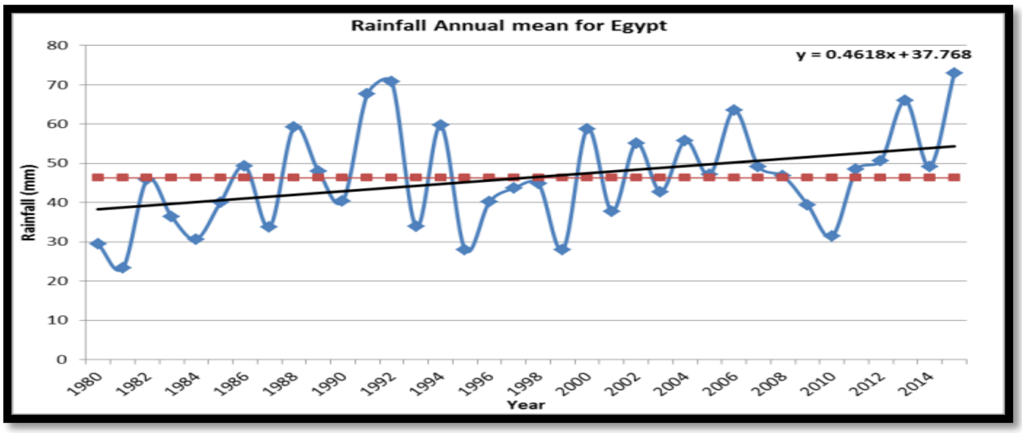

Figure 4: Rainfall trend analysis for whole Egypt, (Elmenoufy et al., 2017)

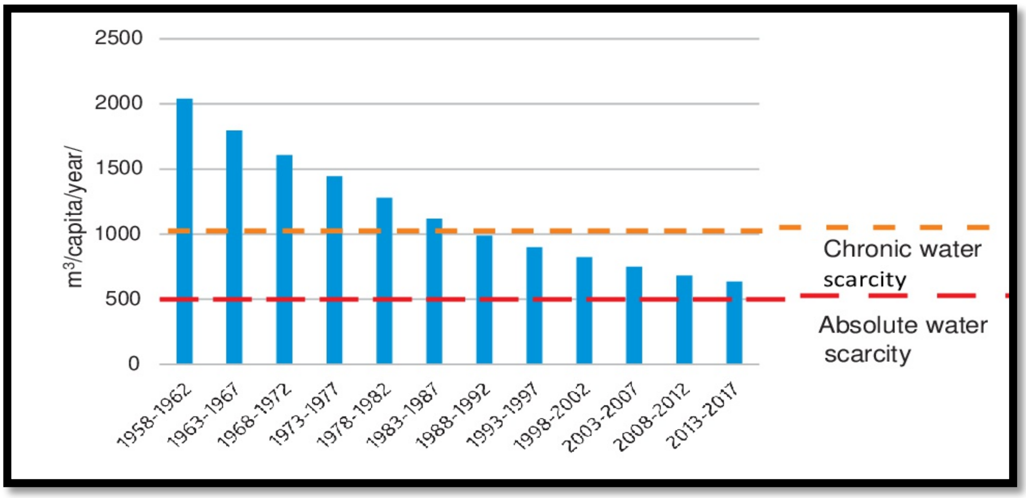

Figure 5. The availability of total renewable freshwater per capita, (Tellioglu and Konandreas, 2017)

The yearly variations of sea level showed there is an increasing trend in the sea level (Figure 6). This increase during the eight years is strongly affected by meteorology such as wind force and atmospheric pressure system (Hussein et al., 2010).

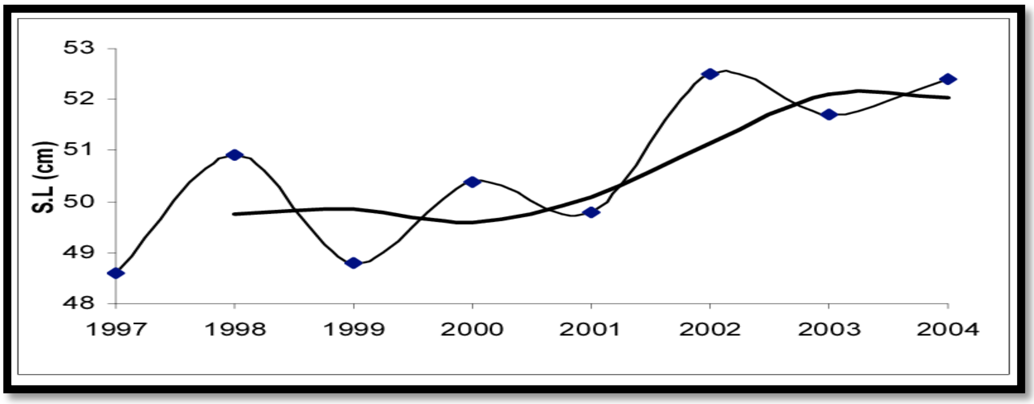

Figure 6. The mean annual sea-levels measured at Alexandria, (Hussein et al., 2010)

Predicted Changes in Egyptian Climate

In the African continent, the warming will be more massive in all seasons than the global annual mean warming. The annual rainfall will also decrease in Mediterranean Africa and the northern Sahara (IPCC, 2007). Most of the existing studies about climate change used GCM models such as (CGCM2, ECHAM4 and HadCM3) to predict climate change scenarios for Egypt (Attia, 2009). In these models, mean annual temperature, precipitation, and potential evapotranspiration presented for two of the SRES emission scenarios (A2 and B2) and three future periods to get a better understanding to the future changes of Egyptian climate (Attia, 2009).

Temperature

CGCM2, ECHAM4 and HadCM3 models indicated that temperature would continue to increase with the times especially in the southern and western regions of Egypt. The CGCM2 model predicts that some areas in southern regions will be warmer due to the increase in temperature by 5.85°C. However, by 2050s the three models predict less warming will be in the northern regions and more in the southern regions. The average increase in temperature will be about 2.5°C. By 2080s, the average increase in temperature will range between (2.3

and 4.2°C) across Egypt. Generally, these models predict less warming in the northern parts and more warming to the southern parts of Egypt (Table 2) (Attia, 2009).

Table 2. Spatially Averaged Temperature Anomalies (°C) over Egypt for three future periods

Model	A2			B2		
	2020s	2050s	2080s	2020s	2050s	2080s
CGCM2	1.38	2.70	4.19	1.32	2.14	2.91
ECHAM4	1.04	2.13	3.81	0.78	1.78	2.70
HadCM3	1.34	2.54	4.26	1.48	2.27	3.19

Source: (Attia, 2009)

Potential evapotranspiration

The three models showed there is a gradual increase in evapotranspiration with the time and this increase ranges between 5 % and 14 % by the 2080s depending on the model and scenario. It should note that evapotranspiration also depends on temperature; so, daily evapotranspiration will increase in the warmer climate especially in southern parts of Egypt, towards Aswan and western desert (Table 3).

Table 3. Spatially Averaged evapotranspiration Anomalies (%) over Egypt for three future periods

Model	A2			B2		
	2020s	2050s	2080s	2020s	2050s	2080s
CGCM2	6%	9%	14%	7%	8%	10%
ECHAM4	1%	4%	9%	1%	4%	5%
HadCM3	4%	7%	12%	4%	6%	8%

Source: (Attia, 2009)

Precipitation

In Egypt, the rainfall is low, irregular and unpredictable. The northern coast is the only region with considerable rainfall. These models also showed the northern coast is the only area with measurable precipitation in Egypt. In general, the three models showed similar precipitation patterns for all periods. The models predict that reductions in rainfall over the northern coast. The predicted reductions in coastal precipitation range between 10 % and 30

% depending on the model and scenario used (Attia, 2009). However, the rainfall is not a valuable water resource in Egypt because it is account 3.5% of its freshwater resources (El-Ramady et al., 2013).

Sea level rise

Many factors can cause sea level rise in the Mediterranean region, and these can be: climate change, local tectonics for subsidence and seasonal oceanographic process (Frihy, 2003). These three causes of sea level rise are applicable to northern areas of Egypt. Frihy (2003) reported that the global sea level rise by 2100 would be between 23 and 96 cm, with a mid-estimate of 55 cm. It is also predicted that global average sea levels may rise between 7 and 36 cm by the 2050s, between 9 and 69 cm by the 2080s and 30–80 cm by 2100. The majority of this change will occur due to the expansion of the warmer ocean water (Roaf et al., 2005). Nicholls and Leatherman (1995) estimated that one meter global sea level rise by 2100 would give rise about 0.37 meter sea level rise at the Nile delta. It is reported that the average sea level rise by 2100 at the Nile delta margin and Alexandria in Egypt would be 26 cm and 10 cm respectively (Milliman et al., 1989). El-Shaer (1997) also estimated that sea level rise in the northern Delta would be ranged between 20 to 40 cm by the next 50 years. El-Raey (2010) estimated that even with a sea level rise of only 50 cm, approximately 30% of the cities of Alexandria and Port Said would be damaged.

Effects of Climate Change

Effects of Climate Change on Agriculture

The Egyptian agriculture sector is more likely to be vulnerable to climate change. Changes in the Egyptian climate such as increasing temperatures can directly affect plant production through heat stress or indirectly through higher plant water demand and increasing transpiration (Eid et al., 2007). High temperatures can also cause a decrease in crops yield due to a shortening of the grain-filling period; and affecting other physiological processes (Attia, 2009). According to Eid et al. (2007), climate change could reduce national production of all crops in Egypt, and this reduction ranges from 11 % for rice, 28 % for soybeans, 15 % for wheat and 19 % for maize by 2050. Also, climate change could increase crops demand water by 6 to 16 % by 2100. At the Upper Egypt site, the yield of all crops can be reduced by about 20 % with the warmer scenario (Eid et al., 2007).

Egyptian agriculture sector depends on the Nile River as the primary water source. Thus, sensitive crops are likely to suffer from drought stress and salt stress because of the limitation in water. High temperatures are likely to increase evaporation rates and crop water requirements; so, this can directly decrease crop water use efficiency and increase irrigation demands of crops (Ludwig, 2006). Irrigation efficiency for Egypt is low due to inefficient flood irrigation practices which are used by Egyptian farmers (Attaher et al., 2010). Increase irrigation will lead to elevate water-table and soil salinization, as well as decrease crop yields because it impedes aeration and leaches nutrients (El-Shaer, 1997).

High evaporation rates may also accelerate soil salinization by accelerating the transport of harmful salts to the soil surface, and as a result of that significant area under poor drainage systems will become unsuitable for agriculture (El-Shaer, 1997). Degradation of water resources, inefficient flood irrigation practices and poor water management are already ill-drained lands at the lower Nile Delta which are already subjected by the water-table rise, and saline-water intrusion (El Raey, 2010). Thus, under these conditions more agricultural lands will indeed become unsuitable for agriculture in the future.

Egypt is one of the most countries that may face risks from the effects of rising sea level in the future (El Raey, 2010). Thus, rising sea level is another change in the Egyptian climate which may affect agriculture sector by losing agricultural lands (Mervat and Yasser, 2016).

The low-lying lands along the northern Delta are the most areas that may be affected by sea level rise and approximately 12.5 % of the existing agricultural land in the Delta may be lost by rising sea level one meter (Elsharkawy et al., 2009). Rising sea level will accelerate the intrusion of saline water into surface bodies of water, and this may increase the tendency toward water logging and salinization of low-lying lands; results in significant areas will become unusable for agriculture (Elsharkawy et al., 2009).

Changing the climate in the future may also affect livestock production in Egypt through harmful heat stress, the availability of fodder and new animal diseases (Sadek et al., 2015). The availability of fodder will decrease due to climate change impacts on crops production, reducing agricultural lands and higher competition for water resources between fodder and cereal crops (Elsharkawy et al., 2009).

Effects of Climate Change on Aquaculture

The effects of climate change on aquaculture are more complicated than those on terrestrial agriculture owing to the much wider variety of species produced (Brander, 2007). Aquaculture in Egypt is currently regarded as the primary source of fish supply accounting for almost 78.8% (1.56 million tons) of the total fish production of the country (Soliman, 2017) (Figure 7).

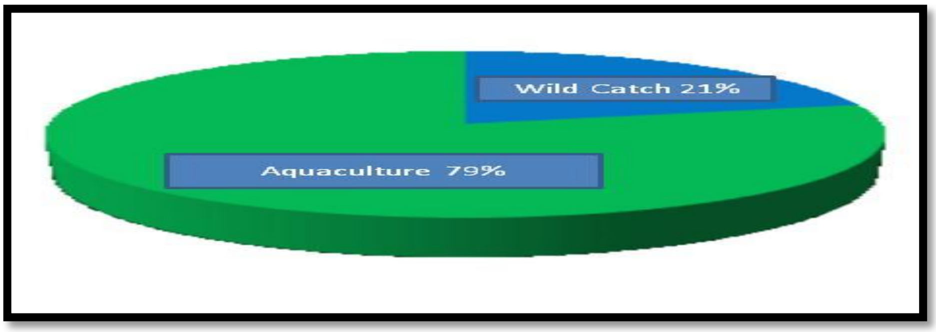

Figure 7. Total annual fisheries and aquaculture production in Egypt, (Soliman, 2017)

Effects of climatic change on aquaculture can be either direct or indirect impacts. It can affect natural resources such as water, land, feed, seed, and energy inputs (Soliman, 2017). Climate change can decrease the water availability in the major rivers and streams; changing in precipitation can also cause a spectrum of changes in water availability ranging from droughts to floods, and this can reduce water quality (IPCC, 2007), leading to significant effects in fish spawning, migration and seed availability. It can also lead to an increase in drought periods, which results in less water retention time in water bodies (Goswani et al., 2006).

Rising sea level will destroy weak parts of the sand belt, which is necessary to protect lakes and the low-lying reclaimed lands and this will have severe effects on fish population and species in these lakes because one-third of Egypt's fish catches are made in these lakes (Elsharkawy et al., 2009). Sea level rise is also expected to change the water quality which caused the fish distribution to move northwards and go into deeper waters (Elsharkawy et al., 2009). Inland freshwater aquaculture practices will threaten by rising sea levels through the movement of saline water further upstream in rivers and salinization of groundwater supplies (IPCC, 2007).

The increase in global warming and temperature will affect on pond aquaculture through solar radiation, air temperature, wind velocity, and water turbidity results to increase in vaporization, cloud cover and subsequent reduction in solar radiation reaching the ponds. As a result, the rate of algal blooms and red tides on the surfaces of water will increase; and can affect on water quality and survival of fish in the ponds (Soliman, 2017). Increasing temperatures can reduce the level of dissolved oxygen and increase metabolic rates of fish, which leads to the decline in production and increase in feed requirements while also increasing the risk of spread of disease and fish deaths (FAO, 2008).

One of the biggest challenges facing the future aquaculture industry is access to proteins, minerals and fatty acids. More than 85% of the world fish stocks are already fully exploited, thus increasing the use of wild-caught fish as ingredients in the aquaculture fish feed is no longer possible (Pile and Barlow, 2002). Loss of productivity from the capture fisheries oils can cause fewer raw materials availability for fish meal and fish oil industry. The changes in the sea circulation patterns can influence fish meal and fish oil supplies (Pile and Barlow, 2002). The ingredients in the feed that fed to fish have already replaced by plant sources. Climate changes could also reduce the agricultural production of soybean, corn and other ingredients that today's fish feeds rely on them. The use of soybean meals and corn meal for the production of biofuel instead of the usual feed formulation poses both economic and social challenges, especially in the aquaculture sub-sector. Therefore, the industry has to search for new and sustainable resources to produce cultured fish, such as algae. The industry is in need of innovative solutions to solve this urgent challenge.

Adaptation to Climate Change

Agriculture Adaptation Options to Climate Change

The future strategies to adapt the impacts of climate change can involve water and land management. First agricultural adaptation can be the improvement of water use efficiency and technical application efficiency (Tellioglu and Konandreas, 2017). This includes maintaining the whole system of supplying water. So, water should be available on demand and be delivered in proper quantities in exact time. The government should provide farmers by guidance about optimal crop selection, irrigation and fertilization to enhance the adoption of water preservation; as well as training programs to farmers who need to modernize their irrigation systems (El-Shaer, 1997). The government should also establish strong rules to avoid excessive water such as establishing water meters to measure the amount of water that used, and water pricing especially when using water more than which is supposed to use

(Attia, 2009). Moreover, modern methods of irrigation should be adopted, and these methods should be based on the high frequency and low volume application of water (Tellioglu and Konandreas, 2017).

On the other hand, adaptations of land should include the management of low-lying lands on the northern border of the Delta. It is clear that when sea level rise will affect agricultural lands in this area (submergence and salinization), and produce the highest damage. One of the adaptation options for these lands is that some lands need to be retired from agricultural practice. As a result, the amount of water which is used to irrigate these lands will be available and can be used to irrigate new lands outside the Nile Valley and the Delta (Eid et al., 2007).

Breeding and selection crops can be another adaptation option; which can help to find crops that tolerant to heat and salinity, efficient in using water, and suitable for different production methods (Attia, 2009). Thus, these crops can minimize water use and maximize the yield. Moreover, extensive efforts should be made by the government to encourage farmers for adopting crops that high return and water conserving instead of growing crops that characterized by water consuming such as rice and sugarcane (Attia, 2009).

The government should put the policy of crops which can give farmers the possibility of adapting the suitable crops in each area (Eid et al., 2007). Changing time of sowing and management practices can also be another option to adapt the impacts of climate change and reduce yield losses in the warmer climate (Eid et al., 2007). Alternative cultivation methods such as hydroponics and the vertical farm systems should be considered to mitigate the damaging effects of climate change on Egyptian agriculture (Kalantari et al., 2017).

It will be essential for Egypt to develop and test new crop models which can help to adapt the impact of climate change. These models can be used to know appropriate crops, varieties, and management strategies for maximizing production and minimizing risks of changing the climate. Furthermore, the adaptation for livestock should be by improving the low productivity, and this can be achieved by developing new breeding and feeding programs (Sadek et al., 2015).

Aquaculture Adaptation Options to Climate Change

The first adaptation step is that developing new strains of aquaculture species that are tolerant to lower water quality and higher levels of salinity to survive with changes driven by climate change. This is a relevant issue for many countries; where freshwater will be a limiting factor by climate change, as seems to be the case for Egypt (FAO, 2008). The development of strains of farmed aquatic organisms with improved salinity tolerance has already been practised (Abu Hena et al., 2005); while, the development of strains tolerant of higher or lower temperatures, and other environmental variables impacted by climate change, need to be studied as solutions for adaptation to climate change**.** Using non-native aquatic species such as euryhaline and estuarine species as well as species tolerant to warmer water also need to be proposed as a means of adaptation to climate change (Harvey et al., 2017).

Recirculation aquaculture systems can be a promising aquaculture method of fish farming that can be used in Egypt aquaculture farming. In recirculation aquaculture systems, fish is cultured under entirely controlled environmental conditions independent of their natural environment. Recirculation aquaculture systems are land-based fish production systems in which water from the rearing tanks is reused after mechanical and biological purification to reduce water and energy consumption as well as reduce discharged water to the environment (Schneider et al., 2010). Moving water-based aquaculture especially cages and pens for finfish onto land and employing recirculating aquaculture system technologies are being proposed like means of reducing exposure to climatic changes. In such systems, water quality, temperature, dissolved oxygen, salinity and pH, can be controlled to meet the biological and environmental requirements of cultured species. Recirculation aquaculture systems, however, remain relatively expensive regarding both capital and operational costs and require high levels of technical expertise (Murray et al., 2014).

Aquaponic systems which refer to the production of fish and plants in an integrated system can be another application to produce food in areas where freshwater is limited (Somerville et al., 2014). Aquaponic systems can be regarded as a particular type of recirculation aquaculture systems, and thus shares many of the same attributes. It is also worth pointing out that neither system is likely to be immune from extreme climate events in small areas developing states or coastal areas vulnerable to such events without further development (Somerville et al., 2014). The integrated aquaculture with agriculture through the use of groundwater and effluent discharge should be developed to mitigate the limitations of freshwater and brackish water (Soliman, 2017).

On the farm level, well-designed and well-built ponds can also help to mitigate some of the effects of climate change. For example, deeper ponds provide a thermal refuge and higher dissolved oxygen reserves for fish, while raised pond embankments can help prevent fish escapes and serve as water storage during droughts (Harvey et al., 2017). Converting flow-through ponds and raceways into more water-efficient techniques is also desirable, as is reducing seepage through the use of pond liners (Harvey et al., 2017).

Recent advances in remote sensing platforms (e.g., drones and satellite constellations) are now being integrated with information and communication technologies; examples include early warning information systems (e.g., weather forecasts and early detection) and communication of risks using mobile communication devices (e.g., smartphones and tablets), cloud-based data systems and useful simulations. Stronger materials and better system designs coupled with the development and implementation of proper technical guidelines can play a role in reducing vulnerability to climate change in the marine aquaculture subsector of the country (Soliman, 2017).

Reducing the amount of imported fishmeal and feed ingredients through the usage of local ones is another important thrust area to be taken care. Research on the utilization of agricultural meals and oils to replace the use of fish meals and fish oil is a significant subject of aquaculture research and development. Furthermore, the focus should be addressed toward reducing the impact of aquaculture industry on climate change and fossil fuels depletion by investigating how to reduce energy utilization through energy conservation, proper energy management in feed manufacturing, and introduce possible renewable energy approaches in aquaculture industry (Soliman, 2017).

Conclusion

The Egyptian agriculture sector is more likely to be vulnerable to climate change because it depended on the Nile River as the primary water source and based on traditional irrigation methods. The main changes in the Egyptian climate are increase temperature, decrease precipitation, increase evapotranspiration and rising sea level. Nile Valley and the Delta regions will be more likely to have potential impacts of these changes.

For the agriculture sector, climate change studies predict a reduction in the productivity of major crops in Egypt because of heat stress, water stress, and increase salinity. In addition, most productive arable land in the Nile Delta will be lost because of sea level rise and saltwater intrusion. Increase temperatures and evapotranspiration are likely to increase crop water requirements and increase irrigation demands of the agriculture sector. The effects of climatic change on aquaculture could be either direct or indirect impacts. It can affect natural resources that are necessary for aquaculture production. These resources include water, land, feed, seed, and energy inputs.

Several adaptation options can help the Egyptian agriculture sector to adapt climate change impacts such as improving the technical water application efficiency and saving water by establishing water metering, water pricing and using modern methods of irrigation. Another adaptation option involves that some lands in Nile Valley and the Delta need to be retired from agricultural practice. The selection and breeding tolerant crops to heat and salinity and water use efficiency can be considered another adaptation option. Furthermore, developing and testing new crop models, changing the time of sowing, encouraging farmers to adopt crops that high return and less water use can consider other agricultural options to adapt to climate change impacts. On the other hand, aquaculture adaptation involves the development of new strains which are tolerant to salinity, temperature and other environmental conditions. Increase the efficient use of land, water, food, seed and energy through intensification systems which use less land and freshwater. The utilization of alternative renewable energy systems and feed sources can be one of the choices for adaption to climate change in the aquaculture sector. Thus, it recommended that enormously efforts should be taken by the Egyptian government to enhance the productivity of the agriculture sector and minimize the risks which associated with climate change.

Acknowledgement

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IDENTIFICATION AND EXPRESSION OF POLLEN ALLERGEN TRANSCRIPTS IN DIFFERENT ORGANS OF THE COMMON RAGWEED (*AMBROSIA ARTEMISIIFOLIA* L.)

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Abstract

An increasing number of individuals are affected in the temperate climate zone by allergy caused by the pollen of the common ragweed (*Ambrosia artemisiifolia* L.). For 11 allergen families of ragweed the polypeptide sequences are available, and for most the nucleotide sequence is also known. However, little is known about the expression of these allergen genes. In the present study transcriptomes of the male and female flowers, as well as of leaves were produced and transcripts with high similarity or identity to the allergen genes were identified. All the analyzed 11 allergen genes could be detected in the male flowers and with the exception of Amb a 6 also in leaves. Amb a 1, Amb a 3, Amb a 8 and Amb a 12 were expressed also in female flowers. The strongest expression was found for Amb a 5 and then for Amb a 3. Our results indicate that expression of ragweed pollen allergens is not restricted to the pollen, but they function also in other organs of the plant. The results presented here contribute to our understanding on the function and role of the genes of pollen allergens in common ragweed.

Keywords

Ambrosia artemisiifolia, common ragweed, pollen allergen, Amb a, transcript, gene expression

Összefoglalás

Az ürömlevelű parlagfű (*Ambrosia artemisiifolia* L.) pollenje által kiváltott allergia egyre több embert érint az e gyomnövénnyel fertőzött területeken. A parlagfű 11 allergén géncsaládja fehérje-szinten ismert, és legtöbbjük nukleotid szekvenciája is feltárásra került. Ugyanakkor, az allergén gének expressziójáról kevés ismeret áll rendelkezésre. A világ különböző régióiból származó parlagfű növényekben azonosított allergén géneknek a hazai populációból való kimutathatósága, illetve működésének tanulmányozása céljából transzkriptomokat készítettünk a parlagfű hím és nő virágjából, valamint leveléből, és azonosítottuk az ismert allergén génekkel azonos vagy nagyfokú hasonlóságot mutató transzkripteket. A hím virágokból mind a 11 allergén gént ki tudtuk mutatni, és az Amb a 6 kivételével a levelekből is. Az Amb a 1, Amb a 3, Amb a 8 and Amb a 12 gének a nővirágokban is mutattak expressziót. A génkifejeződés az Amb a 5, majd az Amb a 3 esetében volt a legerősebb. Eredményeink azt mutatják, hogy az ismert parlagfű allergének, függetlenül attól, hogy fő vagy allergológiai szempontból kisebb jelentőségű allergének, a hazai növényekben is jelen vannak. Továbbá, eredményeink egyértelműen igazolják, hogy a parlagfű pollen allergén génjei nemcsak a hímvirágokban működnek, hanem a növény egyéb részeiben is van szerepük. Itt bemutatott eredményeink hozzájárulnak a parlagfű allergéneket kódoló gének működésének és szerepének jobb megértéséhez.

Introduction

The common ragweed (*Ambrosia artemisiifolia* L.) is a noxious, invasive weed in large areas of the temperate climate zone (Kazinczi et al. 2008). From 1995 it is the most widespread weed in Hungary (Novák et al. 2009). Besides endangering yield safety of crops the pollen of ragweed is the clinically most important seasonal aeroallergen (Rafnar et al. 1991). However, the pollen is the main source of ragweed allergy, it is known that also the green parts of the plant may cause dermatitis in sensitive patients.

Ragweed is wide source of different type pollen allergens which are on different importance regarding their allergenicity. Nomenclature of allergens is approved by the WHO/IUIS (World Health Organization and International Union of Immunological Societies). In the allergen nomenclature database of WHO/IUIS (http://www.allergen.org/) 11 different types of common ragweed allergens are identified. Each represent a different family, which involve an unknown number of isoforms. Two proteins, a pectate lyase (Amb a 1) (Rafnar et al., 1991; Nandy et al., 2011; Augustin et al., 2012; Augustin et al., 2013) and a cysteine protease (Amb a 11) (Bouley et al., 2015) are considered as major allergens with a prevalence of >90% and 66% in sensitization, respectively. Others are minor proteins, like plastocyanins (Amb a 3

(Klapper et al., 1980; Taller et al., 2016) and Amb a 7 (Roebber et al., 1991), defensin (Amb a 4) (Léonard et al., 2010), lipid transfer protein (Amb a 6) (Roebber et al., 1983; Hiller et al., 1998), profilin (Amb a 8), and polcalcins (Amb a 9 and Amb a 10) (Wopfner et al., 2008). For Amb a 7 no protein or DNA sequence is available. Amb a 5 is also a minor ragweed allergen with unknown function, although the 3D structure of the protein has been resolved (Ghosh et al., 1993; Metzler et al., 1992). A recently identified new allergen, Amb a 12 is an enolase (Bordas-Le Floch et al., 2015b). Besides these 11 allergens of the IUIS database a further minor ragweed allergen is known, which is a cysteine protease inhibitor (Amb a CPI) (Rogers et al., 1993).

Comprehensive knowledge of the allergen repertoire of ragweed pollen is prerequisite for accurate diagnosis and efficient immunotherapy. To this end we performed a transcriptome analysis on different developmental stage male and female flowers, as well as on leaves, using pooled samples of six phenotypically different plants. One of the aims of the present study was to explore whether the known allergen families, which were identified in plants from different geographic regions of the world, can be detected in the randomly chosen Hungarian ragweed plants. Further aim of the study was to explore whether the allergens are expressed only in the male flowers or also in female flowers and/or leaves, and to estimate their expression level.

Materials and Methods

Plant material

Six phenotypically different ragweed plants growing under natural conditions in Keszthely, Hungary were used. During the flowering season young shoots were covered with transparent paper bags to protect from pollen contamination. Male racemes were covered separately. Male and female flowers were collected in seven and nine developmental stages, respectively, as follows:

Male flowers: stage 1.: 1 mm raceme; stage 2.: 4-5 mm long raceme; stage 3.: 10 mm long raceme; stage 4.: not yet opened nest from a 10 cm long raceme; stage 5.: opened nest without opened flowers of that 10 cm long raceme; stage 6.: a nest with partially opened flowers; stage 7.: a nest in stage of maximal pollen release.

Female flowers: stage 1.: young flowers without visible pistils; stage 2.: flowers with 1-2 mm pistils; stage 3.: flowers with 3-4 mm pistils; stage 4.: flowers with 5-6 mm pistils; stage 5.: flowers with full length pistils; stage 6.: flowers just after fertilization; stage 7.: fertilized flowers with living pistil; stage 8.: fertilized flowers with dying pistils; stage 9: flowers with clearly developing seeds in them.

Leaf samples were collected in five developmental stages: stage 1. leaves were collected from the youngest shoots, while stage 5. leaves from old branches of the same plant, and the other three stages were collected in between these two.

Collected samples were immediately frozen in liquid nitrogen and stored at -80^{o}C until analysis.

Molecular analyses

A male and a female flower, as well as a leaf sample was created by bulking the subsamples of the different developmental stages, in a way that each stage should be represented equally. Molecular analyses were performed as described in Virág et al. (2016). Briefly:

RNA of these three samples was extracted by TaKaRa Plant Extraction kit (Takara Bio, Japan). For cDNA synthesis the Illumina TruSeq RNA sample preparation kit was used.

RNA-sequencing was performed on an Illumina HiSeq2000 system (Illumina, USA). Each fragment was pair-end sequenced 100 nucleotide deep.

Bionformatics analyses

The http://allergen.org/ and Uniprot databases were used for allergen annotation. For the primary reconstruction of allergen gene expression analysis we used the Trinity *de novo* assembler, followed by transcript abundance calculation using Bowtie short read aligner and Geneious®. Normalized expression level values (RPKM – reads per kilobase per million mapped reads) were assigned with GenoUtils that was developed in Visual Studio and C# programming language (Virág and Hegedűs, 2018). For screening for highly similar sequences in the transcriptome datasets the available DNA sequences of nine Amb a gene families of the IUIS database and the Amb a CPI (Bordas-Le Floch et al., 2015), as well as the Amb a 5 sequence (Gosh et al., 1993) was used.

Results

The transcriptome datasets of male and female flowers, as well as of leaves have been screened with all published DNA sequences of 11 Amb a gene families for highly similar transcripts. Results are summarized in Table 1.

Table 1. Expression of Ambrosia artemisiifolia allergens

Allergen	Identification code[1]	Protein length (aa)[2]	Coverage (%)[3]	Identity (aa)[4]	Identity (%)[5]	Expression in male flowers (RPKM)	Expression in female flowers (RPKM)	Expression in leaves (RPKM)
Amb a 1	TR45018|c2_g2_i13	396	100	396/396	100	2138.04	1.40	27.73
Amb a 3	TR20404|c0_g3_i1	101	97	87/101	90	7958.35	2.39	71.24
Amb a 4	TR30326|c0_g2_i1	129	82	129/135	96	5517.13	0.00	1.33
Amb a 5	TR27249|c0_g1_i1	45	100	45/45	100	17671.01	0.00	6.31
Amb a 6	TR44274|c4_g7_i2	118	100	114/118	97	4673.33	0.00	0.00
Amb a 8	TR37128|c0_g1_i2	133	100	133/133	100	2533.45	2.67	41.92
Amb a 9	TR28466|c1_g1_i1	83	100	81/83	98	662.77	0.00	18.63
Amb a 10	TR38368|c0_g1_i2	160	100	160/160	100	349.50	0.00	20.42
Amb a 11	TR43337|c0_g2_i1	386	100	379/386	98	819.88	0.00	3.01
Amb a 12	TR43144|c0_g1_i1	445	100	445/445	100	671.89	137.50	1358.49
Amb a CPI	TR36272|c0_g1_i1	92	100	90/92	97	131.97	0.00	9.79

[1] The identification code of predicted allergen transcript in Ambrosia artemisiifolia transcriptome database

[2] The size of published allergen proteins (aa: number of amino acids)

[3] The percentage of query covered by alignment to the published allergen sequence

[4] The identity of query covered by alignment to the published allergen sequence (aa: number of amino acids)

[5] The percentage of identity by alignment to the published allergen sequence

Colors refer to the level of gene expression. Non-expressed genes are indicated with green color, while deep red indicates the highest expression level. Yellow color show transition expression value. The numbers show the RPKM value of each expressed genes. High RPKM value show higher gene expression level.

Sequence similarity

Transcripts which exactly matched sequences of allergens were obtained for the Amb a 1, Amb a 5, Amb a 8, Amb a 10 and Amb a 12. These five transcripts had identical sequence with the corresponding Amb a gene and covered them 100%.

Except for Amb a 3 and Amb a 4 we could identify transcripts in all three transcriptome which covered 100% the corresponding Amb a gene. Except for Amb a 3 the sequence similarity between the transcripts and allergen genes was very high, >96%.

In the IUIS database just the amino acid sequence of the Amb a 3 allergen is registered. There was no nucleic acid sequence available. From the transcriptomes used in the present study we recently identified a transcript (Taller et al., 2016) that encodes a protein with 90% similarity to the Amb a 3 amino acid sequence published by Klapper et al. (1980). In Table 1. the Amb a 3 isoform identified by Taller et al. (2016) is compared to the Amb a 3 protein of Klapper et al. (1980).

Expression levels

Expression of the Amb a transcripts of the three transcriptomes are shown in Table 1.

All identified transcripts were expressed in the male flowers, and except Amb a 6 they were expressed also in leaves. In female flowers expression could be detected just for the Amb a 1, Amb a 3, Amb a 8 and Amb a 12 allergen transcripts.

In male flowers the strongest expression was observed for Amb a 5 and then for the Amb a 3 isoform. Strong expression was detected for Amb a 4 and Amb a 6 and then for Amb a 8 and Amb a 1.

In female flowers expression of only of Amb a 12 is notably, since it is relatively high, being about one-fifth of that found in male flowers.

Also in leaves the highest expression was detected for Amb a 12 with an RPKM value about double of that found in male leaves. Except Amb a 12, where expressed, the expression of the Amb a genes is relatively low in leaves.

Discussion

Identification of new allergens from non-model plant species such as *A. artemisiifolia* are classically hindered by the paucity of protein or genomic information available in public databases (Bordas-Le Floch et al., 2015). For Amb a 7 neither a protein, nor a nucleotide sequence is known, and for Amb a 3 just a polypeptide sequence (Klapper et al., 1980) was available. For this later, a transcript coding for a protein with 90% similarity to that polypeptide was identified recently (Taller et al., 2016). However, this Amb a 3 isoform showed the second highest expression in male flowers in the present study, to prove the allergenic nature of it requires further functional and allergological analyses.

Amb a 1, Amb a 3, Amb a 8 and Amb a 12 were expressed in all three organs, indicating a more general functional role for these proteins. Amb a 1 is the major ragweed pollen allergen, since 95% of ragweed-sensitive individuals react to it. Further, the Amb a 1 protein is highly abundant, comprising about 6% of the total protein of pollen (Rafnar et al., 1991). In our study we found strong, but not outstanding expression for Amb a 1 in male flowers. Amb a 1 is a pectate lyase, which act in cell wall softening and have an important role in various plant developmental processes (Marin-Rodrigez et al., 2002). In the style pectate lyases facilitate pollen tube emergence and penetration of the pollen (Taniguchi et al., 1995), which can be a possible reason for abundancy of Amb a 1 in the ragweed pollen.

The function of Amb a 5 is not cleared yet. In male flowers we observed outstandingly high expression for this minor allergen. It needs to be studied what is the role of Amb a 5 in the plant and why is it expressed at such a high level.

Amb a 12 is a newly identified minor allergen belong to the enolases. In this study we found a twice so high expression for Amb a 12 in leaves than in male flowers. In this aspect it is the only one among the studied allergens, where expression in male flowers is lower than in leaves. Functional analysis of enolases in the plant and in the pollen would be necessary to understand the reasons of this phenomena.

Except Amb a 6 all examined allergens were expressed also in leaves, indicating that function of the majority of Amb a allergens is not restricted to the pollen. Many of the pollen allergens are evolutionarily conserved and are involved in stress responses, as the pathogenesis related (PR) proteins, and in metabolic processes, e.g. in cell wall metabolism, while other allergens emerged during evolution (Chen et al., 2016). Expression in leaves and female flowers and the molecular type of the different Amb a allergens indicate that they have a more general role in plants. However, whether allergens in the pollen are produced exclusively in the anther or they are accumulated to the pollen from other parts of the plant needs further investigations.

In this study we could identify each analyzed Amb a gene family in the randomly chosen Hungarian ragweed plants. Originally, the Amb a genes were identified from plants on different geographic origin, like North-America or Western-Europe. This indicates, that the analyzed Amb a allergens can be widespread in ragweed populations and possibly present in every ragweed plant. In ongoing studies we are examining the expression and function of the different allergens in different organs and their role during development of the ragweed plant.

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