ICDRA 2022

6th INTERNATIONAL CONFERENCE ON DUCKWEED RESEARCH AND APPLICATIONS

29 MAY - 01 JUNE 2022

IPK
LEIBNIZ-INSTITUT

Leopoldina
Nationale Akademie der Wissenschaften

ISCDRA
International Steering Committee on Duckweed Research and Applications

AKB STIFTUNG
Stiftung der Familie Carl-Ernst Büchting

DFG Deutsche Forschungsgemeinschaft
German Research Foundation

DBG Deutsche Botanische Gesellschaft

Orgentis Chemicals

1. **ACRON HOTEL** | Gartenstraße 44 A, 06484 Quedlinburg
2. **HOTEL AM BRÜHL** | Billungstraße 11, 06484 Quedlinburg
3. **BEST WESTERN HOTEL SCHLOSSMÜHLE**
   Kaiser-Otto-Straße 28, 06484 Quedlinburg
   Conference - Dinner 31.05 | 20:00
4. **MEETINGPOINT CITY GUIDE TOUR** | Townhall | Markt 1
   31.05 | 18:30
5. **TRAIN STATION**

BUS TRANFERS BETWEEN QUEDLINBURG - IPK GATERSLEBEN - QUEDLINBURG
(Please see conference program)

6. **BUS STOP** | Oeringer Straße / Klinikum H
7. **BUS STOP** | Parking lot / Carl-Ritter-Straße P

WELCOME TO THE IPK.
Use this instruction to join our WIFI “guest”

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Connect to WIFI “guest”
SSID : guest
Wireless-Key : IPK-Key954368

Alternatively use the QR-Code “Step 1” (see below)
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Step 2 :

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Username: konferenz@local
Password: konferenz

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QR-Codes:

Step 1

Step 2

IPK LEIBNIZ INSTITUTE

- 1 Communication Centre
- 2 Casino
- 3 Administration Building
- 4 Library
- 5 Vavilov Building (Gene Bank)
- 6 Genebox Building
- 7 Genome Centre
- 8 Friedrich Miescher Building
- 9 Konrad Zuse Building
- 10 Guesthouse IPK
- 11 Club Room
- 12 Technical Services
- 13 Receiving Department
- 14 Botanical Garden
- 15 Plant Cultivation Hall
- 16 Phenotyping Facility
- 17 Bioinformatics Centre
- 18 Fruit Variety Garden

BIOTECHPARK

- 19 Saaten-Union Biotec GmbH ;
  Dr. Fahrendorf Bioscience UG ;
  IT-Breeding GmbH
- 20 BASF Agricultural Solutions GmbH
  (European Wheat Breeding Center)
- 21 Orgentis Chemicals GmbH

BIOTECH CENTRE

- 22 SGS - TraitGenetics GmbH ;
  Gartenland Produktion GmbH –
  Quality Seed Testing Lab ;
  Bioanalytics Gatersleben UG ;
  Grünes Labor Gatersleben e.V. ;
  InnoPlanta e.V.
- 23 Guesthouse Biotech Centre

Sunday May 29

	17:00	**Bus departure Quedlinburg to IPK, Gatersleben**
	16:00	Registration, poster hanging
	18:00	Opening address
01	**18:30**	**Plenary talk** Eric Lam, Rutgers U, NJ, USA Duckweed hibernation: unraveling the molecular basis of the turion induction switch in *Spirodela polyrhiza*
	19:30	Welcome reception

Monday May 30

GENOMICS & EVOLUTION

	08:00	**Bus departure Quedlinburg to IPK, Gatersleben**
02	**08:30**	**Invited talk** Rob Martienssen, Cold Spring Harbor Laboratory, NY, USA Genomic and epigenomic consequences of clonal growth habit in the Lemnaceae
03	**09:00**	**Invited talk** Shuqing Xu, Muenster U, DE The Giant duckweed, a model system for studying plant evolution in a multitrophic community
04	09:30	Laura Morello, IBBA CNR, Milano, IT TBP fingerprinting unveiled interspecific hybridization in the genus *Lemna*
05	09:50	Ljudmilla Borisjuk, IPK Gatersleben, DE Integrative analysis of growth dynamics in duckweed
06	10:10	Rodolphe Dombey, Gregor Mendel Institute, Austria Epigenetic regulation of transposable elements in duckweeds
	10:30	**Coffee break**

DIFFERENTIATION & STRESS

07	**11:00**	**Invited talk** K. Sowjanya Sree, Kerala Central U, IN Nutrient stress on duckweeds
08	**11:30**	**Invited talk** Alexandra Chavez, Muenster U, DE Non-genetic inheritance alters stress resistance across generations in the Giant duckweed
09	12:00	Osnat Gillor, BGU Negev, IL All in one: the microbiome of a rootless plant
10	12:20	Minako Isoda, Kyoto U, JP Analysis on floral induction of Wolffiella hyalina
11	12:40	Alexander Ware, Nottingham U, UK To root or not to root: evolution of rootlessness in duckweed
	13:00	**Lunch**

ECOLOGY, MICROBIOME & STRESS

12	**14:00**	**Invited talk** Marcel Jansen, Cork U, IE Lemnaceae as a key player in the circular economy; examples of wastewater valorization in Ireland
13	**14:30**	**Invited talk** Quinten Bafort, Ghent U, BE Stress tolerance in tetraploid *Spirodela polyrhiza*
14	15:00	Simona Paolacci, UCC/BMRS, West Cork, IE Wastewater valorization in an integrated multitrophic aquaculture system; assessing nutrient removal and biomass production by duckweeds
15	**15:20**	Masaaki Morikawa, Hokkaido U, JP Dual function of environmental bacteria that enable duckweed prosperity
16	15:40	Conference Photo
	16:00	**Coffee, fruits & cake**

16:30 **POSTER PRESENTATION & VIEWING I (POSTER P01-P13)**
17 18:30 **Public lecture** Klaus-J. Appenroth, FSU Jena, DE
Eine neue Kulturpflanze mit großem Potential für Ernährung, Wasserreinigung und Energie
19:30 **General Assembly**
News from Steering Committee; Suggestions for 7thICDRA, Round-table discussion: “ Duckweed futures”, Joint projects etc.

20:30 **BBQ & beer**

23:00 **Bus departure IPK, Gatersleben to Quedlinburg**

Tuesday May 31

PHYSIOLOGY & METABOLISM I

08:00 **Bus departure Quedlinburg to IPK, Gatersleben**

18 **08:30** **Invited talk** Asaph Aharoni, WIS, Rehovot, IL
The underwater chemical world of duckweed
19 **09:00** **Invited talk** Tokitaka Oyama, Kyoto U, JP
Variability of chronobiological characteristics in duckweed
20 09:30 Cristian Mateo-Elizalde, Cold Spring Harbor Laboratory, NY, USA
Unraveling genetic mechanisms of sexual reproduction in duckweed
21 09:50 Moshe T. Halpern, ARO, Volcani Institute, IL
Using rootless duckweed *Wolffia globosa* as a model to clarify the effect of e[CO_2] on NO_3 photo-assimilation in C3 plants
22 10:10 Philippe Juneau, Quebec U, Montreal, CA
How aminomethylphosphonic acid (AMPA), the main glyphosate metabolite, affects *Lemna minor* photosynthesis?

10:30 **Coffee break**

PHYSIOLOGY & METABOLISM II

23 11:00 **Invited talk** Paul Ziegler, Bayreuth U, DE
The life cycle of *Spirodela polyrhiza*: a model for aquatic plant overwintering?
24 11:30 Darlielva do Rosario Freitas, UFV, BR
Different sources of iron modulate responses in the antioxidant system in plants
25 11:50 Olena Kishchenko, Huaiyin Normal U, CN
The dynamics of NO3 and NH4+ uptake in duckweed are coordinated with the expression of major nitrogen assimilation genes
26 12:10 Shogo Ito, Kyoto U, JP
Development of a cryopreservation protocol for a variety of duckweed meristems by the vitrification-cryo-plate method
27 12:30 Manuela Nagel, IPK Gatersleben, DE
Advances in droplet-vitrification and -freezing of Lemna fronds for long-term preservation of duckweeds

13:00 **Lunch**

GENOME ORGANIZATION, DIVERSITY & IMAGING

28 **14:00** **Invited talk** Nikolai Borisjuk, Huaiyin Normal U, CN
Organization and evolution of duckweeds ribosomal RNA genes
29 **14:30** **Invited talk** Todd Michael, Salk Institute, La Jolla, CA, USA
Duckweed genome architecture
30 15:00 Phuong Hoang, Dalat U, VN & IPK Gatersleben, DE
Chromosome numbers, genome sizes and evolutionary implication for genome evolution and diversity of Lemnaceae
31 15:20 Anton Stepanenko, Huaiyin Normal U, CN
Biodiversity of Lemnaceae in water reservoirs of Ukraine and China assessed by double chloroplast DNA barcoding

32 15:40 Viktor Olah, Debrecen U, H
Family portrait of a duckweed colony: What can imaging-based phenotyping tell us about stress

16:00 Coffee, fruits & cake

16:30 **Poster presentation & viewing II (POSTER P14-P28)**

18:00 **Bus departure IPK, Gatersleben to Quedlinburg**
18:30 Guided tour in Quedlinburg
20:00 Conference dinner (Schlossmühle, Quedlinburg)

Wednesday June 01

WASTE WATER REMEDIATION, FEED, FOOD, BIOFUEL & TRANSFORMATION

08:00 **Bus departure Quedlinburg to IPK, Gatersleben**

33 **08:30** **Invited talk** Britt Schumacher, DBFZ GmbH, Leipzig, DE
Duckweed - Conservation and conversion into biogas
34 **09:00** **Invited talk** Ingrid van der Meer, WUR, Wageningen, NL
Water lentils and derived products thereof for human consumption: overview of the current status of NF approval in Europe
35 09:30 Jurriaan Mes, WUR, Wageningen, NL
Water lentils protein for human nutrition
36 09:50 Anton Peterson, Huaiyin Normal U, CN
Lemna turionifera demonstrates good potential as an alternative system for transient expression of recombinant proteins
37 10:10 Johannes Demann, Osnbrück U, DE
Insights into the nutritional value of duckweed as protein feed for broiler chicken

10:30 Coffee break

LARGE SCALE CULTIVATION & SOCIAL ASPECTS

38 **11:00** **Invited talk** Paul Skillicorn, Skillicorn Technologies, Austin, TX, USA
Duckweeds, the engine of the New Circular Economy (via Zoom)
39 **11:30** **Invited talk** Tsipi Shoham, GreenOnyx, IL
Securing fresh nutrition via a breakthrough technology for growing duckweeds
40 12:00 Finn Petersen, UAS Osnabrück, DE
Development and scale-up process from a small duckweed culture to a large in-door vertical farm for duckweed biomass production
41 12:20 Wisuwat Songnuan, Mahidol U, Bangkok, TH
Carbon dioxide sequestration potential of two Thai duckweed species, *Wolffia globosa* and *Lemna aequinoctialis*, under open-air greenhouse condition
42 12:40 tba

13:30 Best Poster Award (founded by CLF PlantClimatics GmbH)
Best PhD Presentation Award (founded by MDPI Plants) and Closing remarks

14:00 **End of conference**

14:00 Bus departure IPK, Gatersleben to Quedlinburg

POSTER PRESENTATION

Monday May 30

16:30 POSTER PRESENTATION & VIEWING I

P01	Alexej Sonnenfeld
P02	Leone E. Romano
P03	Dylan Jones
P04	Anton Stepanenko
P05	Tram Tran
P06	Kellie E. Smith
P07	Olena Kishchenko
P08	Olena Kishchenko
P09	Metha Meetam
P10	Avital F. Yosef
P11	Marie Lambert
P12	Manuela Nagel
P13	Daniel Buendía

Tuesday May 31

16:30 POSTER PRESENTATION & VIEWING II

P14	Martin Höfer
P15	Rachel O'Mahoney
P16	Claire Smith
P17	Adelaide Iannelli
P18	Timo Stadtlander
P19	Martin Schäfer
P20	Fernanda V. da Silva Cruz
P21	Sajjad Kamal Shuvro
P22	Linda Klamann
P23	Jannis von Salzen
P24	Reindert Devlamynck
P25	Hassana Ghanem
P26	Johan A. Pasos-Panqueva
P27	Javier Espinosa-Montiel
P28	Javier Espinosa-Montiel

01

Duckweed hibernation: unraveling the molecular basis of the turion induction switch in Spirodela polyrhiza

Eric Lam

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Over 15 families of aquatic plants are known to use a similar strategy of developmental switching upon environmental stress to produce a dormant propagule called a turion. We carried out RNA-seq analysis of mature turions from the Greater Duckweed Spirodela polyrhiza and compared its transcriptome to that of fronds, the actively growing leaf-like tissue that turions derive from. Our study revealed about 3,000 high-confidence expressed transcripts that displayed more than a 4-fold difference between these two states. Orthogroup analysis of these genes informed on the pathways related to dormancy, stress tolerance and metabolism that are mobilized to reprogram frond meristems for turion differentiation. Furthermore, we identified genes involved in rewiring the biosynthetic pathways to drive increased starch and lipid, as well as to prepare for their metabolism upon germination. Lastly, comparison of genomewide cytosine methylation levels revealed evidence for epigenetic control in the transition between frond and turion tissues.

02

Genomic and epigenomic consequences of clonal growth habit in the Lemnaceae

Evan Ernst[1], Todd Michael[2], John Shanklin[3], Eric Lam[4] and Rob Martienssen[1]

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The Lemnaceae are the world's smallest but fastest growing flowering plants, with a drastically reduced morphology that allows a perpetual clonal growth habit. Chromosome-resolved assembly of Lemnaceae species by single molecule nanopore sequencing has revealed gene family loss and frequent hybrid polyploidy. Lemnaceae genomes have selectively lost genes required for RNA-directed DNA and histone methylation, which are necessary for transposon silencing, and for post-zygotic reproductive isolation (the triploid block) in other flowering plants. Triploid hybrids commonly arise among Lemna, presumably by hybridization with unreduced gametes, and divergent "ZMM class" mismatch repair genes could support polyploid meiosis. Functional mapping of centromeres indicates that polyploid karyotypes are maintained as triploid clones, making Lemna a useful model for subfunctionalization in recently emerged polyploid genomes. Phylogenomic orthology analysis has uncovered genetic contrasts between duckweeds and other aquatic and terrestrial plant families that can inform the ongoing development of Lemnaceae as a platform for protein, starch, and oil micro-cropping, and for remediation of dissolved nutrients and atmospheric CO2.

03

The giant duckweed, a model system for studying plant evolution in a multitrophic community

Shuqing Xu

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In nature, plants interact with other organisms and form communities. Understanding how plants evolve in multitrophic communities is a major challenge in evolutionary ecology. The giant duckweed, Spirodela polyrhiza, which has a small genome size and short generation time, is an ideal model system to address this challenge. During the last few years, my group has been developing genomic, metabolomic and molecular tools and resources, which established S. polyrhiza as an ideal model system for conducting experimental evolution experiments both indoors and outdoors. In this talk, I will summarize these resources and tools. I will also present some preliminary results from our ongoing long-term experimental evolution experiments that aim to study plant evolution in multitrophic communities.

04

TBP fingerprinting unveiled interspecific hybridization in the genus Lemna

Laura Morello[1], Luca Braglia[1], Floriana Gavazzi[1], Silvia Gianì[1], Massimiliano Lauria[1], Klaus-J Appenroth[2], Manuela Bog[3]

[1]Istituto Biologia e Biotecnologia Agraria CNR, Milano, Italy; [2]Matthias Schleiden Institute of Plant Physiology, University of Jena, Germany; [3]3Institute of Botany and Landscape Ecology, University of Greifswald, Greifswald, Germany; morello@ibba.cnr.it

The taxonomy and phylogenesis of Lemnaceae, previously based on morphologic and biochemical markers, has been recently updated with the help of molecular markers, such as AFLP and barcoding sequences. Plastid regions are presently the gold standard for species discrimination when close morphological similarity makes it challenging. Nevertheless, some doubtful assignment of species was not clearly resolved. In the genus Lemna, even barcoding sequences are not able to discriminate Lemna minor from Lemna japonica.

With the aim of finding a reliable alternative for full species delimitation, we applied a fingerprinting method based on TBP (tubulin-based polymorphism), a nuclear multilocus marker developed at IBBA-CNR, to the genetic profiling of duckweed species. Profiles are species-specific and allowed drawing similarity trees by cluster analysis, with clear separation of species. TBP also provided clear evidence that the species described by Elias Landolt in 1980 as Lemna japonica is in fact a hybrid species between L. minor and Lemna turionifera, as confirmed by tubulin sequence analysis. By analyzing a large number of clones from the Landolt Collection, recently transferred to Milan, we identified many hybrid clones that were misclassified as L. minor for their morphologic similarity, suggesting a geographic distribution of L. × japonica wider than originally thought. The genetic structure of the hybrid species and its parental species, which show variable genome size, remains to be elucidated.

L. × japonica is not a unique case of hybridization found within the genus Lemna, as a second interspecific hybrid L. gibba × L. minor, represented by at least six different clones from the Landolt collection, was identified, suggesting low reproductive isolation among species of the Lemna section Lemna. These findings pose new interesting questions about the contribute of hybridization in shaping duckweed phylogenesis.

TBP genotyping of the whole Landolt Collection, is ongoing and may disclose other interesting findings.

05

Integrative analysis of growth dynamics in duckweed

Stefan Ortleb[1], Twan Rutten[1], Alexander Hilo[1], Andreas Guendel[1], Hardy Rolletschek[1], Eric Lam[2], Ljudmilla Borisjuk[1]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research, IPK-Gatersleben, Germany;
[2]Department of Plant Biology Rutgers, The State University of New Jersey, USA;
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Various species of duckweed are the fastest-growing plants in the world (Acosta et al., 2021). Data about the Wolffia genome already provide important insights into the interplay between how plants develop their body plan and how they grow (Michael et al., 2021). In our current work, we are focusing on structural modeling and metabolic analysis of various Duckweed strains, which are distinct in their growth behavior.

First of all, we investigated the process. proliferating daughter fronds in W. australiana, W. globosa and Lemma gibba by applying light and confocal microscopy. Intrinsic events of budding were documented, starting from very early stage onwards during formation of the developing daughter fronds. Next, we generated 3D models which display intern architecture and some details of the inter structure of the plant body, and also visualize the budding pouch and successive generation of daughter fronds. This approach also allowed to perform volumetric analysis, to estimate cell numbers and to characterize gradients in cell size. This altogether is used to understand the functional arrangement of tissues.

To identify metabolic cues underlying the dynamics of biomass accumulation in Wolffia, we performed an untargeted metabolome analysis (LC/MS) and spatially resolved infrared imaging (FTIR) in five genotypes with distinct growth characteristics. The comparative analysis was performed under controlled light/dark conditions and sampling was done during the logarithmic growth phase. We identified substantial differences in metabolite composition between the individual strains. Clustering analysis of metabolome data highlighted three distinct groups characterized by differences in accumulation of key metabolites. The first cluster was characterized by accumulation of central sugar intermediates, TCA-cycle intermediates and energy metabolites. The second cluster showed accumulation of various cofactors, PPP-intermediates, as well as other central and energy metabolites. The third group was distinct by significantly less energy metabolites and had the highest concentration of ureides, indicatory for stagnated assimilation and stress/senescence.

Altogether, the integrative analysis of structural organization, meristematic activity and metabolism can improve our understanding of growth dynamics in duckweed.

References

Acosta K, Appenroth K J, Borisjuk L, Edelman M, Heinig U, Jansen M A K, Oyama T, Pasaribu B, Schubert I, Sorrels S, Sree K S, Xu S, Michael T P, Lam E: Return of the Lemnaceae: Duckweed as a model plant system in the genomics and post-genomics era. Plant Cell 33 (2021) 3207-3234.

Michael T P, Ernst E, Hartwick N, Chu P, Bryant D, Gilbert S, Ortleb S, Baggs E L, Sree K S, Appenroth K J, Fuchs J, Jupe F, Sandoval J P, Krasileva K V, Borisjuk L, Mockler T C, Ecker J, Martienssen R A, Lam E: Genome and time-of-day transcriptome of Wolffia australiana link morphological minimization with gene loss and less growth control. Genome Res. 31 (2021) 225-238. https://dx.doi.org/10.1101/gr.266429.120

06

Epigenetic regulation of transposable elements in duckweeds

Rodolphe Dombey, Veronica Barragan, Arturo Marí-Ordóñez

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Transposable elements (TE) are mobile DNA sequences capable to colonize their host genome. Despite being a source of genetic variation and innovation, TE mobilisation is often associated with loss-of-function mutations. Hence, TEs activity must remain tightly regulated by their host. In plants, this is achieved by the formation the heterochromatin, which is characterized by high level of DNA methylation (5mC), as well as repressive histone post-translational modifications (e.g., H3K9me2). 5mC is first installed at new TE insertions by the RNA-directed DNA methylation pathway (RdDM), which relies on the generation of loci specific 24nt small-interfering RNA (siRNAs). However, duckweeds, a family of aquatic flowering plants, have seemingly lost RdDM. Indeed, previous report of both siRNAs and gene expression profiling have suggested the absence of this central pathway rising questions, on how duckweeds effectively regulate TEs. To tackle these questions, we perform several omics analyses. Transcriptomic data revealed the absence and non-expression of key components including the RdDM methyltransferase (DRM2), as well as the lack of siRNA associated with TEs. Despite the non-functional pathway, TEs remain methylated in most cases and silenced. These results suggest the presence of an uncharacterized pathway to deposit methylation in duckweeds, to in fine silence TEs.

07

Nutrient stress on duckweeds

Sowjanya Sree Kandregula[1], Klaus-J. Appenroth[2]

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Duckweeds are fast growing aquatic plants that can efficiently take up nutrients from the aqueous medium. However, in conditions of nutrient deficiency, duckweeds can depend on several mechanisms to alleviate the stress. One of them is to form specialized bodies called turions. One of the species in which turions have been well studied is Spirodela polyrhiza. Not as a surprise, several other species of duckweeds also rely of similar strategy. In the genus of Wolffia, all species form turions except for W. microscopica which has not been reported to form these structures. Here, we present the first report of the formation of turions in W. microscopica. Further, the stress caused by nutrient deficiency (phosphate, nitrate and sulphate) as investigated in different clones belonging to all five genera of duckweeds, induces accumulation of starch in the fronds. Maximum amounts of starch on dry weight basis of the whole plant biomass, 40 - 50 %, were obtained after application of phosphate- or nitrogen-lacking nutrient media for two weeks, and deficiency of sulphate showed only minimal effects on the plants.

08

Non-genetic Inheritance alters Stress Resistance across Generations in the Giant Duckweed

Alexandra Chávez Argandoña, Meret Huber

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Non-genetic traits, like microbiota, nutrients and epigenetic mechanisms would produce additional phenotypes, increasing species diversity. In the absence of genetic change, species may acquire resistance to stress through the inheritance of non-genetic induced phenotypes or selected non-genetic phenotypic variants. However, it is challenging to identify the contribution of these two mechanism to stress resistance due to the number of generations required for such evaluations and the difficulty to disentangle non-genetic from genetic effects. We used Spirodela polyrhiza, a clonal species with low mutation rate, to characterize non-genetic inheritance and their role in the resistance to natural-occurring biotic (Rhopalosiohum nymphaea) and abiotic (CuSO4) stress. We subjected clones into stress and control conditions for multiple generations, and we found that stress exposure affects offspring fitness for at least five generations after stress release, both in the absence of stress and under recurrent stress. Across 56 genotypes analyzed, we showed that non-genetic transgenerational resistance is associated to the clone genotype, and that ancestral stress exposure can cause fitness costs besides fitness benefits. Additionally, assessment of large S. polyrhiza monoclonal populations in outdoor conditions showed that clones under natural selection have a higher resistant offspring to recurrent stress than those clones grown in the same environment, but in the absence of selection. Thus, suggesting that selection of non-genetic phenotypic variants alters population resistance to stress across generations. Currently, we are investigating the molecular mechanisms that underlie these events. Our data provides evidence that S. polyrhiza would acquire stress resistance through non-genetic induced phenotypes and selection of non-genetic phenotypic variants, both passed to the offspring for multiple generations. These findings highlight the role that non-genetic inheritance may have on stress adaptation in clonal plants.

09

All in one: the microbiome of a rootless plant

Tarun Pal, Osnat Gillor

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Plants provide different niches each occupied by a unique microbiome that serve different functions. Plats microbiomes vary significantly between the rhizosphere, endophytes and the phyllosphere each enriched by different bacterial phyla. However, the plant body of duckweed species, has undergone reduction and simplification, especially members of the genus Wolffia that are rootless and represent the smallest and most reduced plants on earth. The morphology, anatomy, genome, and function of Wolffia species were explored yet their microbiome was never explored. We hypothesized that the microbiome of this rootless plants will engulf unique endophytic community that will combine properties usually reserved to different plants compartments. To test our hypothesis, we assessed the composition and function of W. globosa microbial community, focusing on nutrient uptake, metabolites production and protection from pathogens via population-resolved metagenomic analysis. W. globosa endophytes significantly differed from the epiphytes and the communities in the surrounding media. The microbiome was dominated by bacteria (~90% in relative abundance) and almost devoid of fungi, protists, and archaea. The diversity of the endophytic community was low, thus we assembled full genomes of 32 species mostly affiliated with Alpha- and Gamma- Proteobacteria. The potential to utilize atmospheric nitrogen, usually reserved to the rhizosphere, was detected in three species, while other members of the community enhanced iron and phosphors uptake. We mostly found genomes with a strictly heterotrophic genetic repertoire, yet six species encoded CO2 fixation pathways, indicating potential independence from the plant. The synthesis of the essential metabolite, vitamin B12, is complex containing over 30 genes. The full pathway was encoded in four of the endophytes, yet sections of the B12 pathway were present in almost all the endophytic species, suggesting an exchange of precursors and metabolites. Network analysis of the endophyte's community confirmed high interconnectivity among species. Our results assign functional potential to W. globosa endophytic species and delineate their close relations. This in-depth description of the community allows for the generation of hypotheses on the dynamics and interactions in a rootless plant microbiome.

10

Analysis on floral induction of Wolffiella hyalina

Minako Isoda, Tokitaka Oyama

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Regulation of flowering time is one of the most important strategies in life history of plants. In this study, we analyzed the floral induction of Wolffiella hyalina. First, we examined the flowering of three accessions (7378, 8640, 9525) of W. hyalina under long-day (15 h light/9 h dark) and short-day (9 h light/ 15 h dark) photoperiodic schedules in a culture medium with/without 5 µM salicylic acid (SA) or benzoic acid (BA). Consistent with previous studies, the 7378 accession flowered in two weeks under the short-day photoperiodic schedule in the culture medium with SA and BA. The 9525 accession flowered under long- and short-day photoperiodic schedules in culture medium with SA. The 8640 accession did not flower under any of these conditions. These results suggest variation of floral inductive pathways between accessions in W. hyalina. Next, we examined the effective period of SA or BA treatment for the floral induction. We found that BA induced flowering in a shorter period of treatment than SA. Furthermore, we found that the flowering of W. hyalina was induced when co-cultured with W. hyalina with flower even in the absence of SA or BA in the medium. This result suggests the plant-to-plant communication for floral induction in a population.

11

To root or not to root: evolution of rootlessness in duckweed

Alexander Ware, Dylan Jones, Anthony Bishopp

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Duckweeds are unusual angiosperms as they possess either an extremely reduced root system or do not have one at all. Molecular mechanisms controlling organ loss and vestigiality have been explored in animal systems but are unprobed in plants. Duckweeds thus represent a unique untapped system for exploring the key evolutionary question of why and how an organ would become vestigial. However, clarity on whether the duckweed root is vestigial, as historically suggested by authors such as Landolt and Gorham, is needed before employing them as a model system for investigating organ loss.

We systematically tested this to better resolve the status of the duckweed root as a vestigial organ. Our approach investigated root anatomy, physiology and transcriptomics. Sectioning roots of nearly all rooted duckweed species revealed a highly reduced anatomy, with greater simplification in the more recently diverged genus Lemna, notably in traits such as number of cortical cell layers. PCoA of root anatomical traits revealed 5 distinct clusters which corresponding to existing genera. We compared duckweed to another free-floating member of the Araceae, Pistia stratiotes, which retained a more complex root anatomy.

We showed that roots are dispensable for normal growth in both Spirodela polyrhiza and Lemna minor, and tested whether roots were required for nutrient uptake in these species. Subsequent analyses of 13 mineral ions in these fronds revealed almost no impact of root removal. Removal of Pistia roots depressed growth, but did not eliminate it, and generated broad changes in the mineral profile of aerial tissues. Our data showed that roots were no longer required for nutrient uptake in duckweeds, so we hypothesised that expression of nutrient transporters might be elevated in the frond. We developed an organ-specific transcriptome for S. polyrhiza and used this to follow the spatial regulation of putative nutrient transporters. Analyses indicate a frond-ward shift in the expression pattern of orthologues of nutrient transporters compared to species with non-vestigial roots. Investigation of the biochemical properties of these may reveal novel changes that have evolved specifically in the duckweeds and may not be present in terrestrial plants.

Our results take a holistic approach and consider a broad spectrum of nutrients, species and morphologies. They strongly support historical observations that duckweed roots are vestigial. Recent advances in duckweed genomics and molecular approaches, alongside our own data, will permit investigation into the molecular genetics underlying the loss of specific structures in duckweed roots like root hairs and lateral roots. We can leverage this, drawing on deep understanding of molecular genetic regulation of root development in other species, to probe fundamental mechanisms underlying organ simplification. We therefore propose that duckweeds represent a powerful, novel model system with which to understand organ loss and vestigiality.

12

Lemnaceae as key players in the circular economy; examples of wastewater valorisation in Ireland

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The circular economy (CE) advocates long-term value retention and reductions in the linear use of raw resources. In cases where waste is produced, the CE focuses on waste valorisation, i.e., the use of waste as a resource. Members of the family of Lemnaceae are particularly suited for waste valorisation, contributing both to recycling of plant nutrients, as well as the generation of valuable protein-rich biomass. In this presentation, two duckweed-based applications will be explored. Firstly, as part of an Integrated MultiTrophic Aquaculture (IMTA) farm, duckweeds are used to control ammonia levels. A low input water regeneration system, comprised of one hectare of duckweed covered channels, is integrated with fishponds containing up to 30 tonnes rainbow trout and perch. The closed-loop, IMTA approach nullifies environmental impacts of aquaculture wastewater. Indeed, the duckweed system effectively controls ammonium levels as part of the IMTA farm, while generating an estimated 30 tonnes of dry-weight plant biomass. This effective, but technically basic, system is contrasted with a high input system used to manage dairy processing wastewaters. Globally, dairy processing waste is one of the largest waste streams by volume, estimated at 0.2 to 11 litres of wastewater per litre of milk processed. Dairy processing plants typically have on-site facilities to treat wastewater, and this constitutes a major cost to the industry. A pioneering, cascading system for valorisation of dairy wastewater couples microbial-based technologies of anaerobic digestion (AD) and/or aerobic dynamic feeding (ADF) with duckweed (Lemnaceae) cultivation. Each step in this integrated, cascading system contributes a value-added output, ranging from volatile fatty acids for manufacture of, inter alia, bioplastics, to duckweed biomass, while concomitantly remediating used water. As part of the indoor system, duckweed is grown in a stacked (multitiered) flow-through system to minimise the spatial footprint. The system operates independently from seasons and climatic factors, and matches expertise and facilities present in the industrial setting of a modern dairy wastewater treatment plant. Thus, it is argued that Lemnaceae can be effective components of a CE approach but that bespoke solutions (i.e., low or high technology) are required for different industrial applications.

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Stress tolerance in tetraploid Spirodela polyrhiza

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Polyploidisation or genome duplication, is one of the main drivers of plant evolution. Often such a duplication coincides with considerable morphological, physiological and even phytochemical changes. Polyploid plants are often larger and sturdier than diploids, and some are more resistant to abiotic stress e.g. drought. Not surprisingly, many economically important crops are polyploid e.g. common wheat, cotton, sweet potatoes, coffee, banana, oat, watermelon, strawberries. Nevertheless neopolyploids face many challenges e.g. interploidy competition, low genetic diversity, minority cytotype exclusion, genomic shock and meiotic/mitotic irregularities, making polyploid establishment one of the biggest mysteries in polyploid research. There is increasing evidence that the fate of neopolyploids depends strongly on the environment. We used genetically diverse neopolyploid strains of the greater duckweed Spirodela polyrhizha to investigate the phenotypical impact of polyploidisation, and its impact on fitness (growth) over a range of environmental conditions. Our results are relevant to both polyploidy research and applied duckweed science.

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Wastewater valorisation in an integrated multitrophic aquaculture system; assessing nutrient removal and biomass production by duckweed species

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Freshwater aquaculture in Europe is an underexploited sector that could represent an important source of high-quality food. The limited development of the sector is associated with the competition with countries that can produce fish at lower cost. Moreover, there are concerns about the impacts of this activity on natural waters. Coupling freshwater aquaculture with duckweed-based remediation systems could provide a valuable by-product of the fish farm and prevent release of eutrophic water in the environment

This study presents quantitative information on the nitrogen and phosphorus removal efficiency of a duckweed-based Integrated Multitrophic Aquaculture (IMTA) system located in Ireland. The pilot system includes four fishponds for the farming of rainbow trout Oncorhynchus mykiss and European perch Perca fluviatilis, and 16 canals (total area 1.2 Ha) where the aquaculture effluents are treated by Lemna gibba and Lemna minor. After restoration by duckweed, the water is sent back to the fishponds.

The system is the first of its kind built at large-scale in Europe. This study creates a scientific basis to determine the balance between aquaculture and extractive species.

Duckweed remediation capacity was assessed in three different ways. Assessment of water quality pre and post treatment with duckweed showed that the system can remove 0.78 and 0.38 T y-1 1 of Total Nitrogen (TN) and Total Phosphorus (TP), respectively. Analyses of the nitrogen and phosphorus content of newly grown duckweed biomass showed that 1.71 and 0.22 T y-1 1 of TN and TP can be removed, respectively. Extrapolation based on laboratory established nitrogen and phosphorus uptake rates determined that 0.88 and 0.08 T y-1 1 of TN and TP can be removed by the system. There is substantive agreement between the three assessments, and the study confirms that duckweed can maintain good quality water in an IMTA system, while yielding biomass with a high protein content (21.84 ± 2.45%).

Part of the duckweed biomass produced in this IMTA system was used to produce experimental fish feeds tested on Salmo salar and Oncorhynchus mykiss as part of Aquatech4feed, a project funded by BlueBio ERA-NET COFUND, aiming to improve aquaculture sustainability.

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Dual function of environmental bacteria that enable the duckweed prosperity

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Microalgal bloom imbalances aquatic ecosystems and damages not only animals but plants. The health of duckweeds is also impaired by microalga mainly due to nutrient competition. Bacteria are the most abundant and diverse organisms on the earth. Some of them form ecological relationships, such as competition, mutualism, or commensalism with microalgae. It is known that bacteria can control microalgae growth by directly attacking cell to cell or indirectly by releasing extracellular substances and competing for nutrients. Genus Bacillus is one of the most reported algicidal bacteria. Bacillus strains produce several compounds including surfactin that inhibit the growth of cyanobacterium, Microcystis aeruginosa. It can also disintegrate cyanobacteria colonies, making them susceptible to environmental pressures. These facts indicate that Bacillus group plays significant role in controlling M. aeruginosa blooms.

Besides the genus Bacillus, Chryseobacterium is also reported as a potential algicidal bacterium where it attacks M. aeruginosa by producing potent compounds, diketopiperazines. Those compounds increased intracellular reactive oxygen species (ROS) levels while decreasing the activities of antioxidases, effective quantum yield, and electron transport rate of M. aeruginosa. In our previous study, we isolated Chryseobacterium sp. 27AL from a food factory wastewater that can promote the growth of duckweed Lemna gibba (Khairina et al., 2021). Strain 27 AL produces some growth-promoting factors, such as indole acetic acid (IAA) and siderophore. Moreover, its unique limited inorganic nitrogen metabolisms enables 27AL to avoid nutrient competition when co-existing with duckweed in wastewater condition. In this study, we asked if 27AL can benefit duckweed growth in the presence of M. aeruginosa.

It was found that 27AL, indeed functions to inhibit the growth of M. aeruginosa, especially in the bacterial suspension condition. The growth yield of L. gibba was successfully recovered by 27AL in the presence of harmful microalga, M. aeruginosa. On the other hand, Bacillus sp. MRB10, isolated from the duckweed surfaces, inhibited the growth of M. aeruginosa without giving adverse effects on L. gibba only in the bacterial attachment condition. The health of L. gibba was significantly impaired by MRB10 in suspension condition. It was found that there exists an appropriate population range of each duckweed associated bacterium for maintaining healthy growth of the host plant.

References

Yeni Khairina et al. (2021) Chemosphere, 268, 129247.

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Eine neue Kulturpflanze mit großem Potenzial für Ernährung, Energie und Wasserreinigung

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Wasserlinsen, landläufig auch als Entengrütze bezeichnet, wird von den meisten Menschen nicht gerade mit der Verwendung für Ernährung, Erzeugung von Energie oder Wasserreinigung in Verbindung gebracht werden. Die Möglichkeiten der praktischen Anwendung und die Verwendung in der biologischen Grundlagenforschung wurden bereits im Jahr 2011 bemerkt, als die Zitationen mit Bezug auf Wasserlinsen (lateinisch Lemnaceae) in wissenschaftlichen Zeitschriften erstmalig die Zahl 70.000 überschritt. Entsprechend der Datenbank Web_of_Science ist die Zahl der Zitationen gegenwärtig, zehn Jahre später, auf insgesamt 180.000 gestiegen. Es ist sicher auch kein Zufall, dass 2011 die 1. Internationale Konferenz zur Förderung von Wasserlinsenforschung und -anwendung (ICDRA) stattgefunden hat und zwar in Chengdu/China. Das dazugehörige Komitee, ISCDRA, wurde 2013 an der Rutgers University in New Jersey, USA während der 2. ICDRA gegründet. Die Ursache für enorme Entwicklung dieses Forschungsgebietes liegt am Potential, dass diese kleine Pflanzenfamilie (bestehend aus 36 Arten in 5 Gattungen) bietet: Es stellt die am schnellsten wachsenden Blütenpflanze überhaupt dar. Diese Eigenschaft ist wichtig für die Erzeugung großer Mengen Biomasse. Je nach Kultivierungsführung kann Biomasse mit einem hohem Proteingehalt für Futter- oder Nahrungsmittel erzeugt werden oder, alternativ, mit einem hohen Stärkegehalt für die Energiegewinnung produziert werden. Im Weiteren können diese Pflanzen zur Reinigung von Abwasser eingesetzt werden, da sie Nährstoffe zusammen mit Kontaminationen während des Wachstums schnell aufnehmen. Diese praktische Verwendbarkeit hat auch das akademische Interesse beflügelt. Inzwischen sind die Genome mehrerer Arten vollständig sequenziert. Viele Entwicklungsprozesse können somit auf genetischer und molekularer Ebene leichter und effizienter untersucht werden. Das gilt z.B. für die Blütenbildung (sexuelle Vermehrung) oder die Bildung von Überdauerungsorganen, sog. Turionen. Die Mechanismen, die zum rasanten Biomassezuwachs oder zur Vereinfachung der Pflanzenstrukturen während Evolution geführt haben sind interessante Aspekte der gegenwärtigen Forschung. Zudem ist die geringe Größe der Pflanzen (geringer Platzbedarf im Labor!) und die Kultivierbarkeit unter sterilen Bedingungen besonders vorteilhaft für wissenschaftliche Untersuchungen. Die Verwendung der rasant wachsenden Blütenpflanze hält auf jeden Fall noch einige Überraschungen in Zukunft bereit.

Emerging crop plant with high potential for nutrition, energy and water purification

Many people will find it difficult to correlate the word duckweed (if they know it at all) with its use in nutrition, production of energy or water purification. The increasing awareness of the practical applications of duckweeds and its use in basic biology research was noted already in 2011 wherein the duckweed-related citations in scientific journals exceeded for the first time the number 70,000. According to Web_of_Science, the number of citations is presently, ten years later, approximately 180,000 in total. It cannot be by accident that in 2011 the 1st International Conference on Duckweed Research and Applications (ICDRA) was held, in Chengdu/China. The related steering committee (ISCDRA) was founded in 2013 at the Rutgers University, NJ, USA during the 2nd ICDRA. The reason for the enormous development of this area of research has to be the potential that this small family of water plants (comprising 36 species in 5 genera) offers: it represents the fastest growing flowering plants on earth. This property is important to produce large amounts of biomass. Depending on the method of cultivation, biomass with high protein content (suitable for feed and human nutrition) or, alternatively, biomass with high starch content (suitable for the production of energy) can be produced. Also, duckweeds can be used for wastewater purification because the plants can efficiently take up minerals for fast growth, together with other contaminants present in the wastewater. These practical applications spurred for sure the academic interest (see the number of publications). Meanwhile, the genomes of many species are completely sequenced which makes the use of methods of molecular biology much easier and more effective. Many developmental processes can be investigated at the molecular level, e.g. flowering (sexual propagation) or the formation of perennial organs, so-called turions. The mechanism that makes the fast growth possible or the simplification of plant structures are interesting aspects of the present duckweed research. The small size of the plants (space requirement in the lab!) and the opportunity to cultivate the plants under sterile conditions play an important role. The use of these very fast growing flowering plants will offer some more surprises in the future.

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The Underwater Chemical World of Duckweed

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Information regarding root-bacterial interactions in aquatic plants environments is currently scarce. The simple structure of Lemna minor, its rapid growth combined with the availability of genomic resources and molecular tools such as stable transformation, makes it an ideal model organism to study plant-bacteria interactions in aquatic settings. The chemosphere surrounding roots, either in soil or water, plays a key role in the dynamic communication between plants and their associated microbiome. Roots exude an extensive and diverse repertoire of metabolites including hormones that play a dominant role in attracting or deterring specific bacteria. Yet, we know little with respect to how hormones affect root exudation of secondary metabolites, and how exudate composition impacts the plant-associated microbiome. In my presentation I will describe our research examining the hormonal effect on L. minor root exudation and its link to microbiome community composition and colonization. We anticipate that investigating duckweed-bacteria chemical interactions might unravel the key metabolites and metabolic pathways stirring plant root-microbiome interaction in the aquasphere.

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Variability of chronobiological characteristics in duckweed

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The natural environment for plants has a large number of elements showing differences between daytime and nighttime. Light, temperature, moisture and feeding behavior of herbivores are examples of them. The circadian clock plays an important role in adaptation to the day-night cycle. Circadian traits such as the period length, entrainability (ability to synchronize the clock to the cyclic environment), temperature dependency, show a range of intra- and interspecific variation. Even in the plant body, these properties show a variation between cells and between tissues/organs. Physiological and evolutionary relevance of the variations are largely unknown partly because quantitative analyses on the variation of circadian properties are experimentally challenging.

In my laboratory, we have developed a number of methods to measure the circadian rhythm of duckweed. A bioluminescence monitoring system combined with transient reporter-gene expression by a particle bombardment transfection method has been effectively used to detect the circadian rhythm of any species of duckweed1. Using this system, the interspecific variation of the circadian traits were investigated in Lemna and Wolffiella genera2. Compared with species in Lemna, those in Wolffiella showed more unstable rhythms and species in a clade of Wolffiella showed arrhythmia under constant light at a high temperature. Thus, the stability of the circadian rhythm reflects a phylogenetic relationship in duckweed.

Intraspecific variation of the circadian traits are found in plants but its physiological and evolutionary relevance is poorly understood. We analyzed 72 strains of Lemna aequinoctialis from 20 populations in Japan (from 31.5°N to 43.8°N) and found a variation of circadian periods and of critical day lengths3. Interestingly, a significant correlation between circadian periods and locally adaptive critical day lengths is detected, suggesting that the variation in the circadian trait underlies the local adaptation.

We further studied the variation/heterogeneity of circadian traits between fronds and also between cells in the duckweed plant4,5,6. I will discuss physiological and evolutionary relevance of the variations in duckweed.

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Acknowledgement: We thank the Rutgers Duckweed Stock Cooperative and the Landolt Duckweed Collection for providing us accessions of duckweed.

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Unraveling the genetic mechanisms of plant sexual reproduction in duckweed

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Duckweeds are known as the smallest flowering plants, although many clones barely produce flowers either in nature or in laboratory conditions. This was one of the reasons that left Lemna behind with the advent of the molecular genetic era during the 1990's. Recent advances in high-throughput genomic technologies have opened up an interesting opportunity to study different aspects of the biology of duckweed and the number of scientific publications on Lemna have raised exponentially over the last 20 years, from 17 in 2000 to 107 articles in 2021. Today, we find ourselves in what many authors have defined as "The return of the Lemnaceae" (Acosta et al., 2021).

Duckweed sexual reproduction is still one of the most unknown aspects in this plant family. Several recent transcriptomic and physiological studies regarding Lemna flowering development, allowed researchers to identify conditions required for duckweed flowering (Fouronjian et al., 2021) and key flowering genes such as LgFT1 and LaFTL1 (Fu et al., 2020; Yoshida et al., 2021).

Here, we present our transcriptomic analysis conducted on duckweed pistils, anthers, seeds and cotyledons of the short-day plant L. aequinoctialis with the aim of providing the first specific and global characterization of the duckweed transcriptome related to flowering. Our study contributes to the further characterization of not only flower development, but also of the alternating sexual and clonal reproduction strategy, shedding light on this unexplored area of plant physiology and development.

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Using rootless duckweed Wolffia globosa as a model plant to clarify the effect of e[CO_2] on NO_3 photosassimilation in C3 plants.

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There have been many studies showing that elevated [CO2] (e[CO2]) reduces protein concentration in leaves and grain. This could have negative ramifications for human and animal health in expected future [CO2]. Therefore, it is important to understand the mechanisms behind this protein reduction. However, there has been some unresolved controversy about the mechanism behind this reduction in protein concentration. One hypothesis is that e[CO2] reduces NO3 photoassimilation in C3 plants. There is a large body of evidence to support this claim. However, two major predictions based on this hypothesis have not been shown consistently: a) e[CO2] should lead to a buildup of unassimilated NO3. b) Plants should respond better to e[CO2] when provided with NH4 as opposed to NO3.

There are 3 reasons why these predictions may not be consistently fulfilled: 1) While NO3 assimilation is thought to be reduced in the leaves due to e[CO2], it may be simultaneously increased in the roots, changing the N form being transported to the leaves and complicating the picture. 2) NH4 nutrition often has negative effects on plant growth, so even if there is an increased positive effect of CO2 when given NH4, it is less convincing, since overall growth often goes down. 3) It can be difficult to maintain NH4 concentration in soil, due to nitrification processes by soil microorganisms.

Wolffia globosa is a useful model plant to isolate this mechanism, since it has 3 main advantages: 1) It does not have roots, so the NO3 assimilation can only occur in the leaves. 2) It is tolerant or even prefers NH4 over NO3. 3) It can be grown in a sterile hydroponic medium, which should minimize any nitrification.

Two experiments will be discussed in this presentation. I) Wolffia globosa are grown in high NO3, and then transferred to an NO3-free medium in ambient [CO2] and e[CO2]. Nitrate concentration in the fronds are monitored to determine how e[CO2] affects NO3 assimilation. II) Wolffia globosa are grown in ambient and e[CO2] at different NO3:NH4 ratios. Growth and is monitored and frond N concertation is measured to determine whether reducing the reliance of the plant on NO3 assimilation will increase its response to e[CO2].

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How aminomethylphosphonic acid (AMPA), the main glyphosate metabolite, affects Lemna minor photosynthesis?

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Aminomethylphosphonic acid (AMPA) has been proposed to interfere with photosynthetic processes in plants, but its mechanism of action is not yet elucidated. Here, the effects of AMPA were investigated on photosynthesis (and related processes) of the aquatic macrophyte Lemna minor. At environmentally relevant concentrations (35-50 µg l-1) of AMPA had no effect on growth and photosynthesis but significantly decreased chlorophyll concentration. Photosynthesis was only reduced in plants exposed to AMPA concentrations ≥ 50 µg l-1. We showed that by depriving plants from glutamate (GLU), due to its interference on glycine decarboxylase (GDC), AMPA prevented the synthesis of δ-aminolevulinic acid (ALA) in chloroplasts, and thus blocked chlorophyll synthesis. This negative effect was reversed by the addition of GLU or ALA to the growth media. Our results indicate that the negative effects of AMPA on photosynthesis is related to the interference on chlorophyll biosynthesis.

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The life cycle of Spirodela polyrhiza: a model for aquatic plant overwintering?

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Spirodela polyrhiza is widely distributed in temperate climates that may give rise to winter cold that would be lethal to the fronds proliferating on the water surface during the growing season. To cope with low winter temperatures, the primordia of newly formed fronds switch from producing further fronds toward the end of the growing season to the formation of overwintering buds, or turions, which separate from the mother fronds when mature. The turions are compact structures with a high content of starch that accordingly sink and spend the winter on the bottom of the body of water inhabited by the duckweed. In the spring they surface and germinate and sprout to form new fronds that can again proliferate on the water surface. The life cycle of S. polyrhiza has been subjected to numerous studies over many years that have revealed several key processes involved in turion formation and function. For instance, the developmental switch from frond propagation to turion formation is not primarily due to the decreasing day lengths and temperatures responsible for many developmental issues associated with the coming of winter, but rather to nutrient depletion of the water habitat. The newly formed turions are dormant and require a prolonged exposure to cold water to be able to germinate. Turions surface in the spring with the aid of an endogenously produced gas bubble: the germination and sprouting of the surfaced turions correspond to two distinct phytochrome responses to light and are fuelled by two distinct carbohydrate reserves. And the breakdown of the turion starch upon sprouting is initiated in a manner not typical of vegetative plant tissues. These findings are interesting in their own right in providing a comprehensive view of the events involved in the overwintering of a cold-sensitive surface aquatic plant in a purely vegetative mode. But how representative is this overwintering scheme of those of other aquatic plants? Given the key role of turions in S. polyrhiza overwintering, this question should be restricted to turion-bearing species of vascular aquatic plants (or “macrophytes”). True turions are found in three genera of the duckweeds, or Lemnaceae (Spirodela (one species), Lemna (two) and Wolffia (six)), and one further Lemna and two further Wolffia species exhibit turion-like resting fronds. Turions also occur in 11 genera of 9 other families of aquatic plants.

The present contribution examines to what extent the features characterizing the S. polyrhiza turion life cycle are known to be mirrored in the other turion-bearing genera and families. If the S. polyrhiza features are in widespread accordance with those of other turion-bearing macrophytes, S. polyrhiza could be a model organism for investigating turion-based macrophyte overwintering. However, the findings respective of S. polyrhiza may be restricted to the other turion-bearing duckweeds, in which case the model role would be limited to the Lemnaceae. The turion life cycle of S. polyrhiza may also represent a unique solution to the problem of turion-bearing macrophyte overwintering, or available data on other turion-bearing macrophytes may not be sufficient for comprehensive comparisons with S. polyrhiza. In this latter case, the turion life cycle of S. polyrhiza could be a model in the sense of pointing to investigations to be done on other turion-bearing macrophytes in order to determine if the use of S. polyrhiza as a representative reference for investigating turion-based macrophyte overwintering is justified.

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Different sources of iron modulate responses in the antioxidant system in plants

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Large-scale anthropogenic activities, such as mining, can generate waste and contaminate the environment with high concentrations of metals and metalloids, such as arsenic, chromium, lead, aluminum and cadmium, which can result in trophic biomagnification. In iron (Fe) mining, residues also contain significant concentrations of this element. Fe is an essential nutrient for plants, participating in the processes of photosynthesis, respiration and as a cofactor of enzymes. Several technologies can be implemented to reduce the impact of these pollutants on biological systems, among which, phytoremediation. This method consists of the use of plants to assist in the removal of contaminants, both in soils and in the aquatic environment. In the aquatic environment, there is the possibility of using plants capable of tolerating and removing different contaminants. Species of the Lemnaceae family stand out for their high capacity to absorb and accumulate pollutants from water bodies and, therefore, with the potential to be used in phytoremediation programs. This work seeks to evaluate the possibility of Fe, in the ionic form and as nanoparticles, to attenuate the biological damage caused by cadmium (Cd), which is considered one of the most toxic contaminants in the aquatic environment. For this, plants of the Lemna valdiviana species were exposed to Cd and, simultaneously, to different sources of Fe, in the ionic form and of zero valent nanoparticles (nZVInps), for 5 days, followed by evaluation of the alterations in the cellular antioxidant system. Results indicated that the different sources of Fe, associated or not with Cd, modulated differently the antioxidant capacity of the plant. There was an increase in the activity of the enzyme superoxide dismutase (SOD), which acts on the removal of the superoxide ion, mainly associated with the exposure of nZVInps. Enzymes such as catalase (CAT), which catalyze the removal of hydrogen peroxide, stood out for the increments associated with the joint exposure of Fe in the ionic form with Cd. However, the exposure of plants to treatments did not result in increases in lipid peroxidation, represented by the detection of thiobarbituric acid reactive substances (TBARS) levels. The observed effects on the response of antioxidant enzymes may be related to (1) synergistic relationship between Fe sources with Cd, and (2) discrepancies in absorption and distribution of Fe in the ionic form and nZVInps, since they have differences of size and charge that can affect access to cellular compartments and the induction of reactive oxygen species, substrates of antioxidant enzymes. In addition, there was no reduction in the relative growth rate of the plants, demonstrating the absorption capacity and tolerance to Cd, evidenced by the catabolism of reactive oxygen species.

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The dynamics of NO_3- and NH_4+ uptake in duckweed are coordinated with the expression of major nitrogen assimilation genes

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Duckweeds are promising aquatic plants for sustainable wastewater remediation because of their high growth rate and efficient absorption of water contaminants, such as agricultural fertilizers leached out of the nearby fields. Despite the recent intensive studies of various duckweed species for wastewater remediation and biomass production, there is not much information on duckweed’s preferences for different nitrogen sources and the regulation of nitrogen utilization.

Here we present our data on the utilization of two major sources of inorganic nitrogen, nitrate and ammonia, by six duckweed species Spirodela polyrhiza, Landoltia punctata, Lemna aequinoctialis, L. turionifera, L. minor and Wolffia globosa. Taking advantage of the available sequence of the S. polyrhiza genome, we monitored the expression levels of six genes involved in nitrogen assimilation using real time quantitative PCR and performed bioinformatic analysis of promoter sequences of the nitrogen assimilation genes. Integration of the nitrogen utilization data with the gene expression patterns has revealed the follows:

1. All six analyzed duckweed species can grow using both NO_3^- and NH_4^+ as a single nitrogen source. Duckweeds grew well on NO_3^- supplied at high concentrations up to 30 mM, while the NH_4^+ concentrations greater than 2 mM caused rapid drop in the medium pH and required additional buffering to support duckweed growth.

2. When NO_3^- and NH_4^+ were present in the medium together, all duckweed species first used NH_4^+, and started using NO_3^- only when NH_4^+ concentration dropped below 2.1 μM.

3. The expression of genes encoding nitrate reductase (NR) and nitrite reductase (NiR) increased about 10-fold when NO_3^- was supplied and decreased when NH_4^+ was supplied. NO_3^- and NH_4^+ induced the glutamine synthetase (GS) genes GS1;2 and GS2 by 2- to 5-fold, respectively, but repressed GS1;1 and GS1;3. NH_4^+ and NO_3^- up-regulated the genes encoding ferredoxin- and NADH-dependent glutamate synthases (2-2.5 times for Fd-GOGAT and 8-11 times for NADH-GOGAT).

4. A survey of nitrogen assimilation gene promoters suggested complex regulation of nitrogen assimilation genes, with major roles for NRE-like and GAATC/GATTC cis-elements, TATA-based enhancers, 〚(GA/CT)〛_n repeats, and G-quadruplex structures.

The presented data shed new light on the mechanisms of nitrogen assimilation in aquatic duckweed plants and paves ground for further investigations of the involved gene network and the regulation of plant nitrogen metabolism at molecular level.

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Development of cryopreservation protocol for a variety of duckweed meristems by vitrification-cryo-plate method

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Duckweeds are aquatic monocots classified as the subfamily Lemnoideae, which comprises 37 species grouped into five genera. They are used for both basic research and applications (biofuel, phytoremediation etc.). Huge numbers of clones collected from across the globe were maintained by successive culture of the clonal plants. Moreover, we have reported a stable transformation method by which many transformants are produced. Keeping many clones by vegetative propagation is time-consuming and the mixing samples, microbial contaminations and accumulation of genetic alteration are also concerned.
Here, we explored the feasibility to apply a reliable storage method to a variety of duckweeds using vitrification-based cryo-plates at -196℃. We have tried several parameter conditions (preculture, osmoprotection, dehydration, rewarming etc.) and analyzed regrowth rates. Duckweed clones that were collected in relatively higher latitude area showed the high regrowth rate of excised frond meristems. (e.g. Spirodela, Landoltia and Lemna genera), however the regrowth rate after cooling was currently still low in Wolffia and Wolffiella genera. Establishing cryopreservation protocols will facilitate the research using duckweeds for both basic and applied research.

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Advances in droplet-vitrification and -freezing of Lemna fronds for long-term preservation of duckweed

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The species of the Lemnaceae family are aquatic plants that typically reproduce asexually by developing meristematic pockets from which clonal daughters successively bud off and detach. To conserve the Lemnaceae genetic diversity as resource for scientific research and future applications, cryopreservation can only be used to preserve viable fronds over long-term periods. For successful cryopreservation of living organs, often vitrification approaches including application of dehydration, cryoprotection, ultra-low temperatures (-196°C) and pre- and post-cryopreservation treatments are applied. However, these treatments induce various stresses such as mechanical stress provoked by excision, osmotic stress induced by the pre-culture or cryoprotection of the explants and thermo-mechanical stress that occur during cooling and rewarming. All these stress factors affect the ability of explants to survive cryopreservation.

To study and overcome the sensitivity of the duckweed fronds towards dehydration and other stress factors, we used differential scanning calorimetry (DSC) and a range of different cryopreservation protocols and media compositions. These included different combinations of non-axenic/ axenic culture, non-excised/excised fronds, non/low/moderate sucrose concentrations, DMSO/Plant Vitrification Solution 2 (PVS2)/ PVS3 and various light treatments. Although duckweed fronds tolerate only short-term dehydration and show ice crystallization during DMSO droplet freezing, sterile fronds of Lemna minuta and Lemna gibba survived DMSO freezing and PVS3 droplet vitrification. Based on these first promising results, we continue to develop a stable and reliable cryopreservation protocol that is applicable to a range of Lemnaceae species.

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Organization and evolution of ribosomal RNA genes in duckweed

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Ribosomal DNA (rDNA) plays a pivotal role in organisms by producing the RNA components required to form ribosomes, the riboprotein structures responsible for protein synthesis in eukaryotic cells. Plants typically have two types of rDNA loci: 35S rDNA loci containing clusters of tandemly repeated units composed of conserved sequences encoding three linked rRNA genes (18S-5.8S-25S) separated by more diverse intergenic spacers (IGSs), and 5S rDNA loci containing tandem copies of 5S rRNA genes separated by non-transcribed spacers (NTS). Due to its high copy number, conserved coding sequence, and more rapidly evolving spacer sequences, the rDNA has become a favorite subject for studies related to plant systematics, evolution, and biodiversity. Our research on rDNA in duckweed (Lemnaceae) showed a number of specific features compared to other plants. Genome surveys of two ancient duckweed species, Spirodela polyrhiza and Spirodela intermedia, revealed a very low representation of 35S and 5S rRNA genes, at around 100 copies per genome, compared to the thousands of copies present in other plants. Analysis of 35S rDNA IGS in these two species demonstrated relatively low intra- and inter-genomic heterogeneity, high GC content, and an unorthodox molecular structure of the usually conserved rRNA transcription initiation site (TIS). The latter finding is intriguing considering that the TIS in other characterized duckweed species representing two younger genera (Lemna and Wolffia) has a canonic TATAGGGG signature characteristic of other plants. Comparison of 35S rDNA in two Spirodela species, Landoltia punctata, Wolffia globosa, and three Lemna species showed that the length of the IGS positively correlates with genome size, with S. polyrhiza (~157 Mbp) having the shortest IGS, about 3.2 kb, and Wolffia globosa (~1295 Mbp) having the longest IGS, about 10 kb. Characterization of 5S rDNA revealed two major length variants of the gene repeat units in genomes of S. polyrhiza, S. intermedia, and Landoltia punctata. Analyses of 5S rDNA in twenty S. polyrhiza ecotypes from five continents showed that European ecotypes contain a predominant NTS type of about 1060 bp, whereas the Asian and American ecotypes predominantly have a 400-bp NTS. The two NTS length variants differ in their cis element repertoire, which may influence transcription of the linked 5S rRNA genes. Comprehensive analysis of 5S rDNA units in Landoltia punctata demonstrated an unusual, mosaic arrangement of genes with different types of NTS within the locus, further challenging the postulated homogenization of rDNA repeats within the rDNA loci. In summary, our findings put duckweeds in the spotlight for research on the molecular evolution of the rDNA, promising new insights into basic principles of organization and regulation of rRNA genes in plants.

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Duckweed genome architecture

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Duckweed were key to the elucidation of many fundamental aspects of plant biology such as flowering time and the phytohormone auxin. These ubiquitous plants were a model plant of choice for many years due to their small size, reduced morphology, fast clonal growth, direct contact with media, and physical response to the environment. The genomics era presented an opportunity to leverage duckweed once again for many areas of research such as single cell sequencing, synthetic biology, and genome evolution. Spirodela polyrhiza was the first duckweed genome sequenced revealing a highly streamlined genome with the fewest protein coding genes of any known flowering plant at the time. An additional reference S. polyrhiza revealed a genome under rapid evolution actively purging transposable elements and an extremely low level of DNA methylation. Sequencing of the only other species in the Spirodela genera, revealed that S. intermedia and S. polyrhiza share a similar genome structure. In contrast, genomes for Wolffia and Lemna revealed dynamic evolutionary trajectories, whole genome duplications (WGDs) events and polyploidization. Now with genomes for the final two genera Landoltia and Wolffiella, the complete evolutionary trajectory of duckweed unraveled key innovations to plant form, function, and growth. Duckweed will provide insight into future crops not even imagined by this generation of farmers, scientists, and climate warriors.

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Chromosome numbers and genome sizes for duckweed species and implication for genome evolution and diversity of Lemnaceae

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Usually, chromosome sets (karyotypes) and genome sizes are rather stable for distinct species and therefore of diagnostic value for taxonomy. In combination with (cyto-)genomics, both features provide essential cues for genome evolution and phylogenetic relationship studies within and between taxa above the species level. We present for the first time a survey on genome size measurement for all 36 duckweed species, on chromosome counts for 31 species and on the evolutionary impact and peculiarities of both parameters in duckweeds.

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Biodiversity of duckweed (Lemnaceae) in water reservoirs of Ukraine and China assessed by double chloroplast DNA barcoding

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Monitoring and characterizing species biodiversity is essential for germplasm preservation, academic studies, and various practical applications. Duckweeds represent a group of tiny aquatic plants that includes 36 species divided into five genera within the Lemnaceae family. They are an important part of aquatic ecosystems worldwide, often covering large portions of the water reservoirs they inhabit, and have many potential applications, including in bioremediation, biofuels, and biomanufacturing. Here, we evaluated the biodiversity of duckweeds in Ukraine and Eastern China by characterizing specimens using the two-barcode protocol with the chloroplast atpH–atpF and psbK–psbI spacer sequences. In total, 69 Chinese and Ukrainian duckweed specimens were sequenced. The sequences were compared against sequences in the NCBI database using BLAST. We identified six species from China (Spirodela polyrhiza, Landoltia punctata, Lemna aequinoctialis, Lemna minor, Lemna turionifera, and Wolffia globosa) and six from Ukraine (S. polyrhiza, Lemna gibba, L. minor, Lemna trisulca, L. turionifera, and Wolffia arrhiza). The most common duckweed species in the samples from Ukraine were L. minor and S. polyrhiza, accounting for 17 and 15 out of 40 specimens, respectively. The most common duckweed species in the samples from China was S. polyrhiza, accounting for 15 out of 29 specimens. Landoltia punctata and Lemna aequinoctialis were also common in China, accounting for five and four specimens, respectively. According to both atpH–atpF and psbK–psbI barcode analyses, the species identified as L. aequinoctialis does not form a uniform taxon similar to other duckweed species and therefore, the phylogenetic status of this species requires further clarification. By monitoring duckweeds using chloroplast DNA sequencing, we not only precisely identified local species and ecotypes, but also provided background for further exploration of native varieties with diverse genetic backgrounds. These data could be useful for future conservation, breeding, and biotechnological applications.

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Family portrait of a duckweed colony: What can imaging-based phenotyping tell us about stress responses of mother and daughter fronds

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Owing to their unique traits, duckweeds are popular models for the broader plant research community to study basic plant processes, including responses to environmental factors. A commonly overlooked aspect in duckweed research is, however, that despite their genetic homogeneity, laboratory cultures consist of fronds of different ages. As fronds go through consecutive ontogenetic stages, different physiological states and responses can be hypothesized as function of their maturity. When focusing on population-level data, this ontogeny-related heterogeneity may be considered as noise obscuring valuable information. Yet, detailed analysis of spatial response patterns can provide important additional knowledge.

To present the potential information that can be acquired by studying frond-level heterogeneity, we assessed 1-1 clone of Spirodela polyrhiza, Landoltia punctata and Lemna minor, treated with either nickel (2.5 mg l-1), or hexavalent chromium (4 mg l-1), respectively; and analyzed responses along longitudinal frond transects by means of in vivo chlorophyll fluorescence imaging, after 3 days-long exposure. Heavy metal-induced photosynthetic responses were strongly metal- and frond size-dependent. Moreover, different species showed diverging patterns: in Sp. polyrhiza, the smaller fronds showed lower relative tolerance to Cr(VI), but higher to Ni than the larger ones, while in La. punctata opposite trends were observed. In Le. minor, smaller fronds were more sensitive to both heavy metals then larger ones. More detailed analyses of within-frond chlorophyll fluorescence patterns indicated that mature duckweed fronds can basically be divided into a basal and an apical region separated by the node zone.

In the second study, we exposed the same Sp. polyrhiza clone to 1.25 mg l-1 Ni, 2 mg l-1 Cr(VI), 4 g l-1 NaCl, or nutrient limitation for 3 days, and spatial patterns in frond responses were analyzed in terms of chlorophyll fluorescence, anthocyanin accumulation and starch accumulation, respectively. Results confirmed that different stressors induced distinct response patterns across mother and daughter fronds. A characteristic group of mother fronds -usually one in each colony- underwent enhanced senescence marked by lacking starch accumulation. Stress-induced anthocyanin accumulation, on the other hand, was more characteristic for daughter fronds.

These results highlight that various imaging-based phenotyping methods are not only suitable for rapid gathering of data on larger duckweed populations, but also facilitate detailed analyses of “within colony” and “within frond” duckweed responses.

This study was funded by the National Research, Development and Innovation Office of the Hungarian Ministry for Innovation and Technology, grant number OTKA FK 134296.

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Duckweed – Conservation and conversion into biogas

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Duckweed´s conservation and conversion into biogas was examined within the project “Generation of energy using aquatic biomass demonstrated by co-cultivation of duckweed and cyanobacteria (LemnaGas)” (sub-project 2: “Conservation and conversion of aquatic biomass into biogas”) by DBFZ. Sub-project 1: “Cultivation of aquatic biomass” was carried out by GMBU. The project (duration: 08/2014 – 07/2017) was supported by the German Federal Ministry of Food and Agriculture (funding code 22401514).

The project´s focus was the examination of duckweed in lab scale as an alternative substrate or energy crop for biogas plants. It included the development of processes with broadly closed material cycles enabling the production of biomass by aquacultures. Aquacultures could be implemented on areas, which are actually unused like large roofs or uneconomic for cultivation of other crops due to e. g. aridity or poor soil quality.

Samples of duckweed were investigated regarding its methane yields in Biochemical Methane Potential (BMP) tests in laboratory scale in triplicates each. Automatic Methane Potential Test System (AMPTS®) devices (Bioprocesscontrol, Lund, Sweden, temperature set on 39 ± 1 °C) were used. BMP tests were conducted in accordance with the guideline VDI 4630 (2006). The methane yields were standardized (dry gas, 273.15 K, 1013.25 hPa). Approximately 40 g fresh duckweed and 400 g inoculum were used in BMP test. As inoculum served digestate from the research biogas plant of DBFZ, which was fed with energy crops and manure. Total solids (TS) and volatile solids (VS) were measured in accordance with DIN EN 12880 (2001) and DIN EN 12879 (2001).

As a result of the lab scale BMP tests it has been found that the conservation of a natural duckweed mixture (mainly Lemna minor, Lemna gibba and Lemna turionifera from a pond near Schkopau, Germany) via drying halved the biochemical methane potential after 48 days from 204 (fresh, macerated, TS 5.84 %, VS 85.28 % TS) to 100 mL/g VS (dried, milled, TS 96.43 %, VS 84.61 % TS). Ensiling was also investigated as an alternative process of the conservation of duckweed. The first test with preserving jars failed due to volume expansion in the first hours, which led to leakage and faults fermentation afterwards. The second ensiling test (with mainly Lemna minuta = Lemna minuscula from a pond in Lützschena, Germany) using preserving jars was successful with 2.1 % loss of fresh matter at the end of 10 month of storage. Another BMP experiment for 38 days using a natural duckweed mixture (mainly Spirodela polyrrhiza, Lemna minor, Lemna turionifera from a pond in Machern, Germany, TS 7.28 %, VS 82.43 % TS) revealed the necessity of maceration (Ultraturrax) for a sufficient conversion into methane. For untreated duckweed a BMP of 144 mL/g VS and for macerated duckweed 214 mL/g VS was measured, respectively.

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Water lentils and derived products thereof for human consumption: overview of the current status of NF approval in Europe

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Plants from the duckweed family have been consumed in Asian countries already for a long time. Very often the specific species are not documented but often classified as Wolffia arrhiza and Wolffia globosa (also known by the name Mankai). In Europe all duckweed plants and derived products were seen as novel food (NF; not consumed to a significant level in Europe before 1997) for which approval via EFSA/EC applications is required before it can enter the European market. This need for approval is mandatory for each individual species or product that is based on duckweed, such as fresh or dried material, protein, fibres etc.

Based on the 'Traditional Foods from a third country' pursuant to Article 14 of the EC Regulation 2015/2283, the company GreenOnyx Ltd from Israel submitted an application for two Wolffia species. In a published technical report, the EFSA announced that it does not raise safety objections to the placing on the market of the fresh plants of Wolffia arrhiza and Wolffia globosa within the EU [1]. However, the dried Wolffia material for which a separate NF application was submitted, was recently rejected by EFSA as the Panel concluded that an increase in manganese and vitamin K1 intake from the dried material used as food ingredient or food supplements is of safety concern [2]. The same rejection was given by the EFSA to the dried Lemna product for which a NF dossier was submitted by Parabel; again because of the levels of manganese. Parabel Ltd. received a GRAS (Generally Recognized as Safe) status in the US and their duckweed product is approved as a novel food in Australia and New Zealand.

Currently, two EFSA Novel Food applications are still pending for Lemna gibba and Lemna minor: Wageningen University & Research submitted a dossier on heat-treated plants as vegetable, and ABCkroos (currently under the name RUBISCO Foods) submitted a dossier on protein concentrate from a mixture of Lemna gibba and Lemna minor. As part of these dossiers we performed extensive research including initiation of a cultivation system, composition analysis of both fresh and heat-treated plants, intake assessments, digestibility studies based on human volunteers [4,5] were performed, the potential allergens in water lentils was studied, a tolerance trial in which subjects consumed Lemna vegetables based products 11 days on a row [6] was performed, and the shelf life and quality of fresh and frozen products was studied. Next to that we collaborated with chefs that developed very tasty meals and dishes containing substantial amounts of water lentils plant material. The products were evaluated by a consumer taste panel and were well appreciated.

An overview will be provided on the composition of the different water lentil based products, the specification, all supportive analyses, and the flaws in the risk assessment as requested by EFSA. In addition results of the product developments and taste analysis will be discussed.

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Water lentils protein for human nutrition

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Plants from the duckweed family can produce high amounts of biomass via vegetative growth. Next to that protein concentration of 30-45% on dry matter base in duckweed is high for plant leaf material. The combination of these two qualities of duckweed results in a high production capacity of protein per hectare using this plant. By cultivating duckweed about 7 times more protein could be produced on an annual base per ha compared to soy. When grown in multiple layers via vertical farming this can even be further increased. In the light of the ongoing Protein Transition, that directs towards a shift in the human intake of more plant based proteins, the interest in purified proteins from duckweed increases. For a broad spectrum of applications of duckweed protein with versatile use in food products, a white protein concentrate with neutral taste and good product functionalities is favoured. On top of that, a good nutritional value would even support application for people at risk with a low intake of nutritious protein or having higher requirements in case of intensive sport activities, or diseased or recovering from a disease. Protein extraction from leafy material with high percentage of water is, however, much different than isolation of proteins from dry seeds or beans. Wageningen Food and Biobased Research owns a patent for the extraction of proteins (mainly RuBisCO) from leafy material, such as duckweed [1, 2]. Recently we analysed the composition of this duckweed protein concentrate and several of its food functionalities [2]. Next to that we studied the nutritional value and digestibility of duckweed protein by a human in vivo method [3]. Details on the extraction method, functionalities and protein quality will be presented and discussed, together with the future steps that are required to develop this product and its applications further.

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Lemna turionifera demonstrates good potential as an alternative system for transient expression of recombinant proteins

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Plant-based transient expression systems have recognized potential for use as rapid and cost-effective alternatives to expression systems based on bacteria, yeast, insect, or mammalian cells. Duckweeds as the plants with compact structures and high growth rates provide a feasible opportunity for production of high-value recombinant products in fully contained, controlled, aseptic and bio-safe conditions in accordance with the requirements for pharmaceutical manufacturing and environmental biosafety.

Following-up our recent demonstration of successful transient expression in Spirodela polyrhiza and Landoltia punctata (Peterson et al., 2021), in this study we explore the established protocol for a number of other duckweed species, in particular: Lemna turionifera and Wolffia globosa. Biomass of aseptically cultivated Chinese ecotypes of Lemna turionifera and Wolffia globosa (Zhou et al, 2022), were subjected to infiltration by Agrobacterium suspension bearing pCAMBIA-based non-replicating vector harboring coding sequence for Green Fluorescent Protein (GFP) under the control of 35S promoter of cauliflower mosaic virus.

Compared to Landoltia punctata, the resulted GFP accumulation in the L. turionifera fronds was 3 – 6 times higher, reaching 0.12 – 0.23 mg/g fresh biomass, with substantial increased levels of the tissue involvement in the process of transient expression. However, in a number of cases, we observed almost complete death of infiltrated L. turionifera biomass after a few days post-infiltration and no GFP accumulation, this was not observed for L. punctata. In Wolffia globosa, GFP specific fluorescent spots visible by naked eye on the surface of fronds were observed extremally rare. The major part of the infiltered biomass usually died soon after infiltration.

Despite of substantial deviations observed among biological replicas, L. turionofera performed as a very promising platform for transient expression of recombinant proteins and as a model plants for functional genomics of monocotyledonous plants. Our results suggest that after certain optimization steps the efficiency of Agrobacterium-mediated transient expression in duckweed could potentially match the level reached for Nicotiana benthamiana, the best-established transient expression plant system at present time, providing the additional advantage of full containment.

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Insights into the nutritional value of duckweed as protein feed for broiler chickens

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Projections estimate rising demand for animal protein leading to a global protein gap and increasing demand for animal feed. Especially, soybean meal is widely used as protein feedstuff for livestock and particularly broiler chickens. Soy production is often associated with deforestation and the decoupling of land farming and livestock production has caused global nutrient imbalances and long transport distances. However, the growth potential for common protein plants is limited. Therefore, a nutrient and space efficient protein production is needed in areas with intense livestock production, to handle nutrient surpluses. Duckweed is a possible alternative protein crop, because production in closed vertical indoor vertical farms is space and nutrient efficient and is almost globally feasible. The nutritional value of duckweed as feedstuff has already been confirmed in several experiments with broiler chickens. However, contrasting effects of using duckweed in complete diets on growth and especially feed intake have been determined.

To find indications for these effects, different duckweed qualities were tested in broiler chickens. The digestibility of crude protein and 20 amino acids differed significantly among the tested duckweed batches, with a digestibility of methionine ranging from 68.5 to 90.4 %. Lower digestibilities could be attributed to high tannin levels of up to 2943 mg/kg. First findings show a reduced feed intake in association with the use of some duckweed qualities, which could be related to high fiber contents and increased intestine filling.

Because todays feed formulation is based on digestible nutrients, the results represent a contribution to the practical application of duckweed in broiler diets as an alternative protein-rich feedstuff. Nutrient composition and other abiotic factors strongly influence plant composition and thus nutritional properties. Therefore, this study can provide indications for the optimization of duckweed production with respect to a standardized product.

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Duckweeds, the engine of the New Circular Economy

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The term The New Circular Economy provides semantic differentiation from the commonly misused term Circular Economy and the plethora of narrow, typically linear applications to which it is applied. The New Circular Economy encompasses the universe of volatile wastes produced by humans and farm-animals, industrial volatile wastes and farm-crop residuals – as well as the waters that accompany them. Here, we explore systems by which these wastes and their accompanying fluids are parsed as mineralized grit, renewable energy, clarified nutrients and streams of clean water; and then monetized as flows of value added foods, foodstuff and feeds, pure water, on-demand renewable power and advanced materials.

The critical role of duckweeds in effecting The New Circular Economy is examined in some detail. The plants' extraordinary utility in stripping nutrients, salts, toxin vectors and trace organic compounds (TOrCs) from wastewaters, and its role as a feedstuff in manufacture of high-valued foods, feeds and advanced materials are described. The plants' potential role(s) in cleaning up the global environment; helping to remove carbon from the atmosphere; solving global malnutrition; providing rural jobs and bestowing wealth on smallholder farmers, the world over, are explored. The presentation concludes by presenting some "numbers" that suggest what Duckweed's role in the global economy can quickly come to represent.

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Securing fresh nutrition via a breakthrough technology for growing Duckweeds

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Fresh wholesome phytonutrients are crucial to promote cellular strength and protect ourselves from chronic (as cancer, cardiovascular, diabetes, stroke) and infectious diseases. Yet, there are significant challenges in consuming even the minimum recommended daily portion. Following years of development, GreenOnyx is introducing a breakthrough farming technology to grow and deliver duckweed vegetables thus, realizing the promise of fresh phytonutrients to the global mass population.

Our farms are designed to secure year-round local supply our fresh duckweed vegetables, called Wanna greens®. Based on an array of compact-modular growing systems that are sealed and sterile, we introduce a fully automated supply chain, from seed production to packaging. Farms are designed for diversity, enabling to grow any species or strain of the duckweed family, and for "Copy Smart" to enable a fast expansion at global scale.

Wanna greens are delivered at the farm gate as fresh vegetables ready to eat – just ”scoop & use” to ‘vegetelize’ any of your food choice. We have identified huge global market potential and established strategic partnerships with leading food brands in Israel, Europe, and US. We are now accelerating our production capacity and initiate sales.

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Development and scale-up process from a small duckweed culture to a large indoor vertical farm for duckweed biomass production

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Duckweeds are promising plants with a variety of possible applications in the future, such as human and animal nutrition, biotechnology or wastewater treatment. Protein contents of up to 45 % with an amino acid distribution close to WHO recommendations, starch contents of up to 65 % in the turions (both based on dry weight) and fast growth rates make these plants highly interesting. Additionally, it is possible to influence the nutritional composition of the biomass by changing the composition of the growth medium.

To reliably cultivate large quantities of a defined product quality, a standardized production process is needed. A stable and year round production, independent of the climatic conditions, of duckweed biomass with a high protein content in an automated system with little space requirement and fertilizer use was the aim. Therefore, two parallel steps were necessary:

1. Investigation of abiotic factors

The nutrient medium was modified and light conditions were investigated to find the optimal growth conditions and highest protein contents for Lemna minor and Wolffiella hyalina.

2. Development and scale up of a duckweed cultivation system

The concept of indoor vertical farming (IVF) was chosen, because the production area per m2 is increased. When used in controlled environments, it allows for a high level of automation. During the scale-up process, different duckweed cultivation systems were tested, increased in size and level of automation. In the end, this resulted in a nine-layer IVF with a total production area of 25 m2. The identified cultivation conditions were then transferred to the IVF. The water is re-circulating within the system, while nutrients, temperature and light settings are controlled and regulated automatically. Per day more than 900 g of fresh biomass are harvested. It can be concluded, that the production of duckweed biomass in a large-scale IVF is feasible. Next development steps are a further automation of the production and harvesting processes and an evaluation of the energy, water and fertilizer balance.

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Carbon dioxide sequestration potential of two Thai duckweed species: Wolffia globosa and Lemna aequinoctialis under, open-air greenhouse

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This study aims to evaluate the carbon dioxide (CO2) sequestration potential of two duckweed species: Wolffia globosa and Lemna aequinoctialis, under commercial-scale cultivation. W. globosa AGF-W6 and L. aequinoctialis AGF-L1 were seeded at the initial density of 300 g fresh weight m-2 in a 2.4 m x 4.8 m pond containing proprietarily formulated culture medium at 20 cm depth in an open-air greenhouse at the research facilities of Advanced Greenfarm Co., Ltd., Thailand. Two replicate ponds were included for each duckweed species. The cultures were grown for 6 days (Nov 22 – Nov 28, 2021) under the natural light with 50% shading. The culture density was monitored at 3 and 6 days. The dried biomass samples harvested at Day 0, 3 and 6 were determined for carbon composition using a CHNS/O elemental analyzer. Based on the relative growth rates and the carbon contents, the CO2 sequestration rates were estimated at 27.9 (95% CI 18.2 to 37.6) t CO2/ha/y and 19.4 (95% CI 18.7 to 20.1) t CO2/ha/y for W. globosa AGF-W6 and L. aequinoctialis AGF-L1, respectively. These results indicate high CO2 sequestration potential of the duckweed species compared to common crop plants.

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High-performance-Lemna minor cultivation: LemnaCore 1.0 as an innovative indoor vertical aquafarming concept

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LemnaCore 1.0 is a novel duckweed cultivation system. Based on recycling and utilization of industrial process water and waste water. We launched an vertical aquafarming technology that uses aquatic plants which cultivated biomass vertically. In the process, we use environmentally critical wastewater constituents, like nitrate and phosphate, and the biomass can bound them similar to a filter system.

Our innovative indoor aqua-farming concept is able to scale current and future problems and makes an important contribution. For this purpose, the problems caused by infrastructural change, increased industry, population growth and high resource consumption, we try to exploited. The increasing demand for valuable C-sources (e.g. petroleum) and the increasing demand for important amino acid, proteins leads us to more sustainable circular economy approaches - “cradle to grave” principle. By feeding waste water and industrial waste heat in the system, CO_2 emissions can be significantly reduced by capturing CO_2 in the generated biomass. The carbon capture and storage (CCS) can be defined with an framework of 1 t biomass (dry matter) = 1 t CO_2 (bound in biomass). The combination of a low-tech system plus KI, can create a climate-positive high-performance duckweed cultivation.

LemnaCore 1.0 uses duckweed (Lemnoideae), which floats and grows in tiers on the water surface. The grown biomass can be gathered and be processed further. The biomass is characterized by a variety of important nutrients (fat, proteins, carbohydrates, fiber) and good compounds that we can use in agriculture, e.g. as feed or fertilizer. The harvested biomass can also be used in the human food or cosmetics industry as an additive or extract.

The system is characterized by his closed structure. Its allows automated and scalable cultivation and harvesting. Important is the appropriate selection of waste water, optimal combination of nutrients, visual monitoring (controlling) and evaluation of cultivation conditions (data situation). The optimal interaction and control of all factors in the ecosystem inevitably leads the increase of the value of the final product, or growing biomass. The goal is to create an environment for duckweed in which it can grow well, the production is reproducible and automated. In addition, the greater goal is to create a regional production concept and a supra-regional duckweed network.

P01

Putative Hybridisation between Lemna minor and Lemna turionifera

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The Lemnaceae family is gaining more and more attention both in research and application due to developments in molecular methods. To be able to exploit the plant to the fullest, it is beneficial to understand the taxonomy of the 36 species. In 1986 Landolt already described the species Lemna japonica as a possible hybrid of Lemna minor and Lemna turionifera. In this study, this hypothesis is investigated on one hand by a cultivation experiment with determination of frond areas and relative growth rates. On the other hand, molecular plastid (atpH - atpF, psbK - psbI) and a nuclear DNA marker for the partial coding sequence of the malate synthase are used for sequencing and haplotype and allele type determination. A method based on tubulin-based polymorphism fingerprinting is used as an additional identification tool. This method exploits the intron length polymorphisms of the β-tubulin loci. Both plastid markers distinguish the possible pairs of parents, making it clear that both pairs of parents can act as pollen recipients. The newly constructed nuclear marker allows a subdivision into the two parents L. minor and L. turionifera and therefore the identification of the hybrid L. × japonica. With the allocation of the different intron lengths of the β-tubulin loci the same results are obtained, except for six clones where the results differ. The frond area measurements indicate a dominant effect of the parent L. minor on the frond areas of L. × japonica. After all a clear division into L. minor, L. turionifera and L. × japonica is possible. These results complement previous studies and provide further evidence that L. × japonica clones are hybrids of L. minor and L. turionifera. To further unravel the complex taxonomy within the Lemnaceae, further studies with larger data sets and precise methods such as GISH or GBS are needed.

P02

A new machine learning method to quantify growth in Lemnaceae

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Lemnaceae are on the verge of becoming a model plant in numerous research fields. Thanks to their biological simplicity and great nutritional values, they are gaining world wild attention in multiple areas. New advancements in the Lemnaceae research should push forward the constraints that limit the exploitation of these fascinating tiny plants.

For many years, understanding the growth patterns of Lemnaceae plants have been challenging. Before the introduction of Neuman's growth rate equation, scientists have struggled to quantify the growth rates of these tiny aquatic plants. The introduction of the Newman equation has led to innovation in the research field across different scientific disciplines. Furthermore, some computed analyses have been performed to quantify the growth rates of Lemnaceae; although effective and reliable, these methods required the intervention of the human operator. We present a newly developed method that uses machine learning to substitute human intervention in estimating Lemnaceae growth. Our approach exploited machine training to learn how to recognise plants from their surrounding environments. Results showed a high correlation between the new and gold standards.

The machine training can be stored and reutilised when needed. Compared to the old methods, our approach reduced time consumption drastically. Furthermore, to be widely adopted, we managed the technique to be inexpensive. We tested the process to be successful by employing a high-definition or phone camera and did not require any new laboratory equipment.

The "click of a button" approach can boost researchers' data production and save time. We are confident that the newly described method could find applications across numerous research fields.

P03

Developing approaches to investigate anatomical reduction in duckweed

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As the duckweed genera have become morphogenically reduced, they present a model for investigating the impact of organ reduction and loss on plant function. To examine duckweed anatomy in a comprehensive and high throughput manner necessitates the development of new techniques, adapted to suit the specifics of duckweed and the requirements of a very diverse set of species.

To capture anatomical information for whole plants a variety of microscopy techniques were used, however limitations of these made it difficult to accurately measure many major traits of interest. To overcome some of these difficulties a novel approach of µCT scanning was used. This allowed for 3D visualisation of the internal and whole plant anatomy without any need for fixation, clearing or sectioning. From these scans it was possible to distinguish between intra and extra cellular regions and identify which of those are filled with gas or high density tissue. This also enabled the quantification of gas space size and interconnectivity in the root and frond.

In tandem with this approach, a survey of duckweed root anatomy was conducted using vibratome sectioning and confocal microscopy to assess the extent to which cellular anatomy of duckweed roots is reduced alongside the reduction root number. This included Pistia stratiotes (main and lateral roots) as an ancestral relative, multiple accessions from every species of Spirodela and Landoltia, and the majority of Lemna species.

This survey showed a trend in reduction of anatomical complexity; embodied by a loss of traits, reduction in root diameter and number of cells, from the more ancestral genera to the more recently diverged.

This combination of methods proved to be a very powerful approach in understanding duckweed anatomy. The high resolution and throughput of sectioning and microscopy combined with the 3D context and compositional data of µCT scanning enabled us to deepen our understanding of duckweed anatomy and presents new opportunities in understanding developmental processes in duckweed.

P04

Molecular diversity of 5S rDNA genes in eight geographic ecotypes of Landoltia punctata

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The ribosomal DNA (rDNA) encodes the RNA components of ribosomes and represents a significant part of plant genomes. The plant rDNA is represented by tandemly repeated sequences of 35S rDNA, coding for 18S–5.8S–25S ribosomal RNAs, and 5S rDNA encoding 5S rRNAs. The highly conservative coding sequences are intertwined with more rapidly evolving spacer sequences. Based on their high copy number in plant genomes, and structural features, the 35S rDNA and 5S rDNA loci have been broadly studied in terms of plant systematics, evolution, and biodiversity. The 5S rDNA is especially well suited for such studies, due to the smaller size of its repeat units, and to the higher variability exhibited by 5S rDNA non-transcribed spacer (NTS).

Dotted duckweed (Landoltia punctata), the sole species in genus Landoltia, is one of the most resilient duckweed species of the Lemnaceae family. In this study, we investigated the molecular diversity of the 5S rDNA NTS locus in eight L. punctata ecotypes originating from China, USA, Brazil and Australia based on the characterization of independent sequences derived from cloned PCR products. The estimation of 5S rRNA gene copy number by quantitative PCR, showed the range from 137 ±17 gene copies for ecotype Ya2 (China) to 305±56 for ecotype DWC022 (USA). At least twenty individual clones obtained by cloning 5S rDNA genes amplified by PCR were sequenced and analyzed for each ecotype. The sequences encoding 5S rRNA showed high conservation across all cloned, whereas the sequences of NTS allowed to divide the clones into two major classes. Both classes were characterized by specific conserved sequences at their 5'ends, started with the transcription termination sequence TTTT. Both classes also shared a more variable, TC-rich region in the middle of NTS. The variable central region of both NTS classes is composed of either 12 bp or 9 bp subrepeats specific for class I and class II NTS, respectively. Also, both NTS classes shared a 6-bp long TC-rich core element (T-C-T/C-T/C-T/C-T/C).

Based on arrangement of the 12-bp units, class I NTSs are further divided into four main subclasses: A, B, C and ABbr. Each geographic ecotype of L. punctata is characterized by a predominant type of class I NTS, with subclass I-B dominating in ecotypes from the USA, Australia and Brazil, while I-A being dominant in Chinese ecotypes. Subclass I-C is a minor NTS variant in most of the studied genotypes.

The 9-bp subrepeats of the class II NTS are represented by a single copy followed by a 12-bp sequence resembling an almost perfectly duplicated 6-bp core, tCTTCT-cCTTCC, followed by 2 to 22 copies of the 9-bp element.

In addition to these diverse subrepeats, class I and class II NTSs differed in their representation of cis-elements and the patterns of predicted G-quadruplex structures, which may influence the transcription of the 5S rRNA. Furthermore, analysis of clones containing double 5S rDNA units showed that different classes of NTS are present in neighboring genes in the three ecotypes representing USA and China. This finding challenges the basic concept of extended repeat homogenization along the rDNA arrays.

The obtained data will further stimulate research on the evolution of plant rDNA and discussion on the molecular forces driving homogenization of rDNA repeats.

P05

Morphological variation, chromosome number, and DNA barcoding of Giant Duckweed (Spirodela polyrhiza) in Vietnam

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Our persent study is the first systematic survey on duckweed biodiversity in Vietnam. More than 100 samples of Spirodela, Lemna, and Wolffia were collected throughout Vietnam and maintained under laboratory conditions. In this report, we focused on studying the morphological variation, chromosome number, and DNA barcoding on Spirodela samples. S. polyrhiza and S. intermedia are the only two species of Spirodela genus - the most ancient genus among the five duckweed genera. The obtained DNA sequences of atpF- atpH and psbI – psbK regions showed that all 24 Spirodela samples collected from different regions along Vietnam are S. polyrhiza. Specific SNPs of S. polyrhiza groups in Northern, Southern, and Central Vietnam were identified and evaluated. The differences in genome size, frond size, frond shape, root system among 24 S. polyrhiza clones were investigated. In addition, the chromosome number of six representative S. polyrhiza clones was 40 chromosomes.

P06

Ionomic analysis across the duckweeds reveals pervasive accession-level variation and genus-scale trends

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To date, only a fragmented literature exists on elemental accumulation in duckweeds, with studies typically limited to a handful of accessions and elements. We therefore fill this gap by performing an integrated ionomic assessment of 21 species across all genera, with additional within species sampling to look also at interspecies variation. We observe broad variation in elemental composition both between and within species, with a robust trend of calcium and magnesium levels decreasing from the ancestral representative Spirodela genus towards the derived rootless Wolffia genus, and with the latter also accumulating cadmium. We also identify hyperaccumulators of specific suites of elements in the Lemna and rootless genera. Integrating these findings with previous work underscores the promise of duckweeds for the study of natural variation, in particular the loss of roots, as well as diverse future applications including the use of this anatomically reduced plant as a chassis platform for synthetic biology.

P07

Identification and characterization of C-repeat binding factor (CBF) genes in duckweed

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C-repeat binding factors (CBFs), also named as dehydration-responsive element binding proteins (DREB1), belong to the AP2/ERF (APETALA2/ethylene-responsive element-binding factor) family and play a central role in plant cold response and facilitate plant cold acclimation and resistance to freezing. The CBF genes have been identified and verified in Arabidopsis thaliana, maize, rice, tomato, soybean, cotton and tea plant. In this study, genome-wide analysis was carried out to identify CBF genes in duckweed species with already sequenced genomes: Spirodela polyrhiza 7498, Spirodela intermedia 7747, Landoltia punctata 5562, Lemna gibba 7742, Lemna minor 8627, and Wolffia australiana 8730.

Two putative CBF genes were identified for S. polyrhiza, S. intermedia, La. punctata, and Wo. australiana. Le. gibba 7742 has three putative CBF genes: one localized on chromosome 5 and two on chromosome 13. The tetraploid Le. minor 8627 has 8 homologues of CBF genes: one on chromosome 5, two on chromosomes 8 and 13, and three on chromosome 29. The expansion of CBF genes in L. gibba and L. minor by tandem duplications can be considered as a mechanism of genomic adaptation to a changing environment.

The CBF protein sequences of duckweed, rice, maize, tea plant, Brachypodium and Arabidopsis were analyzed using neighbour-joining method of ClustalW2 for multiple alignment and phylogenetic tree construction. All of the duckweed CBFs had a conservative AP2/ERF DNA-binding domain and a nuclear localization signal (NLS) sequence like other species. The phylogenetic examination of protein sequences demonstrated that duckweed CBFs form a single sub-tree consisting of two sister clusters. The first cluster included one CBF of S. polyrhiza, S. intermedia, La. punctata, Le. gibba, Wo. australiana, and three CBFs of Le. minor which were translated from genes located on chromosomes 5 and 8. The second cluster contained one CBF of S. polyrhiza, S. intermedia, La. punctata, Wo. australiana, and CBFs of Le. gibba and Le. minor which were translated from tandemly duplicated genes.

To further investigate the possible regulatory mechanism of duckweed CBFs, sequences 2,000 bp upstream of the start codon of the CBF genes were extracted from corresponding genome databases. The analysis reveals the presence of both E- and G-boxes in the duckweed CBF promoters that indicates the possibility of regulation of their expression by low temperature, light and during cryopreservation. All promoters of duckweed CBF genes contained multiple elements responsive to water stress and dehydration (MYC), as well as to methyl jasmonate, abscisic acid (ABRE), and ethylene (ERE).

P08

Transcriptional profiles of Mn-stressed duckweed, Spirodela polyrhiza, are affected by the source of nitrogen

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Duckweed Spirodela polyrhiza have great potential for phytoremediation due its ability to remove ammonium, nitrates, phosphates, heavy metals, arsenic, selenium, boron and organic xenobiotics from different type of wastewater. Manganese Mn, being essential micronutrient for live organisms, can be toxic in high concentration for plants, animals and humans. In this study, we research influence of elevated concentration of Mn on the plant's transcriptome in dependence of the duckweed growth in medium supplied with nitrate and/or ammonium as source of nitrogen.

The Mn-dependent toxicity were manifested most profoundly in the S. polyrhiza cultivated in nitrate-based medium when the plants uptaked 91.69% of the supplied Mn (3.25 mg/l). To the contrary, the ammonium-based media was shown to alleviate Mn stress symptoms in compared to the media containing nitrate. Acidification of the media induced by ammonium seems to cancel Mn uptake and even to induce its efflux.

Comparative studies of gene expression patterns by transcriptome analysis were performed to reveal the key pathways that can be involved in the homeostatic cross-talk between ammonium and Mn uptake. A total of 5381 differential expression genes (DEGs) were determined in S. polyrhiza grown in nitrate-based medium in response to the treatment with elevated level of Mn (162.5 mg/l). However, when duckweed was grown on mixture of nitrate and ammonium supplemented with the same concentration of Mn, the number of DEGs was 538, 10-fold lower compared with nitrate-contained medium.

In response to elevated Mn concentration, the most abundant DEGs were genes involved in oxidative stress, defense response, plant hormone signal transduction, MAPK signaling pathway, amino acid biosynthesis, phenylpropanoid biosynthesis, alfa-linolenic acid metabolism, as well genes coding different kind of transporters. The obtained data demonstrated that ammonium enhanced expression of genes coding for metal transport proteins (MTPs) and vacuolar iron transporter homologs 1-like (VITs), responsible for heavy metal export from cytosol. The expression of genes encoding multidrug and toxin extrusion carriers (MATEs) with plasmalemma or tonoplast subcellular localization was remained stable or was up-regulated, whereas the expression of genes MATEs specific for endosome or peroxisome membranes were down-regulated. Elevated concentration of Mn activated phenylpropanoid pathway, biosynthesis of phenolic compounds such as lignin and suberin, considered to be important barriers against pathogen invasion. The transcriptome analysis also confirmed previously published data that Mn affects biosynthesis and signal transduction pathways of jasmonic and salicylic acid, both involved in plant defense responses.

P09

Duckweed holobiont research in Thailand under Japan-Thai collaboration through SATREPS, JICA

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Duckweeds are commonly found in freshwater reservoirs around Thailand including Spirodela polyrhiza, Landoltia punctata, Lemna aequinoctialis, and Wolffia globosa. Traditionally, W. globosa has been consumed in Thai cuisine for centuries, while others have been commonly used as high protein poultry feeds to increase egg yolk color. Towards the Bio-Circular-Green (BCG) policy of Thailand, duckweeds are regarded as a key national bioresource that is ideally aligned with the BCG goals and the UN Sustainable Development Goals (SDGs) to transform value-based to innovation-driven sustainable economy. The 5-year (2021-2026) joint research between Thailand and Japan under a multi-disciplinary project entitled "Development of the Duckweed Holobiont Resource Values towards Thailand BCG Economy' implemented under the "Science and Technology Research Partnership for Sustainable Development (SATREPS)" supported by Japan International Cooperation Agency (JICA) has been awarded to Hokkaido University, Japan and Kasetsart University, Thailand in collaboration with other Thai and Japanese partners. Duckweed holobiont specimens in Thailand have been intensively investigated for their biodiversity, ecology, genomics, microbiomes, and interaction with associated microbes. Duckweed-microbe interaction helps promotion of biomass, protein/starch, nutrition, active compounds, and stress tolerance of the duckweeds. Utilizations of duckweed holobionts include production of high-value active substances, high-nutrition chicken feed and functional foods. Furthermore, duckweeds have been applied for wastewater treatment and their biomass have been circularly used for biogas (methane) and bioplastic production. Duckweed Holobiont Resource & Research Center (DHbRC) will be established at Kasetsart University to host duckweed holobiont collections, a pilot duckweed indoor factory, and research facilities.

P10

The domestication of novel crops in Israel: isolation, cultivation, and characterization of Wolffia species

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Duckweed's high growth rate, efficient water utilisation, and myriad applications, ranging from biofuel to fodder and food source, promise a novel and sustainable agricultural crop. Typically, duckweed are found in temperate and equatorial regions. Seldom have they been described in the Middle East and particularly in Israel, a unique juncture of temperate Mediterranean and Arid climates. To address this gap in knowledge, we surveyed aquatic systems, and collected a variety of duckweed samples from natural populations across Israel. We then isolated the different strains and characterised their genetic diversity, protein and starch content, fatty acid composition and vitamin B12 concentrations. Twenty four strains were categorised morphologically, while barcoding analysis using sequences of plastidic DNA fragments revealed four species in two genera. Strains affiliated with Lemna minor and Wolffia arrhiza dominated the duckweed populations throughout Israel. The genetic divergence of W. arrhiza strains was highest, while L. minor strains were more genetically uniform. Likewise, the genetic diversity within L. gibba and W. globosa strains, was limited. The fatty acid profile of the four duckweed species differed between genera but was uniform among strains. The total fatty acids in plants are dominated by three fatty acids, palmitic acid, linoleic acid, and linolenic acid. Protein and starch content varied among species, with W. arhiza showing the highest contents and L. minor showing the lowest. Lastly, all the isolated strains contained vitamin B12, a rare occurrence in plants, with W. arrhiza containing the highest concentrations. Our results suggest that two species dominate the aquatic systems in Israel tolerating the high temperatures and long droughts. W. arrhiza was demonstrated to possess high nutritional values including high protein, vitamin B12, and starch contents, highlighting its potential as a world novel crop.

P11

Ion specific salt stress of Lemna minor and the implications on biological effluent of pig manure treatment

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Feeding the growing population within the limits of our planet (e.g., agricultural land) is one of the biggest challenges we are facing. As a result of a more intensive agriculture, the demand for high quality protein for feed and the cost of processing the excess of manure is expected to rise. Duckweed (Lemna) can be a possible solution for the treatment of aqueous waste streams and simultaneous provision of a protein-rich biomass. Duckweed is a small, floating, aquatic freshwater plant and the fastest growing Angiosperm in the world. It has a balanced amino acid composition, which makes it very suitable for use as feed. Biological effluent of the manure treatment has been used before as a potential growing medium for duckweed. However, the high concentrations of dissolved salts are typically a problem for this application (EC between 7 and 24 mS/cm). Therefore the effluent must be diluted with water, which can be done in batch or continuous systems. In continuous systems, buildup of K and other elements occur when systematic application of biological effluent is performed in an amount equal to the N and P treatment capacity. Therefore, the objective of this research was to unravel the main drivers of salt stress to indicate which parameters should be monitored to predict accumulation and avoid culture crashes. Additionally, the extent to which the optimal and maximum concentrations recommended in literature apply to biological media was studied, since this toxicity data was obtained by increasing one nutrient concentration at a time whereas this is far from the reality of biological media, in which multiple nutrient concentrations have an impact on toxicity. Furthermore, salt tolerance tests were conducted on synthetic and biological media for comparison of toxicity to the duckweed and the monitoring of EC and Cl- and/or SO42- concentrations in the medium was carried out. A pilot setup was monitored in-situ to evaluate the variation of EC over time and an experiment was conducted to simulate a continuous system in which duckweed is grown on a diluted biological stream. In the salt tolerance tests in synthetic media, a significant higher growth inhibition was observed after adding 46.6 mM Cl- to the medium (up to EC = 6.51 - 6.65 mS/cm), compared to the reference medium (0 mM NaCl added – EC = 1.5 mS/cm). Remarkably, when adding a combination of Cl- and SO42- ions, a significantly higher tolerance was observed as full growth inhibition was only obtained after the addition of 37.3 mM Cl- and 9.32 mM SO42- to the medium (up to EC = 8.18 – 8.43 mS/cm). Furthermore, the effect of salt was investigated in real wastewater by testing different dilutions of biological effluent from the pig manure treatment. Surprisingly undiluted biological effluent (EC = 9.8 mS/cm) showed no inhibition compared to the reference medium. From the in-situ monitoring of a commercial biological treatment facility, it was observed that the EC of biological effluent varied through the season between 8.8 and 15.0 mS/cm. There

fore the EC was artificially increased by adding a saline solution and also by evaporation that was sped up due to heating. Also, in the medium with increased salinity, no significant growth inhibition was seen compared to the reference, but in the evaporated biological effluent a higher significant growth inhibition was observed. Previous tray tests, focused on an initial dilution of the wastewater, however, in a continuous system, accumulation of ballast salts can occur. An attempt to simulate the concentration of a wastewater after 175 growing days with different buffering capacities was performed. These simulations were furthermore tested in a tray test, and from this, it was suggested that a buffering capacity of at least 1 m depth was required for biological effluent which must meet Flemish discharge standards after 175 days. The most important finding of this study was that duckweed grown on biological medium is significantly less salt sensitive than duckweed grown on synthetic medium. This can be partly explained by the composition of the anions, as, in a synthetic medium, duckweed was less inhibited when an equal amount of Cl-/SO42- was added than when only Cl- was added.

P12

Effects of red-light and pre-culture periods on duckweed regeneration after cryopreservation

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For the long-term preservation of unique accessions, mutants and reference clones, a simple and rapid cryopreservation protocol is required. To study the effects of osmotic stress during droplet-vitrification and -freezing as well as light quality during regeneration, we used different cryoprotectants (PVS2, PVS3, DMSO), pre-culture periods with sucrose and evaluated the effect of red light (670 nm) on the regrowth of Lemna gibba and L. valdiviana fronds. The result was that the fronds of both, L. gibba and L. valdiviana cryopreserved using DMSO and regenerated with red light achieved highest regrowth. Droplet vitrification with PVS2 resulted in some viable clones while none of the duckweed fronds multiplied after PVS3-droplet vitrification. A 17 hours pre-culture period in sucrose prior to DMSO droplet-freezing, and a 4 hours period prior to PVS2 droplet-vitrification strongly promoted the proliferation of duckweed fronds after cryopreservation. Although aseptic cultures were used, the experiments were compromised by contaminations, especially after application of PVS2 and PVS3. In summary, cryopreservation of L. gibba and L. valdiviana genetic resources is achievable; and red light and longer pre-culture periods have positive effects on regeneration and multiplication of fronds. However, considerations should be given to the microbiome of duckweed which can significantly affect the success of long-term preservation.

P13

How does Wolffia (Lemnaceae) silence Transposable Elements?

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Transposable elements (TEs) or transposons, are mobile DNA sequences capable of replicate within host genomes. TEs are silenced via the action of numerous epigenetic mechanisms, being the deposition of DNA methylation one of the most relevant in plants. Although the RNA directed DNA methylation (RdDM) pathway plays an important role in angiosperms as it deposits de novo DNA methylation on TEs, there seems to be an exception within the duckweed (Lemnaceae) family. One of its members (Spirodela polyrhiza) have greatly lost most RdDM components (An et al., 2019) and displays global decrease of DNA methylation levels, while TEs remain silenced. Whether this is a common feature of all duckweeds and the mechanisms behind TE silencing, remains unknown. In this work I focus on one of the most recent lineages of the family: Wolffia brasiliensis. I will hereby describe the attempts to generate and annotate its genome, characterize the epigenetic landscape and the silencing pathways present in this specie. This would shed some light in these organisms silencing biology, and hopefully expand our knowledge of the diversity of TE silencing pathways, especially in plants with prominent clonal asexual reproduction such as duckweeds

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P14

Non-targeted site resistance mechanisms to diquat in Spirodela polyrhiza

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Diquat is a bipyridylium herbicide that acts by generating reactive oxygen species through diversion of electrons from photosystem I. Although diquat resistant weeds have been reported in recent years, the genetic basis underlying the resistance remains largely elusive. Here, we address this challenge using Spirodela polyrhiza as a model system. First, we screened the levels of inhibition among 138 genotypes, among them 114 genotypes were sequenced. The most resistant genotype is 8.54 -fold more resistant than the most susceptible genotype. Among the 138 genotypes, the diquat concentration in fronds and diquat toxicity levels are positively correlated ($p = 0.018$). Using genome-wide association (GWA) analysis, we identified a SNP located in the purine permease gene 11 (EmrE), affecting a I225V substitution, to be associated with both the diquat concentration in fronds and diquat resistance. This suggesting that differences in diquat transportation contributes to intra-specific variations of diquat resistance. However, when comparing the most susceptible and resistant genotypes, the differences in diquat resistance is much higher than differences in diquat tissue concentrations, indicating that, in addition to uptake or efflux, other mechanisms also contributed to the variations of diquat resistance in S. polyrhiza. Currently, we are sequencing the transcriptomes of resistance and susceptible genotypes and are developing reverse genetic tools to validate the identified candidate genes.

P15

Cultivation of L. minor on industry derived dairy processing wastewater

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Agriculture produces substantial quantities of nutrient-rich wastewaters. Dairy processing wastewater is, worldwide, one of the largest waste streams by volume. It has been suggested that use of dairy industry wastewaters as a cultivation medium for plants could support greater valorisation. Duckweeds such as Lemnaceae, may be particularly suitable for cultivation on this medium. In the present study, we explored whether dairy processing wastewater, that had been processed through anaerobic digestion to generate biomethane, could subsequently be used as a medium for duckweed growth. The growth of Lemna minor cultivated on a concentration range of pH-standardized post-anaerobic dairy processing wastewater (1%, 5%, 10%, 50% and 100%) was quantified. Plants were maintained in 1) stationary vessels in an indoor setting, under controlled light and temperature conditions; and 2) within a 35-litre recirculatory system in a semi-outdoor setting. Concentration had a significant effect on biomass yield and the relative growth rate (RGR) in both the indoor and the semi-outdoor assays over a seven-day growing period. The 10% concentration proved to be the optimal for growth in both settings. Higher wastewater concentrations were found to negatively affect L. minor survivability and growth. A long-term assessment of L. minor growth on dairy wastewater for 6 weeks demonstrated the potential for continuous growth of L. minor on the wastewater, as there was no difference in the measured growth parameters between the first and final week within each concentration group. The phytoremediation capacity of L. minor for Total Nitrogen and Total Phosphate when grown on two concentration ranges (5% and 10%) of dairy wastewater in the recirculatory outdoor system was also determined. The nutrient removal rates of L. minor in the 10% conditions, with 248.46 mg TN and 126.54 mg TP removed per m2 of duckweed cover per day, were optimum in comparison with the lower concentration of 5%, with 43.72 mg TN/m2d-1 and 53.76 mg TP/m2d-1, which reflects the relationship between plant growth and nutrient uptake.

Results indicate that L. minor grows best on lower concentrations of anaerobically digested dairy processing wastewater (i.e., 10%) compared to higher concentrations, in both the indoor and outdoor experimental environments. The data presented supports the use of L. minor for phytoremediation of dairy processing wastewater. Furthermore, given the produced biomass may be used as, for example, animal feed, thus resulting in the valorisation of dairy processing wastewaters.

P16

Resurrecting an ancestral gene network in duckweed

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Recently derived genera of duckweed have undergone genome duplications and subsequently lost the ability to develop roots. In the model plant Arabidopsis thaliana, the root pole forms during embryogenesis and relies on Auxin Response Factor 5 (ARF5)/MONOPTEROS (MP). Arabidopsis embryos lacking ARF5/MP do not develop basal structures. ARF5/MP also regulates other developmental processes, such as the formation of vascular tissue. The derived rootless duckweed Wolffia australiana lacks an ARF5/MP orthologue, although it possesses other components of the ARF5/MP network, such as ARF5/MP's direct regulator, BODENLOS (BDL). In comparison, the ancestral Spirodela polyrhiza (which produces both roots and vascular tissue) possesses both ARF5/MP and BDL. ARF5/MP-dependent targets have been well studied in Arabidopsis but it is currently unclear which, if any, are present in Spirodela and Wolffia. Initially I will investigate the morphological changes associated with auxin response in Wolffia and Spirodela. I have already undertaken a preliminary study of the effects of exogenous auxin on W. australiana and S. polyrhiza. This experiment demonstrated that while S. polyrhiza is sensitive to exogenous concentrations of 1µM and stronger, W. australiana is only sensitive to the extreme concentration of 50µM. I will then profile the auxin-dependent transcriptome in Spirodela and Wolffia. We expect to see a broader set of auxin responsive genes in Spirodela to explain the increased sensitivity. I then aim to drive missing auxin regulatory components, such as SpARF5/MP in Wolffia using Agrobacterium mediated transformation. I will repeat the RNASeq in these lines to test whether this is sufficient to activate a subset of previously dormant targets. In depth bioinformatic studies will be undertaken to determine if ARF5/MP downstream targets are already present in W. australiana and phylogenetic studies across the duckweed genera will illuminate how the ARF5/MP network in duckweeds has evolved. This project will also include reciprocal experiments to determine the effects of exogenous cytokinin and auxin transport inhibitors, and their effects on the auxin-dependent transcriptome. This project is the first step in a long path to understanding core signalling networks controlling root growth and reintroducing a key ancestral gene network.

P17

Differential phytotoxic effect of silver nitrate ($AgNO_3$) and bifunctionalized silver nanoparticles (AgNPs-Cit-L-Cys) on Lemna plants (duckweeds)

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Duckweeds are aquatic plants often used in phytotoxic studies for their small size, simple structure, rapid growth, high sensitivity to pollutants and ease of maintaining under laboratory conditions. In this study, induced phytotoxic effects were investigated in Lemna minor and Lemna minuta after exposition to silver nitrate (AgNO3) and silver nanoparticles stabilized with sodium citrate and L-Cysteine (AgNPs-Cit-L-Cys – AgNPs) at different concentrations (0, 20 and 50 mg/L) and times (7 and 14 days). Lemna species responses were evaluated analysing plant growth (mat thickness, fresh and dry biomass, Relative Growth Rate) and physiological parameters (chlorophyll – Chl and malondialdehyde – MDA). Ag+ ions concentration was measured in the fronds of the two Lemna species. AgNO3 and AgNPs produced phytotoxic effects on both duckweeds (plant growth and Chl reduction, MDA increase) that enhanced in response to increasing concentrations and exposure times; AgNPs caused much less alteration in the plants compared to AgNO3. Therefore, bifunctionalized AgNPs could be considered a promising solution to reduce the phytotoxic effect of silver ions released into water. Based on the physiological performance, L. minuta plants showed a large growth reduction and higher levels of chlorosis and stress respect to L. minor plants, probably due to greater Ag+ ions accumulation in the fronds. Albeit with some differences, both Lemna species were able to uptake Ag+ ions from the aqueous medium, especially over a period of 14 days, thus resulting aquatic plants adapt as phytoremediation agents for decontaminating water polluted by silver ions.

P18

Duckweed grown in recirculating systems on different dilution rates of cattle slurry: Productivity and protein production

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Two different duckweed species, Spirodela polyrhiza and Landoltia punctata, were grown in five different cow slurry in water dilutions (substrates) in recirculating systems fed from a storage tank. Each system comprised of 10 boxes filled with 4 l substrate and a storage tank with 110 l. Via a submersible pump, the growing boxes were supplied with the substrates from the storage tanks. In each system, five boxes each were inoculated by 20 g fresh matter of either S. polyrhiza or L. punctata. Once a week, the storage tank was emptied, cleaned and refilled with the respective substrate. The cow manure was diluted with water to receive 1:20, 1:10, 1:8, 1:6 and 1:4 substrates. The dilution rates corresponded to 5.57, 14.2, 19.4, 26.3 and 48.6 mg l-1 TAN, respectively.

The duckweed fresh matter (FM) gain was measured weekly by harvesting 50% of the individual box area and the fresh and 1-week old substrates were measured for pH, total ammonia nitrogen (TAN, NH4-N + NH3-N + NO2-N) and phosphorous (P) after the first, third and fourth week. At the end of 4 weeks or 4 production cycles, all duckweed was harvested and frozen before determination of dry matter (DM) and crude protein (CP) content. Amino acids were determined in pooled samples per treatment. Fresh and dry matter production, crude protein production and amino acid profiles have been determined for both duckweed species in the five different treatments.

The FM production ranged between 48.2 and 116.1 g m-2 d-1 and was generally higher for S. polyrhiza. The DM content ranged between 5.69 and 9.38% and was generally higher for L. punctata. This resulted in similar DM production for both species which ranged between 3.81 and 5.83 g m-2 d-1. A clear and significant positive correlation between TAN and CP content of both duckweed species was found and CP ranged between 29.3 and 37.9%. While the highest CP contents were found in the 1:4 dilution for both species, the highest DM productions were achieved in the 1:8 dilution, also for both species. All production data combined resulted in the highest dry CP production in the 1:6 dilution for S. polyrhiza (1.92 g m-2 d-1) and in the 1:8 substrate for L. punctata (1.95 g m-2 d-1). Average reductions between 98.5% (1:10) and 71% (1:4) were observed for TAN and for P the average observed reduction was between 33.4% (1:20) and 64.2% (1:6) in the substrates during a 1-week production period.

With this study, we can show a clear optimum TAN concentration for dry protein production, which also incorporates a high TAN and P reduction from the substrates. While increasing the TAN concentration does also increase the CP content of the duckweed biomass, it reduces the FM and DM productivity of the systems. Furthermore, our results show, that both duckweed species, while showing a different FM productivity, achieve similar dry CP productivity due to different DM contents.

P19

Aphid herbivory changes growth and metabolism of the aquatic plant, Spirodela polyrhiza

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Plants that grow on the surface of freshwaters ecosystems, such as duckweeds, represent important primary producers that are accessible not only for aquatic, but also terrestrial herbivores. Therefore, they represent important keystone species that directly link aquatic and terrestrial ecosystems via trophic interactions. However, while herbivory-induced responses in terrestrial plants are well-understood, the effects of herbivory on freshwater plants, in particular at the metabolic level, remain unclear. Here, we analyzed the interaction between the giant duckweed, Spirodela polyrhiza and the waterlily aphid, Rhopalosiphum nymphaeae, that switches between terrestrial and aquatic host plants within the year. We analyzed the effect of aphid herbivory in the controlled lab environment, as well as under outdoor conditions. Aphid herbivory significantly reduced the growth of S. polyrhiza, altered phytohormone levels and restructured the primary and secondary metabolism. Interestingly, the herbivory response of S. polyrhiza caused further changes in the aquatic system, e.g., in the nutrient levels of the water. Together, we show that the interaction between S. polyrhiza and its terrestrial herbivore plays a central role for the interaction between the terrestrial and aquatic ecosystem.

P20

Different physiological and biochemical responses of Lemna minor and Spirodela polyrhiza exposed to s-metolachlor

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Today's agriculture involves excessive use of herbicides, which makes agriculture an important player in water pollution. S-metolachlor is among the 20 most widely used pesticide active ingredients in the world and has been found in groundwaters and in surface and drinking waters in many countries. Once in the water, the remaining herbicide and their residues can be toxic to non-target aquatic organisms such as duckweeds. Despite their recognized ecosystemic value, little attention has so far been paid to the susceptibility of these organisms to s-metolachlor, an inhibitor of the activity of the very long chain fatty acid (VLCFA) synthases which catalyze the reactions forming the VLCFAs. Two duckweeds (Lemna minor and Spirodela polyrhiza) were exposed to nine s-metolachlor concentrations ranging from 0 to 200 µg/L at a density of 1.27 g (fresh weight) per liter of growth media for 7 days. The concentration-effect curves were adjusted by nonlinear modelling with the R software and the concentration causing 50% inhibition of the growth rate (EC50) for each species was derived from these models. The maximal photosystem II quantum yield (ΦM), operational photosystem II quantum (Φ'M) and relative quenching parameters (qPrel, qNrel and UQFrel) of these plants were calculated from the fluorescence induction curves obtained with a Pulse Amplitude Modulated (PAM) fluorometer. Growth rate (fresh weight) was used for the EC50 calculation. Spirodela polyrhiza (EC50= 15.79 ± 2.55 µg/L) was more sensitive to s-metolachlor than Lemna minor (EC50= 128.90 ± 7.21 µg/L) when growth rate was evaluated. We did not observe changes in quantum yield parameters (ΦM and Φ'M) in the presence of the herbicide. However, for S. polyrhiza we observed a decrease in the photochemical quenching (qPrel) compensated by a higher loss of energy through heat (qNrel) in the presence of s-metolachlor, which corroborates our lower growth rate EC50 for this species. In addition, protein concentration and biomarkers of oxidative stress such as hydrogen peroxide (H2O2), enzymatic activity of catalase (CAT) and ascorbate peroxidase (APX) were also evaluated. A higher protein concentration was induced by an increased herbicide concentration. However, for S. polyrhiza, protein concentration returned to control values when treated to 100 and 200 µg/L. CAT activity was not affected by the herbicide for S. polyrhiza and decreased at concentrations of 100 and 200 µg/L for L. minor. APX activity was lower at concentrations of s-metolachlor higher than 5 µg/L and 50 ug/L for S. polyrhiza and L. minor, respectively. Despite the lower enzymatic activities at high concentrations of s-metolachlor, an increase in hydrogen peroxide concentration was not observed for these treatments. Furthermore, for L. minor, H2O2 concentrations were lower in the treatment with 100 and 200 µg/L of metolachlor compared to the control, which indicates the action of other H2O2 scavenging processes. Our findings provide useful insights into the tolerance and defense mechanisms of duckweeds exposed to s-metolachlor for their potential uses as s-metolachlor phytoremediators.

P21

A soil bacterium Azotobacter vinelandii contributes to growth promotion of duckweed through nitrogen fixation, bacterial synergism and EPS production

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Plant growth-promoting bacteria (PGPB) have direct and indirect positive effect on the growth of host plants. Hence, PGPB is considered as an efficient and environmentally sustainable alternative to inorganic nutrient solutions or fertilizers. Here we asked if a duckweed non-associated diazotrophic bacterium Azotobacter vinelandii benefits on the growth of duckweed Lemna minor.

Nitrogen fixation: Nitrogen is in some cases a limiting factor for L. minor biomass production. In order to explore if nitrogen fixing bacteria can coexist and provide fixed nitrogen to the duckweed, we have co-cultured L. minor with surface attached A. vinelandii NBRC 13581 in nitrogen-free Hoagland medium. Acetylene reduction assay of bacteria attached L. minor biomass showed that A. vinelandii NBRC 13581 fixed 2.88 µmole equivalent N2 /g fresh weight. It was further observed that nitrogen fixation occurs in bacteria associated L. minor while the bacterial attachment increased the frond number and dry weight of L. minor by 2.0 and 2.2-folds, respectively. Moreover, A. vinelandii NBRC 13581 exhibited stable association with L. minor and increased its cell numbers up to 300-folds over 30 days, which suggests the formation of mutualisms with L. minor. Thus, indicating that active nitrogen fixation of A. vinelandii NBRC 13581 is responsible for promoting the growth of L. minor in extremely scarce nitrogen condition.

Bacterial synergism: A. vinelandii NBRC 13581 co-inoculated with another PGPB isolated from Spirodela polyrhiza named Ensifer sp. SP4 increased L. minor frond number and dry weight by 1.7 and 2.0-folds, respectively in Hoagland medium. A. vinelandii CA12 (nif gene deleted strain) did not show such a synergistic effect. Biofilm production assay and alginate lyase assay suggested a coupled mechanism between A. vinelandii NBRC 13581 and SP4 where the hydrolyzed products of alginate from both or either A. vinelandii NBRC 13581 acted as a PGP factor for L. minor.

EPS: A. vinelandii NBRC 13581 produces popular plant growth-promoting factors (IAA, siderophore, phosphate solubilization). Although alginate comprises of ~90% of the dry weight of its extracellular polysaccharide, EPS, the alginate didn't show PGP effect on L. minor. Rather A. vinelandii CA6, a less alginate producing (~34%) and less efficient nitrogen fixer produced an EPS that increased the frond number and dry weight of L. minor by 3.3 and 4.1-folds, respectively at 50 µg/ml.

It was found that A. vinelandii can proliferate and promote the duckweed growth in more than one mechanism. A more in depth understanding of the mechanisms may provide us an oppurtunity to maximize duckweed biomass with lowered environmental impact.

P22

Prevalence of vitamin B12 in a novel crop: developing methods and monitoring B12 in duckweed species

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Vitamin B12, also known as cobalamin, is a water-soluble vitamin that acts as an important co-factor in DNA synthesis, methionine synthase and fatty acid degradation. B12 has a complex ring system with a cobalt atom in the center and an upper and lower ligand. The upper ligand defines several forms of cobalamin, whereas the lower ligand defines bioavailability to humans. For humans, the bioavailable form of cobalamin is only found in animal-based food, though B12 is synthetized de novo by bacteria and archaea. The bioavailable form of B12 was recently detected in the edible duckweed, Wolffia globosa. This species was recently domesticated and cultivated to serve as a highly nutritious leafy green, sold in grocery stores throughout Israel. We hypothesized that bioavailable B12 may be prevalent in other duckweed species and that B12 production in the plant could be boosted by enhancing bacterial production. To test our hypothesis, we developed an extraction and separation protocol of B12 from duckweed plants. The concentration of the extracted B12 was then evaluated by LC/MS-MS that identifies and quantifies the mass and fragmentation of the different cobalamin molecules. In addition to the estimation of the B12 concentration, this protocol has enabled the differentiation of human bioavailable forms of cobalamin from the non-bioavailable pseudocobalamin (prevalent in the cyanobacteria Spirulina and the algae Chlorela). To identify and quantify the cobalamin using LC/MS-MS, protocols for plant extraction and cobalamin concentration were developed and applied resulting in a recovery rate of 99.5 ± 2.5 % with a limit of detection of 1 ppb. The developed protocol was applied to evaluate B12 concentrations and bioavailability in species of the genera Wolffia, Lemna and Landolita (4, 2 and 1 species, were tested, respectively). Bioavailable B12 was detected in all tested species, albeit at different concentrations ranging from 3.8 ± 0.54 to 0.74 ± 0.12 µg B12 per 100 g dry weight in W. globosa and L. gibba, respectively. Using our developed protocol, we showed that human bioavailable B12 is widespread in duckweed species and attains the highest value in the edible genus Wolffia. Our results present a unique plant-based source of human bioavailable vitamin B12.

P23

Efficient lightning in indoor vertical concepts – Effect of light intensity on limit density in sigmoidal growth models

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Cultivation of duckweed in indoor vertical farms (IVF) could be an interesting production method for duckweed in the future. With controllable, constant environmental factors, consistently good product qualities and high yields could be achieved. In IVFs artificial lighting normally represents the largest energy and cost factor. To make the cultivation and harvesting processes as efficient as possible, sigmoidal growth models as developed by Monette et al. (2006) or Driever et al. (2005) can be used as a basis for calculations (Calicioglu et al. 2021). These models calculate the growth dynamics of duckweed as a function of their plant density, their intrinsic growth rate and their limit density. The influence of light intensity on the intrinsic growth rate has already been investigated (Van Dyck at al. 2021). However, an investigation of the influence of light intensity on limit density would be an interesting approach to extend such models and make them more accurate. This could increase the efficiency of artificial light usage.

In the presented study, this relationship was investigated in an IVF pilot plant by measuring the biomass production of Lemna minor at four different light intensities (50, 100, 150, 200 µmol·m-2·s-1) and at six different starting biomasses (10, 42, 74, 105, 138, 170 g drymatter·m-2) after a culture period of seven days.

It was found that the limit density increased between 50 and 100 µmol·m-2·s-1. This continued, albeit in a weaker form, up to 200 µmol·m-2·s-1. It is therefore likely that the relationship between limit density and light intensity follows a saturation curve. Consideration of this relationship between light intensity and limit density should be included in growth dynamics model calculations for an accurate calculation basis of IVFs. This way, the highest possible efficiency of light use might be obtained.

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P24

What is the feasibility of duckweed cultivation on pig farms in Flanders? And how to put the insights in a global context?

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The animal production sector in Flanders and Europe is under pressure. The environmental impact of soy protein imports and nutrient excess in the form of manure contributes largely to climate change. Local and circular feed sources have a potential large effect on the environmental sustainability of animal production systems. For this reason, duckweed is investigated in a Flemish situation. Despite environmental benefits, a technology needs to be economically feasible to be adapted in a competitive market.

A cost-benefit analysis was performed within chapter 5 of the author's PhD thesis. A production and opportunity cost of 2.98 and 0.17 €/kg dry duckweed was estimated, while the feed value ranges between 0.19 and 0.28 €/kg. Feasibility can however be achieved when manure treatment is taken into account, but also when sustainability is valorized in the form of subsidies.

The aim of the presentation is to give a short overview of the economic balance in Flanders. To reach a broad audience, the main conclusions will be discussed in a broad global context. Therefore, online interactive software (Mentimeter) will be used to poll for ideas, solutions, and problems. It is hoped that this presentation can inspire the researchers to place the importance of their research within their own economic context and lead to new research questions.

P25

Phytoremediation potential of duckweed for heavy metals from polluted waterbodies; the case of Lebanon

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Lebanese watercourses are subjected to heavy pollution by a wide range of pollutants which increases the need for sustainable remediation. Phytoremediation presents a nature-based solution that has the potential to provide efficient treatment to remove diverse contaminants using the ability of macrophytes to uptake, bioaccumulate and degrade pollutants. This presentation considers the phytoremediation potential of Lebanese Lemna minor L. and Lemna gibba L. of heavy metal water pollution of waterbodies with emphasis on both in situ and in vitro bioaccumulation and tolerance capacities. In situ bioaccumulation assessment in several locations of fresh water bodies in Upper Litani River Basin revealed varied Bioconcentration Factors (BCFs) of Pb, As, Cd, Cr, Cu, Mn, Zn and Ni falling in the range of 35.25 and 1068. The BCFs showed high variability between locations and species with significantly higher values for L. gibba reaching 212, 359, and 1068 for Pb, Cr, and Cu, respectively. In a comparative evaluation under in vitro conditions after exposure to increasing heavy metal concentrations, both Lemna species indicated high BCFs and tolerance responses. Among the tested metals, Cd attained the highest BCF of 2121 at concentration of 0.5 mg/L by L. gibba and 1841 at 0.1 mg/L by L. minor whilst the morphological and physiological responses of both species fell within normal ranges. Significant reductions of relative growth rates of both species were recorded beyond these concentrations. Further, both species showed high variations in tolerance capacities and oxidative stress responses under increasing concentrations of the tested heavy metals. Findings suggest the promising potential of both L. minor and L. gibba in the phytoremediation of heavy metal pollution of Lebanese water bodies and its possible applications in the case of industrial water pollution.

P26

Simultaneous nutrient control and duckweed biomass production from wastewater using Wolffia angusta: A kinetic study

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Global population growth is triggering an increasing demand of fertilisers and feedstock to support food production, which leads to an increase in the discharge of nutrient-rich wastewaters. For instance, the rapid expansion of aquaculture has led to numerous questions about the possible impacts in terms of waste management and long-term sustainability of the sector. The search for low-cost, decentralized systems for remediation of wastewaters from fish farms have led to the study of different natural wastewater treatment processes that harness the use of aquatic plants, like duckweeds. However, the performance of such treatment processes depends upon environmental conditions under which water remediation occurs. In this work, the effect of temperature and nutrient availability (phosphorus and nitrogen) on biomass growth and nutrient removal by Wolffia angusta was studied under simulated tropical climate conditions. Three different experiments were conducted in 150 mL plastic pots containing 100 mL of Hoagland's culture media (with nitrate as N source) under non-sterile conditions. Experiments were conducted in triplicate for 7 days; duckweed growth and water quality parameters were monitored every 2 days. To study the effect of temperature, the initial concentrations of P and N were kept at 15 mg P L-1 and 100 mg N L-1, respectively, and the temperature was varied between 20 and 30°C. The effects of P and N concentrations were examined using a fixed temperature of 25°C. The effect of N was evaluated using a constant concentration of P (15 mg P L-1), while N concentration was varied (0.5, 5.0, 10.0, 15.0 20.0 mg N L-1). On the other hand, to evaluate the effect of P, N concentration was fixed at 100 mg N L-1, and P concentration varied between 0.2 and 8 mg P L-1. Our findings suggest that W. angusta is more sensitive to changes in temperature than to changes in the supply of nutrients. Also, an increment of 5°C improved the relative growth rate (RGR) of this duckweed species by 62%, compared to plants grew at 25°C. The estimated low P and N saturation constants (0.028 and 0.325 mg/L, respectively) imply that W. angusta growth is only affected at very low provision of external P and N. The higher removal (uptake) of nitrogen confirms the higher N content in duckweed biomass and suggest that P can be considered as the limiting nutrient for duckweed growth. Our findings confirm that W. angusta can be a suitable option for simultaneous nutrient control and nutrient rich-biomass production in fish farms and further discussions will be presented from a case of study in South-East Asia.

P27

FT-MIR-PLSR simultaneous determination of total nitrogen and nitrate in duckweeds

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From both nutritional and sanitary perspectives, is necessary to know the quality and safety of duckweeds as a novel protein-rich source by determining total nitrogen (TN) and nitrate (NO3-N). An important constraint for the routine analysis of TN and NO3-N is the high cost of analysis. Fourier Transformed Mid Infra-Red Spectroscopy (FT-MIR) has advanced rapidly as a new, fast, easy to operate, inexpensive and reliable method of analysis in plant science, to a great extent due to the introduction of chemometric predictive modelling using regression analysis and typically Partial Least Squares (PLS) algorithms.

The objective of the present research was to investigate the feasibility of simultaneous determination of TN and NO3-N in duckweeds by means of FT-MIR-PLSR. To do so, a set of duckweed samples obtained from both field and controlled environment was prepared to obtain the widest variation possible in TN and NO3-N content. All the samples were dried, milled and analysed by both “reference” methods (i.e. Dumas combustion with LECO CN628 for TN and salicylic nitration with spectrophotometric determination at λ410 for NO3-N), and by FT-MIR-PLSR (Tensor II and OPUS-QUANT II software, Bruker). Corrections of the raw spectra were made using the first derivative, with 17 smoothing points using the Savitsky–Golay algorithm. Assessment of the predictive performance of the chemometric models was made with the calculation of the coefficient of determination (R2), which gives the percentage of variance present in the true component values and is reproduced in the prediction; the Root Mean Squared Errors of Cross Validation (RMSECV); and the Residual Prediction Deviation for the rank (RPD = SD/SECV), which allows comparison of model performance across different data sets.

For TN, the chemometric model was built with 155 samples comprising an interval of TN between 0.77 and 6.62 %; a mean of 3.72 % and a median of 3.60 % TN in dry weight. The reliability of the model for TN was confirmed with a R2 of 94.66; a RMSECV of 0.37 and a RPD of 4.33 with a Rank of 6 components. Regarding NO3-N chemometric model, 73 samples were used comprising an interval of NO3-N between 0.03 to 0.59 %; with a mean and median of 0.20 % NO3-N in dry weight. A R2 of 92.29; RMSECV of 0.31 and RPD of 3.61 for a Rank of 6 components confirmed the reliability of the model for NO3-N prediction.

These results indicate that FT-MIR is a reliable technique to simultaneously predict the content of TN and NO3-N in duckweeds. With this method, the cost of analysis is reduced dramatically allowing an affordable routine analysis of both TN and nitrate. This method could also allow to a more accurate nitrogen to protein conversion calculation in duckweeds. We propose the standardization of FT-MIR as the routine method of analysis for TN and NO3-N in duckweeds.

This work was funded by European Research Development Fund for Agri-Tech Research Innovation Accelerator (AgRIA-CO2iRP10297-24).

P28

Biomass, protein and nitrate accumulation in duckweeds supplied with 3 different sources of nitrogen

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The selection of the nitrogen (N) source for duckweed cultivation can have a direct impact on productivity. Our objective was to investigate the effect of 3 different nitrogen sources (nitrate, ammonium nitrate and urea-nitrate) on the biomass, total protein % and nitrate content of 2 representative duckweed species (i.e. Lemna japonica and Lemna gibba).
Lemna japonica (clone 7868 RDSC Collection) was provided by DryGro Ltd and named as DG8. Lemna gibba was obtained from a local pond in Harpenden, (Hertfordshire, UK; 51.807793, -0.33942981) and named as clone SD. Both clones were grown in a glasshouse with controlled temperature (21/18 oC day/night) and light supplement (16h, LED), in containers with 20 L of Rorison medium that was renewed every week. After 30 days, 5g of fresh weight duckweed were transferred to individual trays of 22 x 16 cm. Three different N sources (nitrate, ammonium nitrate and urea-nitrate) were applied at 2 different N doses (4 and 12 mM N) making a total of 6 different N treatments (3 sources x 2 doses) with 3 replicates for 14 days. After 2 days the medium of each tray was topped up to compensate for evaporation and at day 7 the medium was renewed. After 14 days, the pH of the medium was measured and all the samples were collected, weighed for fresh weight, dried, milled and scanned by FT-MIR (Tensor II, Bruker). Total nitrogen (TN) and nitrate nitrogen (NO3-N) were predicted using OPUS-QUANT II software (Bruker). Total protein % (TP) was calculated using the formula TP = (TN – NO3-N) x 6.25.
The ammonium nitrate treatment showed a substantial decrease of the pH with both N doses at harvest. At the same time, a decrease in the biomass production was observed for ammonium nitrate treatments at both N doses, although more significant in L. gibba, where the biomass production was more than 25% less than the respective nitrate and urea-nitrate treatments. The SD clone showed a higher NO3-N accumulation than DG8, and for both clones, increasing the nitrate dose produced a significant increase of NO3-N. The maximum NO3-N level observed in the SD clone supplied with 12mM nitrate (5614 mg NO3-N/kg DW) would exceed the maximum NO3-N level allowed by the EU for human consumption. Despite differences in NO3-N, no significant effects of N sources were found in duckweed TN and TP %.
These results show that the N source does not have a significant impact on the total protein concentration of representative duckweed species (i.e. L. japonica and L. gibba). Ammonium as an N source needs to be carefully managed, as it can affect duckweed growth. NO3-N should be routinely analysed in duckweeds. Also, we propose Rorison medium for duckweed experiments regarding N uptake.

This work is funded by European Research Development Fund (ERDF), Hertfordshire Local Enterprise Partnership's Single Local Growth Fund, for Hertfordshire Knowledge Exchange Partnership Project.

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