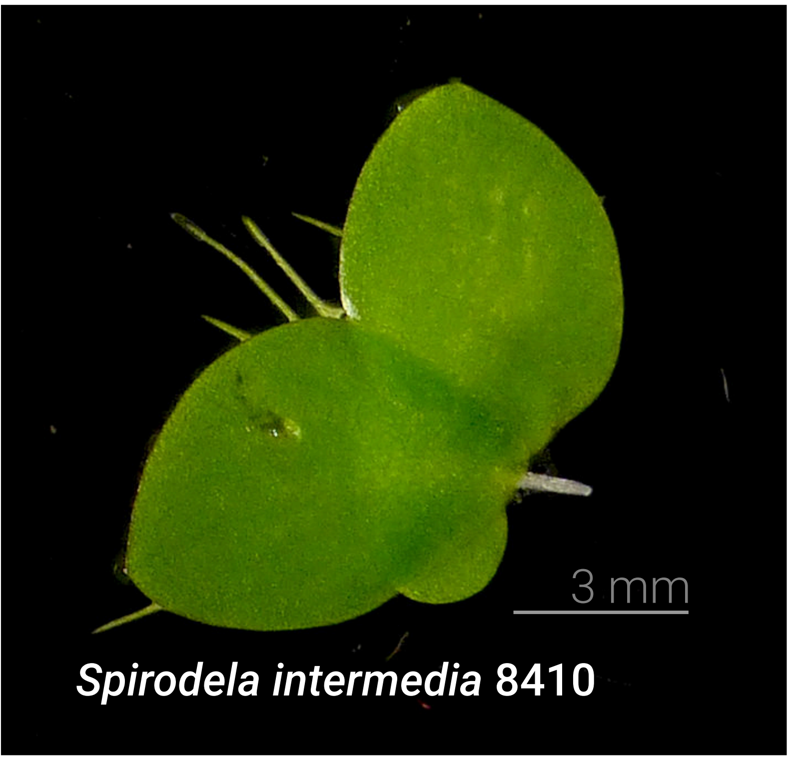
Newsletter of the Community of Duckweed Research and Applications, edited by the ISCDRA

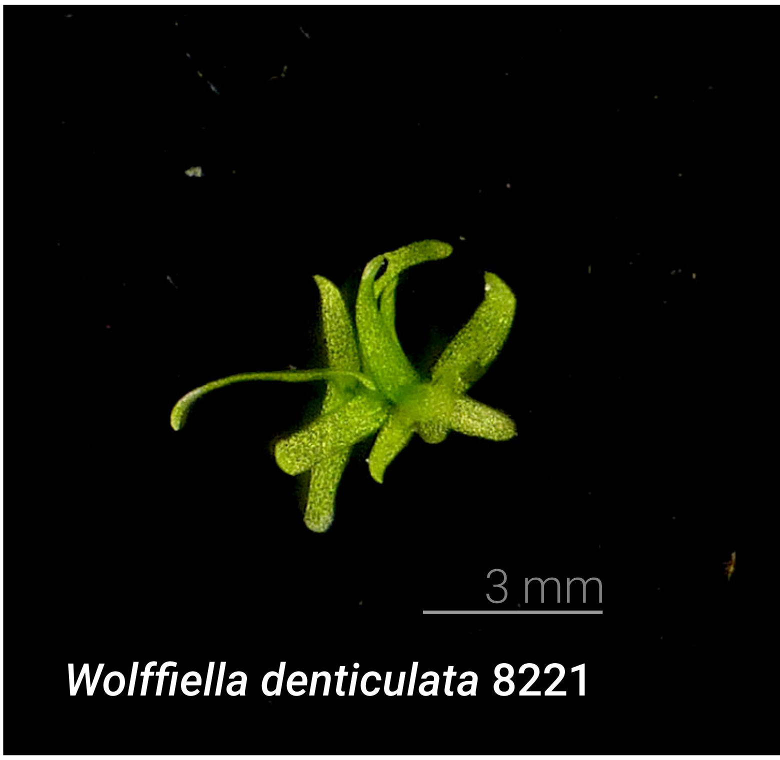
DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

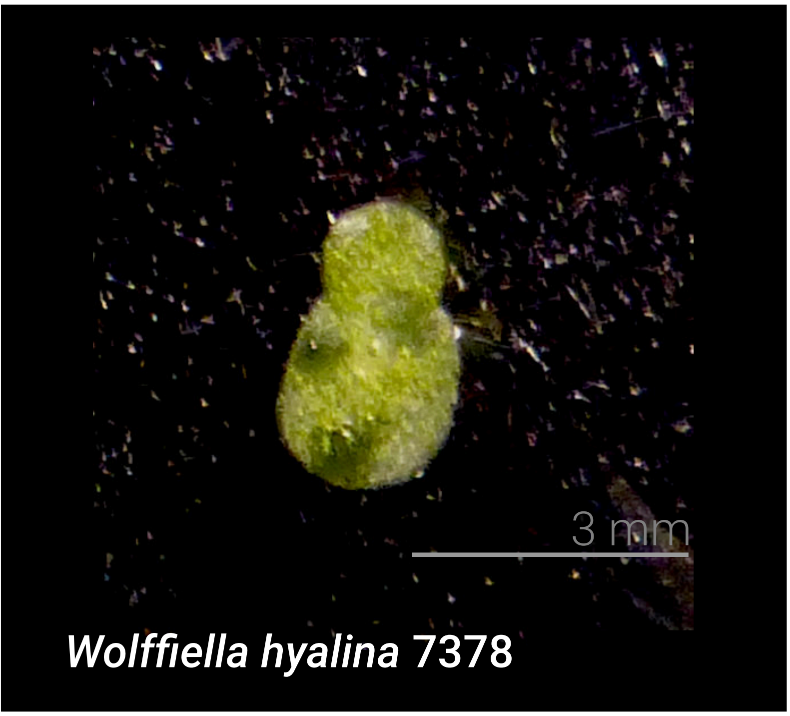
Volume 4 (4), issue 15, pages 304 - 326 (2016)



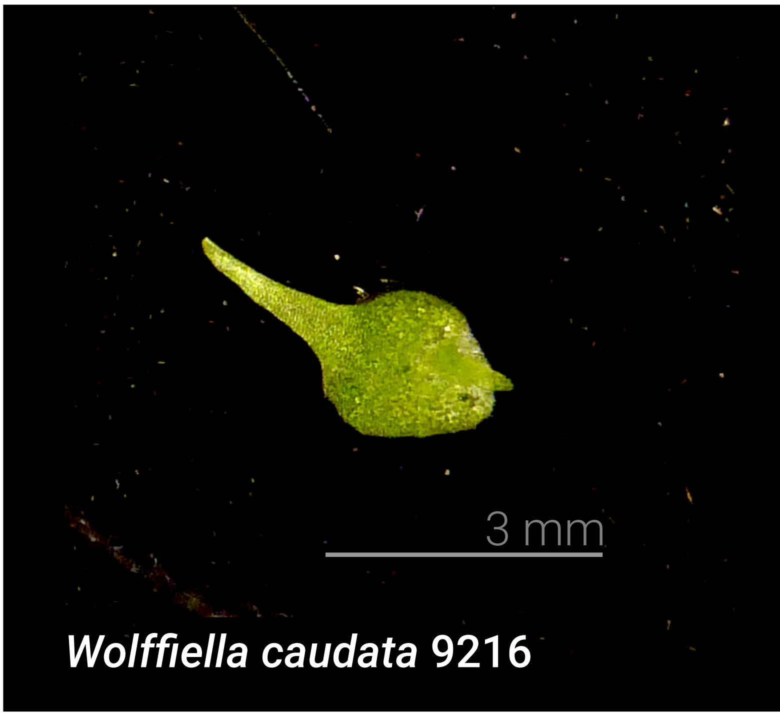
Spirodela intermedia 8410



Wolffiella denticulata 8221



Wolffiella hyalina 7378



Wolffiella caudata 9216

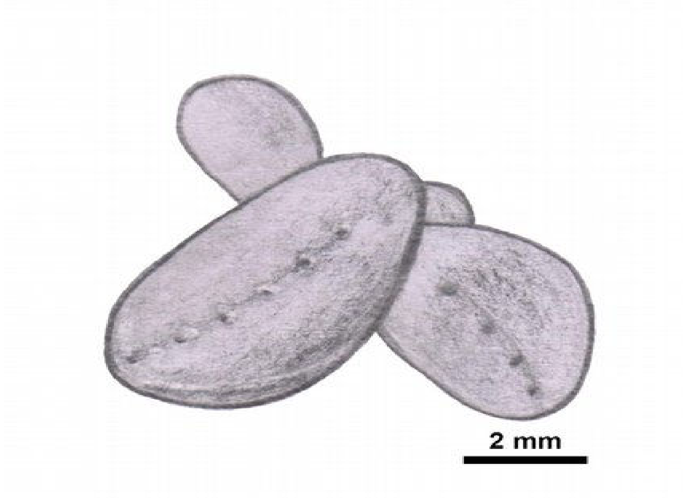
Spirodela intermedia is the lesser-known sibling of *Spirodela polyrhiza* but endemic between northern Argentina and Venezuela. Clone 8410 is currently being used by the lab of Prof. Ingo Schubert (IPK, Gatersleben, Germany) to carry out comparative cytogenetics with *S. polyrhiza*. *Wolffiella denticulata*, here shown as a colony of several fronds, is endemic in southwestern Africa and is considered an endangered species. Clone 8221 is the only one left among all known stock collections. *Wolffiella caudata* was described for the first time by E. Landolt in 1992 and was found in the Bolivian Amazon region, and in the Amazon region of Brazil. *Wolffiella hyalina* is common in dryer regions of Africa, e.g. in Egypt and Tanzania. It might have been introduced in India (Hyderabad and Mumbai) through human activities. Clone 7378 is of significance since it was used as a model system to study the effects of salicylic acid on flowering by the group of Prof. Maheshwari (Delhi, India) in the 1980's. Photographs taken by Dr. Eric Lam at the Rutgers Duckweed Stock Cooperative (Rutgers University, NJ).

In this issue

International Steering Committee on Duckweed Research and Applications Members

Information about the ISCDRA and all prior issues are available at http://lemnapedia.org/wiki/Duckweed_Forum.

Science meets art: *Landoltia punctata*



Landoltia punctata (G.Mey.) Les & D.J.Crawford is characterized by a distinct row of “papillae” on the dorsal side of the frond. The genus *Landoltia* was introduced in 1999 by Les and Crawford in honour of Elias Landolt (1926-2013). This is one of the duckweed species, which had undergone series of changes in nomenclature in the recent past; *Spirodela oligorrhiza* and *Spirodela punctata* are now synonyms (invalid names) of this species. Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India.

Letter from the editor

Dear friends of duckweed,

This is the latest issue, number 15, of our Newsletter "Duckweed Forum" 2016. The focus is on the use of duckweed for human nutrition. K. Sowjanya Sree (Central University Kerala, India) and I introduced a very recently published paper in "Food Chemistry" about this topic, together with a contribution by Tsipi Shoham (GreenOnyx, Israel) about the legal situation of using duckweed as human food. Both contributions extend our report about "Duckweed science and food excursion in Thailand" from the previous issue No. 14, downloadable from http://lemnapedia.org/wiki/Duckweed_Forum#2016-07.

In order to support newcomers, we gave the references of 20 reviews and review-like articles about essential fields of duckweed research and applications. We need more such reviews to help each other and interested people in learning about duckweed. Of course, this issue also have the newest duckweed publications under "From the Database".

In the chapter "Student Spotlight", Zhong Yu from the Peking University, China, explained her interest in duckweed. Tamra Fakhoorian, Executive Director of the ILA and member of our committee, outlined the problem of treating algae in duckweed populations in our "Discussion Corner". We invite you to contribute your suggestions and experience related to this issue because this problem is essential for all people in the applied field of duckweed production. We need to learn from experience by different duckweed users from different parts of the world.

On the cover page, you will find photos of *Spirodela intermedia* and three interesting species from the genus *Wolffiella* (Eric Lam, Rutgers University). A drawing of *Landoltia punctata* (Sowjanya Sree) under "Science meets Art" contributes to the present issue.

We invite readers to contribute in future issues of our "Duckweed Forum" about all aspects of research and applications for the duckweed community. To make such contributions easier, we wrote "Author Guidelines" hoping that these rules may help in our communication and production of the Newsletter.

Best wishes to all of you.

On behalf of the Steering Committee (ISCDRA),

Klaus-J. Appenroth, Chair

Twenty reviews or review-like papers about Lemnaceae

Reviews help newcomers to get quickly informed about a topic without reading completely the original literature. The following 20 reviews or review-like papers were compiled to inform the reader about selected fields of duckweed research. It should be emphasized that some references are not principally about duckweeds but are rather reviews of general topics, which include duckweeds.

1 Duckweed as a model organism for investigating plant-microbe interactions in an aquatic environment and its applications

Appenroth, K-J ; Ziegler, P ; Sree, KS

Endocytobiosis and Cell Research 27: 94-106

http://zs.thulb.uni-jena.de/receive/jportal_jparticle_00453025; Published: AUG 2016

2 The Uses of Duckweed in Relation to Water Remediation

Ziegler, P ; Sree, KS ; Appenroth, K-J

DESALINATION AND WATER TREATMENT

In press; Accepted for publication: JUL 2016

3 Interactions of metal-based engineered nanoparticles with aquatic higher plants: A review of the state of current knowledge

Thwala, M ; Klaine, SJ ; Musee, N

ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 35: 1677-1694

DOI: 10.1002/etc.3364; Published: JUL 2016

4 Taxonomy of duckweeds (Lemnaceae), potential new crop plants

Sree, KS ; Bog, M ; Appenroth, KJ

EMIRATES JOURNAL OF FOOD AND AGRICULTURE 28: 291-302

DOI: 10.9755/ejfa.2016-01-038; Published: MAY 2016

5 Aquatic ecotoxicity of lanthanum - A review and an attempt to derive water and sediment quality criteria

Herrmann, H ; Nolde, J ; Berger, S ; Heise, S

ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 124: 213-238

DOI: 10.1016/j.ecoenv.2015.09.033; Published: FEB 2016

6 Duckweeds for water remediation and toxicity testing

Ziegler, P ; Sree, KS ; Appenroth, K-J

Toxicological & Environmental Chemistry

http://dx.doi.org/10.1080/02772248.2015.1094701; Published online: 21 Jan 2016

7 Utility of Duckweeds as Source of Biomass Energy: a Review

Verma, R ; Suthar, S

BIOENERGY RESEARCH 8: 1589-1597

DOI: 10.1007/s12155-015-9639-5; Published: DEC 2015

8 Resurgence of duckweed research and applications: report from the 3rd International Duckweed Conference

Appenroth, K-J ; Sree, KS ; Fakhoorian T ; Lam E

Plant Molecular Biology 89: 647–654

DOI: 10.1007/s11103-015-0396-9; Published: DEC 2015

9 Plant sulfur nutrition: From Sachs to Big Data

Kopriva, S

PLANT SIGNALING & BEHAVIOR 10, Issue: 9, Article Number: e1055436

DOI: 10.1080/15592324.2015.1055436; Published: SEP 2015

10 How do magnetic fields affect plants in vitro?

Teixeira da Silva, JA; Dobranszki, J

IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY-PLANT 51: 233-240

DOI: 10.1007/s11627-015-9675-z; Published: JUN 2015

11 Lipids and proteins-major targets of oxidative modifications in abiotic stressed plants

Anjum, NA ; Sofo, A ; Scopa, A ; Roychoudhury, A ; Gill, SS ; Iqbal, M ; Lukatkin, AS ; Pereira, E; Duarte, AC ; Ahmad, I

ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH 22: 4099-4121

DOI: 10.1007/s11356-014-3917-1; Published: MAR 2015

12 Status of duckweed genomics and transcriptomics

Wang, W ; Messing, J

PLANT BIOLOGY 17 (Suppl. 1): 10-15

DOI: 10.1111/plb.12201; Published: JAN 2015

13 Growing duckweed for biofuel production: a review

Cui, W ; Cheng, JJ

PLANT BIOLOGY 17 (Suppl. 1): 16-23

DOI: 10.1111/plb.12216; Published: JAN 2015

14 Darwin-Wallace Demons: survival of the fastest in populations of duckweeds and the evolutionary history of an enigmatic group of angiosperms

Kutschera, U ; Niklas, KJ

PLANT BIOLOGY 17 (Suppl. 1): 24-32

DOI:10.1111/plb.12171; Published: JAN 2015

15 Relative in vitro growth rates of duckweeds (Lemnaceae) - the most rapidly growing higher plants

Ziegler, P ; Adelmann, K ; Zimmer, S ; Schmidt, C ; Appenroth, KJ

PLANT BIOLOGY 17 (Suppl. 1): 33-41

DOI: 10.1111/plb.12184; Published: JAN 2015

16 Assessment, validation and deployment strategy of a two-barcode protocol for facile genotyping of duckweed species

Borisjuk, N ; Chu, P ; Gutierrez, R ; Zhang, H ; Acosta, K ; Friesen, N ; Sree, KS ; Garcia, C ; Appenroth, KJ ; Lam, E

PLANT BIOLOGY 17 (Suppl. 1): 42-49

DOI: 10.1111/plb.12229; Published: JAN 2015

17 Characterisation of circadian rhythms of various duckweeds

Muranaka, T ; Okada, M ; Yomo, J ; Kubota, S ; Oyama, T

PLANT BIOLOGY 17 (Suppl. 1): 66-74

DOI: 10.1111/plb.12202; Published: JAN 2015

18 Telling Duckweed Apart: Genotyping Technologies for the Lemnaceae

Appenroth, KJ ; Borisjuk, N ; Lam, E

Chinese Journal of Applied and Environmental Biology 19: 1-10

DOI: 10.3724/SP.J.1145.2013.00001; Published: FEB 2013

19 The Lemna bioassay: Contemporary issues as the most standardized plant bioassay for aquatic ecotoxicology

Mkandawire, M ; Teixeira da Silva, JA; Dudel, EG

CRITICAL REVIEWS IN ENVIRONMENTAL SCIENCE AND TECHNOLOGY 44: 154-197

DOI: 10.1080/10643389.2012.710451; Published: JAN 1 2014

20 Is flowering in Lemnaceae stress-induced? A review

Pieterse, AH

AQUATIC BOTANY 104: 1-4

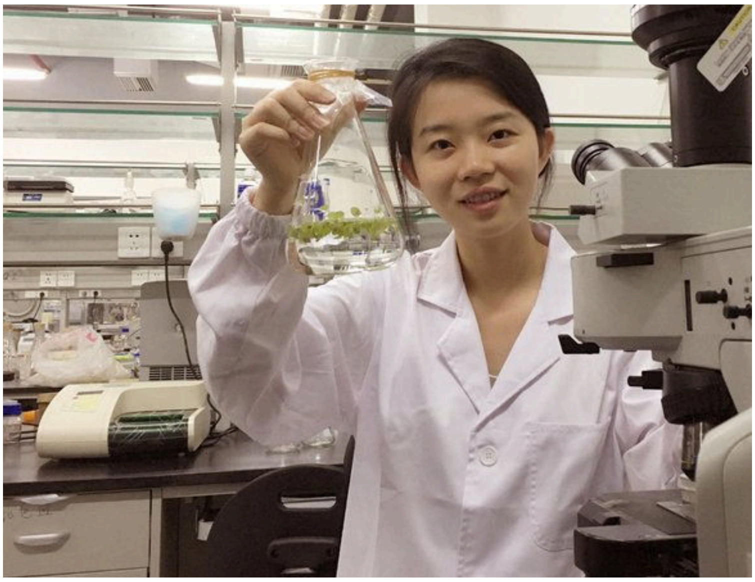
DOI: 10.1016/j.aquabot.2012.08.002; Published: JAN 2013

Student Spotlight: Ms. Zhong Yu

Email: zhongyu@pku.edu.cn

I have always been fascinated by all kinds of natural plants and in particular their beautiful flowers since I was a young girl in kindergarten. When I was a teenager, gathering these beautiful flowers became my hobby and I dreamed of becoming a botanist to study those plants. Although I ended up double-majoring in clinical medicine and psychology in my college, my enthusiasm for natural plants has never faded away. During my senior year in college, a teacher asked me to write a review article about mental health of patients with carcinoma. Through reading literature, I found that the active compounds in natural plants can be potentially used for prevention and treatment of various cancers with minimal side effects. I became interested immediately since I have always been enthusiastic about studying natural plants.

With this idea in mind, I went to graduate school to purse my Master's degree in Hydrobiology. This was my first time to have the opportunity to perform independent research. Marine-based natural products have a great potential as natural pharmaceuticals but they have been largely under-explored. My research involved the study of macroaglae *Ulva fasciata*, which has been reported to have antitumor effects from its crude extract.



By the time I finished my study, I had an opportunity of pursing a PhD degree with Prof. Jay J. Cheng at the Graduate School of Peking University in Shenzhen, China. Professor Cheng is a world renowned scientist in duckweed research. He described to me the fantastic features of duckweed, a rapid growing aquatic plant globally adapted across a broad range of climates. Due to its high starch content, duckweed can potentially be used a feedstock for bio-energy productions.

The protein content of duckweed can also be high, which makes this biomass a potentially good source of animal feed. Duckweed is easily harvested as a free-floating plant in nutrient-rich water. Intrigued by such a great potential and also by the desire to fulfill my teenager dream as a botanist, I immediately decided to continue my study as a PhD student in Peking University with Dr. Cheng as my advisor. Additionally, Dr. Klaus Appenroth also gave me a lot of helpful guidance while I was doing my research.

My PhD project is to study the physiological and biochemical characteristics and antitumor activity of selenium-enriched duckweed. Selenium (Se) can be used in human biomedicine as well as in the diet of domesticated animals. At low concentrations, Se also exhibits immune functions and anti-carcinogen effects, and may slow the aging process. Duckweed has a good affinity to accumulate selenium. It can turn inorganic selenium into its organic form, Selenomethionine, which has an enhanced bioavailability for human and various animals. In the environment, duckweed can bioaccumulate toxic compounds from water.

Duckweed can also be used as an indicator for water body toxicity and provides an effective way for cleaning wastewater. My current research focuses on *Landoltia punctata* 7449 as the model organism for its response to Se. The nutrition value of the *L. punctata* biomass will be explored as a food product with antioxidant properties or as an animal feed.

Author Guidelines

"Duckweed Forum" is the newsletter of the International Steering Committee on Duckweed Research and Applications (ISCDRA), published quarterly for scientists and others who are interested in the duckweed plant family Lemnaceae. The newsletter is free of charge for authors and readers, subscription can be initiated by request and all issues can be downloaded from http://lemnapedia.org/wiki/Duckweed_Forum.

Contributing authors to Duckweed Forum are requested to follow the following guidelines:

- "Duckweed Forum" has sections, which may be chosen by a prospective author(s) to contribute to: main text, discussion corner, useful methods, student experiments, student focus, "Science meets Art" and cover photos.
- The topic should be given in a Title followed by the names and addresses of all authors including their Email addresses.
- 1,000 words are suggested as the upper limit but can be extended on request to the ISCDRA if the reason for the waiver request is warranted.
- Any format readable by Microsoft Word or LibreOffice Writer programs may be used to send the text.
- Photos or diagrams are welcome and recommended to make the contribution more clear, understandable, visually interesting and informative. Format of the photo or diagrams could be .jpg, .png or .tiff for raster (bitmap) images, or .pdf, .svg or .ai (for vectors). Figures should be submitted separately and not only wrapped into the text. They should have a minimum resolution of 300 dpi.
- Potential contributions should be submitted two weeks before the next issue will be published or the contribution will be postponed to the next edition. Deadlines are 15th of March, 15th of June, 15th of September and 15th of December. Members of the ISCDRA collectively decide on the acceptance of a submitted contribution.

In case of doubts or other inquiries, please write us to steering-committee@lemnapedia.org.

Discussion Corner: Natural Approaches to Treatment of Algae in Duckweed Production

By Tamra Fakhoorian, Exec. Director, International Lemna Association, President GreenSun Products, LLC Mayfield, KY USA

Nature abhors a vacuum. We in the duckweed community can especially appreciate that bit of wisdom as we experience it first-hand on a daily basis. Algae is naturally present in open duckweed ponds. It thrives in the same nutrient loads and conditions that duckweed thrives in. A wide variety of algal species can be found in the water column and/or on the surface and can take advantage of a duckweed pond very quickly in the heat of summer. Species that predominately reside in the water column itself quickly absorb the nutrients that are intended for duckweed. Population explosions of algae form surface mats that crowd out and entangle duckweed, strip the available nutrients within duckweed's reach, and can take over a pond within days if left undisturbed. Toxins released by algal blooms can render duckweed biomass not fit for biofertilizer, much less animal or human consumption. Chemical control *can* curb algal growth but will weaken or kill duckweed in the process. This introduction to basic control of algae serves as a jumping-off point for this discussion. You are invited to share your experience and commentary on this critical topic.

Duckweed pond overrun with filamentous algae in the Philippines. Described techniques restored pond to healthy duckweed status but required ongoing vigilance of mat and nutrient loads.

My practical experience: subsurface algal control in the water column starts with a wind-protected pond surface. Watch for wind direction and place wind barriers across the side of the pond that receives the majority of the breezes. This will insure the duckweed does not blow off to one side, exposing the water column to sunlight.

Initiate shading of lower water column in early production season by introducing a solid mat of duckweed, roughly 500g/m2 fresh weight. Do not add full strength nutrient loads until duckweed mat is firmly established over the entire pond. Harvest enough duckweed on a monitored basis to keep this density at all times.

Here is a technique that I have used for nutrient-loaded small pools or narrow pilot ponds that have "gone green" before you even seed them with duckweed starter. If you do not have enough duckweed starter to obtain the above mat density, try confining your duckweed starter with floating Styrofoam pool tubes strung together in an effort to achieve the above density. Then use black plastic stretched over the balance of the pond to get full shading effect. In this situation, you will have a delicate dance with the subsurface algae as it has quite ably taken up most of the nutrients already for the first few days. The algae will begin to die. As duckweed begins to grow, enlarge its confines and pull back the black plastic until you can eventually remove it altogether. Mantra: Keep your mat dense enough to insure a "win" over subsurface algae.

Surface filamentous algae can be approached two ways- hand removal (highly recommended in small ponds) and follow up with "smothering" with additional duckweed. I have brought pilot ponds back from extreme filamentous algal takeover by diluting the water to remove excess phosphates, hand removal of as many algal clumps as possible, and finally introduction of healthy duckweed in a large enough quantity to smother the surface algae.

Much remains to be understood about beneficial algal roles in duckweed cultivation. We can borrow cues from other industries like fisheries to develop our own criteria for possible toxin types and limits in resulting duckweed biomass. By tackling the challenge of algae in duckweed production head-on, we can get past the roadblock. What is your experience?

Duckweed for human nutrition

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

[1]University of Jena, Institute of General Botany and Plant Physiology, Jena, Germany

[2]Central University of Kerala, Department of Environmental Science, RSTC, Padannakad, India

Having reported about the use of duckweed in the local Thai kitchen in the previous issue (Sree and Appenroth, 2016), it would be interesting to learn about the nutritive value of these monocots. These small aquatic plants have been reported to be poor man's food since ages and are known to be eaten in Thailand, Burma (Myanmar) and Laos (Landolt and Kandeler, 1987). *Wolffia globosa* is the dominating *Wolffia* species in many Asian countries and is the species mostly used for human consumption (Sree and Appenroth, 2016). Surprisingly, there are no reports to our knowledge of duckweed being eaten in the Indian subcontinent, i.e. India, Bangladesh or Pakistan, although the environmental conditions for growth of duckweeds in these countries are ideal. Already in 1971, Bhanthumnavin and McGarry reported that duckweeds are rich in protein, mentioning them as "a possible source of inexpensive protein". Food in many Asian countries is rich in starch (based on rice as staple food) but poor in protein. Therefore, duckweed would be a very useful supplement to their traditional diet and would be one of the components of vegetarian / vegan food in the western countries. Further, Rusoff et al. (1980) reported that the amino acid composition of duckweed protein meets the nutritional demand of humans. Although there is a need for in-depth scientific investigation, there are no reports of harmful effects of eating duckweeds.

These two papers mentioned above were published more than 30 years ago. More recently, Yan et al. (2013) investigated the fatty acid composition of 30 different species of duckweed. They reported the contents of saturated, monounsaturated and polyunsaturated fatty acids in these species, which can be used to evaluate the nutritive value of duckweeds in human food. In our newest paper (Appenroth et al., 2017), a more holistic approach was employed, investigating various components of human diet in different genera of duckweed: protein content (20 – 35 % per dry weight) and its amino acid composition, fat content (4 – 7 %) and the fatty acid composition, as well as the starch content (4 - 10 %). The proteins contain ca. 5 % lysine, 3 % methionine + cysteine (the sulphur containing amino acids) and 8 % phenylalanine + tyrosine. All these critical amino acids are above the WHO recommended levels for human nutrition. The fat contains between 48 and 71 % polyunsaturated fatty acids and the ratio of omega-6 to omega-3 fatty acids is at the dream level of 0.5 or even less. Winners for the nutritional composition were the species *Wolffia microscopica* (endemic to the Indian subcontinent) and the rarely investigated *Wolffiella hyalina* (found in Africa and India).

Wolffia microscopica was selected for a more detailed study. This species revealed a good mineral composition concerning the ratios of potassium/ sodium and magnesium/ calcium. Moreover, the mineral composition is easy to manipulate just by selecting a suitable nutrient medium. In this way, plants with high Zinc or Selenium content can easily be produced. The high content of lutein and zeaxanthin (antioxidants) makes this species even more interesting for prevention of age-related macular degeneration. Further, the content of phytosterol, important for maintaining the blood cholesterol level, is unusual high in this species.

These data recommend duckweed as an excellent constituent of human food. In some of the Asian countries, the high protein content and the high-quality amino acid composition together with the composition of fatty acids of duckweeds are attractive. On the other hand, the low starch profile fulfils the requirement for low-energy food and together with the properties of proteins and fats, duckweeds would be appealing to many in the developed countries. These nutritive properties are also desirable for vegetarian or vegan eating behaviors.

References

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The present legal status of using duckweed as human food

Tsipi Shoham, PHD, Email: tsipi@greenonyx.biz

GreenOnyx CTO and co-founder; Israel; http://www.greenonyx.biz/

Food standard agencies across the globe have a statutory objective to protect public health and consumers' interests in relation to any food and drink. Accordingly, to commercialize a new produce, it must comply with all local laws, regulations, codes of practice and guidance that are related to its production, processing, packaging, distribution and labeling.

In many markets in the world, Duckweed plants will be considered as a new produce, or a New Dietary Ingredient (NDI), thus will need to gain a local regulatory status.

In GreenOnyx, we set a strategy that focused first on the known edible Duckweed strains and the regulation into the USA market. As the company and market acceptance develops, we could expand the regulatory process for these strains to other regions and further bring on board new suitable duckweed strains. Thus, GreenOnyx has designed a scalable approach that could eventually bring any edible Duckweed strain of interest into multiple target markets.

Under the USA Food and Drug Administration (FDA) guidelines, a dietary ingredient that was not marketed in the United States before 1994, is considered a New Dietary Ingredient (NDI) subjected to the regulation under New Dietary Ingredient notification (NDIN) process. An alternative path is the GRAS (General Recognized As Safe) affirmation, which is available only for an ingredient that can demonstrate an historical consumption as food for extended periods (several generations) by a significant number of people.

GreenOnyx has successfully completed the GRAS affirmation status for all its natural strains and clones that belong to the *Wolffia arrhiza* or *Wolffia globosa* species (also know as Khai-Nam in Thailand), thus enabling their introduction to the USA market as edible vegetables.

The eligibility for classification as GRAS and a Non-NDI regulatory status was based on our ability to demonstrate the long history of safe food use, the consumption in more then one country outside of the USA, and the consumption as a part of the local customary diet for a significant number of people. Further more, the following aspects had to be documented:

- A specific description of the traditional Khai-Nam consumption: Use of strains found in nature, excluding clones that were modified either under conventional breeding or GMO (Genetically Modified Organisms)
- A detailed description of the composition as traditional food: Used as a food ingredient and as a dietary supplement at the levels not exceeding the traditional servings and as related to the intended purpose.
- A specific description of the cultivation system demonstrating its alignment with the plant's natural habitat conditions its ability to support the Khai-Nam normal reproduction and life cycle.
- A specific description for the conditions of intended use and for specific labeling requirements, which do not mislead the consumer.
    - Documentation regarding nutritional value, all based on FDA certified analytical laboratories' tests.

    - The intended use as a food ingredient and as a dietary supplement for humans and animals, including the ability to consume the GreenOnyx Khai-Nam as a fresh wholesome produce or as a dried, frozen or ground ingredient, the ability to integrate the GreenOnyx Khai-Nam into any food category and that it may be used as a supplement derived from any form or part of the plant.
    - When added to food or used as a dietary supplement, it will be limited by the amount not higher than to achieve its intended purpose and not exceeding its traditional consumption levels.
- Pollution remediation and potential toxin accumulation needed special attention when certifying duckweeds, including Khai-Nam. Our cultivation output had to show that it could meet modern food grade regulation standards, both related to the growing medium (water & fertilizer) and the harvested plant biomass. For both, we needed to present the appropriate data related to safety and toxicity, including our microbiological profile, heavy metal profile, potential human toxicants, and allergens. The concerns raised were due to the following:
    - Wolffia and larger duckweeds can act as a bioremediator of excess phosphorus and nitrogen thus show promise for use in sustainable wastewater treatment systems. As such, Wolffia plants can accumulate toxic heavy metal such as lead, cadmium, chromium, and arsenic.
    - Pond waters, reservoirs, and slow moving streams - where *Wolffia* grow naturally - may be contaminated with Cyanobacteria, also known as blue-green algae. The Cyanobacteria species produce a group of toxins called microcystins. *Wolffia* in the wild have been known to accumulate microcystins.
    - Duckweed species are also able to transform phenols from pesticides. Limited work has been done to characterize the secondary metabolites in specific species of duckweed, but oxalic acid has been identified that can be toxic to animals and humans.

Considering that *Wolffia* plants may accumulates toxins only when exposed to these substances in its habitat, the GreenOnyx closed system approach includes careful control and monitoring of its inputs and special harvest technology, which address these safety concerns. For example, test results show that GreenOnyx Khai-Nam, grown over a year in our closed controlled system, contained less than 10 ppb heavy metals.

Exclusion of pesticides from the growth media and continuous control of the growing solution precludes contamination with phenols and subsequent formation of oxalic acid. Analytical test results of GreenOnyx Khai-Nam plants showed oxalic acid present at very low levels along with all other organic acids that were evaluated.

The GreenOnyx Khai-Nam is also isolated from any allergen compound that could enter either through the growth media or subjected air, and is therefore allergen free

As part of its market expansion strategy, GreenOnyx has developed the ability to adjust its automated closed systems to any natural strain and clone of the duckweed family, thus developing the capacity to exploit the potential of many other duckweeds plants as food. This is done via the closed platform's ability to adjust its cultivation parameters and to bio-mimic almost any natural conditions of specific duckweed plants. Furthermore, the GreenOnyx platform is designed to enable the compliance of various duckweed plants with the strict food safety regulatory constrains across the globe.

From the Database

Biotechnology

A Nanopore-Structured Nitrogen-Doped Biocarbon Electrocatalyst for Oxygen Reduction from Two-Step Carbonization of *Lemna minor* Biomass

Guo, CZ; Li, ZB; Niu, LD; Liao, WL; Sun, LT; Wen, BX; Nie, YQ; Cheng, J; Chen, CG

NANOSCALE RESEARCH LETTERS 11: Article Number 268 (2016)

So far, the development of highly active and stable carbon-based electrocatalysts for oxygen reduction reaction (ORR) to replace commercial Pt/C catalyst is a hot topic. In this study, a new nanoporous nitrogen-doped carbon material was facilely designed by two-step pyrolysis of the renewable *Lemna* minor enriched in crude protein under a nitrogen atmosphere. Electrochemical measurements show that the onset potential for ORR on this carbon material is around 0.93 V (versus reversible hydrogen electrode), slightly lower than that on the Pt/C catalyst, but its cycling stability is higher compared to the Pt/C catalyst in an alkaline medium. Besides, the ORR at this catalyst approaches to a four-electron transfer pathway. The obtained ORR performance can be basically attributed to the formation of high contents of pyridinic and graphitic nitrogen atoms inside this catalyst. Thus, this work opens up the path in the ORR catalysis for the design of nitrogen-doped carbon materials utilizing aquatic plants as starting precursors.

Activated carbons for the hydrothermal upgrading of crude duckweed bio-oil

Duan, PG; Zhang, CC; Wang, F; Fu, J; Lu, XY; Xu, YP; Shi, XL

CATALYSIS TODAY 274: 73-81 (2016)

This study examined a two-stage (noncatalytic pretreatment followed by catalytic upgrading) hydrothermal processing of crude bio-oil produced from the hydrothermal liquefaction of duckweed. The activities of six activated carbons (ACs)-pine wood AC, coconut shell AC, bamboo stem AC, apricot pit AC, peach pit AC, and coal AC-toward the deoxygenation and denitrogenation of the pretreated duckweed bio-oil were determined in supercritical water at 400 degrees C for 1 h with the addition of 6 MPa of H-2 and 10 wt% AC. All of the ACs exhibited activity similar to Ru/C toward the denitrogenation and deoxygenation of the pretreated duckweed bio-oil. Of the ACs tested, bamboo stem AC produced an upgraded bio-oil with the highest yield (76.3 wt%), the highest fraction (90.13%) of material boiling below 350 degrees C, and the highest energy density (44.1 MJ/kg). Decreased ash and acidic groups in the pre-treated AC disfavored the production of upgraded bio-oil but aided denitrogenation and desulfurization. The ACs are suspected to leach ions and weak acids into the reaction solution, which would catalyze denitrogenation and desulfurization. The gases mainly consisted of unreacted H-2, CO2 and CH4 together with small amounts of CxHy (x <= 5, y <= 12) hydrocarbon gases produced from the cracking of the upgraded bio-oil.

Succinic acid production from duckweed (*Landoltia punctata*) hydrolysate by batch fermentation of *Actinobacillus succinogenes* GXAS137

Shen, N; Wang, QY; Zhu, J; Qin, Y; Liao, SM; Li, Y; Zhu, QX; Jin, YL; Du, LQ; Huang, RB

BIORESOURCE TECHNOLOGY 211: 307-312 (2016)

Duckweed is potentially an ideal succinic acid (SA) feedstock due to its high proportion of starch and low lignin content. Pretreatment methods, substrate content and nitrogen source were investigated to enhance the bioconversion of duckweed to SA and to reduce the costs of production. Results showed that acid hydrolysis was an effective pretreatment method because of its high SA yield. The optimum substrate concentration was 140 g/L. The optimum substrate concentration was 140 g/L. Corn steep liquor powder could be considered a feasible and inexpensive alternative to yeast extract as a nitrogen source. Approximately 57.85 g/L of SA was produced when batch fermentation was conducted in a 1.3 L stirred bioreactor. Therefore, inexpensive duckweed can be a promising feedstock for the economical and efficient production of SA through fermentation by *Actinobacillus succinogenes* GXAS137.

Computation-aided separation of seven components from *Spirodela polyrrhiza (L.)* via counter-current chromatography

Ren, DB; Han, BS; Xin, ZQ; Liu, WB; Ma, SS; Liang, YZ; Yi, LZ

SEPARATION AND PURIFICATION TECHNOLOGY 165: 160-165 (2016)

In this study, seven flavonoids were successfully separated from *Spirodela polyrrhiza (L.) Schleid*. via highspeed counter-current chromatography (HSCCC) with elution-extrusion and online recycling elution modes. The first step for successful HSCCC separation is the crucial solvent system selection. In this work, nonrandom two-liquid segment activity coefficient (NRTL-SAC) method, a computational strategy, was used to facilitate the solvent system selection process. According to the NRTL-SAC method, a suitable solvent system can be rapidly predicted and screened by calculating the partition coefficient rather than by performing tedious experimental measurements. Based on the method, two solvent systems of hexane/ethyl acetate/ethanol/water (1:3:1:3 and 1:9:1:9, v/v) were predicted as the most suitable systems for HSCCC separation. To avoid unnecessary waste of the stationary or mobile phase, both the mobile and stationary phases were prepared independently based on the calculated phase compositions by thermodynamic method. Consequently, seven compounds were successfully separated with high purity (>90%). The separated compounds were identified through UV spectra and high-resolution tandem mass spectroscopy. These compounds include orientin (1), vitexin (2), luteolin 7-O-glucoside (3), apigenin 7-O-glucoside (4), luteolin 8-C-(2 "-O-feruloyl-) glucoside (5), apigenin 8-C-(-2 "-O-feruloyl-) glucoside (6), and luteolin (7). This work demonstrates that HSCCC is suitable for separation of natural products, and computational strategy is very helpful for enhancing the efficiency of CCC experiments.

Ecology

Colonization dynamics of the alien macrophyte *Lemna minuta Kunth*: a case study from a semi-natural pond in Appia Antica Regional Park (Rome, Italy)

Ceschin, S; Della Bella, V; Piccari, F; Abati, S

FUNDAMENTAL AND APPLIED LIMNOLOGY 188: 93-101 (2016)

Most studies describing biomass production of Lemna species (duckweeds) underline high growth rates of these species under controlled conditions. *L. minuta*'s growth, as well as its colonizing capability under natural conditions, has never been investigated, despite this species being the most widespread and invasive alien duckweed in Europe. In this study, the colonization dynamics of *L. minuta* were analysed through a 5 month field-experiment carried out in a semi-natural pond of 26.3 m(2) in the city of Rome. Variations in plant coverage (m(2)), biomass produced (g) and Relative Growth Rate (RGR, day(-1)), show that *L. minuta* has an growth of exponential type, consisting of three main phases: (1) an initial phase (40 days), in which *L. minuta* grows moderately by covering just under 15 % of the pond surface; (2) an exponential phase (45 days), in which the duckweed grows intensively, until it completely covers the entire pond surface with a monolayer floating population; (3) an apparently stationary phase perpetuated in time, in which, instead, the species continues to grow in biomass, giving rise to a dense multilayer population 2.5 cm thick. *L. minuta* was able to colonize the entire pond surface in a few months of experiment, increasing its total biomass over 600 times respect to that initial. This underlines the species' high biomass production in natural conditions. The RGR of *L. minuta* estimated at the end of the experiment amounts to 0.041 day(-1) (0.046 day(-1), 0.006 day(-1), 0.126 day(-1) as average, minimum and maximum RGR, respectively) more than double of that calculated in similar conditions for the native congeneric *L. minor*, showing a higher colonization capability of the alien species respect to the native ones. The characterization of the growth dynamics in field of the alien *L. minuta* provides information that could be useful for a better planning of management programs of natural wetlands invaded by this alien species, also with respect to its invasion control. Our findings suggest that in lentic environments, it is necessary to remove the species every 30-40 days for controlling this duckweed and prevent its future outbreaks locally. However, the complete removal of an entire *L. minuta* population from a natural site can result difficult and demanding but, combining a physical control of *L. minuta* with a subsequent biological one, it could make more effective the eradication action of the species from a site.

Feed and Food

Nutritional value of duckweeds (Lemnaceae) as human food

Appenroth, K.-J; Sree, K.S.; Bohm, V.; Hammann, S.; Vetter, W.; Leiterer, M.; Jahreis, G.

FOOD CHEMISTRY 217: 266-273 (2017)

Duckweeds have been consumed as human food since long. Species of the duckweed genera, *Spirodela*, *Landoltia*, *Lemna*, *Wolffiella* and *Wolffia* were analysed for protein, fat, and starch contents as well as their amino acid and fatty acid distribution. Protein content spanned from 20% to 35%, fat from 4% to 7%, and starch from 4% to 10% per dry weight. Interestingly, the amino acid distributions are close to the WHO recommendations, having e.g. 4.8% Lys, 2.7% Met + Cys, and 7.7% Phe + Tyr. The content of polyunsaturated fatty acids was between 48 and 71% and the high content of n3 fatty acids resulted in a favourable n6/n3 ratio of 0.5 or less. The phytosterol content in the fastest growing angiosperm, *Wolffia microscopica*, was 50 mg g-1 lipid. However, the content of trace elements can be adjusted by cultivation conditions. Accordingly, *W. hyalina* and *W. microscopica* are recommended for human nutrition.

Interaction with other organisms

Duckweed as a model organism for investigating plant-microbe interactions in an aquatic environment and its applications

Appenroth, K.-J.; Ziegler, P.; Sree, K.S.

ENDOCYTOBIOSIS AND CELL RESEARCH 27: 94-106 (2016)

Duckweeds, small, floating aquatic plants that constitute the family Lemnaceae, are well established as model organisms for phytotoxicity testing and show great promise for wastewater remediation and useful biomass production. In laboratory experiments, they are often investigated in sterile conditions, but in outdoor environments, they are associated with other biota, especially, microorganisms, and the significance of the interactions between duckweed and bacteria for the growth and the remediative performance of the plants is gradually becoming apparent. Bacteria may stimulate the growth of duckweeds, and can enhance the rate of nutrient removal from eutrophic waters by duckweeds. The interaction of bacteria with duckweeds can also improve the removal of heavy metals and organic xenobiotics from contaminated waters and decrease the emission of greenhouse and odorous gases from water bodies. This review discusses the presently available experimental results on these topics, and points to the value of using duckweeds to evaluate higher plant-microbe interactions. The ease of cultivation and analysis of duckweeds, in conjunction with increasing availability of genomic sequence data and the establishment of genetic transformation techniques for these plants make duckweeds plausible model organisms for the investigation of such interactions in an aquatic context.

The abundance and diversity of heterotrophic bacteria as a function of harvesting frequency of duckweed (*Lemna minor L.*) in recirculating aquaculture systems

Ardiansyah, A; Fotedar, R

LETTERS IN APPLIED MICROBIOLOGY 63: 53-59 (2016)

Duckweed (*Lemna minor L.*) is a potential biofilter for nutrient removal and acts as a substrate for heterotrophic bacteria in recirculating aquaculture systems (RAS). Here, we determined the effects of harvesting frequency of duckweed on heterotrophic bacteria in RAS. Twelve independent RAS consisting of fish-rearing tank, biofilter tank and waste-collection tank were used to study the interactions between duckweed harvest frequencies up to 6days and the composition, abundance and diversity of heterotrophic bacteria. After 36 days, heterotrophic bacteria in the biofilter tank were primarily Gram-negative cocci or ovoid, coccobacilli, Gram-negative bacilli and Gram-positive bacilli. Most bacterial genera were *Bacillus* and *Pseudomonas* while the least common was *Acinetobacter*. Duckweed harvested after every 2 days produced the highest specific growth rates (SGR) and total harvested biomass of duckweed, but harboured less abundant bacteria, whereas 6-day harvests had a higher growth index (GI) of duckweed than 2-day harvests, but caused a poor relationship between SGR and biomass harvest with the abundance and diversity of heterotrophic bacteria. Stronger correlations (R-2>065) between duckweed SGR and biomass harvest with the heterotrophic bacteria diversity were observed at 4-day harvest frequency and the control.

Quantification and enzyme targets of fatty acid amides from duckweed root exudates involved in the stimulation of denitrification

Sun, L; Lu, YF; Kronzucker, HJ; Shi, WM

JOURNAL OF PLANT PHYSIOLOGY 198: 81-88 (2016)

Fatty acid amides from plant root exudates, such as oleamide and erucamide, have the ability to participate in strong plant-microbe interactions, stimulating nitrogen metabolism in rhizospheric bacteria. However, mechanisms of secretion of such fatty acid amides, and the nature of their stimulatory activities on microbial metabolism, have not been examined. In the present study, collection, pre-treatment, and determination methods of oleamide and erucamide in duckweed root exudates are compared. The detection limits of oleamide and erucamide by gas chromatography (GC) (10.3 ng mL(-1) and 16.1 ngmL(-1), respectively) are shown to be much lower than those by liquid chromatography (LC) (1.7 and 5.0 mu g mL(-1), respectively). Quantitative GC analysis yielded five times larger amounts of oleamide and erucamide in root exudates of *Spirodela polyrrhiza* when using a continuous collection method (50.20 +/- 4.32 and 76.79 +/- 13.92 mu g kg(-1) FW day(-1)), compared to static collection (10.88 +/- 0.66 and 15.27 +/- 0.58 mu g kg(-1) FW day(-1)). Furthermore, fatty acid amide secretion was significantly enhanced under elevated nitrogen conditions (>300 mg L-1), and was negatively correlated with the relative growth rate of duckweed. Mechanistic assays were conducted to show that erucamide stimulates nitrogen removal by enhancing denitrification, targeting two key denitrifying enzymes, nitrate and nitrite reductases, in bacteria. Our findings significantly contribute to our understanding of the regulation of nitrogen dynamics by plant root exudates in natural ecosystems.

Molecular Biology

Expression of anti-tumor necrosis factor alpha (TNF) single-chain variable fragment (scFv) in *Spirodela punctata* plants transformed with *Agrobacterium tumefaciens*

Balaji, P; Satheeshkumar, PK; Venkataraman, K; Vijayalakshmi, MA

BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY 63: 354-361 (2016)

Therapeutic antibodies against tumor necrosis factor alpha (TNF) have been considered effective for some of the autoimmune diseases such as rheumatoid arthritis, Crohn's diseases, and so on. But associated limitations of the current therapeutics in terms of cost, availability, and immunogenicity have necessitated the need for alternative candidates. Single-chain variable fragment (scFv) can negate the limitations tagged with the anti-TNF therapeutics to a greater extent. In the present study, *Spirodela punctata* plants were transformed with anti-TNF through in planta transformation using *Agrobacterium tumefaciens* strain, EHA105. Instead of cefotaxime, garlic extract (1mg/mL) was used to remove the agrobacterial cells after cocultivation. To the best of our knowledge, this report shows for the first time the application of plant extracts in transgenic plant development. 95% of the plants survived screening under hygromycin. ScFv cDNA integration in the plant genomic DNA was confirmed at the molecular level by PCR. The transgenic protein expression was followed up to 10months. Expression of scFv was confirmed by immunodot blot. Protein expression levels of up to 6.3% of total soluble protein were observed. -Glucuronidase and green fluorescent protein expressions were also detected in the antibiotic resistant plants. The paper shows the generation of transgenic *Spirodela punctuata* plants through in planta transformation.

Physiology

Characterization of starch-accumulating duckweeds, *Wolffia globosa*, as renewable carbon source for bioethanol production

Fujita, T; Nakao, E; Takeuchi, M; Tanimura, A; Ando, A; Kishino, S; Kikukawa, H; Shima, J; Ogawa, J; Shimizu, S

BIOCATALYSIS AND AGRICULTURAL BIOTECHNOLOGY 6: 123-127 (2016)

The growth and starch accumulation ability of two types of duckweeds (*Wolffia globosa*), designated as duckweeds J and B, respectively, were investigated under different nutrition conditions using HYPONeX. Both duckweeds J and B grew better in rich nutrient condition (5,000-fold diluted HYPONeX solution) than in poor nutrient condition (80,000-fold diluted HYPONeX solution). In terms of starch accumulation, duckweed J accumulated more starch in the rich nutrient condition, whereas duckweed B accumulated more starch in the poor nutrient condition. In the rich nutrient condition, the dry weight of duckweed J increased by about 5.1 folds and the accumulated starch content was about 22% (w/w) of dry duckweed after 1-week cultivation. In the poor nutrient condition, the dry weight of duckweed B increased by about 5.0 folds and the accumulated starch content was about 28% (w/w) of dry duckweed after 1-week cultivation. Furthermore, ethanol production from the duckweeds was investigated using *Saccharomyces cerevisiae* NBRC0224. The most effective pretreatment of duckweeds for ethanol production was treatment with 1% hydrogen peroxide for 1 h, followed by treatment with 1% sodium hydroxide for 1 h. In the case of the duckweed J, 69 g/L ethanol was produced from 30% (w/v) of the pretreated duckweed with 20 mM urea or 0.1% yeast extract and 30 mM ammonium sulfate. In the case of the duckweed B, 30 g/L ethanol was produced from 30% (w/v) of the non-pretreated duckweed without nitrogen source. In conclusion, the duckweeds, *W. globosa*, were found to be a promising renewable carbon source for the production of third-generation bioethanol.

Synthesis of the (9R,13R)-isomer of LDS1, a flower-inducing oxylipin isolated from *Lemna paucicostata*

Takayasu, Y; Ogura, Y; Towada, R; Kuwahara, S

BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY 80: 1459-1463 (2016)

The first synthesis of the (9R,13R)-stereoisomer of LDS1, a flower-inducing oxylipin isolated from *Lemna paucicostata*, has been achieved from a known allylic alcohol by a seven-step sequence that involves the Horner-Wadsworth-Emmons olefination to construct its full carbon framework and an enzymatic hydrolysis of a penultimate methyl ester intermediate to provide the target molecule.

Phytoremediation

Two-stage phyto-microremediation of tannery effluent by *Spirodela polyrrhiza (L.) Schleid*. and chromium resistant bacteria

Singh, A; Vyas, D; Malaviya, P

BIORESOURCE TECHNOLOGY 216: 883-893 (2016)

Two-stage sequential treatment of tannery effluent was conducted employing a wetland plant, *Spirodela polyrrhiza (L.) Schleid.*, and chromium (Cr) resistant bacterial strains. The bacterial strains were isolated from Cr-enriched environmental matrices and rhizosphere of *Spirodela polyrrhiza*. The phytorhizoremediation of tannery effluent by *Spirodela* and its rhizospheric bacteria (*Cellulomonas biazotea* APBR1-6, *Bacillus safensis* APBR2-12, *Staphylococcus warneri* APBR3-5, *Microbacterium oleivorans* APBR2-6), followed by microremediation by Cr resistant bacteria (*Micrococcus luteus* APBS5-1, *Bacillus pumilus* APBS5-2, *Bacillus flexus* APBE3-1, *Virgibacillus sediminis* APBS6-1) resulted in reduction of pollution parameters [COD (81.2%), total Cr (97.3%), Cr(VI) (99.3%), Pb(II) (97.0%), Ni (95.7%)]. The LC-MS analysis showed that many pollutants detected in untreated tannery effluent were diminished after bioremediation or long chains of alcohol polyethoxylates viz. C18EO6 in untreated effluent were broken down into smaller unit of alcohol polyethoxylate (+HHO-[-CH2-CH2-O-]-H), indicating that bacteria and *Spirodela polyrrhiza*, along with its rhizospheric associates utilized them as carbon and energy source.

Lemna minor tolerance to metal-working fluid residues: implications for rhizoremediation

Grijalbo, L; Becerril, JM; Barrutia, O; Gutierrez-Manero, J; Garcia, JAL

PLANT BIOLOGY 18: 695-702 (2016)

For the first time in the literature, duckweed (*Lemna minor*) tolerance (alone or in combination with a consortium of bacteria) to spent metal-working fluid (MWF) was assessed, together with its capacity to reduce the chemical oxygen demand (COD) of this residue. In a preliminary study, *L. minor* response to pre-treated MWF residue (ptMWF) and vacuum-distilled MWF water (MWFw) was tested. Plants were able to grow in both residues at different COD levels tested (up to 2300mgl(-1)), showing few toxicity symptoms (mainly growth inhibition). Plant response to MWFw was more regular and dose responsive than when exposed to ptMWF. Moreover, COD reduction was less significant in ptMWF. Thus, based on these preliminary results, a second study was conducted using MWFw to test the effectiveness of inoculation with a bacterial consortium isolated from a membrane bioreactor fed with the same residue. After 5 days of exposure, COD in solutions containing inoculated plants was significantly lower than in non-inoculated ones. Moreover, inoculation reduced +-tocopherol levels in MWFw-exposed plants, suggesting pollutant imposed stress was reduced. We therefore conclude from that *L. minor* is highly tolerant to spent MWF residues and that this species can be very useful, together with the appropriate bacterial consortium, in reducing COD of this residue under local legislation limits and thus minimise its potential environmental impact. Interestingly, the lipophilic antioxidant tocopherol (especially the sum of +

isomers) proved to be an effective plant biomarker of pollution.

Poultry Effluent Bio-treatment with *Spirodela intermedia* and Periphyton in Mesocosms with Water Recirculation

Basilico, G; de Cabo, L; Magdaleno, A; Faggi, A

WATER AIR AND SOIL POLLUTION 227: Article Number 190 (2016)

Industrial production of poultry meat is associated with indirect environmental impacts such as contributing to climate change and deforestation and other direct impacts such as the deterioration of the quality of surface waters. Poultry industry effluents are rich in organic matter, nitrogen, and phosphorus; nutrients can be removed from wastewater through the use of macrophytes and periphyton. An essay in mesocosms with poultry industry wastewater recirculation was developed in the presence and absence of a native macrophyte *Spirodela intermedia* and periphyton from a lowland stream (La Choza stream, Buenos Aires) where the effluent is poured. The diffusion of O-2, increased by water recirculation, had the effect of increasing the concentration of dissolved oxygen in wastewater, The presence of *S. intermedia* and algae periphyton significantly contributed to the removal rates (%) of solids (69.7 +/- 3.9), ammonium nitrogen (84.0 +/- 3.4), and total phosphorus (38.1 +/- 1.8) from residual water and favored nitrification. The dominance of *Bacillariophyceae* on other groups of algae of periphyton and the low representation of *Euglenophyceae* indicated an advanced stage of the effluent treatment process at the end of the assay.

Phytotoxicity

Arsenic toxicity in the water weed *Wolffia arrhiza* measured using Pulse Amplitude Modulation Fluorometry (PAM) measurements of photosynthesis

Ritchie, RJ; Mekjinda, N

ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 132: 178-185 (2016)

Accumulation of arsenic in plants is a serious South-east Asian environmental problem. Photosynthesis in the small aquatic angiosperm *Wolffia arrhiza* is very sensitive to arsenic toxicity, particularly in water below pH 7 where arsenite (As (OH)(3)) (AsIII) is the dominant form; at pH > 7 AsO42- (As (V) predominates). A blue-diode PAM (Pulse Amplitude Fluorometer) machine was used to monitor photosynthesis in *Wolffia*. Maximum gross photosynthesis (Pg(max)) and not maximum yield (Y-max) is the most reliable indicator of arsenic toxicity. The toxicity of arsenite As(III) and arsenate (H2AsO42-) As (V) vary with pH. As(V) was less toxic than As(III) at both pH 5 and pH 8 but both forms of arsenic were toxic (> 90% inhibition) at below 0.1 mol m(-3) when incubated in arsenic for 24 h. Arsenite toxicity was apparent after 1 h based on Pg(max), and gradually increased over 7 h but there was no apparent effect on Ymax or photosynthetic efficiency (alpha(0)).

Toxicity assessment of boron (B) by *Lemna minor L.* and *Lemna gibba L.* and their possible use as model plants for ecological risk assessment of aquatic ecosystems with boron pollution

Gur, N; Turker, OC; Bocuk, H

CHEMOSPHERE 157: 1-9 (2016)

As many of the metalloid-based pollutants, the boron (B) toxicity issues have aroused more and more global attentions, especially concerning drinking water sources which flow through boron-rich areas. Therefore, feasible and innovative approaches are required in order to assess B toxicity in aquatic ecosystems. In this study, the toxic effects of B on *Lemna minor L* and *Lemna gibba L* were investigated using various endpoints including number of fronds, growth rates, dry biomass and antioxidants enzymatic activities. *Lemna* species were exposed to B concentrations of 2 (control), 4, 8, 16, 32, 64 and 128 mg L-1 for a test period of 7 days. The results demonstrated that plant growth was significantly reduced when the B concentration reached 16 mg L-1. Furthermore, our results also concluded that among the anti oxidative enzymes, SOD, APX and GPX can serve as important biomarkers for B-rich environment. The present results suggested that *L. minor* and *L. gibba* are very useful model plants for phytoremediation of low-B contaminated wastewater and they are also suitable options for B biomonitoring due to high phototoxic sensitivity against B. In this respect, the scientific insight of the present study is to fill the gaps in the research about the use of *L. minor* and *L. gibba* in ecotoxicological research associated with B toxicity.

Molecular distribution and toxicity assessment of praseodymium by *Spirodela polyrrhiza*

Xu, T; Su, CL; Hu, D; Li, FF; Lu, QQ; Zhang, TT; Xu, QS

JOURNAL OF HAZARDOUS MATERIALS 312: 132-140 (2016)

Aquatic macrophytes are known to accumulate and bioconcentrate metals. In this study, the physiological, biochemical, and ultrastructural responses of *Spirodela polyrrhiza* to elevated concentrations of praseodymium (Pr), ranging from 0 to 60 mu M, were investigated over 20 d exposure. The results showed that the accumulation of Pr in *S. polyrrhiza* occurred in a concentration-dependent manner. The accumulation of Pr in biomacromolecules decreased in the order of cellulose and pectin (65-69%), crude proteins (18-25%), crude polysaccharides (6-10%), crude lipids (3%-4%). Significant increases in malondialdehyde (MDA), and decreases in photosynthetic pigment, soluble protein, and unsaturated fatty acids showed that Pr induced oxidative stress. Inhibitory effects on photosystem 11 and the degradation of the reaction center proteins D1 and D2 were revealed by chlorophyll a fluorescence transients, immunoblotting, and damage to chloroplast ultrastructure. Significant increases in cell death were observed in Pr-treated plants. However, *S. polyrrhiza* can combat Pr induced oxidative injury by activating various enzymatic and non-enzymatic antioxidants. These results will improve understanding of the biological consequences of rare earth elements (REEs) contamination, particularly in aquatic bodies.

The different response mechanisms of *Wolffia globosa*: Light-induced silver nanoparticle toxicity

Zou, XY; Li, PH; Huang, Q; Zhang, HW

AQUATIC TOXICOLOGY 176: 97-105 (2016)

Silver nanoparticles (AgNPs) have emerged as a promising bactericide. Plants are a major point of entry of contaminants into trophic chains. Here, the physiological responses of *Wolffia globosa* to AgNPs have been probed using different light schemes, and these data may reveal new insights into the toxic mechanism of AgNPs. *W. globosa* was grown in culture medium and treated with different concentrations of AgNPs for 24 h under pre- and post-illuminated conditions. However, fluorescence quenching, the accumulation of sugar and the reduction of Hill reaction activity were found in response to the AgNP-stresses. In the pre-illuminated condition, oxidative damage was obvious, as indicated by the higher malondialdehyde (MDA) content and an up-regulation of superoxide dismutase (SOD) activity. The maximum increases of MDA content and SOD activity were 1.14 and 2.52 times the respective controls when exposed to 10 mg/L AgNPs. In contrast, in the post-illuminated condition, the alterations in photosynthetic pigment and soluble proteins content were more significant than the alterations in oxidative stress. The contents of chlorophyll a, carotenoids and soluble protein decreased to 77.7%, 66.2% and 72.9% of the controls after treatment with the highest concentration of AgNPs (10 mg/L). Based on the different physiological responses, we speculated that in the pre-illuminated condition, oxidative stress was responsible for the decline in the oxygen evolution rate, while in the post-illuminated condition, the decrease in the Hill reaction activity could be attributed to the blocking of electron transfer and an insufficient proton supply. Our findings demonstrate that environmental factors regulate the physiological responses of plants to AgNPs through distinct mechanisms.

The first toxicological study of the antiozonant and research tool ethylene diurea (EDU) using a *Lemna minor L.* bioassay: Hints to its mode of action

Agathokleous, E; Mouzaki-Paxinou, AC; Saitanis, CJ; Paoletti, E; Manning, WJ

ENVIRONMENTAL POLLUTION 213: 996-1006 (2016)

The antiozonant and research tool ethylene diurea (EDU) is widely studied as a phytoprotectant against the widespread pollutant ground-surface ozone. Although it has been extensively used, its potential toxicity in the absence of ozone is unknown and its mode of action is unclear. The purpose of this research was to toxicologically assess EDU and to further investigate its mode of action using L. as a model organism. Application of EDU concentrations greater than 593 mg L-1 (practically 600 mg L-1) resulted in adverse inhibition of colony growth. As no-observed-toxic-effects concentration (NOEL) we recommend a concentration of 296 mg L-1 (practically 300 mg L-1). A hormetic response was detected, i.e. stimulatory effects of low EDU concentrations, which may indicate overcompensation in response to disruption in homeostasis. Growth inhibition and suppressed biomass were associated with impacted chlorophyll a fluorescence (Phi(PSII) q(P) and ETR). Furthermore, EDU increased mesophyll thickness, as indicated by frond succulence index. Applications of concentrations >= 593 mg L-1 to uncontrolled environments should be avoided due to potential toxicity to sensitive organisms and the environment.

Links for Further Reading

http://www.ruduckweed.org/ Rutgers Duckweed Stock Cooperative, New Brunswick, New Jersey State University. Prof. Dr. Eric Lam

http://www.InternationalLemnaAssociation.org/ Working to develop commercial applications for duckweed globally, Exec. Director, Tamra Fakhoorian

http://www.mobot.org/jwcross/duckweed/duckweed.htm Comprehensive site on all things duckweed-related, By Dr. John Cross.

http://plants.ifas.ufl.edu/ University of Florida's Center for Aquatic & Invasive Plants.

http://www.Lemnapedia.org Online developing compendium of duckweed research & applications, founded by the ISCDRA.

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