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|  | Contract/COMMISSIONED research agreement |

Between

Chr. Hansen A/S

Bøge Alle 10-12

DK-2970 Hørsholm

Denmark

(“Chr. Hansen”)

and

**Global Change Research Institute CAS** (CzechGlobe)  
Bělidla 986/4a

603 00 Brno

Czech Republic

represented by prof. RNDr. Ing. Michal V. Marek, DrSc. dr. h. c., director

(“Researcher")

(individually the "Party" and collectively the "Parties")

###### Purpose and background

Chr. Hansen has extensive global activities within research, production and marketing in the field of microbial product and product development for agriculture and possesses extensive know-how and other intellectual property rights in relation hereto.

Researcher has special interest and expertise in the field of Adaptive Biotechnologies and notably the cultivation of higher plant suspension cultures, the use of noninvasive fluorescence detection techniques and other analytical techniques to determine responses of plant cells to external stimuli.

Chr. Hansen wishes to purchase Researchers services within the Field by conducting the Project and Researcher wishes to provide such services based on the mutual interest to obtain a proof of concept in a pre-project for the usefulness of plant suspension cultures as fast and simple test system for beneficial microbes as basis for a possible future extended collaboration within this Field.

###### definitions

In the Agreement, the following words and expressions have the meanings stated below, unless the context requires otherwise.

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| “Affiliate” | any legal person that, directly or indirectly, controls, is controlled by or is under common control with a Party, where control means ownership of more than 50% of the shares in another legal person; (ii) ownership of more than 50% of the voting rights in another legal person; or (iii) the right to appoint or dismiss more than 50% of the members of the supreme management body of another legal person. |
| “Agreement” | this agreement with exhibits as amended from time to time. |
| “Background IPR” | all IPR which is (i) owned or controlled (whether by license or otherwise) by a Party as of the Effective Date; or (ii) independently developed by or on behalf of a Party (or such Party’s Affiliates) outside the Project during or after the Term; or (iii) licensed or acquired from a third party by a Party (or such Party’s Affiliates) during or after the Term. |
| “Budget” | the budget set out in **Exhibit 1**. |
| “Confidential Information” | any and all information disclosed directly or indirectly to the receiving Party by the disclosing Party, whether disclosed orally or in written, graphic or electronic form under this Agreement. Confidential Information shall also mean any and all technical or non-technical information obtained in any form by the receiving Party during observation or examination of confidential information provided by the disclosing Party which may include, but is not limited to, technical processes, specifications, instrumentation, formulae, assays, manufacturing techniques, sales and marketing information, Material, raw data, or research and development activities, including but not limited to information generated in the course of the Project. |
| “Effective Date” | the date of last signature of this Agreement. |
| “Field” | the field described in **Exhibit 2** and covering the Project. |
| “Force Majeure” | any event that either Party has not reasonably been able to predict, control, avoid or remedy, including – depending on the circumstances – defects or breakdowns of telecommunications networks or lines, server or computer breakdowns (e.g. due to virus or hacker attacks), disruptions of electricity supply, public authority prohibition or enforcement notices, strikes and lockouts, acts of terrorism, wars, civil wars, riots, natural disasters, nuclear accidents, epidemics, pandemic diseases, fires, floods, storms, sabotage, criminal damage and similar events. |
| “Material” | any biological, chemical or other materials, including progeny and unmodified derivatives from said material, described in the Project Plan, or such other compounds and/or tangible material delivered by a Party to the Project. |
| “Project” | the development work and other work subject to this Agreement to be performed by the Researcher in accordance with the Project Plan. |
| “Project Directors” | the persons appointed by the Parties as project directors and listed in **Exhibit 3**. |
| “Project Plan” | the project plan attached as **Exhibit 4**. |
| “Term” | the period from the Effective Date to the expiry or termination of the Agreement. |

###### Research work

Researcher shall provide the services described in the Project Plan.

Researcher shall commence the performance of the Project promptly after the Effective Date and shall perform the services fully in accordance with the terms and conditions of this Agreement including the Project Plan. The Parties may at any time amend the Project Plan by mutual written agreement.

Researcher shall carry out the Project in accordance with good scientific practice, using the knowledge available at the Researcher and the facilities at the Researcher’s disposal.

Researcher shall comply with the time schedule for the execution and delivery of the work indicated in the Project Plan.

Researcher’s performance is completed when Chr. Hansen has received and approved the agreed final performance. The final performance shall be considered approved if Chr. Hansen has not indicated otherwise to the Researcher no later than thirty (30) days from receipt of the final performance.

###### project Management

Each Party shall appoint a Project Director.

The Project Directors are responsible to oversee and secure the performance of the services stipulated in the Project Plan.

The Project Directors may select and supervise other project staff as needed.

###### Independent contractor

In the performance of all services according to the Project Plan:

1. Researcher shall be deemed to be and shall be an independent contractor and, as such, Researcher shall not be entitled to any benefits applicable to employees of Chr. Hansen.
2. Neither Party is authorised or empowered to act as an agent for the other for any purpose and shall not on behalf of the other enter into any contract, warranty, or representation as to any matter. Neither shall be bound by the acts or conduct of the other.

###### Material Transfer

Chr. Hansen will deliver any Material agreed in the Project Plan at the agreed time and place.

Material delivered by Chr. Hansen to Researcher shall remain the sole property of Chr. Hansen.

Researcher undertakes not to divulge, give, or otherwise transfer to any third party any of the Material received from Chr. Hansen, as well as any products derived from Material, information emanating from Material or its products, and/or any and all Project related results, without Chr. Hansen's prior written approval.

Material provided under this Agreement by Chr. Hansen shall not be analyzed, decompiled, reverse engineered, modified in any way by e.g. genetic engineering, mutagenesis, transformation, conjugation or transduction or used except to the extent necessary to carry out the Project.

Material is provided to Researcher without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. Researcher agrees to hold harmless and indemnify Chr. Hansen for any claim, including, but not limited to, Chr. Hansen's attorneys' fees, arising from Researcher's use of Material.

At Chr. Hansen sole discretion, Recipient shall destroy or return to Chr. Hansen all Material and all products derived from Material at the termination of Project or this Agreement. Researcher shall not later than one (1) month after the termination of Project send a written confirmation that the above obligation has been fulfilled.

###### Reporting

Written reports shall be provided by Researcher to Chr. Hansen for each independent analysis as detailed in the Project Plan.

During the term of this Agreement, representatives of Researcher will meet or arrange for conference or video calls when deemed required with representatives of Chr. Hansen at times and places mutually agreed upon to discuss the results of the analysis, ongoing plans, action plans for follow-up experiments or changes therein.

###### Payment

The total costs to be paid by Chr. Hansen for the work undertaken by Researcher shall not exceed € 27.707. The fee is based on the Budget prepared by the Researcher and approved by Chr. Hansen.

If the Budget cannot be met and this is not due to errors or omissions by the Researcher, the Parties shall jointly reassess the Project and the costs required to finalise the Project. Chr. Hansen will subsequently decide whether the work shall be carried out at a higher price or be terminated as is.

Payment shall be made by Chr. Hansen into the account designated by Researcher, and in accordance with the payment schedule in clause 8.4.

The Researcher will invoice Chr. Hansen for completed work. 80 % of the Fee (€ 22.165,60) will be paid at Project start and 20 % (€ 5.541,40) will be paid at Project end as set out in Clause 3.4. The amount will be paid by Chr. Hansen no later than thirty (30) days from the date of the invoice. Invoices shall be sent by email to dklmb@Chr-Hansen.com and kreditor@Chr-Hansen.com and be marked for the attention of Lars Moelbak, Plant Health, Chr. Hansen A/S, Tax/VAT Nº DK12516479.

In the event of early termination of this Agreement by Chr. Hansen pursuant to clause 13, Chr. Hansen shall pay all costs accrued by Researcher as of the effective date of termination, including non-cancellable obligations incurred prior to said date of termination.

###### intellectual property rights

Background IPR and Material

Each Party will retain the rights to its Background IPR and Material. Neither Party will acquire rights to the other Party's Background IPR or Material by way of license or otherwise, except as required to fulfil its obligations under this Agreement.

Foreground IPR

Researcher shall promptly inform Chr. Hansen of any generated Foreground IPR.

Researcher is obliged to take the necessary steps to acquire all Foreground IPR from its employees. If costs are incurred to obtain such rights, Researcher shall bear costs related to its employees.

Researcher shall own any Foreground IPR which is solely related to Researcher’s Background IPR, i.e. the technology owned and used by Researcher to perform the Project.

Chr. Hansen shall own the final report and raw data delivered to Chr. Hansen and any Foreground IPR which is solely related to Chr. Hansen’s Background IPR and Material.

The Parties shall jointly own in equal shares any Foreground IPR within Field which is not governed by Clauses 9.2.3 and 9.2.4.

Any disposal and use, including patenting, of Foreground IPR jointly owned which has not been acquired or licensed by Chr. Hansen, requires agreement between the Parties.

Licenses and transfer of ownership

Provided and to the extent a Party is legally entitled to do so, each Party shall grant to the other Party a royalty-free, non-exclusive license to use its Background IPR and Foreground IPR for the sole purpose of carrying out the Project. Such license will terminate without further notice upon the termination or expiry of this Agreement.

Chr. Hansen is granted an option to acquire or obtain an exclusive or non-exclusive, royalty free, sub-licensable, perpetual and worldwide license to any of the Researcher’s Foreground IPR, including the Researcher’s shares in jointly owned Foreground IPR, to develop, manufacture, have manufactured, use and sell products under the licensed Foreground IPR within microbial product and product development for agriculture. Chr. Hansen shall exercise its option to purchase or license by written notice to the Researcher within sixty (60) days from they have received information of the generated Foreground IPR.

###### PATENTING AND PROSECUTION OF FOREGROUND IPR

The right to take out patents based on Foreground IPR and to prosecute Foreground IPR accrues to the Party who owns the relevant Foreground IPR.

In the event that jointly-owned Foreground IPR can form the basis for a patent, the right to take out such patent shall accrue to the Parties jointly, unless Chr. Hansen has exercised its option in Clause 9.3.2 to purchase the Foreground IPR or the Parties have otherwise agreed in writing. The Parties shall enter into a patent co-ownership agreement regarding the protection, prosecution, maintenance and commercial use of jointly-owned Foreground IPR.

Provided that one of the Parties do not wish to patent jointly-owned Foreground IPR, the other Party may unilaterally decide to seek patent protection thereof in the name of that Party alone and that Party shall be entitled to defend, prosecute and maintain the patent without further notice to the other Party. Any damages or other compensation obtained by the Party from a third party infringer etc. shall belong to that Party.

All expenses related to patent applications and patents are held by the Party filing the patent application. This includes all direct and indirect expenses related to the drafting, filing and the maintenance of the patent application and the subsequent patent(s), unless otherwise agreed by the Parties.

Where a Party is pursuing patent protection for jointly-owned Foreground IPR on behalf of both Parties the following applies:

1. The Party seeking patent protection will include specific claims and wording in the patent application at the request of the other Party as far as such claims and wording are not contradictory to the claims and wording to be included in the application by the Party seeking patent protection.

Where a Party is pursuing patent protection for jointly-owned Foreground IPR without the participation of the other Party the following applies:

1. The Party pursuing patent protection can at any time with two (2) months written notice to the other Party decide not to continue the financing of a patent or patent application, in which case the other Party can decide to continue the patent application or patent compensating the Party that had pursued patent protection for the expenses already held. The conditions for the transfer of the patent application or patent are to be agreed upon between the Parties.

###### publication

Each Party shall be entitled to publish own Foreground IPR, except that Researcher will not publish any results included in the final report or raw data delivered to Chr. Hansen. Any publication of jointly owned Foreground IPR requires prior agreement by the Parties.

The Party who wishes to publish its Foreground IPR shall notify the other Party at least sixty (60) days prior to the intended time of publication and forward the text and any additional material the Party wishes to publish for the other Party’s review. Until forty-five (45) days after receipt of the notice with the intended publication, the receiving Party can request that the publication be postponed by up to four (4) months from the date of the request to postpone the publication is made provided that the postponement is important for the Party’s prospects of acquiring intellectual property rights protection of the Foreground IPR.

Publication shall always take place with due respect for the duty of confidentiality described in Clause 12 and a Party is entitled to demand that Confidential Information of the Party is removed from any publication. Such demand shall be made within the sixty (60) day deadline mentioned in clause 11.2.

Chr. Hansen will not use the name of Researcher, nor of any member of Researcher's project staff, in any publication, advertising or press release without the prior written approval of an authorised representative of Researcher.

Researcher will not use the name of Chr. Hansen or any employee of Chr. Hansen in any publication without the prior written approval of Chr. Hansen.

###### Confidentiality

Researcher undertakes from the date of disclosure during the term of this Agreement and for a period of ten (10) years after expiry or termination, for whatever reason, of this Agreement to treat all received Confidential Information as strictly confidential and therefore not to disclose it to any third party and to make no commercial use of it without the express written consent of Chr. Hansen.

The obligations set forth in clause 12.1 do not apply to Confidential Information which:

1. at the time of disclosure is already in the public domain;
2. after disclosure, becomes part of the public domain through no violation of this Agreement;
3. Researcher is able to prove to have been in possession of prior to disclosure by Chr. Hansen. In this case, Researcher will, in writing and within forty-five (45) days from date of disclosure, demonstrate to the satisfaction of Chr. Hansen that it was in possession of such Confidential Information;
4. is hereafter lawfully disclosed by a third party to Researcher, which Confidential Information such third party did not acquire under a still effective obligation of confidentiality to Chr. Hansen; and
5. is disclosed to the extent required by law or regulation provided that Researcher gives Chr. Hansen prompt written notice and sufficient opportunity to object, time permitting, to such disclosure.

Researcher may disclose Confidential Information only to reliable employees who need to know in order to carry out the obligations under this Agreement and provided that such persons are bound by obligations of confidentiality and non-use to Researcher which are equal to the terms of this Agreement. Researcher shall ensure that such employee(s) be fully aware of the obligations of this Agreement and shall be responsible for any breach of these provisions by its employees.

Researcher shall keep Chr. Hansen fully and effectively indemnified against any and all losses, expenses, and damages suffered by Chr. Hansen arising from any unauthorised disclosure or use of any part of Confidential Information by Researcher or Researcher’s employees, including, but not limited to, reasonable attorney’s fees and costs.

###### Term and termination

The Agreement shall be effective as of the Effective Date and expires on the date the Project has been completed in accordance with the Project Plan unless sooner terminated in accordance with the provisions of this clause 13.

Chr. Hansen is entitled to terminate the Agreement at any time with one (1) month’s written notice against payment of the full fee indicated in the Budget less any savings made by Researcher as a result of the termination.

Termination of this Agreement by either Party for any reason shall not affect the rights and obligations of the Parties accrued prior to the effective date of termination of this Agreement. No termination of this Agreement, however effectuated, shall release the Parties from their rights and obligations under clause 5-7, 9-16 and 18.

###### Breach

A Party shall be entitled to terminate the Agreement, if the other Party commits a serious breach or repeatedly breaches its obligations under this Agreement and the conduct that constitutes the breach has not come to an end within thirty (30) days from a request by the other Party to do so.

If a Party is prevented from fulfilling its obligations under the Agreement as a result of Force Majure, this shall not be considered a breach. However, the other Party shall be entitled to terminate the Agreement if the Party’s failure to fulfil its obligations results in a material delay in the completion of the Project. A delay of more than three (3) months compared with the time schedule agreed in the Project Plan shall always be deemed material.

Unreasonable delay of the Project ascribable to one of the Parties shall constitute default in the terms and conditions of this Agreement and the Party not in default may terminate this Agreement in accordance with clause 14.1.

If Chr. Hansen terminates the Agreement, Chr. Hansen shall be entitled to claim compensation for the loss caused by the breach in accordance with clause 15.1.

###### Liability

Researcher shall be liable for defects in its execution of the Project and a delay in delivering the agreed performance provided the defect or delay is a result of a negligent or intentional act or omission on the part of the Researcher.

Researcher shall also be liable for the wrongful acts or omissions of its employees in accordance with applicable law.

Researcher is not liable for a failure to comply with its obligations under the Agreement if the failure to perform is due to force majeure as set out in clause 14.2.

###### Insurance

Researcher warrants and represents that Researcher has adequate liability insurance, such protection being applicable to officers, employees, and agents while acting within the scope of their employment by Researcher, and Researcher has no liability insurance policy as such that can extend protection to any other person.

###### Assignment

Subject to clause 17.2 this Agreement shall not be assigned by either Party without the prior written consent of the other Party.

Chr. Hansen may assign this Agreement without consent in connection with the transfer or sale of all or substantially all of its assets or business, its merger or consolidation with another company.

###### Notices

Any notice, report, request, approval, consent or other communication required or permitted to be given under this Agreement shall be in writing and shall for all purposes be deemed to be fully given and received if delivered in person or sent by registered mail or e-mail (with an appropriate transmission receipt) to the respective Parties at the following addresses:

If to Chr. Hansen: Chr. Hansen A/S

Bøge Alle 10-12

DK-2970 Hørsholm

Denmark

Attn: Lars Moelbak

E-mail: dklmb@chr-hansen.com

If to Researcher: **Global Change Research Institute CAS**

CzechGlobe

Bělidla 986/4a

603 00 Brno

Czech Republic

Attn: Jan Červený

E-mail: [cerveny.j@czechglobe.cz](mailto:cerveny.j@czechglobe.cz)

Either Party may change its forwarding address for the purpose of this Agreement by giving the other Party written notice of its new address.

###### Governing law and venue

This Agreement is governed by and will be interpreted in accordance with Danish law. However, without recourse to conflict of laws rules.

The Parties must seek to settle amicably any dispute arising out of the Agreement, including any dispute concerning the existence or validity of the Agreement.

Any dispute arising out of the Agreement, including any dispute concerning the existence or validity of the Agreement, that cannot be settled amicably between the Parties will be decided by the competent Danish court.

Each of the Parties are entitled to request that disputes related to intellectual property rights or Confidential Information is finally settled by arbitration in accordance with the procedural rules of the Danish Institute of Arbitration. The Danish Institute of Arbitration will apply the rules in force when the application for arbitration is submitted.

Each Party will appoint one (1) arbitrator. The Danish Institute of Arbitration will appoint one (1) additional arbitrator who will be chairman of the arbitration tribunal. If either Party fails to appoint an arbitrator no later than thirty (30) days after submitting an application for arbitration or receiving notice of arbitration, the Danish Institute of Arbitration will also appoint that arbitrator.

The arbitration proceedings will take place in Copenhagen, and the language of the proceedings will be English.

Neither Party is entitled to disclose confidential information about the arbitration proceedings to others, including information about any decision or award made by the Danish Institute of Arbitration unless the other Party has consented to any such disclosure of information in writing. Either Party is entitled, however, to disclose information about the arbitration proceedings to others if such disclosure is made to protect the Party's interests against the other Party in the best possible manner, to comply with current legislation or public authority decisions or is required by stock exchange listing agreements.

The arbitration provisions in clause 19.4 - 19.7 do not prevent a Party from making use of any interim remedies (preliminary injunction etc.).

The parties are aware that this contract meets the requirements specified in Act No 340/2015 Coll. and therefore it is subject to the obligation to be published in the register of contracts. The Researcher undertakes to enter the contract in the register of contracts within the statutory period and send a confirmation that the contract was published to the Chr. Hansen upon its request.

###### Signatures

This Agreement has been executed in two (2) originals, each Party receiving one (1) copy.

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| **Chr. Hansen A/S**  Signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  Title: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |  | **Chr. Hansen A/S**  Signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  Title: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |
| **CzechGlobe representative**  Signature: :\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  Name: prof. RNDr. Ing. Michal V. Marek, DrSc. dr. h. c.  Title: Director  Date: :\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |  | **CzechGlobe responsible researcher**  Signature: :\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  Name: Ing. Jan Červený, Ph.D.  Title: Head of Department  Date: :\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |

**Exhibit 1 – Budget**

**(III) Budget:**

(1) CzechGlobe

Technician, 3 month: 4.800 EUR

Postdoc, 1 month: 1.970 EUR

Senior staff: 0,5 month 1.170 EUR

Consumables: 2.000 EUR

Sum 9.940 EUR

Overhead 37% 3.678 EUR

Total Sum CzechGlobe 13.618 EUR

(2) PSI

Rental of MultiColor FluorCam 7.089EUR

(3) Transcriptome Analyses including bioinformatics at Novogene

27 samples, ca. 6.665 EUR

**Total project sum 27.707 EUR (207.300 DKK)**

**Exhibit 2 – Field**

**Response of plant suspension cultures to bacteria**

**Exhibit 3 – Project Directors**

Chr. Hansen:

|  |  |  |
| --- | --- | --- |
| **Name** | **Title** | **Contact Details** |
| Lars Mølbak | Head of PH Innovation | Phone: +4553390373  Email: [dklmb@chr-hansen.com](mailto:dklmb@chr-hansen.com) |
| Jacob Baelum | Project Manager | Phone: +4526819847  Email: [DKJABM@chr-hansen.com](mailto:DKJABM@chr-hansen.com) |

Researcher:

|  |  |  |
| --- | --- | --- |
| **Name** | **Title** | **Contact Details** |
| Jan Červený | Ing., PhD; Head of Department of Adaptive Biotechnologies | Phone: +420 775 171 968  Email: [cerveny.j@czechglobe.cz](mailto:cerveny.j@czechglobe.cz) |
| Thomas Roitsch | Prof. Dr.; Head of Research Group: Molecular Physiology & Phenomics | Phone: +45-35331526  Email: [Roitsch@plen.ku.dk](mailto:Roitsch@plen.ku.dk) |

###### Exhibit 4 – Project PLAN

**High-throughput screening of response of plant suspension cultures to plant beneficial microbes by fluorescence imaging**

**Proof of concept pre-project**

**to assess the responsiveness of plant suspension cultures to plant beneficial microbes**

**(I) Background**

In plant suspension cultures plant cells grow as individual cells or as small clusters in liquid medium. Photoautotrophic cultures have the special feature of higher plant source tissues and heterotrophic cultures have the special feature of higher plant sink tissues. They are an experimental system that combines the easy handling and possibility for high-throughput screening of bacterial or yeast cultures with the analyses of true higher plant responses (e.g. reviews from my lab: Segecova et al., 2017; Sinha and Roitsch, 2000).

Plant suspension cultures have been successfully used to study various plant responses to diverse stimuli and identify novel regulatory or signal transduction mechanisms. The lab of Thomas Roitsch has in particular pioneered the application of photoautotrophic cultures and has unique experience since 1990 and has established some unique cultures. Notably this lab has been able to identify novel regulatory mechanisms with suspension cultures that lead to new discoveries with real plants. E.g. (i) on the coordinated regulation of source-sink relation and defense responses (Ehneß et al. (1997) Glucose and stress independently regulate source/sink relations and defense mechanisms via signal transduction pathways involving protein phosphorylation. Plant Cell 9: 1825-1841), (ii) metabolic signaling (Sinha et al. (2002) Metabolizable and non-metabolizable sugars activates different signal transduction pathways in tomato. Plant Physiol.128, 1480-1489 with many more published examples. A proof of concept has been also obtained in a PhD thesis to use photoautotrophic cultures to establish a high-throughput screening platform for environmental toxicants in microtiter plates using fluorescence imaging (Segečová et al., (2017) Stress Response Monitoring of Photoautotrophic Higher Plant Suspension Cultures by Fluorescence Imaging for High-Throughput Toxic Compound Screening. Journal of Environmental Protection 8: 678-692).

**(II) Concept:**

(1) PAM Chlorophyll-Fluorescence (Chl-F) and Multicolor fluorescence(MCF) imaging could be used to assess basic responses of plant primary and secondary metabolism.

(2) Fluorescence labelled reporter dyes could be used to assess specific plant responses such as changes in pH, redox status and accumulation of reactive oxygen species (ROS).

(3) Generic response pathways such as phytohormone signaling could be assessed using transgenic lines expressing corresponding promoter-fluorescence reporter gene constructs.

(4) Based on the identification of potential plant marker genes within other projects the promoters of the corresponding marker genes could be used to engineer fusions with the coding sequence of genes encoding auto-fluorescence reporter proteins. The suspension cultures could then be transformed either with single constructs or super-transformed with multiple, staked constructs. E.g. a transformation with 4 constructs linked to different fluorescence reporter proteins with different fluorescence emission colors could yield a fluorescence signature.

(4) The approaches (1) through (4) could be combined to yield even more complex and possibly more specific signatures. This approach could be also combined with the determination of additional physiological and/or molecular biomarkers.

The various suspension cultures used in the lab of Thomas Roitsch are currently cultivated, within a collaboration, at the CzechGlobe institute in Brno, a national Research Institute of the Czech Academy of Sciences.

**(III) Experimental and analytical approaches**

1. Tomato culture, autotroph

1.1 Analyses by fluorescence-imaging

* Untargeted, non-invasive analyses of responses of primary and secondary metabolism
* Treatments: Mock, LS1, LS1-boiled, LS-2, LS-2-boiled, LS-3, LS-3-boiled
* Preliminary dose response: 12h
* Analyses time course: 90min, 3h, 24h, 48h, 72h

1.1.1 Measurements: Chl-F- and MCF-imaging

Biological replicates: 3

2. Chenopodium rubrum culture, autotroph

2.1 Analyses by fluorescence-imaging

* Untargeted, non-invasive analyses of responses of primary and secondary metabolism
* Treatments: Mock, LS1, LS1-boiled, LS-2, LS-2-boiled, LS-3, LS-3-boiled
* Preliminary dose response: 12h
* Analyses time course: 90min, 3h, 24h, 48h, 72h

2.1.1 Measurements: Chl-F- and MCF-imaging

Biological replicates: 3

3.Arabidopsis thaliana culture, mixotroph

3.1 Analyses by fluorescence-imaging

* Untargeted, non-invasive analyses of responses of primary and secondary metabolism
* Treatments: Mock, LS1, LS1-boiled, LS-2, LS-2-boiled, LS-3, LS-3-boiled
* Preliminary dose response: 12h
* Analyses time course: 90min, 3h, 24h, 48h, 72h

3.1.1 Measurements: Chl-F- and MCF-imaging

Biological replicates: 3

4. Arabidopsis thaliana culture, autotroph

4.1 Analyses by fluorescence-imaging

* Untargeted, non-invasive analyses of responses of primary and secondary metabolism
* Treatments: Mock, LS1, LS1-boiled, LS-2, LS-2-boiled, LS-3, LS-3-boiled
* Dose response: 12h
* Analyses time course: 90min, 3h, 24h, 48h, 72h

4.1.1 Measurements: Chl-F- and MCF-imaging

Biological replicates: 3

4.2 Determination of enzyme activity signatures

* Targeted analyses of responses at the level of activities of key enzymes of carbohydrate and antioxidant metabolism
* Treatments: Mock, LS1, LS1-boiled, LS-2, LS-2-boiled, LS-3, LS-3-boiled
* Analyses time course: 0, 6h, 24h

4.2.1 Measurements: selected carbohydrate and antioxidant enzymes

Biological replicates: 3

Number of samples: 3 + (3 x 2 x 7) = 45

4.3 Non-targeted metabolom analyses

* Untargeted analyses of responses at the level of metabolites
* Treatments: Mock, LS1, LS1-boiled, LS-2, LS-2-boiled, LS-3, LS-3-boiled
* Analyses time course: 0, 6h, 24h

4.3 Extraction

4.3.2 Instrumental analyses by cooperation partner at CzechGlobe

4.3.3 Determination of differential peak profiles at CzechGlobe

Biological replicates: 3

Number of samples: 3 + (3 x 2 x 7) = 45

4.4 Non-targeted expression analyses

* Untargeted analyses of responses at the level of gene expression
* Identification of responsive promoters as basis to engineer promoter – fluorescent reporter gene fusions for the future transformation of plant suspension cultures as reporter lines
* Treatments: Mock, LS1, LS1-boiled,
* Analyses time course: 0, 6h, 24h

4.3.1 RNA isolation

5.4.3 RNAseq by company

5.4.4 Bioinformatic analyses of differentially expressed genes by cooperation partner

Biological replicates: 3

Number of samples: 3 + (3 x 2 x 3) = 21

**(II) Time line**

28.08.2019 – 31.03.2020