

CASS III Program Delivery and Management

Phase II/Periode 5 - Project Meeting 2024

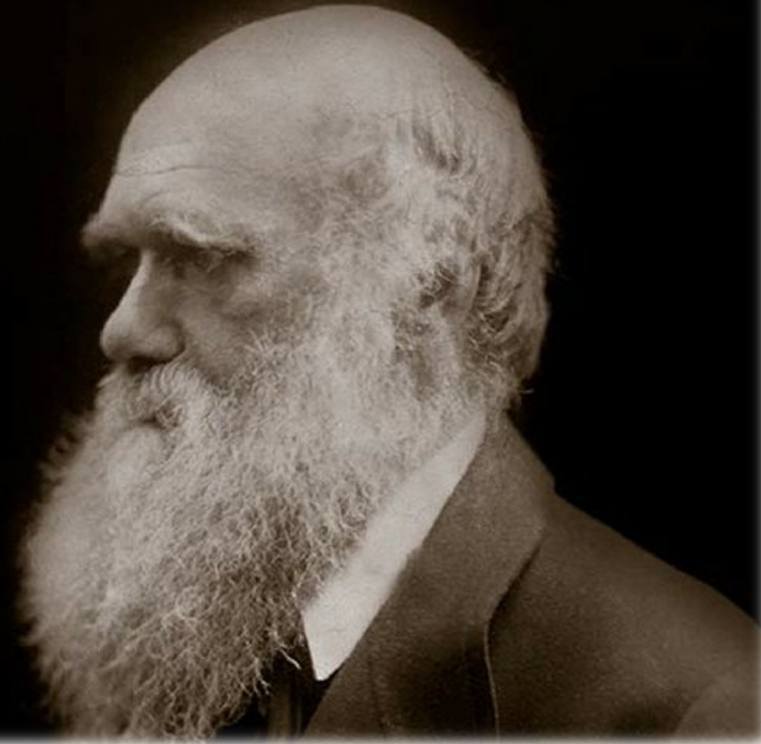
Zürich, 9th April 2024

with contributions from Catherine Espinosa (AgOne),
Christian Rogers (ENSA), and Wolfgang Zierer (CASS)

CASS III – Changes are coming

"It is not the
strongest species
that survives, nor the
most intelligent, but
the most responsive
to change."

Charles Darwin



Cathy working on CASS video

2024-04-04 22:12 UTC

Recorded by

Catherine Espinoza

Organized by

Catherine Espinoza

CASS III Program Structure

TECHNICAL MODULES

TM1-6

MANAGEMENT
CONSTRUCTS
TRANSFORMATION
FIELD TESTING
PHENOTYPING
DATABASE

ENGINEERING MODULES

EM1

OPTIMIZING
ASSIMILATE
ALLOCATION TO
STORAGE ROOTS

EM2

INCREASING SINK
STRENGTH
BY OPTIMIZING
XYLEM
PARENCHYMA CELL
FORMATION IN
STORAGE ROOTS

EM3

INCREASING SINK
STRENGTH
BY OPTIMIZING
METABOLIC FLUX IN
STORAGE ROOTS

CASS III Team Contributions

EM1_OPTIMIZING ASSIMILATE ALLOCATION TO STORAGE ROOTS

EM1.1

Manipulating shoot architecture

Lead:

Uwe Sonnewald
(FAU)

EM1.2

Limiting lateral transport losses through active phloem (re-)loading

Lead:

Ekkehard Neuhaus
(RPTU)

EM1.3

Transport and allocation modelling

Lead:

Lee Sweetlove
(OU)

EM2_INCREASING SINK STRENGTH: XYLEM PARENCHYMA

EM2.1

Manipulation of cytokinin/thermospermine metabolism or signaling

Lead:

Yjrö Helariutta
(HU)

EM2.2

Manipulation of downstream factors involved in xylem parenchyma cell formation

Lead:

Ari Pekka Mähönen
(HU)

EM3_INCREASING SINK STRENGTH: METABOLIC FLUX

EM3.1

Optimization of sucrolytic cleavage and glycolysis

Lead:

Alistair Fernie
(MPI-MPP)

EM3.2

Optimization of starch-precursor delivery to amyloplasts

Lead:

Wolfgang Zierer
(FAU)

EM3.3

Starch granule initiation and growth

Lead:

Sam Zeeman
(ETH)

EM3.4

Model-based predictions

Lead:

Lee Sweetlove
(OU)

CASS III Team Contributions

Technical Modules

TM1

Project management
and central services

Lead:

Uwe Sonnewald
(FAU)

TM2.1

Cassava routine
transformation

Lead:

Simon Bull
(ETH)

TM2.2

Chemical and genetic
boosters

Lead:

Christian Lamm
(FAU)

TM2.3

Transformation of
farmer-preferred
genotypes

Lead:

Ihuoma Okwuonu
(NRCRI)

TM3.1

Testing of identified
promoter candidates

Lead:

Wolfgang Zierer
(FAU)

TM3.2

Single cell/nuclei
sequencing

Lead:

Ari-Pekka Mähönen
(HU)

TM4.1

NCHU field testing

Lead:

Wilhelm GUISSEM
(NCHU)

TM4.2

IITA field testing /
crossing

Lead:

Ismail Rabbi
(NCHU)

TM5

Cassava plant and field
phenotyping

Lead:

Onno Muller
(FZJ)

TM6

Research data
management

Objective Lead:

Lukas Mueller
(BTI)

**All objective leads (EM & TM) have
reporting duties!**

CASS III Work Plan

[illegible]

- The current work plan is outlined in the CASS III GANTT chart
- The work plan is not set in stone but changes need to be reasoned and agreed upon (with FAU and AgOne)
- Quaterly reporting against the current workplan (or simplified version)

Communication and Meetings

- Regular reporting promotes information flow to GatesAgOne, but also within CASS
- Quarterly reports to FAU to monitor progress based on proposed timelines
- FAU and Gates Ag One Quarterly meetings
- IP Committee meetings (+X) held on demand.
- Regular meetings between Cathy, Uwe, and Wolfgang
- CASS annual meeting facilitates exchange, scientific presentations, and discussion of challenges

1. Quarterly Reports to FAU

2. Annual Financial Reports

3. CASS Annual Meeting

Meetings and Reports

QUARTERLY

ALL OBJECTIVE LEADS

Module leaders report
to FAU



CASS MANAGEMENT OFFICE

Quarterly Report
submitted to Gates Ag
One



FAU + Gates Ag One

CASS
Quarterly Meeting

*No more than 2 hours work
for objective leads per
quarter*



ANNUALLY

CASS Annual Meeting

Annual Financial
Reporting

+

Annual Scientific
Report

CASS Management Office



Uwe Sonnewald

CASS Program Leader



Wolfgang Zierer

CASS Program Manager



Catherine Espinoza

Gates Ag One Technical Program
Lead, Bill & Melinda Gates
Agricultural Innovations



Christian Rogers

Cambridge Discovery
Year 1 - program
management support.



Iris Hammer

CASS Finance Manager

CASS Data Manager (TBH)

Supports data transfer to central platforms
Facilitates data transfers.
Assists with data provision for IP and CFT
applications.

Impact Manager (TBH)

Monitors progress across program areas.
Represents CASS team in IP committee.
Handles communication with the public.

Reporting Framework

A quarterly reporting system requires three actions and should take no more than 2 hours per team, per quarter.

Objective leaders provide two scores for each task:

% Complete

Progress Score

Objective Leader answers three questions:

1. Progress Update
2. Challenges and Mitigations
3. Next Steps in the Action Plan

Objective Leader provides links to Cassavabase or other relevant data resources

New key data made available

Shared with Gates Ag One Team

- Template documents will be made available facilitating reporting across the project each quarter
- Edited into a report each quarter by FAU Management office team
- Communication from CASS team members on project progress will be achieved throughout the year

Reporting Framework

No more than 2 hours reporting work for Module Leaders per quarter

1. **Percent Complete:** indicates towards our objective
2. **Progress Scores:** Scored 0.1 – 1.0, Green (on track for target date), orange (risks identified), red (problem)
 - ◆ 0.9: Excellent progress with delivery assured by the target date.
 - ◆ 0.7: On track and likely to deliver by the target date.
 - ◆ 0.5: Lower than anticipated progress with some challenges or delays.
 - ◆ 0.3: Low progress with significant challenges or delays.
 - ◆ 0.1: Very low progress or no progress at all.

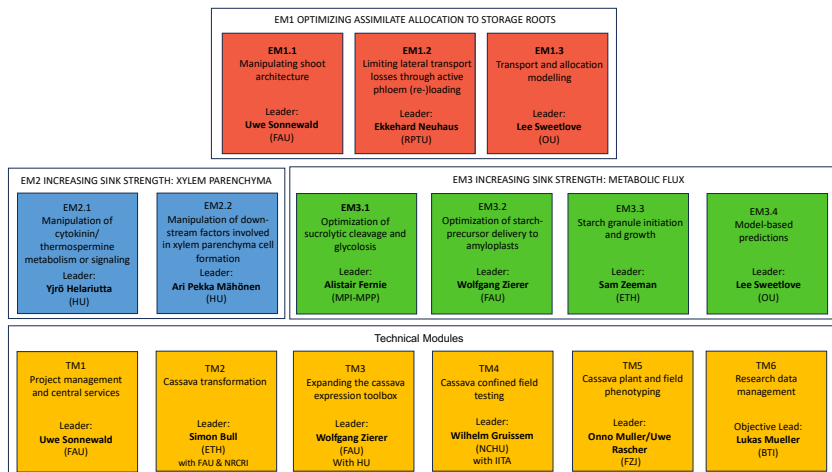
3. Justification Statements

Progress Update: A brief update on the progress made toward each objective. This includes key accomplishments and milestones achieved during the quarter.

Challenges and Mitigations: The challenges or obstacles encountered during the quarter that affected progress. We explain the steps taken or strategies implemented to mitigate these challenges and minimize their impact on the project.

Next Steps in the Action Plan: Provides an outline of the planned activities or next steps that will be undertaken in the upcoming quarter to continue progress toward the objective. It includes specific tasks, milestones, or experiments that will be conducted.

Reporting system similar to ENSA



Excel

Objective leaders provide two scores for each task:

% Complete

Progress Score

Word

Objective Leader answers three questions:

1. Progress Update
2. Challenges and Mitigations
3. Next Steps in the Action Plan

CassavaBase Links

Objective Leader provides links to Cassavabase or other relevant data resources

New key data made available

Shared with Gates Ag One Team

OBJECTIVE 1: KEY RESULTS, TARGET DATES AND DEPENDENCIES

Understand the function of **native symbiotic LysM-RLKs** in target cereal crops

	PC	PS	TEAM OBJECTIVE	KEY RESULT (Milestone)	Assigned	Species
1.1.1	10.0%	0.0%	Create selected receptor mutants in maize with 1 independent allele per gene with at least 100 seeds per line.	Provide receptor null-mutant maize lines with homozygous mutations in 3 key receptor genes with 1 independent allele per gene with at least 100 seeds per line.	Cambridge with GAO transformation partner	Maize
1.1.2	10.0%	0.0%	Characterize cereal receptor mutants for immunity and symbiotic responses.	Characterize barley mutants in 10 RLK genes and maize mutants in 5 single RLK genes in coordination with ENSA, assessing mycorrhizal and pathogen colonisation, markers of symptoms and immunity signaling.	Cambridge	Barley, Maize
Engineer efficient Nod factor perception in target crops						
1.2.1	10.0%	0.0%	Assess impact of nanobody induced receptor complex fusion in barley.	Assess symbiosis signaling and transgenic impacts in T1 homozygous lines of receptor complex fusion in barley to determine if additional downstream components are needed for engineering.	Nature	Barley
1.2.2	10.0%	0.0%	Define structures and biochemical properties of barley and maize RLK10 and RLK15 receptor ectoproteins.	To identify the residues in cereal receptors based on structural understanding for engineering efficient LCO perception. To engineer 2 receptors (per RLK10 and RLK15 type) of barley and maize and achieve 100% of the specificity and affinity for LCOs as obtained in native and Medicago receptors.	Nature	Barley, Maize
1.2.3a	10.0%	0.0%	Optimize the engineering of native barley and maize receptors for strongest LCO recognition and activation of the Nod pathway.	The activation of the common symbiosis pathway should be comparable to that in nodulating plants (legumes and/or non-legumes), measured with calcium and gene reporters.	Nature	Barley
1.2.3b	10.0%	0.0%	Optimize trans receptor contributions to maximize LCO signaling in cassava.	Optimized receptor combination resulting in activation of the NIN domain response gene in 3 independent stable transformed cassava lines. These transgenic lines should show the following: 1. Stronger or enhanced NRC activation compared to the A. Albogones ParH9P1Pac1X42 roots. 2. No negative side effects due to ectopic receptor expression using growth differences to wild type as a proxy. 3. Precise LCO specificity. Availability of 3 independent transgenic lines with optimized receptor pairs but no GUS reporter construct. Repetitions: These lines are used for gene stacking (see 1.3.3).	Algeringen and Althea	Cassava
Engineer Nod factor induction of nodule organogenesis in target crops.						
1.3.1a	10.0%	0.0%	Engineer a rhizobium LCO induced NIN regulatory loop in nodules and target crops.	Establish a NIN construct that can functionally complement legume and/or Parasitica on nodules giving nitrogen fixing nodules and is induced upon host factor signaling to cassava and barley in response to symbiosis signaling.	Algeringen and Cambridge	Cassava
1.3.1b	10.0%	0.0%	Engineer NIN downstream signaling critical for nodule organogenesis.	Function of one or more modules in lines of cassava and barley as a function of LCO regulation of NIN.	Algeringen, Cambridge, and Thünen	Barley, Cassava
1.3.2a	10.0%	0.0%				
1.3.2b	10.0%	0.0%				

OBJECTIVE 1.1: JUSTIFICATION OVERVIEW

Progress Update: Understand the function of **native symbiotic LysM-RLKs** in target cereal crops

1.1.1. In summer 2023, we have isolated homozygous mutants of *cerk1*, *cerk2*, *nr5a*, *nr5b*, *nr11* and *symrk*. In agreement with our previous findings, these mutants display a complete loss of symbiosis. We have initiated a full collection of maize mutants. In addition, as a clearer strategy to identify "sign coding" sequences are being synthesized for a nanobody-driven fusion approach to identify the key receptors capable of driving

1.1.2. Progress in characterising the barley *rlk* mutants: over several experiments, we have analysed the full suite of single *rlk* responses. We have now completed all remaining studies and clarified the mutants that had initially ambiguous phenotypes. Phytophthora palmivora perception and signalling, none of the mutants display a complete loss of symbiosis. We have initiated a full collection of maize mutants. In addition, as a clearer strategy to identify "sign coding" sequences are being synthesized for a nanobody-driven fusion approach to identify the key receptors capable of driving

Challenges and Mitigations: Bulking of maize mutant lines will occur in 2024-2025, to increase the currently low stocks of seeds per line. These mutant lines will be bulked in the background other than the RLK of interest. Further backcrosses to B73 (original wild-type background) and other appropriate lines will be initiated now that the material containing the transposon insertion has been identified. Maize growth and therefore crossing in the Cambridge glasshouse facilities, so this has been excluded as an option. To fully deliver on 3 independent alleles for *cerk1* clarification and certainty from the identified maize transformation partner will be needed, especially to obtain the required colonisation experiment in addition, require clean uncontaminated seeds but the recent experiments for Phytophthora and NIN with serious fungal contamination of the seeds. This is currently being dealt with by a deep clean of the glasshouse in Cambridge. However, this seed contamination issue has seriously impeded progress and resulted in experiments with sub-optimal sets of

Next Steps in the Action Plan: We will complete the list of maize mutants and state of selection (heterozygous or homozygous) and attempt to initiate some feasible plan/pipeline is decided by GAO together with ENSA. For the barley mutant work, we aim to complete the analyses to illustrate the mutant phenotypes in a comprehensive analysis. In conjunction with a detailed microscopy analysis, we will repeat the infection experiments in the glasshouse. Plans to similarly screen through the Magnaporthe oryzae infection experiment followed by detailed microscopy analyses of mutants that show increased/reduced susceptibility is also underway.

OBJECTIVE 1: ENGINEER RHIZOBIUM-INDUCED NODULE ORGANOGENESIS IN CROPS



	Species	Percent Complete	Progress Score	TEAM OBJECTIVE
1.1.1	Maize	10.0%	0.0%	Create selected receptor mutants in maize
1.1.2	Barley, Maize	10.0%	0.0%	Characterize cereal receptor mutants for immunity and symbiotic responses.
1.2.1	Barley	10.0%	0.0%	Assess impact of nanobody induced receptor complex fusion in barley.
1.2.2	Barley, Maize	10.0%	0.0%	Define structures and biochemical properties of barley and maize RLK10 and RLK15 receptor ectoproteins.
1.2.3a	Barley	10.0%	0.0%	Optimize the engineering of native barley and maize receptors for strongest LCO recognition and activation of the Nod pathway.
1.2.3b	Maize	10.0%	0.0%	Optimize trans receptor contributions to maximize LCO signaling in cassava.
1.3.1	Cassava	10.0%	0.0%	Engineer a rhizobium LCO induced NIN regulatory loop in nodules and target crops.
1.3.1b	Cassava	10.0%	0.0%	Engineer NIN downstream signaling critical for nodule organogenesis.
1.3.2a	Cassava	10.0%	0.0%	
1.3.2b	Barley	10.0%	0.0%	

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Quartely report created by FAU

Time schedule

	Week 1	Week 2	Week 3	Week 4
April		CASS 3 Launch meeting April 9, 2024		
May	CASS Phase 3 START May 1st 2024		Ramp up and recruitment period	
June	Management office and objective teams complete			
July			First Quarterly Meeting CASS - GAO	
Aug				
Sept				
Oct	CASS Q3 2024 Reporting		CASS Q4 2024 Quarterly Meeting	
Nov				

	Week 1	Week 2	Week 3	Week 4
Dec				
Jan 2024	CASS Q4 2024 Reporting		CASS Q1 2025 Quarterly Meeting	
Feb				
Mar				CASS3 Period 1 END April 30 th 2025
Apr	CASS Q1 2025 Reporting	CASS Annual Meeting 2025	CASS Q2 2025 Quarterly Meeting	
May		Annual Reporting		
June				
July	CASS Q2 2025 Reporting		CASS Q3 2025 Quarterly Meeting	

Thank you!

Questions? Comments?

Q2 2024 - Critical activities to move forward

FAU $\leftrightarrow$ EM1/EM2/EM3/TM3

Cloning

May 2024 – July 2024

Transformation

Aug 2024 – April 2025

OU $\leftrightarrow$ RPTU/MPI/FZJ/ETH

Modeling data generation

Should be concluded October 24 / April 25

Discussion session

IITA $\leftrightarrow$ RPTU

IITA CFT / Crossing activities

Discussion session

Plants should be growing at IITA next month /
Open IP issue for *AKT2*

BTI $\leftrightarrow$ Users

Database user training

HU

Single cell / Single nuclei RNA-Seq