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A Roadmap For Photosynthesis To Drive Crop Improvement

CAPITALISE photosynthesis: concepts2crops · GAIN4CROPS · BestCrop · engineering PHOTO BOOST next level photosynthesis

European Strategic Research Agenda and Road Map to 2030

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European Strategic Research Agenda and Roadmap to 2030

Including contributions from CAPITALISE, GAIN4CROPS, PhotoBoost and BestCrop EU H2020 projects

Acronym:	CAPITALISE Combining Approaches for Photosynthetic Improvement To Allow Increased Sustainability in European agriculture
Grant Agreement Number:	862201
H2020-NMBP-BIO-2019:	BIOTEC-02-2019 Boosting the efficiency of photosynthesis (RIA)
Start date:	01/04/2020
Duration:	56 months

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Compiled by	**Louisa Dever, Ritchie Head, Christina Olsen**
Contributors	**This Deliverable includes contributions from multiple organisations and experts who were invited to contribute.**
Dissemination Level	**Public**
Status	**Final**

The CAPITALISE project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 862201.

Table of Contents

1 Executive Summary.

The executive summary is comprised of a 4-page policy brief and a 28-page brochure in the following pages. These two summary documents were prepared and presented to the European Commission on 29th November 2024 at a workshop entitled:

1.1 Workshop: Improving Crop Yield and Resilience in a Changing Climate

Our ambition with this deliverable is to provide both the summary and the technical detail to inspire future breeding research and innovation programmes. We outlined multiple photosynthesis linked strategies that are available for exploitation. We believe the most efficient way forward is through Public Private Partnerships at the European level. This will enable a strategic approach for the plant science and industry to cross the “translation gap” and take promising results from research to crop breeding programmes.

Figure 1 Dr Dever (Ceratium) Presenting the SRA and Roadmap at Euroseeds Offices in Brussels.

“*Investing in improving photosynthetic efficiency is not only a strategy to increase productivity but also a concrete response to the need for a more sustainable agriculture integrated into new production contexts. At a time when traditional agriculture is evolving toward new forms of cultivation, such as vertical farming, and facing climates with increasingly higher temperatures, the seed companies that integrate these aspects into their research programs can drive innovation and contribute significantly to the future of the sector*”

Anna Giulia Boni & Massimiliano Beretta at ISI Sementi, Italy.

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A Roadmap For Photosynthesis To Drive Crop Improvement

Society needs more resilient crops

Climate change is driving abiotic stresses that negatively impacts crop health and yields. This reduces primary production, threatening food, feed and energy security, and the bioeconomy. New climate resilient crops are urgently needed.

Photosynthesis - an underexploited trait

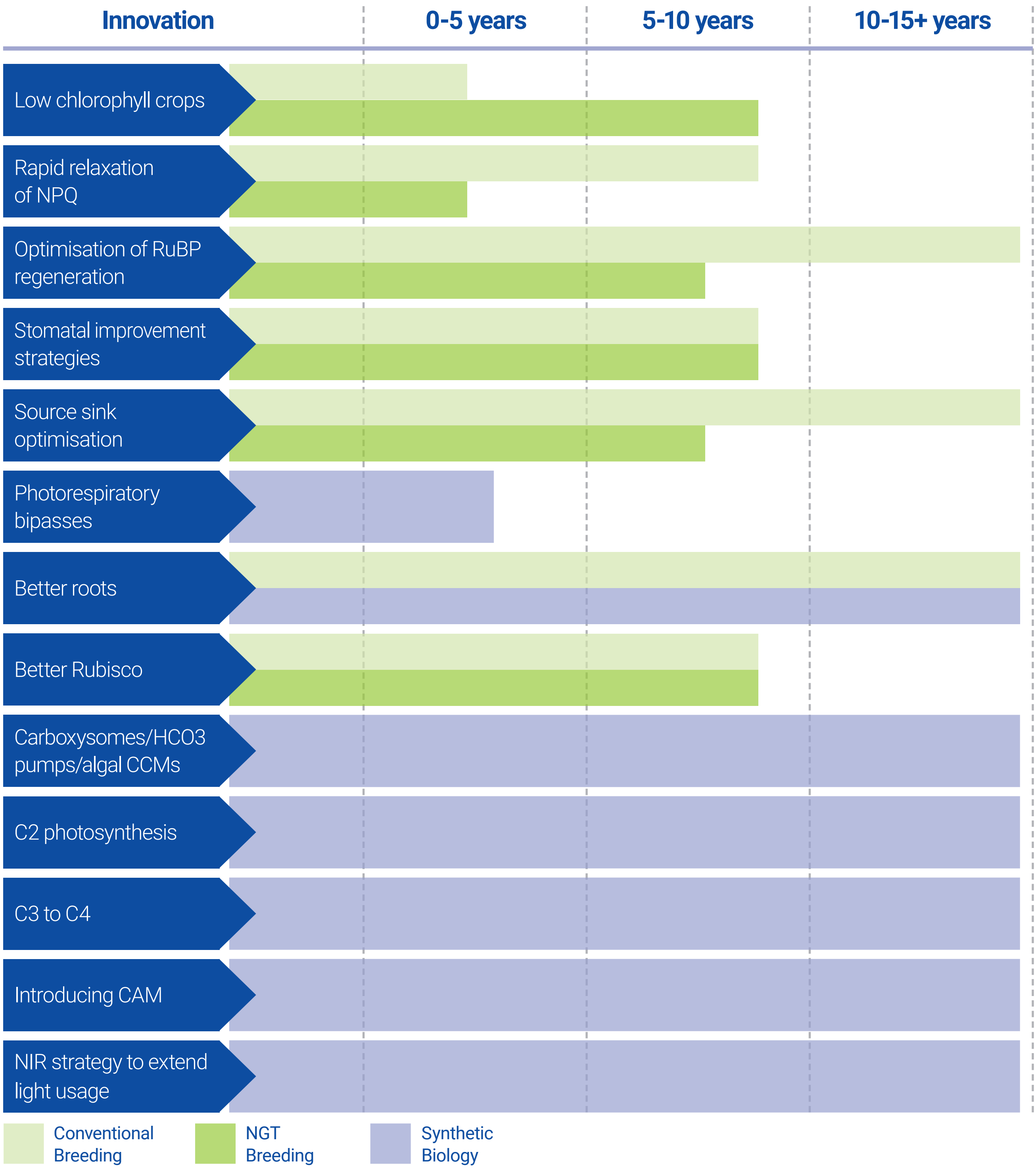
Photosynthesis is a complex process but has many underexploited traits with great potential to improve crop yield and resilience to climate change. Recent scientific advances have demonstrated significant improvements in crop productivity through improving photosynthesis efficiency. Multiple innovations have been developed by the research community to varying Crop Technology Readiness Levels (TRL).

Industry has a strong interest in photosynthesis-driven crop improvement, but collaborative projects and an enabling environment are needed to bridge the translation gap for 'smart' crop development.

Conventional modern breeding and New Genomic Techniques (NGTs) provide pathways to exploit these innovations. The time to translate is now.

European Strategic Research Agenda and Road Map to 2030

Timeline for strategies to reach crop TRL7



Crop TRL	Definition	
TRL1	Basic principles for improving target crop(s) identified	**Preliminary evaluation**
TRL 2	Crop improvement concept formulated	
TRL 3	Experimental proof of concept (laboratory level)	**Experimental testing**
TRL 4	Improvement validated in a crop model (laboratory level)	
TRL 5	Improvement validated in a field/glass house environment	**Pre-commercial assessments**
TRL 6	Pre-breeding with improved traits in a relevant environment	
TRL 7	**Improved prebreeding crop line demonstration in a grower/farm environment**	**Commercial Deployment**
TRL 8	Breeding in elite crop line achieved and qualified	
TRL 9	Elite crop line incorporating trait(s) proven in commercial growing environments	

Evidence for dramatic crop improvements

Advances in genomics, phenotyping, and modelling now enable scientists to demonstrate the yield benefits of improving photosynthesis in genetically modified plants.

Barley	Chlorophyl tuning: A pale green barley line showed a 40-50% reduction in transpiration rate under drought stress and 40% increased photosynthetic efficiency under high light conditions (BestCrop).[1]
Potato	Expression of a glycolate dehydrogenase polyprotein (DEFp) led to 12-45% increases in potato tuber yield (PhotoBoost).[2]
	Introduction of algal carbon concentration mechanism components into potato chloroplast led to 17-42% enhanced tuber yield (PhotoBoost).[3]
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Soybean	Accelerating plant recovery from photoprotection delivered a 33% improvement in seed yield.[4]
Rice	The overexpression of a transcription factor that regulates photosynthetic capacity led to a 41-68% yield increase.[5]
	Overexpressing Rubisco in rice enhanced yields by 17-28%.[6]

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Recommendations

- ✔ Crop development can be a 10–15 year investment, this demands a systematic approach. Research on relevant germplasm, improved genetic resources, tools, computational models and an innovative culture that embraces biotechnology, is needed to accelerate crop improvements.
- ✔ Translation of Key Exploitable Results is a priority area. Collaborative working is urgently needed between industry and the science base to overcome market failures translating research to develop climate resilient crops.
- ✔ Short term projects (3-5 years) produce fragmented 'islands' of research, a more strategic approach is needed. Public Private Partnerships (PPP) represent the best option to exploit the knowledge base to deliver 'future proof' crops.
- ✔ Low level and declining public investment in crop breeding programmes should be reversed. Crop research needs a reinvigorated strategic programme at the European level. Collaborative research and innovation projects need to be longer term (5+ years) and well-funded (€8M+) to drive translational crop research effectively.
- ✔ An enabling regulatory environment to support NGTs should be a short-term priority to accelerate the broader application of biotechnology. This will compliment conventional crop improvement pathways, to develop some new plant varieties faster, and in a more precise manner.
- ✔ In parallel, environmental risk assessments should be undertaken, and literacy programmes developed and implemented, to educate citizens and stakeholders about NGTs. This should be linked to sustainability issues, and making informed risk assessments.
- ✔ Barriers to translating public research to industry need to be better understood and addressed. Life Cycle Analysis represents an important tool to address the socioeconomic costs, risks and benefits of the proposed approaches. This will support commercial decision making.
- ✔ Issues regarding IP and benefits sharing need to be resolved for maximal use of research outputs by Industry.

Strategic research priority areas:	
Phenotyping and validation	Chlorophyll Tuning
Translation of QTLs	Moving to field trials
Computational model improvement	Phenotyping tool development
Genomic toolbox expansion	Synthetic biology approaches

‘Smart’ Crops Align with EU policy

Focusing on crop breeding to enhance photosynthesis supports the **Green Deal** and **Farm to Fork** goals, promoting sustainable production, food security, the bioeconomy and climate action. Biotechnologies are crucial for advancing both conventional and new breeding techniques, with the proposed **Biotech Law** aiding progress. Enhancing photosynthesis aligns with the **Strategic Dialogue on the Future of EU Agriculture** recommendations for innovative breeding. This approach increases productivity, reduces fertilizer use, enhances water efficiency and supports biodiversity, contributing to multiple SDGs.

Photosynthesis is positioned to be a crucial technology for crop improvement, enhancing European competitiveness and economic prosperity.

This roadmap has been developed based on:

- Literature reviews.
- Results from the H2020 projects: CAPITALISE, Gain4Crops, PhotoBoost and BestCrop.
- Opinions from online breeder and grower surveys.
- Workshops with 20+ stakeholder representatives to identify: (1) The needs of Industry & (2) The barriers to translating crop research.
- A Translational Photosynthesis workshop with 50+ academics and industry representatives.
- Social Sciences Stakeholder Engagement with consumers, farmers and breeders.

This Roadmap has been developed by the CAPITALISE project (EC Grant Agreement 862201), with contributions from the GAIN4CROPS (ECGA 862087), PhotoBoost (ECGA 862127) and BestCrop (ECGA 101082091) projects and key stakeholders. This is a summary of the more detailed European Strategic Research Agenda and Road Map to 2030.
This document can be accessed using the QR code.

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A roadmap for Photosynthesis to drive crop improvement

This roadmap has been developed based on:

- Literature reviews.
- Results from four H2020 photosynthesis projects: CAPITALISE, Gain4Crops, PhotoBoost and BestCrop.
- Stakeholder opinions from online breeder and grower surveys (70 respondents)
- Two workshops to identify: (1) The needs of Industry and, (2) The barriers to translating academic research to crop breeders. This engaged 20 representatives from breeders, growers, vertical farmers, academics/EPSO representatives, consumers, crop genetic engineers, horticulturists, trade organisations, Euroseeds and the Plants for the Future Technology Platform.
- A Translational Photosynthesis workshop, co-organised by CAPITALISE and the French Groupment de Recherche. This brought together 50+ academics and industry representatives.
- Social Sciences Stakeholder Engagement with consumers, farmers and breeders.

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A call to action: the benefits of public private partnerships

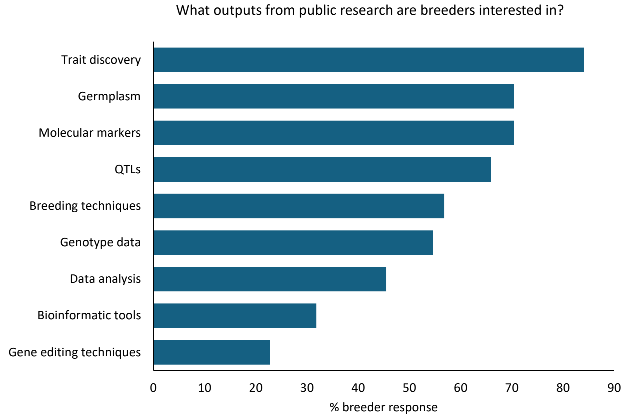
The European crop breeding sector consists of approximately 7000 businesses including numerous small to medium-sized enterprises (SMEs). The crop breeding sector plays a critical role in agricultural sustainability in Europe, and SMEs contribute significantly to the resilience of regional agriculture. These companies need to be innovative to remain competitive, but many lack significant research and innovation capacity. In contrast, larger companies have resources to invest in R&D but still benefit from collaborations with the public research base.

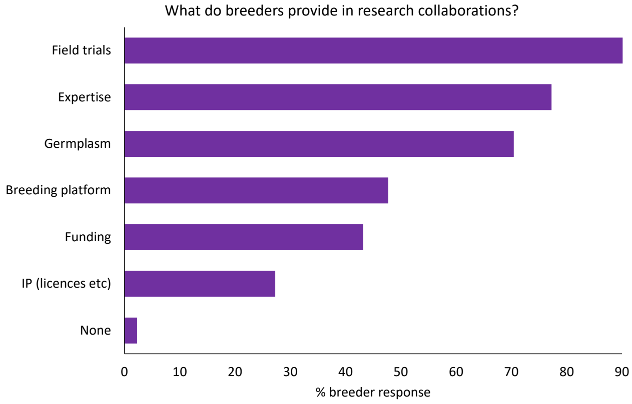
The current fragmented nature of research funding in plant and crop sciences is creating unnecessary barriers to rapid translation of promising results.

To strengthen European competitiveness in agriculture and the bioeconomy, an integrated strategy is needed to advance biotechnologies and accelerate crop research. Conventional crop development cycles of 10–15 years are mismatched with the 3–5 year research funding schemes. This limits opportunities to quickly reach high crop Technology Readiness Levels (TRL). A European approach can streamline the research-to-application pipeline, addressing inefficiencies and fragmentation that hamper responses to climate stresses, food security, and feedstock shortages.

While industry can support higher TRL innovations, short-term public funding often prioritises exploratory academic research and publications. This may discourage translation of results. Stronger collaboration between academia and industry is essential to advance promising strategies such as enhancing photosynthesis.

A survey of breeders highlighted specific research outputs desired from public research, and industry contributions to collaborations.





Declining public investment in crop research needs to be reversed

Reversing declining public investment in crop research and encouraging private funding through Public-Private Partnerships (PPPs) is critical for strategic collaborations in crop improvement. Despite EU commitments, the CAPITALISE project only identified 229 H2020 projects as relevant to "crop breeding" **with less than 0.5% of the €80 billion budget allocated to this research**. *Analysis by the Plants for the Future* ***European Technology Platform*** (ETP) revealed a decline in funding for plant breeding research, although Horizon Europe funds rose by 42% compared to FP7. Plants for the Future and others have already called for a new partnership "Optimising genetic potential for resilient and diverse production systems". This report confirms the need to harness discovery science and precompetitive breeding for improved crops and algal systems and accelerate promising areas including the multiple options to improve Photosynthesis discussed here.

KEY MESSAGES

Climate change is driving abiotic stresses that negatively impacts crop health and yields, reducing primary production and threatening food, feed and energy security. New climate resilient crops are urgently needed.

- ✔ Crop development is a long term investment taking 10-15 years and requiring a strategic approach. Time is of the essence. Research on relevant germplasm, improved genetic resources, tools, models and an innovative culture that embraces biotechnological advances are critical to accelerate the required improvements to crops.
- ✔ Public private partnership represent the best option to develop the tools and knowledge base to deliver a new generation of resilient sustainable climate adapted crops that address the emerging threats to primary production for food and the bioeconomy.
- ✔ Low level and declining public investment in crop breeding programmes needs to be reversed. Crop research needs a reinvigorated strategic programme, at the European level, to implement longer term (5+ years) well-funded (€8M+) collaborative research and innovation projects creating enabling environments to drive translational crop research.
- ✔ Photosynthesis is a complex process but has many underexploited traits with significant potential to improve crop yield and resilience to climate change. Recent scientific advances have demonstrated significant improvements in crop productivity through improving photosynthesis efficiency.
- ✔ Translation of Key Exploitable Results represents a priority research area. Collaborative working is needed between industry and the science base to overcome market failure in developing photosynthesis driven climate resilient crops.
- ✔ An enabling regulatory environment to support NGTs should be a short-term priority to accelerate the broader application of biotechnology. This will compliment conventional crop improvement pathways to develop some new plant varieties faster, and in a more precise manner to exploit promising traits and approaches.
- ✔ In parallel, environmental risk assessments should be undertaken, and literacy programmes developed and implemented, to educate citizens about NGTs and making informed risk assessments.
- ✔ Barriers to translating public research to industry need to be better understood and addressed. Life Cycle Analysis represents an important tool to address the socioeconomic costs, risks and benefits of the proposed approaches and will form a basis for commercial decision making. Issues regarding IP and the Nagoya protocol need to be resolved for maximal use of research outputs by Industry.

Society needs more resilient crops

There is an urgent need for improved climate resilient crops to mitigate the effects of abiotic stresses linked to a changing climate. Future food security to meet the needs of a growing global population and the shift to a biobased economy both require efficient sustainable agricultural production. To protect biodiversity, it is important that yields are increased to meet future demands without needing more land, leaving space for nature.

Addressing these challenges requires a combination of adaptation and mitigation strategies, technological innovation, and changes in agricultural practices to ensure food security and environmental sustainability. Future crops, irrespective of farming systems, need to respond effectively to multifactorial changes related to a changing climate and environment. A coherent, multifaceted approach is needed, supported by investment, cross sector collaborations and a supportive regulatory and policy environment that encompasses sustainability challenges and citizen concerns.

Four EU projects: CAPITALISE, Gain4Crops, PhotoBoost and BestCrop, have taken different approaches to improving the efficiency of photosynthesis in key European crops. Each project is making significant progress towards increasing the crop TRLs for breeders. This is not a simple task, significant barriers to translation exist.

This roadmap highlights how improved photosynthesis in crops is part of the solution. It explores the challenges faced by researchers and industry in taking research results to the grower and consumer, and highlights the potential future directions for photosynthesis research to meet breeder, grower and societal needs. This includes recommendations for strategic research and innovation approaches and timescales to inform key actors in the value chain, policy makers, and public/private sector actors and funders.

Plant breeding is reliant on introducing genetic diversity to improve crop characteristics. This can be achieved by Conventional Breeding or New Genomic Techniques.

Modern Conventional Plant Breeding is a high-tech selective process involving phenotyping, genotyping, molecular markers, and genomic selection. It expands the gene pool over multiple generations but is time-consuming (10-15 years) and lacks precision, as undesirable traits can be inherited with target traits.

New Genomic Techniques (NGTs) like CRISPR-Cas9 and synthetic biology enable precise gene editing to enhance traits such as drought tolerance or disease resistance, without unwanted traits. NGTs are faster (1-5 years), more sustainable, and can overcome barriers to gene flow that limit conventional breeding.

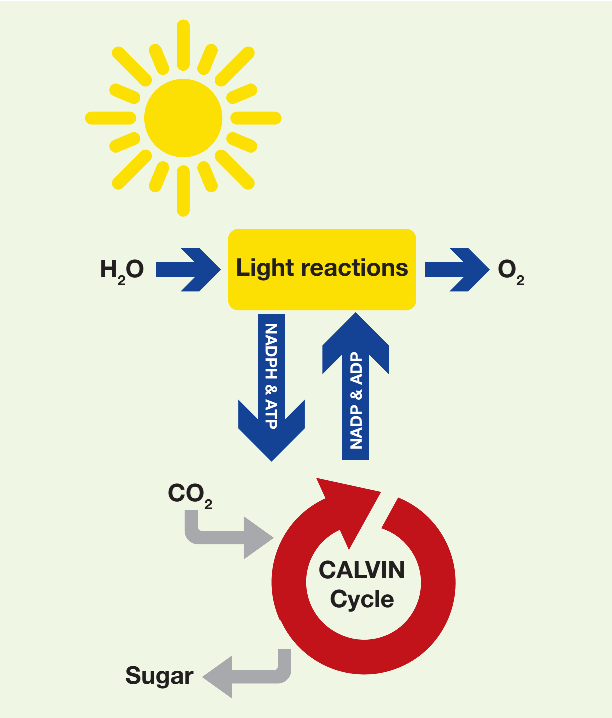
Photosynthesis driving crops of the future

Photosynthesis is a fundamental process that drives plant growth and productivity by converting light energy into chemical energy. In the context of plant breeding, scientific advances have shown photosynthesis-related traits have significant potential to enhance crop yields and resilience in the face of increasing abiotic stresses linked to climate change.

While photosynthesis is a promising science driven approach and has attracted the interest of commercial breeders, it has not gained traction in breeding programmes in part due to complexity and difficulties for selection that require better tools.

The complexity that has prevented exploitation in the past is increasingly well understood and is now opening up multiple promising crop improvements. This includes options to combine and stack traits in plants to address different abiotic stresses that reflect changing real world environments.

Improving photosynthesis is one of the most promising options to improve crop yields and achieve global food security

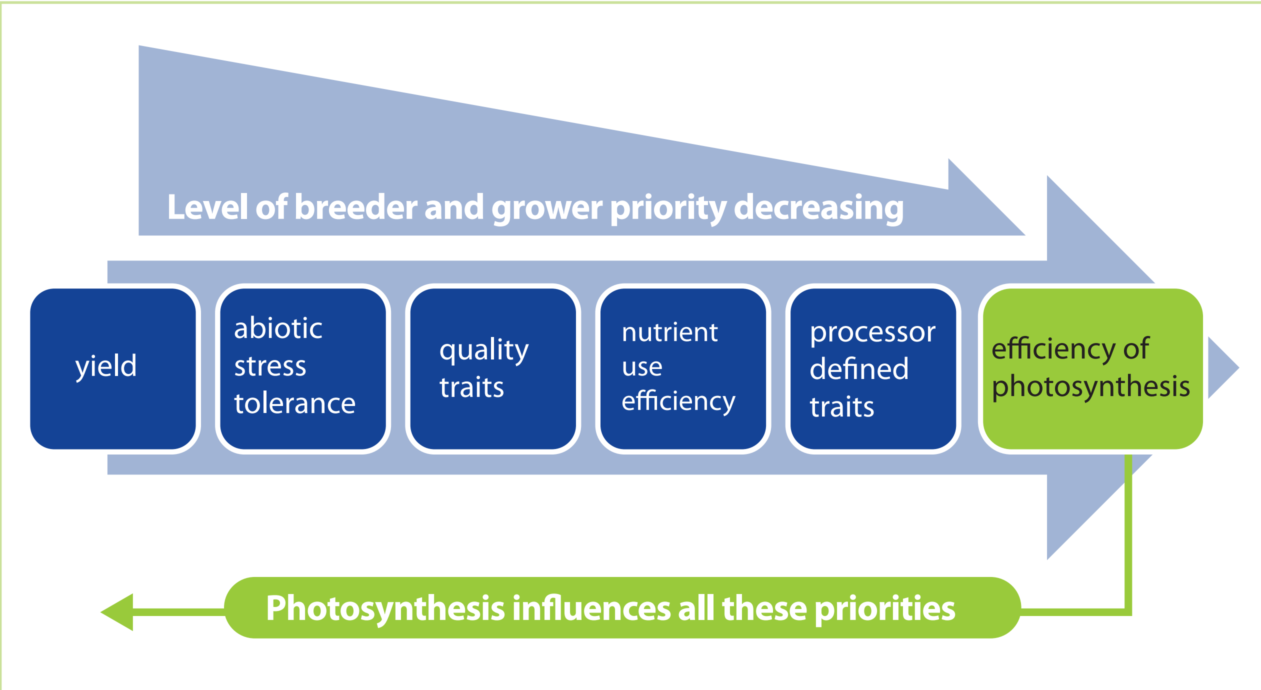


Over 50 years of research is providing exciting new approaches for climate-smart crop breeding. Timely translation is needed to deliver food and energy security, and to provide plant derived biomaterials, and climate change mitigation. Despite these advances, developing new resilient high-yielding varieties typically still takes 10-15 years. Given the increasing pressure on agricultural systems, speed is of the essence.

To accelerate the translation of photosynthesis requires investment and cross sector interdisciplinary working. Strategic initiatives are needed in the short term through Public Private Partnerships to transfer the knowledge from academic plant sciences to the crop breeding sector.

Photosynthesis: an undervalued solution?

A CAPITALISE survey asked seventy breeders and growers to prioritise their current breeding goals for their crops of interest. Overall, the three highest priorities identified across all the crops were yield, abiotic stress tolerance and quality traits, by both breeders and growers. This figure represents an overview of these results.



Efficiency of photosynthesis was rated a relatively low priority. The breeders and growers surveyed did not seem to recognise that improved photosynthetic efficiency has been shown to influence their other priorities.

To date, policy drivers for EC investment in photosynthesis research were primarily linked to population growth, increasing yield and long-term food security. Unsurprisingly, industry breeding goals and grower crop priorities were focussed on more immediate economic challenges linked to abiotic stresses associated with climate change and market demands.

The full advantages of improving photosynthetic efficiency are not fully appreciated by many value chain actors

Dramatic crop improvements achieved by exploiting photosynthesis

Quantifying the benefits of improved photosynthesis to crop yield has been difficult to demonstrate. Recent advances in genomics, high-throughput phenotyping, and modelling now enable scientists to demonstrate the yield benefits of improved photosynthetic efficiency in genetically modified plants through lab and field trials.

Barley	Chlorophyl tuning: A pale green barley line showed a 40-50% reduction in transpiration rate under drought stress and 40% increased photosynthetic efficiency under high light conditions (BestCrop).[1]
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Integrating photosynthesis into crop improvements

Multiple processes are coupled to photosynthesis from the cellular to the canopy scale. A plethora of other factors, including water, nutrients, pathogens, temperature, light, and management practices affect photosynthesis and co-limit yield. An integrative research approach is essential to understand and exploit photosynthesis within a complex agroecological context for improving yield and resilience.

Selected features of such an integrative photosynthesis research programme are outlined below:

- ✔ ***A whole-plant perspective:*** Research should consider photosynthetic traits in the context of the whole plant, including its non-photosynthetic parts such as roots, and the plant holobiont, i.e. the plant and its associated microbiome.
- ✔ ***A product-centric perspective:*** Prioritizing desired qualities like mineral nutrient or protein content, ease of processing, and addressing diverse value chains (food and non-food applications) may require distinct strategies tailored to optimize product quality.
- ✔ ***A climate-centric perspective:*** This will require expanding the pool of genetic variation available for breeding from landraces, crop wild relatives, in addition to novel variants through new breeding technologies and chromosome engineering.
- ✔ ***Photosynthesis in the context of different cropping systems:*** Optimal photosynthetic traits will vary for different cropping systems and may need to be adapted to new cropping schemes, including multi-cropping and other agroecological types of farming, or for combining crop production with renewable energy production (agrophotovoltaics). Indoor agriculture under constant, controlled environments eliminates the need for adaptive photosynthetic traits, allowing for higher conversion efficiencies. Novel photosynthetic ideotypes will be required to fully exploit the potential benefits of these alternative growing systems.
- ✔ ***Photosynthesis in multi-purpose crops:*** Different qualities are needed to provide cost-efficient food, fibre and renewable resources. Crops can also be modified to better provide important ecosystem services and contribute to climate change mitigation, e.g. Carbon Farming.
- ✔ ***Integrative research requires a Multi-Actor Approach:*** Collaboration with breeders and growers will identify key knowledge gaps, while participatory research will enhance field trial design and analysis. This integrated approach aims to accelerate the development of novel germplasm, benefiting farmers and consumers, and effectively disseminating findings.

Contribution to EU policy

This Roadmap aligns with the **Green Deal** and **Farm to Fork** priorities, focusing on crop breeding to enhance photosynthesis for higher yields with lower inputs, supporting sustainable farming and the Common Agricultural Policy (CAP) goals. The ambition is to deliver sustainable production within planetary boundaries, contributing to food security, climate action, and multiple **Sustainable Development Goals** (2, 3, 6, 7, 12, 13, 15). Biotechnologies are key to accelerating progress supporting conventional and new breeding techniques. This strategy will benefit from the proposed EU Biotech Law and improved regulatory environments.

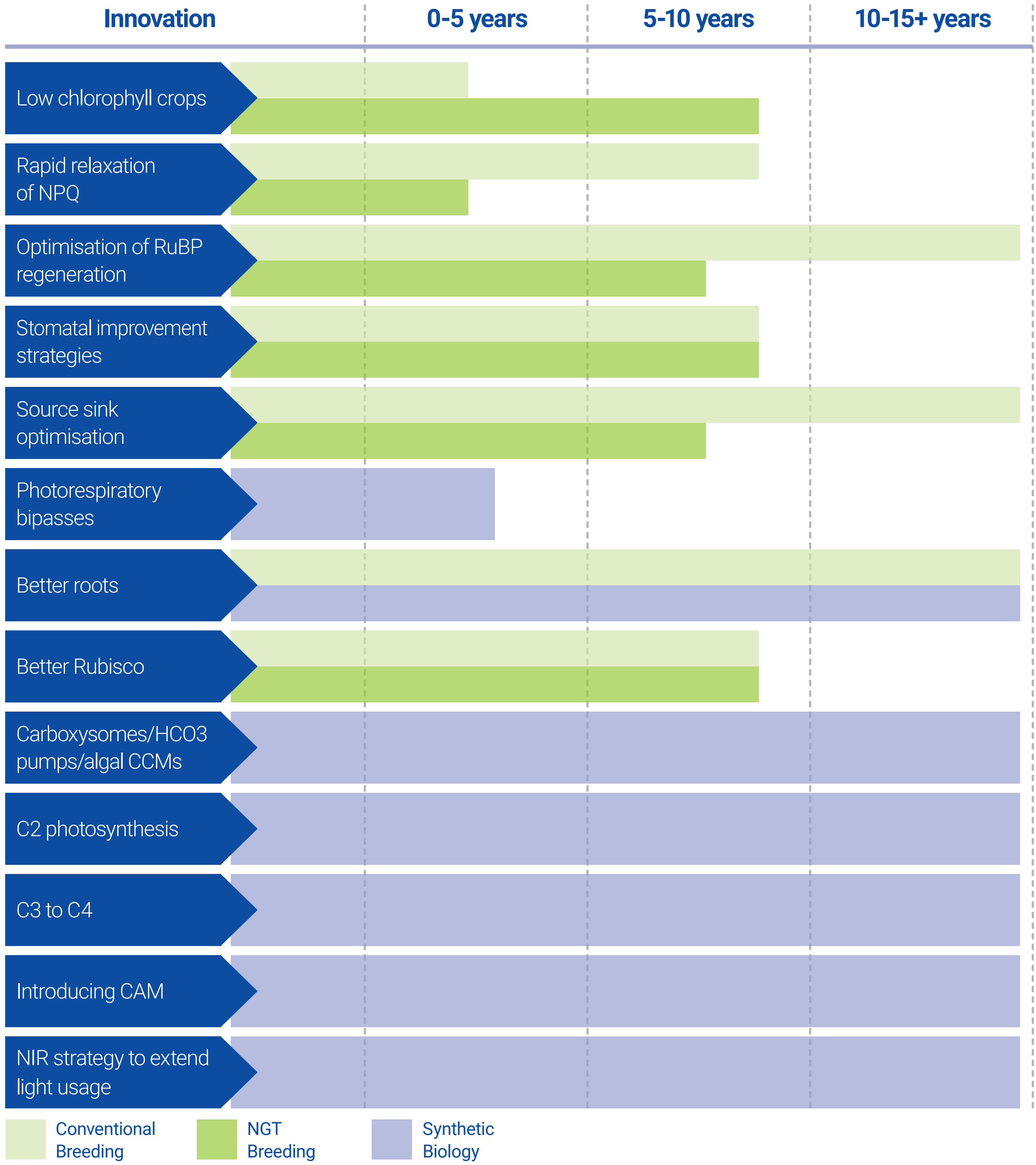
Stakeholder dialogues have emphasized the need for investment in innovation and knowledge-sharing to maintain crop yields under challenging conditions. Enhancing photosynthesis aligns with the Strategic Dialogue on the Future of EU Agriculture recommendations, promoting better farmland management, water-resilient agriculture, and innovative breeding. This approach boosts productivity without expanding farmland, supporting the Biodiversity 2030 strategy by protecting natural spaces. It also reduces fertilizer use and improves water use efficiency.

New bioeconomy optimised crops will strengthen the bioeconomy and meet predicted expansion by delivering cost-effective products for diverse uses including food, bioenergy, and biomaterials. This future proofed sustainable primary production will support the EU's Green Transition and circular economy goals, as outlined in the European Bioeconomy Strategy (2018).

Photosynthesis Improvement Strategies

There are a number of well-known strategies for improvement of photosynthesis efficiency, at various levels of advancement and translation level, many of which are used in CAPITALISE, GAIN4CROPS, PhotoBoost and BestCrop. Each strategy presents different challenges and benefits, and suit particular environments and type of agriculture. No single strategy is applicable to all crops, but combinations of some strategies may give wide ranging benefits to crop resilience and efficiency. Modelling can give insights into the most optimal combinations.

Timeline for strategies to reach crop TRL7



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1 Lower Chlorophyll crops

Crop TRL 5-6

Decreasing the chlorophyll concentration at the top of a crop allows more light to reach the lower canopy, boosting overall plant photosynthetic efficiency. Since chlorophyll complexes are nitrogen rich, better nitrogen use efficiency is achieved. Natural variation has been identified for this trait.

PYI	20%	Timeline for Translation	2-5 years	Benefits

Challenges
Improved field instrumentation, robust model predictions. Stacking with other strategies.

N_2

2 Rapid Relaxation of NPQ

Crop TRL 6

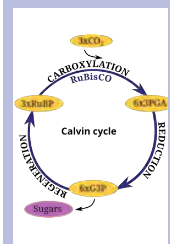
Light intensity can be quite changeable within crop canopies. Sharp fluctuations due to wind movement, overtopping leaves and changing solar angles, lead to less efficient photosynthesis. This strategy looks at minimizing loss of photosynthetic efficiency when leaves are exposed to changes in light intensity. Natural variation has been identified.

PYI	10-20%	Timeline for Translation 5-10 conventional breeding, 0-5 NGTs	Benefits

Challenges - Low-cost sensors for phenotyping needed. Crop models. Understanding NPQ regulation under different environmental conditions.

3 Optimisation of regulation of Calvin cycle and Electron Transport Chain

Crop TRL 5



The Calvin cycle is the primary photosynthetic pathway for assimilation of atmospheric CO_2 in the majority of C3 crops. Manipulating the expression of key enzymes in this cycle and the photosynthetic electron transport chain (ETC) has been shown to enhance photosynthesis and growth.

PYI	Up to 70%	Timeline for Translation	5-10 years NGT, 10+ Conventional breeding

Challenges
Remote sensing systems for selection in the field needed. Acceptance of NBTs.

Benefits

4 Better Rubisco

Crop TRL 1-4

Rubisco activity is highly regulated and coordinated in tandem with other photosynthetic reactions and plant processes. Rubisco is a very inefficient enzyme, its abundance, catalytic properties and regulation of activity are all targets for improvement. Natural variation has been identified for some Rubisco traits. GE is needed for many manipulations.

PYI	60%	Timeline for Translation	5-10 years

Challenges
Predictive modelling, computational and AI tools needed. Acceptance of GM technologies.

Benefits

N_2

5 Adding cyano carboxysomes, HCO3 pumps algal CCMs

CROP TRL 1-2

Introducing carbon concentrating mechanisms (CCMs) into C3 crops is a high-risk, high-reward strategy to boost yields and resilience. CCMs concentrate CO_2 around Rubisco, reducing photorespiration and favouring CO_2 assimilation to boost yield.

PYI	up to 60%	Timeline for Translation	10-15 years

Challenges
Specific expression and accumulation of CCM-related proteins, correct assembly and functionality of protein complexes. Acceptance of GM technologies.

Benefits

CSeq

6 NIR strategy to extend light usage

Crop TRL 2

Green plants absorb light energy in a limited range (400-700nm). Extending the ability of plant pigments to absorb energy from a wider range of the spectrum to 750nm into the far red range can increase light absorption by 20%.

PYI	20%	Timeline for Translation	10-15 years	Benefits

Challenges
Challenges - Modelling to predict far red light dependent gene expression in plants. Cellular models. Acceptance of GM technologies.

7 Stomatal strategies

Crop TRL 2-5

Stomata, are small pores on the surfaces of leaves. The behaviour of stomata determines CO_2 uptake for photosynthesis and water loss through transpiration. There are numerous targets that can be exploited in crops to manipulate stomatal behaviour such as their density, sensitivity and speed of response.

PYI	20%	Timeline for Translation 3-5 years for density, 5-10 years for rapidity	Benefits

Challenges - Identification of the key genes involved. Development of tools for deep phenotyping.

CSeq

8 Synthetic Photorespiratory Bipasses

Crop TRL 4-6

Synthetic Photorespiratory Bypasses circumvent natural photorespiration, a wasteful process involving the loss of 25% of the previously assimilated carbon as CO_2. Photorespiratory bypasses have been extensively developed in Europe and been demonstrated in field trials to significantly increase yield, biomass and grain quality in crops.

PYI	30-50%	Timeline for Translation 0-5 years	Benefits
Challenges - Regulatory approval and acceptance of GM technologies			CSeq

9 C3 to C4

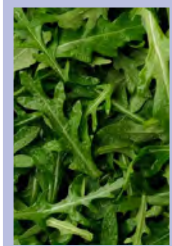
Crop TRL 2-3

C_4 plants employ a biochemical carbon pump that elevates the concentration of CO_2 available to Rubisco, achieving around 50% higher energy conversion efficiency compared to C3 plants. Converting C3 crops to utilize C4 photosynthesis would significantly enhances crop productivity.

PYI	50%	Timeline for Translation 10-15 years	Benefits
Challenges - The genetic blueprint for C_4 photosynthesis. Introduction of multiple transgenes - scientifically and technically highly challenging. Regulatory approval and acceptance of GM technologies.			

10 C2 Photosynthesis

Crop TRL 3



C2 photosynthesis is a simple carbon concentrating mechanism (CCM) that recycles CO_2 released from photorespiration. This has only been identified in Rocket. This natural CO_2 recycling mechanism allows C_2 plants to avoid the significant carbon costs of photorespiration, to ultimately improve carbon-, water-, and nitrogen - use efficiency compared to C_3 close relatives.

PYI	>20%	Timeline for Translation	10-15 years

Challenges

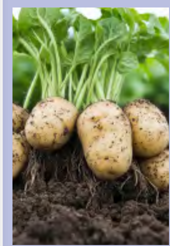
Predictive modelling, computational and AI tools needed. Acceptance of GM technologies.

Benefits

CSeq N₂

11 Source Sink interactions

Crop TRL 3-4



Sinks are the rapidly growing parts of the plant that receive sugars and other compounds from the source, the green photosynthesizing leaves and shoots. Reducing, sink limitations; and better timing and control of source – sink activities help to maximise photosynthetic source production and yield.

PYI	30-50%	Timeline for Translation	5-15 years

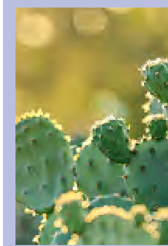
Challenges

Assays to identify sink limitation, better tools for measuring transport limitation in the phloem. Assays for screening in the field.

Benefits

12 Integrating CAM

Crop TRL 2



CAM plants are incredibly water use efficient because they capture CO_2 at night, rather than during the day like C3 and C4 plants. Introducing drought inducible CAM in to C3 plants will significantly reduce transpiration and improve WUE in water stressed conditions.

PYI	yield maintained under drought stress	Timeline for Translation	10-15 years

Challenges

Improved modelling. CAM gene expression and regulation. Significant anatomical changes needed. Low cold tolerance

Benefits

CSeq

13 Better Roots

Crop TRL 2-3

Rhizospheric interactions are a key component of crop performance needed to support improved photosynthesis. Better roots support all other improvement strategies.

PYI	to maintain rather than raise yield	Timeline for Translation	10-15 years	Benefits
Challenges A complex trait, difficult to phenotype in the field.				CSeq

INDEX KEY

Increase in Yield	Synthetic biology
Water Use Efficiency	New Genomic Techniques
CSeq Carbon Sequestation	Conventional breeding
N₂ Nitrogen use efficiency	PYI Potential yield increase expected

Gain4Crops PhotoBoost BestCrop CAPITALISE

Four projects with four approaches to exploiting Photosynthesis

The EC recently invested €27M into four early-stage projects exploring different promising approaches to improve crop photosynthesis and increase yield and crop quality. This strategic approach has advanced the science to early field trials. CAPITALISE, PhotoBoost, GAIN4CROPS and BestCrop present different approaches to improving the efficiency of photosynthesis in a range of different crops using a variety of promising strategies.

CAPITALISE photosynthesis: concepts2crops

The CAPITALISE project is exploiting natural variation in core elements of photosynthesis in diverse collections of Barley, Maize and Tomato, to identify and develop new genetic resources, models and support tools. By using a conventional modern breeding approach, the majority of CAPITALISE outputs can be used directly in the EC without regulation.

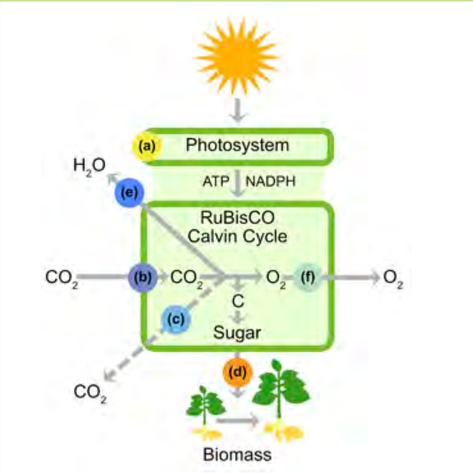
Using a highly multidisciplinary approach CAPITALISE is advancing the following most promising strategies:

i) Tuning the Calvin Cycle (CC) via a) Rubisco activity and activation state & b) activity of the RuBP regeneration phase of CC. ii) Kinetics of photosynthetic responses to changes in irradiance a) kinetics of build-up of CC after an irradiance increase; b) activation of Rubisco after an irradiance increase & c) deactivation of qE component of non photochemical quenching after an irradiance decrease. iii) Tuning leaf chlorophyll via a) Photosystem II antenna size & b) Photosystems density.

The **GAIN4CROPS** project aims to increase photosynthetic energy conversion efficiency using a complementary approach that combines natural variation in photosynthetic carbon assimilation strategies with new-to-nature approaches to overcome photorespiration, the main limitation of energy conversion efficiency in C3 plants, and replace it with a carbon-neutral or carbon-fixing synthetic bypass.

The approach is using high-precision genome editing and extensive interspecific crosses between phylogenetically related C3 crops and their wild C3-C4 intermediate relatives in Sunflower. This is expanding the access to genetic variation beyond the species borders. In a parallel, complementary approach, GAIN4CROPS is designing nature-inspired synthetic versions of C3-C4 intermediates that require fewer genes for implementation, and developing novel pathway designs that are more efficient than naturally occurring variations in photosynthetic carbon assimilation.

GAIN4 CROPS



The **PhotoBoost** approach involves six new and improved strategies that have been exploited to boost photosynthetic efficiency by at least 30% and biomass yields by at least 40%.

PhotoBoost has met their goal by developing enhanced C3 crops (Potato and Rice) that combine one or more of the following strategies: (a) the optimisation of light reactions during photosynthesis; (b) the integration of an algal CCM; (c) the introduction of an engineered photorespiratory bypass mechanism; (d) the optimisation of source–sink capacity (only in potato); and (e) the adaptation of stomatal conductance by the introduction of a hexokinase gene, to improve water-use efficiency. PhotoBoost have also explored the integration of an oxygen scavenging mechanism as a novel strategy (f) to further reduce photorespiration and boost the efficiency of photosynthesis.

The **BestCrop** project adopts the most promising strategies to improve the photosynthetic properties and ozone assimilation capacity of barley by:

i) Tuning leaf chlorophyll content and modifying canopy architecture; ii) Increasing the kinetics of photosynthetic responses to changes in irradiance; iii) Introducing photorespiration bypasses; iv) Modulating stomatal opening, thus increasing the rate of carbon dioxide fixation and ozone assimilation.

In parallel, the resulting barley straw is tailored to: i) increase straw protein content to make it suitable for the development of alternative biolubricants and feed sources; ii) control cellulose/lignin contents and lignin properties to develop straw-based construction panels and polymer composites.

Strategic Research Agenda priority areas

SRA Priority 1: Phenotyping and Validation

Identification of genomic control coefficients

- ✔ Validation of naturally derived innovations with transgenic/genome-edited lines with modified expression of traits underlying genetic determinants. This will explore the genomic basis for established variation in selected traits and the potential for enhancing a trait by altered gene expression in situ e.g. modifying a promotor. The aim is to rapidly establish genomic control coefficients for key physiological pathways. Identification of key genes is the first step i.e. genes underpinning natural variation for a trait.

Identification of diagnostic signatures

- ✔ Identification of novel diagnostic signatures for combinations of traits which improve photosynthetic performance and yield.
- ✔ Ongoing phenotyping to feed back into QTL and GWAS mapping, integrating trait data to allow finer characterisation / confirmation of loci discovered in previous mapping rounds.

SRA Priority 2: Translation of QTL/QTN

Validation in inbred backgrounds

- ✔ Survey elite germplasm (ex PVP)/breeding material, for allele/haplotype variation in candidate genes/QTLs (ideally validated genes) that affect selected traits previously detected as QTLs.
- ✔ Near Isogenic lines (NILs) or similar (e.g. overexpressor of trait gene) in relevant parents.
- ✔ Characterise performance of selected traits in prebreeding and elite lines in controlled & field conditions.

Fine-mapping / candidate gene identification

- ✔ New recombination in offspring
- ✔ Genome editing
- ✔ Physiological characterisation

Facilitate implementation in breeding

- ✔ Develop diagnostic markers

Genomic selection

- ✔ Exploiting advanced understanding of the genetic make-up of crop plants and measurable photosynthesis traits to increase genetic gain of (complex) target traits. Molecular markers will be a key tool to reduce time and costs.
- ✔ Activities based on the recognition that a trait maybe under multi-locus control for which improvement by genomic selection may be necessary.

Building on Project' Key Exploitable Results (KERs) the following "next steps" represent a Strategic Research Agenda (SRA) to speed up the translation of promising photosynthesis strategies towards the breeding sector. Implementing this SRA will require cross sector strategic efforts through Public Private Partnerships (PPP) to develop new collaborations. This should build interdisciplinary synergies, exploit efficiencies of scale, and accelerate research and innovation pathways.

Additional funding from public and private sources including industry, philanthropy, Horizon Europe and follow-on European programmes will be critical to facilitate future crop improvements. This includes exploiting advances in photosynthesis science.

SRA Priority 3: Model-development and improvement

Model-development

- ✔ Use of existing, and generation of new datasets to identify the genetic architecture of plasticity in photosynthetic efficiency and its genetic correlation to growth.
- ✔ Dynamics of fluxes in photosynthesis-related pathways: this will call for new ways for flux profiling and can be used to specify missing regulators.
- ✔ Integration into i) canopy models and ii) crop models.

Model improvement cycle

- ✔ Use models to identify key regulatory steps as a function of relevant environmental conditions.
  i) Genotype to phenotype: Find sequence variation in genes involved in key steps/traits and phenotype corresponding plant material (diversity panels, breeding material).
  ii) Phenotype to genotype: Phenotype variation in critical steps/traits across diversity panels and breeding material and correlate with sequence variation.
- ✔ Feed results into the model-improvement and start the next cycle.

SRA priority 4: Genomic toolbox/Tuning the CBC - using transgenic/ non-transgenic approaches

Advance emerging alternative strategies to provide novel approaches to modify target genes known from transgenic work to improve photosynthesis:

- ✔ Newly identified twelve base pair palindromic sequence from the octopine synthase gene can be used to upregulate multiple genes simultaneously (cis-genic).
- ✔ Deletion of upstream open reading frames with stop codons, this would offer a non-GM approach. Targets are easy to identify and multiple genes can be targeted simultaneously.

SRA Priority 5: Chlorophyll Tuning

- ✔ Demonstrate improved yield and/or increased efficiency in water and nitrogen use.
- ✔ Investigate the potential of increased albedo to mitigate climate change and to increase the efficiency of bifacial photovoltaic systems.
- ✔ Identify novel strategies that allow a chlorophyll gradient concentration through the canopy.
- ✔ Investigate the potential of "staygreen" phenotypes, i.e. delayed senescence on crop yield.
- ✔ Use of new phenotyping tools to quantitatively assess variation and performance.
- ✔ Application of gene editing or conventional breeding to transfer to elite lines.

SRA Priority 6: Into the Field-Crop Performance to advance improvement strategies and deliver data to support Life Cycle Analysis, Socio-economic and Environmental assessments

- ✔ Demonstrate a yield increase of the photosynthetically optimized plants (prebreeding and elite lines) for target crops.
- ✔ Assessment of the inputs required to achieve target crop performance (>10%): fertilizer and/or irrigation vs standard lines.
- ✔ Assess the susceptibility of photosynthetically improved crop varieties to biotic/abiotic stresses vs standard lines.
- ✔ Assess impacts on the soil microbiome of roots e.g. impact of increased root exudates or below ground root biomass and necromass.
- ✔ Assess the impact of the soil microbiome and root properties on above ground photosynthetic activity.
- ✔ Determine the characteristics of the optimized lines for end use e.g., harvest/ processing and identify desirable traits to improve.
- ✔ Assess the suitability of improved varieties for different (changing) environments and farming practices.
- ✔ Acceptability to CITIZENS = CONSUMERS evaluated across different European regions and demographics.
- ✔ Explore the possibilities for stacking of traits.

SRA Priority 7: Phenotyping Tool Development

- ✔ Awareness raising and training about new agile and high throughput phenotyping tools.
- ✔ Exploitation of the chlorophyll a/b ratio non-destructive analysis devices developed by CEA and PSI for use by breeders and growers and researchers in field environments.
- ✔ Improved tools suitable for assessing target traits in field trials and crop breeding programmes.
- ✔ Development of 'breeders eye' tool for assessment of crop health for breeders and grower use in crop breeding and crop management strategies. This is expected to utilise photosynthesis traits as measures of crop health and performance.

SRA Priorities 8: Synthetic Biology approaches

- ✔ Transfer successful synthetic biology strategies (e.g. developed and demonstrated by PhotoBoost) to other food and feed crops.
- ✔ Perform further field trials to confirm robustness and sustainability of generated plant lines showing improved photosynthesis and biomass yield.
- ✔ Perform studies to investigate nutritional value and processing of generated plant lines showing improved photosynthesis and biomass yield.
- ✔ Test further stacking strategies and alternative synthetic biology approaches.
- ✔ Develop an evidence base to support regulatory approval and market adoption of suitable tools and approaches.

Short Term Topic suggestions for Horizon Europe Cluster 6

Building on recent progress in EU projects, follow-on funding in future Cluster 6 work programmes is recommended to advance promising results. Proposed 2026-2027 topics align with the CropBooster Roadmap (CropBooster-P, Grant 817690) and the EPSO Working Group Photosynthesis, Abiotic Stress, Input Use Efficiency.Budgets should build on the earlier calls to reflect costs of inflation.

Photosynthetic resilience of crops in a changing climate (RIA TRL2-4)

Photosynthesis and its connection with plant development, yield, source/sink dynamics and respiration should be key considerations of plant breeding. This needs to be carried out in increasingly challenging field conditions with multiple limitations. This calls for the development of a selection of genetic variants associated with enhanced photosynthetic performance using fine-mapping, validating these variants in elite inbred and heterotic backgrounds and developing diagnostic markers. Use of model-guided germplasm improvement should simultaneously enhance model performance and speed up the development of improved accessions.

1. Non-destructive phenotyping of photosynthesis in response to stress (RIA TRL3-6)

The precision of phenotyping remains a limiting factor in genetic selection approaches. The development of genetic strategies to identify genes of interest, along with the description of increasingly detailed traits, requires the development of non-destructive phenotyping tools with higher resolution. Instrumentation and analysis methods must be developed to achieve quantitative and contextualised measurements for phenotyping of photosynthetic efficiency in plants whether in controlled and instrumented environments or in the field. Additionally, tools for data acquisition, storage, access, and modelling are needed. These spatio-temporal studies are crucial for providing data for model design and plant ideotype research.

2. Improved nitrogen fixation for increased photosynthetic CO_2 assimilation (RIA TRL3-6)

To sustainably enhance agricultural productivity, it is important to improve both photosynthesis and nitrogen fixation. This approach would boost the productivity of existing nitrogen-fixing crops by providing them with more energy for nitrogen fixation and more carbon for root biomass alongside and more carbon for above ground growth and more nitrogen for photosynthesis. By leveraging increases in nitrogen fixation and photosynthesis, we can establish a foundation for high-yielding and sustainable agriculture.

3. Redesigning photosynthesis for crops of the future (RIA TRL4-6)

Recent advances in protein engineering allow the design of new-to-nature enzyme activities that outperform existing enzymes in terms of kinetic properties, selectivity and, when combined into novel metabolic pathways, substrate conversion efficiency. The transfer of new-to-nature and/or new-to-crop pathways into crops enables step changes in photosynthetic carbon conversion efficiency, as well as water and nitrogen use efficiency, that are unlikely to be achievable through canonical breeding approaches. A research programme aimed at achieving sustainable crop improvement should be accompanied by a research theme that addresses the inherent limitations of conventional crop breeding.

Key enabling technologies: tools to support translation

Recent innovations and emerging technologies are transforming plant science and crop breeding. Key enabling technologies include lab-based phenotyping devices; high throughput devices for screening in laboratory/indoor growth facilities; field devices for researchers and breeders to rapidly screen traits; and large-scale deployable sensors and satellite imaging.

Science and technology discussions at two CAPITALISE Workshops focussed on predicting the current and future trends shaping plant breeding, and highlighting innovations needed to accelerate the translation of results from lab to field. The main insights are summarized below.

Innovations/Technologies	Reasoning
GE and precision breeding	Essential to speed up the breeding process enabling incorporation of improved traits to crops in response to rapidly changing climatic conditions.
Synthetic biology for whole pathway development	An active research area enabled through gene editing. Examples include rewiring photorespiration (GAIN4CROPS and BestCrop) as well as the use of plant systems as cell factories.
Expanding the genetic diversity of crops	Allelic diversity in modern crops has been severely reduced during the selection process. Genetic diversification of crops can be improved by exploiting crop genetic resource collections to mine for improved stress resilience, adaptivity and productivity traits.
Improved multi-trait breeding approaches required	Conventional or NGT breeding methods to achieve resilience to multiple (but potentially short term) stresses associated with climate change to improve yield, resiliencies and resource use efficiencies, that will enable photosynthetic improvements to be 'expressed' under wide agro-ecological scenarios.
Life Cycle Analysis	To quantify the real cost-benefits of the adoption of agronomic innovations promoting better decision making for investment.
"On-farm" living labs to advance from controlled environments to field phenotyping	There is an urgent need to facilitate the rapid translation of research towards breeding programmes. Field phenotyping is a vital step to validate outputs and progress crop TRLs. Suitable sites, including "on-farm" living labs are needed to allow for testing under real world and experimental conditions.
Sensors, digitisation, AI data platforms and Envirotyping	Rapidly emerging technologies in agriculture will enable better field phenotyping, as well as improved mechanisation and robotics in plant breeding and farming practices. Plant breeding is increasingly 'big data driven' and innovations in AI and modelling are providing tools to collect, process and analyse large data sets. Better and cheaper sensors will allow 'Envirotyping' i.e. the use of environmental factors eg. soil, climate, local ecology to compliment genotyping and phenotyping to improve crop modelling, phenotype predictions and management practices.

The need for better phenotyping towards field-based photosynthesis

Advances in tools and technologies are now enabling the identification, quantification, and application of photosynthesis traits in field-based breeding programs. The tools currently used by researchers are not always user friendly enough to be embraced by breeders and growers. Scaling up effective translation to the field requires new breeder-friendly phenotyping tools using standardized tools & methods to measure photosynthesis. This will complement the expertise of breeders, facilitating faster adoption of scientific advances.

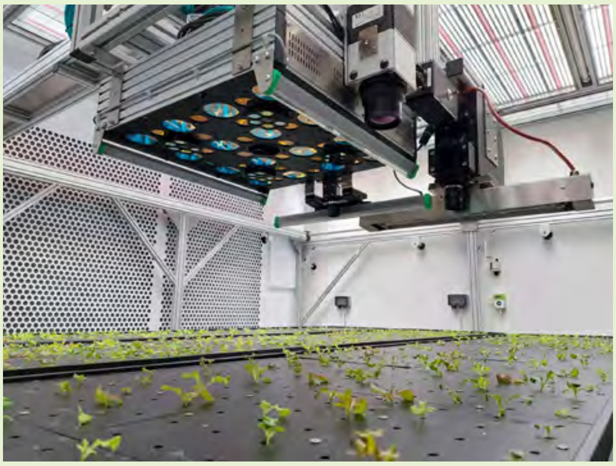
Credit PSI

A photosynthesis based accelerated breeding toolbox for next generation crop resilience breeding programmes.

Rapid phenotyping of photosynthesis in the field is critical to understand the limits to crop performance under changing environmental conditions. Easy-to-use sensor arrays for rapid phenotyping are integrated with advanced predictive modelling. This quantitative "Breeders Fitness Eye 2.0" is a new approach for breeders to access the wealth of information on plant performance.

High-throughput phenotyping infrastructures are becoming available in crop research institutes across Europe. This expands the scale and robustness of experimental work. The new NPEC system at Wageningen allows high-throughput phenotyping to assess plant performance. At IPK, the recently installed Phenosphere represents an advanced indoor high-throughput phenotyping system using strictly controlled environmental conditions to run climate simulations. These systems represent a new level of indoor phenotyping to accelerate translation of research.

The PhenoSphere at IPK - Credit IPK



NPEC at Wageningen - Credit WUR

Ground robotics and state of the art field phenotyping

Field phenotyping using manual methods is labor-intensive and inefficient. Advanced field phenotyping systems are increasingly vital, enabling the collection of extensive data to support analysis and decision-making. At the CAPITALISE workshop in France, the PhenoMobile at Arvalis showcased high-throughput field phenotyping capabilities. This fully automated, ultra-precise system continuously monitors multiple crop traits non-destructively in field conditions. The integration of AI and robotics is anticipated to play a growing role in advancing crop science and improving phenotyping efficiency.

Two types of phenotyping devices : the large phenomobile, and the 'literal' (a light and easy-to-carry system). Credit : Arvalis and Hiphen

Remote sensing for Photosynthesis Crop Improvement and Carbon Sequestration

Advances in remote sensing have enhanced photosynthesis monitoring for crop improvement. Optical, thermal, and microwave sensing at canopy scale provide key agronomic data, including height, leaf area index, albedo, temperature, and soil moisture. Sensor platforms range from proximal (e.g., towers, UAVs) to intermediate (aircraft) and satellite levels. Applications include phenotyping, land-use monitoring, yield forecasting, optimisation, and ecosystem service estimation, such as carbon sequestration. Satellites support precision agriculture by estimating nitrogen deficiency via chlorophyll content and using thermal imaging for irrigation management. Integrating satellite data, local observations, expert insights, and models is critical for robust crop improvement strategies amid climate change.

A Data Platform for Photosynthesis research

Targeting complex and dynamic processes such as photosynthesis in next generation crop breeding will require a highly integrated approach. Data platforms to collect, manage and model direct and indirect photosynthesis data will be an important future resource. The recently established Jan IngenHousz Institute (JII), based in Wageningen (NL), has a mission to address this issue on a global scale to accelerate cohesion at multiple levels. An open science platform is being developed to probe photosynthesis at scale, under field conditions. This will link the development and application of novel, high-throughput sensors, data science, and genetic resources at previously unattainable scales. These tools will be deployed among a community of researchers to link the EU with teams in many international countries, across disciplines, from basic to highly applied science and engineering, to understand the conditions limiting photosynthesis. Through sharing resources and knowledge, rapid progress is expected to realize true gains in productivity.

JII Jan IngenHousz Institute

The potential of computational models to accelerate translation of improved photosynthesis to crops

Modelling provides the possibility to guide the identification of modifications (e.g. gene knock-outs, mix-and-match strategies, protein engineering) that can modify photosynthesis in a desired direction, to pinpoint genes that control photosynthesis-related traits, and to identify genotypes with improved photosynthesis under specific conditions.

Current mechanistic models of photosynthesis often lack accurate species-specific parameterization due to challenges in generating high-quality data, that can increase identifiable parameters and/or reduce variability. Resolving this calls for new measuring technologies to obtain data that facilitate model parameterization. These models hold significant potential to identify breeding targets by: (i) relying on the coupling of mechanistic models with machine / deep learning approaches via (bio)physics-constrained neural networks, (ii) advances in in *silico* design of metabolic engineering strategies, based either on enzyme engineering, mix-and-match strategies, and novel chemistries, and (iii) hybrid models that integrate photosynthesis in larger metabolic and developmental contexts. In addition, developing genotype-specific photosynthesis models will pave the way for identification of condition-specific limitations to photosynthesis, that can be tested with advances in measurement technologies.

To fulfill the potential of photosynthesis models, future projects must prioritise testing model predictions from the outset, embedding a robust model-test-refine cycle throughout their duration. Integrating expertise across scales will benefit from involving multiple modelers in projects. Establishing an Integrative Photosynthesis Modelling Network could coordinate synergistic efforts across projects.

Barriers to translation of research

Photosynthesis improvement strategies in crops offer significant potential to increase or stabilise yields and often reduce inputs. These strategies address multiple societal challenges and policy objectives. But most photosynthesis research remains underutilised by industry. Through stakeholder workshops and surveys, we examined the barriers to industry translation from both researcher and value chain perspectives.

Challenges	Problem	Possible solutions
Historical lack of funding for plant breeding innovation by Europe.	We estimated that less that 0.5% of the Horizon 2020 budget was used for crop improvement.	Inclusion of more photosynthesis and resilience crop related calls in Cluster 6 and FP10.
Inconsistent (3-5 years) funding streams in plant research.	Funding too limited to deliver significant increases in TRLs for crops; disruption of successful consortia slows progress and disrupts pathways to impact.	Conditional longer funding available for successful consortia to develop innovation for successful projects delivering the Key Exploitable Results requested.
Access to germplasm sources	Industry would benefit if public researchers worked on more modern germplasm. Industry would like better access to germplasm collections	Collaboration with industry encouraged in PPP. Funding for better curation and characterisation of germplasm collections for use.
Adoption of photosynthesis as a key trait by breeders.	Survey showed that the benefits of improved photosynthesis were not known by many breeders	Education around what improved photosynthesis can do in crops. More research collaborations encouraged with Industry.
Infrastructure problems for carrying out field trials in public research organisation.	Lab to field is problematic for public researchers due to the limited facilities, and personnel for field trials	Promote collaborations with industry partners. Promote the use of the network of EC Living Labs.
A need for better sensors and phenotyping tools, for field work	Photosynthesis is a complex trait, user friendly devices for breeder/grower communities are needed if promising traits are to be adopted.	Sensor development and AI are key for quickly advancing photosynthesis translation and should be invested in. CAPITALISE is developing field devices.
IP and Industry collaborations	IP and the freedom to operate is a significant issue for breeders to take up research results	More clarity in collaborative agreements between public researchers and industry.
Acceptance of NGT and genetically modified crops and derived products.	The EC funds innovative research using NGTs and genetically modified plants. Uptake by industry is hampered by perceived public opinion.	A need to speed up developing policy for more lenience towards NBTs in Europe. Education around the need for NGTs as solutions to growing societal needs.

LCA assessments

Life Cycle Assessment (LCA) supports sustainability-focused breeding by evaluating how target traits impact the production chain. It aids decision-makers and breeders in anticipating environmental, social, and economic effects, requiring detailed data on inputs, outputs, and scaling effects from small to large processes. LCA aims to guide the development of sustainable crops with enhanced photosynthesis. Under this scenario, there is a challenge to perform a prospective analysis of a low-to-mid TRL, and to scale up to higher TRLs. Field trials must capture real-world environmental and economic data to refine models. Educational tools, including gamification, can engage decision-makers and citizens, illustrating the costs, benefits, and trade-offs of photosynthesis strategies and breeding methods.

The need for societal acceptance

In Europe today crop breeding is primarily based on conventional modern breeding techniques. The acceptance of New Genomic Techniques is important for tackling global challenges such as food security, sustainability, and climate change. NGT derived crops are controversial due to concerns about safety, ethics, and environmental impacts. For these crops to gain broader acceptance, it is crucial to address concerns through clear communication, rigorous regulation, and education on risk.

The CAPITALISE, PhotoBoost, and Gain4Crops projects explored different aspects of photosynthesis research. The social sciences teams focussed on the attitudes of downstream users - consumers, farmers, and the agri-food industry - towards enhanced photosynthesis, using modern breeding techniques, and the potential adoption of more controversial New Breeding Tools (=NGTs). These biotechnology tools enable direct gene editing or in synthetic biology create greater levels of genetic modification to advance crop breeding cycle. Openness to biotechnology and NGTs increased when the drivers were linked to societal challenges, such as improving crop resilience to climate change.

The work across the projects identified the need for engagement with value chain actors and citizens to improve literacy on photosynthesis, and the use of biotechnology in crop breeding to effectively enhance understanding and acceptance of NGTs.

The following recommendations are based on consultations from the three projects:

- **Develop an EU-wide communication strategy in concert with the new policy on NGTs and perceived risks**
- **Ease regulation for sustainability-focussed crop improvement that utilises SDN-1 techniques**
- **Balance research funding between discovery research and applied plant breeding programmes that capture societal needs and maximise sustainability**
- **Involve farmers in early stages of problem identification and in formal field trials and phenotyping.**

This brochure, and a longer version of this Roadmap can be accessed here.

Contributors to the roadmap

The Consortia from 4 photosynthesis projects:

CAPITALISE photosynthesis: concepts2crops; GAIN4 CROPS; BestCrop; PhotoBoost

CAPITALISE Workshops: 1&2: The needs of Industry; The barriers to translating academic research to crop breeders:

Nick Vangheluwe (Euroseeds); Amrit Nanda (Plants for the Future ETP); Frank Ludewig (KWS); Massimiliano Beretta & Anna Giulia Boni (ISI Sementi); Carlos Baixauli Soria (Fundación Cajamar); Padraic Flood (InFarm); Alan Schulman (EPSO); Bill Wirtz (Consumer Choice Centre); Dimitri Tolleter (Gain4Crops); Jonathan Menery (PhotoBoost); Laurens Pauwels (VIB-UGENT); Lauren Chappell (VeGIN);Julia Hammermeister (German Farmers association); Francesco Pascolo (INsociety); Anna Santoro (HaDEA); Tomasz Calikowski (DG R&I); Jeremy Harbinson (Wageningen University); Louisa Dever & Ritchie Head (CERATIUM BV)

Workshop 3 Translational Photosynthesis, co organised by CAPITALISE and the French Groupment de Recherche, involved the 4 photosynthesis projects and invited experts.

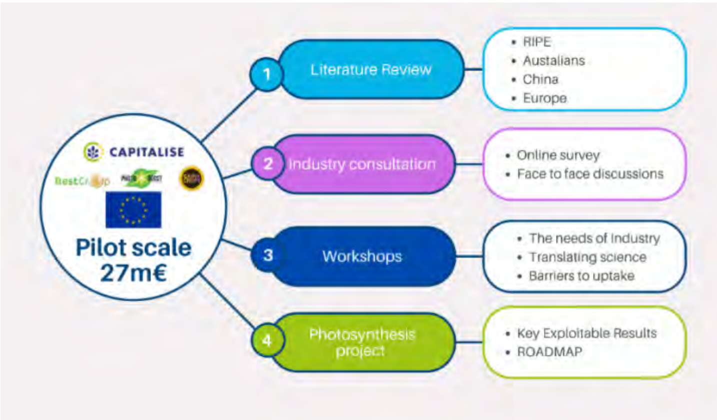
Mark Aarts (Wageningen University), Jean Alric (CEA), Latfi Amel (CNRS), Massimiliano Beretta (ISI Sementi), Florian Busch (University of Birmingham), Mirko Busto (INsociety), Stefano Caffarri (Aix-Marseille University), Fabien Chardon (INRAE), Nicholas Cheron (CNRS), Laurent Cournac (INRAE) Roberta Croce (Free University Amsterdam), Etienne Delannoy (INRAE), Matteo Dell'Acqua(Scuola Superiore Santa'Anna Pisa), Louisa Dever (CERATIUM BV), Sylvie Dinant (INRAE), Steven Driever (Wageningen University), John Ferguson (University of Essex), Benjamin Field (CNRS/CEA), Inaki Garcia de Cortazar (INRAE), Bernard Genty (CEA), Anna Guila Boni (ISI Sementi), Jeremy Harbinson (Wageningen University), James Hartwell (University of Liverpool), Michel Havaux (CEA), Ritchie Head (CERATIUM BV), Michael Hodges (CNRS), Stefane Jézéquel (Arvalis), Xenie Johnson (CEA), Steve Kelly (University of Oxford), David Kramer (Jan Ingenhousz Institute), Anja Krieger-Liszkay (CEA), Johannes Kromdijk (University of Cambridge), Helene Launay (Aix-Marseille University), Nathalie Leonhardt (CEA), Yonghua Li-Beisson (CEA), Marjorie Lundgren (Lancaster University), Fabienne Maignan (CEA), Anne Marmagne (INRAE), Antoine Martin (CNRS), Jonathan Menary (University of Oxford), Tomas Morosinotto (University of Padova), Erik Murchie (University of Nottingham), Zoran Nikoloski (University of Potsdam), Greta Nölke (Fraunhofer IME), Paolo Pesaresi (University of Milan), Stefan Schillberg (Fraunhofer IME), Pallavi, Singh (University of Essex), Samuel Taylor (Lancaster University), Dimitri Tolleter (CEA), Alessandro Tondelli (CREA) , Stefania Viola (CEA), Andreas Weber (Heinrich-Heine University Düsseldorf).

Additional Contributions: Gemma Molero (KWS), Petra Jorasch (Euroseeds).

2 Introduction

This roadmap outlines the three different approaches in photosynthesis improvement strategies by the 3 EC funded sister projects CAPITALISE, PhotoBoost and GAIN4CROPS, and benefits from additional input from the BestCrop consortium. These projects have the joint goal of improving the efficiency of photosynthesis in crops. This roadmap discusses how improving photosynthesis can play a crucial role in developing sustainable climate resilient crop varieties to meet the needs of a growing population in the midst of a climate crisis. The outputs of these projects address industry and consumer needs for plant-based products. This roadmap identifies knowledge gaps that need to be filled to take promising results for the benefit of society as a whole. This includes milestones, challenges and the forward thinking needed to get the timely translation of this cutting-edge research towards use by breeders, farmers and eventually society. It also outlines how crop breeding for photosynthesis addresses EC policy as well as offering solutions to the growing current climate changes crisis and mitigating its devastating effects on crop productivity.

2.1 The Road mapping process



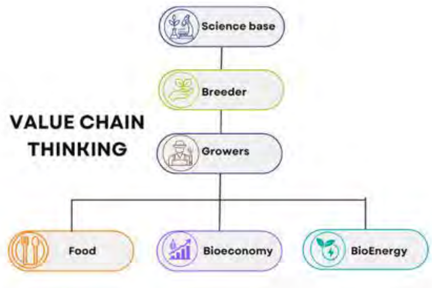


Figure 2 An overview of the multiple steps in the design of the Strategic Agenda and Roadmap.

This roadmap has been developed based on:

- Literature reviews.
- Results from four H2020 photosynthesis projects: CAPITALISE, Gain4Crops, PhotoBoost and BestCrop.
- Stakeholder opinions from both online breeder and grower surveys and direct inputs (over 70 respondents).
- Two workshops to identify: (1) The needs of Industry and, (2) The barriers to translating academic research to crop breeders. This engaged 20 representatives from breeders, growers, vertical farmers, academics/EPSO representatives, consumers, crop genetic engineers, horticulturists, trade organisations, Euroseeds and the Plants for the Future Technology Platform.
- A Translational Photosynthesis workshop, co-organised by CAPITALISE and the French Groupment de Recherche. This brought together 50+ academics and industry representatives.
- Social Sciences Stakeholder Engagement with consumers, farmers and breeders.

2.2 Contributors

This roadmap and strategic agenda has been a joint effort with multiple contributions. This has included many people. Here we list the consortia and many of the attendees and writers who have brought their knowledge and expertise to the process. We offer our sincerest apologies for any oversights.

The Consortia from 4 photosynthesis projects:

CAPITALISE Workshops: 1&2: The needs of Industry; The barriers to translating academic research to crop breeders:

Nick Vangheluwe (Euroseeds); Amrit Nanda (Plants for the Future ETP); Frank Ludewig (KWS); Massimiliano Beretta & Anna Giulia Boni (ISI Sementi); Carlos Baixauli Soria (Fundación Cajamar); Padraic Flood (InFarm); Alan Schulman (EPSO); Bill Wirtz (Consumer Choice Centre); Dimitri Tolleter (Gain4Crops); Jonathan Menery (PhotoBoost); Laurens Pauwels (VIB-UGENT); Lauren Chappell (VeGIN); Julia Hammermeister (German Farmers association); Francesco Pascolo (INsociety); Anna Santoro (HaDEA); Tomasz Calikowski (DG R&I); Jeremy Harbinson (Wageningen University); Louisa Dever & Ritchie Head (CERATIUM BV)

Workshop 3 Translational Photosynthesis, co organised by CAPITALISE and the French Groupment de Recherche, involved the 4 photosynthesis projects and invited experts.

Mark Aarts (Wageningen University); Jean Alric (CEA); Latfi Amel (CNRS); Massimiliano Beretta (ISI Sementi); Florian Busch (University of Birmingham); Mirko Busto (INsociety); Stefano Caffarri (Aix-Marseille University); Fabien Chardon (INRAE); Nicholas Cheron (CNRS); Laurent Cournac (INRAE) Roberta Croce (Free University Amsterdam); Etienne Delannoy (INRAE); Matteo Dell'Acqua(Scuola Superiore Santa'Anna Pisa); Louisa Dever (CERATIUM BV); Sylvie Dinant (INRAE); Steven Driever (Wageningen University); John Ferguson (University of Essex); Benjamin Field (CNRS/CEA); Inaki Garcia de Cortazar (INRAE); Bernard Genty (CEA); Anna Guila Boni (ISI Sementi); Jeremy Harbinson (Wageningen University); James Hartwell (University of Liverpool); Michel Havaux (CEA); Ritchie Head (CERATIUM BV); Michael Hodges (CNRS); Stefane Jézéquel (Arvalis); Xenie Johnson (CEA); Steve Kelly (University of Oxford); David Kramer (Jan Ingenhousz Institute); Anja Krieger-Liszkay (CEA); Johannes Kromdijk (University of Cambridge); Helene Launay (Aix-Marseille University); Nathalie Leonhardt (CEA); Yonghua Li-Beisson (CEA); Marjorie Lundgren (Lancaster University); Fabienne Maignan (CEA); Anne Marmagne (INRAE); Antoine Martin (CNRS); Jonathan Menary (University of Oxford); Tomas Morosinotto (University of Padova); Erik Murchie (University of Nottingham); Zoran Nikoloski (University of Potsdam); Greta Nölke (Fraunhofer IME); Paolo Pesaresi (University of Milan); Stefan Schillberg (Fraunhofer IME); Pallavi; Singh (University of Essex); Samuel Taylor (Lancaster University); Dimitri Tolleter (CEA); Alessandro Tondelli (CREA) ; Stefania Viola (CEA); Andreas Weber (Heinrich-Heine University Düsseldorf).

Workshop 4 Improving Crop Yield and Resilience in a Changing Climate

Jean Alric CEA; Tomasz Calikowski DG RTD; Frédéric Debode Walloon Agricultural Research centre ; Louisa Dever Ceratium BV; Jeremy Harbinson Wageningen University; Ritchie Head Ceratium BV; Götz Hensel Heinrich-Heine University; Petra Jorasch Euroseeds; Giulia Meloni DG RTD; Amrit Nanda Plants for the Future; Greta Nölke Fraunhofer IME; Paolo Pesaresi University of Milan; Mara Sgroi DG Sante; Stefan Schillberg Fraunhofer IME; Urte Schlüter Heinrich-Heine University; Laia Segura Broncano Heinrich-Heine University; Katrijn Van Laere ILVO; Nick Vangheluwe Euroseeds; Lukas Varnas EC project officer.

Additional Contributions: Gemma Molero (KWS); Christina Olsen (Ceratium).

3 Society Needs More Resilient Crops

There is an urgent need for improved climate resilient crops to mitigate the effects of abiotic stresses linked to a changing climate. Future food security to meet the needs of a growing global population and the shift to a biobased economy both require efficient sustainable agricultural production. To protect biodiversity, it is important that yields are increased to meet future demands without needing more land for growing crops, leaving space for nature.

Addressing these challenges requires a combination of adaptation and mitigation strategies, technological innovation, and changes in agricultural practices to ensure food security and environmental sustainability. Future crops, irrespective of farming systems, need to respond effectively to multifactorial changes related to a changing climate and environment. A coherent, multifaceted approach is needed, supported by investment, cross sector collaborations and a supportive regulatory and policy environment that encompasses sustainability challenges and citizen concerns.

Science driven crop improvements are making huge steps towards addressing these societal challenges, however there is a major bottle neck in the pipeline between research breakthroughs and take up by industry. **This roadmap highlights how improved photosynthesis in crops is part of the solution.** It explores the challenges faced by researchers and industry in taking research results to the grower and consumer and highlights the potential future directions for photosynthesis research to meet breeder, grower and society needs. This includes recommendations for Research and Innovation and timescales to inform key actors in the value chain, policy makers, and public/private sector funders.

Improving photosynthesis is one of the most promising options to improve yields, increase sustainability, and achieve global food security

4 Photosynthesis driving crops of the future

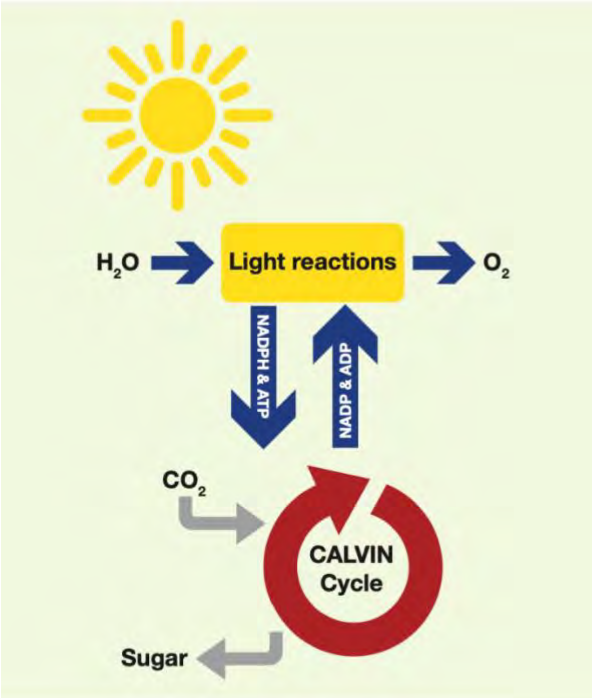


Figure 3 A simplified schematic of photosynthesis

Photosynthesis is a fundamental process that drives plant growth and productivity by converting light energy into chemical energy. In the context of plant breeding, photosynthesis-related traits have the potential to significantly enhance crop yields and resilience (Croce et al., 2024). While photosynthesis is a promising science driven approach and has attracted the interest of commercial breeders it has not gained traction in breeding programmes in part due to complexity and difficulties for selection. Recent scientific advances have empirically shown photosynthesis has the potential to increase or sustain yields in the field and has the potential to do so the face of increasing abiotic stresses linked to climate change. A key attraction is multiple promising strategies to exploit and options to combine and stack traits to address different stresses that reflect real world environments. By improving crop efficiency there will be opportunities to maintain or increase yields with lower inputs.

Recent advances in tools and technologies is enabling improvements in photosynthesis traits to be identified, quantified and exploited

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Science driven approaches can now supply key outputs for use by breeders for climate smart crop development. Despite this, the crop breeding sector estimates that developing new varieties from research outputs for key resilience and yield traits can take 10-15 years. Given the increasing pressure on agricultural systems, **the time to translate photosynthesis research is now.**

4.1 Why Photosynthesis is part of the solution

Photosynthesis is a complex multi-component and a very inefficient process. Multiple bottlenecks have been identified for potential optimisation. It is the final unexploited trait from the Yield equation with room for improvement in crops.

The potential yield (YP) a crop can attain without abiotic stresses is dependent on 4 components.

- ✓ Ei - solar radiation interception efficiency,
- ✓ Ec - solar energy conversion efficiency into biomass,
- ✓ Ep - harvest index and
- ✓ Q - the solar radiation received over a growing season per unit area of land (Long et al., 2015).

Where

$$YP=Q.Ei.Ec.Ep$$

Successful breeding programmes have significantly improved **Ep** and **Ei** through optimising crop architecture, (e.g. shorter stemmed cereals to avoid lodging, better angled leaves to maximise light absorption), and are now close to their theoretical maximum. However, **Ec** solar energy conversion efficiency (photosynthesis), is operating at around a fifth of its theoretical maximum and is therefore the last remaining trait left for significant improvement regarding **YP** (Zhu et al., 2010, Long et al., 2015).

Until relatively recently, quantifying the actual benefits of improved photosynthesis to crop yield has been difficult to demonstrate. Recent advances in genomics, high throughput phenotyping, field phenotyping, modelling and other -omics has allowed scientists to demonstrate improved photosynthetic efficiency and its benefits in laboratory grown plants and field trials in genetically modified plants. Table 1 below outlines some recent photosynthesis improvement breakthroughs in field trials for crop plants.

Table 1 Recent crop improvements through improved photosynthesis in field trials

 Barley	Pale green barley line with 40-50% reduced transpiration rate under drought stress and 40% increased photosynthetic efficiency under high light conditions. (Persello *et al*., 2024). BestCrop project
 Potato	12-45% enhanced tuber yield by expressing of a glycolate dehydrogenase polyprotein (DEFp). (Nölke and Schillberg 2020) PhotoBoost project
	17-42% enhanced tuber yield by introduction of algal carbon concentration mechanism components into potato chloroplast. (PhotoBoost project, unpublished results)
	25-31% enhanced yield and photosynthesis related metabolites by integration of a novel oxygen scavenging pathway. (PhotoBoost project, unpublished results)
 Soybean	33% seed yield by accelerating recovery from photoprotection (De Souza *et al.,* 2022).
 **Rice**	41-68% yield increase through overexpression of a transcription factor that regulates photosynthetic capacity (Wei *et al*., (2022)
	17-28% enhanced yield by overexpressing Rubisco (Yoon *et al.,* 2020).

The benefits of improved photosynthesis to crops are not simply increased yield (or biomass). By improving carbon gain, other closely related traits such as improved water use efficiency, nitrogen use efficiency and enhanced climate resilience traits are additional important benefits (Smith *et al*., 2023, Long *et al*,.2015, Baslam *et al*.,2020).

5 Four projects with four approaches

The EC recently invested €27M into four early-stage projects exploring different promising approaches to improve crop photosynthesis and increase yield and crop quality. This strategic approach has advanced the science to early field trials. CAPITALISE, PhotoBoost, GAIN4CROPS and BestCrop present different approaches to improving the efficiency of photosynthesis in a range of different crops using a variety of promising strategies.

5.1 CAPITALISE

The *CAPITALISE* project is exploiting natural variation in core elements of photosynthesis in diverse collections of Barley, Maize and Tomato to identify and develop new genetic resources and support tools. CAPITALISE is using a conventional modern breeding approach which means the majority of their outputs can be used directly in the EC without regulation.

The CAPITALISE project addresses 3 core themes to boost photosynthetic efficiency by more than 10%:

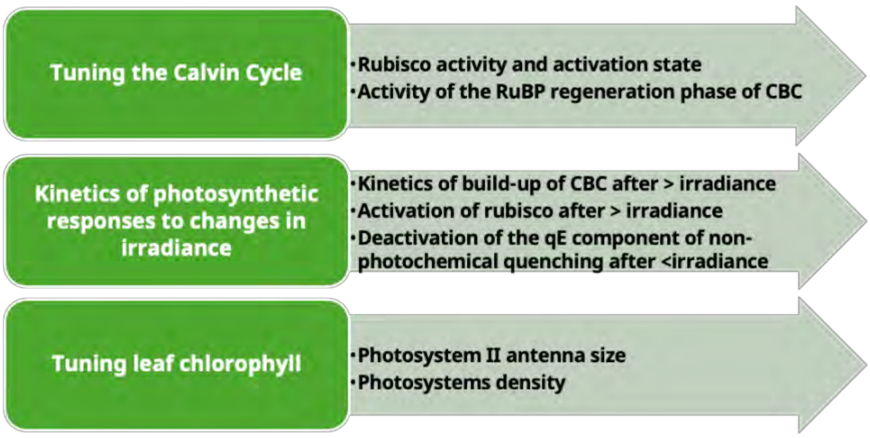


Figure 4 Most Valuable Players - MoVAPS

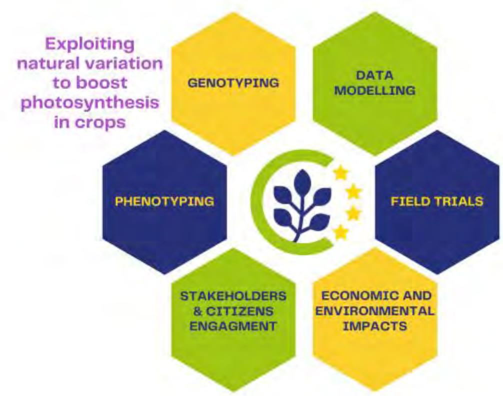


Figure 5 CAPITALISE Approach.

CAPITALISE is delivering the following outputs associated with improved photosynthetic efficiency to provide breeders with new translatable knowledge and tools .

- Advanced genetic materials (germplasm),
- Sequence data,
- Extensive phenotyping data
- QTLs
- Molecular markers
- Whole plant performance traits
- Physiological characterisation of contrasting genetic variants
- Field trials for chlorophyll tuning/leaf greening as a demonstration of a MoVaP translation
- Advanced models of the Calvin Benson Cycle (CBC) and C4 photosynthesis using integrated multi-trait and time series analyses.
- A novel tool-box to guide users about promotor analysis to identify motifs that could be targeted for crop improvement.
- Novel germplasm, to be used as proofs of concepts.
- Two non-invasive methods for the estimation of total chlorophyll and chl a/b ratio and advanced devices that can be adapted for non-destructive field sampling.

5.2 GAIN4CROPS

The GAIN4CROPS project aims to increase photosynthetic energy conversion efficiency using a complementary approach that combines natural variation in photosynthetic carbon assimilation strategies with new-to-nature approaches to overcome the main limitation of energy conversion efficiency in C3 plants, photorespiration, and replace it with a carbon-neutral or carbon-fixing synthetic bypass.

GAIN4CROPS is unravelling the genetic blueprint of C3-C4 intermediate photosynthesis (also known as C2 photosynthesis) with the goal of introducing the genetic traits that control this more efficient form of photosynthesis into sunflower. The approach is using high-precision genome editing and extensive interspecific crosses between phylogenetically related C3 crops and their wild C3-C4 intermediate relatives. This is expanding the access to genetic variation beyond the species borders. In a parallel, complementary approach, GAIN4CROPS is designing nature-inspired synthetic versions of C3-C4 intermediates that require fewer genes for implementation, and developing novel pathway designs that are more efficient than naturally occurring variations in photosynthetic carbon assimilation. Through its technology-agnostic approach, GAIN4CROPS avoids the constraints on efficiency improvement imposed by intraspecific genetic and trait variation.

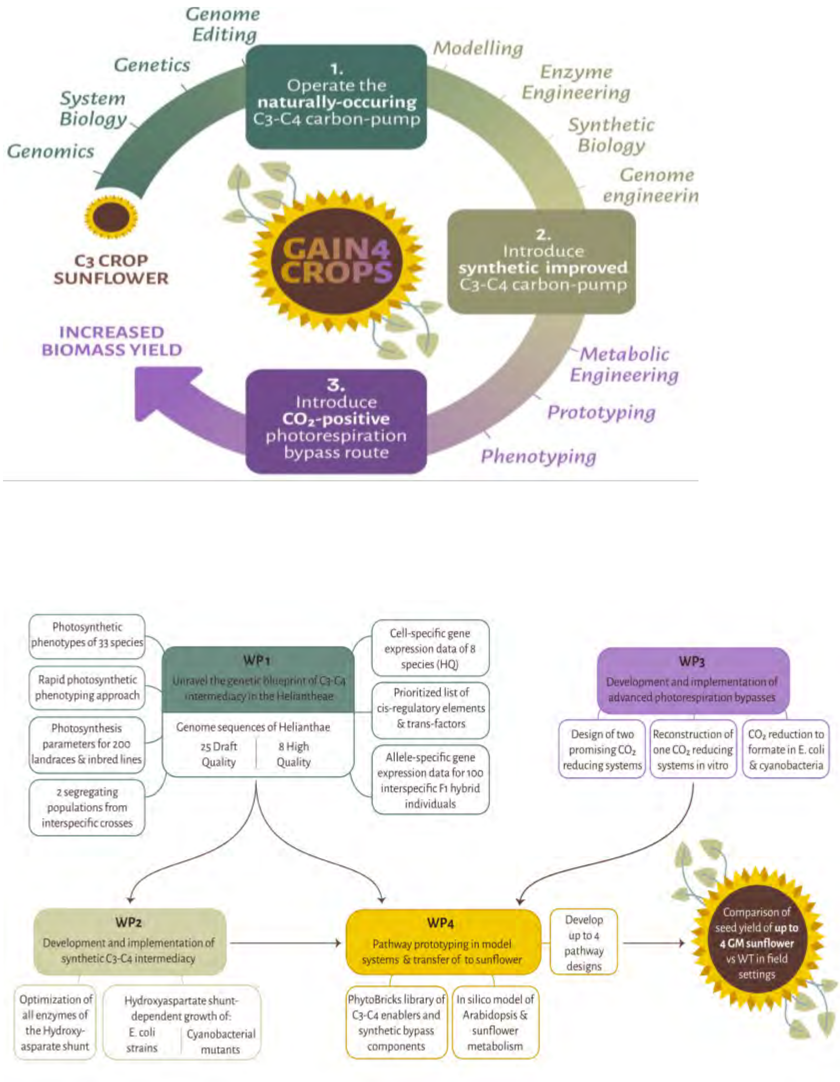


Figure 6 Schematic overview of the GAIN4CROPS project.

GAIN4CROPS is delivering the following outputs associated with improved photosynthetic efficiency.

- Genome sequences of 35 C3, C3-C4 intermediate (C2) and C4 relatives of sunflower
- Deep genotyping data on sunflower cultivars with different photosynthetic efficiencies
- Photosynthetic phenotyping data on 33 Asteraceae species to map the photosynthetic trait landscape in the phylogenetic neighbourhood of sunflower
- Field and laboratory photosynthetic phenotyping data on 200 sunflower landraces and inbred lines
- 2 segregating populations from Interspecific crosses between sunflower and related and related wild species with contrasting photosynthetic efficiencies
- Cell-specific gene expression data of 8 C3, C2, and C4 relatives of sunflower
- Novel phenotyping methods that enable rapid screening of cultivars for photosynthetic efficiency
- Computational models of various synthetic photorespiration bypass designs
- Libraries of genetic parts for the construction of synthetic bypasses and of cis- and trans-regulatory elements that control subtraits of C2 photosynthesis

- Proof-of-concept for synthetic pathway designs in prokaryotic and land plant prototyping systems
- A sunflower prototype expressing a synthetic photorespiration bypass that shows at least 10% increase in photosynthetic efficiency

5.3 PhotoBoost

The PhotoBoost **Project** has significantly improved the efficiency of photosynthesis in plants. This optimisation of photosynthesis has been achieved by capitalising on multidisciplinary approaches including computational biology, metabolic modelling, systems biology, enzyme and pathway engineering, synthetic biology, and the multigene transformation of two major C3 crops: potato and rice. The "PhotoBoost" approach involves six new and improved strategies. These have been exploited to boost photosynthetic efficiency by at least 30% and biomass yields by at least 40%. The successful implementation of the "PhotoBoost" concept has either been, or will be, validated by performing greenhouse and field trials.

The consortium met their goal by developing enhanced C3 crops that combine one or more of the following strategies:

- ✓ (a) the optimisation of light reactions during photosynthesis;
- ✓ (b) the integration of an algal CCM;
- ✓ (c) the introduction of an engineered photorespiratory bypass mechanism;
- ✓ (d) the optimisation of source-sink capacity (only in potato); and
- ✓ (e) the adaptation of stomatal conductance by the introduction of a hexokinase gene, to improve water-use efficiency.
- ✓ (f) the integration of an oxygen scavenging mechanism as a novel strategy to further reduce photorespiration and boost the efficiency of photosynthesis was also explored.

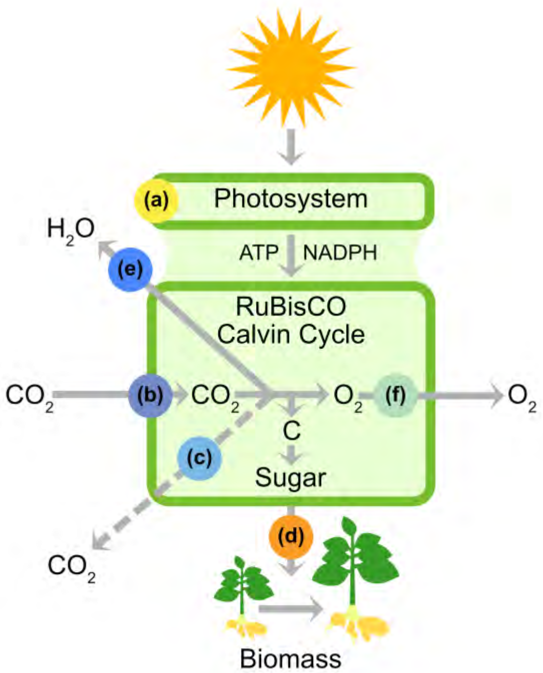


Figure 7 A Schematic of the PhotoBoost project approaches

PhotoBoost has delivered the following specific outcomes:

1. Development, refinement and application of metabolic models that simulate complex photosynthetic pathways/networks to predict the effects of proposed pathway modifications, support the selection of suitable combinations of photosynthetic enhancement strategies, and provide a rational basis for enzyme optimisation.

2. Development and implementation of a novel oxygen scavenging pathway in potato and rice (strategy **f**, above) to enhance the CO_2/O_2 ratio at the RuBisCO site and limit photorespiration, thus complementing the photorespiratory bypass.

3. Generation of potato and rice plants featuring different combinations of the PhotoBoost approaches (strategies a-f, above) with improved photosynthesis and biomass yield in the greenhouse and the field.

5.4 BestCrop

The *BestCrop* project adopts promising strategies to improve the photosynthetic properties and ozone assimilation capacity of barley by:

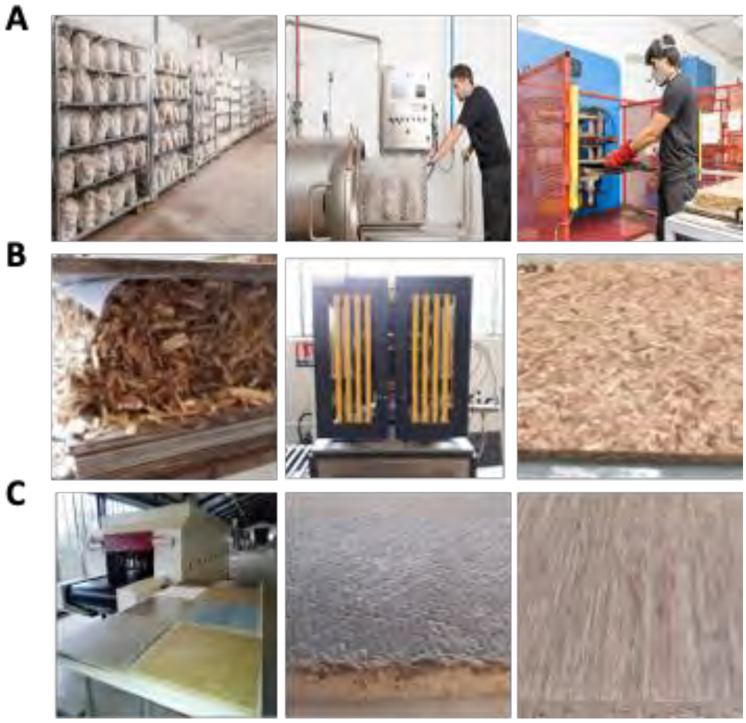
- ✓ i)tuning leaf chlorophyll content and modifying canopy architecture;
- ✓ ii) increasing the kinetics of photosynthetic responses to changes in irradiance;
- ✓ iii) introducing photorespiration bypasses;
- ✓ iv) modulating stomatal opening, thus increasing the rate of carbon dioxide fixation and ozone assimilation.

Improving the targeted traits is expected to achieve increases in above ground total biomass production without modification of the harvest index, with added benefits in sustainability, via better resource-use efficiency of water and nitrogen. In parallel, the resulting barley straw is tailored to: i) increase straw protein content to make it suitable for the development of alternative biolubricants and feed sources; ii) control cellulose/lignin contents and lignin properties to develop straw-based construction panels and polymer composites.

Figure 8 Schematic overview of BestCrop

Overall, by exploiting natural- and induced-genetic variability as well as gene editing and transgenic engineering, BestCrop will lead to multi-purposes next generation barley cultivars supporting sustainable agriculture and capable of straw-based applications. Specifically, BestCrop is expected to deliver:

- Barley lines with either improved photosynthesis efficiency or ameliorated canopy architecture validated at growth-chamber/greenhouse level
- Barley lines with increased O_3 uptake validated at growth-chamber/greenhouse level
- Barley lines with tailored straw composition, i.e. either optimized lignin content and properties or increased protein amount
- Barley lines carrying single traits or trait combinations evaluated under different European agroclimatic scenarios and growing practices
- Demonstrators at pilot scale suitable for barley straw transformation into high-value bio-based products



A) Mycelium technology for acoustic insulation panels at partner MOGU. Growing room where the mycelium is let to colonize the substrate under controlled environmental conditions (left); Steam autoclave for substrate sterilization (middle); Mechanical press for panel construction (right); B) Barley straw panels with biobased binders obtained using thermo-mechanical press at lab scale at partner FRD-Codem; C) Sandwich panels manufacturing using a laminated press at industrial scale for prototype at partner FRD-Codem (left), sandwich panels with mycelium panel similar to MOGU product with flax fabrics and coated layer (middle) and flax particle board with a layer of flax/PP composite (right).

Figure 9 Strategies used in BEST-CROP for manufacturing barley straw-based panels.

6 Next Steps: the Strategic Research Agenda priority areas

The following "next steps" represent a Strategic Research Agenda (SRA) to maximise the translation of the newly gained photosynthesis knowledge towards the breeding sector. This will require collective European, and ideally international, efforts through Public Private Partnerships (PPP) to develop future research and innovation collaborations and translate results. Additional funding from public and private sources including Horizon Europe and follow-on European programmes will be critical to facilitate future crop improvements exploiting advances in photosynthesis science.

The current fragmented approach to translating science for crop improvement needs to be replaced by a longer-term strategic programme. This can build interdisciplinary synergies, exploit efficiencies of scale, and accelerate research and innovation pathways.

Photosynthesis research has a leading role to play in such a programme.

SRA Priority 1: Phenotyping and Validation

Identification of genomic control coefficients

- ✓ Validation of naturally derived innovations with transgenic/genome-edited lines with modified expression of traits underlying genetic determinants. This will explore the genomic basis for established variation in selected traits and the potential for enhancing a trait by altered gene expression *in situ* e.g. modifying a promotor. The aim is to rapidly establish genomic control coefficients for key physiological pathways. Identification of key genes is the first step i.e. genes underpinning natural variation for a trait.

Identification of diagnostic signatures

- ✓ Identification of novel diagnostic signatures for combinations of traits which improve photosynthetic performance and yield.
- ✓ Ongoing phenotyping to feed back into QTL and GWAS mapping, integrating trait data to allow finer characterisation / confirmation of loci discovered in previous mapping rounds.

SRA Priority 2: Translation of QTL/QTN

Validation in inbred backgrounds

- ✓ Survey elite germplasm (ex PVP) / breeding material for allele/haplotype variation in candidate genes/QTLs (ideally validated genes) that affect selected traits which we detected as QTLs or which have been identified elsewhere.
- ✓ Near Isogenic lines (NILs) or similar (e.g. overexpressor of trait gene) in relevant parents.
- ✓ Characterise performance of selected traits in prebreeding and elite lines in controlled and field conditions.

Fine-mapping / candidate gene identification

- ✓ New recombination in offspring
- ✓ Genome editing
- ✓ Physiological characterisation

Facilitate implementation in breeding

- ✓ Develop diagnostic markers

Genomic selection

- ✓ Exploiting advanced understanding of the genetic make-up of crop plants and measurable photosynthesis traits to increase genetic gain of (complex) target traits in novel breeding programmes. The identification and use of molecular genetic markers will be a key step in reducing time and costs.
- ✓ Activities based on the recognition that a trait maybe under multi-locus control for which improvement by genomic selection may be necessary.

***SRA Priority 3: Model-development** and model-guided improvement*

Model-development

- ✓ Use of existing, and generation of new datasets to identify the genetic architecture of plasticity in photosynthetic efficiency and its genetic correlation to growth.
- ✓ Dynamics of fluxes in photosynthesis-related pathways: this will call for new ways for flux profiling and can be used to specify missing regulators.
- ✓ Integration into i) canopy models and ii) crop models.

Model improvement cycle

- ✓ Use models to identify key regulatory steps as a function of relevant environmental conditions.
  i) *Genotype to phenotype*: Find sequence variation in genes involved in key steps/traits and phenotype corresponding plant material (diversity panels, breeding material).
  ii) *Phenotype to genotype*: Phenotype variation in critical steps/traits across diversity panels and breeding material and correlate with sequence variation.
- ✓ Feed results into the model-improvement and start the next cycle.

SRA priority 4: Genomic toolbox/Tuning the CBC - using transgenic/non-transgenic approaches

Emerging alternative strategies that would provide novel approaches to modify target genes known from transgenic work to improve photosynthesis:

- ✓ Newly identified twelve base pair palindromic sequence from the octopine synthase gene can be used to upregulate multiple genes simultaneously – this would be cis-genic as these sequences are widespread in a number of plant species.
- ✓ Deletion of upstream open reading frames with stop codons, this would offer a non-GM approach and targets are easy to identify in genes of interest, and multiple genes can be targeted simultaneously.

SRA Priority 5: Chlorophyll Tuning

- ✓ Demonstrate improved overall yield in chlorophyll tuned plants.
- ✓ Demonstrate increased efficiency in use of water and nitrogen.
- ✓ Investigate the potential of increased albedo to mitigate climate change and to increase the efficiency of bifacial photovoltaic systems.
- ✓ Identify novel strategies that allow a chlorophyll gradient concentration through the canopy: from pale-green leaves on top of the canopy to dark green leaves at the bottom of the canopy.
- ✓ Investigate the potential of "*staygreen*" phenotypes, i.e. delayed senescence, on crop yield in new chlorophyll tuned plants
- ✓ Use of newly developed phenotyping tools to quantitatively assess variation and use a range of tools to measure performance.
- ✓ Application of gene editing or conventional breeding to transfer to elite lines from natural or existing mutants.

SRA Priority 6: Into the Field-Crop Performance to advance crop improvement strategies and deliver data to support Life Cycle Analysis and Socio-economic and Environmental assessments

- ✓ Demonstrate a yield increase of the photosynthetically optimised plants (prebreeding and elite lines) for target crops.
- ✓ Assessment of the different inputs required to achieve target crop performance (>10%): fertilizer and/or irrigation vs standard lines.
- ✓ Assess the susceptibility of photosynthetically improved crop varieties to biotic/abiotic stresses vs standard lines.
- ✓ Assess impacts on the soil microbiome of roots (-> soil richness/health) e.g., impact of increased root exudates or below ground root biomass and necromass.
- ✓ Assess the impact of the soil microbiome and root properties on above ground photosynthetic activity.
- ✓ Determine the characteristics of the optimised lines for end use e.g., harvest/ processing and identify desirable traits to improve.
- ✓ Assess the suitability of improved varieties for different (changing) environments and farming practices.
- ✓ Acceptability to *CITIZENS = CONSUMERS* evaluated across different European regions and demographics.
- ✓ Explore the possibilities for stacking of traits: combining traits to fully exploit the yield potential and to tailor the quality of agriculture residues to the needs of circular economy, closing the loop on resource use and waste generation in agroecological systems.

SRA Priority 7: Phenotyping Tool Development

- ✓ Awareness raising and training about new agile and high throughput phenotyping tools.
- ✓ Exploitation of the chlorophyll a/b ratio non-destructive analysis devices developed by CEA and PSI for use by breeders and growers and researchers in field environments.
- ✓ Improved tools suitable for assessing target traits in field trials and crop breeding programmes.
- ✓ Development of ‘breeders eye’ tool for assessment of crop health for breeders and grower use in crop breeding and crop management strategies. This is expected to utilise photosynthesis traits as measures of crop health and performance.

SRA Priorities: Synthetic Biology approaches
✓ Transfer successful synthetic biology strategies (e.g. developed and demonstrated by PhotoBoost) to other food and feed crops. ✓ Perform further field trials to confirm robustness and sustainability of generated plant lines showing improved photosynthesis and biomass yield. ✓ Perform studies to investigate nutritional value and processing of generated plant lines showing improved photosynthesis and biomass yield. ✓ Test further stacking strategies and alternative synthetic biology approaches. ✓ Develop an evidence base to support regulatory approval and market adoption of suitable tools and approaches.

7 Gaps in the knowledge/challenges to overcome

Recent workshops and consultations identified the **Gaps in the Knowledge** needing be addressed to take improved photosynthesis research forward. Five pertinent themes were identified and debated by photosynthesis researchers and crop breeders at the CAPITALISE 3rd workshop in round table discussions in June 2024. These discussions aimed to identify the current state of the art, the most promising options, the biggest technical challenges to overcome, and next steps and milestones in the field. The 5 themes are

- **Integrative photosynthesis**, what questions should we be asking? What tools are we missing?
- **Sink-Source Interactions**: Will improving source activity improve yields?
- **Resilience** – maintaining photosynthesis in changing environments
- **Omnigenomics,** genomic selection, genetics of breeding
- **The potential of computational models** to accelerate the research and innovation programmes

7.1 Integrative photosynthesis, what questions should we be asking? What tools are we missing? - Andreas Weber

Photosynthesis is the interface between the atmosphere and the biosphere, using light energy to convert atmospheric carbon dioxide into the biomolecules that support life on Earth. In addition, photosynthesis is the basis of most food chains and thus the primary energy input to most natural ecosystems, as well as the agro-ecosystem that has been the foundation of human civilization since the Neolithic Revolution. In crops, photosynthesis can limit yield, as demonstrated by free-air CO_2 enrichment (FACE) experiments (Ainsworth and Long, 2021), most C_3 crops show increased yield in FACE environments, (on average 18% yield increase, Ainsworth and Long, 2021) suggesting that the amount of photosynthate produced is an important determinant of yield. However, it is not straightforward to infer yield from measurements of maximal assimilation or electron transport rates or steady-state photosynthetic rates. Furthermore, it is not clear which aspects of photosynthesis are particularly important for yield and resilience in a given environment. This is because photosynthesis is a complex trait and multiple processes are coupled to photosynthesis from the cellular to the canopy scale, and because a plethora of other factors such as water and nutrient availability, pathogen pressure, temperature and light fluctuations, and management practices affect photosynthesis and co-limit yield. Thus, we need to go beyond the silos of disciplinary research and take an integrative approach to photosynthesis research that considers the role of photosynthesis in yield and resilience in the context of a complex agroecological framework.

Selected features of an integrative photosynthesis research programme are outlined below. Many of these dovetail with other knowledge gaps and research needs addressed in other sections below, such as source-sink interactions or resilience:

- *A whole-plant perspective*: Integrative photosynthesis research considers photosynthetic traits in the context of the whole plant, including its non-photosynthetic parts such as roots, and the plant holobiont, i.e. the plant and its associated microbiome.
- *A product-centric perspective*: Considering desired product qualities, such as mineral nutrient or protein content, and value chains, such as food and non-food purposes, which may require different photosynthetic strategies that should be tailored to achieve optimal product quality.
- *A climate-centric perspective*: Crop domestication occurred under historically low atmospheric carbon concentrations, and intense selection since then has depleted genetic variation that would be potentially beneficial for adaptation to anthropogenic climate change. Photosynthesis is particularly vulnerable to climate change because of the temperature dependence of the Rubisco oxygenation reaction and the trade-off between CO_2 uptake and water loss (Long *et al*., 2006). However, this will require expanding the pool of genetic variation available for breeding, e.g. through introgression from landraces but also from wild crop ancestors, in addition to targeted introduction of novel variants through new breeding technologies and chromosome engineering.
- *Photosynthesis in the context of different cropping systems*: Optimal photosynthetic traits will vary for different cropping systems and may need to be adapted to new cropping schemes, including multi-cropping and other agroecological types of farming (e.g. light use and distribution in the canopy), or for combining crop production with renewable energy production (agrophotovoltaics). Indoor agriculture under constant, controlled environments eliminates the need for adaptive photosynthetic traits, allowing for higher conversion efficiencies. Novel photosynthetic ideotypes will be required to fully exploit the potential benefits of these alternative cropping systems.
- *Photosynthesis in multi-purpose crops*: An exciting vision for crops of the future is that, in addition to providing food, fibre and renewable resources, such crops will also provide important ecosystem services and contribute to climate change mitigation, e.g. through carbon sequestration. This places new demands on photosynthetic efficiency, as additional carbon assimilation is required to support root exudation, for example, without compromising yield per unit area.
- *Photosynthesis beyond conventional agricultural crops*: Conventional agriculture depends on large amounts of arable land, water availability and permissive climate parameters. Some of these constraints can be overcome by cyanobacteria and algae, also in combination with fermentation processes that convert cyanobacterial or algal biomass into products that can replace canonical agricultural value chains. This will require an adaptation and optimization of photosynthetic traits for contained environments.
- *Integrative photosynthesis research includes integrating various stakeholder dimensions*: The way forward requires close interaction with breeders to identify the fundamental questions that need to be solved to achieve impact in the development of novel germplasm that benefits farmers and consumers. Participatory research and development approaches should include farmers as equal partners, for example in design and analysis of field trials, and in the process of dissemination of research.

The key requirement for the successful implementation of an integrative photosynthesis research programme is the initiation of a new farm-to-lab research paradigm:

From lab-to-farm to farm-to-lab: The prevailing research paradigm in (not only) photosynthesis research is hypothesis-driven basic research in the laboratory, followed by translation of knowledge into applications in the field. Given the highly complex interactions between plants and their environment, and the plasticity of physiological and architectural traits, it is often difficult to translate laboratory findings to the field. To overcome this challenge, hypothesis-free, data-driven approaches are needed, (Yanai and Lercher, 2020) with data sampling across multiple field environments and climates, and across organisational scales from molecular and cellular to canopy phenotypes, over entire growing seasons, in genetically well-described panels. Climate and soil parameters, pathogen pressure, and management interventions must be recorded with high density and precision. Specific hypotheses developed through integration of such high-dimensional phenotyping data with genomic data through data science approaches should then be tested in controlled laboratory environments to establish causal relationships between genotypes and phenotypes in the context of varying environments. Such causal relationships between genotype and phenotype will enable a step change from current genomic prediction approaches (which are limited to known genetic, trait, and environmental variation) to knowledge-based predictions for future environments and, through generative AI, even the ab initio design of novel genotypes with desirable traits in future environmental conditions.
The farm-to-lab approach also offers new opportunities for participatory science, where farmers can be involved in experimental design and knowledge generation. In addition, the farm-to-lab approach provides farmers with an additional source of income through compensation for time and land used for research.

A network of research farms across Europe: A farm-to-lab approach requires a network of farms across climate zones, soil types and a latitudinal gradient across Europe. Large-scale experiments, genetic panels, sampling and analysis designs, data management and access should be developed in collaborative efforts by several laboratories with complementary expertise. Farmers and breeders should be involved in experimental design and data analysis and should be compensated for their efforts.

An Open Data and Open Science Framework: As demonstrated by the development of large generative language models, data is knowledge, and data is the basis for monetising such models. Large international players are acquiring large amounts of field and phenotyping data through their business networks which, if not countered by public infrastructures, will lead to a monopolisation of the database for predictive and generative models in crop science. A coordinated and adequately funded European infrastructure is therefore needed to enable open science and to remain competitive in an increasingly data-driven economy.

Other requirements are mostly identical with those described in the following sections.

The next steps/milestones

Short-term:

- Define the concept and aims of an Integrative Photosynthesis research program
- Establish a network of stakeholders and develop a roadmap for implementation of the program

Medium-term:

- Pilot funding for a small network of three farms and two crops
- Develop experimental design guidelines, data management structure, analytical pipeline
- Refine, adapt, and revise concept based on outcome of pilot

Long-term:

- Large-scale funding for European-scale network
- Include major European crops and future crops
- European Integrative Photosynthesis Open Data Center
- Predictive and generative models for crop design

The funding landscape for farm-to-lab research is currently non-existing, which is to be expected for a novel research paradigm. It will hence require a substantial investment to kick off the program. However, existing infrastructure can be integrated. To advance this new research paradigm, it will be essential to engage new stakeholders, such as European farmers, in the research endeavour. Data management and data analysis policies and protocols must be established before implementation of a larger-scale network. Experimental design to be developed in a participatory and integrative process.

7.2 Sink-Source Interactions: Will improving source activity improve yields? - Pallavi Singh

The source-sink relationship in plants is recognised as a complex trait that involves dynamic resource allocation significantly influenced by environmental conditions. This relationship is critical for determining plant productivity and, consequently, agricultural yields. Currently, efforts are being directed towards enhancing sink activity to improve water use efficiency (WUE) and nitrogen use efficiency (NUE), with a substantial focus on improving photosynthesis. Understanding the dynamics and complexity of source-sink interactions necessitates a sophisticated approach to resource allocation and a deep understanding of species-specific complexities. Transport mechanisms, particularly those reviewed by the CropBooster project, highlight the importance of focusing on these interactions to maximise yield. This underscores that improving source activity alone may not suffice unless there is a concomitant enhancement in sink capacity, emphasising the need for a balanced approach to source-sink dynamics.

Below is the list of several promising options that can be pursued to underpin the factors affecting source-sink relations. This is not an exhaustive list:

- *Optimising anatomical capacity for transport*: Enhancing the anatomical features of plants to improve the efficiency of resource transport between source and sink organs is crucial. This can lead to better allocation of photosynthates, nutrients, and water, thereby improving overall plant productivity and yield resilience.

- *Developing advanced measurement tools and sensors*: Accurate measurement of source-sink dynamics requires sophisticated tools and sensors that can provide real-time data on resource allocation. These tools are essential for modeling and understanding the dynamic correlations between source and sink.
- *Modeling source-sink interactions*: Creating detailed models to predict and analyse the interactions between source and sink can help in identifying key factors that influence yield. These models can incorporate environmental variables, plant physiological traits, and genetic factors to provide a comprehensive understanding of source-sink dynamics.
- *Carbon farming*: This innovative approach focuses on using agricultural practices to sequester carbon in the soil, thereby mitigating climate change while improving soil health and crop yields. Carbon farming represents a dual benefit for climate mitigation and global food security.

The biggest technical challenges to overcome that could help unravel the complexities of source-sink dynamics include:

- *Enhancing phenotyping capabilities*: There is a need for fast, automated, portable, affordable, and non-destructive phenotyping techniques. Such advancements would allow for large-scale screening of crop varieties for desirable source-sink traits.
- *Adapting NMR techniques*: Nuclear Magnetic Resonance (NMR) techniques, currently used in tree studies, could be revolutionary if adapted for crop research. These techniques can provide detailed insights into internal plant processes and help in understanding source-sink dynamics.
- *Bridging the gap from 'Lab to Field' measurements*: Translating findings from controlled laboratory settings to field conditions remains a significant challenge. Field measurements need to account for environmental variability and other stress factors that affect source-sink interactions.
- *Understanding rate of flux*: Measuring the rate of resource flux between source and sink, including diurnal and nocturnal variations, is critical. This requires sophisticated techniques to monitor and analyse these fluxes in real-time.
- *Using C/N Ratio as a Proxy*: The carbon/nitrogen (C/N) ratio can serve as a proxy for understanding carbohydrate status and balance within the plant. This ratio can help in assessing the efficiency of resource allocation and identifying imbalances that affect yield.
- *Managing trade-offs with elevated CO_2 (eCO_2)*: Elevated levels of CO_2 can affect source-sink interactions. It is essential to identify and optimise trade-offs to ensure that increases in source strength do not compromise other aspects of plant quality and productivity.

The next steps/milestones

Short-term:

- Define clear targets for source-sink dynamics across various species and environmental conditions.
- Identify limiting factors to inform molecular targets, such as trehalose 6-phosphate (T6P), transporters, peptides, and non-coding RNAs.

Medium-term:

- Optimise these molecular targets in elite crop varieties.
- Diversify sink phenotypes to enhance carbon farming.
- Translate leaf-level understanding of source-sink interactions to the whole-plant level.

Long-term:

- Investigate the interdependency between yield and quality.
- Improve soil quality through sustainable agricultural practices.
- Enhance public engagement to gather support for these systems biology initiatives.

The funding landscape for source-sink interaction research is currently limited. The International Wheat Yield Partnership (IWYP) funds research on source-sink interactions in wheat, particularly during reproductive stages under heat stress. In France, some funding is directed towards 'Fair Carbon' initiatives, focusing on carbon farming in trees. To advance this research, it is imperative to establish collaborative networks that channel ideas, identify limitations, and develop achievable goals. Engaging industrial and commercial partners, as well as forming strategic multi-institutional academic-industrial networks, will be essential for securing the necessary funding and resources.

The primary species of focus include wheat, rice, potato, sugar beet, and forest trees (for latex and biomass production). However, the research should not be limited to these species as understanding

C/N balance and dynamics in other models like alfalfa and peas can be beneficial. The current research landscape is fragmented, emphasising the need for strong partnerships with industry and commercial stakeholders to drive targeted research.

The future of this research will hinge on securing funding opportunities, addressing and optimising limitations, and navigating the regulatory and policy landscape. Identifying new model systems with higher WUE/NUE and increased photosynthesis capabilities, coupled with dynamic source-sink interactions, will be critical. Additionally, future agricultural management practices, including the integration of AI and robotics, will influence the architectural needs of plants, particularly the need for stronger stems to support increased panicle load.

Overall, improving source activity holds significant promise for enhancing agricultural yields. However, it necessitates a multi-faceted systems biology approach that includes the development of advanced measurement tools, robust phenotyping techniques, and strong collaborative networks. Strategic funding and interdisciplinary partnerships will be pivotal in overcoming the technical challenges and translating these advancements into practical agricultural practices. These practices must meet the demands of climate change and global food security, ensuring sustainable and resilient agricultural systems for the future.

7.3 Resilience – maintaining photosynthesis in changing environments - Stephan Schilberg

Photosynthesis is responsible for the sustainable growth and productivity of plants and secures our food supply. However, stressful environments and change in global climate patterns affects photosynthesis reducing crop yield and poses threat to food security. Increasing soil degradation, water scarcity and rising temperatures and CO_2 levels cause severe alterations in a wide range of physiological, biochemical, and molecular processes including photosynthesis in plants. Not only do plants have to cope with rising temperatures and greenhouse gases, but the accumulation of rapidly changing weather extremes also puts them under great stress. The gaining of new agricultural land through rising temperatures in the northern and southern regions of the northern and southern hemisphere is not an alternative either, as the low sunlight intensities and times and the strong temperature fluctuations between day and night are not favourable for plant growth there. Therefore, new agricultural and breeding technologies are required to maintain or even improve photosynthesis in changing environments.

This is a very difficult task, as the number of stress factors with a direct or indirect effect on photosynthesis is likely to increase and favourable cultivation periods will become less stable and shorter. Nevertheless, there is knowledge on how multiple stresses affect photosynthesis and various techniques have been used to improve resilience in crop plants, e.g., QTLs for breeding, priming to induce memory in acclimation, and pathway engineering. But growing conditions have also been optimised so that plants can cope better with stress, e.g. through the use of chemicals that act as biosafeners/bioregulators to improve plant health and fitness, or through alternative cultivation systems such as agroforestry, intercropping, agrophotovoltaics and water-saving irrigation systems. Vertical farming even eliminates abiotic and biotic stress factors by growing plants resource-efficiently indoors under artificial light (LEDs).

These examples show that knowledge of the molecular mechanisms involved in photosynthesis and future climate change is crucial for the creation of resilient crops. The most promising options to achieve this goal are (i) utilising gene pools to identify new targets for breeding and genetic engineering, (ii) identifying external factors (e.g. compounds, cultivation techniques) that improve plant health and stress resistance, (iii) increasing root biomass and improving root architecture, (iv) improving and introducing new measurement/analysis/software tools for analysing genetic and phenotypic traits, and (v) using mathematics/models/computing/deep learning/AI to reduce the complexity of understanding and manipulating photosynthesis.

Changing environmental conditions make it necessary to focus more on the development of plants with improved and resilient photosynthesis in order to maintain crop productivity under changing cultivation and climate scenarios. To achieve this goal, we recommend the following methods:

- Identify further QTLs and target genes involved in photosynthesis by utilizing genetic information from biodiversity
- Expand knowledge of factors that influence photosynthesis and the interplay with molecular and cellular functions in the plant
- Improve tools to measure photosynthetic performance from molecular to the phenotypic level and in the lab to the field
- Provide more and better tools (databases, software, AI, infrastructure) for data analysis
- Improve data standardization and quality through more cooperation and environmental measurements
- Improve translation of research findings to breeding and field application
- Provide more funding from national public funding bodies, plant breeding sectors, and philanthropic organisations.

7.4 Omnigenomics, genomic selection, genetics of breeding - Matteo Dell'Acqua

The omingenomics concept speculates that complex traits are determined by the entire set of genetic factors present in an organism, in other words, all genes expressed within a cell affect the expression of a given trait, with infinitesimal cumulative effect. Acknowledging this concept, that is increasingly supported by observational data, raises a compelling question: if trait determination is infinitely complex, how to effectively master it and translate it into genetic gain? We believe that genomics and modelling may provide an answer to this question and open a new era of translational photosynthesis research, inspiring crop plant breeding.

In recent years, scientific efforts led by groups from the EU achieved significant advancements in understanding key target mechanisms that contribute to photosynthesis. Researchers have identified associated genetic targets and have developed knowledge about allelic diversity linked to these targets. This diversity is crucial as it forms the basis for developing crop varieties with improved photosynthetic performance. This diversity contributes to photosynthesis either positively or negatively, *i.e.* increasing or decreasing the trait value. This knowledge is important regardless of the direction of the effect of the allelic variant: photosynthesis can be improved by increasing the positive effect on the trait or reducing the negative effect on it. Thanks to these projects, not only do we understand better what the molecular determinants of photosynthetic effects are, but there is now proof of concept showing the linkage between photosynthesis and agronomic traits, providing a solid foundation for future research. These projects collected unprecedented data from both field and controlled environments, that is crucial in advancing our understanding of how to translate photosynthetic efficiency in an actionable target for crop improvement. This linkage is essential for translating improvements in photosynthetic efficiency into tangible agricultural benefits.

Despite the progress, several technical challenges remain, including a clearer understanding of how genetic diversity and environmental factors interact to affect photosynthesis. The complexity of trait determination, we have seen, goes beyond a handful of molecular factors that can be identified to be linked with the trait. Then what is the contribution of genome-wide variation to photosynthesis? And how this contribution varies across time and plant developmental stage in the different environmental and management regimes in which the crop may be grown? This knowledge is critical for developing robust crop varieties that can perform well under a wide range of environmental conditions, and in which photosynthetic improvement translates in improved agronomic traits, such as yield and resilience. To this end, it is particularly important to understand how photosynthesis-related traits interact with other agronomic traits, and determine the synergies and trade-offs which must be considered to avoid unintended consequences of photosynthesis improvement.

We think that to overcome these challenges it is essential to work *with* breeders and farmers rather than *for* them, ensuring that the research aligns with the practical needs of breeders and farmers, including with regards of traits to be improved. Farmers may favour traits such as Resource Use Efficiency (RUE) over yield, which instead is a classical target of breeding. Neither probably targets photosynthesis: but both can rapidly adopt photosynthesis insofar as it becomes clear how this trait contributes to agronomic performance. In this sense, it may be worth concentrating on actionable improvement targets that can be easily achieved by breeding, rather than chasing exceedingly complex and refined traits with limited understanding and uncertain impact. Providing clear and practical targets, supported by appropriately straightforward measurements approaches, will facilitate the adoption of research outcomes, ensuring that the benefits of research are realized in the field.

We see two main avenues to translate photosynthesis research into genetic gain in the hands of breeders. The **first avenue** is that of the development of better crop growth and crop architecture models that can more effectively link genomic information to photosynthesis and agronomic traits. Improved models have the potential to enhance the understanding of pleiotropic effects, which occur when one gene influences multiple traits, hence defining synergies and trade-offs. Furthermore, such models will better incorporate crop development over time, including response to environmental variations occurring at different moments in crop development. These models bear the promise to link the expression of traits with specific environmental conditions, opening the possibility of projecting genetic gains across different environments to ensure the robustness of new crop varieties. Such models are being developed, thanks to the wealth of phenomics data developed via large-scale experiments in field and greenhouse and to the identification of key molecular mechanisms influencing photosynthetic capacity. The **second avenue** is that of the identification of better genetic targets for improvement, that is intimately connected with the modelling approach. Prioritizing genetic targets based on their practical importance and relevance, rather than their novelty, will ensure that research efforts are focused on the most actionable targets. Increasing genotyping efforts and expanding the genomic information available will also enhance the genetic information, providing a richer resource for research, and supporting the use of genomic selection strategies linking photosynthetic performance with field traits. Expanding collections of allelic variation under study and conducting comprehensive trait measurements on species related to breeding targets but capturing distant allele pools (e.g. wild relatives) will further contribute to the definition of molecular targets of improvement.

Therefore, what are the next steps at hand?

Short term:

- The community needs to provide convincing proof that 'improving' photosynthesis is relevant to reach targets desired by growers and aimed for by breeding. This has not happened yet, although we are getting closer to the point that this can be confidently stated. For this to be achieved, we need to show that photosynthetic improvement targets are tractable, *i.e.* can be used by breeding;
- We need to show that trait improvement works in practice, in the field, for crops that are relevant to breeding, rather than in model species such as Arabidopsis or tobacco;

- We need to make full use of the data available in a modelling dimension to assess whether connections exist across photosynthetic and performance traits.

Mid-term:

- The community needs to build on the connection made between photosynthesis and agronomic performance,
- Cast a larger net on more species and plant genetic resources,
- Identify where are the limits of photosynthetic efficiency across the spectrum of the species available to crop improvement. This requires identifying targets that are easy to track and that are amenable in day to day breeding operations, screening these targets in larger and larger breeding populations.

Long term,

- These targets and variation should be applied in breeding programs, screening what is available and creating new allele mixes and, if required, and legally approved, even new alleles via new breeding technologies (NBTs).

Current research in photosynthesis has the means to achieve these ambitious goals. The big data revolution in genomics and phenomics, combined with the advances in molecular biology, can confidently identify targets for improvement and predict their contribution by modelling. Philanthropists in the EU and USA have seen this opportunity and are supporting avenues to enable breakthroughs in this area of research, which has potential to contribute to the sustainable intensification of agriculture not only in the western world but also in the global south. Finally, it is worth pointing out that photosynthesis research is also aligned to biodiversity conservation and climate change mitigation via CO_2 fixation.

It is time for translation of photosynthesis research into crop improvement. This needs the research to align with what breeding needs: amenable innovation. Also, to put it where breeding wants it: in relevant genetic materials and commercially viable products. This objective is in reach, if we combine crop models with genetic innovation to improve the prediction ability of photosynthesis impacts on agronomic traits. There is a plethora of tools making this possible in a way it was not conceivable until a few years ago: singe cell -omics, coupled with time-resolved data on short- and long-term fluctuations of light, bears the promise of observing molecular mechanisms in the act of contributing to trait expression. This be coupled with genebank genomics and pangenomes, to resolve individual genomic and allelic setups contributing to phenotypic diversity that may eventually be taken up by breeders and innovators.

7.5 The potential of computational models to accelerate the research and innovation programmes - Zoran Nikoloski

The state of the art of modeling photosynthesis and its effects on plant lifecycle traits can be roughly divided into mechanistic and data-driven models. The mechanistic models make use of biochemical and biophysical principles / constraints to simulate and make predictions about a process of interest (e.g. binding of ligand to protein, rate of biochemical reactions, photosynthesis rate). In contrast, data-driven modeling aims to identify an empirical relation between quantities / traits rooted in multivariate statistical, machine learning, and deep learning approaches. The latter type of models arises in quantitative genetics, where photosynthesis-related traits are used alongside genetic markers to identify genes associated with these traits or predict traits for genotypes not used in training. The mechanistic and data-driven approaches converge in problems that deal with parameter estimation. In the context of photosynthesis, the latter type of problems include: (i) estimation of reaction fluxes by using atom transition maps, metabolic models, and labelling patterns of metabolites, (ii) estimation of enzyme parameters given a metabolic model alongside quantitative proteomics and metabolomics data.

These model types provide the possibility to guide the identification of modifications (e.g. gene knock-outs, mix-and-match strategies, protein engineering) that can modify photosynthesis in a desired direction, to pinpoint genes that control photosynthesis-related traits, and to identify genotypes with improved photosynthesis under specific conditions.

The biggest technical challenges to overcome: Photosynthesis takes place throughout the lifecycle of a plant, with cumulative effects on yield over spatio-temporal scales. Environmental factors change may alter plant development at different stages of the lifecycle. As a results, while recent advances have allowed us to make short-term changes in photosynthesis-related traits, it remains extremely challenging to accurately predict the cumulative effects of photosynthesis; this is coupled with difficulties in improving the precision in predicted outcomes.

The existing mechanistic models of photosynthesis lack proper parameterization for specific species. The reasons underpinning this issue is the difficulty in generating data of sufficient quality that can increase the identifiability of parameters and/or lower the variability. Resolving this issue calls for development of new measuring technologies to obtain data that facilitate model parameterization.

Directly related to the problem of parameterization is expansion of the models with adequate mathematical descriptions of regulation of photosynthesis by different environmental factors (e.g. temperature, water availability, atmospheric CO_2, light intensity and quality). Even if these models are developed, it will remain challenging to expand their applicability over longer time scales, allowing to make predictions about acclimation of photosynthesis. A related issue is the integration of these models of photosynthesis with crop models, allowing their usage for decision-making purposes in precision agriculture.

The major opportunity for models of photosynthesis and related processes is their usage in the identification of targets for breeding. This opportunity can be realized by: (i) relying on the coupling of mechanistic models with machine / deep learning approaches via (bio)physics-constrained neural networks, (ii) advances in *in silico* design of metabolic engineering strategies based either on enzyme engineering, mix-and-match strategies, and novel chemistries, and (iii) hybrid models that integrate photosynthesis in larger metabolic and developmental contexts. Yet another opportunity is provided by development of genotype-specific photosynthesis models that will pave the way for facile identification of condition-specific limitations to photosynthesis that can be tested with advances in measurement technologies.

Realizing of the promises of photosynthesis models will be possible if future projects are shaped to start by testing predictions from modeling. This approach will ensure that the model – test – refine cycle is fully embedded in the lifetime of the project. In addition, future projects on photosynthesis will benefit from integrating more than a single modeler, thus representing the necessary expertise to cover the different scales. This can be achieved by initiating an Integrative Photosynthesis Modeling Network to coordinate future contributions across multiple projects.

Intense discussions suggested that philanthropy will likely be the most promising source for future funding of projects with the above-mentioned structure.

The next big thing we expect is the transition into a so-called “Post FvCB era” that will move away from limitation-driven steady-state Farquhar-vonCaemmerer-Berry model, that has been useful in making qualitative predictions about photosynthesis across scales (from single cells to leaves and canopies). The post FvCB era is expected to be marked by the development of kinetic and/or hybrid models that provide: (i) accurate and precise quantitative predictions of photosynthesis-related traits in specific genotypes, (ii) the short- and long-term dynamics of these traits upon changes in environment, and (iii) the genotype-by-environment (GxE) interaction in shaping photosynthesis-related traits. These models will make use of different data generative technologies, not only measuring photosynthesis-related traits, but also relying on proxies for these traits (via machine / deep learning approaches), to bridge the gap between lab and field.

8 Work programme topic suggestions for Horizon Europe Cluster 6

Based on recent progress in EU projects, several research topics are of immediate interest to advance promising results. Providing follow-on funding as part of future Cluster 6 work programmes will build on the momentum developed by the current portfolio of projects. The following topics are proposed for 2026-2027 calls. They have also been proposed to the European Plant Science Organisation (EPSO) for their support. This aligns closely with the CropBooster Roadmap from CropBooster-P (Grant Agreement 817690) and the remit of the EPSO Working Group Photosynthesis, Abiotic Stress, Input Use Efficiency.

There was some support for a new Cluster 6 area around *Enabling sustainable crop improvement.*

Title	Scope	Type of action
Photosynthetic resilience of crops in a changing climate	Future plant breeding should consider aspects of plant biology that have newly emerged as having "room for improvement" such as photosynthesis and its connection with plant development, yield, source/sink dynamics and respiration. In addition, this needs to occur with increasingly challenging field conditions where multiple limitations may occur. This calls for the development of a selection of genetic variants associated with enhanced photosynthetic performance using fine-mapping, validating these variants in elite inbred and heterotic backgrounds and developing diagnostic markers. Use of model-guided germplasm improvement should simultaneously enhance model performance and speed up the development of improved accessions. To meet the crop yield expectations described by the EU road map, plant phenotyping must put a greater emphasis on establishing connections between measured traits and crop performance.	RIA (TRL 2-5)
Improved nitrogen fixation for increased photosynthetic CO_2 assimilation	Carbon and nitrogen are essential components for agricultural productivity, and both can be sourced from the atmosphere. However, the conversion of carbon dioxide and nitrogen into biologically useful forms relies on two crucial processes: photosynthesis and nitrogen fixation. These are connected: photosynthesis provides the energy for nitrogen fixation and nitrogen fixation provides the nitrogen for photosynthesis. To	RIA (TRL 3-6)

	sustainably enhance agricultural productivity, it is important to improve both photosynthesis and nitrogen fixation. This approach would boost the productivity of existing nitrogen-fixing crops by providing them with more energy for nitrogen fixation and more carbon for root biomass alongside and more carbon for above ground growth and more nitrogen for photosynthesis. By leveraging increases in nitrogen fixation and photosynthesis, we can establish a foundation for high-yielding and sustainable agriculture. Such an agriculture model would rely on harnessing the energy of sunlight and utilizing the abundant carbon dioxide and nitrogen in the atmosphere, thus achieving more yield with fewer resources.	
Non-destructive phenotyping of photosynthesis in response to stress	Genomic resolution has significantly increased in recent decades due to sequencing efforts. However, the precision of phenotyping remains a limiting factor in genetic selection approaches. The development of genetic strategies to identify genes of interest, along with the description of increasingly detailed traits, requires the development of non-destructive phenotyping tools with higher resolution. These tools enable spatial and temporal analysis of physiological and developmental responses. Instrumentation and analysis methods must be developed to achieve quantitative and contextualized measurements for phenotyping of photosynthetic efficiency in plants whether in controlled and instrumented environments or in the field. Additionally, tools for data acquisition, storage, access, and modelling are needed. These spatio-temporal studies are crucial for providing data for model design and plant ideotype research. Advanced machine learning methods have also been proposed, which could be integrated into a plant phenotyping pipeline.	RIA (TRL 3-6)
Redesigning photosynthesis for crops of the future	Although crop breeding has been successful for centuries, it can only act on the genetic variation that exists within a given species. Even through extensive interspecific crosses, crops have limited access to the global gene space. In addition, recent advances in protein engineering allow the design of *new-to-nature* enzyme activities that outperform existing enzymes in terms of kinetic properties, selectivity and, when combined into novel metabolic pathways, substrate conversion efficiency. The transfer of new-to-nature and/or new-to-crop pathways into crops enables step changes in photosynthetic carbon conversion efficiency, as well as water and nitrogen use efficiency, that are unlikely to be achievable through canonical breeding approaches. Traditional breeding uses existing (or induced) genetic variation within a species to select for desired traits, such as yield or water use efficiency. This can lead to local maxima in trait performance; global maxima, which can only be reached via	RIA (TRL4-6)

	fitness troughs, are not accessible to canonical breeding. Breeding gain is also limited by pleiotropy and epistasis, linkage drag, and limited recombination in certain regions of the genome. All of these constraints can be overcome by genetic engineering, providing access to additional gains that are inaccessible through conventional breeding. Therefore, a research programme aimed at achieving sustainable crop improvement should be accompanied by a research theme that addresses the inherent limitations of conventional crop breeding.	

9 Alignment to EU policy

Crop breeding for climate resilience, especially through enhancing photosynthesis, can play a key role in improving yields, crop quality, and resilience to abiotic stresses. The challenge of biotic stress was beyond the scope of this work.

This Roadmap aligns with the European Green Deal and Farm to Fork strategy priorities, focusing on cropbreeding to enhance photosynthesis for higher yields with lower inputs, supporting sustainable farming and the Common Agricultural Policy (CAP) goals. The ambition is to deliver sustainable production within planetary boundaries, contributing to food security, climate action, and multiple Sustainable Development Goals (2, 3, 6, 7, 12, 13, 15). Biotechnologies are key to accelerating progress supporting conventional and new breeding techniques. This strategy will benefit from the proposed EU Biotech Law and improved regulatory environments. Stakeholder dialogues have emphasized the need for investment in innovation and knowledge-sharing to maintain crop yields under challenging conditions. Enhancing photosynthesis aligns with the Strategic Dialogue on the Future of EU Agriculture A shared prospect for farming and food in Europe recommendations. Enhancing photosynthesis aligns with **Recommendation 10**, which calls for better farmland management, water-resilient agriculture, **and innovative plant breeding**. This approach boosts productivity without expanding farmland, supporting the Biodiversity Strategy to 2030 by protecting natural spaces. It also reduces fertilizer use and improves water use efficiency. New bioeconomy optimised crops will strengthen the bioeconomy and meet predicted expansion by delivering cost-effective products for diverse uses including food, bioenergy, and biomaterials. This future proofed sustainable primary production will support the EU's Green Transition and circular economy goals, as outlined in the European Bioeconomy Strategy (2018).

Figure 10 Sustainable Development Goals contributed to by plant breeding projects

10 Key enabling technologies - The need for tools to support researchers and Industry

Recent innovations and emerging technologies are transforming plant science and crop breeding. Key enabling technologies include lab-based phenotyping devices; high throughput devices for screening in laboratory/indoor growth facilities; field devices for researchers and breeders to rapidly screen traits; and large-scale deployable sensors and satellite imaging.

Science and technology discussions at two CAPITALISE Workshops focussed on predicting the current and future trends shaping plant breeding, and highlighting innovations needed to accelerate the translation of results from lab to field. The main insights are summarized below.

Innovations/Technologies	Reasoning
GE and precision breeding	Essential to speed up the breeding process enabling incorporation of improved traits to crops in response to rapidly changing climatic conditions.
Synthetic biology for whole pathway development	Highlighted as active research area enabled through gene editing. Example projects include rewiring photorespiration (GAIN4CROPS and BestCrop) as well as the use of plant systems as cell factories to meet the need for sustainably derived raw materials.
Expanding the genetic diversity of crops	Allelic diversity in modern crops has been severely reduced during the selection process. Genetic diversification of crops can be improved by exploiting crop genetic resource collections to mine for improved stress resilience, adaptivity and productivity traits.
Improved multi-trait breeding approaches required	Conventional or NGT breeding methods to achieve resilience to multiple (but potentially short term) stresses associated with climate change to improve yield, resiliencies and resource use efficiencies, that will enable photosynthetic improvements to be ‘expressed’ under wide agro-ecological scenarios.
Life Cycle Analysis	To quantify the real cost-benefits of the adoption of agronomic innovations promoting better decision making for investment.
“On-farm” living labs to advance from controlled environments to field phenotyping	There is an urgent need to facilitate the speedy translation of research towards breeding programmes. Field phenotyping is a vital step to validate outputs and progress TRL Suitable sites, including “on-farm” living labs are needed to allow for testing under real world and experimental conditions.
Sensors, digitisation, AI data platforms and Envirotyping	Rapidly emerging technologies in agriculture will enable better field phenotyping, as well as improved mechanisation and robotics in plant breeding and farming practices. Plant breeding is increasingly ‘big data driven’ and innovations in AI and modelling are providing tools to collect and process large data sets. Better and cheaper sensors will allow ‘Envirotyping’ i.e. the use of environmental factors eg. soil, climate, local ecology to compliment genotyping and

	phenotyping to improve crop modelling, phenotype predictions and management practices.

10.1 Enabling Phenotyping devices

Translation to the field needs suitable phenotyping tools. Recent advances in the support tools and technologies now available allow improvements in photosynthesis traits to be identified and quantified in the field. The tools currently used by researchers are not always user friendly enough to be embraced by breeders and growers.

Scaling up effective translation to the field requires new breeder-friendly phenotyping tools using standardized tools & methods to measure photosynthesis. This will complement the expertise of breeders, facilitating faster adoption of scientific advances.

The development of tools such as the “Breeders Fitness Eye 2.0” handheld device by PSI are linking new devices with advanced modelling and AI aims to give breeders and growers an easy plant health check through measurement of photosynthesis, complimenting experienced breeders' ability to assess plants.

Development of breeder friendly tools is an important segway to easily phenotype photosynthetically superior plants in breeding programmes

Figure 11 Handheld device developed by PSI in the CAPITALISE project. Picture credit PSI.

A photosynthesis based accelerated breeding toolbox for next generation crop resilience breeding programmes. Rapid phenotyping of photosynthesis in the field is critical to understand the limits to crop performance and under dynamically changing environmental conditions. The innovation exploits easy-to-use sensor arrays for rapid phenotyping of photosynthetic performance. The sensors are integrated with advanced predictive modelling. This quantitative “Breeders Fitness Eye 2.0” is a new approach for breeders to access the wealth of information on plant performance.

High-throughput phenotyping infrastructures are becoming available in crop research institutes across Europe. This expands the scale and robustness of experimental work. The new NPEC system at Wageningen allows high-throughput phenotyping to assess plant performance. At IPK, the recently installed PhenoSphere represents an advanced indoor high-throughput phenotyping system using strictly controlled environmental conditions to run climate simulations. Manipulations of variables including air and soil temperature, relative air humidity, vapor pressure deficit (VPD), light quality and quantity, CO_2 levels, and wind simulation can be automatically controlled and to mimic seasons, day length, day and night cycles, and field-like frequency and amplitude dynamics of conditions (Heuermann et al.,2023). These systems represent a new level of indoor phenotyping to accelerate translation of research.

Figure 12 The PhenoSphere at IPK (picture credit IPK)

Figure 13 NPEC at Wageningen (picture credit WUR)

Ground Robotics and the state of the art field phenotyping

Field phenotyping using manual methods is labor- intensive and inefficient. Advanced field phenotyping systems are increasingly vital, enabling the collection of extensive data to support analysis and decision-making. At the CAPITALISE workshop in France, the PhenoMobile at Arvalis showcased high-throughput field phenotyping capabilities. This fully automated, ultra-precise system continuously monitors multiple crop traits non- destructively in field conditions. The integration of AI and robotics is anticipated to play a growing role in advancing crop science and improving phenotyping efficiency.

Figure 14 Two types of phenotyping devices : the large phenomobile, and the 'literal' (a light and easy-to-carry system). Credit : Arvalis and Hiphen.

The European Strategy Forum for Research Infrastructures (ESFRI) identified "Plant Phenotyping" as a priority for the European research area, programmes like EMPHASIS (European Infrastructure for Plant

Phenotyping) enable the use of State-of-the-Art phenotyping facilities such as the PhenoSphere for researchers.

10.2 Remote sensing for Photosynthesis, Crop Improvement and Carbon Sequestration - Fabienne Maignan

Remote sensing in the optical, thermal and microwave spectral domains provide crucial information on various agronomic variables at the canopy scale such as height, leaf area index (LAI), albedo, temperature, and soil moisture. The altitude of sensors can range from close (proximal sensing with fixed or mobile structures like towers and unmanned aerial vehicles) to intermediate levels (e.g., using aircrafts) and up to satellite platforms. The applications of remote sensing depend on the spatial scale of the observed area, and include phenotyping, land-use monitoring, yield forecasting, yield optimization, and the estimation of ecosystem services like carbon sequestration (Weiss et al., 2020).

The introduction of the ESA Copernicus Sentinel satellites has greatly expanded the applications in the field of crop studies, particularly with Sentinel-2 (S2) data in the optical domain. With a decametric resolution, and a global coverage with a revisit of 5 days since 2018, these freely available datasets can be processed on powerful cloud platforms, such as Google Earth Engine (Gorelick et al., 2017). S2 data facilitate the monitoring of crop phenology at the parcel scale, across various cropping systems. For instance, the Sen2-Agri near-real time system has already produced crop type maps and vegetation status indicators for entire countries, using machine-learning algorithms (Defourny et al., 2019).

Figure 15 The Netherlands by satellite imaging

The LAI and gross primary production (GPP) can be estimated from S2 data using vegetation indices or machine learning methods (e.g., Wolanin et al., 2019). Above-ground biomass can be further estimated based on process-based crop or land surface models (e.g., He et al., 2021). Solar-induced fluorescence, a radiation emitted by plants under sunlight, provides insights into the plant photosynthetic activity and its physiological status, serving as an early stress detector. The upcoming

ESA FLEX mission (Fluorescence Explorer, to be launched in 2026) will provide such data at a 300m resolution.

Satellites also contribute to precision agriculture. For example, S2 optical data can be used to estimate chlorophyll content, which is related to nitrogen content and may inform on nitrogen deficiency (Xie et al., 2019). Thermal remote sensing, which provides information on evapotranspiration, is useful for irrigation management. In this regard, the upcoming Indo-French satellite TRISHNA, scheduled for launch in 2026, will offer estimates at unprecedent high spatiotemporal resolution (60m, 3-day revisit), suitable for most agricultural plots (Roujean et al., 2021).

The soil organic carbon (SOC) content cannot be directly estimated from remote sensing data. Most models are developed based on the spectral characteristics of bare soil images, primarily from optical sensors (e.g., S2), sometimes coupled with radar imagery (e.g., from Sentinel-1). However, additional information, such as crop type, and agricultural practices, is necessary. This remains an evolving research field (Vaudour et al., 2022).

Above all, an integrated approach that combines satellite data, local observations, expert knowledge, and models, each with its own associated uncertainty, is essential for providing robust solutions for crop improvement in the context of climate change.

10.3 A Data Platform for Photosynthesis research- Ambitions of the JII - Dave Kramer

The Jan IngenHousz Institute represents an important strategic partner in Europe. The overall goal of the EU Roadmap is to increase the resilience and productivity of crops while reducing impacts on the environment and natural resources, resulting in a more robust agricultural system that relies less on imports, minimize resource loss, and contributes to sustainability.

Previous research has shown that photosynthesis is central to these optimizations, and recent work, in part supported by the EU programs, has provided proofs of concept that selective modifications of photosynthesis can, indeed, improve crop performance by taking advantage of rapid advances scientific knowledge, genetic resources, plant phenotyping and data science.

However, targeting such complex and dynamic processes as photosynthesis will require a highly integrated approach. We must consider a range of biochemical and physiological traits, ranging from light energy capture, CO_2 fixation, carbon allocation, source-sink and plant-soil feedback limitations to maximize yield while minimizing water loss through, fertilizer application and impact from extremes of temperature and other environmental fluctuations. Moreover, the genetic control of these traits must be identified and controlled for each crop in each growing region.

No single approach, laboratory or crop improvement program can address these questions on their own. Instead, realizing real gains will require novel, highly integrated scientific approaches that innovate across disciplinary and cultural boundaries.

The JII Institute is established to address precisely this issue to accelerate cohesion at multiple levels that all will be addressed at JII:

1. Data platform to collect, manage and model direct and indirect photosynthesis data,
2. Standardized tools & methods to measure photosynthesis,
3. Setup and coordinate (EU) Living labs for collaborative and iterative testing, and
4. Physical space (the photosynthesis Clubhouse) to meet and interact.

In order to build the key “missing piece”, a unified, open science platform is being developed to probe photosynthesis at scale, under field conditions. This will link the development and application of novel, high-throughput sensors, data science, and genetic resources at previously unattainable scales. Most importantly, these tools must be deployed among a large cadre of researchers to link the EU with teams in many international countries, across many disciplines, from basic to highly applied science and engineering.

The platform will be applied to answer critical the following questions:

- ✓ In what crops and under what conditions is photosynthesis limiting?
- ✓ What processes are involved? Which of these processes are the best targets for crop improvement?
- ✓ Which of these can be immediately be incorporated into crop improvement pipelines?
- ✓ Which represent the next generation of opportunities?

Only with full sharing of resources and knowledge can we make the rapid progress needed to realize true gains in productivity. For example, only by working closely with crop experts can sensor developers, physiologists and modelers know what traits are likely to be the most impactful.

11 The benefits of public private partnerships

The European seed and crop breeding sector is made up of a highly diverse range of businesses from large corporates and numerous small to medium-sized enterprises (SMEs). The seed sector plays a critical role in agricultural sustainability and innovation in Europe. The SMEs contribute significantly to the resilience of regional agricultural systems. Many of these companies need to be innovative to remain competitive but lack the research and innovation resources and capacity of the large businesses. In contrast several large multinational corporations, such as Bayer, Syngenta, and Limagrain, have the resources to invest heavily in research and development for new breeding technologies.

The current fragmented nature of research funding in plant and crop sciences is creating unnecessary barriers to rapid translation of promising results

To strengthen European competitiveness in agriculture and the bioeconomy, an integrated strategy is needed to advance biotechnologies and accelerate crop research. Conventional crop development cycles of 10–15 years are mismatched with the 3–5 year research funding schemes. This limits opportunities to quickly reach high crop Technology Readiness Levels (TRL). A European approach can streamline the research-to-application pipeline, addressing inefficiencies and fragmentation that hamper responses to climate stresses, food security, and feedstock shortages. While industry can support higher TRL innovations, short-term public funding often prioritises exploratory academic research and publications. This may discourage translation of results. Stronger collaboration between academia and industry is essential to advance promising strategies such as enhancing photosynthesis.

Overall, the balance between SMEs and larger companies allows the sector to remain both innovative and adaptable to diverse agricultural needs across. Encouraging new partnerships between industry and academic plant scientists to take the most promising strategies forward is now required to accelerate progress.

A survey of breeders outlined some clear research outputs they would like from the academic sector:

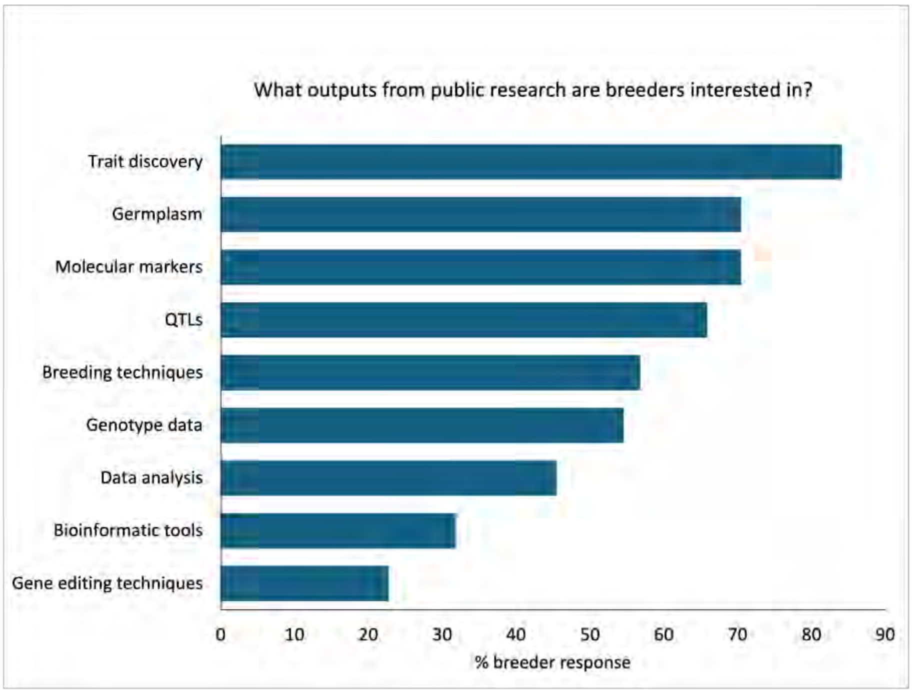


Figure 16 Online survey results exploring the interest from breeders in public researchers outputs.

In developing new collaborations there are clear opportunities for synergistic projects. Industry can provide multiple high value services to collaborate with the academic sector and advance the TRL of target crops.

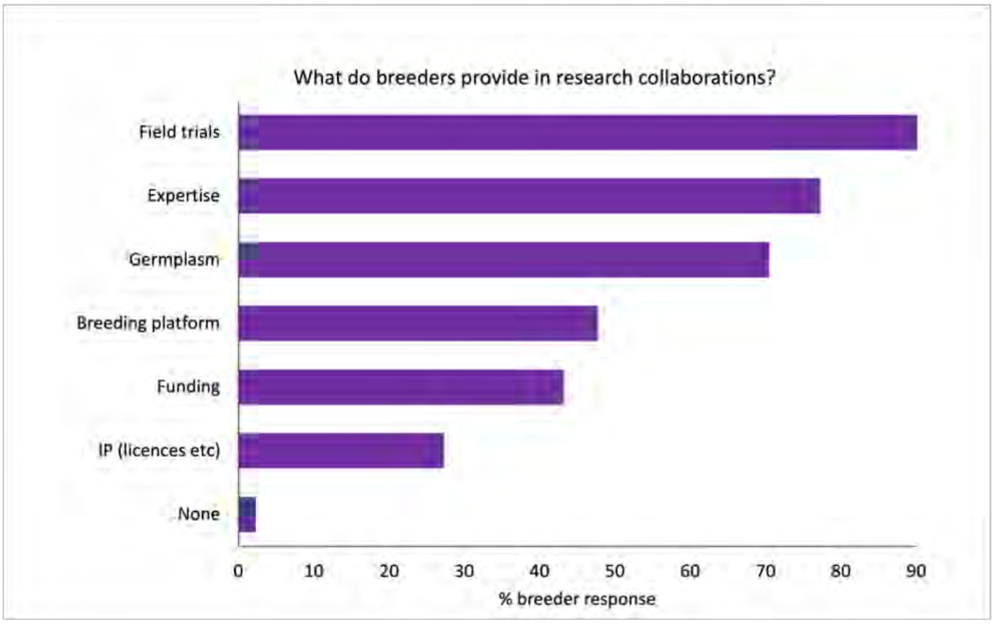


Figure 17 Online survey results exploring what breeders provide in research collaborations

There is a perception from some participants that public funders are more interested in funding new and exciting research rather than further funding aimed at improving TRL levels of promising research. At the same time there is little incentive for academics to translate results outside of the lab – in academia the number of publications are typically valued more highly than the translation of results to industry and their application to real world problems.

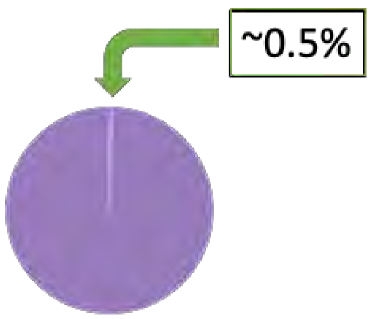


Figure 18 Our estimate indicates less than 0.5% of the ~€80 billion H2020 research and innovation budget targetted crop research.

Despite recognising the important role of crop improvement and commitments at EU level, the funding levels for crop research in the European Framework Programmes have been surprisingly limited. The Horizon 2020 programme that preceded the current Horizon Europe programme had a budget estimated to be around €80 Billion. Based on a H2020 search for projects using terms "crop breeding"; "crop improvement"; "agriculture"; "photosynthesis"; "photosynthesis+algae"; we identified 229 projects and estimate that the costs of all equate to less than 0.5% of the 80 billion Euros for research funding. Further analysis by Plants for the Future ETP indicated a decline in funding for plant breeding research although Horizon Europe funding rose by 42% compared to FP7.

Crop funding is currently fragmented across multiple projects without a clear strategy despite multiple policy drivers and clear market failure.

The effects of climate change and population growth, even within Europe are being readily felt. Data from our surveys backs up the need for climate tolerant crops. Given the 10-15 years needed to develop a new variety – speed is of the essence. Further funding in Europe for harnessing photosynthesis as a tool for crop improvement is critical for improving yield resilience and sustainability for the future.

We recognise that to address the current fragmentation in plant breeding research and innovation, and given the long timelines, a critical mass of funding and coordinated European approach is needed. This will allow public private partnerships to effectively exploit discovery science and precompetitive breeding ***to produce new and improved crops and algal production systems*** delivering more sustainable agri-food systems and a circular bioeconomy***. This is in agreement with the current calls from the*** **Plant European Technology Platform (TP)** and other primary production TPs for a new partnership "**Optimising genetic potential for resilient and diverse production systems**".

12 Photosynthesis: an undervalued solution?

A CAPITALISE survey asked breeders and growers to prioritise their current breeding goals for their crops of interest. Overall, the three highest priorities identified across all the crops were yield, abiotic stress tolerance and quality traits, by both breeders and growers.

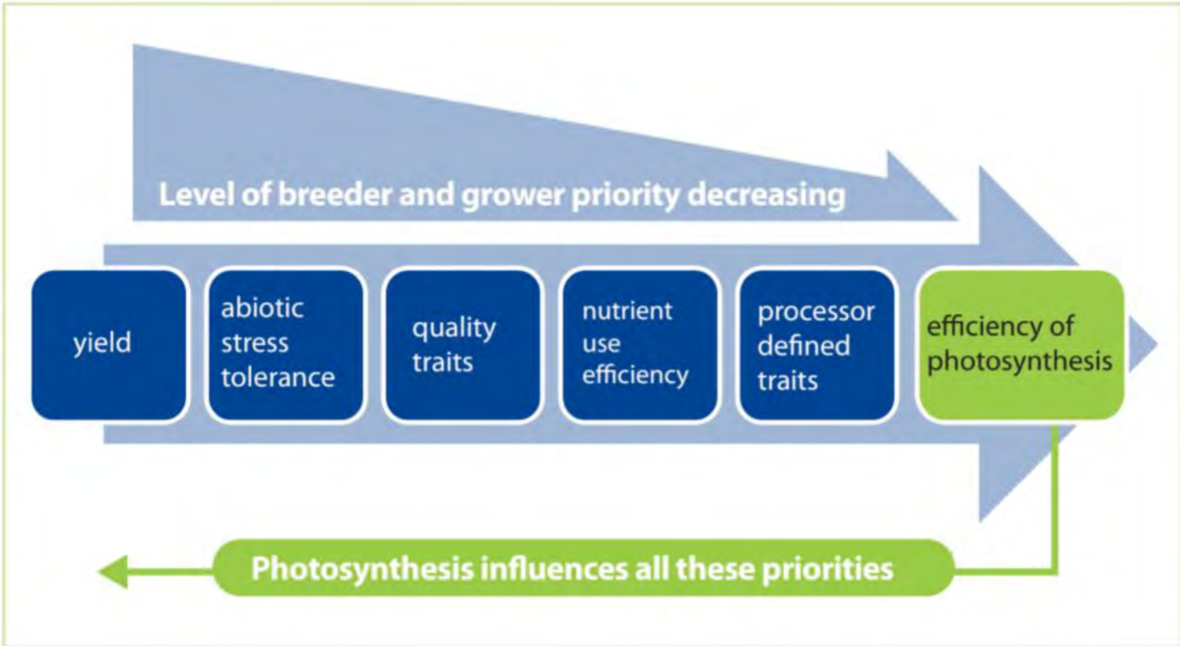


Figure 19 In our survey 70 breeders and growers scored traits for the crops they worked with. The role of photosynthesis was rated a low priority.

The efficiency of photosynthesis was rated a relatively low priority. The breeders and growers surveyed did not seem to recognise that improved photosynthetic efficiency has been shown to influence their other priorities.

The full advantages of improving photosynthetic efficiency are not fully appreciated by many value chain actors.

The policy drivers for EC investment in photosynthesis research were primarily linked to population growth, increasing yield and long-term food security. Unsurprisingly, industry breeding goals and grower crop priorities were focussed on more immediate challenges, primarily linked to abiotic stresses associated with climate change and market demands.

13 Challenges to using Photosynthesis related traits in a breeding context - A viewpoint - Gemma Molero, Group Lead Wheat Research and Pre-Breeding at KWS

Breeders have been interested in photosynthesis-related traits for many years, but their adoption has been limited due to several bottlenecks. These include the complexity of the traits, the need for sophisticated equipment to measure them, and the lack of clear association with key performance characteristics in target environments. Additionally, there is often a lack of significant genetic variation and workable repeatability/heritability for these traits.

Despite these challenges, CAPITALISE and others are developing better field phenotyping devices and high-throughput phenotyping (HTP) platforms that can provide meaningful data for breeders

(Kuhlgert et al., 2016; Silva-Perez et al., 2017; Furbank et al., 2019; Reynolds et al. 2020; Robles-Zazueta et al., 2021). These tools can help in screening diverse sets of genetic resources and elite lines for good expression of traits like final biomass and radiation use efficiency (RUE) (Molero et al., 2019; Joynson et al., 2021). These integrative traits (biomass and RUE) together with canopy temperature have been already used in wheat breeding programs (Reynolds et al. 2020) while specific and more complex traits (e.g. spike or leaf photosynthesis) are more difficult to be integrated due to the lack of fast phenotyping tools available (Molero and Reynolds 2020). Public researchers could maximise the adoption by breeders of their research outputs if they consider that a publication is not an end product; the results need to be connected with breeding programs to be useful. By ensuring that traits show significant genetic variation, good heritability estimates, and a correlation with yield and/or yield components, researchers can increase the likelihood of their adoption by breeders. In addition, most public and commercial breeders do not use sophisticated equipment in their programs due to the large number of lines (>1,000's) they need to phenotype each cycle. However, they do use markers, genomic selection tools and meaningful data from HTP platforms. Advancements on these areas will also contribute to the adoption of photosynthesis related traits in breeding. Additionally, researchers should connect their results with breeders through collaborative projects such as CAPITALISE (www.capitalise.eu/photosynthesis/) or translational research platforms such as IWYP (www.iwyp.org). Doing this they will ensure that they work with relevant plant material for breeders, and they address their needs. Breeders want outputs that are relevant to their breeding targets, which are determined by market requirements and the demands and needs of farmers, processors, and consumers.

Projects focusing on photosynthesis can improve the translatability of their results by ensuring that the traits are easy to measure, have validated markers, and show a clear association with key performance characteristics.

14 Barriers to translation of photosynthesis research

Faced with the urgent need for more resilient higher yielding and sustainable crops, plant translational research must step up to meet these challenges and move from the lab to field to the breeder. Stakeholder workshops and an online breeder and grower survey identified the following barriers to taking research outputs forward from a researcher and stakeholder point of view.

Infrastructure and tools for carrying out field trials Taking research from the lab to field is problematic for public researchers due to the limited facilities for field trials. The European commission are addressing this with a network of Living Labs. Promoting collaborations with industry partners is also a key step to overcoming this barrier.

Photosynthesis is a complex trait, encompassing a set of sub traits that determine resource use efficiency and resilience e.g. stomata, reactive oxygen generation and the development of better sensors and phenotyping tools are needed, for field work, for use by researchers but also user-friendly devices aimed at the breeder and grower community are needed if these traits are to be adopted into breeding programmes. Sensor development and AI is a key milestone for taking photosynthesis translational research forward. The CAPITALISE project have been addressing the need for user friendly tools, such as PSI's development of a 'breeders eye' device to measure 'plant health' aimed at breeders and growers, a proxy for photosynthetic efficiency traits.

Acceptance of NBT and genetically modified crops Although the EC funds much innovative research using NBTs and genetically modified plants, there is little provision for continued translation of this work in crops outside of the lab. Legislative problems in carrying out trials using gene edited plants in Europe (including red tape in the UK where they will be permitted soon) is a problem for taking research results forward. Alongside this is the issue of acceptance by consumers for NBT derived products. Although currently in discussion in the EU it may take some time for acceptance of NBT derived breeding methods to be allowed happen in practice for researchers (and breeders). Given the length of time needed to bring an improved variety to market, the translational research required for many NBT derived innovations needs to continue for strategies to be ready to address the changing needs of society. NBT derived products should be in the pipeline in the (not unlikely) event that global change requires solutions that can only be provided by NBTs and/or GMOs. This is a question of being prepared, not of what may or may not be marketable under the current regulatory framework. It may be that NBT or GMO solutions are not needed, but if so, we need to have them on the shelf to roll out quickly. Things become acceptable very quickly if other solutions are not available. From a policy standpoint (and also scientifically), it would be irresponsible to exclude viable solutions to problems exclusively on the basis of an outdated regulatory framework that dates back to the last century.

Short term research funding for long term projects Inconsistent (3-5 years) funding streams in plant research, without conditional follow-on funding are too short and fragmented to allow significant rapid increases in TRLs for crops. Current European funding leads to disruption of successful research consortia, interrupting progress, and creates disjointed 'islands of research' (Borrell and Reynolds 2017). Schemes such as Horizon Europe have the potential to provide a route to take successful project results forward with winning teams. But the current strategy needs to be redesigned to support longer term Public Private Partnerships ensuring continuity and accelerate progress in crop innovation through longer term collaborations.

IP and Industry collaborations IP and the freedom to operate is a significant issue for breeders. Researchers need an appreciation that a) publications may need to be delayed in some cases to allow invested industry partners rights to initial exploitation and b) without an IP agreement, results are very unlikely to be translated by Industry. Around 37% of breeders cited IP as a barrier to working with public researchers in our online survey. CAPITALISE and Euroseeds co-organised two webinars aimed at researchers, to address this. These were delivered on 15th and 22nd November 2024 . Speakers Szonja Csörgö (World Seed) presented on the Nagoya protocol with a case study from our partner BASF, and Francesca Garbato (Euroseeds) on IP.

43% of breeders in our survey highlighted complications in coming to a collaboration agreement and (39%) a lack of interest by public researchers to develop end products/apply their research to commercial products were issues in working with academia.

Germplasm Good access to germplasm sources, was highlighted as a key barrier by breeders. See below for a breeder point of views on this. Breeders also highlighted that often public researchers worked on irrelevant/old varieties of crops, rather than modern varieties used by industry making translation more difficult. Access to modern germplasm by researchers could be facilitated through closer collaborative working of public researchers with industry.

14.1 Germplasm an industry view point - Anna Giulia Boni and Massimiliano Beretta at ISI Sementi

The Industry's Need for Better-Characterised Germplasm in Germplasm Banks for the Vegetable Business

The vegetable industry and the seed companies rely heavily on the availability of diverse and well-phenotyped germplasm. Germplasm banks, which store seeds material for breeding and conservation purposes, play a critical role in this process. However, despite the existence of these vast repositories, the horticultural industry is facing the lack of detailed information that would allow breeders to exploit the stored allelic variability for breeding programs, ultimately leading to the development of new varieties. Right now, the current lack of projects dedicated to genotyping and high throughput phenotyping in many germplasms accession, makes it difficult for breeders to identify the best candidates for developing better performing varieties or find the proper source of traits.

The need for well characterized germplasm is driven by several factors, including:

- Climate change: unpredictable weather patterns, increased temperatures, and shifting rainfall patterns are causing stress on crop yields and quality. To mitigate these challenges, breeders need access to well-documented germplasm with specific traits such as drought tolerance, disease resistance, and temperature resilience.
- Market demands for new and improved varieties: consumers are increasingly looking for high-quality, diverse, and nutritious horticultural products. To meet these market needs, breeders require access to germplasm that has been accurately characterised in terms of morphological, biochemical, and genetic traits.
- The need for sustainable agricultural practices: the shift toward this kind of agriculture, is possible only through the development of varieties that are naturally resistant to pests and diseases or require fewer resources to grow. Well-characterised germplasm, with information on resistance to biotic and abiotic stresses, is essential in this process.

Unfortunately, many germplasm banks currently hold large collections of genetic material that are poorly documented: very often the accessions have never been evaluated or the information available is outdated/incomplete. This lack of information leads to inefficiencies in breeding programs and it reduces the exploitation of new potential private-academical projects. For these reasons, breeders often have to invest considerable time and resources in evaluating the germplasm themselves; but, since only the big companies have the resources to develop proper pre-breeding programs, small-medium enterprises find themselves penalized.

To address this issue, there is a need for increased investment in germplasm characterization efforts. This includes conducting comprehensive evaluations of existing collections using modern tools such as molecular markers, genomic sequencing and high-resolution phenotypic analysis. Additionally, better data management systems and databases are required to ensure that breeders and researchers can easily access and utilize the information available in germplasm banks. Collaborative efforts between researchers, breeders, and industry stakeholders are also essential to ensure that the characterization process is aligned with the needs of the horticultural industry.

Shifting Leadership: The Impact of Limited Public Funding on Genetic Resource Development and characterization

In recent decades, the public research has significantly reduced its involvement in the development of genetic resources, primarily due to limited funding. Because of a constrained budget, public institutions have shifted their focus towards genomic and other "-omic" studies, as these offer faster and more immediate results compared to the laborious process of creating and characterizing new genetic resources. This shift has led to a decline in public involvement in producing and preserving germplasm.

Conversely, seed companies have intensified their efforts in genetic resource development, increasingly taking the lead in the selection, characterization, and commercial exploitation of genetic material. These companies possess the infrastructure and financial resources to undertake long-term projects, such as creating new varieties through crossbreeding with wild relatives. This process, which can take years, requires dedicated personnel, specialized facilities (like test fields and greenhouses) and advanced data management systems to efficiently handle phenotypic and genotypic information.

As a result of this process, the leadership in the germplasm development field, has shifted from public to private entities. The players who control and characterize seed accessions, not only have access to critical genetic information but also the know-how to commercially exploit it. This has reduced the influence of the public sector in favor of seed companies, who now dominate the field of genetic resource management.

The investment required to develop new genetic resources is substantial. It involves long-term efforts (often spanning decades) and relies on specialized human resources, infrastructure, and sustained funding. Without strong public sector involvement and international strategic coordination, there is a risk that genetic diversity will increasingly fall under the control of a few private actors, limiting access to these resources in the future.

This scenario highlights the need for balance between public and private sectors. While private companies may have the economic and technological edge to drive research and development forward, the public sector should continue to play a critical role in the conservation and creation of genetic resources, ensuring they remain accessible for the scientific community and for the broader public good.

Academia's Role in Using More Modern and Relevant Germplasm in the Vegetable Field

In the field of vegetable research, academic institutions have traditionally relied on a limited number of model plants for scientific studies. While these varieties have contributed significantly to our understanding of plant biology and genetics, there is a growing recognition that they may no longer be sufficient to address the modern challenges facing vegetable breeding and production.

The introgression of beneficial genes from model plant varieties into modern commercial vegetable crops, presents significant challenges despite advancements in plant breeding techniques. Academic research often focuses on model plants like *Arabidopsis thaliana* or other simplified vegetable varieties, which provide valuable insights into gene function. However, transferring these discoveries into commercially viable crops is far from straightforward. As a result, the gap between academic discovery and practical application in the vegetable industry remains significant.

Note the EU projects recognise the importance of publically funded research to further blue skies research is important but there is also an important role in solving societal problems. Industry would like. academic institutions to also prioritize collaboration with the vegetable industry to ensure that their research remains practical and relevant. Too often, academic research is disconnected from the immediate needs of farmers and breeders. Stronger partnerships between academia and industry will ensure that the germplasm being developed is directly aligned with the challenges and opportunities faced in commercial vegetable production. Engaging with industry stakeholders can provide valuable insights into market trends, regulatory issues, and practical challenges that breeders and farmers face. These collaborations also ensure that research outcomes are more rapidly translated into commercial products that benefit both producers and consumers.

Successful example of public-private cooperation in germplasm characterization

A successful example of public-private cooperation is the EVA (European Evaluation Network) project: this is linked to the evaluation of European maize, lettuce, carrot, and pepper germplasm, which has led to significant results in crop genetic improvement. Thanks to the collaboration between public and private partners, the project evaluated 600 maize accessions and numerous accessions of lettuce, carrot, and pepper conserved in European gene banks: phenotypic and genotypic traits were analysed under various environmental conditions. These data were used to identify the accessions more adapted to climate change and resistant to biotic/abiotic stresses, providing a solid foundation for the development of more resilient varieties. The EVA project demonstrates how the collaboration between researchers and private companies is essential to improve the exploitation of genetic diversity in crops, overcoming traditional barriers that hinder the efficient utilization of genetic material available in gene banks. By sharing these data via the EURISCO platform, breeders across Europe could benefit from critical information for plant breeding, leading to a speed up in their breeding programs (https://www.ecpgr.org/eva).

The Importance of Publicly Accessible Introgression Lines in Advancing Plant Breeding and Agricultural Research

The creation of available introgression lines by public institutions (RILs, MAGIC, ILs, BILs), distributed free of charge for research projects, represents a vital opportunity for studying new alleles and phenotypic traits, offering practical solutions to the challenges faced by modern plant breeding and agriculture. To address these issues, it is crucial to implement introgression lines (RILs), including those from wild species, to provide easy access to detailed information on important genes and traits.

The value of RILs, lies in their ability to provide precise genetic mapping due to the high level of recombination achieved through introgression. These lines allow to study new alleles and to better understand the relationship between genes and complex phenotypes, such as resistance to abiotic/biotic stresses, drought tolerance, nutritional quality. For the plant breeding sector, RILs are a crucial tool to quickly identify sources of useful traits without the need for significant investments in phenotyping and genotyping existing collections.

Moreover, the free distribution of RILs would promote the collaboration between public institutions, academia and industry, speeding up both the research and the rapid transfer of knowledge from basic science to practical applications. This not only supports the agricultural sector in creating more resilient and sustainable crop varieties, but also answers the growing demands from the market for higher-quality and more diverse products. Finally, free access to these data encourages innovative, multidisciplinary projects, fostering sustainable and adaptive agriculture, which is essential to address the global challenges of climate change.

In summary, the establishment of publicly available introgression lines represents a crucial investment in germplasm characterization, filling a gap that currently limits the potential of genetic improvement programs and providing essential tools for advancing agricultural research and the development of more efficient and sustainable crop varieties.

14.2 Life Cycle Assessment - Monique Branco-Vieira and Jovanka Saltzmann

LCA can support decision making around photosynthesis driven crop breeding strategies and adoption by growers/the market and to support the environmental sustainability of the technologies. Evidence indicates that breeding advancements significantly contribute to mitigating climate change by reducing the carbon footprint of cereals. However, in the initial phase of breeding programs, only limited information is available regarding the potential sustainability of future crops. Life cycle assessment (LCA) is a tool that can promote a sustainability-focused design process by enabling a thorough evaluation of how target traits in breeding programs might affect the entire production chain. It is a robust methodology that helps decision-makers and breeders anticipate potential environmental, social, and economic impacts. When LCA is linked to physiological plant growth models, it becomes possible to visualize the effects of cellular-level changes on the field scale, allowing the impacts of innovations to be anticipated at a very early stage of development.

Assessing potential impacts during breeding will aid in selecting more sustainable crop varieties that meet future needs, streamline agricultural processes, and strengthen both regional and global bioeconomy's. LCA methods typically assess present or near-future product scenarios, requiring primary process data from field trials or datasets from various assays. However, not all primary data are useful for conducting an LCA. Project partners must define the objectives of the project regarding sustainability analysis *a priori* and ensure that their assays and trials include datasets relevant for use in LCA methods.

Usually, field data are often lacking in the early stages of breeding programs, making it necessary to use *in silico* analysis to evaluate the potential future impacts of new cultivars. For conducting these analyses, it is crucial to use a primary dataset, ranging from biochemical to process data provided by project partners, to feed different models and gain insights into yield prediction and the relationship between resource use and productivity. Acquiring detailed information about all inputs and outputs in a process, such as materials, energy, and emissions (known as inventory data) presents a challenge in prospective LCAs due to significant scaling effects when moving from small to large process scales. This bottleneck is particularly pronounced when evaluating new bio-based products, where there is often a nonlinear relationship between resource use and biomass production.

Addressing this gap requires a multidisciplinary approach that captures the complex nonlinear interactions among genotype, environment, and management in crop development, growth, and yield. The quality and availability of primary data are crucial in this process and their robustness can contribute to bridge this gap more effectively.

In addition to use in crop breeding programmes, society may be engaged through developing a user-friendly interface and/or interactive dashboard that the citizen/stakeholder can use to predict yield in different challenging environments (such as climate change scenarios), and by choosing different parameters for crop improvement. This educative tool can elucidate differences between types of breeding process (traditional X GMO) and clarify misunderstand science concepts and show how important is the crop improvement research.

15 Current Strategies of Photosynthesis Improvement

There are a number of well-known strategies for improvement of photosynthesis efficiency, at various levels of advancement and translation level, many of which are used in CAPITALISE, GAIN4CROPS, PhotoBoost and BestCrop. Each strategy presents different challenges and benefits and suit particular environments and type of agriculture. No single strategy is applicable to all crops, but combinations of some strategies may give wide ranging benefits to crop resilience and efficiency. Modelling can give insights into the most optimal combinations. The strategies are described in here highlight the benefits, the trade-offs and the challenges and next steps. Translation readiness levels (TRL) of each strategy is also estimated.

15.1 Transmit more light to lower canopy- A CAPITALISE strategy (TRL 5-6) - Jean Alric and Paolo Pesaresi

Figure 20 Examples of chlorophyll mutants. Credit: CEA and The photosynthetic mutant library http://pml.uoregon.edu/photosyntheticml.html

A significant advantage of reducing chlorophyll content in the upper canopy of crops is enhanced light penetration. By decreasing the chlorophyll concentration at the top of a crop, more light can reach the lower canopy, potentially boosting overall photosynthetic efficiency across the crop as a whole. Additionally, this approach is expected to lower nitrogen input requirements, as chlorophyll and its associated macromolecular protein complexes are nitrogen-rich. Managing crops with lower chlorophyll content can lead to reduced nitrogen inputs, cutting fertilizer costs and environmental impact and contributing to more sustainable cropping systems. In fact, low soil nitrogen fertilization can serve as a selection pressure for optimizing low-chlorophyll crops. This reduction in chlorophyll content can also complement structural traits, such as erect leaf architecture, that improve light penetration throughout the canopy. By combining traits with reduced chlorophyll, crops can achieve higher light penetration and faster relaxation from non-photochemical quenching (NPQ), potentially leading to higher yields.

Optimising crop architecture can lead to yield improvements of up to 20% in crops like wheat. There are also potential improvements in water use efficiency due to optimized light distribution.

One of the strengths of this approach lies in the ease of selection for traits related to chlorophyll content and plant architecture, it is also achievable through traditional breeding or genetic engineering. Numerous handheld instruments are available to measure leaf chlorophyll, often alongside other traits, such as those developed by the CAPITALISE project.

Challenges, milestones and the way forward: There are, however, some limitations to this strategy. Achieving a proper developmental chlorophyll gradient, or "smart canopy," for optimal light capture and utilization is complex and not fully understood. Introducing and maintaining lower chlorophyll traits can have pleiotropic effects, such as increased photosensitivity and altered stress resistance. Moreover, targeted modifications or the combination of traits might necessitate genetic modification, which faces regulatory and public acceptance challenges.

Identifying and manipulating genes responsible for lower chlorophyll content without causing adverse effects is crucial. Additionally, field instrumentation must be developed to measure and monitor light penetration and chlorophyll content effectively. Ensuring that overall light harvesting efficiency is maintained is essential, as is the accurate detection of nitrogen deficiency to optimize fertilization strategies.

Given the significant potential, particularly in crops such as wheat and maize, breeding for these traits is worth pursuing. Advanced crop models, such as 3D radiative transfer models, can help predict outcomes and guide breeding efforts. These in silico crop models are beneficial for simulating various scenarios and predicting the impacts of genetic changes on light distribution and yield. However, these models require accurate input data and validation against real-world conditions. Existing models, including 3D radiative transfer and ray tracing models, need refinement for this specific application. In the short term, existing models can be used, while in the long term, more sophisticated models incorporating photosynthesis and light distribution dynamics should be developed.

Currently, this concept is at TRL 5-6, indicating that it has been validated in the field and has been introgressed into KWS elite breeding materials. It has yet to be widely adopted in commercial breeding programs. This technology is available now but will take around 10 years for elite varieties to be developed.

To translate this concept into practice, several challenges need to be addressed. These include introducing traits into elite lines and ensuring their broad applicability across different environments.

In the frame of the BestCrop project, the *chlorina* mutant collection obtained by chemical mutagenesis in the cultivars *Tron*, *Bonus* and *Donaria*, has been screened by a forward genetics approach searching for mutants with reduced leaf chlorophyll content and increased fluorescence yield of photosystem II (Y_{II}). Seventeen mutants with the desired traits have been selected and the causal mutations identified in most of them. In detail, mutations affect multiple pathways, including factors involved in antenna biogenesis, chlorophyll biosynthesis, chloroplast division, thylakoid membrane biogenesis, and chloroplast integrity. Recently, the *xan-h.chli-1* mutant, which carries a missense mutation in the *Xan-h* gene for subunit I of Mg-chelatase (HvCHLI), the first enzyme in the chlorophyll biosynthesis pathway, has been fully characterised. Intriguingly, *xan-h.chli-1* is the only known viable homozygous mutant at the *Xan-h* locus in barley. The Arg298Lys amino-acid substitution in the ATP-binding cleft causes a slight decrease in HvCHLI protein abundance and a marked reduction in Mg-chelatase activity. Under controlled growth conditions, mutant plants display reduced accumulation of antenna and photosystem core subunits, together with reduced photosystem II yield relative to wild-type under moderate illumination, and consistently higher than wild-type levels at high light intensities. Moreover, the reduced content of leaf chlorophyll is associated with a stable reduction in daily transpiration rate, and slight decreases in total biomass accumulation and water-use efficiency, reminiscent of phenotypic features of wild barley accessions and landraces that thrive under arid climatic conditions (Persello *et al.* 2024).

The timeline for translation of this strategy is relatively short, estimated at 5-10 years compared to multi-gene GM traits. Acceleration can be achieved through advanced breeding techniques, improved field instrumentation, and robust model predictions.

15.2 Rapid relaxation of NPQ-CAPITALISE (TRL6) - Johannes Kromdijk

Figure 21 A example of the changes in light intensity across leaves.

Changes in light intensity occur frequently in crops during short term cloud cover or light flecks between leaves through wind movement in the canopy. This strategy addresses the significant loss of photosynthetic efficiency when leaves are exposed to changes in light intensity.

Plants convert energy from intercepted sunlight to biochemical energy-carriers via photochemistry. While an increased cross-section of the light-harvesting antenna allows photochemistry to proceed with enhanced efficiency, light can easily also be absorbed in excess, this leads to excitation pressure beyond the capacity to process photochemically. When leaves are light-saturated, the probability of formation of damaging reactive oxygen species (ROS) increases. To avoid damage from ROS accumulation, pathways for nonphotochemical energy dissipation are induced in response to high light.

Non-photochemical quenching (NPQ) is like a sun screen for the sensitive light absorbing antennae, it is protective under high light, but is relatively slow to switch off, leading to **decreased light use efficiency** for prolonged periods following high light exposure. Since plant canopies are characterized by fluctuating light conditions, leaves are exposed to a range of light intensities, spanning from strongly limiting to highly saturating. The induction and relaxation of NPQ provide a balance between avoidance of photodamage at the high light range, versus loss of efficiency at the low light range. Rapid NPQ response times are therefore key to maintain efficient photosynthesis.

Speeding up the time taken for the induction and relaxation of photoprotection (NPQ) response times can improve photosynthetic efficiency and therefore crop yields.

Early theoretical studies predicted 9% (Long *et al*. 1994) and 6% (Werner *et al*. 2001) for canopy carbon losses due to photoinhibition. In work specifically focused on the temporal responses in NPQ, the potential losses were corrected upwards to **10-30%** (Zhu *et al*. 2004). These latter predictions were confirmed by proof of concept studies using transgenic tobacco (Kromdijk *et al.,* 2016) and soybean plants (De Souza *et al*., 2022), both using the same approach to speed up NPQ response time via overexpression of three genes, violaxanthin de-epoxidase, zeaxanthin epoxidase and photosystem II subunit S, leading to **14-20% enhanced biomass productivity** (tobacco) and **10-15% seed yield** (soybean) in field experiments. Overexpression of only the photosystem II subunit S gene in rice enhanced biomass accumulation and yield under greenhouse conditions (Hubbart *et al.,* 2018) and in tobacco led to **increased water use efficiency** (Głowacka *et al*., 2018, Turc *et al.,* 2024). Based on these results, we tentatively put the technology readiness level of this manipulation strategy currently at **TRL 6 "Field prebreeding**". Interestingly, in the microalgae *Nannochloropsis gaditana* and *N. oceanica*, overexpression of zeaxanthin epoxidase also gave rise to increased biomass productivity (Perin *et al*., 2023), suggesting that manipulating NPQ has scope to work across a large phylogenetic range.

Key benefits of this strategy are increased biomass 14-20% and increased seed yield 10-15%, and increased water use efficiency**.**

Challenges, milestones and the way forward: One potential downside of this strategy is that there may be strong variation in the effect between different species, since similar manipulations did not achieve beneficial effects in *Arabidopsis thaliana* (Garcia-Molina and Leister 2020) and *Solanum tuberosum* (potato, Lehretz *et al*., 2022). This may be a result of species-specific differences in native NPQ regulation, perhaps in line with differences in canopy architecture, but may also stem from differences in the testing environment, with results from the latter two studies being derived from controlled environments, but the results in tobacco and soybean from field testing.

One of the main challenges for incorporating the genetic determinants of NPQ into crop models remains the limited understanding of the benefits and drawbacks of specific NPQ regulation patterns as a function of environmental conditions. NPQ in crops should be regulated to optimize the balance between photoinhibition and photosynthetic efficiency across space and time, i.e. across the canopy throughout the growing season. The complexity of this optimization problem mandates the use of advanced computer models that incorporate realistic depictions of the canopy light environment as well as sufficiently mechanistic representation of photosynthesis and NPQ in predictions of crop performance. However, while the NPQ relaxation trait is a key example of a trait predicted by crop modelling (Zhu *et al*., 2004), no suitable crop models currently exist that have this trait incorporated. In addition, the genetic variation determined in crop germplasm thus far has been collected on old materials, and for incorporation of genetic marker information into breeding programs, screening would need to be performed on more relevant elite material.

Knowledge of the molecular determinants of NPQ induction and recovery has allowed design of transgenic strategies as detailed above. More recently, multiplexed CRISPR-Cas9 editing of the genomic region upstream from the PsbS coding sequence in rice allowed generation of non-transgenic heritable variation in gene expression (Patel-Tupper *et al*., 2024). Genetic variation in NPQ has been known for a while to exist between Arabidopsis accessions (Jung and Niyogi, 2009; Rungrat *et al.,* 2019). Research efforts to determine this across crop germplasm collections have expanded substantially, showing significant heritable variation in rice (Wang *et al*., 2017), soybean (Burgess *et al*., 2020), sorghum (Ortiz and Salas-Fernandez, 2021; Vath, 2023), maize (Sahay *et al.,* 2023) and wheat (McAusland *et al*. 2020) and data collected by the CAPITALISE consortium for maize (Ferguson *et al*., 2023a), wild barley and tomato backcrossed inbred lines (manuscripts in preparation). One major advantage for screening of genetic variation in NPQ is the precision and practicality of chlorophyll fluorescence as a non-invasive, high-throughput phenotyping technique. In addition, with confirmation by the CAPITALISE project that photosynthetic traits can be screened on detached parts of field-grown plants (Ferguson *et al*., 2023b), the ease of HTP chlorophyll fluorescence measurements allows screening of 10,000s of individual field-grown plants with limited technological investment. Planned development of low-cost sensors at the newly established Jan Ingenhousz Institute may offer scope to further alleviate the phenotyping bottleneck to create temporal and spatially resolved genetic variation in photosynthesis and NPQ and relevance for crop performance.

15.3 Optimisation of regulation of RuBP/CBB cycle/Electron and Proton Transport-(TRL5) - Christine Raines

Crop species are already being produced with targets in electron transport and RuBP regeneration therefore a 5-10 year time frame is feasible for this strategy.

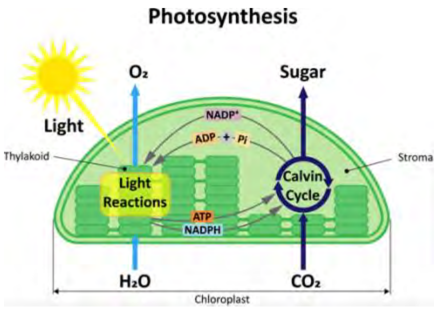


Figure 22 A simplified view of the Calvin Cycle in the Chloroplast

The Calvin-Benson-Bassham (CBB) cycle is the primary photosynthetic pathway for assimilation of atmospheric CO_2 in over 85% of C3 plant species. The CBB cycle involves eleven enzymes and the biochemical steps have been divided into three stages; i) carboxylation carried out by Rubisco, ii) reduction, and ii) RuBP regeneration. Under light saturating and CO_2-limiting conditions Rubisco activity is the major determinant of the efficiency of carbon fixation but, as CO_2 levels rise and light intensity decreases, this balance shift towards both the reductive and regenerative phases of the CBB cycle that catalyse the synthesis of the CO_2 acceptor molecule, RuBP. A major focus of efforts to improve photosynthesis is the enzyme Rubisco, through the application of protein engineering strategies and also by manipulating of expression in transgenic plants (see 15.4 below). However, it has been shown that manipulating the expression of other enzymes of the CBB cycle can also enhance photosynthesis and growth. In the 1990's antisense technology demonstrated that Rubisco did not have total control over CO_2 assimilation under all conditions, and identified sedoheptulose 1,7-bisphosphatase (SBPase), fructose 1,6-bisphosphate aldolase (FBPA) and transketolase (TK) as promising targets for improvement of photosynthesis. Based on these studies a transgenic overexpression approach has shown that increasing the levels of SBPase can improve photosynthesis and growth in algae and a number of plant species including: tobacco (in the field and greenhouse), wheat, and Arabidopsis, in contrast no positive effect was observed in rice. Furthermore, tomato plants with increased SBPase activity were found to be more chilling tolerant with increased photosynthetic capacity, (Ding et al. 2017).

Figure 23 Transgenic wheat with increased levels of the CBB enzyme sedoheptulose-1,7-biphosphatase exhibit improved photosynthesis, increased total biomass and dried seed yield under greenhouse conditions. (Modified Driever et al., 2017)

Overexpression of FBPA in tobacco also resulted in positive effects on photosynthesis and biomass and in tomato an increase in seed weight in both optimal and sub-optimal temperatures was observed. Introduction of the bifunctional cyanobacterial CBB enzyme sedoheptulose 1,7-bisphosphatase/ fructose 1,6 bisphosphatase (SBPase/FBPase) into tobacco plants, lettuce and soybean (in elevated CO_2), has also resulted in improved CO_2 assimilation and growth.

The CBB cycle is dependent on photosynthetic electron transport chain for the production of ATP and NADPH. Manipulation of the photosynthetic electron transport chain is therefore another potential option for improving photosynthetic carbon assimilation and yield. The first demonstration that increases in electron transport can drive improvements in plant growth showed that the expression of the algal (*Porphyra yezoensis*) cytochrome (Cyt) c_6 in the chloroplasts of Arabidopsis leads to an increase in chlorophyll and starch content as well as an increase in ATP and NADPH. These changes were accompanied by an increase in CO_2 assimilation, efficiency of photosynthetic electron transport, and biomass (Chida *et al.*, 2007). Similar results were also observed when the Cyt c_6 from *Ulva fasciata* was overexpressed in tobacco.

The Cyt b_6f complex is a central component of photosynthetic electron transport and is located in the thylakoid membrane where it acts in both cyclic and linear electron transport mediating electron flow between PSII and PSI, providing ATP and NADPH for photosynthetic carbon fixation. Previous studies have shown that by reducing the accumulation of the Rieske FeS protein, it is possible to manipulate the levels of the Cyt b_6f complex First, Cyt b_6f inhibitors were used and then antisense studies suppressing the Rieske FeS protein (PetC) have shown that the Cyt b_6f complex is a key determinant of the electron transport rate. These findings suggested that the electron transport chain, and specifically the Cyt b_6f complex, is a limiting step in photosynthetic carbon assimilation and that increasing electron transport could increase photosynthesis and yields. This was demonstrated by the overexpression of the Rieske FeS protein in Arabidopsis where it was shown to lead to substantial increases in CO_2 assimilation and relative electron transport rates, and importantly, to contribute to a **27–72% increase in biomass and up to a 51% increase in seed yield**.

Importantly as atmospheric CO_2 rises, theoretical models predict that the limitation of carbon assimilation shifts from Rubisco to RuBP regeneration (Long *et al*., 2004). Therefore, modifications that improve RuBP regeneration are predicted to stimulate photosynthesis and yield under elevated atmospheric CO_2. This is supported by experimental evidence using plants grown in Free-air CO_2 enrichment (FACE) facilities, when grown at 585 ppm CO_2, transgenic tobacco plants overexpressing SBPase have greater yield increases at elevated CO_2. When both CO_2 and temperature are manipulated, transgenic overexpression of cyanobacterial bifunctional FBP/SBPase in soybean protects against temperature-induced yield loss under elevated CO_2 (Köhler *et al.,* 2017). These results indicate that improving RuBP regeneration in food crops can mitigate the effects of climate change on yield, and also demonstrate the importance of testing future manipulations under future climate conditions. The benefit from improving RuBP regeneration is likely to be a **10-20% yield improvement**.

Advances in kinetic flux and multiscale modelling have provided novel predictions on how to further enhance RuBP regeneration. Testing these outputs will require the application of rapid high throughput and iterative approaches to identify the best candidates to achieve improvements to photosynthesis.

Challenges: This strategy would be difficult to breed for as selection of this trait in the field would be needed, however, remote sensing systems in the future could help by monitoring the canopy over the day and through the season. A key translational challenge is the availability of field trial sites for testing transgenic manipulations and availability of multilocation trial sites to follow up on successes. Remote sensing to identify the most productive events would be needed.

Next steps in this work: New approaches enabling identification of genetic factors and mechanisms involved in regulating the expression of CBB cycle genes will underpin the application of gene editing technologies to modify this pathway. Synthetic biology may also allow the building of a completely synthetic, more efficient CO_2 fixation pathway to operate in parallel with the endogenous cycle or to introduce improved enzymes to operate within the existing cycle.

15.4 Rubisco adapted to todays (elevated) CO_2 (TRL 1-4) - Elizabete Carmo Silva

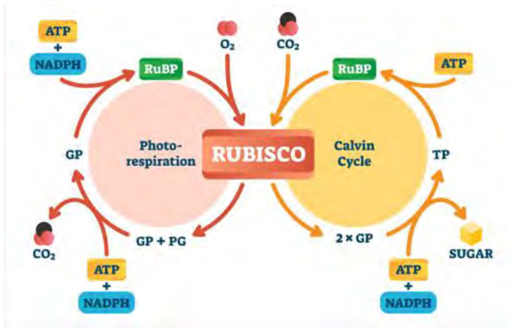


Figure 24 The dual reactions of Rubisco

Rubisco fixes atmospheric carbon into sugars that plants use for growth and is therefore critical for agricultural crop production. The enzyme evolved in a very different atmosphere and, despite being remarkable and essential for life on earth, it is characterized by several inefficiencies. Making Rubisco better to enhance photosynthetic productivity requires consideration of the plant species and target environment, the same solution is unlikely to fit all crops.

In addition to the enzyme's abundance, the catalytic properties (how fast or how specific the enzyme is), as well as the regulation of Rubisco activity (how it interacts with other chloroplast components), need to be considered in the context of specific environments and crop canopies for maximum gains in agricultural productivity, sustainability and climate resilience (Amaral *et al*., 2024; Croce *et al*., 2024). Importantly, in addition to increased CO_2 concentrations in the atmosphere, warmer temperatures are likely to demand a more thermotolerant version of Rubisco's activating enzyme, Rubisco activase (Qu *et al.,* 2023; Sparrow-Muñoz *et al.,* 2023; Amaral *et al.,* 2024).

Key benefits of this strategy are NUE, WUE**.** For C_3 crops like wheat, barley, beans, tomato, potato and sunflower, the cost of efficient photosynthesis means investing up to 30% of leaf nitrogen to Rubisco protein in leaves (Carmo-Silva et al. 2015; Evans and Clarke, 2018), which has a critical influence on crop nitrogen requirements. In C_4 crops like maize and sorghum, a pump-like mechanism increases the CO_2 supply to Rubisco, thereby reducing the competing reaction with O_2 and the energy-costly photorespiration. As a result of the CO_2 concentrating mechanism, C_4 plants typically have a lower requirement for high abundance of Rubisco and for stomata to be wide open, resulting in higher efficiencies of nitrogen and water use compared to C_3 plants (Ghannoum *et al.,* 2002). **A faster Rubisco that is better able to exploit increasing atmospheric CO_2 concentrations has potential to reduce both the nitrogen and water requirements of crop photosynthesis and/or increase energy capture through increased rates of carbon assimilation.**

Limitations: the need for GM acceptance. C_4 plants provide an excellent example of natural variation in Rubisco catalytic properties, typically showing higher catalytic rates (k_{cat}) but lower specificity towards reaction with CO_2 rather than O_2 (S_{CO}) than their C_3 counterparts (Sharwood *et al.,* 2006). Variation in catalytic properties of Rubisco also exists among C_3 species, including within closely related germplasm (Hermida-Carrera *et al.,* 2016; Orr *et al.,* 2016; Prins *et al.,* 2016), but due to the relatively small differences offers limited scope for substantial improvements in crop yields in current and future climates. Enhancing Rubisco's efficiency by increasing its maximum catalytic rate (k_{cat}), improving its specificity towards reaction with CO_2 rather than O_2 (S_{CO}), and/or ensuring efficient regulation of its activity through removal of sugar phosphate inhibitors and quick re-activation (Amaral *et al.,* 2024) may be possible though gene editing, but given the number of genes involved is more likely to be achievable through genetic manipulation. In crops where increasing the abundance of Rubisco is feasible and likely to produce substantial gains, this is also likely to require upregulation of multiple genes, including those encoding the Rubisco large and small subunits, as well as proteins required for proper folding and assembly of the enzyme (e.g. Salesse-Smith et al. 2018). Therefore, this strategy is dependent on progress towards acceptance of genetically engineered crops.

Over the last two decades, progress towards improved Rubisco has been achieved through identification of key amino acid residues that alter its catalytic properties (Whitney *et al.,* 2015), manipulation of subunits comprising the Rubisco protein using novel bioengineering approaches in *E. coli* (Aigner *et al.,* 2017; Gionfriddo *et al.,* 2024), identification of high-temperature tolerant forms of Rubisco activase (Scafaro *et al.,* 2019; Degen *et al.,* 2020), and exploration of the impacts of sugar phosphatases on Rubisco activity (Orr *et al.,* 2023). These fundamental studies (TRL1) are complemented by modelling work that has repeatedly suggested that improvements in Rubisco function can yield step changes in crop photosynthetic potential (Wang *et al.,* 2021; Vijayakumar *et al.,* 2024), and position Rubisco researchers with a toolkit that is ready to be deployed across a range of crop systems. Rubisco research depends on a global community, with particular foci in North America, Australia and Europe.

Challenges: Through its central role in the Farquhar von-Caemmerer Berry (1980) model of photosynthesis, our understanding of Rubisco catalysis fundamentally underpins current mechanistic models of global vegetation and crop production. Crop simulators based on this model predict increases in **productivity up to 60% as a result of modified Rubisco catalysis** (Long *et al.,* 2006; Wu *et al.,* 2019; Harbinson and Yin 2022). Yet, we lack models with which to explore the multilevel controls influencing Rubisco activity, including interactions with Rubisco activase, inhibitory sugar phosphates, and binding of CO_2 and magnesium that are needed as co-factors for catalysis of CO_2 fixation. Predictive modelling of these effects will be critical to recover losses thought to arise during non-steady-state photosynthesis that is common in crop canopies (Long *et al.,* 2022). In addition, the fast development of computational and AI tools is set to dramatically enhance our ability to predict the impact and understanding which amino acid residue changes in Rubisco and its regulatory proteins are key to enhance carbon assimilation in crops via gene editing.

Given its critical role in central carbon metabolism it is perhaps not surprising that Rubisco activity is highly regulated and coordinated in tandem with other photosynthetic reactions and plant processes. Efforts to model and enhance Rubisco activity in crops must consider Rubisco assembly pathways as well as its regulatory protein partners (Rubisco activase and phosphatases). A photosynthetic and crop model including dynamic metabolic regulation via interaction of enzymes with metabolic adjustments would enable tailoring Rubisco improvement for optimal performance in variable environments as experienced by crops in current and future environments. Approaches for Rubisco enhancement include a minimum of 3 genes to be manipulated. Simpler approaches can be translated into crop improvement in a short timeline whereas more complex yet potentially more rewarding approaches will require a longer timeline. In C3 rice (Yoon *et al.,* 2020) and C_4 sorghum (Salesse-Smith *et al.,* 2024), increasing the abundance of Rubisco has resulted in increased biomass and yield in single location field trials (TRL4), which represents the most straightforward approach to enhance Rubisco function in the short term. Other approaches focused on improving Rubisco catalysis and regulation remain dependent on additional fundamental research and are likely to yield substantial outputs in the longer term but offer potential for greater gains for efficient and climate resilient crop production.

15.5 Adding cyano carboxysomes, HCO_3 pumps algal CCMs (TRL 1-2) - Luca Tadini

This strategy is still at the stage of basic research and investigation of basic principles suitability for application. There is a medium/long-term translational timeline.

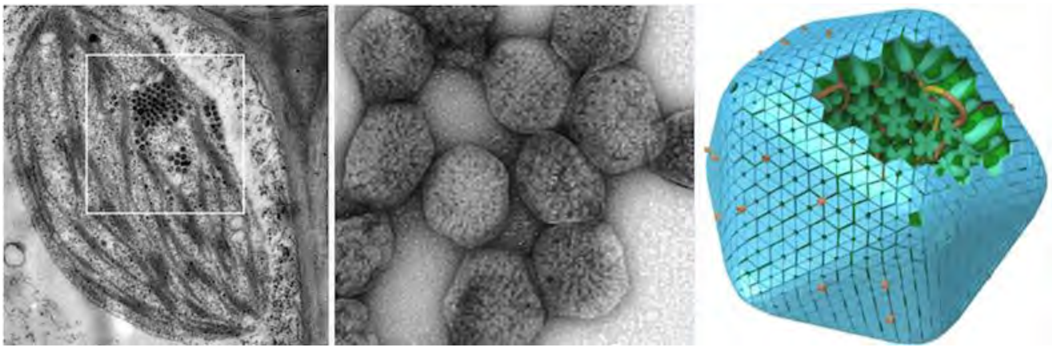


Figure 25 Carboxysomes built in Tobacco chloroplasts, adapted from the original (Long et al., 2018).

The growth of many photosynthetic organisms, including most crops, is limited by Rubisco's CO_2 assimilation and competition with O_2, leading to wasteful photorespiration (Bauwe *et al.,* 1987). Some photosynthetic clades have evolved carbon-concentrating mechanisms (CCMs) to supply Rubisco with concentrated CO_2, favouring CO_2 assimilation over photorespiration. Biophysical CCMs increase intracellular inorganic carbon (Ci) through channels or active pumping, present in prokaryotic autotrophs, algae, hornworts, and seagrasses (Capó-Bauçà *et al.,* 2022 He *et al.,* 2023, Lafferty *et al.,* 2023, Nguyen *et al.,* 2023). Cyanobacteria and some chemoautotrophic bacteria physically sequester Rubisco in carboxysomes, while most algae and some hornworts condense Rubisco into pyrenoids, both supplied with HCO_3^- by localized carbonic anhydrase (CA) activity. Introducing CCMs into C3 crops is a high-risk, high-reward strategy to boost yields and resilience. Reconstituting functional CCMs in C3 plants remains complex, with specific challenges for each type. The most efficient biophysical CCMs are carboxysome-based (cCCMs) and pyrenoid-based (pCCMs), increasing CO_2 concentrations around Rubisco by up to 1,000-fold and 40-fold, respectively (Price *et al.,* 2013; *Fei et al.,* 2022). cCCMs are predicted to boost yields in C3 crops (Nguyen *et al.,* 2023) due to cyanobacteria's fast form Rubiscos (Ang *et al.,* 2023). Progress includes reconstructing α- and β-carboxysomes in plants. Recently, α-carboxysomes from *Halothiobacillus neapolitanus* were reconstructed in tobacco chloroplasts with active Rubisco and CA enzymes (Chen *et al.,* 2023). However, functional cCCMs in bacteria require active HCO_3^- uptake and CA activity restricted to carboxysomes (Price *et al.,* 2013). Testing carboxysome-enhanced plant growth will require removing native chloroplastic CA activity and introducing functional HCO_3^- transporters in chloroplasts. New screening tools for HCO_3^- transporters and channels in plants may help (Förster *et al.,* 2023). Pyrenoids (pCCMs) have diverse morphologies (Barrett *et al.,* 2021). pCCM in *Chlamydomonas reinhardtii* features a liquid-like matrix of Rubisco, thylakoid-derived tubules supplying Ci, and starch as CO_2 diffusion barrier (Ang *et al.,* 2023; He *et al.,* 2023). Modeling suggests a functional Chlamydomonas pCCM in C3 plant chloroplasts could triple CO_2 assimilation rates (Fei *et al.,* 2022). While pCCMs are less efficient than cCCMs, they may be more compatible with plants, as they do not require chloroplastic CA activity removal or active HCO_3^- transport at ambient CO_2 levels (Fei *et al.,* 2022). Rubisco condensation into a "proto-pyrenoid" matrix has been achieved in Arabidopsis (Atkinson *et al.,* 2020), with ongoing work to reconstitute other features. Studies in diatoms and hornworts show various solutions for functional pCCMs in plants, like using a pyrenoid protein shell instead of starch layers (Lafferty *et al.,* 2023; Nam *et al.,* 2023; Oh *et al.,* 2023).

The potential to enhance yields by genetically engineering a CCM into C3 crops is supported by both theoretical models and experimental data. CO_2 enrichment experiments have demonstrated that C3 crops produce higher biomass when grown in elevated CO_2 concentration. Co-engineering of CO_2 transport mechanisms together with synthetic CCM in crop plants could boost also plant performance at environmental CO_2 concentrations.

Models estimate potential photosynthetic efficiency **increases from 30% to 60%** (Ort *et al.,* 2015) Although the extent of productivity gains is debated, considering factors like sink demand (Paul, 2021) a recent model predicts an **8% increase** in wheat yields from successful cyanobacterial CCM introduction (Wu *et al.,* 2023). There are also possible improved WUE features.

Recent efforts to introduce prokaryotic CCMs into crops have focused on tobacco plants. Tobacco is advantageous because its chloroplast genome can be manipulated. Therefore, tobacco remains the best option short term, until similar technologies are developed for other crops.

Challenges: Introducing CCMs into C3 crops is a high-risk, high-reward strategy to boost yields and resilience. Despite the significant progress already made in this strategy, players required for the introduction of CCMs into plants still need to be entirely identified and tested experimentally. *In silico* models give hints where to concentrate the engineering effort. However, assumptions and models should be tested experimentally. Moreover, whilst only a few dicotyledonous plant species can be manipulated in chloroplast genomes, no monocots (i.e. cereals), are suitable. This could be overcome by nuclear genome transformation and post-translational protein targeting to chloroplasts. Considering unicellular origins of CCMs, plant multi-cellularity must be also considered.

CCMs do not exist in C3 plants, therefore it is not possible to breed for this trait. The acceptance of GMOs by the general public, along with GMO-related restrictions, could represent a major issue with this strategy. This is despite the fact that the most recent genome editing techniques are comparable to natural evolution in terms of genome modification. Fully enhancing plant metabolism through synthetic CCMs should likely be accompanied by additional genetic alterations that can support increased carbon fixation and sugar production.

Models estimate that potential improvements in leaf photosynthetic efficiency could range from **30% to 60%**, representing some of the most significant gains predicted through engineering approaches. Recently, a conservative model predicted an 8% increase in wheat yields with the successful introduction of the cyanobacterial CCM.

Expression and accumulation of CCM-related proteins in proper compartments, correct assembly and functionality of protein complexes is still a significant technical challenge in this area.

To accelerate translation basic biotechnology should be implemented and proved to be effective in laboratory conditions. *E. coli*, can be used as chassis organism for identifying minimal gene set required for carboxysome structures.

In introducing CCM in crops, challenges are still very significant. Milestones are: 1) Introgression of bicarbonate pumps able to achieve HCO_3^- mobilization across plant cell membrane systems without interfering with endogenous metabolic pathways (5 years). 2) Generation of algal pyrenoid-like structures in crop (5-10 years). 3) Development of protocols for chloroplast genome transformation in other species, especially in monocots and/or identification of CCM gene and sequences suitable for transformation of nuclear genome and post-translational import into chloroplast (5-10 years).

This strategy is suitable as a carbon storage strategy. Developing C3 crops with CCMs and enhanced CO_2 absorption will help carbon farming approaches and contribute to the EU Soil Strategy for 2030 and is directly aligned to the climate change commitments of the Paris (COP 21) and Glasgow (COP

26) Agreements. This strategy is also suitable for the sustainable bioeconomy (feedstock) through Oceanic photosynthesis- as feedstock for fish. Production of feedstock for the bioeconomy, production of plant-based materials (biomanufacturing).

15.6 Expanding the Photosynthetic Spectrum in Crops to the far-red (TRL2) - Roberta Croce

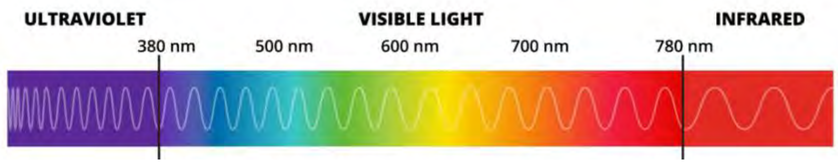


Figure 26 Spectra availability for photosynthesis.

Light is the primary energy source for photosynthesis, but most plants only utilize photons in the 400–700 nm range (photosynthetically active radiation, PAR), leaving out a substantial portion of solar energy. This limitation reduces the efficiency of solar energy utilization to less than 50% of the sunlight reaching the Earth's surface (Zhu *et al.,* 2010). Crop canopies exacerbate this inefficiency, as upper leaves absorb most visible light, leaving the lower canopy exposed predominantly to far-red (FR) photons (700–800 nm), which plants cannot use effectively for photosynthesis. This results in a near-zero photosynthetic rate in the lower canopy (Srinivasan *et al.,* 2017).

Recent modeling studies highlight the transformative potential of expanding the photosynthetic spectrum in crops (Yu *et al.* submitted). Using a 3D canopy model of soybean reconstructed from field data, it was demonstrated that introducing far-red-absorbing pigments into crops could significantly enhance CO_2 assimilation. This adaptation could boost photosynthesis in the visible-light-starved lower canopy and increase crop productivity by up to **26%,** without raising the risk of photodamage. Adjusting far-red absorption through phytochrome-regulated mechanisms further optimized light use, making this approach highly promising for agricultural applications.

Expanding the photosynthetic spectrum of crops to include FR photons has long been considered unfeasible due to the lower energy of these photons. However, cyanobacteria capable of FR acclimation have shown that FR light can effectively drive photosynthesis (Miyashita *et al.,* 1996; Chen *et al.,* 2010). This provides a roadmap for introducing FR utilization in plants, with the potential to increase energy capture and productivity, especially in dense canopies.

Requirements for far-red photosynthesis: For an efficient use of far-red light in photosynthesis several requirements need to be met *i)*Harvesting FR photons *ii)* Efficiently transfering their energy to the reaction center (RC) *iii)* Utilizing this energy for charge separation and stabilization, limiting losses.

Cyanobacteria achieve FR absorption using specialized chlorophylls (Chl d and Chl f) that absorb in the far-red spectrum. These pigments differ from Chl a, the primary chlorophyll in plants, by having a formyl group that shifts their absorption properties (Gan *et al.,* 2014; Ho *et al.,* 2017). The enzyme responsible for Chl f synthesis has been identified and successfully expressed in cyanobacteria incapable of FR acclimation (Shen *et al.,* 2019b). This demonstrates that introducing FR-absorbing pigments into new organisms is achievable. The enzyme responsible for the Chl d synthesis still needs to be identified.

In addition to Chl d and Chl f, some cyanobacteria and plants utilize “red-shifted” Chl a, which absorbs in the FR region due to strong pigment-pigment interactions (Croce and van Amerongen, 2013). This strategy could also be adapted in plants without requiring new chlorophyll types. However, this will require the careful design of the binding sites to favour interactions between pigments that lead to the strong red-shift of their absorption.

For efficient photosynthesis, absorbed energy must be transferred to the RC for charge separation. In plants, this process relies on the modular assembly of pigment-protein complexes, such as Photosystem I (PSI) and Photosystem II (PSII). However, integrating far-red pigments can create energy traps, slowing transfer rates and reducing efficiency (Mascoli *et al.,* 2020). Careful design of pigment-protein complexes, with strategic placement of far-red chlorophylls, is critical to maintaining efficiency (Mascoli *et al.,* 2022).

Additionally, the introduction of far-red chlorophylls into RCs requires ensuring that these pigments support charge separation without reducing efficiency or increasing photodamage risks. Cyanobacteria provide valuable insights into how these challenges can be overcome, as studies indicate that PSI containing Chl f can function efficiently with Chl a in the RC (Tros *et al.,* 2021).

Smart Canopy Design - A Targeted Approach: The "smart canopy" concept proposes selectively introducing far-red absorption capabilities in lower leaves while leaving upper leaves optimized for visible light (Ort *et al.,* 2015). This would maximize light use efficiency across the canopy without compromising charge separation efficiency in upper leaves exposed to full sunlight. Phytochrome-mediated acclimation, as seen in far-red acclimating cyanobacteria, could enable plants to dynamically adjust pigment and protein expression based on light quality (Gan *et al.,* 2014; Gisriel *et al.,* 2022).

Conclusion: Harnessing far-red light for photosynthesis in crops represents a transformative strategy to enhance productivity. Insights from cyanobacteria and advanced canopy models highlight the feasibility and value of this approach. While challenges remain, such as engineering efficient energy transfer and photochemsitry, ongoing research offers a promising foundation for developing far-red absorbing crops capable of more effectively utilizing solar energy

15.7 Stomatal strategies (TRL 2-5) - Tracy Lawson

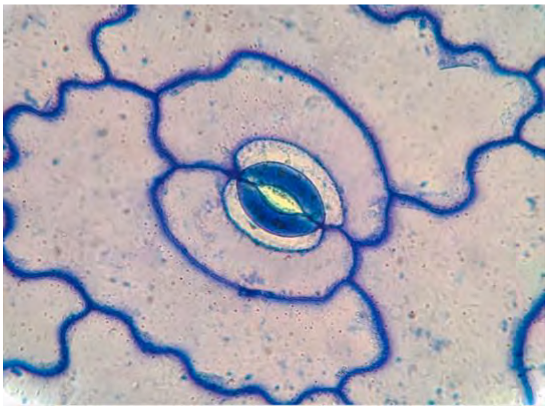


Figure 27 Microscopy image of a stomata on a leaf

Stomata, are small pores on the surfaces of most aerial parts of plants, that change in aperture to regulate gaseous exchange between the external atmosphere and internal leaf airspaces. The behaviour of stomata is therefore critical in determining CO_2 uptake for photosynthesis (*A*) and water loss through transpiration and the ratio of these processes is often referred to as intrinsic water use efficiency.

Stomatal conductance (g_s), a measure of the ease of gaseous exchange, is determined by both stomatal anatomy, density and function, and therefore there are numerous targets that can be exploited to manipulate stomatal behaviour, with impacts on photosynthesis and water use. Stomatal movements are brought about in response to a number of environmental stimuli and internal signals, however the sensitivity and speed with which stomata respond are not identical in different crops or even cultivars of the same species, neither is the overall stomatal conductance achieved (McAusland *et al.,* 2016; Lawson & Vialet-Chabrand, 2019).

Maximising photosynthesis: In order for photosynthesis to take place CO_2 must enter the leaf from the external atmosphere, the ease of which is determined by stomatal conductance. In dynamic field environments, plants are subject to rapid fluctuations in conditions; in particular light can alter due to cloud cover, overlapping leaves, time of day and season, and both photosynthesis and stomata respond to these changes. Photosynthesis adjusts in response to light intensity in a matter of seconds, however stomata are considerably slower, taking tens of minutes. Slow stomatal opening in response to increasing light or at the start of day can therefore limit CO_2 uptake and assimilation rate (Long *et al.,* 2022), whilst slow closure (due to decreasing light) can lead to unnecessary water loss for no carbon gain (Lawson & Blatt, 2014).

Novel targets: The speed of stomatal responses has been linked to guard cell morphology, including the size and shape of these cells and the surrounding subsidiary cells, as well as signal pathways and guard cell metabolism. These represent a number of unexploited targets to improve the rapidity of stomatal responses, with several studies having demonstrated the potential positive impact of such manipulations on *A* and plant growth. For example, Horaruang *et al.,* (2022) engineered sensitivity in K+ channels to enhance stomatal kinetics and demonstrated improved WUE and *A*.

The density of stomata (SD) is also a target for manipulation and there is now considerable understanding of the regulatory pathways that determine stomatal density (see Zhu *et al.,* 2020; Bertolino *et al.,* 2019). Alteration in the expression of epidermal patterning factor genes (EPFs) and their related families (ERECTA, EPFL etc.) have altered stomatal densities on leaves of a number of species, with over expression of EPF1 and 2 decreasing stomatal density which saves water (Caine *et al.,* 2019) whilst OE of EPFL9 increases stomatal density and improves rates of photosynthesis (albeit at the expense of greater water loss)(Tanaka *et al.,* 2013).

Limitations: The above studies have demonstrated the potential to manipulate SD with expected impacts on CO_2 uptake and water loss and the pathways and gene targets are well understood. However, although stomatal metabolism has been a subject of study for over a century and a great deal is known, there are still many gaps. For example, it remains inconclusive which osmoregulatory processes occur in which species and if these change throughout the day. It is also well established that there is close correlation between *A* and g_s, however there is also limited understanding of what co-ordinates g_s and mesophyll demands for CO_2. Although internal CO_2 concentration (C_i) is clearly involved, it is not the only signal, and if we are to manipulate stomata or photosynthesis a better understanding of what links these two processes is crucial. As stated above, several studies have demonstrated variations in the speed of stomatal responses, however the underlying mechanisms are less understood and therefore targets are less clear. For this reason, considerable fundamental research is still required if we are to exploit stomatal morphology and function to improve A and water use efficiency.

Significant variation exists in stomatal morphology and behaviour both within and between leaves and plants, and this also changes temporally depending on the surrounding conditions. This means that measurements of g_s are often “noisy” and considered unrepeatable. Therefore, there is a need for a large number of measurements to accurately capture stomatal behaviour. These measurements are often conducted using IRGAs, however new techniques and equipment are needed to allow measuring a large number of plants and or individual stoma apertures. Thermography has shown promise as a proxy for g_s for large numbers of leaves and for understanding temporal behaviour (Vialet-Chabrand & Lawson, 2019; 2020) and new AI tools for counting and measuring stomata are also looking promising (Gibbs and Burgess 2024). It is essential that these tools are developed in a way that makes them easy to use and understand by the plant science community.

Some specific mechanisms such as manipulating SD via EPFs and EPFLs have been identified and are at TRL4/5 and some field trials have been conducted. TRL levels for manipulating the rapidity of stomata is only at TRL3/4 with ongoing and further research needed. The genes that regulate stomatal size are still unknown, along with the those that co-ordinate g_s and *A* and are therefore at much earlier TRL stages 2/3.

Benefits: Depending on the crop and system, yield may be increased by **20%** if stomatal limitations on photosynthesis are removed (McAusland *et al.,* 2016) whilst improvements in water use and maintaining soil water status for critical periods could make further improvements (Faralli *et al.,* 2019; 2024).

There are risks that manipulating stomata can either increase *A* at the expense of water, or vice versa. However, stacking stomatal traits to account for the complexity of stomatal behaviour could be extremely beneficial. This will require more research and understanding of the signalling pathways, guard cell metabolism, the causes of underlying variation, and what co-ordinates *A* with g_s. Climate change means that not all crops or environment may need the same traits, so there is a need to consider developing idiotypes for different environments.

Modelling: There are many stomatal models, but many of these rely on steady state conditions. Several new models have been developed for modeling stomatal dynamics, however many of these tend to be over parameterized. There is scope for the development of new models that integrate stomatal traits with photosynthetic performance.

Translation Challenges: include i) identification of the key genes involved in the rapidity of guard cell responses and regulation of stomatal sizes, as well as those that are responsible for the signals that link g_s and *A*; ii) development of tools and assays to accurately measure g_s and rapidity of changes in g_s under a range of different conditions. Exploring natural variability in germplasm and gene banks of current crops, and also in wild relatives could help to accelerate translation.

Key targets for some traits are known whilst other need to be identified and the impact of environments on these determined. Translation time is short for density and medium to long term for rapidity. Temperature regulation and stacking stomatal traits with other key traits for improving photosynthesis could be extremely beneficial. There are also other stomatal targets that could be exploited that are beyond the scope of this. Since stomata control carbon fluxes this strategy is also suitable for Carbon Farming/Sequestration. Improved stomata is a trait worth breeding for if we identify the correct genes and targets, and develop the correct measuring technologies.

15.8 Synthetic Photorespiratory Bipasses (TRL 4-6) - Andreas Weber

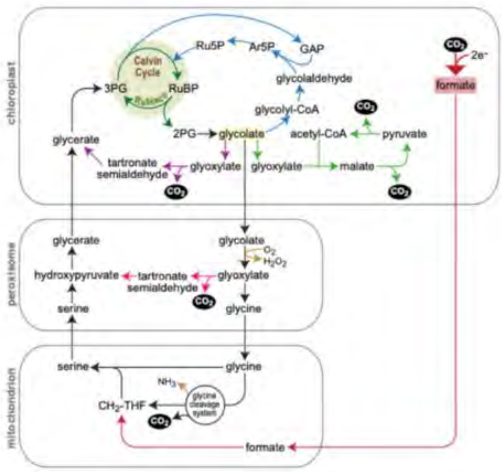


Figure 28 A photorespiratory bipass option

Photorespiration is a biochemical pathway that removes 2-phosphoglycolate, a toxic byproduct of the Rubisco oxygenase reaction (Segura Broncano *et al.,* 2023). In this process, 75% of the carbon from two molecules of 2-phosphoglycolate is recovered, but 25% of the previously assimilated carbon is lost as CO_2. Because of this carbon loss, photorespiration reduces the energy conversion efficiency of C_3 crops by 30-50%, depending on environmental conditions, thereby decreasing their yield potential (Walker *et al.,* 2016). Synthetic bypasses that circumvent natural photorespiration have been demonstrated in field trials with several crop species to significantly increase yield per unit area (Smith *et al.,* 2023).

Natural photorespiration requires enzymatic steps in chloroplasts, peroxisomes, mitochondria, and cytoplasm. During this process, CO_2 and NH_3 are released in the mitochondria, and their reintegration into metabolism consumes ATP and NAD(P)H, thereby decreasing the efficiency of photosynthesis. To address these inefficiencies, the first photorespiratory bypass designs were developed in 2007 (Kebeish *et al.,* 2007) and 2012 (Maier *et al.,* 2012). A common principle of most current photorespiratory bypass designs is to complete all necessary enzymatic reactions within the chloroplasts. This approach avoids NH_3 release, and the associated costs of its reassimilation, and ensures that CO_2 is released in the chloroplasts, preventing loss to the environment by diffusion and reducing the oxygenation reaction of Rubisco.

South *et al*., (2019) compared the designs of Kebeish *et al*., (2007) and Maier *et al*., (2012) in field trials with transgenic tobacco lines. They further modified the original designs by using a single-subunit glycolate dehydrogenase from a green alga as the initial step, in contrast to earlier designs that used either a multi-subunit bacterial glycolate dehydrogenase or an H_2O_2-releasing glycolate oxidase. Additionally, they reduced the leakage of glycolate to native photorespiration by minimizing its export from the chloroplast. Their results demonstrated that a modified version of the Maier *et al*., (2012) pathway showed the strongest biomass increase in field trials. Field trials with rice lines expressing photorespiratory bypasses have also shown significantly **increased yields—over 15%** as reported by Shen *et al*., (2019) and Wang *et al*., (2020)—along with improvements in grain quality, such as **increased protein content** and **reduced chalkiness** (Zhang *et al*., 2022). Thus, the bypasses enhanced both yield and nutritional quality.

However, a disadvantage of these pathway designs is that they have higher energy costs than natural photorespiration and, at least for the Maier *et al*. pathway, completely convert 2-phosphoglycolate to CO_2. To overcome these limitations, more recent bypass designs aim to reduce CO_2 loss (Roell *et al.,* 2021) or even serve as auxiliary carbon-assimilation routes (Scheffen *et al.,* 2021), thereby turning photorespiration into a carbon-capturing rather than a carbon-losing process.

A major advantage of all currently known bypass designs is that they require only a small number of genes (fewer than 10) and hence are straightforward to implement in transgenic lines.

Limitations: Several challenges remain for the implementation of photorespiratory bypasses in crops, particularly in the European Union. The regulatory environment poses a significant limitation, as pathway implementation requires transgenesis, and the resulting crops are classified as regulated genetically modified organisms that necessitate a costly and lengthy approval process. Since all currently known pathway designs require genes encoding enzyme activities not present in the target crops, implementation cannot be achieved through genome editing technologies alone. Moreover, it is uncertain whether the introduced pathways are beneficial under all environmental conditions, such as varying soil types, climate zones, or water availability; more comprehensive field trials are needed to assess their performance across different environments. The bypasses will also need to be tested in the genetic backgrounds of elite cultivars, and the stability of pathway expression over multiple generations is uncertain and requires evaluation. Additionally, most pathway designs are protected by patents, so their introduction into breeding programs will require licensing agreements and associated fees.

Risks of this strategy are predominantly associated with the regulatory environment. Since plants carrying synthetic photorespiration bypasses would be classified as regulated genetically modified organisms. It is uncertain whether plants carrying synthetic photorespiration bypasses would find acceptance in the agricultural value chain, despite their significant increase in photosynthetic efficiency and associated sustainability traits, such as increased yield per unit area of land.

Modelling: Recent computational models have thoroughly evaluated the current designs of photorespiration pathways, with the most comprehensive analysis conducted by the GAIN4CROPS project (Smith *et al.,* 2024 bioRxiv). This analysis indicates that all bypasses are beneficial under low-stress or CO_2-limited conditions, such as those resulting from reduced water availability and decreased stomatal conductance. However, the modeling results show that fully decarboxylating bypasses—specifically the Maier *et al.* pathway design and its derivatives—perform worse than natural photorespiration under energy-limited conditions, such as shaded areas of the canopy or overcast skies. In contrast, carbon-assimilating designs (e.g., Scheffen *et al.,* 2021) consistently outperform natural photorespiration across all simulated environmental conditions (Smith *et al.,* 2024).

Overall, computational modeling strongly suggests that synthetic photorespiratory bypasses can enhance yield, a conclusion supported by earlier field trials. Future directions for computational modeling include coupling bypass models with crop growth models, which would allow for more comprehensive simulations and assessments of growth and yield benefits—or potential penalties—across different environmental scenarios.

Translation: Field trials under realistic conditions with different crop species, such as tobacco and rice, have demonstrated that synthetic photorespiration bypasses are ready for translation into agricultural practice. These trials have shown significant increases in yield and improvements in crop quality, indicating the practical viability of implementing these bypasses in crops. However, the main hurdle for translation, particularly in the European Union, is regulatory approval. Since the implementation requires transgenesis, the resulting crops are classified as regulated genetically modified organisms, which necessitates a costly and lengthy approval process. This regulatory environment could potentially lead to a competitive disadvantage for European agriculture compared to regions with more permissive regulations, hindering the adoption of technologies that could enhance crop yield and sustainability.

Considering the significant yield increases observed in field trials and supported by computational modeling, implementing photorespiratory bypasses in C_3 crops appears highly advantageous. Expected yield improvements are at least 15% and potentially higher with carbon-assimilating bypass designs. These increases not only enhance productivity but also improve nutritional quality, as evidenced by higher protein content and reduced grain chalkiness in rice. Therefore, from both agronomic and economic perspectives, it is worth integrating synthetic photorespiratory bypasses into breeding programs.

Key development milestones include demonstrating the pathway benefits in key European crops, such as wheat, rye, barley, oilseed rape, and leguminous crops, particularly within elite cultivar backgrounds.

The translation time is expected to be short since proof-of-concept already exists; however, the main constraint is the process of regulatory approval.

Implementing synthetic photorespiration bypasses is suitable for carbon farming and carbon sequestration. The increased carbon assimilation resulting from these bypasses generates additional photosynthetic products that can be allocated to roots and exuded into the soil, enhancing soil carbon content. Furthermore, higher yields reduce the need for expanding agricultural land, thereby minimizing land use change and its associated carbon emissions. The surplus biomass produced can also be converted into biochar, which improves soil quality and serves as a means for long-term carbon sequestration. Thus, synthetic photorespiration bypasses not only enhance crop productivity but also contribute to sustainable agricultural practices by capturing and storing atmospheric carbon.

Photorespiratory bypasses have been extensively developed in Europe, with much of the intellectual property held by European universities and research institutions. Despite this technological lead, field testing and practical applications of these bypasses in crops are primarily taking place in the United States and China. This shift is largely attributed to the regulatory environment in the European Union concerning biotechnology and genetically modified organisms (GMOs). The stringent regulations in Europe make it challenging to conduct field trials and commercialize genetically modified crops, including those enhanced with photorespiratory bypasses. These regulations require a lengthy and costly approval process for GMOs, which can deter research institutions and companies from pursuing development within the EU. As a result, European stakeholders may face competitive disadvantages, and the region risks falling behind in the adoption of technologies that could improve agricultural sustainability and productivity. The situation highlights a paradox where Europe, despite being at the forefront of developing innovative agricultural technologies, sees the implementation and monetization of these advancements occurring elsewhere. This outcome not only affects the potential economic benefits for European entities but also limits the adoption of technologies that could contribute to more sustainable agricultural practices within Europe.

15.9 C3 to C4 (TRL 2-3) - Andreas Weber

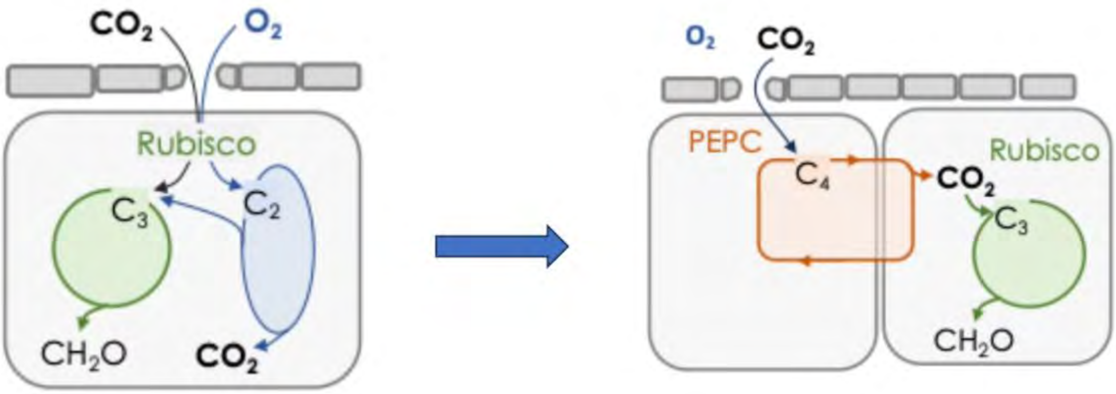


Figure 29 Schematic showing the conversion of C3 to C4

C_4 photosynthesis exhibits increased photosynthetic efficiency compared to C_3 photosynthesis because C_4 plants employ a biochemical carbon pump that elevates the concentration of CO_2 available to Rubisco. This enhancement is achieved by partitioning photosynthetic carbon assimilation between two specialized cell types: mesophyll (M) cells and bundle sheath (BS) cells. Mesophyll cells capture atmospheric CO_2 and convert it into C_4 acids, which then diffuse into the bundle sheath cells. In the BS cells, these C_4 acids are decarboxylated, releasing CO_2 and thereby increasing the CO_2 partial pressure within these cells. Since Rubisco is confined to the bundle sheath cells, it operates in this high-CO_2 environment, substantially reducing photorespiration. Consequently, C_4 plants have approximately 50% higher energy conversion efficiency compared to C_3 plants (Zhu, Long, and Ort, 2010). This increased efficiency leads to higher yields per unit area of land and per unit of nitrogen and water.

C_4 photosynthesis has independently and convergently evolved approximately 70 times across both monocotyledonous and dicotyledonous land plants (Sage 2004). This frequent evolution of a complex trait suggests that, given certain anatomical prerequisites, the transition from the C_3 to the C_4 state is relatively accessible, resembling a Mount Fuji-shaped evolutionary landscape (Heckmann *et al.,* 2013). Phylogenetic, anatomical, biochemical, and physiological studies have demonstrated that C_4 photosynthesis often evolves from C_3 photosynthesis via C_3–C_4 intermediary states, also known as C_2 plants (Schlüter and Weber 2020; also see Section 15.11). In many cases, these intermediates represent evolutionarily stable ecological adaptations in their own right.

The advent of next-generation DNA sequencing technologies has enabled detailed genomic and transcriptomic comparisons among closely related C_3, C_4, and C_3–C_4 intermediate (C_2) species. These comparative analyses have led to the identification of numerous candidate transcription factors and cis-regulatory elements involved in the C_4 trait (Schlüter and Weber, 2020). The EU FP7 project "3to4" (https://3to4.org) pioneered this approach by identifying several genes and genetic regulators that underpin C_4 photosynthesis. Building upon this foundation, the H2020 GAIN4CROPS project has expanded the database to include independent origins of C_4 and C_3–C_4 intermediate photosynthesis within the Asteraceae family, which includes the important oilseed crop sunflower.

Large international consortia, such as the Bill & Melinda Gates Foundation-supported C_4 Rice Project (https://c4rice.com), have spent over a decade developing strategies to engineer the C_4 trait into the C_3 crop rice and have produced initial prototypes. Consequently, there is a substantial body of knowledge and experience in trait engineering aimed at converting C_3 crops to utilize C_4 photosynthesis (Wang *et al.,* 2016; Furbank *et al.,*2023).

Limitations: Currently, all efforts to convert C_3 crops into C_4 crops rely on genetic engineering techniques, resulting in organisms classified as genetically modified organisms (GMOs). Implementing C_4 photosynthesis in C_3 plants requires transgenesis, which involves introducing foreign genes into the plant genome. As regulated GMOs, these engineered plants must undergo a costly and lengthy approval process before they can be commercially cultivated. Furthermore, the complete list of genes necessary to establish C_4 photosynthesis is still incomplete, and it is estimated that more than 20 genes may need to be introduced to confer the trait. This presents a significant engineering challenge that is both complex and time-consuming.

Alternative strategies, such as introducing genetic C_4 enablers into C_3 plants through wide interspecific crosses, as explored by the H2020 project GAIN4CROPS,show promise. However, these methods require a decade-long time horizon due to the necessity of multiple backcrossing and inbreeding steps, compounded by the long generation times of the species involved.

The most promising approach to date, which is also explored by GAIN4CROPS, involves employing genome editing technologies to alter gene regulatory (cis-regulatory) regions, effectively "preconditioning" a C_3 species for C_4 evolution (Schuler *et al.,* 2016). This would be followed by strong selection for biomass production under low CO_2 environments to select for synthetic C_3–C_4 intermediates and, eventually, fully functional C_4 lines. This strategy could be combined with wide interspecific crosses to introduce additional, currently unknown, C_4 enablers. However, it is likely that the number of gene edits required will exceed the limits permitted under recent drafts of the New Genomic Techniques (NGT) regulations. Therefore, even the genome editing approach may face regulatory challenges.

Engineering C_4 photosynthesis into C_3 crops holds immense potential but is highly challenging, both scientifically and technically. It is also likely to encounter regulatory hurdles. Moreover, this approach is time-consuming and would require a long-term funding commitment to achieve practical results.

Risks are predominantly associated with the regulatory environment. Engineering C_4 photosynthesis would require the introduction of multiple transgenes or a relatively large number of gene edits, resulting in plant lines classified as regulated genetically modified organisms (GMOs). This classification entails stringent regulatory hurdles, including costly and time-consuming approval processes. Furthermore, the complete genetic blueprint for C_4 photosynthesis is still unknown. Consequently, engineering efforts must proceed with an incomplete understanding of the trait's genetic makeup, adding scientific and technical challenges to the endeavor.

Modelling: Computational models have explored the evolutionary transition from C_3 to C_4 photosynthesis (Heckmann *et al.,* 2013; Williams *et al.*, 2013; Mallmann *et al.,* 2014; Blätke and Bräutigam, 2019), providing conceptual blueprints for evolutionary-guided approaches to develop the C_4 trait de novo in C_3 plants. While these models are informative and important, they do not precisely identify the specific genes or gene regulatory regions that need to be modified to steer a C_3 plant toward C_4 photosynthesis. Addressing these gaps requires phylogenetically informed genetic, genomic, and pan-genomic comparative studies, such as those currently conducted by the GAIN4CROPS project.

Overall, computational modeling strongly indicates that C_4 photosynthesis is accessible from the C_3 state via engineered preconditioned or intermediary stages. Future modeling approaches should incorporate large-scale genomic data to identify signals of selection and co-occurrence patterns across multiple independent origins of C_4 photosynthesis, thereby constraining the nucleotide space for engineering efforts. As the volume of available data approaches levels suitable for machine learning applications, these advanced computational methods are likely to dominate the next wave of modeling approaches.

Translation: Given that C_4 engineering is currently at an early Technology Readiness Level (TRL), it will likely require at least a decade of research and development to produce prototypes suitable for field trials. A significant hurdle to translation, particularly within the European Union, is regulatory approval. Implementing C_4 photosynthesis in C_3 plants necessitates transgenesis, resulting in crops classified as regulated genetically modified organisms (GMOs), which entails a costly and lengthy approval process.

Despite these challenges, the potential gains are immense. Therefore, efforts to translate the growing body of knowledge on the evolution of C_4 photosynthesis into practical applications in crops should continue. It is also probable that the effects of anthropogenic climate change on our agricultural systems will become so severe that a more lenient regulatory approach may need to be adopted to develop crops adapted to future climatic conditions. Initiating these approaches now is essential to ensure they are ready when needed in the not-too-distant future.

High-quality modeling studies and experimental data obtained from free-air CO_2 enrichment (FACE) experiments indicate that engineered C_4 crops could deliver yield gains of 30% or higher. Such substantial yield increases are challenging to achieve through conventional breeding methods, and additional gains may be possible by further breeding of engineered lines. Moreover, C_4 crops are more water- and nitrogen-use efficient than C_3 crops and produce higher yields per unit area of land, which are important sustainability traits. Therefore, from both agronomic and economic perspectives, it is worthwhile to continue efforts to introduce C_4 photosynthesis into C_3 crops. Engineering C_4 photosynthesis into C_3 crops holds enormous potential for increasing yields and improving sustainability traits such as water and nitrogen use efficiency. Therefore, despite the scientific, technical, and regulatory challenges involved, it is both agronomically and economically prudent to continue efforts to introduce C_4 photosynthesis into C_3 crops.

Key development milestones include demonstrating a C_4-like carbon isotope signature in engineered lines. Intermediate milestones would include demonstration of a reduced carbon compensation point (as compared to the C_3 ancestors) and incorporation of carbon from C_4 acids into the Calvin Benson Bassham cycle. Anatomical milestones would include the establishment of Kranz anatomy in a C_3 background.

Time to translation is likely over one decade, however, the relatively long time horizon is offset by the step-change in yield increase that outpaces what is possible in traditional breeding schemes. Implementing C_4 in C_3 is suitable for carbon farming and carbon sequestration. The increased carbon assimilation resulting from increased photosynthetic efficiency in C_4 generates additional photosynthetic products that can be allocated to roots and exuded into the soil, enhancing soil carbon content. Furthermore, higher yields reduce the need for expanding agricultural land, thereby minimizing land use change and its associated carbon emissions. The surplus biomass produced can also be converted into biochar, which improves soil quality and serves as a means for long-term carbon sequestration. Thus, engineering C_4 into C_3 not only enhances crop productivity but also contributes to sustainable agricultural practices by capturing and storing atmospheric carbon.

Engineering C_4 photosynthesis into C_3 crops is a “moonshot” project due to its significant risk of failure and the substantial scientific and technical challenges involved. However, the potential rewards for sustainable agriculture are immense. C_4 crops exhibit higher photosynthetic efficiency, water-use efficiency, and nitrogen-use efficiency compared to C_3 crops, leading to higher yields per unit area of land.

The long development timeline for this endeavour necessitates sustained and reliable funding commitments. Projects of this scale and complexity are challenging to conduct within a corporate environment, much like ambitious initiatives in the energy sector such as nuclear fusion. Achieving the goal of converting C_3 crops to C_4 photosynthesis requires long-term vision and dedication from the public sector to advance to a stage where translation into public-private partnerships becomes feasible.

In the interim, synthetic photorespiratory bypasses offer a promising short-term solution. These bypasses can provide similar efficiency gains and require the introduction of only a small number of genes, making them more straightforward to implement. By enhancing photosynthetic efficiency with synthetic bypasses, we can improve crop productivity and sustainability in the near term while continuing to develop the more challenging C_4 engineering approach for long-term benefits.

15.10 Optimise Source Sink Interactions (TRL 3-4) - Erik Murchie

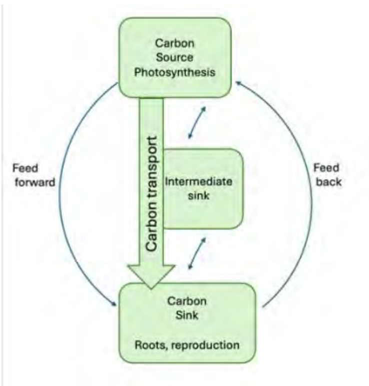


Figure 30 Schematic of source - sink activities

Sinks are the rapidly growing parts of the plant that receive sugar and essential molecules from the *source,* the green photosynthesizing leaves and shoots. Sinks are often portrayed as the harvestable organ, like the grain, fruit or tuber but sinks can also be exploratory roots, expanding leaves or a temporary storage such as stems. It is an essential feature of plant growth that the source export, transport and capacity of the sink organ must be coordinated to avoid feedback inhibition. The circumstance is complex because it intimately connects multiple primary processes during growth, leading to the proposal of a 'wiring diagram' for wheat to describe the system (Murchie *et al.,* 2023). Plants are sometimes labelled as source or sink limited when one or other is not operating efficiently or affected by environmental conditions. Development of organs must be matched to source capacity. While this is a complex whole plant process, there have been recent advances demonstrating that their coordination may be optimised and new approaches for monitoring and screening can be devised (White *et al.,* 2016).

This is an undervalued whole plant trait that uniquely enables the successful translation of enhanced photosynthesis to the field. It needs to be noted that there is substantial species - specificity: crop species, that differ according to phenology, for example, determinate cereals (wheat, barley, rice) are generally source limited before flowering to allow construction of a large reproductive sink. After flowering the size of the sink seems to be critical. In other determinate species like tomato and potato source or sink limitation can change throughout their life cycle.

Maximising photosynthesis: Far from being fixed entities, sources and sinks are dynamic, co-regulated and offer opportunities for intervention and improvement. Targeted research on elements of this control system has highlighted that it can be used to 'remove the brakes' on yield caused by sub optimal export and deposition of extra photosynthate (Miret *et al.,* 2024), *making a strong argument that it needs to be part of translational photosynthesis research.*

Novel targets: To allow (potential) source activity to increase the harvestable yield there has to be a matching increase in the appropriate sink activity. This does not always occur and while breeding for sink size is an ongoing concern in some species such as wheat (e.g. spike size and number), *there has* *been no breeding to specifically target source sink co-regulation.* Promising results have been obtained by targeting regulatory components such as trehalose 6 phosphate / snrk1 have been identified and are at TRL3/4**.** While promising field data exists (Lyra *et al.,* (2021) this has not been allied with enhanced leaf photosynthesis interventions.

Combined stress tolerance and carbon sequestration: Environmental conditions can change sink preference, for example during water and temperatures stress more roots will be needed in poor, dry soils and a greater reliance on stored carbohydrate rather than current photosynthesis. This is seen in cereal yields which accumulate substantial pre heading storage which becomes the source. These provide opportunities that exploit enhanced photosynthesis *but have not yet formed part of crop breeding programmes.*

Limitations: It is essential to better coordinate source and sink activity to increase yield, but it is complex. The regulatory components combine plant development, metabolic control and stress tolerance. It is environment dependent, with multiple sinks including the undervalued roots and secondary storage sinks which may need to be exploited more by the plant to enhance sequestration and prevent source limitation.

Recent advances in phenotyping plant transport need to be rapidly developed and applied. Common measurements / proxies of sink activity include harvest index and the ratio of leaf area to grain weight. These can work but have severe limitations, we need to move on to measurement of photosynthate transport directly and under field like conditions. New advances in NMR have been shown to show real time grain filling (Coleman *et al.,* 2023) and these need further development. A means to measure CH_20 transport *in-vivo* in realistic conditions is needed. Understanding source-sink capacity also requires developing a targeted combination of assays that can be used for long term responses, developmental descriptions and environment responses and can be combined with photosynthesis models such as the FvCB model in the leaf to provide understanding necessity of nutrient/water inputs and effects on sink/source capacity. If we can develop a means to measure leaf carbohydrate level (NIR spec), photosynthetic kinetics (eg. via 820nm), whole plant growth dynamics it is possible to develop the next generation of sink limitation non-destructively.

Depending on the crop and system, **yield may be increased 30 – 50 %** according to some trials (Paul *et al.,* (2017)

This is a complex trait that feeds into abiotic stress tolerance and carbon sequestration and will require an interdisciplinary team of agronomists, molecular biologists, physiologists and engineers. It already has proof of concept and phenotyping technology advancement. It is worth breeding for if we identify the correct technologies and mapping populations.

Modelling: Due to complexity and a lack of understanding of the underlying mechanisms, fixed sink size and source activity has been part of models but the co-regulation has not, so it is under utilised (White *et al.,* 2016). Modelling therefore can be exploited further and the FvCB model may be a good place to start with further development of models for different crops with various strategies for managing C storage and integration with crop based models.

Translation: Challenges include i) the development tractable assays to identify sink limitation properly in target species in the field; ii) to develop tech further for measuring transport in the phloem; iii) Integrated system/whole plant carbon economy understanding; iv) Plant architecture (phloem transport limitation), v) to exploit existing regulatory components such as T6P but must be fully aware that target species will optimise; vi) to deploy sophisticated assays in targeted screens in field and simulated conditions in a wide range of environments.

The TRL of this strategy is estimated at 4-5 for existing components but phenotyping using newly developed methodology may identify further novel gene targets at TRL3.

Translation time is estimated at 5-10 years, but could be accelerated by developing of phenotyping for transport and metabolic sink activity with environmental responses. Milestones are species-dependent but would in each case include development and validation of phenotyping methodology for sink transport, identification of underlying genes and markers and field validation across multiple sites.

This strategy is suitable for carbon farming/sequestration since roots are sinks, C-seq will exploit their sink activity

15.11 C2 Photosynthesis (TRL 3) - Marjorie Lundgren

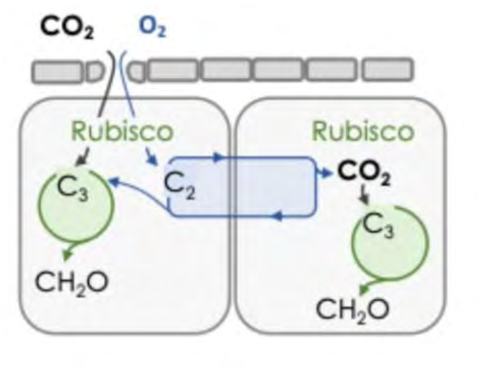


Figure 31 Schematic of C2 photosynthesis

C_2 photosynthesis, also called the glycine shuttle and photorespiratory CO_2 pump, is a simple carbon concentrating mechanism (CCM) that recycles CO_2 released from photorespiration. This natural CO_2 recycling mechanisms allows C_2 plants to avoid the significant carbon costs of photorespiration that hinder C_3 plants, to ultimately improve carbon-, water-, and nitrogen- use efficiency compared to C_3 close relatives.

The C_2 CCM functions only in the presence of photorespiration, via a simple glycine shuttle addition to the C_3 plant phenotype. Leaf anatomy and biochemistry is largely similar between C_3 and C_2 plants, meaning that engineering C_2 photosynthesis into C_3 crops is relatively simple compared to other photosynthesis crop engineering programmes (Lundgren 2020).

C_2 plants have very broad geographical and ecological ranges, as a result of their inherent physiological flexibility (Lundgren & Christin 2017). That is, C_2 plants function the same as a C_3 plant under cool and wet conditions when rates of photorespiration are low yet will engage their glycine shuttle to enhance efficiency under warmer and dryer conditions as rates of photorespiration increase. Engineering C_2 photosynthesis into C_3 crops, therefore, would convey valuable physiological flexibility to help crops maintain yields under a broader range of environments, as well as under the problematic variable weather patterns manifesting with climate change. Despite the clear promise of C_2 crop engineering programmes, the technology remains at TRL3**.** More research is desperately needed to exploit the full potential of C_2 photosynthesis engineering and to push this technology forward.

Benefits: The benefits are C_2 photosynthesis engineering are currently being investigated. A comparison of C_2 species with their close C_3 relatives across diverse plant lineages found that C_2 plants had overall **approximately 159% higher dried biomass, 120% greater leaf area, 19% thicker leaves, 21% higher rate of photosynthesis, 13% greater water use efficiency, and 38% greater photosynthetic nitrogen use efficiency** under 25°C glasshouse conditions (Lundgren *et al.* unpublished data). Furthermore, modelling work predicts that engineering C_2 photosynthesis into C_3 rice would convey consistent improvements to net rates of photosynthesis across a broad environmental envelope (Bellasio & Farquhar 2019). Specifically, this study predicted that an engineered C_2 rice would have, for example, enhanced rates of photosynthesis across a wide range of growing temperatures (15-45°C) under high light. We conclude that engineering C_2 photosynthesis into C_3 crops should convey enhanced growth, physiology, and resource use efficiency to maintain or increase yields across a broad range of environment, potentially expanding current agricultural ranges.

Limitations: Due to potentially high nitrogen requirements, C_2 plants may, in theory, require greater fertiliser investments to realise their full benefits. This remains to be experimentally confirmed, however, as does hypothesized symbiotic relationships between C_2 plants and nitrogen fixing bacteria that would provide C_2 plants with their potentially higher nitrogen requirements.

Modeling: While leaf level physiological models exist for C_2 photosynthesis (*e.g.,* von Caemmerer 2000; Bellasio 2017), advanced crop simulation models are still needed to predict the impacts of C_2 photosynthesis across diverse environments and inform the selection and design of the most promising bioengineering strategies.

Translation: A C_2 photosynthesis crop engineering program remains a long-term strategy. However, several advances would accelerate C_2 photosynthesis translation. First, because characterizing C_2 phenotypic components is laborious, development of a rapid C_2 photosynthesis identification tool would go a long way to speeding up this research program. Second, we need a clearer understanding of the precise phenotypic and genotypic modifications that are required to recreate a functional C_2 phenotype in C_3 plants. Finally, a global C_2 photosynthesis consortium has potential to create a step change in the field by bringing together experts and early career scientists in the field to identify progress, challenges, and work together to solicit funds to support a large-scale, collaborative global C_2 photosynthesis engineering program. Research is currently underway to test the hypothesis linking C_2 photosynthesis with enhanced nutrient content (Walsh *et al.* 2023), especially under climate change (Walsh and Lundgren 2024).

Plants using C_2 photosynthesis have enhanced biomass and larger root systems compared to C3 plants, which would likely translate into an effective carbon farming and sequestration strategy. Furthermore, C_2 photosynthesis is already prevalent in the grass family and, as such, may be readily adopted in grassland and seagrass carbon banks.

15.12 Rhizosphere endophytes root exudates (TRL 1-3/6-8) - Laurent Cournac

Figure 32 The rhizosphere

The ability of plants to interact with soil fungi and microorganisms is one of the key components of root function in plants in the real world. Besides root architecture, which is well known as a key component of plant water and nutrient foraging, there is increasing evidence that these biological rhizospheric interactions are a key component of crop performance, especially in water- or nutrient- limited conditions, under organic cultivation, or in terms of plant resilience. Unsurprisingly, these root/soil/microorganisms interactions are for a large part under dependency of plant genetic characteristics.

Such characteristics can be linked to signaling compounds that are either excreted by the plant into the soil or present at root surface and shape connection with microorganisms communities, eventually selecting favourable ones. They can also be promoted by a variety of “bulk” organic compounds which are excreted in large quantity into the soil during root growth, and serve as substrate for microbial metabolism. This phenomenon constitutes a significant carbon sink for plants (amounting up to ca. 15 % of fixed C), and there is accumulating evidence that this excretion is under genetic control. It may be viewed as C loss, but can also be considered as an "investment", as C deposition and transformation by microbial communities improves soil properties in root vicinity, notably in terms of water retention, aggregate structure, or nutrient accessibility. In terms of GxE (genotype x environment) formulation, this is a way by which G can profitably modify and improve E!

Precise identification of underlying genetic determinants and of their impact on plant performance in actual, agronomic conditions, is still lacking although identification of plant beneficial microorganisms in soils is an old story. Indeed, numerous attempts to isolate and inoculate such organisms exist and are promoted by biotech companies, with varying success. But on the plant side, identification of plant determinants such as composition and quantity of exudates which may promote thriving of such beneficial microorganisms without need of external inoculants (and would therefore enhance the "return on investment" of excreted C) is an alternative strategy to envision and which we propose to explore here as a plant breeding target. Also, the ability of the plant to host beneficial bacterial endophytes (some of them being N fixing), or to develop mycorrhizal associations, may be considered within the same family of root traits to be addressed as breeding goals.

As the underlying phenomena are multifactorial and complex, and as their genetic determinants are far from being completely understood, this constitutes an open field for research, innovation, and plant breeding.

There are an increasing number of strategies on QTL/gene identification for these traits, and molecular tools to analyse the microbial communities that result from these interactions have considerably improved in the last decade. It can be considered that such a strategy is still under rather basic research identification and therefore rather low TRL. However, once genetic determinants identified, improvement and stacking of properties may be quite straightforward, with no particular difficulty.

The USP here is the ability to manipulate the root biome as well as the plant biome with a relatively cheap strategy, by genetically shaping the hosting characteristics of the plant. But this does not prevent one to further stimulate beneficial rhizospheric interactions, either by inoculating desired microbial strains, or by favouring them through agronomic practices for instance.

This strategy may be more beneficial toward yield resilience than yield improvement in the short term. This strategy has certainly a great potential in organic farming where input reduction is key, and where nutrient mobilization stems from organic sources, which inherently requires activation of microbial networks. Moreover, enhancement of soil microbiome dynamics, abundance and diversity is also a lever to regulate potential phytoparasitic soil microorganisms and meso fauna.

Limitations:As this involves a carbon sink, there could possibly be a negative effect on crop yields under some circumstances, especially in conditions where rhizospheric soil properties enhancement does not counterbalance the C "investment". As this cost/benefit ratio is under dependency of very complex interactions, and as measurement of these phenomena are quite difficult to perform in the field, development of suitable proxies and recording of evidence about effects on agronomic performance in the real world are needed to support the strategy.

Translational Challenges: Spatial description of root exudomes (metabolites, peptides etc) in field conditions under different environments is needed to fully understand the rhizospheric effect and its relationship to sink strength, and is quite challenging to measure.

This is a complex trait involving many biotic interactions and is difficult to phenotype in the field. Consequently, the breeding strategy may be to phenotype in controlled conditions for identifying

QTL/genes. And once the genes have been identified, to stack them into elite varieties then check under cultivation cycles how successful they are in bringing crop performance/resilience.

The translation timeline for improved roots is medium long term but by exploring variability in germplasm and gene banks of current crops, and also in wild plants, especially wild relatives of cultivated crops could accelerate translation.

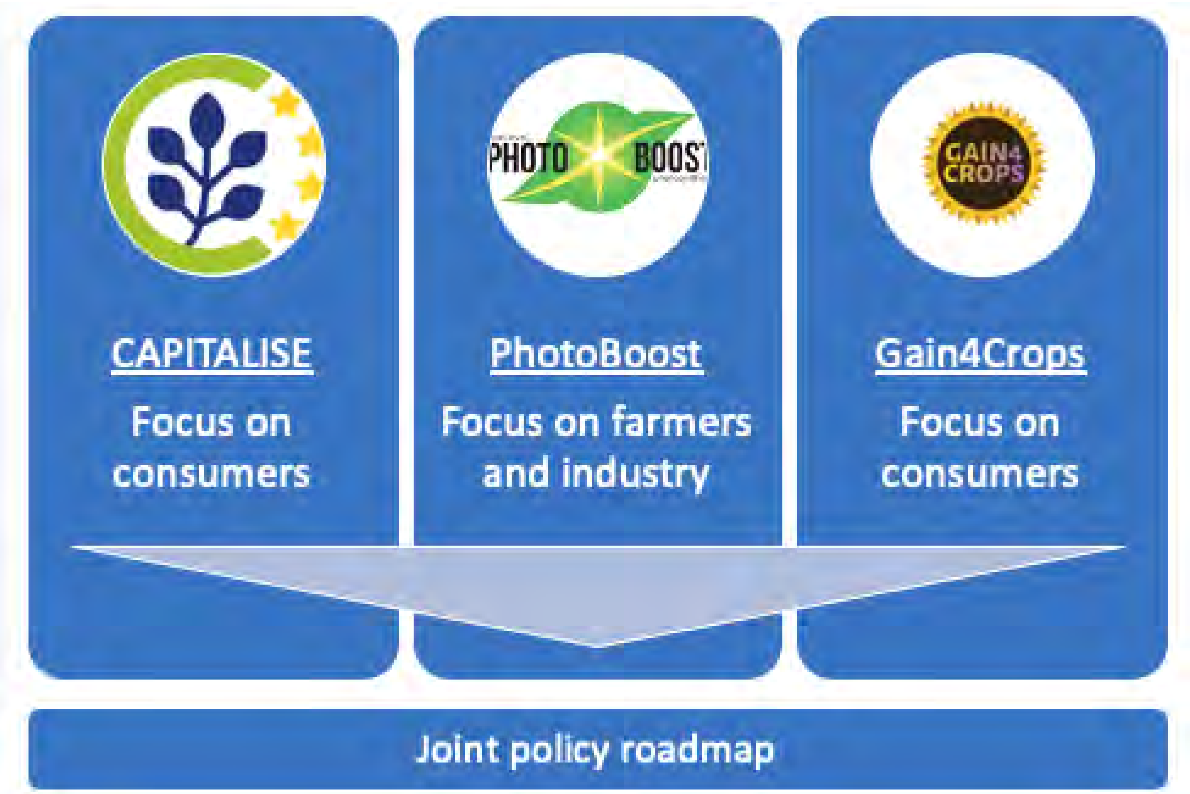
For key development milestones, unravelling molecular targets has to be the first step, then quantifying impacts in actual environments.

Carbon Sequestration suitability is certainly the strongest potential asset of this phenomenon. Indeed, storing carbon in soil is not just a question of injecting biomass but also of transforming organic matter in stable forms (which is essentially due to microbial soil activity) and protecting it (through aggregate formation which is also consequence of rhizospheric soil deposition for a large part), etc.

16 The Social Sciences in Photosynthesis Research - Jonathan Menary, Sebastian Fuller, Paul Nales, Arnout Fischer, Michela Candotti

Three projects were funded in the first-round of the Horizon 2020 funding call “Boosting the efficiency of photosynthesis” in 2019/20: CAPITALISE, PhotoBoost and GAIN4CROPS. Each included a work package dedicated to the social sciences led by *Wageningen University & Research* and *Julius Kühn-Institut*, *University of Oxford* and *IN society* respectively.

Although these work packages have focussed on different aspects of European photosynthesis research, they share the goal of understanding the attitudes of downstream users – consumers, farmers and the wider agri-food industry – towards improved crop photosynthesis and the biotechnology tools commonly used to achieve it.



Below we briefly explain how each project approached these issues and their main conclusions, before providing a consolidated view of the field and recommendations for future science funding in light of our findings across the three projects.

16.1 CAPITALISE

Capitalise used a dual approach.

Environmental and socio-economic modelling On the one hand CAPITALISE has the ambition to create and implement a model to predict environmental and socio-economic impact of introduction of increased photosynthesis plants. The initial environmental modelling considered many factors including change in fuel use needed to transport larger yields, different water and fertiliser needs for the plants to become more productive. While the original intention was to implement needs of individual photosynthesis boosted plants, too few specifics about such plants was known at the time of modelling and hence a more generic model was created.

The model yielded different sweet-spots depending on the value attached to transportation, water, pesticide and fertiliser use suggesting different possible strategies for implementation of increased photosynthesis crops. This needs further detailing once the plants become available.

The socio-economic modelling adopted the same approach and was developed from the environmental models, these models are now in the process being finalised.

Societal acceptance Although not the focus of CAPITALISE *per se*, the need and potential of gene editing for increasing photosynthesis is relevant. Given previous resistance against genetically modified (GM) crops from society and its consumers, the societal work package of project CAPITALISE focuses on understanding the psychology behind consumer perceptions towards gene-editing. Specifically, we focused on spontaneous associations to do so, three major studies have been conducted; 1) focus groups; 2) large scale survey; and 3) online experiments (ongoing).

Focus groups Six face-to-face focus groups (n=6-8 each) were conducted, two each in three European countries (The Netherlands – a north western European coastal country, Italy – a Mediterranean country and Czechia a landlock central European country), to gain insights in the range of associations with and deliberations on breeding techniques. Breeding techniques studied included conventional breeding, gene-editing, genetic modification (cisgenesis and transgenesis), marker-assisted breeding and synthetic biology. The countries represented the North-Western, Mediterranean and Central European regions (Nales and Ficsher , 2023).

Results We found that when participants relied on their spontaneous associations, gene-editing was evaluated similarly as genetic modification. However, after information provision and group discussion, gene-editing was preferred over genetic modification. Perceived naturalness was found to be the main reason for obtaining different levels of acceptance, not only between gene-editing and genetic modification but across all breeding techniques examined. These findings highlight that beliefs about naturalness remain crucial in understanding how consumers evaluate breeding techniques, including the new genome breeding techniques such as gene-editing; as well as showing that initial response of towards gene-editing are closely related to those towards genetic modification.

Survey. This study further examined the role of associations in attitudes towards breeding techniques, either in isolation or embedded into food products that were more natural (corn cobs) and more processed (corn tortilla chips). We examined to what extent associations predict attitudes in comparison to trust and to their memory based attitude of the stimuli. Breeding techniques studied were conventional breeding, gene-editing and genetic modification (see Figure 33) A large experimental survey to capture a more representative view was conducted (N=3027) in 3 countries: the Netherlands (N=998), Italy (N=1010) and Czech Republic (N= 1019).

Figure 33 CAPITALISE survey breeding techniques against corn products

The survey had a 3 (gene-editing, genetic modification, conventional breeding) by 3 (corn cob, chips, none) design, in which participants were asked to write down their associations and provide an evaluation of the breeding technique product combination.

Results We found that associations significantly predicted consumer attitudes and that associations with naturalness have a positive effect on attitude, whereas associations with artificiality have a negative effect on attitude. We found that gene-editing was evaluated more positively than genetic modification, however still less positive than conventional breeding. Finally, we observed contextual effects of food products on overall attitude. When embedded into heavily processed snack products (tortilla chips), the negative effects of genetic modification and in part, gene-editing, start to diminish compared to when these techniques are evaluated in isolation.

Experiments. In another study we will conduct multiple experiments to examine which kind of information has an influence on consumer opinions towards new plant breeding techniques and in particular gene-editing. A first study tests the effect of cues and associations on attitudes towards a food product (potatoes) in which either one of the following breeding techniques are embedded: gene-editing, genetic modification, or conventional breeding. Study 2 aims to partially replicate study 1but replaces an unprocessed product with a processed product – specifically potato chips. Study 3 adds informative text on the products and compares its effect when consumers are and are not overwhelmed by other cues in the environment (cognitive load) as a proxy for common shopping contexts. These experiments are still to be analysed.

16.2 PhotoBoost

There have been calls to better link downstream users with photosynthesis research in recent years (Kohili *et al*., 2020). Photosynthetically-improved crops are a new offering for farmers, although specific benefits of improving photosynthesis, such as improved yield, are not so new. Crop biotechnology is the most transformative means to achieve improved photosynthesis; taken together, there is a need to explore the intersection of photosynthesis research and crop biotechnology amongst farmers, without whom, new and improved varieties will not be planted (Glover et al., 2020).

In PhotoBoost Work Package 6, we employed a three-pronged approach to these issues outlined in Figure 34.

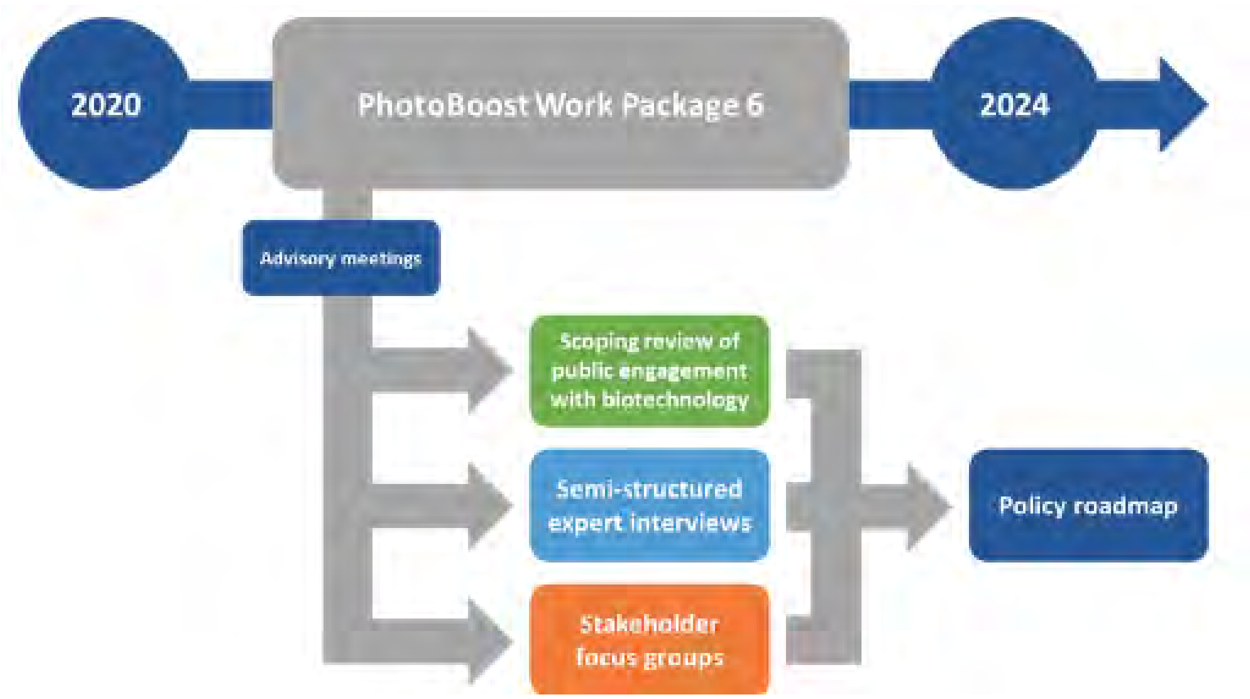


Figure 34 PhotoBoost Work Package 6 Research Process

Each of these methods provides several findings pertinent to the Roadmap.

Scoping review of public engagement with biotechnology. Our review included 37 journal articles, with most of the 2300+ papers captured by our initial search terms excluded due to lack of described public engagement. We found that most engagement has been conducted in high-income countries and, though a wide variety of methods have been employed to engage the public in those countries, most use qualitative research methodology, with focus groups as the primary means of engagement. Our thematic analysis showed that public(s) typically identify more risks than benefits and "unknown" or "unintended consequences" represent the greatest perceived risk for biotechnologies. The broad desire for equity and the specific need for independent assessment of environmental interventions were also prominent themes.

Semi-structured expert interviews We conducted semi-structured interviews with experts from a range of agri-food stakeholder groups in the European Union and United Kingdom to understand current attitudes towards new biotechnology regulation, how they viewed the process of consultation in the EU and the UK as both polities sought to develop regulation around the use of new genomic techniques and what influence they felt they had in shaping regulations. We found that the discussion is similar in both EU and UK, with predictable and fixed opinions determined by attitudes towards the perceived risks associated with direct mutagenesis. Both UK and EU consultations were considered to have the same weaknesses and stakeholders discussed a desire for more dialogic forms of engagement.

Stakeholder focus groups. We organised focus group discussions with farmers, agronomists and other agri-food stakeholders (n=62) in four countries: the UK, Spain, Bangladesh and Philippines. We aimed to understand their attitudes towards improving photosynthesis as a plant breeding aim and the use of biotechnology to achieve it.

We found that farmers were positive about improving photosynthesis if it led to improved yield and water use efficiency or drought stress tolerance. Not all participants considered yield to be the most important trait, though, contrasting this with the ongoing need for pest resistance. Because farmers have to balance competing demands when it comes to varietal choice, any new varieties have to perform at least as well as existing varieties across a range of parameters, including agronomic performance, storage and cooking. Better use of sunlight was also linked to sustainability, as improvements through genetics might reduce the need for agricultural inputs like synthetic fertilizer.

In terms of biotechnology, views varied by location – participants rooted their understanding of PhotoBoost in existing biotechnology crops if commercialised in their country. Participants from low- and middle-income countries were not concerned about the use of genetic modification, although participants were more cautious in Europe due to supermarket and consumer reactions.

We identified a key area of responsibility for breeders in taking PhotoBoost genetics forward and introgressing them into locally-adapted varieties. We have also noted how few formal mechanisms exist for expediting this process between research and industry.

16.3 GAIN4CROPS

The GAIN4CROPS study examined how different communication strategies affect public perceptions and acceptance of New Genomic Techniques (NGTs) in agriculture considering the recent NGTs-related policy revision in Europe. Effective science communication could play a pivotal role in addressing misconceptions and shaping public attitudes towards NGTs in agriculture.

Conducted in Italy, Germany and Romania, the research involved exposing participants to two distinct narratives: one focusing on the sustainability benefits of NGTs and the other emphasizing the safety of NGTs compared to traditional breeding techniques. The goal was to determine which narrative more effectively enhances public understanding and acceptance of NGTs.

Methodology The study recruited approx. 2400 participants across the three countries, ensuring a diverse sample in terms of age, gender and education. Participants were first surveyed to assess their baseline acceptance of NGTs, trust in science, interest in agricultural biotechnology and familiarity with NGT terminology. Then, they were randomly assigned to watch either a sustainability-based or a safety-based video narrative about NGTs or a control video with neutral content. After viewing the videos, participants completed a follow-up survey to measure changes in their perceptions and acceptance of NGTs (see Figure 35).

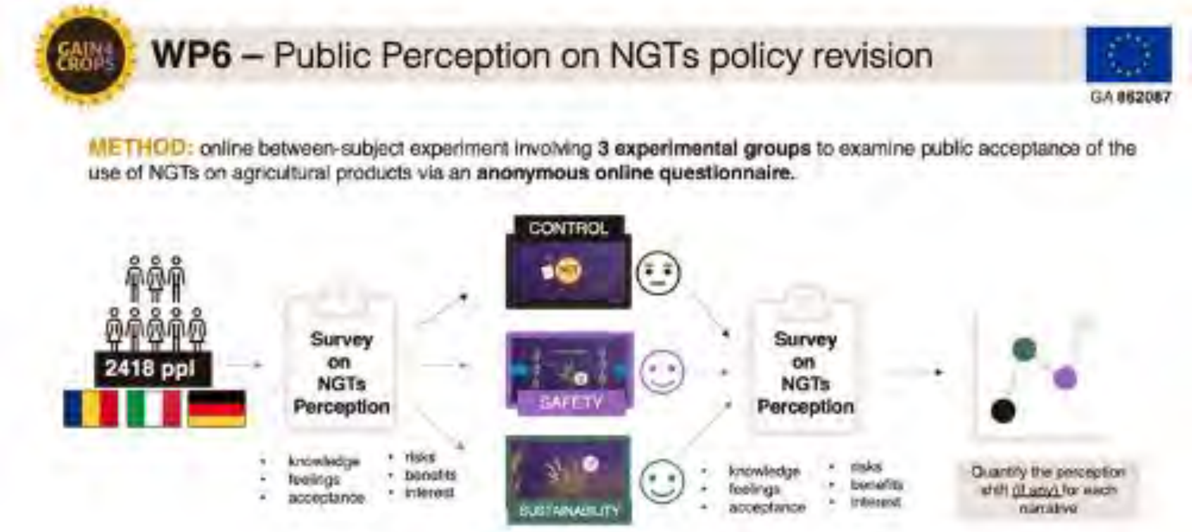


Figure 35 GAIN4CROPS public perception methodology in the framework of Work Package 6 Research Process

Key results The baseline survey revealed a generally positive acceptance of NGTs, with younger participants, those with higher trust in science, greater interest in agricultural biotechnology and better familiarity with NGT terminology being more likely to accept NGTs. This aligns with previous research suggesting that trust in science and familiarity with technology are key determinants of public acceptance (Bearth *et al.*,2024). Furthermore, the relatively high baseline acceptance of NGTs among the surveyed population suggests a general openness to new agricultural technologies that can be leveraged by communicators and policymakers to further promote the benefits of NGTs.

Post-video assessments indicated that both narratives were effective in increasing participants' feelings of being informed about NGTs. However, the sustainability-based narrative led to a more substantial positive shift in acceptance compared to the safety-based narrative. Participants exposed to the sustainability narrative not only felt more informed but also showed increased recognition of the potential benefits of NGTs for sustainable agriculture. This suggests that messages highlighting the environmental and sustainability benefits of NGTs resonate more strongly with the public than those focusing solely on safety and precision.

Impact The findings underscore the importance of narrative framing in science communication. Emphasizing the sustainability benefits of NGTs appears to be a particularly effective strategy for enhancing public acceptance. This is consistent with prior research indicating that positive messaging about new technologies can improve public perception (Baum *et al.,* 2023). However, while the sustainability-based narrative was effective in highlighting the benefits of NGTs, it is important to address public concerns about risks, particularly related to health and environmental impacts. A balanced approach that combines positive messages about sustainability with clear information addressing safety and risk concerns can provide a more comprehensive understanding of NGTs and foster informed public engagement.

These insights are useful for science communicators, policymakers and stakeholders involved in the promotion and regulation of NGTs. By employing communication strategies that highlight both the benefits and address the concerns associated with NGTs, it is possible to foster informed public discussions and facilitate the integration of these technologies into society.

16.4 Consolidated findings

There are several common themes across the three projects.

16.4.1 Acceptance amongst consumers and farmers

Our findings show that NGTs are often perceived (slightly) more positively than first-generation, transgenic techniques, likely owing to their perceived naturalness and when linked explicitly to sustainability. CAPITALISE outcomes show that initial intuitive consumer responses are much closer to the (negative) response towards first generation techniques than to conventional breeding. However, the GAIN4CROPS findings suggest that while environmental concern was significant for all participants, positive environmental impacts were not recognized as a top reason for supporting NGTs *unless explicitly highlighted* in the narrative. This aligns with previous research indicating that consumers do not automatically associate NGTs with environmental benefits (Ferrari *et al*., 2021) and that the framing of emerging technologies is important. (Bauer and Bogner 2020). The findings emphasize the importance of clearly addressing the sustainability impact of NGTs in communication efforts to enhance public acceptance. However, it is crucial to avoid "greenwashing" and ensure that claims about the environmental benefits of NGTs are accurate, transparent and supported by scientific evidence. Although the sustainability-based narrative effectively promoted NGTs, it is essential to also address risk concerns, particularly related to health and environmental impacts. A balanced, 'authentic' communication strategy that combines sustainability benefits with clear information on safety and risks is necessary to foster informed and engaged public attitudes towards NGTs (Prendiville *et al*., 2023).

This indicates a role to play for the European Union if it wants to continue to invest in research projects utilising the latest biotechnology tools. A concern for biologists is that without these tools Europe would lose a key competitive edge in plant breeding (Menary and Fuller, 2024), which serves to highlight the ongoing importance of both clear policy and a coherent communication strategy that supports decisions made on agricultural biotechnology.

Recommendation #1: develop an EU-wide communication strategy in concert with new policy on NGTs

16.4.2 Biotechnology regulation and rights sharing

These findings reinforce the need for clarity on NGT regulation in the European Union, which has remained an issue across successive Parliaments in recent years (Macnaghten and Habets, 2020). Numerous provisions have been proposed to create a regulatory environment that delivers on both safety and innovation (Reichenbecher *et al*., 2024) in the European Union. Following stakeholder and public consultations, the Commission did put forward a proposal for more lenient regulation of gene-edited organisms in 2023 that Parliamentary approval but became "stuck" in the Council – this process has been slow and, at present, constitutes a barrier to specific kinds of plant breeding research and development.

These debates will determine the nature of future photosynthesis research in Europe, as the more transformative options for its improvement rely on crop biotechnology (Long *et al*., 2006). More lenient regulation of gene-edited organisms would provide developers with the tools to accelerate certain breeding aims and create the market conditions for commercialisation of those crops, but does leave transgenic techniques, which are in many respects the regulatory benchmark against which other techniques are judged, facing the existing and more challenging route to commercialisation. PhotoBoost focus groups in low- and middle-income countries did demonstrate that there are means of regulating transgenic crops that manage both approvals and public acceptance through a case-by-case approach, but this looks very unlikely in Europe.

The results of CAPITALISE and GAIN4CROPS' public engagement work indicates that if used to improve traits in crop plants associated with sustainability, then acceptance amongst consumers could be higher than otherwise, provided this is effectively communicated. In addition, CAPITALISE outcomes show that acceptance of these crops in highly processed products is higher than in unprocessed fresh produce, which suggests different marketing, labelling and even possibly regulatory regimes for these products are needed.

A related problem identified in PhotoBoost is 'freedom to operate', whereby the system of intellectual property protection that characterises the crop biotechnology space creates a difficult-to-navigate environment for developers (Luby *et al*., 2015). The view of biotechnology as contributing to corporate control of the food system likewise constitutes a barrier to acceptability amongst certain groups of European stakeholders Menary and Fuller, 2024).

Recommendation #2: ease regulation for sustainability-focussed crop improvement that utilises SDN-1 techniques

16.4.3 LCA data lacking

In CAPITALISE effort was done to model the socio-economic and environmental impacts of the introduction of increased photosynthesis crops. However, the lack of real-world data about potential increases in fertiliser, space, water and crop protection products for improved photosynthesis plants in field conditions makes it currently impossible to estimate larger scale effects of these crops.

16.4.4 Funding

Some of the barriers we have identified through the social sciences work packages in each project are best met at the level of European research funding, where a balance should be struck between discovery, molecular biology research and more applied research focussed on moving photosynthetically-improved crops into the field. The social sciences can also lead research of this kind by providing an anticipatory approach to what locations, crop types, intellectual property regimes and crop breeding targets are most likely to be successful – this can be seen as essential at both the early stages of crop development, but also as potential plant breeding programmes move through the technology readiness levels (TRLs) towards the market. Stipulating the need for a "societal readiness level" (SRL) metric into crop improvement projects could also provide a framework for identifying what societal needs might be *best met* by crop improvement, at what stage of research and development of plants, itself a key priority for agri-food stakeholders in Europe (Stetkiewicz *et al.,*2023).

Research funding can facilitate photosynthesis research by investing in breeding strategies that maximize sustainable outcomes – this serves the dual purpose of 1) aligning research with consumer and stakeholder values and 2) addresses issues around futureproofing European agriculture against climate change and resource depletion. For example, the possibility of improved water use efficiency was received positively by farmers in the PhotoBoost focus groups, as was improved resource use efficiency.

Recommendation #3: balance research funding between discovery research and applied plant breeding programmes that capture societal needs and maximise sustainability

16.4.5 *Addressing translational challenges*

Several of the suggestions above – aligning research with the values of society, creating market conditions for gene-edited crops in Europe – could facilitate better translation of photosynthesis research into the field. However, other measures would also help with this crucial step in the innovation process.

One is involvement of farmers in the development process of photosynthesis crop improvement, at an early stage, for example, through problem identification and articulation of needs, or through crop phenotyping and field trials in the later stages of plant breeding. One possibility to facilitate this is through the concept of 'living labs', such as the FOOD 2030 Connected Lab Network (https://food2030.eu). Small farms are particularly underrepresented at the science-policy interface Šūmane *et al.,* 2021, so ensuring these voices are not left out of future photosynthesis research is also important.

A key barrier here was identified in the PhotoBoost project, whereby farmers gave priority to other crop improvement targets, specifically pest and disease resistance, over the improved yield that better photosynthesis could bring – this suggests that more can be done to highlight the importance of abiotic stress in a warming world.

Recommendation #4: involve farmers in early stages of problem identification and in formal field trials and phenotyping

17 The way forward - Recommendations

Climate change is driving abiotic stresses that negatively impacts crop health and yields, reducing primary production and threatening food, feed and energy security. New climate resilient crops are urgently needed. We need full value chain thinking.

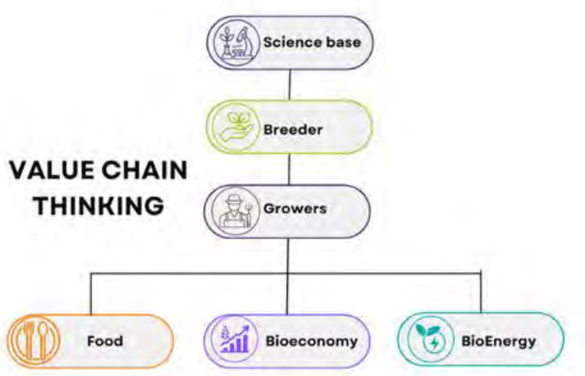


Figure 36 Public Private Partnerships are needed that take into account the full value chain for crop based products

Here we suggest a number of key recommendations towards enabling the effective translation of photosynthesis research for benefit of societal needs.

- ✓ Crop development is a long term investment taking 10-15 years and requiring a strategic approach. Time is of the essence. Research on relevant germplasm, improved genetic resources, tools, models and an innovative culture that embraces biotechnological advances are critical to accelerate the required improvements to crops.

- ✓ Public private partnership represent the best option to develop the tools and knowledge base to deliver a new generation of resilient sustainable climate adapted crops that address the emerging threats to primary production for food and the bioeconomy.
- ✓ Low level and declining public investment in crop breeding programmes needs to be reversed. Crop research needs a reinvigorated strategic programme, at the European level, to implement longer term (5+ years) well-funded (€8M+) collaborative research and innovation projects creating enabling environments to drive translational crop research.
- ✓ Photosynthesis is a complex process but has many underexploited traits with significant potential to improve crop yield and resilience to climate change. Recent scientific advances have demonstrated significant improvements in crop productivity through improving photosynthesis efficiency.
- ✓ Translation of Key Exploitable Results represents a priority research area. Collaborative working is needed between industry and the science base to overcome market failure in developing photosynthesis driven climate resilient crops.
- ✓ An enabling regulatory environment to support NGTs should be a short-term priority to accelerate the broader application of biotechnology. This will compliment conventional crop improvement pathways to develop some new plant varieties faster, and in a more precise manner to exploit promising traits and approaches.
- ✓ In parallel, environmental risk assessments should be undertaken, and literacy programmes developed and implemented, to educate citizens about NGTs and making informed risk assessments. Barriers to translating public research to industry need to be better understood and addressed. Life Cycle Analysis represents an important tool to address the socioeconomic costs, risks and benefits of the proposed approaches and will form a basis for commercial decision making.
- ✓ Issues regarding IP and the Nagoya protocol need to be resolved for maximal use of research outputs by Industry.

17.1 A final word from industry:

ISI Sementi is an example of a breeder inspired by the collaborative work done with CAPITALISE.

"ISI Sementi are examining several traits related to photosynthetic efficiency using a recombinant inbred line population (RIL), obtained from two parental lines of tomato with different chlorophyll content and photosynthetic capacity.

Through the use of advanced tools such as the FluorPen (handheld.psi.cz) which measures chlorophyll fluorescence, and the AtLeaf+ which quantifies chlorophyll content, we have assessed various parameters, including the maximum quantum yield of photosystem II (Fv/Fm) and the efficiency of excess energy dissipation (NPQ). These traits were phenotyped in a population of 152 individuals to identify genetic variants associated with photosynthetic efficiency in tomatoes.

Preliminary results show significant phenotypic variability, which will enable us to identify genotypes with a greater potential for photosynthetic improvement and to define breeding strategies aimed at developing more efficient varieties.

Investing in improving photosynthetic efficiency is not only a strategy to increase productivity but also a concrete response to the need for a more sustainable agriculture integrated into new production contexts. At a time when traditional agriculture is evolving toward new forms of cultivation, such as vertical farming, and facing climates with increasingly higher temperatures, the seed companies that integrate these aspects into their research programs can drive innovation and contribute significantly to the future of the sector"
Anna Giulia Boni & Massimiliano Beretta at ISI Sementi, Italy.

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