



THIS PROJECT IS FUNDED BY THE EUROPEAN UNION HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRANT AGREEMENT 817690



CropBooster-P

Deliverable 4.2

Title: White Paper and Scientific Basis of the Strategic Research Agenda

Start date of the project:	November 1st, 2018 / Duration: 36+6 months
Planned delivery date:	M37 (November, 2021)
Actual submission date:	November 23 rd , 2021
Work package:	WP4 / Tasks: 4.2 & 4.3
Work package leader:	INRAE
Deliverable leader:	INRAE
Version:	1
Date of version:	November 23 rd , 2021
F	

Dissemination type	R
Dissemination level	Public



CONTENTS

No.	Title	Page
1.	Summary of reports of the focus groups and the workshop (June 8/9, 2021)	3
1.1	Introduction	3
1.2	Results elaborated by the focus groups and in the workshop	4
2.	Expert panel assembled to define scientific and technical strategies and their visions (Task 4.2-1)	8
2.1	List of Tasks and coordinators of the focus groups (Task 4.2a)	8
2.2	List of Countries and Institutions to which members of "Focus Groups" are affiliated (Task 4.2a)	9
2.3	Draft of Focus Groups Report (Task 4.2b)	11
	Focus Group Y-1: Optimizing photosynthesis	11
	Focus Group Y-2: Improving source-to-sink relationships	21
	Focus Group Y-3: Optimizing shoot architecture and canopy	29
	Focus Group Y-4: Optimizing root architecture	35
	Focus Group Y-5: Adapting life histories to changing environments	43
	Focus Group N-1: Increasing protein content and quality	47
	Focus Group N-2&4: Improving specialized metabolite contents	53
	Focus Group N-3: Increasing omega 3 fatty acids in oilseeds	60
	Focus Group N-5: Improving biomass digestibility	64
	Focus Group S-1: Improving nitrogen uptake and use efficiency	69
	Focus Group S-2: Improving water uptake and water use efficiency	76
	Focus Group S-3: Improving phosphorus uptake and use efficiency	85
	Focus Group S-4: Improving micronutrient uptake and use efficiency	93
	Focus Group S-5: Improving heat tolerance	103
	Focus Group S-6: Using plants for carbon sequestration	111



1. SUMMARY OF REPORTS OF THE FOCUS GROUPS AND THE WP4 WORKSHOP (JUNE 8/9, 2021)

Norbert Rolland¹, Günter Strittmatter², Peter Westhoff².

1: <u>norbert.rolland@inrae.fr</u> (INRAE, CNRS, Univ. Grenoble Alpes, FR); 2: <u>guenter.strittmatter@hhu.de</u> & <u>west@hhu.de</u> (Universität Düsseldorf, GER)

1.1 Introduction

The world of 2050 will be facing three major, primary challenges: Firstly, the world population will most probably have reached 9 to 10 billion people and will still be growing, particularly in Africa and Asia, although at reduced rates as compared to presently. To fulfil the growing demands for food and feed plant production has to be increased. In order to protect natural ecosystems and biodiversity, the area of agricultural land presently used should not be extended and, hence, the yield per area arable land must be enhanced substantially.



Figure 1: Major challenges for agriculture and general approaches to cope with them

Secondly, the global climate changes will exact its toll. Temperatures remain high and may even increase further, thus shortages in water supply for agriculture will prevail, and extreme weather conditions will occur more frequently. These developments will pose a severe stress to agricultural production leading to substantial decreases in primary plant production. It is, therefore, of prime importance to search for solutions how to stabilize yield.

Thirdly, the current food system has become unsustainable, and there is an urgent need for substantial changes as stressed by the EU's Green Deal and its Farm-to-Fork Strategy. Mineral fertilizers and pesticides are heavily used in agriculture posing risks for human health, but, more importantly, they harm the environment as well as biodiversity. If the reductions in inputs are not to be mirrored at the yield level, the resource use efficiencies of our current crops have to be improved. Alternatively, novel (orphan) crops that are better adapted to the changing environments than the current ones have to be identified, trialled and, if found suitable, introduced into farming. Presently, European diets are still relatively meat-rich necessitating that large amounts of primary plant production have to be used for the feeding of livestock. Achieving a transition towards a plant-



based diet requires that the cultivation of crops have to be intensified that are rich in food constituents such as proteins, vitamin or micronutrients which hitherto are mainly provided by meat consumption.

Coping with these challenges and providing solutions requires an integrative and interdisciplinary approach that has to deal with three pressing issues of crop production, namely yield, nutritional quality, and sustainability. For each of these three topics we have, therefore, set up expert panels whose aim was to look at the three issues from various angles. Each focus group had to review the state of knowledge of their field, to identify the future challenges in the research field that should be addressed with high priority, and finally to outline action points for a future research programme.



Figure 2: Goals, topics and coordinators of focus groups in WP4

1.2 Results elaborated by the focus groups and in the workshop (June 8/9, 2021)

Yield

Yield will continue to be a key goal of plant breeding as well of key importance for the farmers. Yield is a complex trait consisting of various components. The yield potential is defined as the yield obtainable under optimal conditions, i.e. with no limitations in nutrient resources and no abiotic or biotic stresses acting on the crop. It integrates photosynthesis, the allocation of the photoassimilates within the plant, and the effects of canopy.

In the field, however, resources are usually limited and abiotic or biotic stresses are more or less present reducing the final yield obtained. Increasing the sustainability of plant production by reducing inputs or the occurrence of extreme weather conditions caused by the global climate changes will certainly affect yield.

Photosynthesis is a multidimensional process. The core photosynthetic reactions taking place in the plant's green cells are evolutionary conserved. In land plants these cells are predominantly located in the leaves which represent the organ of photosynthesis. The inner anatomy of a leave and its



form, therefore, strongly influence its overall photosynthetic output. Atmospheric carbon dioxide enters the leaf through the stomates, while stomates release the plant's water by evaporation. The availability of water, extracted from the soil by the roots, therefore, affect the degree of stomata opening and, hence, the rate of photosynthesis. Roots are also key players in the uptake of nutrients. Since the photosynthetic apparatus represents a heavy investment of resources, nutrient availability is central for photosynthetic performance.

In the field, but also in commercial greenhouses, it is the photosynthetic output of the cop community that ultimately determines yield. The denser plants can be grown without hindering each other's photosynthetic output, the higher the yield potential of that community is. Plant architecture, for instance the stature of the plants and their leaf angles, is a key factor for canopy structure and, hence, affects the yield potential of a crop community.

The photosynthetic output of a field also depends on the length of the growing season. Increasing the cultivation time of a field to cover as much of the potential growing seasons as possible could for instance be achieved by the use of perennial crop species or of successive crop plantings.

All breeding or agronomic approaches for increasing yield have to be pursued along the lines of sustainability. Given the challenges arising from global climate change the stabilisation of yields rather than its further improvement may be in the focus of the research efforts.

Action points

- Reach a mechanistic understanding of the key factors contributing to or limiting yield and use that knowledge for "Breeding-by-Function".
- Set up common and shared pools of genetic material of current crops and their wild ancestors and use that material for constructing common and shared segregating populations for allowing an easy cloning of major QTLs.
- Exploit underutilized crops with promising traits.
- Set up common and shared experimental stations that are equipped with state of the art tools for phenotyping the relevant traits and allow the field-testing of genetically modified crops.

Nutritional quality

Healthy diets are a key goal of the EU's Green Deal implying that the eating habits of Europe's citizens have to change substantially. The consumption of meat and other animal-derived food has to be substantially reduced and concomitantly be replaced by plant-based products. This change is inevitable, since the current food system is not sustainable anymore by stressing the earth's resources beyond their limits and by producing too many environmental costs, for instance by the release of green-house gases into the atmosphere.

The nutritional quality of food has to be ensured. The basis for a switch to more plant-based nutrition has to be provided by agricultural and horticultural production of sufficient crop plants that fulfil all needs of a healthy diet, with respect to the composition and amount of proteins, carbohydrates, lipids, vitamins and micronutrients.

Action points

- Increase nutrient yield and quality per area arable land used.
- Put a focus on how global climate change impacts on the micronutrient and vitamin content of plant-based foods.



- Understand the mechanistic interrelationship between yield and resource-use efficiency, on one hand, and content and composition of essential nutrients in crops, on the other hand; transfer this know-how to plant breeding and agricultural practices.
- Intensify breeding for organic agriculture.

Sustainability

All "Focus Groups" have carried out a detailed analysis of the scientific status quo in the respective field, have identified scientific questions to be addressed with high priority and have suggested actions to be taken for providing the necessary know-how to reach the goals of reducing resource use or improving resource-use efficiency by crops in agricultural/horticultural plant production, and to adjust to climatic changes.

Ways of uptake, transport and assimilation of the various nutrients, the effect of water potential on metabolic processes and the dependence on soil parameters and temperature have been elaborated. Also, requirements and various concepts to use plants for carbon sequestration have been illustrated. In general, it became obvious that substantial basic research is necessary in all fields considered, led by a (holistic) systems view, to understand causal functional relationships in the expression of the corresponding plant traits and the interdependence of parameters affecting them. This knowledge should provide the basis for more effective and efficient predictive breeding of improved culture crops, not only depending statistical probabilities for phenotypic performance, and the development of innovative plant protection and nutrition concepts. And it should also deliver the fundamental knowledge base for the establishment of novel farming practices and the accurate prediction of ecological effects generated by the various forms of agricultural plant production.

Understanding agro-ecological rules and processes, *i.e.* the interaction of crop plants with organisms in their environment, the development of living communities in various agricultural/horticultural systems, and the impact on that by climatic changes, is seen a key task for future plant sciences. This knowledge base will be essential for the adaption of agriculture to the described challenges.

At the same time, the strong interconnections between the individual traits and their impact on yield potential and yield stability were elaborated. This supports the need for capacities and knowhow in the smart use of big data collections from various research fields, to mechanistically understand complex biological processes. Finally, this should pave the way to breeding approaches based on biological know-how, and also to the synthetic design of required plant phenotypes.

In a more concrete sense, the following action points for designing future research programs in the field of "Sustainability" were proposed:

- Exploitation of natural diversity in the adaptation of plants to varying resource availability (water, macro- and micro-nutrients), and abiotic/biotic stresses; in this context the exploitation of genetic variability existing and accessible in gene bank collections should be supported and strengthened.
- Further development of tools for precision phenotyping, especially under conditions of practical agricultural plant production, also allowing the analysis of root development and function.
- Investigating the impact of soil parameters on plant performance, with a focus on understanding functional processes regulating the interaction of plants with their soil environment.



- Development of know-how in agroecology: Addressing the question what is the impact of agricultural plant production (different types of crops, different types of agricultural practices) on ecosystems of farmland; investigating the development of living communities under various agricultural/horticultural conditions, and its dependence on environmental factors
- Test/development of agricultural and forestry practices for using crop plants and trees in carbon sequestration concepts; this should aim at identifying features of plants that need to be optimized to improve the efficiency of such processes?

Conclusions: Suggestion of Topics for Research Agenda

In summary, the reports of the 15 "Focus Groups" and the presentation and discussion of these reports during an Online-Workshop on June 08/09, 2021, led us to the recommendation of the following high priority topics for a future EU research agenda in the field of plant sciences, all under the headline "Better Crops for Tomorrow's Needs":



Figure 3: The research agenda

This proposal is guided by the aim to provide the scientific basis for the generation of crop plants and the development of agricultural practises that cope with the need for a combination of several characteristics in future agricultural and horticultural plant production: (*i*) maintenance of high yield under reduced application of agrochemicals and reduced use of limited resources (biology for breaking the wall between yield and sustainability), (*ii*) providing sufficient high-quality plant raw material that allows a substantial reduction of meat in healthy diets (for example legumes as plant protein source in European agriculture), (*iii*) using plants in a sustainable manner for the net reduction of carbon dioxide concentration in the atmosphere, and (*iv*) dealing with rapidly changing and extreme weather conditions due to climate change.



2. EXPERT PANEL ASSEMBLED TO DEFINE SCIENTIFIC AND TECHNICAL STRATEGIES AND THEIR VISIONS (TASK 4.2-1)

On the basis of results from WP1 and WP2 individual research fields were defined that are associated with "Yield" (five research fields), "Nutritional Quality" (five research fields) or "Sustainability" (six research fields). Within these research fields, the landscape of interactions in Europe was mapped, between researchers, research institutions, countries and research fields (see WP4, Task 4.1 & Deliverable D4.1).

From these analyses, researchers excelling, in a given research field, where identified and contacted ("Focus Group" coordinators). In order to strengthen interactions between Europe's research institutions and between hubs of plant research in Europe, each "Focus Group" coordinator was solicited to identify and contact experts, with relevant scientific expertise in their respective fields, and expert panels ("Focus Groups") have then been formed for each individual research field (Milestone 15). The ambition of each "Focus Group" was to elaborate and propose strategic research actions that are expected to contribute to reaching the EU strategic goals as outlined in the so called "Green Deal" and the "Farm to Fork" concepts. The reports of these "Focus Groups" (Milestone 17) were pooled and will be distributed to all CropBooster-P Partners and stakeholders. The "Focus Group" coordinators had the opportunity to share their defined strategic research actions at a meeting (WP4, Task 2-2) which took place on June 8-9, 2021. An aggregated final report (WP4, Deliverable D4.2) was forwarded to WP5, as a basis for proposing a detailed future plant research agenda, with priorities set according to the strategic goal setting of the EU Commission.

The implications of this approach ("Focus Groups") for structuring the European plant research landscape are obvious. The coordinators of the 16 "Focus Groups", established contacts with an average of 9 experts per "Focus Group", 46 experts related to "Yield", 37 experts for "Nutritional Quality" and 51 experts for "Sustainability". Altogether, this approach involved more than 130 experts, from 70 institutes or universities and 15 countries.

2.1. List of Tasks and "Focus Groups" coordinators (Task 4.2-1)

WP4 and Task 4.2 leaders:

- Norbert Rolland, INRAE/CNRS, Grenoble, France
- Günter Strittmatter, Univ. Düsseldorf, Germany
- Peter Westhoff, Univ. Düsseldorf, Germany

List of "Focus Groups" coordinators:

- Catherine Bellini, INRAE, Versailles, France & Univ. Umea, Sweden
- Massimiliano Corso, INRAE, Versailles, France
- Sylvie Dinant, INRAE, Versailles, France
- Emmanuel Gaquerel, Univ. Strasbourg, France
- Hermanus Höfte, INRAE, Versailles, France
- Maria von Korff-Schmising, Univ. Düsseldorf, Germany
- Anne Krapp, INRAE, Versailles, France



- Patrick Laufs, INRAE, Versailles, France
- Jacques Le-Gouis, INRAE, Clermont-Ferrand, France
- Loïc Lepiniec, INRAE, Versailles, France
- Pierre Martre, INRAE Montpellier, France
- Céline Masclaux-Daubresse, INRAE, Versailles, France
- Bertrand Muller, INRAE, Montpellier, France
- Johnathan Napier, Rothamsted, UK
- Laurent Nussaume, CEA, Cadarache, France
- Sébastien Thomine, CNRS, Gif sur Yvette/Paris Saclay, France
- Roberto Tuberosa, Univ. Bologna, Italy
- Andreas Weber, Univ. Düsseldorf, Germany
- Peter Westhoff, Univ. Düsseldorf, Germany

2.2. List of Countries and Institutions to which members of "Focus Groups" are affiliated (Tasks 4.2a and 4.2b)

Belgium (Ghent University - VIB Ghent - ULB, Bruxelles - UGent Department of Plant Biotechnology and Bioinformatics, Ghent). Denmark (Department of Plant and Environmental Sciences, University of Copenhagen). Estonia (University of Life Sciences, Tartu). France (Agroécologie INRAE, Dijon -ARVALIS, Institut du Végétal, Paris - ARVALIS, Institut du Végétal, Toulouse - Arvalis Institut du Végétal, Pusignan - ARVALIS, Institut du Végétal, Villiers Le Bacle - BPMP INRAE, CNRS, Montpellier - BIAM, CEA, CNRS, Aix Marseille Univ, Saint-Paul lez Durance - CIRAD, Montpellier - Eco&Sols INRAE, Montpellier - GDEC INRAE, Clermont-Ferrand – I2BC CNRS, Paris-Saclay University - IBMP CNRS, University of Strasbourg - IJPB INRAE, AgroParisTech, Versailles - IPS2 CNRS, INRAE, Paris-Saclay University, Orsay - IRD Montpellier - LPCV CNRS, INRAE, CEA, Univ. Grenoble - Terres Inovia, Thiverval-Grignon Alpes). Germany (Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University Goettingen - Bonn University - CEPLAS, Düsseldorf - Forschungszentrum, Jülich -Department of Molecular Signal Processing, Leibniz Institute of Plant Biochemistry, Halle - Goethe University, Frankfurt am Main - Institute of Plant Breeding, University of Kiel - IPB Halle – IPK Gatersleben - JIC, Jülich - MaxPlanck Golm - University of Bayreuth - University of Düsseldorf -University Erlangen-Nuremberg - University of Hohenheim, Department of Biobased Resources in the Bioeconomy - Saaten-Union Biotec GmbH - ZALF, Muencheberg). Italy (University of Bari "Aldo Moro" - University of Bologna - Università degli Studi di Torino - University of Milan). Nederland (Plant Breeding Department, Wageningen University & Research, Wageningen - Vrije Universiteit Amsterdam - Wageningen University). Poland (PPC ADOB). Portugal (University of Trás-os-Montes and Alto Douro, Vila Real). United-Kingdom (Institute of Aquaculture, University of Stirling - Plant Sciences, Rothamsted Research, Harpenden - Quadram Institute, Norwich - Rothamsted Research -University of Birmingham - University of Essex - University of Nottingham). Sweden (UPSC, Umea). Spain (Centro Nacional de Biotecnologia, CSIC Madrid - Dept. of Crop and Forest Sciences, University of Lleida - Instituto de la Grasa, CSIC, Campus Universitario Pablo de Olavide, Sevilla - Plant Molecular Genetics Department, CSIC, Madrid - Universidad de Córdoba - Universitat de les Illes Balears - Universidad Politécnica de Madrid). Switzerland (Department of Plant Molecular Biology,



University of Lausanne - ETH, Zürich - Structural Plant Biology Laboratory, Department of Botany and Plant Biology, University of Geneva). **Turkey** (Sabanci University, Istanbul). **USA** (Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Berkeley, California - Plant, Soil, and Microbial Sciences, Michigan State University, East-Lansing, Michigan - University of Illinois).



2.3. Draft of "Focus Group" reports assembled to define scientific and technical strategies (Task 4.2b).

Focus Group Y-1: Optimizing photosynthesis

Maeva Baumont¹, Roberta Croce², Jon Falk³, Jeremy Harbinson⁴, Anja Krieger-Liszkay⁵, Eric Murchie⁶, Christine Raynes⁷, Norbert Rolland⁸, Andreas Weber⁹, *Peter Westhoff¹⁰*.

1: <u>m.baumont@arvalis.fr</u> (Arvalis, FR); 2: <u>r.croce@vu.nl</u> (Vrije Universiteit Amsterdam, NL); 3: <u>falk@saaten-union-biotec.com</u> (Saaten-Union Biotec GmbH, GER); 4: <u>jeremy.harbinson@wur.nl</u> (Wageningen University, NL); 5: <u>anja.krieqer-liszkay@cea.fr</u> (CNRS, Université Paris-Saclay, FR); 6: <u>erik.murchie@nottingham.ac.uk</u> (University of Nottingham, UK); 7: <u>rainc@essex.ac.uk</u> (University of Essex, UK); 8: <u>norbert.rolland@inrae.fr</u> (INRAE, CNRS, Univ. Grenoble Alpes, FR); 9: <u>andreas.weber@hhu.de</u> (Universität Düsseldorf, GER); 10: <u>west@hhu.de</u> (Universität Düsseldorf, GER).

Status quo of research

Crop yield is determined by the available solar irradiation energy (St), the efficiencies by which the radiation is intercepted (ϵ_i) and the light energy is converted into biomass (ϵ_c), and which fraction of the total biomass is partitioned into the harvestable part of the crop (ϵ_p). This results in the Monteith equation, which serves here as a quantitative framework to identify potentials for yield improvement: **Yield = St** • $\epsilon_i \cdot \epsilon_c \cdot \epsilon_p$.

The Green revolution raised the yield potential of the major grain crops mainly by increasing the harvest index (ε_p) which is now about 0.6. Also, the light interception efficiency (ε_i) is in modern cultivars is now close to 1. The available evidence suggests that harvest index and interception efficiency have reached a ceiling and further increases in the yield potential are rather unlikely to be achieved; they appear to be close to their biological limits already. However, the best light conversion efficiencies (ε_c) observed in field experiments are far below their theoretical maxima, and therefore this trait is the prime target when aiming to increase crop yield by raising the photosynthetic performance [1-3].

Photosynthesis is an energy-transducing, metabolic process that involves coordinated activities at various levels of biological organisation, i.e. that of cells, organs, whole plants and plant communities.



Fig, 1: Multidimensional organization of photosynthesis



Photosynthesis - the cellular dimension

At first glance, photosynthesis may be described as a cellular trait that uses light energy for the conversion of inorganic carbon into carbohydrates. The underlying biophysical and biochemical processes, i.e., the conversion of light into chemical energy by the reaction centres in the thylakoid membranes of the chloroplasts, the fixation of CO₂ by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), and the reactions of the Calvin-Benson cycle have been well conserved during evolution.

Rubisco evolved more than two billion years ago, when CO₂ concentrations were high and only tiny amounts of oxygen were present. Adaptation to the current atmospheric conditions revealed Rubisco's Achilles' heel, i.e., its affinity to both, oxygen and CO₂. Its enzymatic bi-specificity results in the energetically wasteful process of photorespiration, and its poor catalytic turn-over rate mandates high levels of this protein in leaves. From the economic point of view, Rubisco thus constitutes a major investment of resources for plants [4, 5].

Rubisco thus seemingly constitutes the prime target for optimisation of photosynthesis [6]. The enzyme consists of two different subunits, large (LS) and small (SS), which in all green alga and land plants are encoded by the plastid (LS) and nuclear (SS) genomes, respectively [4, 5]. All engineering efforts, therefore, strictly require the capability of gene transfer or replacement technologies for both genetic compartments. While those technologies are well established for the nuclear genomes of all major crops, they are lacking for the respective plastid genomes[7]. Moreover, the enzyme requires several auxiliary factors for its biogenesis or metabolic repair [5]. Recent results on engineering of Rubisco in model plants indicate that this, in principle, is possible. However, none of these approaches so far resulted in an improvement of the catalytic features of this enzyme [4, 5].

The limitations of Rubisco can be overcome by enriching CO_2 at its location. Thereby the CO_2/O_2 ratio increases which enhances Rubisco's carboxylation rates while repressing its oxygenase activity and consequently photorespiration. Three different biophysical or biochemical approaches have evolved in photosynthetic pro- and eukaryotes to concentrate CO_2 at the site of Rubisco. Cyanobacteria use a series of bicarbonate transporters and carbonic anhydrases to concentrate CO_2 in a specific proteinaceous micro-compartment, the carboxysome, where Rubisco is sequestered together with carbonic anhydrase [8]. Alga rely on pyrenoids, non-membrane-bound structures within the chloroplasts. Densely packed Rubisco in the centre of the pyrenoids is surrounded by a starch sheath and traversed by thylakoid-derived membrane tubules into which bicarbonate is concentrated and then released to Rubisco [8]. Land plants, i.e. angiosperms, have evolved polyphyletically a biochemical CO_2 -concentrating mechanism which requires a distinct leaf anatomy (Kranz anatomy) consisting of two different cell types, mesophyll and bundle-sheath cells. These cells form a joint photosynthetic system designed to pump CO_2 via C_4 acids into the bundle sheath cells where Rubisco is located [9].

Computational modelling and experimental evidence predicts that establishing a CO_2 concentrating mechanism in C_3 crops could theoretically enhance photosynthetic efficiency up to 60 % [3]. Hence, several attempts are underway to implement these three carbon-concentrating mechanisms in model plants or crops [10, 8]. These approaches require oligo-to-multi-gene engineering of target crops or model species for proof of concept. At the present project stages it is not possible to assess whether the pursued strategies will led to increases in photosynthetic efficiencies [10, 8].



The photorespiratory pathway functions as a repair system for detoxifying 2-phosphoglycolate (2-PG) resulting from the oxygenase activity of Rubisco. The pathway involves the metabolic interactions of chloroplasts with the cytosol, peroxisomes and mitochondria. During the process of 2-PG recycling one out of four 2-PG carbons is released as CO₂ by the glycine decarboxylase (GDC) reaction in the mitochondria [11, 12]. Introducing synthetic photorespiratory bypasses to capture the photorespired CO₂ within the chloroplast increased rates of photosynthesis and biomass production in Arabidopsis [13], *Camelina sativa* [14], tobacco [15], potato[16], and rice [17]. Recently developed new-to-nature enzyme activities, such as glycolyl-CoA carboxylase [18], hold potential for turning the CO₂-releasing photorespiratory pathway into an accessory CO₂-fixing system, such as the tartronyl-CoA pathway (see also Trudeau et al. [19] for alternative pathway designs). Surprisingly, also the overexpression of the H and/or L subunits of GDC improved photorespiration somehow controls photosynthesis. The overexpression of these subunits also resulted in enhanced plant growth [20, 21]. Taken together these data provide proof-of-concept that modifying the photorespiratory pathway may enhance photosynthesis and yield [11, 12].

Transgenic manipulations of the carbon assimilatory pathway, i.e. raising Rubisco levels in maize [22] and rice [23] or sedoheptulose-1,7-bisphosphatase and/or fructose-1,6-bisphosphatase amounts in tobacco [24, 25] and wheat [26], also enhanced photosynthesis and biomass yield.

Attempts to modify light energy transduction or light acclimation aiming to improve photosynthetic efficiency were also met with success. Overexpressing components of the photoprotection machinery (PsbS, violaxanthin de-epoxidase, and zeaxanthin epoxidase) accelerated the recovery from photoprotection, and the photosynthetic output of tobacco and rice under field conditions was improved which was associated with increased total biomass [27-29]. Increasing the levels of the cytochrome b_6f complex by overexpression of its Rieske subunit in various species or a ferredoxin-like protein also enhanced photosynthesis [30-32].

Taking together, all these successful examples in improving energy conversion at the thylakoid membrane or the CO_2 assimilatory reactions pinpoint imperfections or bottlenecks in these photosynthetic processes that may be improved by genetic engineering and potentially result in higher yields.

Photosynthesis - the leaf perspective

At second glance, photosynthesis may be viewed as a property of an organ, i.e. the leaf. Leaves of angiosperms are composed of various specialized cell types and tissues, and have been optimised during evolution to ensure that the carbon dioxide from the atmosphere as well as the solar radiation reach the chloroplasts of the green parenchyma cells in the leaf's interior, but that evaporation (of water) is minimized [33]. The inner anatomy of leaves, therefore, plays a decisive role in determining the photosynthetic output of this organ [34, 35]. This involves the organization of the mesophyll tissue into palisade and spongy parenchyma, amount and sizes of mesophyll cells, their intercellular air spaces, the thickness and properties of their cell walls and the intracellular location of the chloroplasts. All these factors strongly affect mesophyll conductance, a key parameter of leaf photosynthesis [36, 37]. Its genetic architecture is largely unknown [38, 39]. In addition, the vascular architecture, particularly vein density, is influencing leaf photosynthesis [40-42]. Finally, the numbers, distributions and functional characteristics of stomates play decisive roles



in photosynthetic gas exchange [43]. Not surprisingly, such leaf characteristics were targets of evolutionary change when the modern leaves of the angiosperms with their superior photosynthetic capacity were shaped [40, 44] and in the oligocene C_4 photosynthesis with its distinct Kranz anatomy and the intimate metabolic coupling with the mesophyll cells evolved polyphyletically within the angiosperms [45, 46].

Dicotyledonous plants display tremendous diversity in the forms and sizes of their leaves [47, 48]. While developmental biologists have uncovered many of the regulatory mechanisms involved in the development of leaf shape and size, the functional significance, i.e. the impact of leaf traits on photosynthetic output are largely unknown. Studies with South African Pelargonium species differing greatly in leaf forms revealed strong links between leaf shape and photosynthetic rates as well as temperature optima [49]. Work on the molecular basis of leaf shape in the Brassicacean model species *Arabidopsis thaliana* and *Cardamine hirsuta* supports this inference [50] and provides, in addition, a mechanistic framework of its genetic architecture [51]. Leaf size, shape and angle is integral to final canopy architectural properties, vertical leaf area distribution and the subsequent distribution of light in time and space, highlighting the importance of leaf shape to scaling photosynthesis.

Photosynthesis - the plant system

Thirdly, photosynthesis is part of a system, namely the whole plant consisting of source and sink organs, as well as the structures connecting these organs. From the system's perspective the production of goods and their utilization have to be coupled. In this respect, mature leaves function as source organs providing photoassimilates, i.e. sucrose, to sink organs for being used in anabolic metabolism or energy conversion and to build structural biomass. It is well documented that leaf photosynthesis can be controlled by the sink demand at particular growth stages. For example, before anthesis wheat growth is often considered to be source driven but after this the photosynthetic capacity becomes less important. However both are needed for driving yield and so it can be considered that the production and utilisation of photoassimilates are balanced [52]. When attempting to enhance photosynthesis for improving yield this interaction of source capacity and sink strength has to be taken into account [53, 54].

Photosynthesis - the field

Fourthly, from the farmer's point of view yield and its dependence on photosynthesis are the properties of the crop communities of the field and not that of individual crop plants. All attempts of enhancing photosynthesis must, therefore, be effective at the canopy level [55]. This sets up a key challenge for undertaking improvements of photosynthesis at the leaf level: It is noted that plant architecture and the leaf/cell/biochemical properties interact in complex ways. For example, the light dynamics, CO₂, temperature of leaves are established by architecture and thus are the dominant environmental constraints on photosynthesis in the field. In this respect, the erectness of leaves plays a critical role [56]. There is evidence that rice as well as maize plants with more erect leaves produce more biomass and corn yield under conditions of dense planting than plants with less erect leaves [57, 58].



Future challenges in the field to be addressed with high priority

The core components of photosynthesis, i.e. the light-driven electron transport chain, Rubisco, and the Calvin-Benson cycle, consist of various multi-partite modules whose components have coevolved more than 2 billions years. Rational engineering of these modules by site-directed mutagenesis or adding heterologous components will hence require a detailed mechanistic understanding of their function and regulation.

In vascular plants, atmospheric carbon dioxide enters the leaf through stomates, while water is lost through evapotranspiration. While the evaporation of water through stomates into the air may constrain plant performance, it is also the driving force for the transport of nutrients from the soil towards the leaves and hence unavoidable. Sufficiently high internal CO₂ concentrations are essential for carbon fixation, and therefore photosynthesis is intimately linked to the plant's water status. However, if CO₂ access or CO₂ assimilation efficiency is enhanced this has the opportunity to both increase carbon gain and enhance leaf integral water use efficiency.

There is an urgent need to integrate cell-based photosynthesis research with field and canopy level, in this sense the research into 'translational ' photosynthesis is key [59] and recognising the key growth stages of each crop where photosynthesis is most limiting.

The natural variation in cellular photosynthesis in crop species is probably substantial. Genetic techniques to identify source of variation such as quantitative trait (QTL) mapping of genome-wide associations studies (GWAS) will enable the harnessing of natural variation for crop improvement. Such work requires a marriage of cell, leaf and field photosynthesis science which is already ongoing.

How to design the future research agenda on "Optimising photosynthesis"

The optimisation of photosynthesis has to be translated into increases in the yield potential of crops. Increases in photosynthetic efficiency may also lead to a reduction in photosynthetic resource use efficiencies with respect to water and nitrogen. Crops, not model plants, should therefore be in the centre of a future research programme on "Optimising photosynthesis".

Improvements in crop photosynthesis must materialise under the growing conditions at which those crops are cultivated, i.e. either in fields with their dynamic changes in irradiation levels, temperature and water supply or in highly protected greenhouse environments. Because of these drastic differences between the two crop production systems the target areas for the optimisation of photosynthesis and hence the strategies pursued will differ, but also the growth conditions for phenotyping.

Genetic engineering approaches ("breeding-by-synthetic design") have proven to be successful in demonstrating that photosynthesis can be improved and that this could lead to increases in yield potential. However, because of the current situation in Europe, commercial plant breeders will, most likely, hesitate to embark on these approaches, at least in the near future. Exploiting the natural or artificially induced genetic diversities within or between nearly related species should therefore be pursued as a complementary strategy in a future research programme.

However, genetic engineering approaches, both gene transfer and gene editing, will play an important role as a tool for fast and reliable verification of the identified beneficial alleles/genes. Such a strategy offers two advantages: (1) the identified loci containing the beneficial alleles/genes can be directly transferred into elite germplasm by marker-assisted introgression and backcrossing;



(2) a directed gene-transfer is still a possible alternative and could be pursued, if conditions allow it.

The research agenda - a balance between conventional and high gain/high risk approaches for improving photosynthesis

The status quo report on research activities in the improvement of photosynthesis reveals that proof-of-concept studies pinpoint research strategies, which will likely lead to improved photosynthesis coupled with increases in yield potential. Work on optimising the light acclimation machinery or on bypassing or redesigning photorespiration has already left the model plant stage and "real crops" are in the focus of the research. Similarly, the genetic work on rice has shown that exploiting the allelic diversity among the various varietal groups can result in increases in photosynthesis that can be translated into yield improvement and variety development. The work in these areas should be continued, since *"we need winners in the race to improve photosynthesis"* [60]. On the other side, there are areas such as Rubisco engineering or the introduction of carbon-concentrating mechanisms into C₃ crops where solid proofs of principle are still lacking, but nevertheless the gains might be high, if at least some of these approaches would be successful. The research programme, therefore, requires a fine balance between conventional approaches that have passed the proof-of-concept stage and high-risk, potentially disruptive approaches that may result in ever higher gains.

Action points for a future research programme

A future research programme for the optimisation of photosynthesis and yield should/could rely on two different, but complimentary approaches:

- Leveraging naturally occurring or induced genetic variation
- Rational re-design of photosynthetic modules and application of new-to-nature concepts



Fig, 2: Two methodological approaches towards optimising photosynthesis



Optimising photosynthesis starts at the leaf and plant level, respectively. However, if those alterations are to be translated into yield gains, they must effective in the field at the canopy level. Moreover, some of the interventions may only materialise at that level:



Fig, 3: Optimising photosynthesis - the targets

References

- 1. Zelitch I (1982). The close relationship between net photosynthesis and crop yield. *BioScience* 32: 796-802.
- 2. Murchie EH, Pinto M & Horton P (2009). Agriculture and the new challenges for photosynthesis research. *New Phytol* 181: 532-552.
- 3. Long SP, Marshall-Colon A & Zhu XG (2015). Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. *Cell* 161: 56-66.
- 4. Parry MAJ, Andralojc PJ, Scales JC, Salvucci ME, Carmo-Silva AE, Alonso H & Whitney SM (2013). Rubisco activity and regulation as targets for crop improvement. *J Exp Bot* 64: 717-730.
- 5. Bracher A, Whitney SM, Hartl FU & Hayer-Hartl M (2017). Biogenesis and Metabolic Maintenance of Rubisco. *Annu Rev Plant Biol* 68: 29-60.
- 6. Orr DJ & Parry MAJ (2020). Overcoming the limitations of Rubisco: fantasy or realistic prospect? *J Plant Physiol* 254: 153285.
- 7. Bock R (2015). Engineering plastid genomes: methods, tools, and applications in basic research and biotechnology. *Annu Rev Plant Biol* 66: 211-241.
- 8. Hennacy JH & Jonikas MC (2020). Prospects for engineering biophysical CO2 concentrating mechanisms into land plants to enhance yields. *Annu Rev Plant Biol* 71: 461-485.
- 9. Schluter U & Weber APM (2020). Regulation and evolution of C4 photosynthesis. *Annu Rev Plant Biol* 71: 183-215.
- 10. Weber APM & Bar-Even A (2019). Update: Improving the Efficiency of Photosynthetic Carbon Reactions. *Plant Physiol* 179: 803-812.
- 11. Eisenhut M, Roell MS & Weber APM (2019). Mechanistic understanding of photorespiration paves the way to a new green revolution. *New Phytol* 223: 1762-1769.
- 12. Fernie AR & Bauwe H (2020). Wasteful, essential, evolutionary stepping stone? The multiple personalities of the photorespiratory pathway. *Plant J* 102: 666-677.



- Kebeish R, Niessen M, Thiruveedhi K, Bari R, Hirsch HJ, Rosenkranz R, Stabler N, Schonfeld B, Kreuzaler F & Peterhansel C (2007). Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in Arabidopsis thaliana. *Nat Biotechnol* 25: 593-599.
- 14. Dalal J, Lopez H, Vasani NB, Hu Z, Swift JE, Yalamanchili R, Dvora M, Lin X, Xie D, Qu R & Sederoff HW (2015). A photorespiratory bypass increases plant growth and seed yield in biofuel crop Camelina sativa. *Biotechnol Biofuels* 8: 175.
- 15. South PF, Cavanagh AP, Liu HW & Ort DR (2019). Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. *Science* 363: eaat9077.
- 16. Nolke G, Houdelet M, Kreuzaler F, Peterhansel C & Schillberg S (2014). The expression of a recombinant glycolate dehydrogenase polyprotein in potato (Solanum tuberosum) plastids strongly enhances photosynthesis and tuber yield. *Plant Biotechnol J* 12: 734-742.
- 17. Shen BR, Wang LM, Lin XL, Yao Z, Xu HW, Zhu CH, Teng HY, Cui LL, Liu EE, Zhang JJ, He ZH & Peng XX (2019). Engineering a new chloroplastic photorespiratory bypass to increase photosynthetic efficiency and productivity in rice. *Mol Plant* 12: 199-214.
- Scheffen M, Marchal DG, Beneyton T, Schuller SK, Klose M, Diehl C, Lehmann J, Pfister P, MCarrillo M, He H, Aslan S, Cortina NS, Claus P, Bollschweiler D, Baret JC, Schuller JM, Zarzycki J, Bar-Even A & Erb TJ (2021). A new-tonature carboxylation module to improve natural and synthetic CO2 fixation. *Nature Catalysis*.
- 19. Trudeau DL, Edlich-Muth C, Zarzycki J, Scheffen M, Goldsmith M, Khersonsky O, Avizemer Z, Fleishman SJ, Cotton CAR, Erb TJ, Tawfik DS & Bar-Even A (2018). Design and in vitro realization of carbon-conserving photorespiration. *Proc Natl Acad Sci U S A* 115: E11455-E11464.
- 20. Timm S, Florian A, Arrivault S, Stitt M, Fernie AR & Bauwe H (2012). Glycine decarboxylase controls photosynthesis and plant growth. *FEBS Lett* 586: 3692-3697.
- 21. Lopez-Calcagno PE, Fisk S, Brown KL, Bull SE, South PF & Raines CA (2019). Overexpressing the H-protein of the glycine cleavage system increases biomass yield in glasshouse and field-grown transgenic tobacco plants. *Plant Biotechnol J* 17: 141-151.
- 22. Salesse-Smith CE, Sharwood RE, Busch FA, Kromdijk J, Bardal V & Stern DB (2018). Overexpression of Rubisco subunits with RAF1 increases Rubisco content in maize. *Nat Plants* 4: 802-810.
- 23. Yoon D-K, Ishiyama K, Suganami M, Tazoe Y, Watanabe M, Imaruoka S, Ogura M, Ishida H, Suzuki Y, Obara M, Mae T & Makino A (2020). Transgenic rice overproducing Rubisco exhibits increased yields with improved nitrogen-use efficiency in an experimental paddy field. *Nature Food* 1: 134-139.
- 24. Lefebvre S, Lawson T, Zakhleniuk OV, Lloyd JC & Raines CA (2005). Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. *Plant Physiol* 138: 451-460.
- 25. Lopez-Calcagno PE, Brown KL, Simkin AJ, Fisk SJ, Vialet-Chabrand S, Lawson T & Raines CA (2020). Stimulating photosynthetic processes increases productivity and water-use efficiency in the field. *Nat Plants* 6: 1054-1063.
- 26. Driever SM, Simkin AJ, Alotaibi S, Fisk SJ, Madgwick PJ, Sparks CA, Jones HD, Lawson T, Parry MAJ & Raines CA (2017). Increased SBPase activity improves photosynthesis and grain yield in wheat grown in greenhouse conditions. *Philos Trans R Soc Lond B Biol Sci* 372: 20160384.
- 27. Kromdijk J, Głowacka K, Leonelli L, Gabilly ST, Iwai M, Niyogi KK & Long SP (2016). Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science* 354: 857-861.
- Glowacka K, Kromdijk J, Kucera K, Xie J, Cavanagh AP, Leonelli L, Leakey ADB, Ort DR, Niyogi KK & Long SP (2018). Photosystem II Subunit S overexpression increases the efficiency of water use in a field-grown crop. *Nat Commun* 9: 868.
- 29. Hubbart S, Smillie IRA, Heatley M, Swarup R, Foo CC, Zhao L & Murchie EH (2018). Enhanced thylakoid photoprotection can increase yield and canopy radiation use efficiency in rice. *Commun Biol* 1: 22.
- 30. Chang H, Huang HE, Cheng CF, Ho MH & Ger MJ (2017). Constitutive expression of a plant ferredoxin-like protein (pflp) enhances capacity of photosynthetic carbon assimilation in rice (Oryza sativa). *Transgenic Res* 26: 279-289.
- 31. Simkin AJ, McAusland L, Lawson T & Raines CA (2017). Overexpression of the RieskeFeS Protein Increases Electron Transport Rates and Biomass Yield. *Plant Physiol* 175: 134-145.
- 32. Ermakova M, Lopez-Calcagno PE, Raines CA, Furbank RT & von Caemmerer S (2019). Overexpression of the Rieske FeS protein of the Cytochrome b 6 f complex increases C4 photosynthesis in Setaria viridis. *Commun Biol* 2: 314.
- 33. Gago J, Carriquí M, Nadal M, Clemente-Moreno MJ, Coopman RE, Fernie AR & Flexas J (2019). Photosynthesis optimized across land plant phylogeny. *Trends Plant Sci* 24: 947-958.



- Lehmeier C, Pajor R, Lundgren MR, Mathers A, Sloan J, Bauch M, Mitchell A, Bellasio C, Green A, Bouyer D, Schnittger A, Sturrock C, Osborne CP, Rolfe S, Mooney S & Fleming AJ (2017). Cell density and airspace patterning in the leaf can be manipulated to increase leaf photosynthetic capacity. *Plant J* 92: 981-994.
- 35. Lundgren MR & Fleming AJ (2020). Cellular perspectives for improving mesophyll conductance. *Plant J* 101: 845-857.
- 36. Berghuijs HN, Yin X, Ho QT, Driever SM, Retta MA, Nicolaï BM & Struik PC (2016). Mesophyll conductance and reaction-diffusion models for CO2 transport in C3 leaves; needs, opportunities and challenges. *Plant Sci* 252: 62-75.
- 37. Ren T, Weraduwage SM & Sharkey TD (2019). Prospects for enhancing leaf photosynthetic capacity by manipulating mesophyll cell morphology. *J Exp Bot* 70: 1153-1165.
- 38. Takai T, Adachi S, Taguchi-Shiobara F, Sanoh-Arai Y, Iwasawa N, Yoshinaga S, Hirose S, Taniguchi Y, Yamanouchi U, Wu J, Matsumoto T, Sugimoto K, Kondo K, Ikka T, Ando T, Kono I, Ito S, Shomura A, Ookawa T, Hirasawa T, Yano M, Kondo M & Yamamoto T (2013). A natural variant of NAL1, selected in high-yield rice breeding programs, pleiotropically increases photosynthesis rate. *Sci Rep* 3: 2149.
- 39. Adachi S, Yamamoto T, Nakae T, Yamashita M, Uchida M, Karimata R, Ichihara N, Soda K, Ochiai T, Ao R, Otsuka C, Nakano R, Takai T, Ikka T, Kondo K, Ueda T, Ookawa T & Hirasawa T (2019). Genetic architecture of leaf photosynthesis in rice revealed by different types of reciprocal mapping populations. *J Exp Bot* 70: 5131-5144.
- 40. Brodribb TJ & Feild TS (2010). Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification. *Ecol Lett* 13: 175-183.
- 41. Feldman AB, Murchie EH, Leung H, Baraoidan M, Coe R, Yu SM, Lo SF & Quick WP (2014). Increasing leaf vein density by mutagenesis: laying the foundations for C4 rice. *Plos One* 9: e94947.
- 42. Rockwell FE & Holbrook NM (2017). Leaf hydraulic architecture and stomatal conductance: a functional perspective. *Plant Physiol* 174: 1996-2007.
- 43. Harrison EL, Arce Cubas L, Gray JE & Hepworth C (2020). The influence of stomatal morphology and distribution on photosynthetic gas exchange. *Plant J* 101: 768-779.
- 44. Feild TS, Brodribb TJ, Iglesias A, Chatelet DS, Baresch A, Upchurch GRJ, Gomez B, Mohr BA, Coiffard C, Kvacek J & Jaramillo C (2011). Fossil evidence for Cretaceous escalation in angiosperm leaf vein evolution. *Proc Nat Acad Sci USA* 108: 8363-8366.
- 45. Sage RF, Sage TL & Kocacinar F (2012). Photorespiration and the evolution of C4 photosynthesis. *Annu Rev Plant Biol* 63: 19-47.
- 46. Sedelnikova OV, Hughes TE & Langdale JA (2018). Understanding the genetic basis of C4 Kranz anatomy with a view to engineering C3 crops. *Annu Rev Genet* 52: 249-270.
- 47. Chitwood DH & Sinha NR (2016). Evolutionary and environmental forces sculpting leaf development. *Curr Biol* 26: R297–R306.
- 48. Nikolov LA, Runions A, Das Gupta M & Tsiantis M (2019). Leaf development and evolution. *Curr Top Dev Biol* 131: 109-139.
- 49. Nicotra AB, Cosgrove MJ, Cowling A, Schlichting CD & Jones CS (2008). Leaf shape linked to photosynthetic rates and temperature optima in South African Pelargonium species. *Oecologia* 154: 625-635.
- 50. Vuolo F, Mentink RA, Hajheidari M, Bailey CD, Filatov DA & Tsiantis M (2016). Coupled enhancer and coding sequence evolution of a homeobox gene shaped leaf diversity. *Genes Dev* 30: 2370-2375.
- 51. Kierzkowski D, Runions A, Vuolo F, Strauss S, Lymbouridou R, Routier-Kierzkowska AL, Wilson-Sanchez D, Jenke H, Galinha C, Mosca G, Zhang Z, Canales C, Dello Ioio R, Huijser P, Smith RS & Tsiantis M (2019). A growth-based framework for leaf shape development and diversity. *Cell* 177: 1405-1418.
- 52. Chang TG & Zhu XG (2017). Source–sink interaction: a century old concept under the light of modern molecular systems biology. *J Exp Bot* 68: 4417–4431.
- 53. Dingkuhn M, Luquet D, Fabre D, Muller B, Yin X & Paul MJ (2020). The case for improving crop carbon sink strength or plasticity for a CO2-rich future. *Curr Opin Plant Biol* 56: 259–272.
- 54. Paul MJ, Watson A & Griffiths CA (2020). Linking fundamental science to crop improvement through understanding source and sink traits and their integration for yield enhancement. *J Exp Bot* 71: 2270-2280.
- 55. Ort DR, Merchant SS, Alric J, Barkan A, Blankenship RE, Bock R, Croce R, Hanson MR, Hibberd JM, Long SP, Moore TA, Moroney J, Niyogi KK, Parry MA, Peralta-Yahya PP, Prince RC, Redding KE, Spalding MH, van Wijk KJ, Vermaas WF, von Caemmerer S, Weber AP, Yeates TO, Yuan JS & Zhu XG (2015). Redesigning photosynthesis to sustainably meet global food and bioenergy demand. *Proc Natl Acad Sci U S A* 112: 8529-8536.



- 56. Richards RA, Cavanagh CR & Riffkin P (2019). Selection for erect canopy architecture can increase yield and biomass of spring wheat. *Field Crops Res* 244: 107649.
- 57. Sakamoto T, Morinaka Y, Ohnishi T, Sunohara H, Fujioka S, Ueguchi-Tanaka M, Mizutani M, Sakata K, Takatsuto S, Yoshida S, Tanaka H, Kitano H & Matsuoka M (2006). Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. *Nat Biotechnol* 24: 105-109.
- 58. Tian J, Wang C, Xia J, Wu L, Xu G, Wu W, Li D, Qin W, Han X, Chen Q, Jin W & Tian F (2019). Teosinte ligule allele narrows plant architecture and enhances high-density maize yields. *Science* 365: 658–664.
- Poorter H, Fiorani F, Pieruschka R, Wojciechowski T, van der Putten WH, Kleyer M, Schurr U & Postma J (2016). Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. *New Phytol* 212: 838-855.
- 60. Long SP (2014). We need winners in the race to increase photosynthesis in rice, whether from conventional breeding, biotechnology or both. *Plant Cell Environ* 37: 19-21.



Focus Group Y-2: Improving source-to-sink relationships

Catherine Bellini¹, Evelyne Costes², Bert De Rybel³, **Sylvie Dinant⁴**, John Lunn⁵, Salomé Prat Monguio⁶, Jenny Russinova⁷, Mark Stitt⁸, Wolfgang Zierer⁹

1: <u>catherine.bellini@inrae.fr</u> (INRAE Versailles, FR & UPSC, Umea, SE); 2: <u>evelyne.costes@inrae.fr</u> (INRAE Montpellier, FR); 3: <u>beryb@psb.vib-ugent.be</u> (VIB Ghent, BE); 4: <u>sylvie.dinant@inrae.fr</u> (INRAE Versailles, FR); 5: <u>lunn@mpimp-golm.mpg.de</u> (MaxPlanck, Golm, GER); 6: <u>sprat@cnb.csic.es</u> (CNB, Madrid, Spain); 7: <u>Eugenia.Russinova@psb.vib-ugent.be</u> (VIB GENT, BE); 8: <u>MStitt@mpimpgolm.mpg.de</u> (MaxPlanck, Golm, GER); 9: <u>wolfgang.zierer@fau.de</u> (University Erlangen-Nuremberg, GER).

General background

For plants to grow effectively, the relationship between resource uptake and resource utilization needs to be finely tuned. In order to sustain their growth, plants take up a range of resources from the environment, including water, carbon dioxide and mineral nutrients. Plant organs responsible for taking up a particular resource are the so-called "source organs", whilst organs using the resource for growth, metabolism or storage are the "sink organs". The main resources translocated between source and sinks are photoassimilates (1-3), in particular sugars and amino acids, whose production depends on the efficiency of carbon fixation from photosynthetic organs and nitrogen uptake from the roots. Source and sink organs must be in balance in order for plants to function effectively in their environment. During a plant's life cycle, the same organ can shift from sink to source and vice versa. For example, young leaves start as carbon sinks and later on will export carbon to other sinks, and become sources until senescence. The same organ can also be simultaneously a source of a particular nutrient, but a sink for another nutrient. For example, fully developed leaves are at the same time sources of carbon and sinks for inorganic nitrogen, which is imported from the roots. Nutrients are transported between the different organs of the plant by the vascular system. The xylem that transports water and nutrients from the roots to the shoots, and the phloem that mostly transports nutrients from the leaves to the remaining parts of the plant. This source to sink transport is tightly regulated and the balance between carbon- and nitrogencontaining metabolites is an important indicator of source-sink status.

Because of the increasing population and rapid changes of the environment, agriculture is facing a massive increase in demand. In recent decades accumulating evidence suggests that the tight regulation between source and sink factors, which is a key to plant adaptation to a changing environment, may limit plant growth and yield. Several strategies have been implemented for a better understanding of the genetic, molecular and metabolic mechanisms that control the source sink relationships, in order to improve yield. Although some of these strategies have proven relatively successful to improve yield, the estimations based on the best yield improvements still do not meet the projected needs by 2050. Moreover, many yield improvements reported in greenhouse trials are unstable under the uncontrolled conditions encountered in the open field. Improving yield by manipulating source-sink interactions is still a challenge which leaves an open field for the development of new strategies of research.



Status quo of research in the field

The notion of source-sink relationships is an old agronomic concept, based on the observations that limiting the number of sinks improves the quality and yield of the remaining sinks. This results in the notion of sink and source strengths, taking place in photosynthetic or heterotrophic organs (4). These two traits result from combined multiscale processes, including the balance between growth of organs, their metabolism and their capacity to export, import or store photoassimilates, and varying along development with source-sink transitions (4, 5). Both traits are difficult to assess separately, due to the key role played by the vascular tissues in transporting photoassimilates between organs, including regulation of subprocesses like their loading, unloading, and lateral transfer to the adjacent tissues. In recent years some of the major breakthroughs in our understanding of source-sink relationships were the identification of key factors acting in the phloem on transport, metabolism and signaling pathways acting either at the cell-to-cell or long-distance level. While some of the underlying processes can be modeled mathematically (6, 7), fine-tuned regulations involved in these mechanisms are associated with feedback on photosynthesis and with competition between sinks and they require complex systemic signaling pathways, of which we still have only a partial vision.

For plant breeding, the storage of photoassimilates in sink organs and source organs are measurable traits, which can be used to estimate source and sink strengths. The first attempts to improve source-sink relationships were achieved by deregulation of factors involved in central metabolism and transport of photoassimilates, with the aim of increasing source strength, sink strength, or both (8, 9). Several approaches have been undertaken, including: 1) Manipulation of source C-metabolism, sink C-metabolism, or both source and sink C-metabolism, 2) Modification of N assimilation, partitioning and remobilization in the phloem, 3) Optimization of phloem sugar loading, unloading and utilization, 4) Optimizing storage organ initiation, 5) Manipulation of factors involved in C and N signaling and cross-talk (5, 8–10). For example, a 'Push and Pull approach' was recently applied using the overexpression of the SUT1 sucrose transporter in pea to enhance carbon allocation and improve seed yield (11). Mathematical modelling of source–sink interactions has also been developed to provide a predictive framework for these approaches (12–15).

At the same time, source-sink relationships are also influenced by developmental switches and plant architecture. For example, vernalization can drastically alter source-sink relationships in sugar beet (16). Branching, which interacts with canopy structure and light absorption, intensity of flowering, extent of flower or seed abortion, are additional factors that can be altered under carbon limitation, revealing intricate source-sink relationships (4, 17, 18). To conclude, long-term use for plant breeders of strategies to modify the expression of key factors remains difficult because of the lack of comprehensive understanding of the complexity of source-sink relationships and their multigenic determinism.

Genetic modifications using multi-gene targeting strategies and recent breakthroughs in C and N signaling and allocation (1–3, 19–22) have widened the possibilities to modify the whole-plant resource allocation for crop yield improvement. For example, new engineering strategies to reduce respiratory carbon loss have been proposed recently to boost crop productivity (23). Processes involved in long distance signalling, sugar signalling and regulation of loading and unloading can also be targeted to enhance carbon allocation and improve yield. Several breakthroughs were recently published, such as manipulation of sensing and signaling mechanisms based on T6P/SNRK1 signaling



in maize (24), manipulation of unloading mechanisms from apoplasmic to symplasmic pathways by targeting protein partners of a SWEET sugar transporter in potato (25) and manipulation of the initiation of new sinks using a interactor of the tuberigen complex in potato (26).

These data illustrate the urgent need for more integrated approaches to improve yield, based on a more comprehensive understanding of interactions between plant development, C and N transport, cross-talk between C and N metabolisms, central metabolism, cellular respiration and photosynthesis. Recent studies of inter-species diversity in photosynthetic metabolism (27, 28) imply that a "one-size fits all" engineering approach to improve photosynthesis may not work for all species of interest, indicating that advances in model species might not be readily transferable to crop species, i.e. we also need fundamental research in target crop species. Importantly, several important factors have been identified or confirmed by quantitative genetics approaches for agronomic traits related to source-sink relationships (29–32), hence the importance of identifying relevant traits to be selected.

Future challenges in the field to be addressed with high priority

Future challenges in the field have been recently discussed in the literature (8): What controls the rate of phloem loading? Are there signals from the sink that regulate loading? What determines the developmental switch between apoplasmic and symplasmic unloading pathways? How is the export of sucrose coordinated with that of amino acids and ions? What else apart from the C supply sets the rate of sink growth? What determines the number of sinks? What determines the differences between vegetative and reproductive growth sinks? What mechanisms and signals coordinate source and sink activity, and how do these respond to the environment? To what extent do non-harvestable sinks use photoassimilates and how to control respiratory C losses and rhizodeposition C losses from the roots?? What is the relationship between short-term signalling, and longer-term signals that may be initiated due to events at one time but have a longer lasting impact on sink-source interactions later in the life of the plants? What are the long-term effects, over consecutive years, of sink-source relationships on the perennial crops and on the stability of their performance?

In addition, it has become clear that many forms of regulation take place at a cellular scale. For example, unloading of sugars and amino acids requires a combination of SWEET sugar or UMAMIT amino acid transporter families acting in different cell types in seeds and in roots (33–35). To make progress on these questions, we need to better understand what is happening at the cell and subcellular levels in the highly specialized cells of the phloem (36). This should provide a more integrative view of spatio-temporal events acting in loading, unloading and transport of sugars and amino acids and on the underlying local and systemic signalling pathways.

At the same time, sink-source relations are also regulated by long distance systemic signals, an early example was the role of *miR156/PHO2* in regulating phosphate movement from the roots to the shoot (37). Recent advances uncovered the role of SWEET transporters in FT signalling (25). It is becoming increasingly realistic to build up a systematic picture of what metabolites, hormones, proteins and RNA species (38, 39) are moving in the plant, what regulates their formation and movement and how and where they act. This will open up radically new strategies to modify regulatory interactions between source and sink organs.

A third important issue for improving resource allocation is the better understanding of the mechanisms underlying the development of the vascular tissues for optimizing phloem and xylem



transport. Plants optimize C allocation by changes in leaf vein density, vascular tissue maturation in the stem, frequency of phloem transfer cells in loading and unloading tissues or frequency of plasmodesmata in the phloem of photosynthetic organs (40–42). These traits are based on developmental and adaptive mechanisms for which we are only beginning to identify factors.

Fourth, there are currently major initiatives to improve the rate and efficiency of photosynthesis. It is very likely that success in this direction will require parallel or closely following improvement in sink-source interactions. Indeed, plants with improved rates of photosynthesis (including existing lines grown in FACE systems) will provide a powerful system to understand sink-source interactions in a holistic context.

Last, abiotic stresses influence C and N resource allocation (43–45), also reflecting the plasticity of carbon metabolism in response to environmental conditions (2) and the coupling between nutritional and hormonal signaling (46). A large diversity exists in source and sink features among species (i.e. C₃, C₄, crops, vegetables or fruits) and their metabolism. Hence the importance of a better understanding of storage organ development and metabolism when it comes to processes beyond starch synthesis or the synthesis of other storage compounds (47). This raises new questions on the coordination of highly conserved mechanisms and adaptive responses and on the diversity of the regulation pathways in source-sink relationships. We need to better understand the mechanisms involved in adjusting source-sink relationships to the plant's environment to ensure the stability of yield gains.

These different subtopics cover broad aspects of plant development, physiology and nutrition that encompass very different research fields and require coordinated research from different research communities, working on plant development, plant physiology and nutrition (Figure 1). It requires important national and international research efforts.

Action points for a future research program in the field

Several action points exist to keep defining the best ways to increase C allocation and C fixation for increasing sink yield while minimizing sink feedback or non-productive accumulation of photosynthate, keeping in mind the potential consequences of modifying sink yield on quality, yield stability and resource use efficiency, in particular nitrogen use efficiency.

Via selective breeding, identification of innovative traits relevant to source-sink relationships

Sink and source characteristics should be used, alone or in combination, for the selection of elite genotypes with enhanced source-sink relationships in addition to yield related traits (48–50). For example in wheat, stem fructan reserves are a major contributor to wheat grain filling, especially under stress conditions that limit current photosynthesis, and there is considerable variation in this trait (51). In winter wheat, source and sink characteristics, such as high grain numbers per spike on the sink side, and high flag-leaf longevity on the source side, in addition to high photosynthetic activity at anthesis and green canopy duration, have been associated to breeding progress (48). Other traits, such as source-sink ratios (52, 53) or crop cycle duration (54) could also be considered for breeding and for association studies exploiting the genetic diversity across crops, vegetables or fruits.



Via genetic modification, associated with dialogue for public acceptance of gene editing technologies

Research to enhance source-sink relationships will provide innovative approaches for yield improvement using genetic engineering and gene editing with single or multigene-targeting strategies. Even if the European authorization process for the cultivation of transgenic plants and the use of gene editing in plant biotechnology is the most restrictive in the world, these strategies can constitute long-term alternatives for sustainable agriculture. Promoting new researches on biotechnology also requires maintaining a dialogue with the general public. Further, for the future debate it will be important to develop transgenic and gene-edited plants that can be deployed to improve yield whilst decreasing chemical applications, and improved sink-source relations would be good examples for this.

Validation of the relevant strategies for plant breeding

Because most research is still carried out in controlled conditions in model species and model genotypes, we now need to validate the results of improved source-sink relations in field conditions using multisite field experimentations, in contrasting field environments (soils and climate), and to test large genotype panels representing genetic diversity. This will include the screening of natural diversity and new breeding populations and the validation of edited material performance that will depends on European and National regulation for field trials of GMOs.

Concerted European approach to progress efficiently on the different subtopics

Because the topic covers broad aspects of plant development, physiology and nutrition, we recommend to propose actions to organize the European community for focusing research on a few model crops to advance our knowledge on the basic mechanisms. Based on past experience, research consortia focused on a specific species help to promote cross-disciplinary research, which is essential to engineer improvements in complex processes and traits, such as sink-source interactions. As noted above, "one-size-fits-all" approaches will probably not work for many species, due to metabolic diversity between species. Therefore, projects focused on one species, or at most a few closely related species, may be preferable to large consortia involving fragmented research on many diverse species. At the same time, it is important to propose actions to support translational research for the transfer of fundamental knowledge to crop plants.

Source-sink relationships also rely on highly adaptive processes leading to complex physiological regulations. In Europe we benefit from large germplasm collections (international, national and commercial), many of which are under-exploited. Advances in omics technologies (especially next generation sequencing) have opened up powerful new ways to explore and exploit these resources to improve source-sink relations.





Fig. 1. Overview of components involved in the source-sink relationships.

References

- 1. Braun DM, Wang L, Ruan Y-L (2014) Understanding and manipulating sucrose phloem loading, unloading, metabolism, and signalling to enhance crop yield and food security. *J Exp Bot* 65(7):1713–1735.
- 2. Griffiths CA, Paul MJ, Foyer CH (2016) Metabolite transport and associated sugar signalling systems underpinning source/sink interactions. *Biochim Biophys Acta* 1857(10):1715–1725.
- 3. Tegeder M, Masclaux-Daubresse C (2018) Source and sink mechanisms of nitrogen transport and use. *New Phytol* 217(1):35–53.
- 4. Patrick JW, Colyvas K (2014) Crop yield components-photoassimilate supply-or utilisation limited-organ development? *Funct Plant Biol* 41(9):893–913.
- 5. White AC, Rogers A, Rees M, Osborne CP (2016) How can we make plants grow faster? A source-sink perspective on growth rate. *J Exp Bot* 67(1):31–45.
- 6. Zakhartsev M, et al. (2016) Metabolic model of central carbon and energy metabolisms of growing Arabidopsis thaliana in relation to sucrose translocation. *BMC Plant Biol* 16(1). doi:10.1186/s12870-016-0868-3.
- 7. Grafahrend-Belau E, et al. (2013) Multiscale metabolic modeling: Dynamic flux balance analysis on a whole-plant scale. *Plant Physiol* 163(2):637–647.
- 8. Fernie AR, et al. (2020) Synchronization of developmental, molecular and metabolic aspects of source–sink interactions. *Nat Plants* 6(2):55–66.
- 9. Sonnewald U, Fernie AR (2018) Next-generation strategies for understanding and influencing source–sink relations in crop plants. *Curr Opin Plant Biol* 43:63–70.
- 10. Yadav UP, Ayre BG, Bush DR (2015) Transgenic approaches to altering carbon and nitrogen partitioning in whole plants: Assessing the potential to improve crop yields and nutritional quality. *Front Plant Sci* 6(APR):1–13.
- 11. Lu MZ, Snyder R, Grant J, Tegeder M (2020) Manipulation of sucrose phloem and embryo loading affects pea leaf metabolism, carbon and nitrogen partitioning to sinks as well as seed storage pools. *Plant J* 101(1):217–236.
- 12. Egli DB (2010) SOYPOD: A model of fruit set in soybean. Agron J 102(1):39–47.
- Larue F, et al. (2019) Modelling tiller growth and mortality as a sink-driven process using Ecomeristem: Implications for biomass sorghum ideotyping. *Ann Bot* 124(4):675–690.
- 14. Belhassine F, et al. (2019) Impact of Within-Tree Organ Distances on Floral Induction and Fruit Growth in Apple Tree: Implication of Carbohydrate and Gibberellin Organ Contents. *Front Plant Sci* 10(October):1–13.
- 15. Reyes F, et al. (2020) MuSCA: A multi-scale source-sink carbon allocation model to explore carbon allocation in plants. An application to static apple tree structures. *Ann Bot* 126(4):571–585.
- 16. Rodrigues CM, et al. (2020) Vernalization alters sink and source identities and reverses phloem translocation from taproots to shoots in sugar beet. *Plant Cell* 32(10):3206–3223.



- 17. Lauxmann MA, et al. (2016) Reproductive failure in Arabidopsis thaliana under transient carbohydrate limitation: Flowers and very young siliques are jettisoned and the meristem is maintained to allow successful resumption of reproductive growth. *Plant Cell Environ* 39(4):745–767.
- 18. Ruan YL, Patrick JW, Bouzayen M, Osorio S, Fernie AR (2012) Molecular regulation of seed and fruit set. *Trends Plant Sci* 17(11):656–665.
- 19. Paul MJ, Gonzalez-Uriarte A, Griffiths CA, Hassani-Pak K (2018) The Role of Trehalose 6-Phosphate in Crop Yield and Resilience. *Plant Physiol* 177(1):12–23.
- 20. Figueroa CM, Lunn JE (2016) A tale of two sugars: Trehalose 6-phosphate and sucrose. Plant Physiol 172(1):7–27.
- 21. Tegeder M, Hammes UZ (2018) The way out and in: phloem loading and unloading of amino acids. *Curr Opin Plant Biol* 43:16–21.
- 22. Fichtner F, Lunn JE (2021) The Role of Trehalose 6-Phosphate (Tre6P) in Plant Metabolism and Development. *Annu Rev Plant Biol* 72(1):1–24.
- 23. Amthor JS, et al. (2019) Engineering strategies to boost crop productivity by cutting respiratory carbon loss. *Plant Cell* 31(2):297–314.
- 24. Oszvald M, et al. (2018) Trehalose 6-phosphate regulates photosynthesis and assimilate partitioning in reproductive tissue. *Plant Physiol* 176(4):2623–2638.
- 25. Abelenda JA, et al. (2019) Source-Sink Regulation Is Mediated by Interaction of an FT Homolog with a SWEET Protein in Potato. *Curr Biol* 29(7):1178-1186.e6.
- 26. Zhang X, et al. (2020) TERMINAL FLOWER-1/CENTRORADIALIS inhibits tuberisation via protein interaction with the tuberigen activation complex. *Plant J* 103(6):2263–2278.
- 27. Borghi GL, et al. (2019) Relationship between irradiance and levels of Calvin-Benson cycle and other intermediates in the model eudicot Arabidopsis and the model monocot rice. *J Exp Bot* 70(20):5809–5825.
- 28. Arrivault S, et al. (2019) Metabolite profiles reveal interspecific variation in operation of the Calvin-Benson cycle in both C 4 and C 3 plants. *J Exp Bot* 70(6):1843–1858.
- 29. Liu E, et al. (2019) Favorable alleles of grain-filling rate1 increase the grain-filling rate and yield of rice. *Plant Physiol* 181(3):1207–1222.
- 30. Bezrutczyk M, et al. (2018) Impaired phloem loading in zmsweet13a,b,c sucrose transporter triple knock-out mutants in Zea mays. *New Phytol* 218(2):594–603.
- 31. Sekhon RS, et al. (2019) Integrated genome-scale analysis identifies novel genes and networks underlying senescence in maize. *Plant Cell* 31(9):1968–1989.
- 32. Pallas B, et al. (2018) Growth and carbon balance are differently regulated by tree and shoot fruiting contexts: An integrative study on apple genotypes with contrasted bearing patterns. *Tree Physiol* 38(9):1395–1408.
- 33. Besnard J, et al. (2016) UMAMIT14 is an amino acid exporter involved in phloem unloading in Arabidopsis roots. *J Exp Bot* 67(22):6385–6397.
- 34. Sosso D, et al. (2015) Seed filling in domesticated maize and rice depends on SWEET-mediated hexose transport. *Nat Genet* 47(12):1489–1493.
- 35. Chen LQ, et al. (2015) A cascade of sequentially expressed sucrose transporters in the seed coat and endosperm provides nutrition for the arabidopsis embryo. *Plant Cell* 27(3):607–619.
- 36. Kim J-Y, et al. (2021) Distinct identities of leaf phloem cells revealed by single cell transcriptomics. *Plant Cell*. doi:10.1093/plcell/koaa060.
- 37. Bari R, Pant BD, Stitt M (2006) PHO2, MicroRNA399, and PHR1 Define a Phosphate-Signaling Pathway in Plants. *Plant Physiol* 141(July):988–999.
- 38. Thieme CJ, et al. (2015) Endogenous Arabidopsis messenger RNAs transported to distant tissues. *Nat Plants* 1(April):1–8.
- 39. Yang L, et al. (2019) m5C Methylation Guides Systemic Transport of Messenger RNA over Graft Junctions in Plants. *Curr Biol* 29(15):2465-2476.e5.
- 40. Adams W, Cohu C, Amiard V, Demmig-Adams B (2014) Associations between the acclimation of phloem-cell wall ingrowths in minor veins and maximal photosynthesis rate. *Front Plant Sci* 5(24). doi:10.3389/fpls.2014.00024.
- 41. Amiard V, et al. (2005) Anatomical and photosynthetic acclimation to the light environment in species with differing mechanisms of phloem loading. *Proc Natl Acad Sci U S A* 102(36):12968–12973.
- 42. Amiard V, et al. (2007) Role of light and jasmonic acid signaling in regulating foliar phloem cell wall ingrowth development. *New Phytol* 173(4):722–731.



- 43. Lemoine R, et al. (2013) Source-to-sink transport of sugar and regulation by environmental factors. *Front Plant Sci* 4(July):1–21.
- 44. Keller I, Rodrigues CM, Neuhaus HE, Pommerrenig B (2021) Improved resource allocation and stabilization of yield under abiotic stress. *J Plant Physiol* 257(November 2020). doi:10.1016/j.jplph.2020.153336.
- 45. Pommerrenig B, et al. (2018) In concert: Orchestrated changes in carbohydrate homeostasis are critical for plant abiotic stress tolerance. *Plant Cell Physiol* 59(7):1290–1299.
- 46. Fichtner F, Dissanayake IM, Lacombe B, Barbier F (2020) Sugar and Nitrate Sensing: A Multi-Billion-Year Story. *Trends Plant Sci* S1360-1385(Dec 3):30350–2.
- 47. Sonnewald U, et al. (2020) The Cassava Source–Sink project: opportunities and challenges for crop improvement by metabolic engineering. *Plant J* 103(5):1655–1665.
- 48. Lichthardt C, Chen TW, Stahl A, Stützel H (2020) Co-Evolution of Sink and Source in the Recent Breeding History of Winter Wheat in Germany. *Front Plant Sci* 10(February):1–15.
- 49. Fabre D, et al. (2020) Genotypic variation in source and sink traits affects the response of photosynthesis and growth to elevated atmospheric CO2. *Plant Cell Environ* 43(3):579–593.
- 50. Wang Y, et al. (2020) Identify QTLs and candidate genes underlying source-, sink-, and grain yield-related traits in rice by integrated analysis of bi-parental and natural populations. *PLoS One* 15(8 August):1–21.
- 51. Esmaeilpour-Jahromi M, et al. (2012) Variation in grain weight among Iranian wheat cultivars: The importance of stem carbohydrate reserves in determining final grain weight under source limited conditions. *Aust J Crop Sci* 6(11):1508–1515.
- 52. Glanz-Idan N, Tarkowski P, Turečková V, Wolf S (2020) Root-shoot communication in tomato plants: Cytokinin as a signal molecule modulating leaf photosynthetic activity. *J Exp Bot* 71(1):247–257.
- 53. Xu JL, et al. (2015) SS1 (NAL1)- and SS2-mediated genetic networks underlying source-sink and yield traits in rice (Oryza sativa L.). *PLoS One* 10(7):1–21.
- 54. Saez J V., Mariotti JA, Vega CRC (2019) Source-sink relationships during early crop development influence earliness of sugar accumulation in sugarcane. *J Exp Bot* 70(19):5157–5171.



Focus Group Y-3: Optimizing shoot architecture and canopy

Pilar Cubas¹, Fabio Fiorani², **Patrick Laufs**³, Leo Marcelis⁴, Hilde Nelissen⁵, Ülo Niinemets⁶, Catherine Rameau⁷, Jenny Russinova⁸, Ningyi Zhang⁹

1: <u>pcubas@cnb.csic.es</u> (Plant Molecular Genetics Department, CSIC, Madrid, Spain); 2: <u>F.Fiorani@Fz-Juelich.De</u> (Forschungszentrum, Jülich, GER); 3: <u>patrick.laufs@inrae.fr</u> (INRAE, Versailles, FR); 4: <u>leo.marcelis@wur.nl</u> (Wageningen University, NL): 5: <u>Hilde.Nelissen@psb.vib-uqent.be</u> (VIB, Ghent, BE); 6: <u>ylo.niinemets@emu.ee</u> (University of Life Sciences, Tartu, Estonia); 7: <u>catherine.rameau@inrae.fr</u> (INRAE, Versailles, FR); 8: <u>Euqenia.Russinova@psb.vib-uqent.be</u> (VIB, Ghent, BE); 9: <u>ninqyi.zhanq@wur.nl</u> (Wageningen University, NL).

General background

From a global, energetic point of view, yield can be defined by Monteith equation **Yield = St** • $\varepsilon_i \bullet \varepsilon_c$ • ε_p . in which plant yield is determined by **St**, the solar irradiation energy reaching the plant, ε_i , the efficiency of the plant to intercept the total light, ε_c , the efficiency of the conversion of the intercepted light into biomass and finally, ε_p , the part of the biomass that is harvested. Shoot architecture and canopy structure together with total canopy leaf area are major determinants of yield as they contribute to the three parameters of Monteith equation.

Alternatively, from a functional and biological point of view, photosynthesis and yield are very integrated and multi-scale processes that go from the level of the molecule (for instance the photosystems and RUBISCO), through the organelles (the chloroplasts), and cell and tissue levels (for instance the mesophyll), to reach the organ (the leaf, but also other organs e.g. stem) and individual plant levels and finally to the plant community in the field. In this focus group we have discussed the levels ranging from the tissue to the canopy. We have excluded the more physiological issue of the sink-source relationship that is discussed by the Y-2 focus group.

Status quo of research in the field

In a very broad sense, the issues of shoot architecture, canopy and yield have been addressed from different stand points by different scientific communities using different approaches.

A fine understanding of the basis of plant development

Through the study of model plants or sometimes crops, molecular geneticists have now gathered a good knowledge of the main molecular and genetic actors controlling the developmental processes leading to shoot architecture development. This is particularly true for the control of branching (Barbier et al., 2019), vasculature patterning and differentiation and leaf growth and shape control for which the interplay of cellular processes (cell proliferation, expansion and differentiation) has started to be elucidated (Conklin et al., 2019). Comparative studies between species that are either distantly related from an evolutionary point of view or that differ in their biological processes (e.g., leaf growth in dicots and monocots) suggest that the major regulators are often conserved but that their regulation or the fine-wiring of the regulatory networks may be species-specific (Nelissen et al., 2016). However, one of the problems highlighted was that much of the information is available for non-crop model species. This consideration stresses the need to gain further fundamental



knowledge in both model species and in crops. The gap between model species and crops is progressively narrowing with the wide-spread genomic resources and the deployment of gene editing approaches. The genetic transformation, in particular plant regeneration, can however still be a major limitation for some species or cultivars and hampers functional validation experiments.

Analysing the basis of domestication and pushing it further

Analysing natural variation and the effects of domestication has also allowed identifying biological processes and their specific subtending genetic factors that are instrumental for the diversification of plant architecture. These factors provide potential targets for new improvement of plant architecture and canopy to further push the progress of plant domestication (eg (Fujita et al., 2013)) that has been argued as being only partly achieved (Abbai et al., 2020).

Towards linking plant structure and photosynthesis

Studies that concentrate on the elucidation of the mechanisms of development, often do not consider how varying plant architecture affects photosynthesis and yield. However, some studies now use molecular genetics tools to precisely modify plant anatomy or architecture and analyse the effects on photosynthesis (Vuolo et al., 2016; Andres et al., 2017; Lehmeier et al., 2017). Until now the studies connecting architectural changes to photosynthetic efficiency remain scarce and do not yet fully exploit the knowledge of the photosynthetic processes to precisely link the developmental modifications and the possible changes in photosynthesis or growth.

Integrating modelling and phenotyping

More comprehensive approaches focusing at the whole plant or community level are developed by eco-physiologists, physiologists and geneticists. Thus, genetic loci and sometimes the underlying genes controlling important architectural parameters, such as leaf angle have been identified. Importantly, these QTLs can vary depending on growth conditions (e.g., isolated plant versus dense plots, greenhouse versus field, atmospheric and pedological conditions) or developmental stages (Mantilla-Perez et al., 2020). Eco-physiological optimization models predicting optimal plant architecture for maximization of photosynthesis, plant growth and yield under specific conditions have been developed (Vos et al., 2010; Sarlikioti et al., 2011). These models have become more and more complex, with an increasing spatial complexity being possible by increasing computing facilities and by more precise phenotyping (eg (Drewry et al., 2014)). In this regard, the capacity for precise phenotyping at appropriate spatial and temporal scales appears now as central for both the evaluation of effects of any manipulation of plant architecture and for the calibration and validation of predictive models. One of the challenges to apply such approaches is consideration of appropriate time-scales for environmental variation and agricultural practices (dense vs. sparse stands with differing degrees of self-shading, agroforestry practices etc.). These types of approaches fully integrate photosynthesis and yield (or at least growth and biomass production) as they are parameters either measured or predicted by models. A drawback of some of machine learning or artificial intelligence approaches is that although they can provide very efficient and accurate predictions, their contribution to the understanding of the underlying mechanisms may be more limited. This limitation could be overcome by iterative cycles combined with molecular tweaking of the system.



Identifying candidate targets to improve photosynthesis and yield

Together, these approaches have identified key factors that affect photosynthesis and yield.

Amongst these, a particular focus at the cellular and tissue levels was for the cell number, size and arrangement, cell wall thickness, stomata distribution, cuticle and epidermal hairs, while at the organ level the focus was on the size and structure of the leaf as well as its shape and leaf margin dissection. These different parameters affect photosynthesis through different aspects as they determine the ratio of the photosynthetic versus support tissue of the leaf, how light is intercepted and diffuses in the leaf, how gas and thermal exchanges occur within the leaf and with the environment through the leaf boundary layer and how CO2, water, nutrient, and assimilates are transported within the leaf (Niinemets et al., 2007; Nicotra et al., 2011; Tholen et al., 2012) At the plant and canopy level, an important outcome of many studies is that a plant is not just the sum of x leaves as the field is not just the sum of y plants (Niinemets, 2007; Zhu et al., 2012). Studies on the control of leaf angle are very relevant with improving light interception and distribution at the canopy level (Falster and Westoby, 2003; Mantilla-Perez and Salas Fernandez, 2017) and are part of the smart canopy concept (Ort et al., 2015). To improve photosynthesis, the entire system has to be considered including external factors such as the varying environment, as well as agricultural management practices (Mathan et al., 2016).

Future challenges in the field to be addressed with high priority

Bridging the scientific communities to provide a global view

As photosynthesis and yield are very interconnected processes, more integrated, multi-scale research bridging the different scientific communities needs to be developed. The community/plant/organ level view of eco-physiologists and physiologists needs to go more down to the involved molecular mechanisms to provide better understanding of the changes at play. On the other hand, studies performed by molecular geneticists have to extend to a physiological level and more generally to a higher-scale phenotypic characterisation, possibly under environmental conditions closer to the ones encountered in the field. Such interdisciplinary approaches are challenging for several reasons including the different scientific culture, approaches and vocabulary of the different communities and sometimes also by the different model species. Modelling approaches supported by precise phenotyping could provide a mid-point where the two types of communities may meet.

Beside the development of such interdisciplinary approaches, specific challenges need to be overcome in several different fields to provide the tools to undertake an integrated research agenda:

Going beyond the studies of model species in controlled conditions

Standing on the current broad understanding of the action of key regulators of plant development and architecture, the next challenge will be to determine how these principles are acting in the species of interest. In addition, while the action of these regulatory genes has often been studied in highly controlled laboratory or greenhouse conditions, a major future challenge will be to determine how they are challenged by varying field conditions and how they may contribute to plant phenotypic plasticity and adaptation under different crop production scenarios.



Preserving, characterizing and exploiting or creating genetic diversity

Next, having identified the key actors by which plant architecture can be manipulated, it is essential to have access to genetic resources to be able to introduce target alleles into the species/cultivar of interest. In this respect, it will be essential to maintain and characterise the natural genetic diversity that can be a source of potential alleles of interest. Alternatively, further reinforcing the genome editing tools (by both providing a more versatile tool box for diverse types of edits and by improving plant transformation and regeneration protocols). Here, the legislation and public acceptance level of genome edited plants will be a central issue that goes beyond the question we are specifically addressing here.

Developing accurate and relevant phenotyping

Reinforcing appropriate phenotyping tools will be essential. Defining appropriate phenotyping protocols depends on the question that will be addressed: a higher scale phenotyping level (community, plant, organ) may be appropriate for the characterisation of growth and yield, while a cellular and molecular level may be required to provide a mechanistic approach or to support some eco-physiological approaches. In the former case, devices such as stereo cameras systems that can be deployed both to field and controlled environment conditions will be of interest. In parallel, approaches aiming at phenotyping under precise environmental conditions, reproducing the biotic and abiotic stresses that can face the plant and the farming practices should be fostered.

Increasing the benefits of the modelling approaches

Our current modelling tools need to be extended to be able to simulate plant architectural development based on knowledge at the molecular level. This could help to identify key processes or genes that are interesting for breeding high-yield cultivars. Artificial intelligence (or machine learning) may help to simulate processes at gene-to-cell level. This brings another challenge, on how to combine artificial intelligence with the current crop models which normally simulate processes from leaf to canopy level.

Action points for a future research programme

All the points discussed above underline that the question of improving plant architecture and canopy structure to improve photosynthesis and yield is a multifaceted question, for which no simple and general answer can be given. This is true for a given species but is further complicated by the environmental conditions set by the place where it grows, by the agricultural practices that are set up and by usage of the plant. Phenotypic plasticity as also to be considered with its complex effects (Niinemets, 2020)

Therefore, case scenario defining the what (plant), the where (is it cultivated), the how (the practices) and the why (the usage) should be selected by all the stakeholder involved. Such scenarios could thus be the base of integrated approaches gathering the diverse scientific communities discussed above and focusing on a specific application. In this respect, the role of industrial partners and plant breeding/seed companies appears of particular relevance.

Beside this, specific questions should be addressed:



- Forster research aiming at characterizing the impact of leaf shape, size and anatomy on photosynthesis. For this fine modulation of specific cellular or morphological parameters should be followed by global phenotyping and characterization of their impact on photosynthesis. This characacterisation should go beyond just quantification of the photosynthesis to provide a mechanistic insight.
- Characterise plant architectural acclimation to light conditions and the feedback effects of architecture on light and other microclimate conditions. Light environment affects plant architectural development, which in turn affects plant light interception and distribution in the canopy, as well as other microclimate factors such as relative humidity and leaf temperature. Light interception and distribution directly affect canopy photosynthesis and thus crop yield. Microclimate conditions both directly affect photosynthesis and indirectly link to crop yield by affecting disease development. A better understanding of plant architectural acclimation to light environment and their feedback relationships could help to optimize crop management such as optimizing plant density and pruning strategy, and crop settings in intercropping systems.

References

- Abbai R, Singh VK, Snowdon RJ, Kumar A, Schnurbusch T (2020) Seeking Crops with Balanced Parts for the Ideal Whole. Trends Plant Sci 25: 1189–1193
- Andres RJ, Coneva V, Frank MH, Tuttle JR, Samayoa LF, Han S-W, Kaur B, Zhu L, Fang H, Bowman DT, et al (2017) Modifications to a *LATE MERISTEM IDENTITY1* gene are responsible for the major leaf shapes of Upland cotton (*Gossypium hirsutum* L). Proc Natl Acad Sci **114**: E57–E66
- **B**arbier FF, Dun EA, Kerr SC, Chabikwa TG, Beveridge CA (2019) An Update on the Signals Controlling Shoot Branching. Trends Plant Sci **24**: 220–236
- Conklin PA, Strable J, Li S, Scanlon MJ (2019) On the mechanisms of development in monocot and eudicot leaves. New Phytol **221**: 706–724
- **Dr**ewry DT, Kumar P, Long SP (2014) Simultaneous improvement in productivity, water use, and albedo through crop structural modification. Glob Chang Biol **20**: 1955–1967
- Falster DS, Westoby M (2003) Leaf size and angle vary widely across species: What consequences for light interception? New Phytol **158**: 509–525
- Fujita D, Trijatmiko KR, Tagle AG, Sapasap MV, Koide Y, Sasaki K, Tsakirpaloglou N, Gannaban RB, Nishimura T, Yanagihara S, et al (2013) NAL1 allele from a rice landrace greatly increases yield in modern indica cultivars. Proc Natl Acad Sci U S A 110: 20431–20436
- Lehmeier C, Pajor R, Lundgren MR, Mathers A, Sloan J, Bauch M, Mitchell A, Bellasio C, Green A, Bouyer D, et al (2017) Cell density and airspace patterning in the leaf can be manipulated to increase leaf photosynthetic capacity. Plant J **92**: 981–994
- Mantilla-Perez MB, Bao Y, Tang L, Schnable PS, Salas-Fernandez MG (2020) Toward "smart canopy" sorghum: Discovery of the genetic control of leaf angle across layers. Plant Physiol **184**: 1927–1940
- Mantilla-Perez MB, Salas Fernandez MG (2017) Differential manipulation of leaf angle throughout the canopy: Current status and prospects. J Exp Bot **68**: 5699–5717
- Mathan J, Bhattacharya J, Ranjan A (2016) Enhancing crop yield by optimizing plant developmental features. Dev **143**: 3283–3294
- Nelissen H, Gonzalez N, Inzé D (2016) Leaf growth in dicots and monocots: So different yet so alike. Curr Opin Plant Biol
 33: 72–76
- Nicotra AB, Leigh A, Boyce CK, Jones CS, Niklas KJ, Royer DL, Tsukaya H (2011) The evolution and functional significance of leaf shape in the angiosperms. Funct Plant Biol **38**: 535–552
- Niinemets Ü (2007) Photosynthesis and resource distribution through plant canopies. Plant, Cell Environ **30**: 1052–1071

Niinemets U, Portsmuth A, Tobias M (2007) Leaf shape and venation pattern alter the support investments within leaf

Niinemets Ü (2020) Leaf Trait Plasticity and Evolution in Different Plant Functional Types. Annu Plant Rev online. doi: 10.1002/9781119312994.apr0714



lamina in temperate species: a neglected source of leaf physiological differentiation? Funct Ecol 21: 28–40

- Ort DR, Merchant SS, Alric J, Barkan A, Blankenship RE, Bock R, Croce R, Hanson MR, Hibberd JM, Long SP, et al (2015) Redesigning photosynthesis to sustainably meet global food and bioenergy demand. Proc Natl Acad Sci U S A **112**: 8529–8536
- Sarlikioti V, De Visser PHB, Buck-Sorlin GH, Marcelis LFM (2011) How plant architecture affects light absorption and photosynthesis in tomato: Towards an ideotype for plant architecture using a functional structural plant model. Ann Bot **108**: 1065–1073
- Tholen D, Boom C, Zhu XG (2012) Opinion: Prospects for improving photosynthesis by altering leaf anatomy. Plant Sci **197**: 92–101
- Vos J, Evers JB, Buck-Sorlin GH, Andrieu B, Chelle M, De Visser PHB (2010) Functional-structural plant modelling: A new versatile tool in crop science. J Exp Bot **61**: 2101–2115
- Vuolo F, Mentink RA, Hajheidari M, Bailey CD, Filatov DA, Tsiantis M (2016) Coupled enhancer and coding sequence evolution of a homeobox gene shaped leaf diversity. Genes Dev **30**: 2370–2376
- Zhu XG, Song Q, Ort DR (2012) Elements of a dynamic systems model of canopy photosynthesis. Curr Opin Plant Biol **15**: 237–244



Focus Group Y-4: Optimizing root architecture

Tom Beeckman¹, Malcolm Bennett², Davide Bulgarelli³, Andrea Carminati⁴, Thierry Desnos⁵, Fabio Fiorani⁶, David Gouache⁷, Frank Hochholdinger⁸, Laurent Laplaze⁹, Bertrand Muller¹⁰, Christophe Salon¹¹, Silvio Salvi¹², Roberto Tuberosa¹²

1: tom.beeckman@psb.vib-ugent.be (VIB, Ghent, BE); 2: malcolm.bennett@nottingham.ac.uk (Nottingham University, UK); 3: d.bulgarelli@dundee.ac.uk (University of Dundee, UK); 4: andrea.carminati@usys.ethz.ch (ETH, Zürich, CH); 5: thierry.desnos@cea.fr (CEA, CNRS, Aix Marseille Univ, Saint-Paul lez Durance, FR); 6: f.fiorani@fz-juelich.de (JIC, Jülich, GER); 7: d.gouache@terresinovia.fr (Terres Inovia, Thiverval-Grignon, FR); 8: hochholdinger@uni-bonn.de (Bonn University, GER); 9: laurent.laplaze@ird.fr (IRD Montpellier, FR); 10. bertrand.muller@inrae.fr (INRAE, Montpellier, FR); 11: christophe.salon@inrae.fr (INRAE, Dijon, FR); 12: silvio.salvi@unibo.it (University of Bologna, IT); 12: roberto.tuberosa@unibo.it (University of Bologna, IT).

Status quo of research in the field

Root traits, also defined as root phenes (1) have long been recognized for their pivotal role in plant growth and crop productivity through their mechanical and functional roles (2-4). Collectively, root traits determine the overall architecture and physiology of the root system, hence defining its spatial configuration in the soil and describing its morphology and shape (5-7). Likewise, evidence recently emerged implicating root traits with the recruitment and maintenance of the rhizosphere microbiome, at the interface between roots and soil. Our current understanding of the genes and molecular mechanisms that shape root system architecture (RSA) were mainly acquired from studies conducted on mutants. However, variability in root traits of field-grown plants is controlled by a plethora of quantitative trait loci (QTLs) and their interactions with the environment and management practices (GxExM) that are starting to be revealed by quantitative genetics approaches. Reviews on the genetic control of root systems architecture (RSA) are available for the model plant *Arabidopsis thaliana* (hereafter Arabidopsis; 8) as well as for staple crops, including rice (9), barley (10), sorghum (11) and maize (12). Several functional-structural models of RSA have been developed over the last two decades allowing one to simulate how various root traits and processes influence water and nutrient uptake (listed in https://www.quantitative-plant.org/, reviewed in 13).

Ample genotypic variability for root traits and their plasticity under different environmental conditions has been documented in most crops (e.g., 14-26). Leveraging this genetic variability, to maximize crop productivity while enhancing water- and nutrient-use efficiency requires a deep understanding of the genetic make-up of RSA and root anatomy traits, their ontogeny and functions, as well as their interaction with soil (5, 7, 25, 27-35). Dwindling fresh water resources and climate change pose serious threats to crop production. Roots sense water gradients in soil and grow towards the source using hydrotropism (36). Genes for controlling root hydrotropism have been identified (37). Root hydrotropism is likely to facilitate plant adaptation to changing water availability, but this trait has not been directly selected for in crops to date (38).

Among RSA traits, root growth angle (RGA) is crucial for optimizing plant's uptake of inorganic phosphorus (Pi) which is mainly found in top soil layers and, unlike N fertilizers, characterized by low mobility. Notably, <20% of applied Pi fertilizers are recovered by crops, but the runoff in fresh water and oceans cause eutrophication and environmental damage (39). Among RSA phenes



targeted in QTL studies, RGA has been frequently investigated, mainly due to the possibility to measure it at an early stage in non-soil conditions and its high heritability (26, 40). *DRO1* and *PSTOL1* are two major RSA QTLs cloned in rice. *DRO1* enhances root gravitropism to increase root growth angle, yet maintains higher photosynthesis and yield performance under severe water-stressed conditions (41). Additionally, *DRO1* homolog improved rice yields in saline paddy fields (42). *PSTOL1* acts as an enhancer of early root growth and promotes this under both high- and low-phosphorus conditions (43). Both genes were identified in landrace germplasm rather than elite breeding lines. Among root anatomical traits, those reducing the metabolic cost of root (such as aerenchyma or root cortical senescence) were shown to confer better tolerance to drought stress and low-nutrient conditions through enhanced development of the root system (1, 44-48). Multiseriate cortical schlerenchyma is associated with better tolerance to soil compaction, a major problem for modern agricultural systems.

Most relevant research results

The residual synteny found between Arabidopsis and eudicot crops is invaluable for comparative studies. Notwithstanding the extensive rearrangements between eudicots and monocots that limit the use of Arabidopsis for synteny-based applications in cereals, genes cloned in Arabidopsis provide candidate genes for root-related QTLs in cereals. Mutant collections in model cereals (rice, barley, maize) and legumes will speed up the characterization of QTLs in related crops. Therefore, root mutants in Arabidopsis and the related database are a treasure trove to identify candidate genes at loci influencing RSA phenes in cereals in response to the availability of nutrients and water in soil (49). Among the different traits that have been investigated in RSA and root anatomy studies (e.g., root number, branching, density, allocation of mass to foraging roots, root cortical aerenchyma, root cortical senescence, aerenchyma, etc.), root hairs, root angle and root length have been most frequently considered for their importance in governing water and nutrients uptake from the rhizosphere.

Root hairs

Root hairs play a pivotal role for absorption of nutrients and water and are thought to improve plant performance under stressful conditions, although their importance might vary across species and soil types (50-53). Accordingly, improving a crop's root system will require a detailed understanding of the regulation of root hair ontogeny and length, as well as a mechanistic understanding of their interactions with the soil matrix (54). For example, increased root hair elongation in rice by low Pi levels has recently been shown to be controlled by auxin and a network of auxin synthesis, transport and response components (55).

Root growth angle

Major QTLs for RGA have been identified in wheat, overlapping with QTLs for yield which are dependent on water availability (15, 33) or the genetic background (56). In rice, Uga and co-workers identified and cloned the RGA locus *DEEPER ROOTING 1* (*DRO1*; 41, 57-59). The *DRO1* narrow-angle allele shows deeper roots and, consequently, higher yield in drought conditions. In sorghum grown under drought conditions, stay-green genotypes have contributed additional evidence for the positive role on yield of QTL alleles for narrow RGA (60). Additionally, RGA impacts acquisition of Pi, a low-mobility nutrient abundant in the upper soil layer as shown by work in bean (61, 62). In barley,


a number of mutant collections have been phenotyped for root phenes (5, 63, 64). A forwardgenetic analysis identified more than 30 root morphology mutants (63) including *enhanced gravitropism 2* (*egt2*) exhibiting steeper seminal and lateral RGA and an auxin-independent higher responsiveness to gravity (65). Notably, *EGT2* was shown to perform an evolutionary conserved role in RGA control between barley and wheat by knocking out *EGT2* orthologs in A and B genomes of tetraploid durum wheat. Therefore, *EGT2* is an evolutionary conserved regulator of RGA in barley and wheat that provides a valuable target for root-based crop improvement strategies in cereals.

Root length

In rice, interdisciplinary genomic studies allowed for the positional cloning of the Pi-starvation tolerance (Pstol1) locus, a major QTL affecting RSA and tolerance to Pi deficiency (43). Lines carrying Pstol1 showed altered root architecture and greater surface area with increased P uptake. Cloning of the P uptake1 (Pup1) locus allowed Neelam et al. (66) to identify novel PSTOL1 haplotypes among accessions of wild rice (Oryza rufipogon) which differed significantly in root length under P-deficient conditions. Additionally, overexpression of the rice root-specific gene RCc3 enhanced the root development, plant growth and salt stress tolerance (67). In chickpea, Varshney and co-workers introgressed QTLs for root depth and drought-tolerance traits into three elite chickpea cultivars (Pusa 362, Pusa 372, DCP 92-3). Backcrossed lines showed longer roots and a 16% yield gain than their respective recurrent parents (68). Soil compaction has a major impact on root length, reducing resource capture and crop yield, which is further enhanced when combined with drought. Compaction is intuitively thought to reduce root growth by physically limiting the ability of roots to penetrate hard soils. However, Pandey et al. (69) recently reported root growth in compacted soil is actively suppressed by the growth regulator ethylene. Moreover, rice roots insensitive to ethylene penetrated compacted soil more effectively than wild-type roots. Ethylene appears to act as an early warning signal for roots to avoid compacted soils, which would be relevant to breeding crops resilient to soil compaction.

Future challenges in the field to be addressed with high priority

High-throughput phenotyping of root traits in:

- Controlled conditions (e.g., aeroponics, semi-hydroponics, hydroponics, soil mesocosms, "novel" artificial substrates)
- Experimental fields with rain shelters and/or Free-Air Carbon dioxide Enrichment (FACE) facility
- Field conditions (e.g., canopy temperature, robot for brace root, soil cores, anatomy using LAT)
- Large rhizotrons (e.g., 'Deep Frontiers' project in Denmark)

Imaging

- Non-invasive imaging in 3/4D soil conditions (e.g., 'Hounsfield CT Facility' in Nottingham)
- Automated image analysis based on artificial intelligence (AI)
- Development of user-friendly image analysis software



Cloning of root mutants and major QTLs governing RSA and root anatomy plasticity in response to

- Flooding/excess water on early season root establishment and growth
- Root rots in a context of dwindling seed treatment solutions, herbivory damages
- Elevated atmospheric CO2
- Soil stresses (drought, Nitrogen, Pi, temperature, salinity, aluminum/pH)
- Soil ionome
- Soil compaction

Effects of management practices on RSA and root anatomy.

RSA traits will be beneficial or not, depending on the balance between their metabolic cost and their pay-back in a given environmental scenario (70, 71). There is thus a crucial need to improve crop models in such a way that the impact of root traits on plant performance in testable in a variety of soil and climate scenarios. Moreover, genotype-specific model parameters must be captured from phenotyping tools (72). An overarching aspect related to the adaptive response of RSA to abiotic stress is the plasticity of the different root phenes consequent to the cues and signaling in response to the intensity and dynamics of stressors (73-76), particularly when multiple stresses occur (77, 78).

Action points for a future research programme

What are the most relevant unsolved questions (scientific questions, societal and economic challenges)?

- We currently lack a system biology appreciation of the hormonal and edaphic cues underpinning RSA, in particular in soils constraining crop yield.
- Study of RSA and root anatomy under excess soil moisture (waterlogging) as the most understudied and impactful climate stress: excess early season moisture is often statistically linked to poor yields and can interact strongly with late season drought by limiting soil exploration early in season.
- Based on haplotype effects, model root traits and root/shoot ratio to optimize plant's performance, water use, WUE, nitrogen uptake, NUE, Pi uptake, PUE in varying soil types and management (e.g., conventional vs organic farming).
- Deciphering plant-to-plant signaling and resource competition at the root level.
- Closer and more effective engagement with seed companies. For example, developing breederfriendly KASP markers for selection of beneficial haplotypes.
- Evaluate the effects of new RSA and root anatomy ideotypes on yield, quality and GHG emission of crops cultivated under conventional and organic farming as well as for various uses (e.g.,food, feed, ecosystem services).
- Evaluate the capacity of crops with different RSA or root anatomy to sequester C within soil (e.g., organic matter), and soil modifications related to roots and exudates.



• Evaluate yield and environmental footprint of crops with different RSA or root anatomy using networks of instrumented fields and models.

What needs to be done to solve the scientific questions and to meet the societal and economic challenges?

Scientific questions

- A strong multidisciplinary approach among the diverse experts able to tackle the issues required to tailor RSA according to the target areas of cultivation, prevailing soil conditions and management practices (e.g., conventional *vs* organic).
- Although Arabidopsis is the key model species for dicots and rice for monocots, the availability of sequence data and other -omics database allow the use of crops as valuable models (e.g., barley for the Triticeae, durum wheat for bread wheat, sorghum for maize).
- Consider forage and grain legumes and their nodulating roots, key for sustainable agriculture and representing a complex model.

Societal and economic challenges

- Outreach activities and engagement to educate and inform the public about the biology of plant roots.
- Key role played by research on roots in agriculture.
- The unprecedented opportunities and advantages offered by biotechnology (e.g., MAS, NBT, editing) applied to modern breeding.

What needs to be done to support the translation of research results into societal and economic value?

- Food shortages associated with the on-going SARS-CoV-2 pandemic have been stark reminders to societies of the fragility of food supply. Akin to the public acceptance of vaccination for global health, public acceptance of translational applications of plant sciences will be key to sustainably ensure food security. Therefore, we identify in winning the trust of policy makers, stakeholders and consumers for modern breeding technologies, including genome editing.
- The economic challenge will be to devote sufficient resources to support transparent and freely accessible scientific investigations and their applications for a more sustainable agriculture able to provide consumers with affordable, nutritious and healthy food to contribute to the 17 sustainable developmental goals set by the UN.

References

- Lynch, J. P. (2011). Root Phenes for Enhanced Soil Exploration and Phosphorus Acquisition: Tools for Future Crops. *Plant Physiol* 156, 1041-1049.
- Wu, W., et al. (2018). Enhancing Rapeseed Tolerance to Heat and Drought Stresses in a Changing Climate: Perspectives for Stress Adaptation from Root System Architecture. *Adv Agron Vol* 151, 87-157.
- Muller, B., Martre, P. (2019). Plant and crop simulation models: powerful tools to link physiology, genetics, and phenomics. *J Exp Bot* 70, 2339-2344.
- Uga, Y. (2021). Challenges to design-oriented breeding of root system architecture adapted to climate change. *Breed Sci*, *71*, 3-12.
- Gregory, P. J., et al. (2009). Root phenomics of crops: opportunities and challenges. Funct Plant Biol 36, 922-929.



Morris, E. C., et al. (2017). Shaping 3D root system architecture. Curr Biol 27, R919-R930.

- Tuberosa, R., et al. (2021). Understanding and exploiting the genetics of plant root traits. In Peter J. Gregory (ed.) Understanding and improving crop root function, Burleigh Dodds Science Publishing, Cambridge, UK. Pp. 395-466.
- Taniguchi, M., et al. (2017). The Arabidopsis *LAZY1* family plays a key role in gravity signaling within statocytes and in branch angle control of roots and shoots. *Plant Cell* 29, 1984-1999.
- Meng, F. N., et al. (2019). Molecular Mechanisms of Root Development in Rice. *Rice* 12, 10.
- Rossini L, et al. (2018) Genetics of Whole Plant Morphology and Architecture. In: N. Stein and G. J. Muehlbauer (eds.), The Barley Genome, Compendium of Plant Genomes, pp. 209-230. SPRINGER, Cham. https://doi.org/10.1007/978-3-319-92528-8
- Zheng, Z. H., et al. (2020). Shared Genetic Control of Root System Architecture between *Zea mays* and *Sorghum bicolor. Plant Physiol* 182, 977-991.
- Hochholdinger, F., et al. (2018). Genetic control of root system development in maize. *Trends Plant Sci* 23, 79-88.
- Postma, J. A., Black, K. (2020). *CH02-Advances in root architectural modeling during the last decade* (No. FZJ-2020-00041). Pflanzenwissenschaften.
- Tuberosa, R., et al. (2002). Mapping QTLs regulating morpho-physiological traits and yield: Case studies, shortcomings and perspectives in drought-stressed maize. *Ann Bot* 89, 941-963.
- Maccaferri, M., et al. (2016). Prioritizing quantitative trait loci for root system architecture in tetraploid wheat. *J Exp Bot* 67, 1161-1178.
- 16 Ryan, P. R., et al. (2016). Plant roots: understanding structure and function in an ocean of complexity. *Ann Bot* 118, 555-559.
- Salvi, S. (2017). An evo-devo perspective on root genetic variation in cereals. J Exp Bot 68, 350-353.

Schoppach, R., et al. (2020). Sleep tight and wake-up early: nocturnal transpiration traits to increase wheat drought tolerance in a Mediterranean environment. *Funct Plant Biol* 47(12), 1117-1127.

Varshney, R. K., et al. (2018a). Can genomics deliver climate-change ready crops? *Curr Opin Plant Biol* 45, 205-211. Varshney, R. K., et al. (2018b). Accelerating genetic gains in legumes for the development of prosperous smallholder

agriculture: integrating genomics, phenotyping, systems modelling and agronomy. J Exp Bot 69, 3293-3312.

Varshney, R. K., et al. (2018c). Progress in understanding drought tolerance: from alleles to cropping systems. *J Exp* Bot 69, 3175-3179.

Wu, W., et al. (2018). Enhancing rapeseed tolerance to heat and drought stresses in a changing climate: perspectives for stress adaptation from root system architecture. *Adv Agron 151*, 87-157.

- Fromm, H. (2019). Root Plasticity in the Pursuit of Water. *Plants* 8.
- Yu, P., et al. (2019). Plasticity of Lateral Root Branching in Maize. Frontiers in Plant Science 10.
- Schneider and Lynch (2020). Should root plasticity be a crop breeding target? *Frontiers in Plant Science* 11, 10.3389/fpls.2020.00546
- Alemu, A., et al. (2021). Genome-wide association analysis unveils novel QTLs for seminal root system architecture traits in Ethiopian durum wheat. *BMC Genomics* 22, 1-16.
- Del Bianco, M., Kepinski, S. (2018). Building a future with root architecture. *J Exp Bot* 69, 5319-5323.

Voss-Fels, K. P., et al. (2018b). Designer roots for future crops. *Trends Plant Sci* 23, 957-960.

Ye, H., et al. (2018a). Genetic diversity of root system architecture in response to drought stress in grain legumes. *J Exp Bot* 69, 3267-3277.

- Ye, H., et al. (2018b). A major natural genetic variation associated with root system architecture and plasticity improves waterlogging tolerance and yield in soybean. *Plant Cell Environ* 3
- Oyiga, B. C., et al. (2020). Genetic components of root architecture and anatomy adjustments to water-deficit stress in spring barley. *Plant Cell Environ 43*, 692-711.
- Fradgley, N., et al. (2020). Effects of breeding history and crop management on the root architecture of wheat. *Plant and Soil 452*(1), 587-600.
- Alahmad, S., et al. (2020). Adaptive Traits to Improve Durum Wheat Yield in Drought and Crown Rot Environments. *Int J Mol Sci* 21.
- Shahan, R., Benfey, P. N. (2020). A co-opted regulator of lateral root development controls nodule organogenesis in *Lotus. Dev Cell* 52, 6-7.
- Wachsman, G., Benfey, P. N. (2020). Lateral Root Initiation: The Emergence of New Primordia Following Cell Death. *Curr Biol* 30, R121-R122.



Kaur, V., et al. (2020). Cloning and characterization of a gene encoding MIZ1, a domain of unknown function protein and its role in salt and drought stress in rice. *Protoplasma* 257, 475-487.

Dietrich, D. (2018). Hydrotropism: how roots search for water. J Exp Bot 69, 2759-2771.

- Miyazawa, Y., Takahashi, H. (2020). Molecular mechanisms mediating root hydrotropism: what we have observed since the rediscovery of hydrotropism. *J Plant Res* 133, 3-14.
- Xia, Y., et al. (2020). Recent advances in control technologies for non-point source pollution with nitrogen and phosphorous from agricultural runoff: current practices and future prospects. *Appl Biol Chem* 63, 1-13.
- Canè, M. A., et al. (2014). Association mapping for root architectural traits in durum wheat seedlings as related to agronomic performance. *Molecular Breeding* 34, 1629-1645.
- Uga, Y., et al. (2013). Control of root system architecture by *DEEPER ROOTING 1* increases rice yield under drought conditions. *Nat Genet* 45, 1097.
- Kitomi, Y., et al. (2020). Root angle modifications by the *DRO1* homolog improve rice yields in saline paddy fields. *Proc Natl Acad Sci USA* 117, 21242-21250.
- Gamuyao, R., et al. (2012). The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. *Nature 488*(7412), 535-539.
- Chimungu, J. G., et al. (2015). Root anatomical phenes predict root penetration ability and biomechanical properties in maize (Zea mays). *J Exp Bot 66*, 3151-3162.
- Galindo-Castañeda, T., et al. (2018). Reduced root cortical burden improves growth and grain yield under low phosphorus availability in maize. *Plant Cell Environ* 41(7), 1579-1592.
- Jia, X. C., et al. (2018). Greater lateral root branching density in maize improves phosphorus acquisition from low phosphorus soil. *J Exp Bot* 69, 4961-4970
- Lynch, J. P. (2013). Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Ann Bot* 112, 347-357.
- Lynch, J. P. (2018). Rightsizing root phenotypes for drought resistance. *J Exp Bot* 69, 3279-3292.
- Ötvös, K., et al. (2021). Modulation of plant root growth by nitrogen source-defined regulation of polar auxin transport. *EMBO* 40, e106862.
- Carminati, A., et al. (2017). Root hairs enable high transpiration rates in drying soils. *New Phytol* 216, 771-781
- Zhang, C. Y., et al. (2018). Do longer root hairs improve phosphorus uptake? Testing the hypothesis with transgenic *Brachypodium distachyon* lines over-expressing endogenous RSL genes. *New Phytol* 217, 1654-1666.
- Ruiz, S., et al. (2020). Significance of root hairs at the field scale modelling root water and phosphorus uptake under different field conditions. *Plant and Soil* 447, 281-304.
- Burak, E., et al. (2021). Root hairs are the most important root trait for rhizosheath formation of barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), and *Lotus japonicus* (Gifu). *Ann Bot*.
- Marzec, M., et al. (2015). Root hair development in the grasses: what we already know and what we still need to know. *Plant Physiol* 168, 407-414.
- Giri, J., et al. (2018). Rice auxin influx carrier OsAUX1 facilitates root hair elongation in response to low external phosphate. *Nat Commun 9*(1), 1-7.
- Hohn, C. E., Bektas, H. (2020). Genetic mapping of quantitative trait loci (QTLs) associated with seminal root angle and number in three populations of bread wheat (*Triticum aestivum* L.) with common parents. *Plant Mol Biol Rep* 1-14.
- Kitomi, Y., et al. (2015). QTLs underlying natural variation of root growth angle among rice cultivars with the same functional allele of *DEEPER ROOTING 1*. *Rice* 8.
- Uga, Y., et al. (2015a). Genetic improvement for root growth angle to enhance crop production. *Breed Sci* 65, 111-119.
- Uga, Y., et al. (2015b). A QTL for root growth angle on rice chromosome 7 is involved in the genetic pathway of *DEEPER ROOTING 1. Rice 8*(1), 1-8.
- Borrell, A. K., et al. (2014). Drought adaptation of stay-green sorghum is associated with canopy development, leaf anatomy, root growth, and water uptake. *J Exp Bot* 65, 6251-6263.
- Miguel, M. A., et al. (2015). Phene Synergism between Root Hair Length and Basal Root Growth Angle for Phosphorus Acquisition. *Plant Physiol* 167, 1430-1439.
- Burridge, J. D., et al. (2019). A case study on the efficacy of root phenotypic selection for edaphic stress tolerance in low-input agriculture. *Field Crops Res* 244, 11.
- Bovina, R., et al. (2011). Identification of root morphology mutants in barley. *Plant Genetic Resources-Characterization and Utilization* 9, 357-360.



Szurman-Zubrzycka, M. E., et al. (2018). HorTILLUS-A Rich and Renewable Source of Induced Mutations for Forward/Reverse Genetics and Pre-breeding Programs in Barley (*Hordeum vulgare* L.). *Front Plant Sci* 9.

Kirschner, G. K., et al. (2021). *ENHANCED GRAVITROPISM 2* encodes a STERILE ALPHA MOTIVE containing protein that controls root growth angle in barley and wheat. *bioRxiv*.

- Neelam, K., et al. (2017). Novel Alleles of Phosphorus-Starvation Tolerance 1 Gene (*PSTOL1*) from *Oryza rufipogon* Confers High Phosphorus Uptake Efficiency. *Front Plant Sci* 8.
- Li, X. X., et al. (2018). Overexpression of RCc3 improves root system architecture and enhances salt tolerance in rice. *Plant Physiol Biochem* 130, 566-576.
- Bharadwaj, C., et al. (2021). Introgression of "QTL-hotspot" region enhances drought tolerance and grain yield in three elite chickpea cultivars. *Plant Genome* e20076.
- Pandey, B. K., et al. (2021). Plant roots sense soil compaction through restricted ethylene diffusion. *Science 371*, 276-280.
- Bruce, W. B., et al. (2002). Molecular and physiological approaches to maize improvement for drought tolerance. *J Exp Bot* 53(366), 13-25.
- Tardieu, F., Tuberosa, R. (2010). Dissection and modelling of abiotic stress tolerance in plants. *Curr Opin Plant Biol 13*, 206-212.
- Muller, B., Martre, P. (2019). Plant and crop simulation models: powerful tools to link physiology, genetics, and phenomics. *J Exp Bot* 70, 2339–2344.
- Motte, H., Beeckman, T. (2019). The evolution of root branching: increasing the level of plasticity. *J Exp Bot* 70, 785-793.
- Motte, H., et al. (2019). Molecular and Environmental Regulation of Root Development. Annu Rev Plant Biol 70, 465-488.
- Velinov, V., et al. (2020). Overexpression of the *NMig1*Gene Encoding a NudC Domain Protein Enhances Root Growth and Abiotic Stress Tolerance in Arabidopsis thaliana. *Front Plant Sci* 11.
- Voss-Fels, K. P., et al. (2018). *VERNALIZATION1* modulates root system architecture in wheat and barley. *Mol Plant* 11, 226-229.
- Vescio, R., et al. (2021). Single and Combined Abiotic Stress in Maize Root Morphology. *Plants 10*, 5.
- Rao, I. M., et al. (2020). Root Adaptations to Multiple Stress Factors. FrontPlant Sci 11.



Focus Group Y-5: Adapting life histories to changing environments

*Maria von Korff-Schmising*¹, Gustavo A. Slafer², Michael Palmgren³, Christian Jung⁴, Laura Rossini⁵

1: <u>Maria.Korff.Schmising@uni-duesseldorf.de</u> (Institute for Plant Genetics, Heinrich Heine University, Düsseldorf, GER), 2: <u>slafer@pvcf.udl.es</u> (Dept. of Crop and Forest Sciences, University of Lleida, Spain), 3: <u>palmgren@plen.ku.dk</u> (Department of Plant and Environmental Sciences, University of Copenhagen, Denmark), 4: <u>c.jung@plantbreeding.uni-kiel.de</u> (Institute of Plant Breeding, University of Kiel, GER), 5: <u>laura.rossini@unimi.it</u> (University of Milan, IT).

Status quo of research in the field

Novel crops and life history adaptation to changing environments

The modification of life history traits has been fundamental for adapting our crop plants to a wide range of different environments and human needs. Notable examples include the selection of widely different forms of domesticated cabbage from a single wild cabbage species or breeding for day length neutrality and rapid cycling in wheat during the "green revolution" (Borlaug 1983, Ruggles 1953).

The adaptability of our major crop species to a wide range of environments has led to a strong concentration on only a few major crop species grown worldwide for animal and human nutrition. Today, more than two-thirds of the global cropland is sown to monocultures of a few crop species, that are annual, i.e. only grow one season, namely rice, wheat, maize and barley. Under conditions of climate change yields are stagnating or decreasing while intensive farming is depleting natural resources. It is therefore important to develop new concepts for a more diversified and sustainable food production that is adapted to face climate change.

Wild crop relatives and locally adapted (proto-domesticated) crop species represent a valuable resource for increasing agrobiodiversity and adapting crop production to changing climates (Tanksley and McCouch 1997, Østerberg et al. 2017, Van Tassel et al. 2020). Locally adapted crop species, also called orphan crops, have received little attention by breeding and research programs in the past. However, today there is increasing interest in genetic improvement of orphan crops for (1) improving the nutritional value of crops, (2) increasing agrobiodiversity, and (3) improving agricultural sustainability under climate change (Jamnadass et al. 2020, Je et al. 2021). Similarly, wild crop relatives can be used for a) a de novo domestication of novel crops and b) for the transfer of novel traits into our major cultivated crops (Quezada-Martinez et al. 2021). This includes, for example, attempts to breed perennial cereal crops that grow over many seasons and therefore require less input, reduce soil erosion, and have a more efficient nutrient utilization and conservation compared to annual crops (Cox et al. 2006; Glover et al. 2010). Furthermore, diverse germplasm collections can be used to adapt the phenology of crop plants to environmental changes. This includes, for example, modifying bud dormancy and the timing of bud burst in fruit trees to prevent frost damages as winter warming is advancing the blooming date (Kozlowski and Pallardy 2002)

Taken together, exotic wild or locally adapted species represent valuable resources to a) improve performance of our major crop plants and b) diversify agricultural production systems and make



them more resistant to environmental perturbations. Life history and plant architecture traits are key to achieving this goal.

Trade-offs between life-history traits

Life history traits such as the timing of germination, transition from vegetative to reproductive development, flowering, seed set and senescence are typically linked by either positive correlations (allometries) or negative relationships, which are termed trade-offs (Stearns 1989). For example, differences in resource allocation between annual and perennial plants result in trade-offs between seed production and life span (Vico et al. 2016, Wang et al. 2018). Similarly, breeding for increased grain number per spike in cultivated crops typically reduces spike number or grain weight (Liller et al. 2015, Gambin and Borras 2010; Sadras 2007; Slafer et al. 2015). Such trait correlations may be imposed by resource limitations, i.e. when resource allocation to grains (reproduction) occur at the expense of allocation to vegetative growth (Obeso 2002). For example, the "green revolution": semidwarf genes increased the allocation to the growing spikes during stem elongation at the expense of reducing the allocation to internode length increasing reproductive output based on a trade-off between vegetative and reproductive growth.

Trait correlations can also evolve in response to natural selection and/or through pleiotropic gene actions. During the last decades the genetic and molecular control of developmental transitions has been elucidated in annual model and crop plants (Blümel et al 2015, Jung et al. 2017, Andres and Coupland 2012, Digel et al. 2014; Campoli et al. 2014). This information provides leads for candidate genes and networks that can be targeted and modified for improving development and shoot architecture in crops (Liu et al. 2021). These studies have also shown that single genes often affect different life cycle transitions, such as seed germination, transition to reproductive development, flowering time and seed set, suggesting that pleiotropic gene action may contribute to the covariation of different life-history traits.

Despite these insights into the genetic networks controlling life cycle transitions, knowledge in important crops is lagging behind and the mechanisms controlling resource allocation and underlying trade-offs between different life history traits are still not well resolved. Furthermore, we know relatively little about the conservation of regulatory networks controlling key life history traits across species and more specifically between crop species and their wild relatives.

Future challenges in the field to be addressed with high priority

The genetic improvement of exotic plant genetic resources offers tremendous potential for diversifying agricultural production systems thereby rendering them more resistant to climate change and land degradation. This requires the modification of life-history traits in order to adapt exotic germplasm to novel environments and agricultural production systems. A fundamental challenge for adapting life-histories to changing environments is to understand their genetic and physiological basis and trade-offs between life history traits to optimize growth and yield in a given environment. Furthermore, we need to understand variation in the genetic and physiological control of life-history traits and trade-offs between them beyond the model and major crop species to harness the potential of exotic germplasm for crop improvement.

Today, high-throughput genome profiling technologies provide unprecedented scope to identify, characterise and utilise genetic diversity in crops and their wild relatives (Chalhoub et al. 2014,



Mascher et al. 2017, Monat et al. 2019). Further, the development of genomic editing tools (CRISPR/Cas) opens up completely novel avenues for altering the genetic makeup of plants in deliberate and precise ways that were unthinkable just a few years ago. This ground-breaking technology uses RNA-guided nucleases that achieve site-specific DNA recognition and cleavage thereby altering, adding or removing DNA (Sander and Joung 2014, Schindle et al. 2018, Zsogon et al. 2018, Zhang et al. 2020). This technology can overcome the incompatibility barriers between species and modify a suite of genes necessary to engineer entirely new traits and trait combinations. Furthermore, the establishment of reference collections tested at different sites and environmental conditions is opening new opportunities to study life history traits under a range of environmental conditions at physiological, genetic and epigenetic level (Cirilli et al. 2020, Jung et al. 2020).

These recent technical advances will significantly contribute towards dissecting the tissue and stage specific effects and regulatory networks of developmental and plant architecture genes to understand the (co-)variation of life-history traits across species. This knowledge is crucial for the targeted (quantitative) modification of individual life history traits.

Action points for a future research program in the field

- Decipher regulatory networks underlying life history variation and plant architecture traits in crop and related wild species. This requires the development of genomic and genetic resources for wild crop relatives and orphan crops and of new methods for inter-species genetics. It also requires the development of robust transformation protocols for different crop and wild genotypes.
- Dissect the genetic, physiological and metabolic basis for life-history traits and resource allocation determining their covariation and quantifying trade-offs. For this purpose, fully sequenced core collections grown under different environmental conditions can be used to map and characterize genes controlling phenological development.
- Engineer/breed for novel life history traits and trait combinations. The outcome will be novel crop genotypes with improved resource efficiency and environmental adaptation, that will be able to provide for a sustainable agriculture in the face of climate change

References

- Blümel, M., Dally, N., Jung, C., 2015. Flowering time regulation in crops—what did we learn from Arabidopsis? Current Opinion in Biotechnology 32, 121–129.
- Campoli, C., von Korff, M., 2014. Chapter Five Genetic Control of Reproductive Development in Temperate Cereals, in: Fornara, F. (Ed.), Advances in Botanical Research. Academic Press, pp. 131–158.
- Chalhoub B, Denoeud F, Liu S, Parkin IA, Tang H, Wang X, et al. 2014. Plant genetics. Early allopolyploid evolution in the post-Neolithic Brassica napus oilseed genome. Science 22;345(6199), 950–3.
- Cirilli M, Micali S, José Aranzana M, Arús P, Babini A, Barreneche T, Bink M, Cantin CM, Ciacciulli A, Cos-Terrer JE, Drogoudi P, Eduardo I, Foschi S, Giovannini D, Guerra W, Liverani A, Pacheco I, Pascal T, Quilot-Turion B, Verde I, Rossini L, Bassi D (2020) The Multisite PeachRefPop Collection: A True Cultural Heritage and International Scientific Tool for Fruit Trees. Plant Physiology 184 (2), 632–646.
- Cox, T.S., Glover, J.D., Van Tassel, D.L., Cox, C.M., DeHaan, L.R., 2006. Prospects for developing perennial-grain crops. Bioscience 56, 649–659.
- Gambín, B.L., Borrás, L., 2010. Resource distribution and the trade-off between seed number and seed weight: a comparison across crop species. Annals of Applied Biology 156, 91–102.
- Glover, J.D., Reganold, J.P., Bell, L.W., Borevitz, J., Brummer, E.C., Buckler, E.S., Cox, C.M., Cox, T.S., Crews, T.E., Culman, S.W., DeHaan, L.R., Eriksson, D., Gill, B.S., Holland, J., Hu, F., Hulke, B.S., Ibrahim, A.M.H., Jackson, W.,



Jones, S.S., Murray, S.C., Paterson, A.H., Ploschuk, E., Sacks, E.J., Snapp, S., Tao, D., Van Tassel, D.L., Wade, L.J., Wyse, D.L., Xu, Y., 2010. Increased Food and Ecosystem Security via Perennial Grains. Science 328, 1638–1639.

Jamnadass, R., Mumm, R.H., Hale, I., Hendre, P., Muchugi, A., Dawson, I.K., Powell, W., Graudal, L., Yana-Shapiro, H.,

- Simons, A.J., Van Deynze, A., 2020. Enhancing African orphan crops with genomics. Nature Genetics 52, 356–360. Jung, C., Pillen, K., Staiger, D., Coupland, G., von Korff, M., 2017. Editorial: Recent Advances in Flowering Time Control. Frontiers in Plant Science 7, 2011..
- Jung, M., Roth, M., Aranzana, M.J. et al. (2020) The apple REFPOP—a reference population for genomics-assisted breeding in apple. Hortic Res 7, 189.
- Kozlowski, T.T., Pallardy, S.G. (2002) Acclimation and adaptive responses of woody plants to environmental stresses. Bot. Rev 68, 270–334.
- Liller, C.B., Neuhaus, R., von Korff, M., Koornneef, M., van Esse, W., 2015. Mutations in Barley Row Type Genes Have Pleiotropic Effects on Shoot Branching. Plos One 10,e0140246.
- Mascher M, Gundlach H, Himmelbach A, Beier S, Twardziok SO, Wicker T, et al. 2017. A chromosome conformation capture ordered sequence of the barley genome. Nature. 26;544(7651), 427–33.
- Monat C, Padmarasu S, Lux T, Wicker T, Gundlach H, Himmelbach A, et al. 2019. TRITEX: chromosome-scale sequence assembly of Triticeae genomes with open-source tools. Genome Biol. 20(1), 284.
- Obeso, J.R., 2002. The costs of reproduction in plants. New Phytologist 155, 321–348. https://doi.org/10.1046/j.1469-8137.2002.00477.x.
- Quezada-Martinez, D., Addo Nyarko, C.P., Schiessl, S.V., Mason, A.S., 2021. Using wild relatives and related species to build climate resilience in Brassica crops. Theoretical and Applied Genetics 134, 1711-1728.
- Ruggles gates, R., 1953. Wild cabbages and the effects of cultivation. Journal of Genetics 51, 363–372.
- Sadras, V.O., 2007. Evolutionary aspects of the trade-off between seed size and number in crops. Field Crops Research 100, 125–138.

Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. 2014. Nat Biotechnol. 32, 347.

Schindele P, Wolter F, Puchta H. 2018 Transforming plant biology and breeding with CRISPR/Cas9, Cas12 and Cas13. FEBS Lett. 592(12), 1954–67.

- Slafer, G.A., Kantolic, A.G., Appendino, M.L., Tranquilli, G., Miralles, D.J., Savin, R., 2015. Chapter 12 Genetic and environmental effects on crop development determining adaptation and yield, in: Sadras, V.O., Calderini, D.F. (Eds.), Crop Physiology (Second Edition). Academic Press, San Diego, pp. 285–319.
- Stearns, S.C., 1989. Trade-Offs in Life-History Evolution. Functional Ecology 3, 259–268.
- Tanksley, S.D., McCouch, S.R., 1997. Seed Banks and Molecular Maps: Unlocking Genetic Potential from the Wild. Science 277, 1063.
- Van Tassel DL, Tesdell O, Schlautman B, Rubin MJ, DeHaan LR, Crews TE, Streit Krug A (2020) New Food Crop Domestication in the Age of Gene Editing: Genetic, Agronomic and Cultural Change Remain Co-evolutionarily Entangled. Frontiers in Plant Science 11, 789.
- Vico, G., Manzoni, S., Nkurunziza, L., Murphy, K., Weih, M., 2016. Trade-offs between seed output and life span a quantitative comparison of traits between annual and perennial congeneric species. New Phytologist 209, 104–114.
- Wang, J., Ding, J., Tan, B., Robinson, K.M., Michelson, I.H., Johansson, A., Nystedt, B., Scofield, D.G., Nilsson, O., Jansson, S., Street, N.R., Ingvarsson, P.K., 2018. A major locus controls local adaptation and adaptive life history variation in a perennial plant. Genome Biology 19, 72.
- Zhang Y, Pribil M, Palmgren M, Gao C (2020) A CRISPR way for accelerating improvement of food crops. Nature Food 1: 200-205.
- Ye, C.-Y., Fan, L., 2021. Orphan Crops and their Wild Relatives in the Genomic Era. Molecular Plant 14, 27–39.
- Zsogon A, Cermak T, Naves ER, Notini MM, Edel KH, Weinl S, et al. 2018. De novo domestication of wild tomato using genome editing. Nat Biotechnol. 36(12):1211-.



Focus Group N-1: Increasing protein content and quality

Agata Gadaleta¹, Benoit Méléard², Bertrand Hirel³, Carlos Guzmán⁴, Catherine Ravel⁵, Friedrich Longin⁶, Gilberto Igrejas⁷, Jacques Le Gouis⁸, Karine Gallardo⁹, Peter Shewry¹⁰

1: <u>Aqata.Gadaleta@Uniba.It</u> (University of Bari "Aldo Moro", Bari, IT), 2: <u>b.meleard@arvalis.fr</u> (Arvalis, Boigneville, FR), 3: <u>bertrand.hirel@inrae.fr</u> (CNRS, Versailles, FR), 4: <u>carlos.quzman@uco.es</u> (Universidad de Córdoba, Córdoba, Spain), 5: <u>catherine.ravel@inrae.fr</u> (INRAE, Clermont-Ferrand, FR), 6: <u>friedrich.longin@uni-hohenheim.de</u> (University of Hohenheim, Stuttgart, GER), 7: <u>giarejas@utad.pt</u> (University of Trás-os-Montes and Alto Douro, Vila Real, Portugal), 8: <u>jacques.le-</u> <u>gouis@inrae.fr</u> (INRAE, Clermont-Ferrand, FR), 9: <u>Karine.gallardo-querrero@inrae.fr</u> (INRAE, Dijon, FR), 10: <u>Peter.Shewry@Rothamsted.Ac.uk</u> (Rothamsted Research, UK).

General background

The domain related to "Increasing protein content and quality" is quite large so it was proposed **to** really focus on the content and composition of the grain proteins for technological and nutritional quality. This may include problems related to protein toxicity although another focus group is concerned with "4. Decreasing negative and toxic compounds (FG N-4)". Most people in the group are concerned with small grain cereals, that are a major staple food for Human consumption, but the discussion also concerned other plants as pulses and maize.

Very briefly, the mean protein Human consumption is 80 g/capita/day (100 g/capita/day in developed countries). Protein consumption has drastically increased with the increase in the standard of living. The negative impact is mainly associated with animal-derived protein with reports that 12% of greenhouse gases (GHG) emissions and 30% of human-induced terrestrial biodiversity loss can be attributed to animal production (Henchion et al 2017). Plant-based proteins are associated with lower levels of GHG emissions. Currently vegetal sources of protein dominate protein supply globally (57%), with meat (18%), dairy (10%), fish and shellfish (6%) and other animal products (9%) making up the remainder (FAO 2010). However, consumption of animal proteins is dominant in developed countries (about two-times higher than that of vegetal proteins). In developing countries, the part of animal proteins tends to increase. As the demand for protein will increase due to the increase in the world's population, two scenarios are emerging: either the protein consumption remains stables (by a decrease of protein consumption per capita) or it continues to progress. To avoid (or limit) the negative environmental effects of this consumption, in both cases, a dietary transition is necessary: the part of vegetal protein must be increased, while that of animal proteins decreases or, at least, remains stables.

EU strategic goals, set and documented in the "Farm to Fork" agenda, state that:

- "The Commission will examine EU rules to reduce the dependency on critical feed materials (e.g. soya grown on deforested land) by fostering EU-grown plant proteins";
- "A key area of research will relate to microbiome, food from the oceans, urban food systems, as well as increasing the availability and source of alternative proteins such as plant, microbial, marine and insect-based proteins and meat substitutes;"
- The goal is to "Reduce significantly the use and risk of chemical pesticides, as well as the use of fertilisers [...]";



• and to "Increase the area under organic farming in Europe."

Status quo of research in the field

Current know-how

- Currently they are good sources of intrinsic technological quality for EU cereals and this is no longer a major limitation. The trend in the UK is to use less strong wheats compared to 20 years ago (this also reduces energy costs for processing).
- Modern cereal varieties are very efficient at using N when applied relevantly (using up to date decision support methods to estimate the amount and timing) and environmental conditions are favorable (water availability): in that conditions any improvements are likely to be incremental rather than step changes.
- Grain protein content (i) is a major criterion for grading products and for exportation, (ii) it highly
 depends on nitrogen availability, (iii) could be expected to compensate for the reduction in the
 use of exogenous gluten in baked goods and more broadly the predictable reduction in the use
 of bakery improvers. If there are limitations in the future due to restrictions of nitrogen
 fertilization; protein quality will have to be elaborated in more details. For instance, the wheat
 protein composition should be finely tuned to obtain gluten structures and properties able to
 give the required end-use quality for processing.

Most relevant research results

- The variation for processing and end-use quality is largely explained by protein quality/composition. And the major loci and genes for both protein content and quality, even for some regulatory genes involved in the protein and starch syntheses (globulins in legume seeds, glutenins and gliadins in wheat, modifiers of the opaque-2 mutation in maize), have been identified.
- A large genetic variability exists for protein content and protein quality from the processing enduse quality point of view for wheat and pea. The wheat genetic variability from the nutritional point of view (i.e., sources rich in Lysine) or from the sensitivity/intolerance point of view described seems less large. In maize however, genetic loci have been found to be implicated in controlling the levels of a protein synthesis factor correlated with lysine.
- Whatever the species, a trend is observed for a lower grain protein concentration for modern cultivars. However, there is the possibility of improving the yield while maintaining seed protein content in grain legumes such as pea and soybean, and possibly maize, as no strong negative correlation between yield and seed protein content was observed. In cereals, the exploitation of the deviation from the negative relationship between grain yield and protein concentration (GPD) is possible as shows its stability and heritability. In addition, some regulators, as the TaNAM transcriptional factor in wheat, gives the hope of being able to increase the protein content without negatively affecting yield.
- The effects of low nutrient (N, S) availability on seed protein content and composition have been studied, notably in combination with drought, and candidate genes that might limit the negative impacts of these stresses on seed development were identified.



- Indeed, in addition to genetic factors already mentioned, the protein content and composition is strongly affected by environmental factors influencing nitrogen availability and cultivar development time (see the review of Johannson et al, 2020). Both factors are impacted by environmental (temperature, precipitation, CO₂, etc.) and agronomic (soil properties, crop management practices (e. g nitrogen fertilisation management) components.
- In grain legume seeds, the presence of several antinutritional factors has already been reduced (trypsin inhibitors, tannins, vicin & convicin for faba bean).

Trends in research, new technology applied

Concerning the trends in research:

- due to the development of low input systems the effects of sustainable field managements on protein content is highly investigated.
- how modern breeding has affected grain quality is investigated. This is the case for cereals with the increase of wheat related disorders (celiac disease, gluten intolerance, etc.). This is also the case for legumes where compounds with negative effects in animal feed were eliminated by selection although they can have positive effects in human health (e.g. tannins which have antioxidant functions).
- in the past, the effect of individual protein fractions or gene alleles were analysed but more and more combinations are looked at: % of glutenins/gliadins/albumins/globulins; glutenins alleles combinations (no single alleles/genes) for the wheat grain, % of 7S globulins/11S globulins/2S albumins in legume seeds.
- it is recognized now that there is a need to understand effects at all levels: from gene expression to the phenotype and the biophysical effects on molecular interactions. Therefore, tools for multi-level data integration are developing.
- translational genomics is proposed when relevant to take advantage of information coming from different species.

Concerning the technologies:

- this is of course not only relevant for grain quality, but the genomes of all the major crops have been sequenced. Very good reference genomes exist and more and more sequences for additional genotypes are now available to describe the pangenome of a species. High density genotyping arrays and genotype by sequencing methodologies have been developed for Genome Wide Association Studies.
- low cost and high throughput proteomic tools are being used and they are particularly adapted to analyzing grain proteins related issues.
- ethylmethanesulfonate (EMS)-induced allelic variations (TILLING populations) have been developed (in maize, wheat and pea) and they are used to improve nutritional quality traits. As some traits are determined by major genes this approach is very efficient.
- genome editing, that can target multicopy genes (as it is almost always the case for polyploid species like bread wheat) is mastered for most crops.



• ecophysiological modeling could be a relevant tool to understand the interplay between environmental and agronomic factors for the establishment of the quality of the seed or the flour.

Future challenges in the field to be addressed with high priority

What are the most relevant unsolved questions (scientific questions, societal and economic challenges)?

- The quality of a production is a very complex and changing concept. It is necessary to understand better in each case (processing, end-use, nutrition health) what are the required criteria and possibly diversify and specialize the varieties.
- It is important to stress that both a high quality (whatever the criterion) and a stable quality are required. In that context the effect of global change is a major challenge (low input systems, drought and heat stresses; elevated CO₂; etc.) for breeding.
- The negative correlation between grain yield and grain protein concentration is known for a very long time in cereals. Genetic gains for grain yield generated lower protein contents. Whatever the species there is need to better understand this negative correlation and possibly identify genes that can alter it (notion of GPD in wheat). For cereals, there is then a need to improve the efficiency of conversion of applied N to grain protein. Other trade-offs may exist, for example the consequences on resistance to biotic & abiotic stresses (cold, drought, fusarium & bruchids in legumes ...) of the elimination by genetics of antinutritional / off-flavor factors.
- In that context, one challenge is to know whether it is possible to keep the processing and enduse quality levels when protein content is going down (because higher grain yields, less fertilization or less efficient N fixation) by improving the protein quality and finely tuned the protein composition.
- The quality of a product is estimated generally only on a few easy and rapid to measure criteria (e.g. grain protein concentration for wheat). Development of rapid tests are needed for baking quality so that breeding and trading for real baking quality and nutritional aspects for humans and animals can replace simpler tests.
- A technological/scientific challenge is the establishment of good data bases for the exploitation of new proteomic tools, as most of the identified proteins are unknown.
- One main societal challenge is probably around the possible acceptance of genetic engineering (GMO) and CRISPR modified crops (targeting protein quality) for commercial use in the long term if positive benefits on environment and health are demonstrated.
- One challenge for cereals is the socioeconomic analyses along crop rotations regarding nutritional proteins, e.g. is it necessary to have wheat with 12% protein when legumes can deliver much more? Studying societal acceptance of the consumption of legumes is needed to develop their production for human nutrition.

Aspects/opportunities for application of research results

• In the context of global change, genetics and breeding could, along better management strategies, offer opportunities to improve and stabilize the quality with reduced levels of inputs,



use of fertiliser (lower costs and environmental footprint), pesticides and energy required for processing.

- In the context of an increase of gluten related disorders, genetics and breeding could also offer opportunities to propose innovative wheats, which solve gluten sensitivities.
- Marker/genomic assisted breeding is fast developing to combine best genetics for yield-protein content-protein quality. Many QTL were previously described but not often used in real breeding programs. The identification of major genes using genome sequences and development of statistical methods may change this.
- Use of translational genomics to transfer knowledge of the regulation of seed protein content and quality from models to crops (e.g. *M. truncatula* or *Arabidopsis* to legume crops) or between crops (e.g. pea-faba bean-soybean-lentil...) is increasing.
- Development of predictive models to estimate grain N content, N translocated to the grain (grain N removal) and N balance in the soil taking into account environmental factors (temperature, water variability) and considering genetic parameters linked to the genotype.

Action points for a future research program in the field

What needs to be done to solve the scientific questions and to meet the societal and economic challenges?

- There is still the need to better understand of the genetics of protein content (nitrogen use efficiency / interaction with microbes) to breed varieties competitive for grain yield, with same or more protein content and that demand less fertilization. This includes identifying the molecular determinants of components responsible for off-flavours of legume-derived ingredients, and of for gluten-related diseases.
- The greatest challenge may be to understand G x E interactions for seed protein content and composition (quality). These may include biophysical effects as well as effects on gene expression. This should include climate change and agroecological practices (e.g. the use of legumes in cereal cropping systems).
- Exploiting the genetic diversity (natural as well as induced by TILLING) to increase the quality through improved pre-breeding / breeding is still a challenge.

What needs to be done to support the translation of research results into societal and economic value?

- Breeding of new varieties is a way to translate research into societal and economic value. Translational mechanisms to deliver traits and markers to breeders may be also through prebreeding materials (e.g. UK BBSRC Designing Future Wheat programme).
- Propose new quality criteria that could integrate the registration system and would then be published when the candidate variety is registered (the GPD is a registration criterion assessed in the French registration system for Bread Wheat. It is measured at two N levels and the information of the response to low N is also published, but not yet taken into account in the score of the candidate variety). This new criterion could also be estimated on genetic resources and made publicly available in a database.



- More projects/collaborations between academia-breeding companies-food industry-extension services-farmers-consumers are required.
- More presentations of the challenges, projects and results regularly on scientific but also applied meetings with stakeholders and consumers using Blogs and social media.
- Developing attractive plant protein-based products with quality labels (environmental and nutritional) is a way to better balance the intake of proteins from animal and plant sources. The complementarity of different sources of proteins (cereals, legumes, Brassicaceae) to meet the requirements for essential amino acids in Human nutrition may be better exploited.

References

- Henchion M, Hayes M, Mullen AM, Fenelon M, Tiwari B (2017) Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. Foods 6:53.
- Johansson E, Branlard G, Cuniberti M, Flagella Z, Hüsken A, Nurit E, Pena RJ, Sissons M, Vazquez D (2020) Genotypic and environmental effects on wheat technological and nutritional quality. In: Igrejas G, Ikeda T, Guzman C (eds) Wheat quality for improving processing and human health. Springer Nature, Switzerland, pp 171-204.



Focus Group N2&4: Improving specialized metabolite contents for improving animal and plant health and agriculture sustainability (i.e. fusion of former FG N-2 & N-4)

Emmanuel Gaquerel¹, Loïc Lepiniec², Massimiliano Corso³, Alisdair Fernie⁴, Alain Goossens⁵, Wilhelm Gruissem⁶, Barbara Halkier⁷, Paul Kroon⁸, Massimo Maffei⁹, Francesca Sparvoli¹⁰, Alain Tissier¹¹, Frank Van Breusegem¹², Dominique Van Der Straeten¹³, Nathalie Verbruggen¹⁴

1: <u>emmanuel.gaquerel@ibmp-cnrs.unistra.fr</u> (Université de Strasbourg, FR); 2: <u>loic.lepiniec@inrae.fr</u> (INRAE Versailles, FR); 3: <u>massimiliano.corso@inrae.fr</u> (INRAE Versailles, FR); 4: <u>Fernie@mpimp-golm.mpg.de</u> (MaxPlanck, Golm, GER); 5: <u>Alain.Goossens@psb.vib-ugent.be</u> (VIB, Ghent, BE); 6: <u>wilhelm_gruissem@ethz.ch</u> (ETH, Zurich, CH); 7: <u>bah@plen.ku.dk</u> (University of Copenhagen, DK); 8: <u>paul.kroon@quadram.ac.uk</u> (Quadram Institute, Norwich, UK); 9: <u>massimo.maffei@unito.it</u> (Università degli Studi di Torino; IT); 10: <u>sparvoli@ibba.cnr.it</u> (CNR, Milan, IT); 11: <u>Alain.Tissier@ipb-halle.de</u> (IPB Halle, GER); 12: <u>Frank.VanBreusegem@psb.vib-</u> <u>ugent.be</u> (VIB, Ghent, BE); 13: <u>Dominique.VanDerStraeten@UGent.be</u> (Ghent University, BE); 14: <u>nverbru@ulb.ac.be</u> (ULB, Bruxelles, BE).

General background

Access to healthy and nutritious plant-based food at fair prices for all the citizens is essential to improve human well-being and to promote the transition to more sustainable agriculture and food systems (see the EPSO "nutritional Security Working Group"¹). Within the CropBooster-P project, increasing health-promoting specialized metabolites (SMs), while reducing others being either toxic and antinutritive, is hence one of the critical goals for the production of tailored food/feed able to satisfy the needs of the consumers and for livestock production (in line with the perspective of scenarios "Plantovation" and "Your Feed, your food, your health") as part of more sustainable agriculture systems relying on crops better adapted to climate change. A cornerstone challenge is however that while the accumulation of beneficial SMs is a desirable trait, it can be counterbalanced by the presence of other natural compounds acting as antinutritional or toxic factors in edible parts of cultivated species. In this respect, the below examples do not intend to be exhaustive, but rather to illustrate various potential interests, current limitations as well as new perspectives in improving SM contents in crops.

Status quo of research in the field

Plants collectively produce hundreds of thousands of SMs (e.g. phenylpropanoids, terpenoids, alkaloids, and various micronutrients, such as vitamins) that notably act as chemical shields part of defence/tolerance processes against environmental stresses^{2–6}. For instance, several phenylpropanoids, such as stilbenes and coumarins and flavonoids such as flavonols, anthocyanins or flavan-3-ols (condensed tannins) accumulate to high levels in fruits, seeds and vegetative tissues of several edible plant species^{7–10} and are part of the arsenal of compounds that protect plants against a wide range of both biotic^{2,11–13} and abiotic stresses, including drought and high/low temperature (e.g. flavonoids¹⁴), UV irradiation (e.g. chlorogenic acid, flavonoids^{15,16}, and flavones¹⁷), mineral excess and deficiency (e.g. coumarins^{18–20}).



Besides being important traits defining crops' stress resilience, SMs are also essential due to their positive impacts on human/animal nutrition and health (e.g. vitamins) or used as important medicinal drugs, such as anticancer, antimalarial, antioxidants limiting major chronic diseases or regulating bacteria infection²¹. For instance, phenolics, by acting as strong antioxidants or enzyme inhibitors, have protective roles against a wide range of human diseases²², including diabetes and cancer²³, and additionally, phenolics have been proposed to display antiviral action²⁴, e.g. as inhibitor of the main protease of SARS-CoV-2 causing COVID-19^{25,26}. The low content of specific SMs in food is a common form of malnutrition and is not confined to the developing world²⁷. For example, anaemia, a condition of suboptimal iron and haemoglobin level often occurring in developed countries as well²⁸ has been linked to pro-vitamin A (β -carotene), thiamine (vitamin B1), pyridoxine (vitamin B6), and folate (vitamin B9)^{29,30} deficiencies. Importantly, vitamin deficiencies are linked to birth defects, cardiovascular and neurological pathologies including Alzheimer's disease, as well as to the occurrence of a range of cancers^{21,30,31}.

SMs can also act as antinutritional factors (ANFs) by reducing the intake, digestion and utilization of nutrients, or directly as toxic compounds. Indeed, many plants accumulate SMs potentially toxic for humans and livestock, such as cyanogenic glucosides, saponins, quinolizidine alkaloids, phenolic aldehydes, goitrogenic glucosinolates or steroidal glycoalkaloids³². Cassava, an important staple food, is neurotoxic if not adequately processed, due to its cyanogenic glucoside content. Several SMs are dually considered for their valuable role as plant defence mechanisms and as potential ANFs. For example, gossypol and related sesquiterpenoids protect cotton against insect attacks but, if accumulated at high levels, make the cotton seed oil inedible³³. Besides antinutritional SMs, proteinaceous ANFs, such as lectins, protease inhibitors and α -amylase inhibitors, are particularly abundant in the seeds of legume crops. Levels of several SMs and other ANF compounds have however been reduced in modern crops as an outcome of domestication, selection and breeding. For instance, in almond, the bitter and toxic amygdalin, a cyanogenic diglucoside, was removed due to a mutation in a transcription factor³⁴. In another example, Cassava with reduced cyanogenic toxicity have been obtained^{35,36}. Nevertheless, ANFs may be important for plant fitness and productivity acting as protecting osmolytes (e.g. the raffinose family oligosaccharides), serving as storage or signalling molecules (phytate), or exhibit a variety of other functions in plant protection (e.g. glucosinolates). Together with ANFs, plants may also accumulate toxic trace metal elements, mainly cadmium and lead, which severely affect biological functions of the organisms that ingest them. These toxic elements are commonly accumulated in edible seeds, leaves, roots and fruits of plants growing in highly metal contaminated area, but are mostly plant family-specific^{37–40}.

Future challenges in the field to be addressed with high priority

Using natural variation, conventional breeding and biotechnological approaches have huge potential to modulate SM contents in specific tissue and/or organs and depending environmental conditions. SM diversity has been, intentionally or not, strongly reduced during domestication^{34,41,42}. Mutations that reduce/abolish the accumulation/activity of the ANFs were detected from screening natural biodiversity or mutant populations^{43–45}. Biotechnological approaches can indeed be employed to down-regulate or suppress the expression of genes associated with the production, accumulation, and/or activation of ANF and toxins in plants. For instance reduction of the solanine content has been obtained in transgenic potato lines^{46,47}. Another example is that of the reduction of the glucosinolate levels achieved by mutating several of their



transporters in the seeds of different *Brassicaceae*, including *Brassica napus* (rapeseed) and *Brassica juncea* (mustard)⁴⁸. Seed-specific RNAi was further successfully engineered to knock-down gossypol levels from cotton seeds without compromising the resistance of the plant against herbivores³³. Similar approaches could be easily adopted to reduce proteinaceous ANFs in the main agricultural crops. A related example is the RNAi-based removal of ricin, a type2 ribosome-inactivating protein making impossible the use of oil cakes after oil extraction from the seeds of castor bean as an edible source for feed sources⁴⁹. In another example, ongoing research on the protein-rich seeds of lupin indicates that the toxic quinolizidine alkaloids, which may accumulate upon stress in selected non-bitter varieties, are specifically imported from other plant tissues into seeds. This exemplifies the possibility of using transport engineering to generate stress-resistant varieties producing quinolizidine alkaloid-free seeds⁵⁰.

In this context, in addition to current research towards the (re)discovery of highly nutritious crops, further efforts should be directed towards genetic modulations of SMs contents in high-priority crops. Strategies based on the combined exploitation of metabolic biodiversity and conventional breeding can be applicable especially when the targeted metabolic traits have a simple genetic basis (e.g. removal of toxic proteins or over-accumulation of health-promoting metabolites). More generally, a major challenge to manipulate SM levels lies in a better understanding of their biosynthetic pathways, regulations and functions in crops and in under-utilized, neglected and wild species. For example, phytate (myo-inositolhexakisphosphate) levels were successfully reduced in many grain cereals and legume crops (maize, barley, rice, wheat, soybean, common bean) through screening of mutagenized populations and identification of low phytic acid (*lpa*) mutants⁴⁴. However, lpa mutants often display altered seed metabolite composition and/or negative pleiotropic effects, such as decreased tolerance to abiotic stresses, reduced germination, stunted growth^{51–53}. However, *lpa* mutants often display altered seed metabolite composition and/or negative pleiotropic effects, such as decreased tolerance to abiotic stresses, reduced germination, stunted growth^{51–53}. It is important to note that good agronomic performance of *lpa* mutants could be restored through breeding or via engineering approaches involving seed specific promoters^{54,55}. Lastly, although numerous SM biosynthetic pathways have been elucidated, little is still known about their regulation by environmental stresses⁵⁶ and their multiple interactions in plants as well as all the way down the food chain to human health⁵⁷. Increasing knowledge on gene functions will favour the use of new breeding technologies (based on precise genome editing) to characterize specific coding and non-coding sequences associated with SM and vitamin biofortification⁵⁸ and enable selection of plant varieties that optimize metabolic balance in various environmental conditions^{59,60}.

The process of gene discovery in SM biosynthetic pathways can be further improved by a more systematic use of different multi-omics strategies, including facilitated use and improvement of database infrastructures for the deposit, and combined analysis of omics data for the targeted crops (see Price *et al.* 2020 ⁶¹). Nevertheless, a main bottleneck remains the time-consuming gene function characterization prior to engineering of genetic modulation strategies. It is additionally crucial to ensure that modulating SM content in specific tissues/organs and/or developmental stages does not compromise major determinants of the plant's fitness, protection and agronomic productivity. As most human health-promoting and antinutritional compounds play important roles in plants' adaptation to the environment and/or derive from complex metabolic pathways, there is a crucial need for developing strategies able to overcome unwanted pleiotropic effects. This can



certainly be achieved by targeting genetic manipulations to specific tissues/developmental stages or specific steps such as metabolite transport and storage. For example, for ANF that are produced in leaves or roots, and subsequently transported to seeds (e.g. glucosinolates), a transport engineering strategy could be implemented to silence specific metabolite transporters⁴⁸ to produce plants that have non-toxic harmful seeds but are still protected in other parts. Conversely, provided detailed understanding of *in planta* SMs biosynthesis, transport and regulation, the CRISPR technology could also be used to enhance SMs content in consumed plant parts⁵⁹. Such SM pathway-informed genome editing strategies are obviously not limited to open reading frames and untranslated region of coding genes. As recently demonstrated for the engineering of disease resistance in crops⁶², adequate reconfigurations of SM biosynthetic and transport capacities with limited pleiotropic effects on a crop fitness could also be efficiently implemented by targeting CRISPR edits to promoter regions as well as non-coding RNAs (ncRNAs) including long ncRNA and microRNA.

Action points for a future research program in the field

Discovery and characterization of high-priority SM metabolic pathways

- Identification and functional characterization of key genes (biosynthesis, transport, regulatory, stability, bioactivity) in high-priority SM metabolic pathways, the products of which have a proven effect on human health at large, hence contributing to the "United Nations Sustainable Development, Goal 2: Zero Hunger".
- Characterization of the multi-level environmental and developmental regulations of SM pathways and of the underlying regulatory mechanisms.
- Exploring the natural chemodiversity existing in crops and their wild relatives at both metabolome and genome levels. High priority efforts could be targeted to investigations into gene and metabolic hubs to flavour, digestibility, toxicity, nutritional and health-beneficial profiles of promising wild species in order to help identify candidate genes or reveal additional biochemical and transport pathways that could be targeted for genetic change.

Developing new resources and tools for gene discovery and breeding

- Genome sequencing of promising orphan crops and wild species.
- Development of novel strategies of *de novo* domestication of wild plants with interesting metabolic characteristics, given the fact that we have already in hand powerful portfolios of domestication-related genes⁶³. This is a powerful solution for designing ideal crops with the optimal nutritional balance.
- Development of gene-editing technologies (e.g. fine-tuned targeted promoter editing) to modulate the expression of endogenous genes (e.g. transcription factors, biosynthetic genes, transporters) involved in health-beneficial and anti-nutritional compounds. This requires detailed knowledge of promoter activities and regulators involved. Also, CRISPR Cas9-induced targeted sequence insertion in promoters would allow using endogenous promoter elements to redirect or boost the expression of SMs pathways in nutritious/consumed tissues.
- Optimize the transfer efficiency of new genomic technologies to improve the modulation of specialized metabolite in underused varieties and orphan crops.



• Using isogenic lines differing for a specific gene / compound to feed animals and elucidate their relevant function in animal nutrition and health.

Characterization of SM functions including the impact of SM modulations on plant protection and productivity

- It will be important to take into account the possible pleiotropic effects of such metabolic modifications, such as the putative negative impact of increased levels of certain SMs on the accumulation of storage compounds in seeds (*e.g.* oil and protein in seeds).
- Implementation of streamlined strategies for the identification and characterization of the health-promoting effects of nutritionally-important plant SMs, and including those targeted to and influenced by the human microbiome.
- Facilitation of the transfer to industry of lab-scale protocols for an efficient recovery at reasonable prices of health-promoting compounds from agro-food side-products and to produce safe dietary supplements based on plant extracts.
- Improve our understanding of the interplay between SM content and organoleptic quality. Novel approaches propelled by gene-editing technologies need strong regulatory control, particularly given the fact that many crop wild relatives accumulate higher levels of harmful or even toxic specialized metabolites.
- Expanding our understanding of the impact of global climate change/environmental conditions on SM accumulation/transport in plants

References

- 1. Schreiner, M., Tonelli, C. & Santino, A. EPSO "nutritional Security Working Group". https://epsoweb.org/workinggroups/nutritional-security/
- 2. Li, D., Halitschke, R., Baldwin, I. T. & Gaquerel, E. Information theory tests critical predictions of plant defense theory for specialized metabolism. *Sci. Adv.* **6**, eaaz0381 (2020).
- 3. Tissier, A., Ziegler, J. & Vogt, T. Specialized Plant Metabolites: Diversity and Biosynthesis. in *Ecological Biochemistry: Environmental and Interspecies Interactions* 14–37 (wiley, 2015). doi:10.1002/9783527686063.ch2
- 4. Arimura, G. & Maffei, M. Plant Specialized Metabolism: Genomics, Biochemistry, and Biological Functions. (2016).
- 5. Corso, M. *et al.* Specialized metabolites in seeds. in *Advances in Botanical Research* 35–70 (2021). doi:10.1016/bs.abr.2020.11.001
- 6. Obata, T. & Fernie, A. R. The use of metabolomics to dissect plant responses to abiotic stresses. *Cell. Mol. Life Sci* **69**, 3225–3243 (2012).
- 7. Zhan, C. *et al.* Selection of a subspecies-specific diterpene gene cluster implicated in rice disease resistance. *Nat. Plants* **6**, 1447–1454 (2020).
- 8. Corso, M., Perreau, F., Mouille, G. & Lepiniec, L. Specialized Phenolic compounds in seeds: structures, functions, and regulations. *Plant Sci.* **296**, 110471 (2020).
- Malacarne, G. *et al.* Regulation of flavonol content and composition in (Syrah×Pinot Noir) mature grapes: integration of transcriptional profiling and metabolic quantitative trait locus analyses. *J. Exp. Bot.* 66, 4441–4453 (2015).
- 10. Maffei, M. Plant Bioactive Molecules. (Cambridge Scholars Publishing, 2018).
- 11. Rajniak, J., Barco, B., Clay, N. K. & Sattely, E. S. A new cyanogenic metabolite in Arabidopsis required for inducible pathogen defence. *Nature* **525**, 376–379 (2015).
- 12. Szymański, J. *et al.* Analysis of Wild Tomato Introgression Lines Elucidates the Genetic Basis of Transcriptome and Metabolome Variation Underlying Fruit Traits and Pathogen Response. *Nat. Genet.* **52**, 1111–1121 (2020).
- 13. Zebelo, S. A. & Maffei, M. E. Role of early signalling events in plant-insect interactions. *J. Exp. Bot.* **66**, 435–448 (2015).



- 14. Nakabayashi, R. *et al.* Enhancement of oxidative and drought tolerance in Arabidopsis by overaccumulation of antioxidant flavonoids. *Plant J.* **77**, 367–379 (2014).
- 15. Tohge, T. *et al.* Characterization of a recently evolved flavonol-phenylacyltransferase gene provides signatures of natural light selection in Brassicaceae. *Nat. Commun.* **7**, 12399 (2016).
- Dolzhenko, Y., Bertea, C. M., Occhipinti, A., Bossi, S. & Maffei, M. E. UV-B modulates the interplay between terpenoids and flavonoids in peppermint (Mentha×piperita L.). *J. Photochem. Photobiol. B Biol.* **100**, 67–75 (2010).
- 17. Peng, M. *et al.* Differentially evolved glucosyltransferases determine natural variation of rice flavone accumulation and UV-tolerance. *Nat. Commun.* **8**, 1975 (2017).
- Rajniak, J. *et al.* Biosynthesis of redox-active metabolites in response to iron deficiency in plants. *Nat. Chem. Biol.* 14, 442–450 (2018).
- 19. Tsai, H. H. & Schmidt, W. Mobilization of Iron by Plant-Borne Coumarins. Trends Plant Sci. 22, 538–548 (2017).
- 20. Briat, J.-F., Dubos, C. & Gaymard, F. Iron nutrition, biomass production, and plant product quality. *Trends Plant Sci.* **20**, 33–40 (2015).
- 21. Fitzpatrick, T. B. et al. Vitamin Deficiencies in Humans: Can Plant Science Help? Plant Cell 24, 395–414 (2012).
- 22. Martin, C. & Li, J. Medicine is not health care, food is health care: plant metabolic engineering, diet and human health. *New Phytol.* 699–719 (2017). doi:10.1111/nph.14730
- 23. Bondonno, N. P. *et al.* Flavonoid intake is associated with lower mortality in the Danish Diet Cancer and Health Cohort. *Nat. Commun.* **10**, 3651 (2019).
- 24. Wei, F. et al. Antiviral Flavonoids from the Seeds of Aesculus chinensis. J. Nat. Prod 67, 650–653 (2004).
- 25. Gogoi, N. *et al.* Computational guided identification of a citrus flavonoid as potential inhibitor of SARS-CoV-2 main protease. *Mol. Divers.* (2020). doi:10.1007/s11030-020-10150-x
- 26. Zhu, Y. & Xie, D.-Y. Docking Characterization and in vitro Inhibitory Activity of Flavan-3-ols and Dimeric Proanthocyanidins Against the Main Protease Activity of SARS-Cov-2. *Front. Plant Sci.* **11**, 1–14 (2020).
- 27. Singh, U., Praharaj, C. S., Singh, S. S. & Singh, N. P. *Biofortification of food crops*. *Biofortification of Food Crops* (Springer India, 2016). doi:10.1007/978-81-322-2716-8
- 28. Scott, S. P., Chen-Edinboro, L. P., Caulfield, L. E. & Murray-Kolb, L. E. The impact of anemia on child mortality: An updated review. *Nutrients* **6**, 5915–5932 (2014).
- 29. Singh, S. P., Gruissem, W. & Bhullar, N. K. Single genetic locus improvement of iron, zinc and β-carotene content in rice grains. *Sci. Rep.* **7**, 1–11 (2017).
- 30. Strobbe, S. & Van Der Straeten, D. Toward eradication of B-Vitamin deficiencies: Considerations for crop biofortification. *Front. Plant Sci.* **9**, 1–19 (2018).
- 31. Vanderschuren, H. *et al.* Strategies for vitamin B6 biofortification of plants: A dual role as a micronutrient and a stress protectant. *Front. Plant Sci.* **4**, 1–7 (2013).
- 32. Mithöfer, A. & Maffei, M. E. General Mechanisms of Plant Defense and Plant Toxins. in *Plant Toxins* 1–22 (Springer Netherlands, 2016). doi:10.1007/978-94-007-6728-7_21-1
- 33. Rathore, K. S. *et al.* Ultra-Low Gossypol Cottonseed: Selective Gene Silencing Opens Up a Vast Resource of Plant-Based Protein to Improve Human Nutrition. *CRC. Crit. Rev. Plant Sci.* **39**, 1–29 (2020).
- 34. Sánchez-Pérez, R. *et al.* Mutation of a bHLH transcription factor allowed almond domestication. *Science (80-.).* **364**, 1095–1098 (2019).
- 35. Jørgensen, K. *et al.* Metabolon formation and metabolic channeling in the biosynthesis of plant natural products. *Curr. Opin. Plant Biol.* **8**, 280–291 (2005).
- 36. Narayanan, N. N., Ihemere, U., Ellery, C., Sayre, R. T. & Blazquez, M. A. Overexpression of Hydroxynitrile Lyase in Cassava Roots Elevates Protein and Free Amino Acids while Reducing Residual Cyanogen Levels. *PLoS One* **6**, e21996 (2011).
- 37. Clemens, S. & Ma, J. F. Toxic heavy metal and metalloid accumulation in crop plants and foods. *Annu. Rev. Plant Biol.* **67**, 489–512 (2016).
- 38. Clemens, S. Safer food through plant science: reducing toxic element accumulation in crops. *J. Exp. Bot.* **70**, 5537–5557 (2019).
- 39. Clemens, S., Aarts, M. G. M., Thomine, S. & Verbruggen, N. Plant science: the key to preventing slow cadmium poisoning. *Trends Plant Sci.* **18**, 92–99 (2013).
- 40. Baker, a. J. M. Accumulators and excluders -strategies in the response of plants to heavy metals. *J. Plant Nutr.* **3**, 643–654 (1981).



- 41. Paauw, M., Koes, R. & Quattrocchio, F. M. Alteration of flavonoid pigmentation patterns during domestication of food crops. *J. Exp. Bot.* **70**, 3719–3735 (2019).
- 42. Fu, Y. B. Understanding crop genetic diversity under modern plant breeding. *Theoretical and Applied Genetics* **128**, 2131–2142 (2015).
- 43. Clemente, A. *et al.* Eliminating Anti-Nutritional Plant Food Proteins: The Case of Seed Protease Inhibitors in Pea. *PLoS One* **10**, e0134634 (2015).
- 44. Sparvoli, F. & Cominelli, E. Seed Biofortification and Phytic Acid Reduction: A Conflict of Interest for the Plant? *Plants* **4**, 728–755 (2015).
- 45. Schmidt, M. A., Hymowitz, T. & Herman, E. M. Breeding and characterization of soybean Triple Null; a stack of recessive alleles of Kunitz Trypsin Inhibitor, Soybean Agglutinin, and P34 allergen nulls. *Plant Breed.* **134**, 310–315 (2015).
- 46. Itkin, M. *et al.* Biosynthesis of Antinutritional Alkaloids in Solanaceous Crops Is Mediated by Clustered Genes M. *Science (80-.).* **341**, 175–179 (2013).
- 47. Sawai, S. *et al.* Sterol Side Chain Reductase 2 Is a Key Enzyme in the Biosynthesis of Cholesterol, the Common Precursor of Toxic Steroidal Glycoalkaloids in Potato. *Plant Cell* **26**, 3763–3774 (2014).
- 48. Hassan Nour-Eldin, H. *et al.* NRT/PTR transporters are essential for translocation of glucosinolate defence compounds to seeds. *Nature* **488**, (2012).
- 49. Sousa, N. L., Cabral, G. B., Vieira, P. M., Baldoni, A. B. & Aragão, F. J. L. Bio-detoxification of ricin in castor bean (Ricinus communis L.) seeds. *Sci. Rep.* **7**, 1–9 (2017).
- 50. Otterbach, S. L., Yang, T., Kato, L., Janfelt, C. & Geu-Flores, F. Quinolizidine alkaloids are transported to seeds of bitter narrow-leafed lupin. *J. Exp. Bot.* **70**, 5799–5808 (2019).
- 51. Stevenson-Paulik, J., Bastidas, R. J., Chiou, S.-T., Frye, R. A. & York, J. D. Generation of phytate-free seeds in Arabidopsis through disruption of inositol polyphosphate kinases. (2005).
- 52. Tong, C. *et al.* Analysis of Lysophospholipid Content in Low Phytate Rice Mutants. (2017). doi:10.1021/acs.jafc.7b01576
- 53. Pilu, R., Landoni, M., Cassani, E., Doria, E. & Nielsen, E. The maize lpa241 mutation causes a remarkable variability of expression and some pleiotropic effects. *Crop Sci.* **45**, 2096–2105 (2005).
- 54. Shi, J. *et al.* Embryo-specific silencing of a transporter reduces phytic acid content of maize and soybean seeds. *Nat. Biotechnol.* **25**, 930–937 (2007).
- 55. Colombo, F. *et al.* MRP Transporters and Low Phytic Acid Mutants in Major Crops: Main Pleiotropic Effects and Future Perspectives. *Front. Plant Sci.* **11**, 1301 (2020).
- 56. Smith, M. R. & Myers, S. S. Global Health Implications of Nutrient Changes in Rice Under High Atmospheric Carbon Dioxide. *GeoHealth* **3**, 190–200 (2019).
- 57. Jiang, L., Strobbe, S., Van Der Straeten, D. & Zhang, C. Regulation of Plant Vitamin Metabolism: Backbone of Biofortification for the Alleviation of Hidden Hunger. *Mol. Plant* **14**, 40–60 (2021).
- 58. Kanchiswamy, C. N., Maffei, M., Malnoy, M., Velasco, R. & Kim, J. S. Fine-Tuning Next-Generation Genome Editing Tools. *Trends in Biotechnology* **34**, 562–574 (2016).
- 59. Van Der Straeten, D. *et al.* Multiplying the efficiency and impact of biofortification through metabolic engineering. *Nat. Commun.* **11**, 1–10 (2020).
- 60. Zhang, Y. *et al.* Multi-level engineering facilitates the production of phenylpropanoid compounds in tomato. *Nat. Commun.* **6**, 8635 (2015).
- 61. Price, E. J. *et al.* Metabolite database for root, tuber, and banana crops to facilitate modern breeding in understudied crops. *Plant J.* **101**, 1258–1268 (2020).
- 62. Zhu, H., Li, C. & Gao, C. Applications of CRISPR–Cas in agriculture and plant biotechnology. *Nat. Rev. Mol. Cell Biol.* doi:10.1038/s41580-020-00288-9
- 63. Fernie, A. R. & Yan, J. De Novo Domestication: An Alternative Route toward New Crops for the Future. *Mol. Plant* **12**, 615–631 (2019).



Focus Group N-3: Increasing *a*3 fatty acids in oilseeds

Jonhnathan Napier¹, Eric Maréchal², Ivo Feussner³, Jean-Denis Faure⁴, Douglas Tocher⁵, Richard Haslam⁶, Monica Venegas Caleron⁷

1: johnathan.napier@rothamsted.ac.uk (Rothamsted Research, UK); 2: eric.marechal@cea.fr (Laboratoire de Physiologie Cellulaire & Végétale, CNRS-CEA-INRAE-Univ. Grenoble Alpes, Grenoble, FR); 3: <u>ifeussn@gwdg.de</u> (Georg-August-University Goettingen, Albrecht-von-Haller-Institute for Plant Sciences, Dept. for Plant Biochemistry, Goettingen, GER); 4: <u>jean-denis.faure@aqroparistech.fr</u> (Institut Jean-Pierre Bourgin, INRAE-AgroParisTech, INRAE-Versailles, FR); 5: <u>d.r.tocher@stir.ac.uk</u> (Institute of Aquaculture, University of Stirling, Stirling, UK); 6: <u>richard.haslam@rothamsted.ac.uk</u> (Plant Sciences, Rothamsted Research, Harpenden, UK); 7: <u>mvc@ig.csic.es</u> (Instituto de la Grasa (CSIC), Campus Universitario Pablo de Olavide, Sevilla, Spain).

Background

Omega-3 fatty acids are generally perceived as health-beneficial, and whilst this is broadly correct, the body of scientific evidence indicates that such claims are restricted to specific forms of omega-3 fatty acids and/or in different dietary states¹. From the outset, it is worth keeping in mind that the classification "omega-3" refers to a number of different fatty acids present in a range of different organisms, including microbes, plants and animals. A precise definition of omega-3 fatty acids is polyunsaturated fatty acids characterized by the presence of a double bond, three atoms away from the terminal methyl group in their chemical structure. The most common example of an omega-3 fatty acid is a-linolenic acid (ALA; 18:3n-3, where 18 denotes the number of carbons, 3 defines the number of double bonds, and n-3, sometime written as w-3, indicates that the first double bond from the methyl end of the fatty acid is located three carbons in, commensurate with the classification as an omega-3 fatty acid). Other well-known examples of omega-3 fatty acids are docosahexaenoic acid (DHA; 22:6n-3) and eicosapentenoic acid (EPA; 20:5n-3), both of which are found in fish oils² but are entirely absent from higher plants, unlike ALA which is ubiquitous in photosynthetic organisms and plays a vital role in both the thylakoid membrane and also acting as a metabolic precursor for the signalling compound jasmonic acid. Historically, ALA is also considered to be an essential fatty acid (EFA) based on animal studies carried out in the 1930s which indicated that ALA was an absolute dietary requirement, in conjunction with an absence of any endogenous biosynthetic capacity for EFAs in most vertebrates. However, given the near universal presence of ALA in most human foodstuffs, there no obvious examples of an ALA/EFA deficiency, nor any recommendation for a minimum level of ALA in our diets³. Having said that, it is widely believed by health professionals that human diets were historically skewed towards being strongly omega-3 rich (predominantly vegetables and seafood) whereas as now it is much more skewed to omega-6 rich, due to the elevated consumption of terrestrial animal protein. This in turn is believed to be responsible for increased metabolic diseases such as CVD, type-2 diabetes etc, as well as the impact on climate associated with large-scale animal production. it widely believed that our diets would benefit from being richer in general in omega-3 fatty acids, and this is in addition to the specific recommendations from various national agencies such as EFSA, FDA etc that our diets should contain a defined amount of omega-3 EPA and DHA, which can reduce the risk of infarction and metabolic syndrome. For all these reasons, there is a consensus amongst health professionals that



our diets should contain omega-3 in both the C18 vegetable form of ALA, as well as the "marine" forms of EPA and DHA^{4,5}.

Status quo of research in the field

The genes encoding the fatty acid desaturases responsible for the insertion of the omega-3 terminal double bond present in ALA have been known for several decades, on the basis of forward genetic screens in Arabidopsis and map-based cloning⁶. Thus, the FAD3 microsomal desaturase is the primary determinant of ALA levels in seeds, whereas the FAD7/FAD8 plastidial desaturase isofroms are responsible for ALA in vegetative tissues. In both cases, ALA is generated through the desaturation of omega-6 linoleic acid (LA; 18:2n-6), and in doing so, the ratio of omega-3: omega-6 is altered (since LA is consumed to make ALA). In seed, there is a strong correlation between both expression and activity of FAD3 with the level of ALA in the oil. This is true for loss of function mutants (such as fad3) which have reduced ALA, but also mutations such as rfc4, where a genome segment spanning the FAD3 gene was duplicated, resulting in elevated levels of this omega-3 fatty acids⁷. Thus FAD3 is the major determinant for ALA and omega-3 in vegetable oils, and as such has been a target for both marker-assisted breeding and biotechnological approaches. Other factors which modulate the expression of FAD3 include microRNAs (167A)⁸ and several transcription factors including LEC2 and bZIP67⁹, thought these can be viewed as wider regulators of seed development. In general, the use of genetic approaches to select for plant varieties with elevated levels of omega-3 ALA has had relatively limited effort, not least of all since there is natural difference in the fatty acid composition of vegetable oils which can provide suitable sources - e.g. linseed oil contains ~50% ALA, camelina 30% ALA, compared with <5% in canola. However, as mentioned below, there are a number of additional factors which represent opportunities to tailor the fatty acid profile of a crop to a particular end-use, such as aquaculture.

Considerably more effort has been directed towards biotechnological approaches to enhancing plants to accumulate the so-called omega-3 "fish oils" EPA and DHA, since no higher plant accumulates these fatty acids and there is a clear value chain associated with these products (predominantly in aquaculture but also in direct human nutrition)^{2,4}. The foundational work in this area was carried out in Hamburg (DE) and Bristol (UK), with subsequent industry support from BASF¹⁰. In that respect, European plant biotechnology was at the forefront of developing useful new products that delivered improved nutrition and increased sustainability. Unfortunately, due to negative regulatory approaches by EU, BASF and other companies completely ceased all Europeanbased research and development in the field of plant biotechnology, moving to N. America, where they continued to develop a GM canola strain which contained EPA and DHA¹¹. Research in the UK, at Rothamsted Research, pivoted from canola to camelina to develop a new crop in which the seed oil is rich in EPA+DHA. The oil from such plants has been used extensively in both aguafeed and human nutrition studies, and the crop has undergone field trialling in both the UK and USA & Canada^{4,12}. BASF's GM canola, and a similar competing canola line from the Australian company Nuseed (originally developed by CSIRO) have both received regulatory approval for cultivation in the US, meaning that these novel omega-3 canola crops can now be grown at any scale¹¹. There are a number of ongoing applications across the world for approval for feed and food use for components (oil, meal) derived from these crops, and it is likely that these will ultimately attempt to include EU, although no timescale can be envisaged for the conclusion of such a process. Irrespective of that, these omega-3 fish oil canola lines represent the first examples of a new wave



of output traits focussed on consumer benefit, and also a much more complex form of genetic modification than previously encapsulated in the HT/Bt input traits (since the transgene pathway for the synthesis of EPA+DHA requires at least 6 different activities)¹³.

Collectively, the advances made in both camelina and canola demonstrate the power of plant biotechnology and also the continued utility of GM approaches. It is important to emphasise that the omega-3 fish oil trait can only be generated by GM – it is not possible to use gene editing techniques to obtain the same results, not is it possible to use any advanced molecular breeding techniques, since the trait is multigene gain-of-function. However, the new tools of CRISPR etc will be useful to be used in conjunction with the transgenic delivery of this trait, for example in using editing to tailor the endogenous fatty acid profile of the host plant to further enhance the flux through to EPA+DHA, or to add further useful features such as improved feed palatability (low glucosinolates)

Future challenges in the field to be addressed with high priority

As described above, the accumulation of omega-3 fish oils EPA+DHA has been demonstrated in the seeds of both camelina and canola, achieved via complex metabolic engineering. However, there are still some profound unanswered scientific questions which indicate that these achievements are the result of multiple iterations, whereas the desired position would be a predictive model in which a single cycle of design-build-test could run. Despite the intensive research for several decades on the biosynthesis of omega-3 fatty acids in plants, we are still a long way from synthetic biology. To move forward to this, we need much more research on the metabolic fluxes present during lipid biosynthesis, combined with using AI to help develop species-specific models to describe the processes we are trying to manipulate. Such advances would represent a paradigm shift in not just the metabolic engineering of the omega-3 trait, but also of wider relevance to plant crop improvement in general.

Given that the omega-3 fish oils trait in canola has already received regulatory approval in the US, plans for the commercial launch of these products are available in the public domain, predominantly focusing on the meeting the needs of the aquaculture industry (which currently consumes 80% of the annual wild capture harvest of fish oils from the oceans)⁴. Thus, the expectation is that these novel GM-derived fatty acids from canola enter the human food chain indirectly, presumably with less consumer resistance than if the oils were used for direct human nutrition. However, it remains an open question as to how the consumer will respond to products that contain, directly or indirectly, omega-3 fish oils that have been derived from a GM plant, and this will likely vary from region to region, with less resistance likely in the Americas compared to Europe. This particular trait provides an interesting a new angle to understanding public acceptance of GM, since it represents a trait that delivers both nutritional benefit and also environmental sustainability. In that way, it is quite different from the previous input traits which had less obvious consumer benefits¹³.

Action points for a future research program in the field

Given the increasing interest in applied synthetic biology (also called Engineering Biology), the importance of having a fundamental knowledge of the processes to be manipulated cannot be overstated. This is true irrespective of the nature of genetic improvements used (mutagenesis, GE or GM). Significant refocusing of understanding plant metabolism at the multiscale (subcellular,



cellular, tissue) levels is required, along with a shift away from the assumption that metabolic engineering can be viewed as "plug and play". Similarly, the genetic toolkit needs significant expansion to allow for a much more nuanced and precise modulation of gene networks and endogenous metabolism – this will require discovery of new regulatory elements (promoters, terminators etc) as well as a deeper investigation into the hierarchical factors which contribute to the regulation of gene expression, not limited to RNA stability and transcript splicing. Similarly, the importance of protein turnover is directly relevant to much of synthetic biology. Perhaps of most importance, the impact of transgenesis on both the genome and the epigenome is now emerging as an area requiring significant further study¹⁴. Collectively, these topics need intensive research, not just at the fundamental level, but also to allow the development of robust predictive models. Equally, from the perspective of regulatory approval, the issue of precise and discrete changes are likely to be important, as it will probably be for consumer acceptance. In that respect, there is very much more work to be done, and this needs to be acknowledged. Similarly, the narrative that accompanies research outputs needs to be much more circumspect in broad claims to be delivering to (e.g.) food security, and ideally have solid economic and social science evidence to support such a position. In the case of the example used here, of making omega-3 fish oils in plants, there is significant peer-reviewed research to demonstrate the benefits of such a trait, in terms of both human nutrition and environmental sustainability^{2,4,10}. This could be further strengthened by LCA analysis, and in general, it would be of benefit for plant scientists to work more closely with social and economic scientists, and to test at an early stage the proposed benefits of any trait they are interested in improving.

One final area which needs effort and change is the regulation of genetically modified crops, especially within EU. Current legislation is arcane and outmoded, and serves as a barrier to innovation, impact and societal benefit. This is true for both GM and GE, and places EU at a technological disadvantage compared to all of the other industrial nations. It is hard to see how such political issues can be changed, but it is important to flag that change they must.

References

- 1. Troesch et al. Nutrients. 2020 12(9):2555. doi: 10.3390/nu12092555.
- 2. Tocher et al. Nutrients. 2019 11(1):89. doi: 10.3390/nu11010089
- 3. Abdelhamid et al. 2020 Cochrane Database Syst Rev 3(2):CD003177. doi: 10.1002/14651858.CD003177.pub5
- 4. Napier et al 2020. Agriculture can help aquaculture become greener. Nature Food, 1(11), 680-683.
- 5. Willet et al. 2019 Lancet 393(10170):447-492. doi: 10.1016/S0140-6736(18)31788-4.
- 6. Somerville et al. 1996 Trends Cell Biol.6(4):148-53. doi: 10.1016/0962-8924(96)10002-7
- 7. O'Neill et al. 2011 Plant J. 68(5):912-8. doi: 10.1111/j.1365-313X.2011.04742.x.
- 8. Na et al. 2019 Plant J. Apr;98(2):346-358. doi: 10.1111/tpj.14223
- 9. Mendes et al. 2013 Plant Cell. 25(8):3104-16. doi: 10.1105/tpc.113.116343.
- 10. Napier et al 2015 Eur J Lipid Sci Technol. 117(9):1317-1324. doi: 10.1002/ejlt.201400452.
- 11. Napier et al. 2019 Plant Biotechnol J. 17(4):703-705. doi: 10.1111/pbi.13045.
- 12. Han et al. 2020 Plant Biotechnol J. 18(11):2280-2291. doi: 10.1111/pbi.13385.
- 13. Napier et al 2019 Nat Plants. 5(6):563-567. doi: 10.1038/s41477-019-0430-z.
- 14 Jupe et al. 2019 PLoS Genet.;15(1):e1007819. doi: 10.1371/journal.pgen.1007819



Focus Group N-5: Improving biomass digestibility

Wout Boerjan¹, Herman Höfte², Iris Lewandowski³, Matthieu Reymond², Henrik Vibe Scheller⁴ and Luisa Trindade⁵

1: woboe@psb.ugent.be (VIB Center for Plant Systems Biology and UGent Department of Plant Biotechnology and Bioinformatics, Gent, BE); 2: hermanus.hofte@inrae.fr matthieu.reymond@inrae.fr (Institut Jean-Pierre Bourgin, INRAE, AgroParisTech, Université Paris-Saclay, Versailles, FR); 3: iris lewandowski@uni-hohenheim.de (University of Hohenheim, Department of Biobased Resources in the Bioeconomy, GER); 4: <u>hscheller@lbl.gov</u> (Joint BioEnergy National Laboratory, Berkelev, Institute, Lawrence Berkeley California, USA); 5: luisa.trindade@wur.nl (Plant Breeding Department, Wageningen University & Research, Wageningen, NL).

Status quo of research

As part of the EU "Green Deal" objective to reach carbon neutrality by 2050, an important goal is to replace fossil resources with renewable biomass. This creates immense opportunities for farmers and their cooperatives to diversify their activities by contributing to the development of advanced biorefineries that produce ingredients for animal feed, fertilizers, chemicals, materials and energy.

A key question is how to ascertain a local production of sufficient biomass, while avoiding direct or indirect competition with food production (direct or indirect land use change, (I)LUC) and with a limited environmental footprint. To this end, multi-purpose food or feed crops as well as dedicated crops are needed that are adapted to the local conditions and can be cultivated on « marginal » land.

While awaiting more ambitious regulations that monetize the real environmental cost of greenhouse gas emissions, the trajectories towards advanced biorefineries can be economically anchored either by using co-products of food- or feed-crops and/or by developing higher added value products for niche markets. Farmers and their cooperatives are expected to play a key role in the diversification of such products, by developing increasingly complex on-farm processing procedures.

For multi-purpose crops, the breeding targets for their different uses may be convergent (e.g. improved biomass digestibility for animal feed and reduced recalcitrance for 2G biofuel production) or conflicting (e.g. conflicting nitrogen management strategies for protein-rich vs nitrogen-poor lignocellulosic production), the latter requiring compromises. The advantage of dedicated crops is that breeding can fully exploit the specific requirements for sustainable carbon production, for instance by focusing on perennial crops (1, 2). Indeed, perennials (e.g. miscanthus, poplar, ...) have important environmental advantages such as the absence of soil disturbance, limiting erosion and favoring long-term associations with microbial soil communities; deep rooting with better access to water and nutrients; soil coverage replacing the use of herbicides; early growth season reducing water stress; late harvest after senescence allowing nutrient recycling and reducing fertilizer requirement; and reduced fuel consumption thanks to a reduced number of interventions per year (3, 4)(5). "Marginal" land is not necessarily unproductive land, it also covers polluted soils, water



catchment areas, poorly accessible land etc. (6). Finally (I)LUC can also be avoided by growing intermediate/catch crops (7).

Basic and applied plant research is essential to help identifying and addressing specific bottlenecks in the various trajectories towards advanced biorefineries. This requires systemic, multi-disciplinary and multi-actor approaches involving, not only the usual partnerships with seed companies, but also farmers, industry and the general public.



Fig.1: Aspects of advanced biorefineries that require specific basic and applied plant research. Blue arrows represent the flow from farm to feedstock to product, the environmental, economic and social performance of which is monitored by Life Cycle Sustainability Assessment (LCSA). Red arrows show that product requirements condition the choice of feedstock ideotypes and associated production systems. Green text refers to the areas where plant science can contribute to optimizing the system by overcoming bottlenecks. Plant breeding exploits natural and induced variation to create feedstocks that perform better both in adapted production systems and biomass conversion processes. Lignocellulosic biomass production and conversion has a number of specific requirements beyond those shared for food or feed production. Aspects of plant science that address these requirements are discussed in this chapter.

Future challenges in the field to be addressed with high priority

Biomass production systems

One critical step in the cultivation of perennials is the establishment of the crop in the field, which requires adapted weed control techniques (in particular in ecologically sensible areas) and specific logistics in particular for rhizome-propagated crops such as miscanthus (5). Replacement of the latter by seed-based varieties reduces the establishment costs and increases the propagation ratio. Seed-based establishment of miscanthus requires the development of varieties for which genetically homogenous seeds can be derived. The establishment from miscanthus seeds is



challenged by their small size and temperature requirements, which may ask for pre-growing them before planting. Also, provisions for avoiding these new varieties becoming invasive should be taken (e.g. avoiding seed dispersal or creeping roots). Optimizing fertilizer use with perennial crops requires the evaluation of nutrient fluxes during the growth cycle (8, 9). Water management is not only critical for biomass yield but also of its quality. For instance, water stress changes lignification patterns and enhances the digestibility of the biomass as observed in maize (10), sorghum (11) and miscanthus (12). Other aspects are the impact of soil microbial communities, including arbuscular mycorrhizae and potentially nitrogen-fixing endophytes on plant performance (13), and conversely the impact of plant genotype on the composition of the rhizosphere microbial communities (14); the carbon storage capacity in the soil; the avoidance of lodging; the fate of pollutants when cultured on polluted soil; the evaluation of the invasiveness risk, and the impact on biodiversity. The prediction of biomass yield and quality in different environments and cultivation conditions requires the development of specific agronomic modeling tools (9). Finally, the use of intermediate cultures also requires adapted agronomic techniques (7).

Biomass products and processing

Added value for biomass utilisation can come from the extraction of specific high added-value fractions or molecules. Biomass deconstruction into fractions with desired properties needs to be optimized to minimize the energy and chemicals required and the environmental footprint (15). These goals require efficient screening methods for secondary metabolites and cell wall polymers in feedstocks; a better understanding of structures and functional properties of cell wall polysaccharides, lignins and small molecules but also insights in polymer interactions, nano- and mesoscale architecture of plant cell walls and the histological organization of plant organs. In addition, the study of microbial enzyme repertoires should expand the bioprocessing toolbox.

Breeding

Plant breeding is a time-consuming process, especially for perennial crops, the success of which can often only be evaluated after several years of culturing. Modern breeding techniques allow speeding up this process and facilitating the construction of ideotypes (for specific agricultural settings and end-uses) (15). The development of genomic tools such as molecular markers or genomic models combined with high-throughput phenotyping tools enable faster breeding steps (16). The precision can be further increased by decomposing the traits, for instance by using eco-physiological modeling parameters (17) or transcriptomic, metabolomic or cell wall composition data. The integration of these data requires informatics tools, including machine learning. The next innovative step is the use of genome editing (18). This approach not only gives access to alleles not present in the available accessions, it also allows combining favorable alleles in a given background, while avoiding linkage drag of deleterious variants, thereby overcoming the limitations of crosses (19)(16). These approaches require a detailed knowledge of critical cell wall biosynthetic and regulatory pathways as well as allelic variants that are favorable for the desired traits. Such information can be obtained, at least in part from studies involving model plants. Finally, synthetic biology approaches allow the engineering of biosynthetic pathways of metabolites, polysaccharides or lignin in specific cell types, avoiding interference with the agronomic performance of the crop (20-24).



Action points for a future research programme

Some specific breeding targets for lignocellulosic biomass crops and corresponding plant research topics are listed below:

Trait	Corresponding plant research
Optimized biomass yield with low water and mineral content for a given latitude	Understanding the major regulators of the time of flowering and senescence.
Optimized NUE	Nutrient cycle, control of senescence, the role of the rhizome in perennial grasses
Optimized WUE	Drought stress response, arbuscular mycorrhizae
Optimized photosynthesis	Optimizing photorespiratory cycles, faster non-photochemical quenching
Growth on "marginal land"	Temperature, salinity, heavy metal, drought stress tolerance. Optimized nutrient and water use efficiency
Creation of seed-propagated but non- invasive hybrids	Investigating causes of invasiveness in different crops (e.g. seed or vegetative dispersal) and corresponding mitigation strategies.
Improved seedling vigor allowing direct sowing in the field	Critical factors (e.g. temperature) controlling seed size and seedling vigor
Crop architecture and stem density adapted for optimal biomass yield and lodging resistance	Ecophysiological modeling of crop architecture; source sink relationships in biomass crops; regulation of secondary cell wall accumulation.
Senescence	Investigating the mechanisms of senescence and how they influence crop establishment success, biomass yield and biomass composition as well as harvestability
The accumulation of specific metabolites or polymers	Biodiversity, biosynthesis and functional properties of secondary metabolites, polysaccharides and lignins.
Downstream processing	Structure function of cell wall polymers; nano- and mesoscale cell wall architectures. Biodiversity screens for cell wall modifying enzymes. Fiber formation and cell separation for improved retting of hemp or flax.
Genetic modification of biomass composition	Understanding the relation between cell wall composition (cellulose/hemicellulose and lignin) and cell wall recalcitrance.



	Transcriptional and post-transcriptional regulation of secondary cell wall deposition. Identification of new genes involved in cell wall polymer biosynthesis. Functional testing of mutants in these genes for increased biomass digestibility. Biodiversity screens for enzymes synthesizing monolignol substitutes that can be incorporated in lignins. Understanding the relation between cell wall modification and plant development and yield.
Interactions of biomass composition with the environment	Impact of environmental factors on biomass histology and cell wall composition. Potential bioactivity of phenylpropanoids and the impact of biomass composition on growth- promoting rhizobacteria and endophytes.

References

- 1. J. Clifton-Brown et al., GCB Bioenergy (2019), doi:10.1111/gcbb.12566.
- 2. T. van der Weijde et al., Front. Plant Sci. 4, 107 (2013).
- 3. J. P. McCalmont et al. GCB Bioenergy (2017), , doi:10.1111/gcbb.12294.
- 4. M. Von Cossel et al., Earth's Futur. (2020), doi:10.1029/2020EF001478.
- 5. B. Winkler et al., Renew. Sustain. Energy Rev. (2020), , doi:10.1016/j.rser.2020.110053.
- 6. F. Pancaldi, L. M. Trindade, Front. Plant Sci. (2020), , doi:10.3389/fpls.2020.00227.
- 7. M. Von Cossel et al., Energies (2019), doi:10.3390/en12163123.
- 8. L. Strullu, S. Cadoux, M. Preudhomme, M. H. Jeuffroy, N. Beaudoin, F. Crop. Res. (2011), doi:10.1016/j.fcr.2011.01.005.
- 9. L. Strullu, N. Beaudoin, I. G. de Cortazar Atauri, B. Mary, Bioenerg. Res. (2014).
- 10. F. El Hage et al., J. Agric. Food Chem. (2018), doi:10.1021/acs.jafc.7b05755.
- 11. D. Luquet et al., GCB Bioenergy (2019), doi:10.1111/gcbb.12571.
- 12. T. van der Weijde et al., GCB Bioenergy (2017), doi:10.1111/gcbb.12382.
- 13. N. Cope-Selby et al., GCB Bioenergy (2017), doi:10.1111/gcbb.12364.
- 14. I. Salas-González et al., Science (80-.). (2020), doi:10.1126/science.abd0695.
- 15. K. Van Der Cruijsen, M. Al Hassan, G. Van Erven, O. Dolstra, L. M. Trindade, 1–28 (2021).
- 16. J. G. Wallace, E. Rodgers-Melnick, E. S. Buckler, Annu. Rev. Genet. (2018), , doi:10.1146/annurev-genet-120116-024846.
- 17. F. Larue et al., Ann. Bot. (2019), doi:10.1093/aob/mcz038.
- 18. K. Chen, Y. Wang, R. Zhang, H. Zhang, C. Gao, Annu. Rev. Plant Biol. (2019), , doi:10.1146/annurev-arplant-050718-100049.
- 19. B. De Meester et al., Nat. Commun. (2020), doi:10.1038/s41467-020-18822-w.
- 20. P. Oyarce et al., Nat. Plants (2019), doi:10.1038/s41477-018-0350-3.
- 21. B. De Meester et al., Plant Physiol. (2018), doi:10.1104/pp.17.01462.
- 22. C. Y. Lin, A. Eudes, Biotechnol. Biofuels (2020), , doi:10.1186/s13068-020-01707-x.
- 23. A. G. Brandon, H. V. Scheller, Front. Plant Sci. (2020), , doi:10.3389/fpls.2020.00282.
- 24. M. S. Belcher et al., Nat. Chem. Biol. (2020), doi:10.1038/s41589-020-0547-4.



Focus Group S-1: Improving nitrogen uptake and use efficiency

Jean-Pierre Cohan¹, Alia Dellagi², Christine Foyer³, Alain Gojon⁴, Anne Krapp⁵, Céline Masclaux-Daubresse⁶, Philippe Nacry⁷, Pascal Ratet⁸, François Taulemesse⁹, Andreas Weber¹⁰, Nicolaus von Wirén¹¹

1: <u>JP.COHAN@arvalis.fr</u> (Arvalis Institut du Végétal, Villier le Bacle, FR); 2: <u>alia.dellaqi@inrea.fr</u> (AgroParisTech, INRAE Versailles, FR); 3: <u>C.H.Foyer@bham.ac.uk</u> (University of Birmingham, UK); 4: <u>alain.qojon@inrae.fr</u> (INRAE Montpellier, FR); 5: <u>anne.krapp@inrae.fr</u> (INRAE Versailles, FR); 6: <u>celine.masclaux-daubresse@inrae.fr</u> (INRAE Versailles, FR); 7: <u>philippe.nacry@inrae.fr</u> (INRAE Montpellier, FR); 8: <u>Pascal.Ratet@Cnrs.Fr</u> (CNRS, IPS2 Orsay, FR); 9: <u>F.TAULEMESSE@arvalis.fr</u> (Arvalis Institut du Végétal, Pusignan, FR); 10: <u>andreas.weber@uni-duesseldorf.de</u> (University of Duesseldorf, GER); 11: <u>vonwiren@ipk-qatersleben.de</u> (IPK Gatersleben, GER).

Status quo of research in the field

Research conducted on NUE have focused on N uptake, assimilation and remobilization mechanisms and their regulation (1), influence of N availability on plant architecture (2), interactions and dependences of carbon and nitrogen metabolisms (3), effects of climate changes and especially high CO_2 on NUE and N metabolism (3).

N uptake, assimilation and remobilization mechanisms and regulation

Knowledge about the mechanisms involved in N uptake, N assimilation and N remobilization has been intensely gained in model plants (Arabidopsis and rice) [1;2;3]. Studies on cereals are still partial and on vegetables almost missing [4].

Inorganic N (ammonium and nitrate) transporters for uptake have been characterized and several actors involved in their local and systemic regulation by inorganic N or C availability have been discovered [5;3;6;7;8;9;10]. However, the regulation of intracellular pools of nitrogen metabolites (nitrate, ammonium, etc) are still fairly unknown. Actors and regulators in the uptake other N forms as urea and organic N are mainly unknown [11;12].

Micro-organisms are important partners for nutrient uptake [13]. However, uptake studies have mostly been performed in laboratory and did not consider how uptake mechanisms/regulations and root architecture respond to the soil microbiote populations. How plant genotypes modify rhizosphere microbiote composition and impact nitrogen uptake remains unexplored.

Key enzymes involved in inorganic N assimilation are mostly the same in all plant species: nitrate and nitrite reductases and glutamine and asparagine synthethases [1]. Their co-factors are identified. Their regulations by some metabolites or circadian rhythm have been identified [14]. However, assimilation of nitrate and ammonia and further amino acid syntheses also rely on interaction/coordination of C and N pathways, and on transfers of metabolite/co-factors/reducing powers between organelles. Such mechanisms and their coordination/regulation remain to be discovered.

When nitrogen availability for growth is not limiting, important organic N pool is stored in proteins and as amino acids in vacuole. During senescence or facing N limitation, protein sources are recycled by autophagy and proteases [15;16;17]. Released amino acids that are not reused in the cell are



exported to other tissues. N remobilization and senescence are strongly correlated. Common regulators have not been found so far, even though the TOR/SNRK1 kinases regulate autophagy and possibly senescence [18;15]. Despite important knowledge on N assimilation and remobilisation, the different levels of their regulations (epigenetic or post translational modifications (PTMs)) and the factors involved (transcription factors, kinase/phosphatases) remain largely unknown [7]. The precise coordination by external and internal factors, the systemic and local signals, the coordination of the anabolic/catabolic steps of N assimilation/recycling remain to be explored. Specificities of N metabolism have been identified in legumes. N uptake and the organic N forms transported are different from non-legumes. Legume specificities especially considering regulation of N metabolism deserve more attention [19].

Although it appears that N uptake efficiency cannot be strongly optimised in the field for cereals grown under high fertilisation conditions in Europe, NUpE is generally considered as a bottleneck, especially if fertilizer inputs are decreased [20;21]. It may be even more the case under increasing CO₂ conditions that could possibly increase N requirements in many plant species. Importantly, nitrogen remobilization efficiency might be an important point for improving plant resistance to stresses, including climate changes, and quality of harvested products.

Influence of N availability on plant architecture

The effect of N supply (quantitively and qualitatively) on root architecture is certainly one of the most spectacular effect of N limitation/starvation on plant morphology. Efforts to reveal the genetic determinants of the regulation of root growth and plant architecture by N availability are needed [22;23;24;25]. These factors might be essential in plant adaptation to different soils and microbe populations. They may also control WUE or PUE [26].

Interactions between C and N metabolisms.

Carbon fixation is essential for N metabolism. Many N assimilation enzymes are regulated at transcriptional or post-translational levels by sugar availability, co-factor redox status and energy status. Photorespiration causes the release of ammonium that has to be reassimilated to avoid its lost into the atmosphere. In many plant species, photorespiration components are incomplete. Link between photorespiration and NUE are not understood and need more investigations [27]. RuBisCo activity, is one important factor in the control of N assimilation as photosynthesis provides carbon skeletons and energy to N assimilation. Moreover, RuBisCo protein represent the largest N pool in the shoot of all the plant species. Less-specific and faster RuBisCo would permit the reduction of RuBisCo content in plants and, as a consequence, the decrease of N requirement, without affecting photosynthesis. But, less RuBisco content also means less N storage available for remobilization, which could be deleterious for some plant species as trees [28].

Impacts of climate change on NUE

We only consider here high $[CO_2]$. It has been shown that high $[CO_2]$ (i) decreases protein, zinc, and iron concentration in grains of cereals; (ii) decreases RuBisCo content (less N requirement but also less N storage), (iii) increases the size of nodules in legumes-rhizobia interaction [29;30;31;32;28]. Indeed, high [CO2] provides the highest benefits in term of carbon assimilation for legumes and root crops and the lowest for C4 plants [33]. How high $[CO_2]$ modify root exudates depending on plant



species and genotypes, remains to be explored. High [CO₂] might have effects on soil and microbe populations and as a result modify N uptake efficiency [34].

Most relevant unresolved questions

About N uptake, assimilation and remobilization mechanisms and regulation

- How is nitrate uptake, nitrate homeostasis and *in planta* transport regulated on the protein level?
- How efficient is the assimilation of alternative N sources as urea, amino acids, small peptides? What are the mechanisms, regulations in different species?
- What are the N storage resources usable for nitrogen remobilization in different plant organs/species?
- Is there a link between the ability of plant to recycle/remobilize N sources in response to stresses and for harvested products?
- How are controlled and coordinated during plant cycle, the different mechanisms involved in each steps of N uptake/ assimilation/ remobilization?
- What are the different regulation translational / post translational levels (transcription, epigenetic, PTMs, protein/protein interactions) under both sufficient and limiting conditions?
- How does N availability shape plant morphology (meristem sizes, root architecture)?
- How do plant genotypes influence soil composition and microbiotes (and vice versa)?
- What is the best strategy to improve NUE using basic research on N metabolism? Identification of allelic variants and segregations? Extensive biotechnology efforts based on gene stacking of favourable variants in different plant species? Playing with plant-microbe-soil interactions?
- How to take into account other factors that influence NUE such as agricultural practices and erratic climate changes?
- Is it possible to transfer knowledge from species to species in the identification of variants?
- Are laboratory results still consistent under field conditions?

About influence of N availability on plant architecture

- What are the genetic bases of the control of root growth by N and how are the controls by other nutrients and water availability integrated?
- Is the genetic variability for root architecture in response to N, a way to also improve WUE and PUE?

About Interactions between C and N metabolisms

- What is the impact of photorespiration (PR) on NUE?
- Are there other yet unknown interconnected regulations/pathways between PR and N metabolism?
- What are the co-regulations of N metabolism, C metabolisms and PR?
- Is it possible to improve NUE by engineering RuBisCo in crops?

Impact of high [CO₂] on NUE



- Does preference for ammonia, nitrate, amino-acid, urea change depending on atmosphere [CO₂]?
- What will be the effect of high [CO₂] on plant/soil/microbe relationships?
- What is the limiting factor that explain that non-legume plants do not benefit importantly from high [CO₂]?
- Does yield-promoting effect of high [CO₂] requires more N input?

What needs to be done to solve the scientific questions

- Enforce the basic knowledge on the regulations of NUE related process in model and crop species.
- Consider post-transcriptional and post-translational regulations of pathways developing further proteomic approaches to identify PTMs and protein-protein interactions.
- Determine the effects of photorespiration and of photosynthesis modes on NUE and N metabolism.
- Multiscale comparison (using multiomics including enzymatic activities, cofactor, PTMs) and modelling the theoretical metabolic limits that control NUE in different species, under different N regimes and in well characterized climate conditions.
- Evaluate the effect of N regimes on root/shoot development, root growth and meristem size.
- Develop dynamic approaches to measure fluxes along plant growth and at different developmental stages.
- Develop molecular tools to enable smooth transfer of signaling and biosynthetic pathways between species.
- Evaluate genetic variability in ancient cereal lines or landgrasses.
- Link the studied metabolic pathways with the NUE components as routinely used by breeders: combine multiscale analyses in a dynamic way, taking into account plant growth and development.
- Consider combinations of abiotic stresses on NUE according to climate change predictions: high temperature, water availability and high [CO₂] in priority.
- Perform meta-analyses on traits, allelic variations of candidate genes, climate situations.
- Confirm in lab results and candidate genes in the field.
- Compare effects of high [CO²] on NupE, NutE, NRE in cereals/dicots; legumes/non-legumes, crucifers/others, C3/C4 and estimate interrelated effects on WUE, NUE, PUE, FeUE etc
- Agroecology issues: Identify helper plants with higher benefit from high [CO₂].

Trends in research, new technology applied

- Multiomics/ metanalyses/ metastudies to predict NUE and identify bottlenecks.
- Gene network analysis to identify regulators and interconnection of regulators and of the response to N and other abiotic and biotic stresses (mainly Arabidopsis and rice)


- Biotechnology approaches (1) Gene stacking and synthetic biology (too few reports to evaluate success; limited due to scarce knowledge of allelic variants/ promoters to be used) (2) elaborated targeted mutagenesis by CrispR Cas technology (no report as far).
- Characterisation of whole plant fluxes to identify bottlenecks (at uptake, assimilation, remobilization levels) in different species.
- GWAS analyses as a potential lever to facilitate the identification of master genes of NUE in crops.

Future challenges in the field to be addressed with high priority

Aspects/opportunities for application of research results

- Transfer of knowledge from model plant to crops (cereals, legumes, vegetable), to identify specific and common actors in N uptake/ assimilation and remobilization.
- Confirm key actors in crops/in field: NBT? Using met-analyses? Using quantitative genetics?
- Application of research results to field experiments: reconsider NBT autorization in the field.
- To analyse bottlenecks in NUE: comparisons of plant species in lab and in field (legume/non-legumes; cereal-dicots, crucifers/other, C3/C4). Need consortium.
- Pay attention to plant/soil/microbes interactions in NUE: plant genotype effects /dependences to soil and microbes.
- Revisit FACE Meta-analyses to identify crop behaviour in high CO2 and estimate opportunities for NUE improvement and consider a second large FACE program that would address more specifically the question of NUE components and the underlying molecular and physiological causes for modified NUE under high CO2

Action points for a future research program in the field

The societal and economic demand is to reduce inorganic N fertilizers maintaining yields and quality of harvested products. Demand of farmers is to consider erratic climate changes and propose solutions that offer robustness and resilience of agriculture systems and stability of crop production under unpredictable extreme climate situations. NUE bottlenecks differ among crop species and specific solutions/strategies have to be considered for different crops. The potential success of different strategies has to be discussed between plant biologists, breeders, farmers and socio-economic researchers to evaluate what are the most efficient (research effort, low cost, feasibility, adaptability, rentability, acceptability by consumers /citizens) strategies to be adopted. Strategies can be traditional plant breeding (how to improve, modify approaches), agroecology (plant associations and plant/microbe associations; agricultural practices; plant genotype requirements), biotechnology (feasibility, cost, genericity, ecological impact, biodiversity impact). Beside efforts to evaluate specific and common bottlenecks in different crops it is essential to predict future impacts of climate changes on strategies proposed to improve NUE. This can be reached by European consortium including breeders and public researchers.



Projects with application relevance

Testing the influence of climate change on the NUE using both elite and ancient varieties in different crop species including trees, vegetables. Consortium agreement on a set of common traits to be evaluated for comparisons.

What needs to be done to support the translation of research results into societal and economic value?

Make research progresses and different strategy acceptable by citizens, providing a readable report of the challenges and of the advantages and disadvantages of each strategy.

References

- 1. Li, et al. (2017) Nitrogen use efficiency in crops: lessons from Arabidopsis and rice. Journal of Experimental Botany 68, 2477-2488, doi:10.1093/jxb/erx101.
- 2. Castro-Rodriguez, et al. (2017) Molecular fundamentals of nitrogen uptake and transport in trees. Journal of Experimental Botany 68, 2489-2500, doi:10.1093/jxb/erx037.
- 3. Hachiya & Sakakibara (2017) Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. Journal of Experimental Botany 68, 2501-2512, doi:10.1093/jxb/erw449.
- 4. Hawkesford, M.J. (2017) Genetic variation in traits for nitrogen use efficiency in wheat. Journal of Experimental Botany 68, 2627-2632, doi:10.1093/jxb/erx079.
- 5. Fan, et al. (2017) Plant nitrate transporters: from gene function to application. Journal of Experimental Botany 68, 2463-2475, doi:10.1093/jxb/erx011.
- Bellegarde, et al. (2017) Signals and players in the transcriptional regulation of root responses by local and systemic N signaling in Arabidopsis thaliana. Journal of Experimental Botany 68, 2553-2565, doi:10.1093/jxb/erx062.
- 7. Jacquot, et al. (2017) Post-translational regulation of nitrogen transporters in plants and microorganisms. Journal of Experimental Botany 68, 2567-2580, doi:10.1093/jxb/erx073.
- 8. Liu, et al. (2017) Ammonium as a signal for physiological and morphological responses in plants. Journal of Experimental Botany 68, 2581-2592, doi:10.1093/jxb/erx086.
- 9. Undurraga, et al. (2017) Nitrate signaling and early responses in Arabidopsis roots. Journal of Experimental Botany 68, 2541-2551, doi:10.1093/jxb/erx041.
- 10. Krapp, A. (2015) Plant nitrogen assimilation and its regulation: a complex puzzle with missing pieces. Current Opinion in Plant Biology 25, 115-122, doi:10.1016/j.pbi.2015.05.010.
- 11. Tegeder & Masclaux-Daubresse (2018) Source and sink mechanisms of nitrogen transport and use. New Phytologist 217, 35-53, doi:10.1111/nph.14876.
- 12. Thu, et al. (2020) Role of ureides in source-to-sink transport of photoassimilates in non-fixing soybean. Journal of Experimental Botany 71, 4495-4511, doi:10.1093/jxb/eraa146.
- 13. Dellagi, et al. (2020) Beneficial soil-borne bacteria and fungi: a promising way to improve plant nitrogen acquisition. Journal of Experimental Botany 71, 4469-4479, doi:10.1093/jxb/eraa112.
- 14. Masclaux-Daubresse, et al. (2010) Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Annals of Botany 105, 1141-1157, doi:10.1093/aob/mcq028.
- 15. Have, et al. (2017) Nitrogen remobilization during leaf senescence: lessons from Arabidopsis to crops. Journal of Experimental Botany 68, 2513-2529, doi:10.1093/jxb/erw365.
- 16. Castro-Rodriguez, et al. (2020) Getting more bark for your buck: nitrogen economy of deciduous forest trees. Journal of Experimental Botany 71, 4369-4372, doi:10.1093/jxb/eraa238.
- 17. Li, et al. (2020) Seasonal nitrogen remobilization and the role of auxin transport in poplar trees. Journal of Experimental Botany 71, 4512-4530, doi:10.1093/jxb/eraa130.
- 18. Gent & Forde (2017) How do plants sense their nitrogen status? Journal of Experimental Botany 68, 2531-2539, doi:10.1093/jxb/erx013.
- 19. Murray, et al. (2017) Nitrogen sensing in legumes. Journal of Experimental Botany 68, 1919-1926, doi:10.1093/jxb/erw405.



- 20. Cohan, et al. (2019) Combining breeding traits and agronomic indicators to characterize the impact of cultivar on the nitrogen use efficiency of bread wheat. Field Crops Research 242, doi:10.1016/j.fcr.2019.107588.
- 21. Lassaletta, et al. (2014) 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. Environmental Research Letters 9, doi:10.1088/1748-9326/9/10/105011.
- 22. Jia & von Wiren (2020) Signaling pathways underlying nitrogen-dependent changes in root system architecture: from model to crop species. Journal of Experimental Botany 71, 4393-4404, doi:10.1093/jxb/eraa033.
- 23. Lay-Pruitt, et al. (2020) Integrating N signals and root growth: the role of nitrate transceptor NRT1.1 in auxinmediated lateral root development. Journal of Experimental Botany 71, 4365-4368, doi:10.1093/jxb/eraa243.
- 24. Luo, et al. (2020) How does nitrogen shape plant architecture? Journal of Experimental Botany 71, 4415-4427, doi:10.1093/jxb/eraa187.
- 25. Maghiaoui, et al. (2020) The Arabidopsis NRT1.1 transceptor coordinately controls auxin biosynthesis and transport to regulate root branching in response to nitrate. Journal of Experimental Botany 71, 4480-4494, doi:10.1093/jxb/eraa242.
- 26. Plett, et al. (2020) The intersection of nitrogen nutrition and water use in plants: new paths toward improved crop productivity. Journal of Experimental Botany 71, 4452-4468, doi:10.1093/jxb/eraa049.
- 27. Bloom, A.J. (2015) Photorespiration and nitrate assimilation: a major intersection between plant carbon and nitrogen. Photosynthesis Research 123, 117-128, doi:10.1007/s11120-014-0056-y.
- 28. Millard, et al. (2007) Environmental change and carbon limitation in trees: a biochemical, ecophysiological and ecosystem appraisal. New Phytologist 175, 11-28, doi:10.1111/j.1469-8137.2007.02079.x.
- 29. Stitt & Krapp (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell and Environment 22, 583-621, doi:10.1046/j.1365-3040.1999.00386.x.
- 30. Bloom, et al. (2010) Carbon Dioxide Enrichment Inhibits Nitrate Assimilation in Wheat and Arabidopsis. Science 328, 899-903, doi:10.1126/science.1186440.
- 31. Bloom, et al. (2014) Nitrate assimilation is inhibited by elevated CO2 in field-grown wheat. Nature Climate Change 4, 477-480, doi:10.1038/nclimate2183.
- 32. Uddling, et al. (2018) Crop quality under rising atmospheric CO2. Current Opinion in Plant Biology 45, 262-267, doi:10.1016/j.pbi.2018.06.001.
- 33. Campbell, et al. (1988) Effects of co2 concentration on rubisco activity, amount, and photosynthesis in soybean leaves. Plant Physiology 88, 1310-1316, doi:10.1104/pp.88.4.1310.
- 34. Rubio-Asensio & Bloom (2017) Inorganic nitrogen form: a major player in wheat and Arabidopsis responses to elevated CO2. Journal of Experimental Botany 68, 2611-2625, doi:10.1093/jxb/erw465.



Focus Group S-2: Improving water uptake and water use efficiency

Bertrand Muller¹, Thierry Simonneau², Matthieu Bogard³, Jean-Charles Deswartes⁴, Jaume Flexas⁵, Jeroni Galmes⁵, Tracy Lawson⁶, Andrew Leakey⁷, Christophe Maurel⁸, Hilde Nelissen⁹, Stacia Stetkiewicz¹⁰

1: **bertrand.muller@inrae.fr** (INRAE Montpellier, FR); 2: Thierry.simonneau@inrae.fr (INRAE Montpellier, FR); 3: m.bogard@arvalis.fr (ARVALIS, FR); 4: jc.deswarte@arvalis.fr (ARVALIS, FR); 5: jaume.flexas@uib.es, jeroni.galmes@uib.es (Univ de les Illes Balears, Spain); 6: tlawson@essex.ac.uk (Univ of Essex, UK); 7: leakey@illinois.edu (University of Illinois, USA); 8: christophe.maurel@cnrs.fr (CNRS Montpellier, FR); 9 : Hilde.Nelissen@psb.vib-ugent.be (VIB Ghent, BE); 10: s.stetkiewicz@lancaster.ac.uk (University of Lancaster, UK).

General background

Demand for agricultural food and non-food products increases while crop production is limited by increasing drought frequency and severity around the globe, further accentuated by the competition among the many water usages. Cultivars that are more efficient in terms of water use are urgently needed in complement to efficient crop management practices (9). In this prospect, Water Use Efficiency (*WUE*) has been introduced as a breeding target with a common use by agronomists and farmers as referring to the ratio of grain yield to the amount of water transpired and sometimes to the ratio of yield to total water use (43). Efficient water use has been further considered at lower levels of plant organisation and shorter timescales (Fig 1): transpiration efficiency (*TE*) was defined at plant level as aerial biomass/water transpired, while instantaneous *WUE* designed the ratio of photosynthesis (A_N) to transpiration rates (E) at leaf or plant level, largely dependent on radiation and vapour pressure deficit (*VPD*), and intrinsic *WUE* (*WUE_i*) was defined as the ratio of A_N to stomatal conductance (g_s). This very active field of research at the different levels and timescales opens multiple avenues to breed for crops with higher *WUE*. These are browsed hereafter disregarding attempts to increase yield (eg via increased harvest index) or net photosynthesis (eg via manipulation of biochemistry, (29) when independent on water use.



Fig. 1. The different dimensions of water use efficiency (34)



Status quo of research in the field

Current know-how

Couplings between growth or An and E

- Adaptive process/evolution has locked together the regulation of water and carbon fluxes in vascular plants via stomata (for CO₂ capture) and leaf area (for CO₂ and light capture), hindering improvement in *WUE* (4).
- Overall, *WUE* increases under drought, primarily because stomatal conductance, and thus water loss, declines more than carbon fixation (17).
- WUE is better conserved across environments for reproductive than vegetative traits (17).
- High turgor is required in growing tissues to trigger expansion (52). This is generally accompanied by efficient root water uptake and high water use with turgid leaves and open stomata.

Margins of progress in WUE have been identified

- Transgenics with differences in A_N do not always couple with g_s (56); 26 but see 10).
- Quantitative genetic approaches applied to water uptake and *WUE*-related traits have identified multiple genetic loci/SNPs (3, 13, 14, 19, 58). Isotopic analysis of ¹³C in plant tissue has been used to reveal genotypic variation in *TE*, and to develop new cultivars with large *TE* (46).
- Advances in mechanisms and traits underlying WUE opened ways to improve WUE by reducing wasteful losses of water (not associated with CO₂ entry for A_N). These have been identified with genetic variation (nighttime stomatal opening, cuticular permeability, notably in growing leaves). Reducing such losses improves *iWUE* (16, 47).

Most relevant research results

WUE at the plant cycle timescale

- Plants growing fast at early stages of development save water by covering soil and weeds, escape terminal drought (and high VPD) situations and result in higher WUE (42)
- Stay-green traits can result in improved WUE under drought conditions (22)

WUE at the daily timescale

- Night-time transpiration can result in significant water loss and reduction of WUE (11)
- WUE is higher in the morning than in the afternoon due to lower evaporative demand in the morning. Therefore, water saving strategy can be to reduce night time transpiration and limit transpiration in the afternoon (38, 49)

WUE and stomata

- Stomatal dynamics and speed of responses differentially influence *An* and *gs*, thereby changing WUE. Smaller stomata facilitate faster response to environmental cues (25).
- In C₄ species, natural variation in *gs* could explain substantially more variation in *iWUE* than A_N (28). This paved the way for improvement in *iWUE* by reducing g_s through reduced stomatal density; eg *EPF1* has been targeted to improve WUE in barley (23), rice (6, 36), wheat (15) and poplar (57). Molecular mechanisms controlling stomatal morphology and patterning have been



elucidated in *Arabidopsis thaliana* (8) offering multiple ways to manipulate stomatal density. However, key stomatal genes can be divergent between the lineages, notably in grasses (1, 45).

- Reduction in stomata size or density, or increase in stomatal responsiveness (through manipulation of ABA biosynthesis or ABA receptors) generally increase WUE although at the expense of A_n (27).
- Higher WUE is relevant only in situations where less water is available whereas more is needed (increasing *VPD* with T elevation, 60)
- WUE is improved under eCO2, but not enough. Mechanistic modeling suggests that enhancing intrinsic WUE (*iWUE*) by reducing stomatal conductance (g_s) while maintaining rates of net CO₂ assimilation (A_N) can increase biomass production across a broad range of environmental conditions (28, 53). However, the optimum relationship A-gs for iWUE often does not work (e.g (28), different species, or (34), different canopy positions).

WUE and canopy architecture

- Plant architecture has differential impacts on transpiration and photosynthesis (34)
- Shaded leaves waste water without benefit for $A_N(2)$
- Optimal use of water requires coordinated, within-plant variations in A_N and g_s with a key role of N redistribution (partly and indirectly controlled by local climate) for the former, and a key role of local climate (more directly) for the latter.

WUE and leaf anatomy

- Optimal use of water requires coordinated development of paths for water and CO2.
- Improving the gm/gs ratio may result in improved WUE (20). Potentially, this could be addressed by improving the tortuosity in the mesophyll (30), cell wall thickness, cell wall composition and/or Sc/S, i.e. the chloroplast distribution (Flexas et al., in press), as well as the presence of trichomes and/or sunken stomata, which affect the boundary layer conductance (21)
- Carbonic anhydrases uncouple CO2 from H2O diffusion in leaves (37)
- Sub-stomatal anatomy influences microscale evaporative conditions without altering stomatal aperture and diffusion of CO2 (5).

WUE, water uptake and water supply to growing tissues

- Several quantitative trait genes linking root hydraulics to water availability and in a broader sense environmental signaling have been characterized (48, 50).
- Aquaporins upward growing zones (18) and/or large contact surface with maturing xylem (31) is favorable to volumetric growth.
- Xylem vulnerability is reached only beyond usual soil water depletion levels (12)

Trends in research, new technology applied

HT Phenotyping and remote sensing

- Optical topometry and machine learning for stomata patterning and leaf gas exchange (59)
- HT chlorophyll fluorescence and thermal imaging (33), including under fluctuating environments (55)



• HT platforms combining transpiration and leaf area measurements (44, 54)

Genetics

- Genome wide analyses help to disentangle the link between WUE related traits (11), (32)
- Use of exotic germplasm (19)
- Use transgenics (eg on stomata kinetics to study coupling between A and Gs (40)

Modeling

- Models coupling soil and root hydraulics help to identify major resistances (7)
- Hydraulic based models have been developed allowing the evaluation of the value of traits on WUE in different G x E x M scenarios (2)

Integration

• Photosynthesis and transpiration traits are part of plant strategy as growth vs. defense (24)

Future challenges in the field to be addressed with high priority

What are the most relevant unsolved questions

On stomata and coupling with A

- What determines the speed of stomatal responses?
- What couples A and gs mechanism(s) (*eg.* using transgenics on A to further study coupling with Tr)

On integration

- What is the Integrated impact of climate change (eCO2 + eTemp) on canopy dynamics and waterconsumption ?
- Under which scenario is WUE improvement desirable (51)?
- Since saving water by closing stomata results in warmer leaves, can it be detrimental under hot climates ?
- What is underlying the fact that few of the lines that work in the lab translate to actual applications? (39)
- What is the link between hydraulic properties at the soil-rhizosphere-root interface and within the plant and WUE ?
- What are the possible links between WUE and other traits of interest (tolerance to anoxia, antioxidant properties, digestibility, nutritional status...)
- What is the impact of sink functions on WUE (through avoidance of photosynthesis feedback)
- How to design G x M systems prepared for the suite of shocks with CC and socio-ecological challenges (water extremes, pest, low input...)

On Genetics / breeding

• What are the genetic basis (loci and genes) underpinning natural variation in crops in traits driving variation in WUE



- Is there enough genetic variation in the current elite gene pool used by breeders to improve WUE in current and future climate/management conditions?
- How to stack (genetically) manipulation of stomata / mesophyll conductance / photosynthesis, and tissue (root) hydraulics to optimize WUE
- What was the role of domestication on WUE related traits (35)

On tools

- Which tools could help screening large breeding populations efficiently for WUE (genetic markers, proxies, phenomics, metabolomics, thermography, other sensors) ?
- How to integrate new HTP methods to capture variation in WUE traits across scales from the cell to the canopy ?

Aspects/opportunities for application of research results

- Guide breeders through crop model based simulation of yield / quality under present and close future environmental scenarios combining eCO2, leaf warming, transpiration, to provide regional scale impact of combinations of WUE related traits
- Define and build water efficient ideotypes by combining improved gm/gs, the tortuosity in the mesophyll, cell wall thickness etc... An ideotype could present high mesophyll porosity and Sc/S coupled with thin cell walls with increased pectin fraction
- Screen (for WUE and/or traits underlying WUE) of already existing large breeding / pre-breeding populations
- Provide of non-transgenic targets (tilling) obtained from current understanding of the regulation of gs and/or A and their links
- Implement remote sensing for Water Use (IRT imaging) and plant growth (NDVI) with breeders
- Use GM and mutants as tools to address stomatal/gs/A links and stomatal dynamics
- Integrate new HTP methods to capture variation in WUE traits across scales from the cellular to the canopy (Target technical solutions where manipulation to reduce leaf water loss is combined with enhancements of photosynthesis driven by greater ϕ CO2)

Action points for a future research program

What needs to be done to solve the scientific questions and meet the societal and economic challenges?

- A concerted effort for phenotyping and model parameterization using experiments in controlled conditions and field trials, all leading to reusable and shared data (eg. through concerted development of EU-wide phenotyping capacities and appropriate instrumentation of fields (ESFRI EMPHASIS)
- A concerted effort towards model improvement, eg. to incorporate steady-state / dynamic processes (g_s...) into models
- Co-design of new G with innovative M (Management practices) for highest benefits in terms of water use



• Develop systems-view of water use, considering trade-offs between potentially opposite objectives such as high WUE / yield penalty (41), including optimization, risk analysis (based on probability of drought occurrence), stabilizing farmer's economic yield.

Projects with application relevance

- Screen large genetic diversity panels in the field (possibly including genetic resources, mutants, GM) for WUE and allelic diversity
- Pyramiding interesting traits to overcome negative impacts of higher WUE (example in Australia with higher early vigor + improved WUEi)
- Manipulating epidermal/stomatal patterning and dynamics to optimize gs to current or future CO2 concentrations
- Develop cheap and quick phenotyping tools for breeders for water uptake, WUE

What needs to be done to support the translation of research results into societal and economic value?

- Large collaborative public x private partnership to tackle complex issues demanding concerted effort and large support (field trials, genetic material, model exchanges)
- NBTs would certainly provide more degrees of freedom on the complex, intertwined system.

References

- 1. Abrash E, Gil MXA, Matos JL, Bergmann DC. 2018. Conservation and divergence of YODA MAPKKK function in regulation of grass epidermal patterning. *Development*. 145(14):
- 2. Albasha R, Fournier C, Pradal C, Chelle M, Prieto JA, et al. 2019. HydroShoot: a functional-structural plant model for simulating hydraulic structure, gas and energy exchange dynamics of complex plant canopies under water deficit—application to grapevine (Vitis vinifera). *in silico Plants*. 1(1):diz007
- 3. Arab MM, Marrano A, Abdollahi-Arpanahi R, Leslie CA, Cheng H, et al. 2020. Combining phenotype, genotype, and environment to uncover genetic components underlying water use efficiency in Persian walnut. *Journal of Experimental Botany*. 71(3):1107–27
- 4. Brodribb TJ, Sussmilch F, McAdam SAM. 2020. From reproduction to production, stomata are the master regulators. *The Plant Journal*. 101(4):756–67
- 5. Buckley TN, John GP, Scoffoni C, Sack L. 2017. The Sites of Evaporation within Leaves. *Plant Physiology*. 173(3):1763–82
- 6. Caine RS, Yin X, Sloan J, Harrison EL, Mohammed U, et al. 2019. Rice with reduced stomatal density conserves water and has improved drought tolerance under future climate conditions. *New Phytologist*. 221(1):371–84
- 7. Carminati A, Javaux M. 2020. Soil rather than xylem vulnerability controls stomatal response to drought. *Trends in plant science*
- 8. Chater CCC, Caine RS, Fleming AJ, Gray JE. 2017. Origins and Evolution of Stomatal Development. *Plant Physiology*. 174(2):624–38
- 9. Condon AG. 2020. Drying times: plant traits to improve crop water use efficiency and yield. *Journal of Experimental Botany*. 71(7):2239–52
- 10. Conesa MÀ, Mus M, Galmés J. 2019. Leaf size as a key determinant of contrasting growth patterns in closely related Limonium (Plumbaginaceae) species. *Journal of Plant Physiology*. 240:152984
- Coupel-Ledru A, Lebon E, Christophe A, Gallo A, Gago P, et al. 2016. Reduced nighttime transpiration is a relevant breeding target for high water-use efficiency in grapevine. *Proceedings of the National Academy of Sciences*. 113(32):8963–68
- 12. Dayer S, Herrera JC, Dai Z, Burlett R, Lamarque LJ, et al. 2020. The sequence and thresholds of leaf hydraulic traits underlying grapevine varietal differences in drought tolerance. *Journal of experimental botany*. 71(14):4333–44



- 13. Des Marais DL, Razzaque S, Hernandez KM, Garvin DF, Juenger TE. 2016. Quantitative trait loci associated with natural diversity in water-use efficiency and response to soil drying in Brachypodium distachyon. *Plant Science*. 251:2–11
- Dhanapal AP, Ray JD, Singh SK, Hoyos-Villegas V, Smith JR, et al. 2015. Genome-wide association study (GWAS) of carbon isotope ratio (δ 13 C) in diverse soybean [Glycine max (L.) Merr.] genotypes. *Theoretical and Applied Genetics*. 128(1):73–91
- 15. Dunn J, Hunt L, Afsharinafar M, Meselmani MA, Mitchell A, et al. 2019. Reduced stomatal density in bread wheat leads to increased water-use efficiency. *Journal of Experimental Botany*. 70(18):4737–48
- Duursma RA, Blackman CJ, Lopéz R, Martin-StPaul NK, Cochard H, Medlyn BE. 2019. On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. *New Phytologist*. 221(2):693–705
- Edwards CE, Ewers BE, McClung CR, Lou P, Weinig C. 2012. Quantitative Variation in Water-Use Efficiency across Water Regimes and Its Relationship with Circadian, Vegetative, Reproductive, and Leaf Gas-Exchange Traits. *Molecular Plant*. 5(3):653–68
- Ehlert C, Maurel C, Tardieu F, Simonneau T. 2009. Aquaporin-mediated reduction in maize root hydraulic conductivity impacts cell turgor and leaf elongation even without changing transpiration. *Plant Physiology*. 150(2):1093–1104
- 19. Ferguson JN, Fernandes SB, Monier B, Miller ND, Allan D, et al. 2020. Machine learning enabled phenotyping for GWAS and TWAS of WUE traits in 869 field-grown sorghum accessions. *bioRxiv*. 2020.11.02.365213
- 20. Flexas J, Niinemets Ü, Gallé A, Barbour MM, Centritto M, et al. 2013. Diffusional conductances to CO 2 as a target for increasing photosynthesis and photosynthetic water-use efficiency. *Photosynthesis research*. 117(1):45–59
- 21. Galdon-Armero J, Fullana-Pericas M, Mulet PA, Conesa MA, Martin C, Galmes J. 2018. The ratio of trichomes to stomata is associated with water use efficiency in *Solanum lycopersicum* (tomato). *Plant J*. 96(3):607–19
- 22. Górny AG, Garczyñski S. 2002. Genotypic and nutrition-dependent variation in water use efficiency and photosynthetic activity of leaves in winter wheat (Triticum aestivum L.). *J. Appl. Genet.* 43((2)):145–60
- 23. Hughes J, Hepworth C, Dutton C, Dunn JA, Hunt L, et al. 2017. Reducing Stomatal Density in Barley Improves Drought Tolerance without Impacting on Yield. *Plant Physiology*. 174(2):776–87
- 24. Karabourniotis G, Liakopoulos G, Nikolopoulos D, Bresta P, Stavroulaki V, Sumbele S. 2014. "Carbon gain vs. water saving, growth vs. defence": two dilemmas with soluble phenolics as a joker. *Plant Science*. 227:21–27
- 25. Lawson T, Blatt MR. 2014. Stomatal Size, Speed, and Responsiveness Impact on Photosynthesis and Water Use Efficiency. *Plant Physiology*. 164(4):1556–70
- 26. Lawson T, Lefebvre S, Baker NR, Morison JIL, Raines CA. 2008. Reductions in mesophyll and guard cell photosynthesis impact on the control of stomatal responses to light and CO2. *Journal of Experimental Botany*. 59(13):3609–19
- 27. Lawson T, Vialet-Chabrand S. 2019. Speedy stomata, photosynthesis and plant water use efficiency. *New Phytol*. 221(1):93–98
- 28. Leakey AD, Ferguson JN, Pignon CP, Wu A, Jin Z, et al. 2019. Water use efficiency as a constraint and target for improving the resilience and productivity of C3 and C4 crops. *Annual Review of Plant Biology*. 70:781–808
- 29. López-Calcagno PE, Brown KL, Simkin AJ, Fisk SJ, Vialet-Chabrand S, et al. 2020. Stimulating photosynthetic processes increases productivity and water-use efficiency in the field. *Nature Plants*. 6(8):1054–63
- 30. Lundgren MR, Mathers A, Baillie AL, Dunn J, Wilson MJ, et al. 2019. Mesophyll porosity is modulated by the presence of functional stomata. *Nature Communications*. 10(1):2825
- 31. Martre P, Durand J-L, Cochard H. 2000. Changes in axial hydraulic conductivity along elongating leaf blades in relation to xylem maturation in tall fescue. *New Phytologist*. 146(2):235–47
- 32. Maurel C, Nacry P. 2020. Root architecture and hydraulics converge for acclimation to changing water availability. *Nature Plants*. 6(7):744–49
- 33. McAusland L, Davey PA, Kanwal N, Baker NR, Lawson T. 2013. A novel system for spatial and temporal imaging of intrinsic plant water use efficiency. *Journal of Experimental Botany*. 64(16):4993–5007
- Medrano H, Tomás M, Martorell S, Flexas J, Hernández E, et al. 2015. From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target. *The Crop Journal*. 3(3):220– 28



- 35. Milla R, Morente-López J, Alonso-Rodrigo JM, Martín-Robles N, Stuart Chapin F. 2014. Shifts and disruptions in resource-use trait syndromes during the evolution of herbaceous crops. *Proceedings of the Royal Society B: Biological Sciences*. 281(1793):20141429
- 36. Mohammed U, Caine RS, Atkinson JA, Harrison EL, Wells D, et al. 2019. Rice plants overexpressing OsEPF1 show reduced stomatal density and increased root cortical aerenchyma formation. *Scientific Reports*. 9(1):5584
- 37. Momayyezi M, McKown AD, Bell SCS, Guy RD. 2020. Emerging roles for carbonic anhydrase in mesophyll conductance and photosynthesis. *The Plant Journal*. 101(4):831–44
- 38. Nelson JA, Carvalhais N, Migliavacca M, Reichstein M, Jung M. 2018. Water-stress-induced breakdown of carbon– water relations: indicators from diurnal FLUXNET patterns. *Biogeosciences*. 15(8):2433–47
- 39. Nuccio ML, Paul M, Bate NJ, Cohn J, Cutler SR. 2018. Where are the drought tolerant crops? An assessment of more than two decades of plant biotechnology effort in crop improvement. *Plant Science*. 273:110–19
- 40. Papanatsiou M, Petersen J, Henderson L, Wang Y, Christie JM, Blatt MR. 2019. Optogenetic manipulation of stomatal kinetics improves carbon assimilation, water use, and growth. *Science*. 363(6434):1456–59
- 41. Passioura J. 2008. Drought resistant crops: wet dream or pipe dream? *Australian Grain*. 17(7):18–21
- 42. Passioura JB. 2020. Translational research in agriculture. Can we do it better? *Crop Pasture Sci.* 71(6):517–28
- 43. Passioura JB, Angus JF. 2010. Chapter 2 Improving Productivity of Crops in Water-Limited Environments. In *Advances in Agronomy*, ed DL Sparks. 106:37–75. Academic Press
- 44. Prado SA, Cabrera-Bosquet L, Grau A, Coupel-Ledru A, Millet EJ, et al. 2017. Phenomics allows identification of genomic regions affecting maize stomatal conductance with conditional effects of water deficit and evaporative demand. *Plant, cell & environment*
- 45. Raissig MT, Abrash E, Bettadapur A, Vogel JP, Bergmann DC. 2016. Grasses use an alternatively wired bHLH transcription factor network to establish stomatal identity. *Proceedings of the National Academy of Sciences*. 113(29):8326–31
- 46. Rebetzke GJ, Condon AG, Richards RA, Farquhar GD. 2002. Selection for Reduced Carbon Isotope Discrimination Increases Aerial Biomass and Grain Yield of Rainfed Bread Wheat. *Crop Science*. 42(3):739–45
- 47. Schoppach R, Sinclair TR, Sadok W. 2020. Sleep tight and wake-up early: nocturnal transpiration traits to increase wheat drought tolerance in a Mediterranean environment. *Funct Plant Biol*. 47(12):1117–27
- 48. Shahzad Z, Canut M, Tournaire-Roux C, Martinière A, Boursiac Y, et al. 2016. A Potassium-Dependent Oxygen Sensing Pathway Regulates Plant Root Hydraulics. *Cell*. 167(1):87-98.e14
- 49. Tamang BG, Sadok W. 2018. Nightly business: links between daytime canopy conductance, nocturnal transpiration and its circadian control illuminate physiological trade-offs in maize. *Environmental and Experimental Botany*. 148:192–202
- 50. Tang N, Shahzad Z, Lonjon F, Loudet O, Vailleau F, Maurel C. 2018. Natural variation at XND1 impacts root hydraulics and trade-off for stress responses in Arabidopsis. *Nature Communications*. 9(1):3884
- 51. Tardieu F. 2012. Any Trait or Trait-Related Allele Can Confer Drought Tolerance: Just Design the Right Drought Scenario. *J. Exp. Bot.* 63(1):25–31
- 52. Tardieu F, Simonneau T, Parent B. 2015. Modelling the coordination of the controls of stomatal aperture, transpiration, leaf growth, and abscisic acid: update and extension of the Tardieu–Davies model. *Journal of Experimental Botany*. 66(8):2227–37
- 53. Truong SK, McCormick RF, Mullet JE. 2017. Bioenergy Sorghum Crop Model Predicts VPD-Limited Transpiration Traits Enhance Biomass Yield in Water-Limited Environments. *Front. Plant Sci.* 8:
- 54. Vadez V, Kholová J, Hummel G, Zhokhavets U, Gupta SK, Hash CT. 2015. LeasyScan: a novel concept combining 3D imaging and lysimetry for high-throughput phenotyping of traits controlling plant water budget. *Journal of Experimental Botany*. 66(18):5581–93
- 55. Vialet-Chabrand S, Lawson T. 2019. Dynamic leaf energy balance: deriving stomatal conductance from thermal imaging in a dynamic environment. *Journal of Experimental Botany*. 70(10):2839–55
- 56. von Caemmerer S, Lawson T, Oxborough K, Baker NR, Andrews TJ, Raines CA. 2004. Stomatal conductance does not correlate with photosynthetic capacity in transgenic tobacco with reduced amounts of Rubisco. *Journal of experimental botany*. 55(400):1157–66
- 57. Wang C, Liu S, Dong Y, Zhao Y, Geng A, et al. 2016. PdEPF1 regulates water-use efficiency and drought tolerance by modulating stomatal density in poplar. *Plant Biotechnology Journal*. 14(3):849–60
- 58. Wang H, Zhao S, Mao K, Dong Q, Liang B, et al. 2018. Mapping QTLs for water-use efficiency reveals the potential candidate genes involved in regulating the trait in apple under drought stress. *BMC Plant Biology*. 18(1):136



- 59. Xie J, Mayfield-Jones D, Erice G, Choi M, Leakey ADB. 2020. Optical topometry and machine learning to rapidly phenotype stomatal patterning traits for QTL mapping in maize. *bioRxiv*. 2020.10.09.333880
- 60. Yuan W, Zheng Y, Piao S, Ciais P, Lombardozzi D, et al. 2019. Increased atmospheric vapor pressure deficit reduces global vegetation growth. *Science Advances*. 5(8):eaax1396



Focus Group S-3: Improving phosphorus uptake and use efficiency

Stéphane Abel¹, Francesca Degan², Thierry Desnos³, L., Delphine Hourcade², Michael Hothorn⁴, *Laurent Nussaume³*, Javier Paz-Ares⁵, Yves Poirier⁶, Claude Plassard⁷ Hatem Rouached⁸, Jinsheng Zhu⁶

1: <u>Stephane.ABEL@cea.fr</u> (Department of Molecular Signal Processing, Leibniz Institute of Plant Biochemistry, Halle, GER); 2: <u>F.DEGAN@arvalis.fr</u> - <u>D.HOURCADE@arvalis.fr</u> (ARVALIS, Institut du Végétal, Paris), FR; 3: <u>thierry.desnos@cea.fr</u> - <u>laurent.nussaume@cea.fr</u> (CEA, CNRS, Aix Marseille Univ, Saint-Paul lez Durance, FR); 4: <u>michael.hothorn@unige.ch</u> (Structural Plant Biology Laboratory, Department of Botany and Plant Biology, University of Geneva, CH); 5: <u>ipazares@cnb.csic.es</u> (Centro Nacional de Biotecnologia, Consejo Superior de Investigaciones Cientificas, Campus de Cantoblanco, Madrid, Spain); 6: <u>vves.poirier@unil.ch</u> - <u>jinsheng.zhu@unil.ch</u> (Department of Plant Molecular Biology, University of Lausanne, CH); 7: <u>claude.plassard@inrae.fr</u> (INRAE, Eco&Sols, Montpellier, FR); 8: <u>hatem.rouached@gmail.com</u> (Plant, Soil, and Microbial Sciences, Michigan State University, East-Lansing, Michigan, USA).

Status quo of research in the field

Pi in soil /Fertilizers

Phosphorus (P) is a major macronutrient limiting plant growth and yield. Plants acquire P exclusively in the form of inorganic phosphate (Pi) from the soil, either directly through the roots or indirectly through the mycorrhizal fungi associated with them. Optimal acquisition of Pi involve specific adaptations of root architecture (e.g., top soil foraging, modification of root hairs, cluster roots), coordinated Pi uptake and distribution in various tissues through complex regulatory mechanisms implicating multigenic families of transporters, and secretion of phosphatase and organic acids to recover organic Pi or solubilize mineral Pi (Chiou and Lin, 2011).

Despite its relative abundance on earth, P remains very unevenly distributed in soils across the world. It is estimated that one third of total cultivated soils are lacking available P for optimal plant growth (MacDonald et al., 2011; Alewell et al., 2020). The reasons for this are multiple. Firstly, Pi has very poor mobility in soils, leading to the majority of the Pi applied from fertilizers being recovered by microorganisms at the expense of the crops. Secondly, Pi forms insoluble complexes with many soil cations or chelates with clays, resulting in reduced bioavailability for roots. Such phenomenon is strongly regulated by pH which favors more or less charged forms. Pi uptake by plants occurs mostly with $H_2PO_4^-$ anionic soluble form present at ~ pH 5-6. In acidic and deeply altered soils, particularly frequent in the tropics, most Pi is found bound to clay minerals and oxy(hydr)oxides of Fe and Al. In such conditions, secretion by plant and/or microorganisms of organic acids is an important mechanism used to modify these associations and enhance Pi availability.

The complexity of Pi interactions with other ions explains the <u>difficulty of measuring Pi</u> <u>bioavailability in soil</u> and to control accurate use of Pi fertilizers (it is assumed that no more than 20% of the Pi fertilizers applied are recovered by plants). And yet this is crucial as excess of Pi fertilizers promote severe environmental damages, such as rivers and lakes eutrophication, due to the leaching of Pi fertilizer in rivers and lakes, leading to toxic cyanobacteria blooms and metal



pollution. The vast majority of the mined P-rich rock contains high levels of toxic metals such as cadmium, chromium, mercury, leads, polonium, uranium or thorium, and some of these metals are found in applied Pi fertilizers. This already impacts the trophic chain and led the EC to reinforce the legislation on the amount of cadmium present in Pi fertilizers (https://ec.europa.eu/growth/content/cadmium-fertilisers_is).

Plant

In the last century, we witnessed an incredible progress in our understanding of plants' adaptation to Pi deficiency, including the discovery of the partially independent Pi signaling pathways. One of these pathway controls <u>root tip</u> growth and metal homeostasis. It is regulated by local extracellular Pi, its intrinsic ionic properties allowing it to chelate Fe³⁺. The iron:Pi ratio determines the level of reactive oxygen species (ROS), which when increased leads to callose deposition, impairing symplastic movement necessary for meristem maintenance and primary root growth. Another pathway controls the remaining Pi starvation responses, and is primarily dependent on intracellular Pi sensing. It is systemically regulated by the overall plant Pi status. More than 20 Pi signaling components have been identified, including sensing mechanisms acting via Pi-containing metabolites (Pi-rich inositol pyrophosphates), whose synthesis is Pi dependent (Zhu et al., 2019). They promote the association of repressors (SPX proteins) with the master transcription factors (PHR1) regulating the Pi starvation responses (Wang et al., 2014; Abel, 2017; Puga et al., 2017).

Many hormonal and nutritional signals are also known to modulate the phosphate starvation responses, including <u>cytokinins</u> (CK), <u>strigolactones</u> (SL), auxins (Aux), ethylene (ET), jasmonates (JA), <u>gibberellins</u> (GA) and <u>brassinosteroids</u> (BR), as well as sucrose, nitrate (N), zinc, and calcium. Crosstalk between Pi starvation and plant defense has also been uncovered (Castrillo et al., 2017). One paradigmatic example of these interconnections is the N-P signaling crosstalk that involves interaction between Pi and nitrate sensors (SPX4 and NTR1.1), and also involves PHO2, a protein involved in proteasome-mediated protein degradation, to ensure coordinated acquisition and use of these key elements (Medici et al., 2015; Hu et al., 2019; Medici et al., 2019; Ueda et al., 2020).

Microbiome

Soil organic P (Po) constitutes a variable fraction of the soil total extractable P (Stutter et al., 2012). In Europe, Po ranged from 25% to 50 % in arable and pasture soils, respectively. Po therefore warrants attention as a quantitatively important soil P resource. To be used by plants (and microorganisms), the Pi group must be released by phosphatases secreted into the external medium. Most of the time, the release of these enzymes by plants, bacteria or fungi depend on Pi availability in soil and are up-regulated at very low levels of Pi in the solution. More importantly, the microorganisms that produce these enzymes are also the primary users of the Pi released, thus decreasing the bioavailability of Pi for plants (Pistocchi et al., 2018).

In addition to phosphatases, numerous microorganisms can also release organic acids that contribute to the solubilization of mineral Pi. Interestingly, fertilization of tropical ferralsol with rock P was found to be as efficient as the more soluble Pi-based fertilizer (triple superphosphate) to enhance the yield of soybean (Ndungu-Magiroi et al., 2015). Analysis revealed that sparingly soluble Pi from rock P stimulated the populations of native Phosphate Solubilizing Bacteria (PSB) explaining this result. This may represent a promising way for minimizing the utilization of mineral P fertilizers.



Besides the microbial communities living in the rhizosphere, about 80% of plant species establish a symbiosis with mycorrhizal fungi promoting primary beneficial effect for plant Pi nutrition. Indeed, mycorrhizal fungi considerably increase the volume of soil that can be exploited by the root, resulting in a better Pi acquisition (Briat et al., 2015; Wipf et al., 2019; Briat et al., 2020). In addition to the efficiency of fungal cells to take up Pi not directly available to root cells, the hyphae can recruit bacterial communities able to solubilize mineral P and/or mineralize organic P (Briat et al., 2020). Given the importance of mycorrhizal symbiosis, several products are now commercially available. A recent arbuscular mycorrhizal fungi (AMF) inoculant benchmarks (Basiru et al., 2020) found that most of the products (84%) provide plant nutrient benefits either using soil application or seed coating. This last technique has thus great potential for increasing inoculation efficiency over largescale production due to minimum inoculum use. Other commercial products are also based on addition of micro-organisms, such as non-mycorrhizal fungi and/or bacteria expected to help Pi solubilization. However it is currently difficult to control bacterial populations out of the laboratories due to competitions with soil microflora and putative ecological consequences (Hinsinger et al., 2011). Field experiments conducted so far with this type of products showed relative little benefits (i.e., Arvallis). However, the situation is completely different in the context of controlled environments, such as hydroponics in greenhouses, where microbiomes can be manipulated at will. Application in this case may be realistic at the medium term (5-10 years).

Future challenges in the field to be addressed with high priority

Scientific challenges

Elucidating Pi signaling and transport

Despite crucial discoveries, our understanding of Pi signaling in plants remains very fragmentary compared to many other signaling pathways such as light, hormones, or pathogen interaction). The exact nature of the signals (Pi, ATP, PP-InsPs...) and the complexity of interconnection with other pathways (such as carbon, nitrogen, metals...) need clearly to be resolved. These studies remain challenging as many Pi signaling genes are arranged in multi gene families whose activity can be regulated in very distinct ways. For example, the SPX domain shown to interact with the signaling molecule inositol pyrophosphates, is found in 15 proteins in Arabidopsis. Similarly, the dynamic Pi transport from soil to roots and between the various tissues involves a multitude of Pi importers and exporters, of which only few have been studied in sufficient detail to understand their impact on Pi homeostasis and crop yield. It is important to rationalize our search for genes candidates for translational biology targets (plant improvement). Besides, if we want to have precision agriculture in the future and spare Pi crucial strategic resource, we clearly need the physiological knowledges of Pi transport and adaptation to Pi deficiency to optimize crops production. This is all the more important since symptoms of Pi deficiency are not easy to detect at an early stage and breeders lack easy tools for rationalizing quantification of this phenomenon.

Deciphering real putative input of microbiomes to plant Pi acquisition

From the state of the art, it is clear that the input of microbial inoculant is not yet firmly established in the field. The first step is to assess whether or not the inoculated microbes are able to develop in the rhizosphere or the roots in the field as a function of (i) the crop species and (ii) the soil conditions, especially Pi availability and pH. Regarding the crop species, they could be separated in



broad categories that are (i) cereals developing a highly ramified and extensive root system, (ii) legumes with a less ramified root system but generally able to explore deeper soil horizons than cereals, (iii) tubercule root system such as potato, and (iv) non-mycorrizal crop belonging to Brassicaceae or Chenopodiaceae. From these characteristics, it can be expected a better effect of microbial inoculants with AMF on legumes and tuberculated species. Alternatively, non-mycorrhizal crops could only rely on PSB microbial inoculants, but these hypotheses should be studied. Soil conditions might also be decisive to determine the effects of microbial inoculants. Specific focus on Pi availability will be necessary as AMF symbiosis does not take place at very low but also at high level of Pi availability. Furthermore, the main geochemical characteristics of soil should be taken into account when applying bio-inoculants based on phosphate-solubilizing organisms that may have difficulties to establish in acid soils. Hence, we are still lacking knowledge linking the performances of microbial inoculants and the crop species, together with soil conditions.

Identify Phosphate Use Efficiency (PUE) traits or select plants adapted to Pi depleted soil

There is a need to distinguish between Pi uptake efficiency, e.g. traits that lead to greater acquisition of Pi from the environment, from phosphate use efficiency (PUE) that encompass traits that not only improve Pi acquisition, but more importantly improve its overall utilization to increase production of the harvestable products (typically seeds) under fixed amount of bioavailable Pi. Important aspects of PUE include: (1) Pi recovery in soils; (2) improved soil exploration by roots, (3) Pi uptake, (4) physiological use of Pi for growth and (5) yield production. Analysis of PUE traits has been performed in many species to compare variety such as for Coffea (Neto et al., 2016) or to map QTL in in rice, maize, wheat, common bean, soybean and oilseed rape (Wissuwa et al., 1998; Chen et al., 2009; Yuan et al., 2017; Wang et al., 2019). However, the identification of the causal genes responsible for these traits remain unachieved and so far, the only exception being the gene PSTOL1, encoding a protein kinase, involved a root architecture in rice (Chin et al., 2011; Gamuyao et al., 2012). PSTOL1 expression has been shown to enhance top soil foraging in other species, such as sorghum, demonstrating interest of translational biology for these approaches (Hufnagel et al., 2014). It is clear that we should increase the quest of targets for PUE traits, identify the underlining causal genes and use potentially them as markers for selecting P efficient crops. Performing analysis of PUE in plant species with different PUE characteristics may help to identify bottlenecks and relevant genes in different genetic backgrounds. Because of interactions among different nutrients (e.g. P, N, Fe), these studies should be performed under different nutrient supply.

In addition to studies with plants grown in controlled conditions (e.g., phytotrons and greenhouses), it will be necessary to perform field trials. For such experiments, it will be crucial to have access to reliable Pi bio-availability data on the long term. Such parameter is difficult to access and rely on assays of soil P extracted with chemical extractants. Among the methods routinely used by analytical laboratories, the Olsen test (Olsen et al., 1954) appears probably as the most suitable. Hence, it would be very fruitful to combine systematically PUE traits quest with Olsen P measurements in soils.

Another way to cope with low Pi containing environment would be to select plant species adapted to such environment. Leguminosae, Proteaceae, Casuarinaceae, Myricaceae, Eleagnaceae, and Betulaceae often develop adaptative traits such as cluster roots (specific root adaptation improving Pi recovery in the environment). Few plants in those families have economical interest (ex Lupinus) for farmers and could provide an opportunity to limit the use of fertilizers within the framework of



a rational agricultural policy or can have interest for culture in Pi depleted soil. Nevertheless, as most important crops do not have such feature this can have only limited applications.

Development of improved fertilizers to improve the efficiency of P nutrition

Different solutions relying on improved P fertilization of soils should be considered:(i) coating of fertilizers (to increase progressive release period in the soil), (ii) improve Pi bioavailability (by identifying process to recover mineral or organic Pi), (iii) precision agriculture (reduce Pi by providing micro dosage of fertilizer in the vicinity of the roots).

Improved recycling of organic P appears as a potential strategy because it could decrease the need for mineral P fertilization and reinforce the bio-economy. Nowadays, the main sources of organic P come from manures that are applied in the field. However, the efficiency of this strategy will probably be limited as it will depend on the efficiency of enzymes to release Pi from organic P compounds. As stated above, phosphatases will be mainly released by microbial populations in case of P deficiency and the released Pi will be mostly taken up by these microbial populations. Improving the competitive advantage of the plant roots towards newly released Pi from organic sources thus remains a challenge.

Precision agriculture should also take into account interactions with others elements. For example, nitrogen fertilization, using either ammonium or nitrate-based fertilizer, could be an efficient way to drive pH changes in the rhizosphere. Indeed, ammonium will induce an acidification while nitrate will induce an alkalinization. Thus, depending on the soil context, mineral Pi solubilization could be strongly enhanced at neutral pH in soil high in cations such as Ca or Mg, just by providing ammonium as fertilizers. In contrast, acidic soils containing mineral P associated with Fe and Al, nitrate fertilization should be favored as these P minerals dissolved with pH increases. But these hypotheses need to be firmly established with field experiments.

In certain agricultural practices, hydroponics is an option to avoid the unpredictable impact of soils. Combined to the use of greenhouses, and applied to grow at high density, it can lead to increases of yields by a factor of 10, including for major crops such as tomatoes (according Global Hydroponics Market Analysis & Trends - Industry Forecast to 2025" report). However, such a cultivation practice is limited to specific high-value crops to be economically profitable. It is also a way to spare Pi resource as plant can better directly access to soluble Pi because of reduced competition from microbial community. In this boosting soil-less cultures technological era, a full grasp of the plant ability to extract nutrients from solutions, including Pi, becomes a central aspect of biomass production. In this context, basic research on Pi uptake remains crucial and define plants to target.

Societal challenges

Reducing Pi fertilizer is an environmental necessity to avoid eutrophication of rivers and lakes, reduce metal contamination in soils and aquifer and spare a limited strategical resources, such as high quality phosphate rock (Jiao et al., 2012). Even if expert disagree on the exact timing of coming shortage, it is expected to take place within next 100 to 150 years (Gilbert, 2009).

Important laws have and will reinforce the regulation of these aspects: in June 2019, the European parliament proposed a text reducing to 60 mg/kg the amount of Cadmium allowed in Pi fertilizers. This limitation should be strength within next 10 to 15 years.



Economic challenges

Sustaining (or even increase) crop production despite reduction of Pi fertilizer use is a clear technical and scientific challenge, which will require to act on all actors involved (soil, agriculture practice, fertilizers and plant selection).

Quantify bio-availability of Pi in soil in the most efficient way is crucial to rationalize Pi fertilizer uses and reduce metal contamination of soils.

Aspects/opportunities for application of research result

Identifying targets for plant breeding and translational biology should be a priority. Indeed, if there has already been many researches on this subject (QTL), we still lack the identification of clear molecular targets that can be used in selection schemes.

Deciphering indicators to monitor reliably bio-available Pi is also crucial to limit and/or rationalize Pi fertilizer management. This go beyond agriculture, indeed computer models predicting changes in lignocellulosic biomass are essential for predicting climate changes ant P nutrition is an important feature (Kvakic et al., 2020). However, they cannot yet take into account at broad scale the problems of phosphate bio-availability.

Action points for a future research program in the field

What needs to be done to solve the scientific questions and to meet the societal and economic challenges?

- A strong input in fundamental biology remains essential to elucidate mechanisms controlling Pi homeostasis (to spare resources we should focus on restricted number of species for fundamental research).
- Importance to take into account impact of many focus groups on Pi nutrition (root architecture, photosynthesis, interaction with other nutrients or micronutrients, soil rhizosphere...)
- Access to field resources or screening facilities well defined to identify PUE traits (we need places where bio-available Pi present in soils are well characterized) to perform middle or long term field trials for plant selection.
- Test novel solution acting on modification of soil parameters or novel fertilizers.

What needs to be done to support the translation of research results into societal and economic value?

Here, one of the main challenge will be to see if biotechnologies and translational biology can be socially accepted and to which extend (GMO/CRISPR/classical selection...). Indeed, if traits identified need to be transferred between species this will provide clue for selection schema to use (gene transfer through biotechnology or search for traits variability in each species and transfer through selection.



References

Abel S (2017) Phosphate scouting by root tips. Curr Opin Plant Biol 39: 168-177

- Alewell C, Ringeval B, Ballabio C, Robinson DA, Panagos P, Borrelli P (2020) Global phosphorus shortage will be aggravated by soil erosion. Nat Commun 11: 4546
- Basiru S, Mwanza HP, Hijri M (2020) Analysis of Arbuscular Mycorrhizal Fungal Inoculant Benchmarks. Microorganisms 9
- Briat J-F, Gojon A, Plassard C, Rouached H, Lemaire G (2020) Reappraisal of the central role of soil nutrient availability in nutrient management in light of recent advances in plant nutrition at crop and molecular levels. Eur J Agron 116: 126069
- Briat JF, Rouached H, Tissot N, Gaymard F, Dubos C (2015) Integration of P, S, Fe, and Zn nutrition signals in Arabidopsis thaliana: potential involvement of PHOSPHATE STARVATION RESPONSE 1 (PHR1). Front Plant Sci 6: 290
- Castrillo G, Teixeira PJ, Paredes SH, Law TF, de Lorenzo L, Feltcher ME, Finkel OM, Breakfield NW, Mieczkowski P, Jones CD, Paz-Ares J, Dangl JL (2017) Root microbiota drive direct integration of phosphate stress and immunity. Nature
- Chen JY, Xu L, Cai YL, Xu J (2009) Identification of QTLs for phosphorus utilization efficiency in maize (Zea mays L.) across P levels. Euphytica 167: 245-252
- Chin JH, Gamuyao R, Dalid C, Bustamam M, Prasetiyono J, Moeljopawiro S, Wissuwa M, Heuer S (2011) Developing Rice with High Yield under Phosphorus Deficiency: Pup1 Sequence to Application. Plant Physiol 156: 1202-1216
- Chiou TJ, Lin SI (2011) Signaling network in sensing phosphate availability in plants. Annu Rev Plant Biol 62: 185-206
- Gamuyao R, Chin JH, Pariasca-Tanaka J, Pesaresi P, Catausan S, Dalid C, Slamet-Loedin I, Tecson-Mendoza EM, Wissuwa M, Heuer S (2012) The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. Nature 488: 535-539
- Gilbert N (2009) Environment: The disappearing nutrient. Nature 461: 716-718
- Hinsinger P, Betencourt E, Bernard L, Brauman A, Plassard C, Shen J, Tang X, Zhang F (2011) P for two, sharing a scarce resource: soil phosphorus acquisition in the rhizosphere of intercropped species. Plant Physiol 156: 1078-1086
- Hu B, Jiang Z, Wang W, Qiu Y, Zhang Z, Liu Y, Li A, Gao X, Liu L, Qian Y, Huang X, Yu F, Kang S, Wang Y, Xie J, Cao S, Zhang L, Wang Y, Xie Q, Kopriva S, Chu C (2019) Author Correction: Nitrate-NRT1.1B-SPX4 cascade integrates nitrogen and phosphorus signalling networks in plants. Nat Plants 5: 637
- Hufnagel B, de Sousa SM, Assis L, Guimaraes CT, Leiser W, Azevedo GC, Negri B, Larson BG, Shaff JE, Pastina MM, Barros BA, Weltzien E, Rattunde HF, Viana JH, Clark RT, Falcao A, Gazaffi R, Garcia AA, Schaffert RE, Kochian LV, Magalhaes JV (2014) Duplicate and conquer: multiple homologs of PHOSPHORUS-STARVATION TOLERANCE1 enhance phosphorus acquisition and sorghum performance on low-phosphorus soils. Plant Physiol 166: 659-677
- Jiao W, Chen W, Chang AC, Page AL (2012) Environmental risks of trace elements associated with long-term phosphate fertilizers applications: A review. Environ Pollut 168C: 44-53
- Kvakic M, Tzagkarakis G, Pellerin S, Ciais P, Goll D, Mollier A, Ringeval B (2020) Carbon and Phosphorus Allocation in Annual Plants: An Optimal Functioning Approach. Front Plant Sci 11: 149
- MacDonald GK, Bennett EM, Potter PA, Ramankutty N (2011) Agronomic phosphorus imbalances across the world's croplands. Proc Natl Acad Sci U S A 108: 3086-3091
- Medici A, Marshall-Colon A, Ronzier E, Szponarski W, Wang R, Gojon A, Crawford NM, Ruffel S, Coruzzi GM, Krouk G (2015) AtNIGT1/HRS1 integrates nitrate and phosphate signals at the Arabidopsis root tip. Nat Commun 6: 6274
- Medici A, Szponarski W, Dangeville P, Safi A, Dissanayake IM, Saenchai C, Emanuel A, Rubio V, Lacombe B, Ruffel S, Tanurdzic M, Rouached H, Krouk G (2019) Identification of molecular integrators shows that nitrogen actively controls the phosphate starvation response in plants. Plant Cell 31: 1171-1184
- Ndungu-Magiroi KW, Waswa B, Bationo A, Okalebo JR, Othieno C, Herrmann L, Lesueur D (2015) Minjingu phosphate rock applications increase the population of phosphate solubilising microorganisms with a positive impact on crop yields in a Kenyan Ferralsol. Nutr Cycl Agroecosystems 102: 91-99
- Neto AP, Favarin JL, Hammond JP, Tezotto T, Couto HT (2016) Analysis of Phosphorus Use Efficiency Traits in Coffea Genotypes Reveals Coffea arabica and Coffea canephora Have Contrasting Phosphorus Uptake and Utilization Efficiencies. Front Plant Sci 7: 408
- Olsen SR, Cole C, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Dept. Agric. Circ. 939, Washington, DC.



- Pistocchi C, Mészáros É, Tamburini F, Frossard E, Bünemann EK (2018) Biological processes dominate phosphorus dynamics under low phosphorus availability in organic horizons of temperate forest soils. Soil Biol Biochem 126: 64-75
- Puga MI, Rojas-Triana M, de Lorenzo L, Leyva A, Rubio V, Paz-Ares J (2017) Novel signals in the regulation of Pi starvation responses in plants: facts and promises. Curr Opin Plant Biol 39: 40-49
- Stutter MI, Shand CA, George TS, Blackwell MSA, Bol R, MacKay RL, Richardson AE, Condron LM, Turner BL, Haygarth PM (2012) Recovering Phosphorus from Soil: A Root Solution? Environ Sci Technol 46: 1977–1978
- Ueda Y, Kiba T, Yanagisawa S (2020) Nitrate-inducible NIGT1 proteins modulate phosphate uptake and starvation signalling via transcriptional regulation of SPX genes. Plant J 102: 448-466
- Wang W, Ding GD, White PJ, Wang XH, Jin KM, Xu FS, Shi L (2019) Mapping and cloning of quantitative trait loci for phosphorus efficiency in crops: opportunities and challenges. Plant and Soil 439: 91-112
- Wang Z, Ruan W, Shi J, Zhang L, Xiang D, Yang C, Li C, Wu Z, Liu Y, Yu Y, Shou H, Mo X, Mao C, Wu P (2014) Rice SPX1 and SPX2 inhibit phosphate starvation responses through interacting with PHR2 in a phosphate-dependent manner. Proc Natl Acad Sci U S A 111: 14953-14958
- Wang W, Ding GD, White PJ, Wang XH, Jin KM, Xu FS, Shi L (2019) Mapping and cloning of quantitative trait loci for phosphorus efficiency in crops: opportunities and challenges. Plant and Soil 439: 91-112
- Wipf D, Krajinski F, van Tuinen D, Recorbet G, Courty PE (2019) Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. New Phytologist 223: 1127-1142
- Wissuwa M, Yano M, Ae N (1998) Mapping of QTLs for phosphorus-deficiency tolerance in rice (Oryza sativa L.). Theoretical and Applied Genetics 97: 777-783
- Yuan Y, Gao M, Zhang M, Zheng H, Zhou X, Guo Y, Zhao Y, Kong F, Li S (2017) QTL Mapping for Phosphorus Efficiency and Morphological Traits at Seedling and Maturity Stages in Wheat. Front Plant Sci 8: 614
- Zhu J, Lau K, Puschmann R, Harmel RK, Zhang Y, Pries V, Gaugler P, Broger L, Dutta AK, Jessen HJ, Schaaf G, Fernie AR, Hothorn LA, Fiedler D, Hothorn M (2019) Two bifunctional inositol pyrophosphate kinases/phosphatases control plant phosphate homeostasis. Elife **8**



Focus Group S-4: Improving micronutrient uptake and use efficiency

Ana Assunçao¹, Ismail Cakmak², Stephan Clemens³, Manuel González-Guerrero⁴, Adam Nawrocki⁵, *Sébastien Thomine⁶*

1: agla@plen.ku.dk (University of Copenhagen, Denmark); 2: cakmak@sabanciuniv.edu (Sabanci University, Istanbul, Turkey); 3: stephan.clemens@uni-bayreuth.de (University of Bayreuth, GER); 4: manuel.gonzalez@upm.es (Universidad Politécnica de Madrid, Spain); 5: adam.nawrocki@adob.com.pl (PPC ADOB, Poland); 6: sebastien.thomine@i2bc.paris-saclay.fr (CNRS, I2BC, Paris-Saclay University, FR).

General background

Plants, as well as human beings, require a range of essential micronutrients, including iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) (World Health Organization and Food and Agriculture Organization of the United Nations, 2004).

Agricultural production

- Micronutrient deficiencies affect crop yields and quality, with sharp regional differences in many areas in Europe (Sinclair and Edwards, 2008).
- Mild or hidden micronutrient deficiency probably limits crop yields in much wider areas than those where obvious symptoms, such as chlorosis, occur.
- Several micronutrients are critical for atmospheric nitrogen fixation and nitrogen assimilation (Fischer *et al.*, 2005; Marschner and Marschner, 2012; Burén *et al.*, 2020) and thus required for minimizing the use of nitrogen fertilizers.

Human health

- Micronutrient deficiencies (i.e., hidden hunger) are the most prevalent dietary deficiencies, affecting over 2 billion people worldwide (Welch and Graham, 2004; FAO, 2020).
- Insufficient levels and bioavailability of micronutrients in plant-based diet represent major causes for the high prevalence of micronutrient deficiencies.
- The Harvest Plus program, based at the International Food Policy Research Institute (IFPRI), developed partnership with universities and farmers, and started to release micronutrient biofortified food crops.
- Low micronutrient availability increases toxic heavy metal accumulation in plants and human beings (Clemens *et al.*, 2013; Chaney, 2015; Ohta and Ohba, 2020).
- In Europe, micronutrient deficiencies are a growing concern mainly among pregnant women, children, teenagers and elderly people (Mensink *et al.*, 2013; Kaganov *et al.*, 2015).
- Alcohol consumption, which is widespread in Europe, amplifies Zn and Fe deficiencies (Barve *et al.*, 2017)
- Even subclinical micronutrient deficiencies alter neural function and impair the immune response (Hambidge, 2000; Read *et al.*, 2019; Vogel-González *et al.*, 2021).



• The foreseeable transition of European diets towards a higher proportion of vegetarianism calls for an increase in micronutrient content and availability in plant edible parts for food and feed (Gibson *et al.*, 2014).

Status quo of research in the field

- Plants need high amounts of micronutrients for biochemical processes such as photosynthesis, nitrogen assimilation or reactive oxygen species detoxification (Marschner and Marschner, 2012).
- A highly specialized and controlled network is in place to optimize uptake from soil, delivery to sink organs, and allocation to specific metalloproteins.
- Micronutrient homeostasis needs to be tightly controlled as there is a narrow range of optimal concentration between deficiency and toxicity.

Micronutrient fertilizers

- Synthetic chelators binding Fe or Zn with high affinity making these metals available for uptake by plants have been designed and are now used in the field (Abadía et al., 2011).
- Agronomic effectiveness of the chelated-micronutrients is much higher than the corresponding inorganic forms especially in calcareous high pH soils (Gangloff et al., 2002).
- Due to their high cost, the application of chelated micronutrients needs to be optimized, for example through seed priming or foliar spray.
- Foliar spray minimizes applied quantities, prevents micronutrient loss by precipitation in soils and allows local application compatible with precision agriculture.

Molecular mechanisms of micronutrient acquisition from the soil

- The molecular mechanisms of micronutrient mobilization and uptake by plants have been elucidated mostly through the use of two model species, *Arabidopsis thaliana* and rice (Olsen and Palmgren, 2014; Connorton *et al.*, 2017).
- Recent studies highlighted the importance of secretion of specialized metabolites by dicot roots in the mobilization of essential micronutrients and in shaping root-associated bacterial communities (Sisó-Terraza *et al.*, 2016; Tsai and Schmidt, 2017; Stringlis *et al.*, 2018).
- The existence of specific mechanisms for micronutrient uptake from arbuscular mycorrhizal fungi is beginning to be uncovered (Senovilla *et al.*, 2020).

Mechanisms of micronutrient distribution within the plant

- Within the plant, micronutrients are delivered through the sap to sink organs, typically growing leaves and seeds.
- The major metal ligands in plants, such as citrate or nicotianamine, have been identified and their role in micronutrient transport between cells and organs characterized (Clemens, 2019).
- Plant endosymbionts associated to nitrogen fixation are being identified as major metal sinks (Tejada-Jiménez et al., 2017; Brear et al., 2020; Escudero et al., 2020).



Regulation of micronutrient acquisition

- Transcription factors controlling responses to Fe, Zn and Cu deficiency have been identified (Assuncao *et al.*, 2010; Bernal *et al.*, 2012; Kobayashi and Nishizawa, 2012).
- The mechanisms through which plants perceive micronutrient status, and on its signaling at the systemic plant level, are being deciphered (Dubeaux *et al.*, 2018; Sinclair *et al.*, 2018; Grillet *et al.*, 2018; Lilay *et al.*, 2021).
- The roles of ubiquitination and phosphorylation have been recently highlighted in the control of Fe homeostasis (Rodríguez-Celma *et al.*, 2019; Kim *et al.*, 2019).
- Under Cu deficiency, miRNA dependent downregulation of Cu metallo-protein transcripts allows sparing this micronutrient (Yamasaki *et al.*, 2009; Bernal *et al.*, 2012; Garcia-Molina *et al.*, 2014).

New technologies for micronutrient quantification, localisation and interactions

- Ionomics screens based on Inductively Coupled Atomic Emission Spectroscopy (ICP AES) as well as Mass Spectrometry (ICP MS) have been used to quantify elements in large collections of plants (Huang and Salt, 2016; Campos et al., 2017; Yang et al., 2018).
- Synchrotron X-Ray have been used to map micronutrient localization in the nm to μm range and obtain information about micronutrient speciation (Ajiboye et al., 2015; Escudero et al., 2020).
- Imaging techniques based on Mass Spectrometry, such as Secondary Ion MS (SIMS), Laser Ablation-ICP-MS or Matrix Assisted Laser Desorption Ionisation (MALDI) MS imaging also reveal micronutrient localization (Cakmak et al., 2010a; Moore et al., 2018; Detterbeck et al., 2020).
- Metal sensitive fluorescent probes are used to dynamically monitor labile micronutrient pools in vivo (Languar et al., 2014).
- Analytical chemistry combining chromatography with MS and elemental analysis has identified metal complexes with small molecules or with proteins (Flis et al., 2016; Persson et al., 2016; Küpper et al., 2019) and will allow the determination of the complete spectrum of micronutrient metal complexes with small molecules (metallome), and the full complement of metallo-proteins (metallo-proteome) for any given biological sample.

Future challenges in the field to be addressed with high priority

Most relevant unsolved questions:

How to promote awareness on the importance of micronutrition among breeders, agronomists and consumers?

- There is a general lack of concern about micronutrients both with respect to sustainability of yield and nutrition quality.
- Micronutrient fertilization is only used if deficiency symptoms, such as leaf chlorosis or necrosis, appear. However, in most cases micronutrient deficiencies are hidden.
- The market value of cereals or other crops should be modulated according to their micronutrient content as it is for their protein content.



• There is an urgent need to better inform the stakeholders, policy makers and population about the importance of micronutrients.

How are metal micronutrients transported, used and perceived in plants?

- Micronutrient compete among each other and with toxic metals for transport and binding to ligands and proteins.
- Deficiency symptoms are often related to nutritional imbalances rather than to a lack of micronutrient per se.
- In depth biochemical knowledge of substrate specificities and relative affinities of proteins and transporters for micronutrient and toxic elements with similar chemical properties (such as Zn and Cd for example) is needed. (link with focus group "decreasing negative and toxic compounds")
- Micronutrients are allocated to distinct cell compartments that have different needs or are specifically involved in processing essential metals into cofactors.
- Micronutrient distribution among plant organs involves loading into the xylem and in the phloem, as well as specific ligands in conducting tissues.
- Micronutrient allocation to young leaves and seeds involves transporters localized in the nodes (Durbak et al., 2014; Shao et al., 2018; Mu et al., 2021) and mechanisms for metal recycling in source tissues (Pottier et al., 2018).
- Specific mechanisms allow plant to sense their micronutrient status to adjust uptake and redistribution of micronutrients according to the need of sink organs.
- The molecular mechanisms controlling micronutrient allocation to specific plant organs, cell compartments and active biomolecules require more investigation. (links with focus group "improving sink-source relationships")
- Strong interactions between micronutrient homeostasis and other mineral nutrients, such as phosphate with Zn and Fe (Briat et al., 2015; Dong et al., 2017), nitrogen with Fe and Mo (Burén et al., 2020), or sulphur with molybdenum (Shinmachi et al., 2010; Maillard et al., 2016a,b) occur.
- The concentrations and localizations of Zn, Fe and proteins are closely correlated in seeds (Cakmak et al., 2010b).
- The regulatory networks and specific metalloproteins involved in the interactions among nutrients require intensive research. (links with focus groups "improving nitrogen uptake and use efficiency" and "improving phosphorus uptake and use efficiency)

How does micronutrition interact with global changes and environmental stress?

- Crops are expected to be more and more affected by abiotic stresses such as heat, drought, high light and radiation.
- The atmospheric CO2 concentration is increasing steadily.
- How micronutrition is affected under these conditions requires investigation to select crops that will cope better with global change.
- Micronutrients are key players in redox reactions (Cakmak, 2000)



- Research on how nutrition with Zn, Mn, Fe and B mitigates climate change-related oxidative stress is required. (links with focus group "improving antioxidant and vitamin content", "improving heat tolerance" and "improving water uptake and use efficiency")
- Key enzymes in nitrogen nutrition, the nitrogenase required for atmospheric N2 fixation and the NO3--reductase which catalyzes the first step in N assimilation into proteins, rely on Fe and Mo for their activity.
- Optimal micronutrient uptake and distribution will be crucial for the transition to a more sustainable agriculture, using less N fertilizers. (link with focus group "increasing protein content and quality")
- Micronutrients are involved in plant immunity against pathogens (Dordas, 2008; Kazemi-Dinan et al., 2014; Aznar et al., 2015; Cesco et al., 2020).
- The mechanisms underlying the involvement of metal micronutrients in plant immunity need to be better understood, as it would provide a means to limit the use of pesticides.

How to manage micronutrition in new cropping systems?

- Agroecology is promoted in the EU to limit the use of fertilizers and pesticides.
- Efficient intercropping systems, such as for example maize and bean used for centuries in central America, should be designed scientifically.
- Micronutrient-rich green fertilizers should be developed.
- Soil-free urban or green house intensive agriculture opens the possibility to fine tune micronutrition, through fully controlled nutrient solutions.
- The determination of plant metal nutritional status in situ using portable X-ray fluorescence equipment, in combination with drone-based hyperspectral analyses, to fine-tune metal nutrition should be developed.

Opportunities for application of research results

- Improve yields and nutritional value of crops in environmentally friendly ways, especially under adverse conditions such as nutrient limitation, drought, heat and pathogen attack.
- Improve the efficiency and environmental value of micronutrient fertilizers by adapting fertilizer formulation to specific crops, soil conditions and mode of application, and developing biomimetics of physiological metal chelators as metal fertilizers.
- Promote better nutritional value of crops by increasing micronutrient fluxes and controlling micronutrient speciation/bioavailability in edible parts of the plants.
- Obtain crops with low toxic element levels and balanced micronutrient levels by preventing accumulation of toxic elements or excessive levels of essential elements.
- Optimize the yield and quality in agroecological and urban farming.



Action points for a future research programme in the field

Map micronutrient deficiencies to reveal regional specificities in the EU

- Map mineral micronutrient abundances through Europe to identify areas where they limit crop production through the following means:
- Data mining and databases of soil related data
- The European Soil Data Centre (ESDAC) runs a project for a harmonised and regular survey of top soils across all Member States, named Land Use/Cover Area frame statistical Survey (LUCAS). A report on a Cu survey has been published (Ballabio *et al.*, 2018). We suggest that the LUCAS survey incorporates analysis of the micronutrients Fe, Zn, Cu and Mn as well.
- Use of indicator plants (possible participative projects)
- Design of remote sensing devices to evaluate micronutrient status (on tractors, drones, planes).

Applications:

• Promote awareness about regional problems of micronutrient deficiencies in the EU and facilitate their targeted alleviation both in cultured crops, human beings and animals. It will also allow for precision agriculture with metal fertilization treatments tailored to local conditions.

Harness micronutrition for sustainable yield and protein production.

- Delineate the molecular and cellular pathways of micronutrients from the soil to the metalloproteins that need them most, and their regulation. These include uptake and all the steps until incorporation as cofactors in photosystems (Fe, Mn, Cu) and nitrogen assimilation enzymes: nitrogenase, nitrate reductase (Fe, Mo, Co, Cu, Zn, B).
- For photosystems, the uptake into leaf cells and transport through the chloroplast envelope and thylakoid membranes must be considered.
- In the case of N assimilation, several pathways need to be considered (1) to endogenous nitrate reductase in the cytosol of leaf cells, (2) to nitrogenase of symbiotic bacteria included in symbiosomes in the nodules of legume species and (3) to nitrogenase in endophytic bacteria that colonize the intercellular space in other species.
- Delineate the pathways of micronutrient transport from leaves to other organs. From absorption by the leaf surface, in the case of foliar fertilizer application, their mobilization during leaf senescence towards growing organs, reproductive organs and seeds that require large amounts or micronutrients.
- Harness the functions of micronutrients in biotic and abiotic stress tolerance. This includes understanding the roles of micronutrients in plant resilience to abiotic stresses, through their antioxidative role for example, and understanding the competition for micronutrients between host and invading pathogens and how it is regulated or manipulated during the immune response.

Applications

- Selection of crops with stable yield and high protein production under micronutrient deficiencies.
- Identification of varieties that respond efficiently to foliar micronutrient fertilization.



• Design sustainable crops resistant to abiotic and biotic stresses.

Identify varieties and species with high micronutrient content and bioavailability.

Several pathways may be proposed to achieve better micronutrient delivery through plant-based products:

- Major crops can be bred for higher micronutrient content and availability. The existing genetic resources such as germplasm collections might potentially offer alleles for improved micronutrient uptake and use efficiency traits.
- Orphan crop species or macro algae can be screened for micronutrient content and availability and introduced in the EU diet. Domestication of novel species and change in habits in the population would be required for this option.
- Plant species with outstanding micronutrient content, such as Zn hyperaccumulating species, may be cultivated and used as additives to other foods.
- Improving micronutrient content in edible part of crop plants requires a better knowledge of the mechanisms of micronutrient distribution to organs and tissues in order to be able to breed for targeted micronutrient allocation.
- Improving micronutrient bioavailability requires a better characterization of micronutrient distribution in plant organs and tissues using elemental imaging approaches and of micronutrient speciation using analytic and spectroscopic methods.
- A better knowledge of the chemical forms of micronutrients after processing, such as sprouting, milling and cooking is key to control micronutrient availability in edible parts of crops.
- Information about the transfer of micronutrients in the human digestive system needs to be obtained in close collaboration with nutrition scientists and physicians to determine how varieties with improved micronutrient content and availability impact human health.

Application:

• Provide micronutrient rich plant derived food and feed for human and cattle to fight deficiencies, especially among children and women, as well as to enhance their immunity.

References

- Abadía J, Vázquez S, Rellán-Álvarez R, El-Jendoubi H, Abadía A, Álvarez-Fernández A, López-Millán AF. 2011. Towards a knowledge-based correction of iron chlorosis. Plant Physiology and Biochemistry **49**, 471–482.
- Ajiboye B, Cakmak I, Paterson D, de Jonge MD, Howard DL, Stacey SP, Torun AA, Aydin N, McLaughlin MJ. 2015. Xray fluorescence microscopy of zinc localization in wheat grains biofortified through foliar zinc applications at different growth stages under field conditions. Plant and Soil **392**, 357–370.
- Assuncao AGL, Herrero E, Lin Y-F, *et al.* 2010. Arabidopsis thaliana transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency. Proceedings of the National Academy of Sciences **107**, 10296–10301.
- Aznar A, Chen NWG, Thomine S, Dellagi A. 2015. Immunity to plant pathogens and iron homeostasis. Plant Science 240, 90–97.
- Ballabio C, Panagos P, Lugato E, Huang J-H, Orgiazzi A, Jones A, Fernández-Ugalde O, Borrelli P, Montanarella L. 2018. Copper distribution in European topsoils: An assessment based on LUCAS soil survey. Science of The Total Environment 636, 282–298.
- **Barve S, Chen S-Y, Kirpich I, Watson WH, Mcclain C**. 2017. Development, Prevention, and Treatment of Alcohol-Induced Organ Injury: The Role of Nutrition. Alcohol Research: Current Reviews **38**, 289–302.



- Bernal M, Casero D, Singh V, et al. 2012. Transcriptome Sequencing Identifies SPL7 -Regulated Copper Acquisition Genes FRO4 / FRO5 and the Copper Dependence of Iron Homeostasis in Arabidopsis. The Plant Cell 24, 738–761.
- **Brear EM, Bedon F, Gavrin A, Kryvoruchko IS, Torres-Jerez I, Udvardi MK, Day DA, Smith PMC**. 2020. GmVTL1a is an iron transporter on the symbiosome membrane of soybean with an important role in nitrogen fixation. New Phytologist **228**, 667–681.
- **Briat J-F, Rouached H, Tissot N, Gaymard F, Dubos C**. 2015. Integration of P, S, Fe, and Zn nutrition signals in Arabidopsis thaliana: potential involvement of PHOSPHATE STARVATION RESPONSE 1 (PHR1). Frontiers in Plant Science **06**.
- Burén S, Jiménez-Vicente E, Echavarri-Erasun C, Rubio LM. 2020. Biosynthesis of Nitrogenase Cofactors. Chemical Reviews 120, 4921–4968.
- **Cakmak I**. 2000. Tansley Review No. 111: Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. New Phytologist **146**, 185–205.
- **Cakmak I, Kalayci M, Kaya Y, et al.** 2010*a*. Biofortification and Localization of Zinc in Wheat Grain. Journal of Agricultural and Food Chemistry **58**, 9092–9102.
- **Cakmak I, Pfeiffer WH, McClafferty B**. 2010*b*. REVIEW: Biofortification of Durum Wheat with Zinc and Iron. Cereal Chemistry Journal **87**, 10–20.
- Campos ACA, Kruijer W, Alexander R, Akkers RC, Danku J, Salt DE, Aarts MG. 2017. Natural variation in Arabidopsis thaliana reveals shoot ionome, biomass, and gene expression changes as biomarkers for zinc deficiency tolerance. Journal of Experimental Botany 68, 3643–3656.
- Cesco S, Tolotti A, Nadalini S, *et al.* 2020. Plasmopara viticola infection affects mineral elements allocation and distribution in Vitis vinifera leaves. Scientific Reports **10**, 18759.
- **Chaney RL**. 2015. How Does Contamination of Rice Soils with Cd and Zn Cause High Incidence of Human Cd Disease in Subsistence Rice Farmers. Current Pollution Reports **1**, 13–22.
- **Clemens S**. 2019. Metal ligands in micronutrient acquisition and homeostasis. Plant, Cell & Environment **42**, 2902–2912.
- Clemens S, Aarts MGM, Thomine S, Verbruggen N. 2013. Plant science: the key to preventing slow cadmium poisoning. Trends in Plant Science 18, 92–99.
- **Connorton JM, Balk J, Rodríguez-Celma J**. 2017. Iron homeostasis in plants a brief overview. Metallomics **9**, 813–823.
- Detterbeck A, Pongrac P, Persson DP, et al. 2020. Temporal and Spatial Patterns of Zinc and Iron Accumulation during Barley (*Hordeum vulgare* L.) Grain Development. Journal of Agricultural and Food Chemistry 68, 12229–12240.
- **Dong J, Piñeros MA, Li X, Yang H, Liu Y, Murphy AS, Kochian LV, Liu D**. 2017. An Arabidopsis ABC Transporter Mediates Phosphate Deficiency-Induced Remodeling of Root Architecture by Modulating Iron Homeostasis in Roots. Molecular Plant **10**, 244–259.
- **Dordas C**. 2008. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. Agronomy for Sustainable Development **28**, 33–46.
- **Dubeaux G, Neveu J, Zelazny E, Vert G**. 2018. Metal Sensing by the IRT1 Transporter-Receptor Orchestrates Its Own Degradation and Plant Metal Nutrition. Molecular Cell **69**, 953-964.e5.
- Durbak AR, Phillips KA, Pike S, O'Neill MA, Mares J, Gallavotti A, Malcomber ST, Gassmann W, McSteen P. 2014. Transport of Boron by the *tassel-less1* Aquaporin Is Critical for Vegetative and Reproductive Development in Maize. The Plant Cell **26**, 2978–2995.
- Escudero V, Abreu I, Tejada-Jiménez M, *et al.* 2020. *Medicago truncatula* Ferroportin2 mediates iron import into nodule symbiosomes. New Phytologist **228**, 194–209.
- **FAO**. 2020. *Transforming food systems for affordable healthy diets*.
- Fischer K, Barbier GG, Hecht H-J, Mendel RR, Campbell WH, Schwarz G. 2005. Structural Basis of Eukaryotic Nitrate Reduction: Crystal Structures of the Nitrate Reductase Active Site. The Plant Cell **17**, 1167–1179.
- Flis P, Ouerdane L, Grillet L, Curie C, Mari S, Lobinski R. 2016. Inventory of metal complexes circulating in plant fluids: a reliable method based on HPLC coupled with dual elemental and high-resolution molecular mass spectrometric detection. New Phytologist **211**, 1129–1141.
- **Gangloff WJ, Westfall DG, Peterson GA, Mortvedt JJ**. 2002. RELATIVE AVAILABILITY COEFFICIENTS OF ORGANIC AND INORGANIC Zn FERTILIZERS. Journal of Plant Nutrition **25**, 259–273.



- Garcia-Molina A, Xing S, Huijser P. 2014. A Conserved KIN17 Curved DNA-Binding Domain Protein Assembles with SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE7 to Adapt Arabidopsis Growth and Development to Limiting Copper Availability. Plant Physiology 164, 828–840.
- **Gibson RS, Heath A-LM, Szymlek-Gay EA**. 2014. Is iron and zinc nutrition a concern for vegetarian infants and young children in industrialized countries? The American Journal of Clinical Nutrition **100**, 459S-468S.
- Grillet L, Lan P, Li W, Mokkapati G, Schmidt W. 2018. IRON MAN is a ubiquitous family of peptides that control iron transport in plants. Nature Plants 4, 953–963.
- Hambidge M. 2000. Human Zinc Deficiency. The Journal of Nutrition 130, 1344S-1349S.
- Huang X-Y, Salt DE. 2016. Plant Ionomics: From Elemental Profiling to Environmental Adaptation. Molecular Plant 9, 787–797.
- Kaganov B, Caroli M, Mazur A, Singhal A, Vania A. 2015. Suboptimal Micronutrient Intake among Children in Europe. Nutrients 7, 3524–3535.
- Kazemi-Dinan A, Thomaschky S, Stein RJ, Krämer U, Müller C. 2014. Zinc and cadmium hyperaccumulation act as deterrents towards specialist herbivores and impede the performance of a generalist herbivore. New Phytologist 202, 628–639.
- **Kim SA, LaCroix IS, Gerber SA, Guerinot ML**. 2019. The iron deficiency response in *Arabidopsis thaliana* requires the phosphorylated transcription factor URI. Proceedings of the National Academy of Sciences **116**, 24933–24942.
- Kobayashi T, Nishizawa NK. 2012. Iron Uptake, Translocation, and Regulation in Higher Plants. Annual Review of Plant Biology 63, 131–152.
- Küpper H, Bokhari SNH, Jaime-Pérez N, Lyubenova L, Ashraf N, Andresen E. 2019. Ultratrace Metal Speciation Analysis by Coupling of Sector-Field ICP-MS to High-Resolution Size Exclusion and Reversed-Phase Liquid Chromatography. Analytical Chemistry **91**, 10961–10969.
- Lanquar V, Grossmann G, Vinkenborg JL, Merkx M, Thomine S, Frommer WB. 2014. Dynamic imaging of cytosolic zinc in *Arabidopsis* roots combining FRET sensors and RootChip technology. New Phytologist **202**, 198–208.
- Lilay GH, Persson DP, Castro PH, Liao F, Alexander RD, Aarts MGM, Assunção AGL. 2021. Arabidopsis bZIP19 and bZIP23 act as zinc sensors to control plant zinc status. Nature Plants **7**, 137–143.
- Maillard A, Etienne P, Diquélou S, Trouverie J, Billard V, Yvin J-C, Ourry A. 2016*a*. Nutrient deficiencies modify the ionomic composition of plant tissues: a focus on cross-talk between molybdenum and other nutrients in *Brassica napus*. Journal of Experimental Botany **67**, 5631–5641.
- Maillard A, Sorin E, Etienne P, *et al.* 2016b. Non-Specific Root Transport of Nutrient Gives Access to an Early Nutritional Indicator: The Case of Sulfate and Molybdate (I Baxter, Ed.). PLOS ONE **11**, e0166910.
- Marschner H, Marschner P (Eds.). 2012. *Marschner's mineral nutrition of higher plants*. London ; Waltham, MA: Elsevier/Academic Press.
- Mensink GBM, Fletcher R, Gurinovic M, et al. 2013. Mapping low intake of micronutrients across Europe. British Journal of Nutrition 110, 755–773.
- Moore KL, Rodríguez-Ramiro I, Jones ER, Jones EJ, Rodríguez-Celma J, Halsey K, Domoney C, Shewry PR, Fairweather-Tait S, Balk J. 2018. The stage of seed development influences iron bioavailability in pea (Pisum sativum L.). Scientific Reports 8, 6865.
- Mu S, Yamaji N, Sasaki A, *et al.* 2021. A transporter for delivering zinc to the developing tiller bud and panicle in rice. The Plant Journal **105**, 786–799.
- **Ohta H, Ohba K**. 2020. Involvement of metal transporters in the intestinal uptake of cadmium. The Journal of Toxicological Sciences **45**, 539–548.
- **Olsen LI, Palmgren MG**. 2014. Many rivers to cross: the journey of zinc from soil to seed. Frontiers in Plant Science **5**.
- Persson DP, Bang TC, Pedas PR, Kutman UB, Cakmak I, Andersen B, Finnie C, Schjoerring JK, Husted S. 2016. Molecular speciation and tissue compartmentation of zinc in durum wheat grains with contrasting nutritional status. New Phytologist 211, 1255–1265.
- Pottier M, Dumont J, Masclaux-Daubresse C, Thomine S. 2018. Autophagy is essential for optimal translocation of iron to seeds in Arabidopsis. Journal of Experimental Botany.
- Read SA, Obeid S, Ahlenstiel C, Ahlenstiel G. 2019. The Role of Zinc in Antiviral Immunity. Advances in Nutrition 10, 696–710.
- Rodríguez-Celma J, Connorton JM, Kruse I, Green RT, Franceschetti M, Chen Y-T, Cui Y, Ling H-Q, Yeh K-C, Balk J. 2019. Arabidopsis BRUTUS-LIKE E3 ligases negatively regulate iron uptake by targeting transcription factor FIT for recycling. Proceedings of the National Academy of Sciences 116, 17584–17591.



Senovilla M, Abreu I, Escudero V, Cano C, Bago A, Imperial J, González-Guerrero M. 2020. MtCOPT2 is a Cu+ transporter specifically expressed in Medicago truncatula mycorrhizal roots. Mycorrhiza **30**, 781–788.

Shao JF, Yamaji N, Liu XW, Yokosho K, Shen RF, Ma JF. 2018. Preferential Distribution of Boron to Developing Tissues Is Mediated by the Intrinsic Protein OsNIP3. Plant Physiology **176**, 1739–1750.

- Shinmachi F, Buchner P, Stroud JL, Parmar S, Zhao F-J, McGrath SP, Hawkesford MJ. 2010. Influence of Sulfur Deficiency on the Expression of Specific Sulfate Transporters and the Distribution of Sulfur, Selenium, and Molybdenum in Wheat. Plant Physiology **153**, 327–336.
- **Sinclair AH, Edwards AC**. 2008. Micronutrient Deficiency Problems in Agricultural Crops in Europe. In: Alloway BJ, ed. Micronutrient Deficiencies in Global Crop Production. Dordrecht: Springer Netherlands, 225–244.
- Sinclair SA, Senger T, Talke IN, Cobbett CS, Haydon MJ, Krämer U. 2018. Systemic Upregulation of MTP2- and HMA2-Mediated Zn Partitioning to the Shoot Supplements Local Zn Deficiency Responses. The Plant Cell **30**, 2463–2479.
- Sisó-Terraza P, Luis-Villarroya A, Fourcroy P, Briat J-F, Abadía A, Gaymard F, Abadía J, Álvarez-Fernández A. 2016. Accumulation and Secretion of Coumarinolignans and other Coumarins in Arabidopsis thaliana Roots in Response to Iron Deficiency at High pH. Frontiers in Plant Science **7**.
- Tejada-Jiménez M, Gil-Díez P, León-Mediavilla J, Wen J, Mysore KS, Imperial J, González-Guerrero M. 2017. Medicago truncatula Molybdate Transporter type 1 (MtMOT1.3) is a plasma membrane molybdenum transporter required for nitrogenase activity in root nodules under molybdenum deficiency. New Phytologist 216, 1223–1235.

Tsai HH, Schmidt W. 2017. Mobilization of Iron by Plant-Borne Coumarins. Trends in Plant Science 22, 538–548.

- Vogel-González M, Talló-Parra M, Herrera-Fernández V, et al. 2021. Low Zinc Levels at Admission Associates with Poor Clinical Outcomes in SARS-CoV-2 Infection. Nutrients **13**, 562.
- Welch RM, Graham RD. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. Journal of Experimental Botany **55**, 353–364.
- **World Health Organization, Food and Agriculture Organization of the United Nations (Eds.)**. 2004. *Vitamin and mineral requirements in human nutrition*. Geneva : Rome: World Health Organization ; FAO.
- Yamasaki H, Hayashi M, Fukazawa M, Kobayashi Y, Shikanai T. 2009. SQUAMOSA Promoter Binding Protein–Like7 Is a Central Regulator for Copper Homeostasis in *Arabidopsis*. The Plant Cell **21**, 347–361.
- Yang M, Lu K, Zhao F-J, *et al.* 2018. Genome-Wide Association Studies Reveal the Genetic Basis of Ionomic Variation in Rice. The Plant Cell **30**, 2720–2740.



Focus Group S-5: Improving heat tolerance

Matthieu Bogard¹, Michael Dingkuhn²; Sotirios Fragkostefanakis³, Christine Granier⁴, Pierre Martre⁵, Daniel Van Damme⁶, Heidi Webber⁷, Zoe. A. Wilson⁸, Xinyou Yin⁹

1: <u>M.BOGARD@arvalis.fr</u> (ARVALIS, Institut du Végétal, Toulouse, FR); 2: <u>michael.dinqkuhn@cirad.fr</u> (CIRAD, Montpellier, FR); 3: <u>fraqkost@bio.uni-frankfurt.de</u> (Goethe University Frankfurt am Main, DE); 4: <u>christine.qranier@inrae.fr</u> (INRAE Montpellier, FR); 5: <u>pierre.martre@inrae.fr</u> (INRAE Montpellier, FR); 6: <u>Daniel.VanDamme@psb.vib-uqent.be</u> (VIB, Ghent, BE); 7: <u>Heidi.Webber@zalf.de</u> (ZALF, Muencheberg, DE); 8: <u>zoe.wilson@nottinqham.ac.uk</u> (University of Nottingham, UK); 9: <u>xinyou.yin@wur.nl</u> (Wageningen University, NL)

Status quo of research in the field

Current know-how

Heat stress in crops occurs when temperatures increase above a threshold or an optimum for processes that determine growth and yield. Even very short heat events (heat shocks) can have dramatic consequences on final crop yield if they occur at critical developmental time points. For instance, a meta-analysis of 600 rainfed field trials in Australia showed that one day with maximum temperature above 35°C at flowering decreases final grain yield of wheat by 33% (1). Threshold or optimum temperatures vary depending on the crop species and genotype. Different processes may also have different temperature thresholds or optima and thus heat stress may occur at different temperatures (2, 3). Moreover, heat stress may have different impacts depending on the time of the day or the plant growth stage at which it occurs. Heat stress often occurs alongside other stress such as drought or high evaporative demand. Heat stress events are also generally associate with diurnal temperature asymmetry which alters the relationships between climate factors and has complex effects on the plant's physiology beyond the sole effect of heat stress (4). A recent review alerts to the fact that heat wave vulnerability of plants remains a poorly understudied process (5).

Reproductive processes are generally considered more heat sensitive than vegetative processes and tend to have threshold-like responses to high temperature. Male reproduction is particularly sensitive to heat stress, with early stages of pollen development, including meiosis and early pollen wall formation being especially vulnerable (6, 7). Alongside this, the pollen release and fertilisation processes can be impacted by high temperatures leading to losses in seed yield, in particular anthesis stages including anther dehiscence, pollen shedding, pollen germination and pollen tube growth.

Mitigation of heat stress impact on reproductive processes can be achieved at least in part by using genotypes which flower at an earlier time of day to provide escape (8), or via transpiration cooling to provide avoidance (9-11). Floral architecture or wax content may also help in protection through shading or reduction in radiation load, respectively (1). Modifying sowing dates (for annual crops) combined with the use of varieties with adapted phenology (for both annual and perennial crops) can be an effective strategy to mitigate the risk of heat stress damage during critical growth stages of reproductive development. However, there is a trade-off between heat avoidance with a shorter growth cycle and biomass and yield production (12). Moreover, early reproductive development may increase the risk of cold temperature damages in the spring (13).



At the cellular level, several mechanisms of heat tolerance have been studied with promising results (production of reactive oxygen species => antioxidant and catalase production, reduced membrane stability => desaturase genes, induced cell death => NBR1-dependent aggrephagy, prevent protein misfolding and aggregation => overexpression of heat shock protein genes). Although, these mechanisms might be beneficial for plant survival, they are not necessarily very effective in maintaining high yields under heat stress conditions, or even yield potential under non-stress conditions.

Plant carbon assimilation and expansive growth processes tend to have optimum-like responses to temperature. The shape of the response curves, and their cardinal temperatures, may be different depending on how they are established (14). The response to short-term changes in temperature have been established for a number of processes and species (15). These response curves generally do not consider acclimation processes, which tend to "flatten" temperature responses (16). They also do not consider processes that occur when plants are subjected to recurrent heat stress scenarios. Plant responses to recurrent heat stresses, i.e. the succession of heat stress events separated by non-stressing periods, may not match the addition of individual responses to each event for at least two reasons: process compensatory (or even over-compensatory) recovery after the heat stress period and acclimation from one stressing period to the other. The physiological and molecular mechanisms of temperature recovery and acclimation processes are still largely unknown. Heat stress transcriptional acquired thermotolerance and more recently thermopriming have been highlighted for a number of physiological processes (17-19). Epigenetic regulation has been proposed as key components of these responses (20, 21). The extent to which metabolic and molecular thermopriming mechanisms contribute to plant adaptation to heat stress in the field need to be ascertained and compared to other well-known physiological effects (e.g. changes in plant cycle duration or light interception via plant architecture).

The impacts of sink/source ratios (22) and signalling on carbon assimilation processes responses to heat stress have received only limited attention. Heat sensing mechanisms have been identified but the molecular cascading of these mechanisms and their interplay with other sensing pathways are still largely unknown (23). Liquid-liquid phase separation has recently been shown to act as a heat sensing mechanism for flowering (24). Trehalose 6-phosphate related feast-famine sensing and signalling mechanisms which control plant carbon sinks are also involved in abiotic stress tolerance, including heat (25, 26). The role of maintenance respiration, sink-source ratio, and leaf senescence in the plant carbon balance under heat (27, 28), and its acclimation, is an under-researched topic and warrant further investments (29, 30).

In the field, heat stress is most often associated with other stress factors. High temperatures associated with heat stress create high vapour pressure deficit (VPD) driving increased plant transpiration (31, 32). How this translates into more or less transpirational cooling will depend on soil water availability and crop responses to VPD, though high VPD can result in midday water stress even when soil water availability is high. Climate change increases the risk of heat stress events because of the partial stomatal closure under elevated atmospheric CO₂ concentration, which drives feedforward loops (elevated CO₂ closes stomata, which increases canopy-air VPD, further closing stomata and increasing the plant's temperature). A better understanding of the physiological and molecular mechanisms of these processes would allow developing reverse genetic strategies to improve crop tolerance to heat stress. These mechanisms have mainly been studied under controlled conditions where climate factors can (to some extent) be decoupled. Validations of these



results under field conditions are missing and would need consideration of trade-offs between maintaining transpiration to avoid heat stress versus delaying drought stress, which can be studied with genotypes with a range of isohydric-anisohydric behaviour.

The reduction of transpiration rate under increased CO₂ is large for irrigated rice (33) but significant in virtually all crops. Stomatal response thereby reduces transpirational cooling and thus can cause significant warming of the canopy (34). This effect is under-studied for the main European crops. Transpiration cooling protects from heat, but has trade-off with water use. Drought tolerant commercial varieties, notably maize hybrids, with increased sensitivity of stomata to high VPD have been developed. These varieties close their stomata at lower VPD threshold values than conventional varieties and thus save water under conditions of high evaporative demand. Although they have significant higher water use efficiency and yields in irrigated systems, they may have higher risks of heat stress damage and yield loss under rainfed conditions. Therefore, we might distinguish water-saving versus water consumptive heat responses as strategies of adaptation.

Several studies have shown that stay-green traits are associated with heat (and drought) tolerance. Functional stay-green phenotypes under heat or drought have been associated with deeper roots but resource independent control mechanisms also exist and can be engineered (e.g. via root cytokinin production). Different stay-green strategies and mechanism should be further investigated as potential heat mitigation strategies (35).

The potential advantages and drawbacks of complex cropping systems and spatial crop arrangements are currently being explored to reduce the environmental impacts of cropping and improve the biodiversity of cultivated areas. Landscape and cropping systems scale management of crop microclimate in these systems and how they can mitigate heat stress impacts (through shading, green wind/heat breakers, soil improvement and water conservation) have received little attention to date.

Trends in research, new technology applied

- Evolutionary genetics approaches: Study which genes evolved when (sub)species adapted to environments with high risks of heat stress.
- Use of exotic germplasms (collection of accessions, wild related species) as genetic sources of genes for the improvement of heat stress tolerance(36-38)
- High-throughput plant phenotyping in the field (e.g. canopy temperature measurement) or under controlled conditions for screening large collections of genotypes in different heat stress scenario (multiple stress combinations, recurrent heat stresses...)
- Use of (multi)-omics and single cell analyses approaches to identify key components of plant response to heat stress and for modelling molecular responses (from DNA to metabolite levels)(39)
- Biotechnological approaches using specific gene targets to modify heat adaptations



Future challenges in the field to be addressed with high priority

Most relevant unresolved research questions

At the agronomic level

- Under which agro-climatic scenarios is heat stress tolerance desirable?
- What are consequences of agroecological/multiservices cropping systems on heat stress tolerance and resilience? (e.g. increased temperature in the open crop part in agroforestry systems because of wind turbulence created by trees). How to develop G x M systems more resilient to heat shocks and better adapted to future climate scenarios with increased risk of extreme climate events?
- What are the trade-offs and compensations between plant products quality and quantity as affected by heat stress?
- Can plant/crop canopy architecture influence microclimate favourably, e.g. by shading of sensitive organs/processes? (links with crop management strategies crop mixture, agroforestry, agrivoltaism,...)

At the physiological level

- How do temperature thresholds or optima change when crops are exposed to other stressors?
- Can we optimize trade-offs between traits for heat and drought adaptation (given uncertain conditions)?
- Is there a need for organ/tissue level protection (e.g. leaf wax) to heat stress or is tolerance at whole plant level needed?
- Where is temperature perception detected to drive plant heat stress responses? Is it the relevant organ temperature or is temperature integrated at the whole plant level or that of another organ (e.g. cereal vernalization)?
- Is heat stress a consequence of impaired metabolism or sensitivity (e.g. photosynthesis) of specific developmental stages/tissues? Should we select for a specific pathway improvement or at whole plant level?
- What is the benefit of heat stress transcriptional acclimation or memory at upper scales?
- How does the interplay between organs (e.g. root/reproductive organs) impact on tolerance to heat and can this be modified to increase crop heat tolerance?

At the genetic level

- Is there enough genetic variation in the current elite gene pool used by breeders to improve heat tolerance in current and future climate/management conditions?
- Can there be cross-generational epigenetic acclimation? Can pre-acclimated seed be produced?
- What is the genetic basis behind species or subspecies heat stress tolerance?
- Did species domestication and breeding affect heat stress tolerance?
- Can gene evolution that appended during species adaptation to environment with high risks of heat stress be re-enacted using the most up-to-date techniques of molecular breeding?



At the cellular and molecular levels

- How is the temperature perceived and signalled, which organs are involved?
- How does heat stress link with other stress? Is cross-adaptation likely among several stresses? (e.g., ROS protection is a generic defence mechanism)
- What is the cellular and molecular basis of thermo sensitivity of reproductive tissues?
- How can we avoid heat effects on membrane fluidity as this will affect general trafficking?
- Can we avoid heat effects on intrinsic protein stability by introducing key factors of resistant plants with increased structural stability into crops?

Most relevant societal and economic challenges

- Move from monoculture to more complex and heat resilient crop production systems. Make these systems economically sustainable. Overcome technical acceptability of mixed/complex cropping systems
- Anticipate and adapt to migrations of crops/systems between latitudes due to climate change (geographic escape)
- Evaluate efficiency of different research investments in adaptation (i.e., breeding, irrigation infrastructure, crop diversification, agronomic solutions, subsidizing insurance, regional redistribution of production) at EU, national levels. How do we handle the risks of heat stress? Do potentially infrequent but severe heat stress periods justify changes?

Aspects/opportunities for application of research results

- Crop monitoring systems to support farmer's decisions
- Deficit irrigation management for heat stress mitigation
- Risk assessment frameworks to understand trade-offs of introducing heat adaptive traits
- Introduction of genetic variation found in exotics into domesticated crops by gene editing approaches
- Develop pre-emptive breeding strategies for heat adaptation that do not compromise current breeding efforts.
- Modification of endogenous transcription factors, down-regulation through mutagenesis/editing or up-regulation of expression by GM or editing
- Transformation of "alien" transcription factors from heat resistant species into crops (40)
- Generation of heat stress atlas regarding molecular responses and thermotolerance from cell to tissue to organ

Action points for a future research program in the field

What needs to be done to solve the scientific questions and to meet the societal and economic challenges?

• Improve crop growth models by considering the response and acclimation (when heat stress is recurrent) of key processes (e.g. fertility) to heat and their genetic variability



- Improve crop growth models by considering the response to combined stress (e.g. heat x drought, heat x high evaporative demand) and their genetic variability
- Analyse risk scenarios to identify relevant heat stress scenarios (crop growth stages, duration, intensity) and possible interactions with other stressors to better target researches
- Develop methods to separate heat and high evaporative demand in the field
- Develop methods and tools to screen large breeding populations efficiently for heat tolerance and resilience (genetic markers, phenomics, metabolomics, sensors)
- Develop methods to apply high temperature treatments in controlled environments more representative of real production conditions (e.g. avoiding artefacts of root heating in pot experiments)
- Develop prebreeding strategies (including new primary hybrids or intraspecific crosses) for heat stress tolerance to efficiently tap in exotic allele pools? (including cross species analyses for trait and physiological/molecular processes identification/characterisation)
- Study transcription factors ("master genes" for adaptation networks) from alien sources (adapted species) as transgenes in crops, to unlock "hidden" adaptation potential through regulation
- Identify targets of aggrephagy upon heat stress as these represent temperature sensitive proteins, for which more robust variants can be identified in temperature resistant species

Projects with application relevance

- Develop new cultivars with synergetic positive interactions with agronomic management practices that can substantially increase crop yield and maintain good end-use value in hot conditions (i.e. cultivars with long coleoptile that can be sown deep to allow early sowing into soil with a warm or dry surface but with a cooler or wet subsoil).
- Increase the frequency and pyramid specific morphological traits to protect crops from heat damage during reproductive phases (e.g. erect leaves, glaucous leaves, long coleoptile, leaf rolling) that exist in current cultivars or breeding germplasms but at low frequency and that are rarely combined in the same genotype (1).
- Establish screening strategies to identify genotypes that are temperature resilient and at the same time do not depend on extensive irrigation. Including prebreeding strategies (introduction of new/locked alleles)
- Identify how crops sense temperature in order to generate an effective strategy to generate microclimate-based approaches that would mitigate the impact of heat stress under field conditions
- Innovations (genetic improvement, crop mixtures, crop arrangement) to improve microclimates of cropping systems to minimize mix of heat and drought stress

What needs to be done to support the translation of research results into societal and economic value?

• Change in the policy of Europe towards the application of new breeding techniques for agriculture


- Deliver decision support tools to help farmers choosing the right sowing date x variety and maximize the probability to avoid deleterious effects of heat stress
- Develop cross-disciplinary research and communications (including participatory and multiactors approaches)

References

- 1. Hunt JR, Hayman PT, Richards RA, & Passioura JB (2018) Opportunities to reduce heat damage in rain-fed wheat crops based on plant breeding and agronomic management. *Field Crops Res.* 224:126-138.
- 2. Porter JR & Gawith M (1999) Temperatures and the growth and development of wheat: a review. *Eur. J. Agron.* 10:23-36.
- 3. Sánchez B, Rasmussen A, & Porter JR (2014) Temperatures and the growth and development of maize and rice: a review. *Glob. Chang. Biol.* 20(2):408-417.
- 4. Cox DTC, Maclean IMD, Gardner AS, & Gaston KJ (2020) Global variation in diurnal asymmetry in temperature, cloud cover, specific humidity and precipitation and its association with leaf area index. *Glob. Chang. Biol.* 26(12):7099-7111.
- 5. Breshears DD, et al. (2021) Underappreciated plant vulnerabilities to heat waves. New Phytol. n/a(n/a):in press.
- 6. Rieu I, Twell D, & Firon N (2017) Pollen Development at High Temperature: From Acclimation to Collapse. *Plant Physiol.* 173(4):1967-1976.
- 7. Begcy K, *et al.* (2019) Male Sterility in Maize after Transient Heat Stress during the Tetrad Stage of Pollen Development. *Plant Physiol.* 181(2):683-700.
- 8. Julia C & Dingkuhn M (2012) Variation in time of day of anthesis in rice in different climatic environments. *Eur. J. Agron.* 43(0):166-174.
- 9. Julia C & Dingkuhn M (2013) Predicting temperature induced sterility of rice spikelets requires simulation of cropgenerated microclimate. *Eur. J. Agron.* 49:50-60.
- 10. Deva CR, Urban MO, Challinor AJ, Falloon P, & Svitakova L (2020) Enhanced Leaf Cooling Is a Pathway to Heat Tolerance in Common Bean. *Front Plant Sci* 11:19.
- 11. Siebert S, Ewert F, Rezaei EE, Kage H, & Graß R (2014) Impact of heat stress on crop yield—on the importance of considering canopy temperature. *Environmental Research Letters* 9(4):044012.
- 12. Asseng S, et al. (2019) Climate change impact and adaptation for wheat protein. Glob. Chang. Biol. 25(1):155-173.
- 13. Zheng B, Chenu K, Fernanda Dreccer M, & Chapman SC (2012) Breeding for the future: what are the potential impacts of future frost and heat events on sowing and flowering time requirements for Australian bread wheat (Triticum aestivium) varieties? *Glob. Chang. Biol.* 18(9):2899-2914.
- 14. Yin X (No need to switch the modified Arrhenius function back to the old form. *New Phytol.* 2021(in press).
- 15. Parent B & Tardieu F (2012) Temperature responses of developmental processes have not been affected by breeding in different ecological areas for 17 crop species. *New Phytol.* 194(3):760-774.
- 16. Cai C, *et al.* (2020) The acclimation of leaf photosynthesis of wheat and rice to seasonal temperature changes in T-FACE environments. *Glob. Chang. Biol.* 26(2):539-556.
- 17. Vijayaraghavareddy P, et al. (2020) Acquired traits contribute more to drought tolerance in wheat than in rice. *Plant Phenomics* 2020:5905371.
- 18. Fragkostefanakis S, *et al.* (2016) HsfA2 Controls the Activity of Developmentally and Stress-Regulated Heat Stress Protection Mechanisms in Tomato Male Reproductive Tissues *Plant Physiol.* 170(4):2461-2477.
- 19. Larkindale J, Hall JD, Knight MR, & Vierling E (2005) Heat Stress Phenotypes of Arabidopsis Mutants Implicate Multiple Signaling Pathways in the Acquisition of Thermotolerance. *Plant Physiol.* 138(2):882-897.
- 20. Lämke J & Bäurle I (2017) Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. *Genome Biology* 18(1):124.
- 21. Oberkofler V, Pratx L, & Bäurle I (2021) Epigenetic regulation of abiotic stress memory: maintaining the good things while they last. *Curr. Opin. Plant Biol.* 61:102007.
- 22. Shi W, Xiao G, Struik PC, Jagadish KSV, & Yin X (2017) Quantifying source-sink relationships of rice under high night-time temperature combined with two nitrogen levels. *Field Crops Res.* 202:36-46.
- 23. Vu LD, Gevaert K, & De Smet I (2019) Feeling the Heat: Searching for Plant Thermosensors. *Trends Plant Sci.* 24(3):210-219.



- 24. Jung J-H, *et al.* (2020) A prion-like domain in ELF3 functions as a thermosensor in Arabidopsis. *Nature* 585(7824):256-260.
- 25. Hwang G, et al. (2019) Trehalose-6-phosphate signaling regulates thermoresponsive hypocotyl growth in Arabidopsis thaliana. *EMBO Rep.* 20(10):e47828.
- 26. Lyu Jl, *et al.* (2018) Enhanced tolerance to heat stress in transgenic tomato seeds and seedlings overexpressing a trehalose-6-phosphate synthase/phosphatase fusion gene. *Plant Biotechnology Reports* 12(6):399-408.
- 27. Bahuguna RN, Solis CA, Shi W, & Jagadish KSV (2017) Post-flowering night respiration and altered sink activity account for high night temperature-induced grain yield and quality loss in rice (Oryza sativa L.). *Physiol. Plant* 159(1):59-73.
- 28. Sadok W & Jagadish SVK (2020) The Hidden Costs of Nighttime Warming on Yields. *Trends Plant Sci.* 25(7):644-651.
- 29. Impa SM, *et al.* (2021) High night temperature effects on wheat and rice: Current status and way forward. *Plant Cell Environ.* n/a(n/a).
- 30. Ferguson JN, Tidy AC, Murchie EH, & Wilson ZA (2021) The potential of resilient carbon dynamics for stabilizing crop reproductive development and productivity during heat stress. *Plant Cell Environ*. n/a(n/a).
- 31. Webber H, et al. (2018) Diverging importance of drought stress for maize and winter wheat in Europe. Nat. Comm. 9(1):4249.
- 32. Webber H, et al. (2018) Physical robustness of canopy temperature models for crop heat stress simulation across environments and production conditions. *Field Crops Res.* 216:75-88.
- 33. Shimono H, et al. (2010) Diurnal and seasonal variations in stomatal conductance of rice at elevated atmospheric CO2 under fully open-air conditions. *Plant Cell Environ*. 33(3):322-331.
- 34. Yoshimoto M, et al. (2011) Integrated micrometeorology model for panicle and canopy temperature (IM²PACT) for rice heat stress studies under climate change. J. Agric. Meteorol. 67(4):233-247.
- 35. Abdelrahman M, El-Sayed M, Jogaiah S, Burritt DJ, & Tran L-SP (2017) The "STAY-GREEN" trait and phytohormone signaling networks in plants under heat stress. *Plant Cell Rep.* 36(7):1009-1025.
- 36. Driedonks N, *et al.* (2018) Exploring the natural variation for reproductive thermotolerance in wild tomato species. *Euphytica* 214(4):67.
- 37. Hu Y, et al. (2020) Natural variation in HsfA2 pre-mRNA splicing is associated with changes in thermotolerance during tomato domestication. *New Phytol.* 225(3):1297-1310.
- 38. Li X-M, *et al.* (2015) Natural alleles of a proteasome α2 subunit gene contribute to thermotolerance and adaptation of African rice. *Nat. Genet.* 47(7):827-833.
- 39. Raza A, Tabassum J, Kudapa H, & Varshney RK (2021) Can omics deliver temperature resilient ready-to-grow crops? *Crit. Rev. Biotechnol*.:1-24.
- 40. Mishra SK, *et al.* (2002) In the complex family of heat stress transcription factors, HsfA1 has a unique role as master regulator of thermotolerance in tomato. *Genes Dev.* 16(12):1555-1567.



Focus Group S-6: Using plants for carbon sequestration

Andreas P.M. Weber¹, Günter Strittmatter², Peter Westhoff³

1: **andreas.weber@hhu.de** (Heinrich Heine University Düsseldorf, GER); 2: guenter.strittmatter@ceplas.eu (CEPLAS, Düsseldorf, GER); 3: west@hhu.de (Heinrich Heine University Düsseldorf, GER).

Status quo of research in the field

CO2 increase and peak carbon

The conversion of chemical energy that is stored in fossil biomass into thermal energy by thermodynamic (combustion) engines, coal- and gas fired power plants, and in a broad range of chemical-synthetic processes caused an increase of the CO_2 concentration in the atmosphere from 280 ppm (pre-industrial) to 420 ppm in only 150 years, since the start of the industrial revolution. Anthropogenic climate change is predominantly driven by these emissions of carbon dioxide. The major consequence of increased atmospheric CO_2 is an increase in the global average temperature by 1.5 K since 1880. The warming climate is associated with altered precipitation patterns, severe weather events, and a less predictable weather pattern. This presents a major challenge to crop production and food security and hence threatens the foundations of human civilization.

The International Panel on Climate Change (IPCC) has set the goal of limiting global warming to less than 2 K. These science-based recommendations were put into legal action by the 2015 Paris Agreement, a legally binding international treaty on climate change, aiming at limiting global warming to below 2 K, preferably to 1.5 K, compared to pre-industrial levels as the reference. The Paris Agreement was adopted by 196 Parties at COP 21 in Paris in the year 2015 and entered into force on 4 November 2016¹. Reaching these goals mandates achieving climate neutrality as soon as possible, *i.e.*, reducing net CO₂ emissions to zero.

Importantly, the atmospheric temperature increase caused by rising carbon dioxide concentrations is not expected to decrease significantly even if carbon emissions were to completely cease 2,3 . That is, the climate effects of atmospheric CO₂ at peak carbon (*i.e.*, the CO₂ concentration of the atmosphere once net zero emission will have been reached) will remain irreversible for at least 1,000 years, if not counteracted by net reductions in atmospheric CO₂. In a nutshell, anthropogenic climate change is irreversible over 10^{ths} of generations, unless immediate measures are taken to withdraw and sequester carbon dioxide from the atmosphere ³.





Fig. 1. The concept of peak carbon. Left: Fall-off of CO₂ atmospheric concentration after a particular peak concentration has been reached. Right: Global average warming at peak CO₂ concentrations shown in left panel. The curves marked in red assume a peak carbon of 550 ppm and an average global surface warming of 2 K. Adopted from Solomon et al. (2009)³.

Dynamics of the global carbon cycle

The concept of the global carbon cycle describes the cycling of carbon between the atmosphere and marine as well as terrestrial ecosystems. The terrestrial whole ecosystem's mean carbon turnover time is in the range of 23 years, with longer residence times in northern latitudes and shorter ones close to the equator ⁴. That is, the exchange of carbon between atmosphere and biosphere is highly dynamic. Oxygenic photosynthesis by cyanobacteria, algae, and plants represents by far the major route for carbon flux from the atmosphere into the biosphere. Terrestrial gross primary production (GPP) is approx. 120 Gt C. 60 Gt C of terrestrial GPP are lost through autotrophic respiration, which results in net terrestrial primary production (NPP) of approx. 60 Gt C. Aquatic GPP is 103 Gt C and aquatic NPP is 45 Gt C. Overall, terrestrial and aquatic net primary production is in the range of 100 Gt C per year. Parts of this primary production are stored in plant structural biomass and in soils, the vast majority though is returned to the atmospheric CO₂ pool via heterotrophic respiration. In short, the global carbon cycle is nearly balanced. However, human activities perturb the global carbon cycle, leading to a continuous increase of atmospheric CO_2 concentration. Anthropogenic annual carbon emissions are in the range of 8-10 Gt C per year and only approx. half of these emissions are absorbed by the biosphere and geosphere. Since the begin of the industrial revolution, 300 Gt C (on average 2 Gt C per year since the begin of the industrial revolution, with increasing trend) have been moved from the terrestrial to the atmospheric carbon pool (see Figure 2).

875 Gt C			
Contained in	450 Gt C	105 Gt C	916 Gt C
Contained in	Contained in	Net primary	Potential for
atmosphere		Net prinary	
(410 ppm CO ₂)	plant blomass	production	plant biomass

300 Gt C added to atmospheric pool since 1850

Fig. 2. Current carbon pools and potential for plant biomass.

The majority of carbon in the biosphere is stored in plant biomass, with terrestrial plants constituting > 80% (450 Gt C) of the total carbon contained in the biomass of the biosphere (550 GT C)⁵. Approx. 60% of biomass are above-ground (320 Gt C), and 40% are belowground biomass (plant roots, 130 Gt C; soil microbes, 100 Gt C)⁵. Total plant biomass on earth has declined by approx. 2-fold since the begin of human civilization. The amount of plant biomass that was existing before anthropogenic reduction began defines the theoretical potential for carbon storage in vegetative biomass, 916 Gt C, i.e., approx. twice the current plant biomass⁶. This figure is roughly equivalent to the amount of carbon that is currently contained in the atmosphere in the form of CO₂ (900 Gt C; IPCC). Each part per million by volume of CO₂ in the atmosphere represents approximately 2 Gt C, or 7.82 Gt C of CO₂. As of 2018, CO₂ constitutes about 0.041% by volume of the atmosphere, (equal to 410 ppm) which corresponds to approximately 3210 Gt of CO₂, containing approximately 875 Gt C. The current increase of CO₂ in the atmosphere is exceeding 2 ppm / year, i.e., > 4 Gt C⁷ (Figure 3).



The differential between the current pool of carbon contained in plant biomass (450 Gt C) and the potential for plant biomass (916 Gt C) exceeds the total amount of carbon released into the atmosphere through human activities over the past 150 years (300 Gt C). Hence, enormous potential exists for the withdrawal of carbon from the atmosphere and its sequestration in plant and soil microbial biomass, thereby contributing to reversing the damage done by human activities since the begin of industrialization.



The global carbon cycle



Future challenges in the field to be addressed with high priority

From the data reviewed above it is obvious that carbon dioxide removal from the atmosphere will be essential to achieve carbon neutrality as soon as possible, and to reverse anthropogenic changes to the CO₂ content of the atmosphere. Negative carbon emission technologies, *i.e.*, technologies that actively remove more CO₂ than they emit through their operation, such as bioenergy with carbon capture and storage (BECCS) are discussed as a possible solution to these challenges⁸. Also, reforestation and afforestation are considered as a negative carbon emission approaches⁹, as well as restoration of peatlands¹⁰. Further, soil carbon sequestration aims at increasing the soil carbon stock, which will require a combination of improved management practice (*e.g.*, no-tilling), selecting suitable plant species (*e.g.*, deep-rooting species), and depositing slow-decomposing carbon compounds (*e.g.*, biochar) in the soil¹¹. In a nutshell, photosynthetic plants can be considered self-replicating carbon capture and storage devices. Whether or not plant-based climate mitigation strategies will work out as envisaged, will very much depend on the inputs required, such as land, nitrogen, phosphorous, and water¹¹. Resource use efficiency is hence a crucial factor in bringing BECCS to reality.

Energy for BECCS is provided by solar radiation, captured through photosynthesis and stored in plant biomass. That is, atmospheric CO_2 is captured and concentrated in the form of plant biomass, driven



by solar energy. Conversion of the chemical energy stored in this biomass into other forms of energy releases CO₂, which is directly captured and withdrawn from the carbon cycle by deposition in geological formations. BECCS is only effective if the overall process is energy-positive, *i.e.*, provides more usable energy (such as electricity or heat) than is required to capture and sequester the releases of CO₂. Importantly, the IPCC scenarios for limiting the CO₂-induced increase in temperature to 1.5 K or less include a very substantial contribution of BECCS, in the range of one quarter of CO₂ emissions¹². Under current productivity scenarios, such massive deployment of BECCS would require between 25% to 80% of current global cropland¹². Thus, productivity per unit area land (*i.e.*, yield) is crucial, *i.e.*, BECCS and other negative emission approached can only make a substantial contribution to the mitigation of climate change if agricultural land use is reduced. The major challenge in the field is hence enabling negative emission technologies, without compromising food security and without increasing the combined land-use footprint of agriculture and negative emission approaches.

Action points for a future research program in the field

According to the Food, Agriculture, Biodiversity, Land Use and Energy (FABLE) consortium, three pillars must be balanced to align land use and food production to conserve biodiversity, reduce environmental impact of agriculture, and deliver sufficient amounts of healthy foods¹³: i) Efficient and resilient agriculture systems; ii) Conservation and restoration of biodiversity; and iii) Food security and healthy diets. We here add a fourth pillar, iv) Carbon sequestration and storage through photosynthesis to mitigate anthropogenic climate change. A major component of achieving a balance between these conflicting objectives is the production of more plant biomass per unit area arable land¹³, to avoid negative consequences associated with land-use change.¹²

As defined by Monteith¹⁴, yield can be expressed as a fraction of the total incident solar energy available throughout a growing season (*S*), multiplied by the light interception efficiency (ε_i), the partitioning efficiency (η), and the conversion efficiency (ε_c), as given in equation (1) below:

$$(1)W_h = S \times \varepsilon_i \times \varepsilon_c \times \eta$$

The factors of this equation define some of the action points for future research in the field. Interception efficiency and conversion efficiency is crucial for capturing the maximal amount of solar energy in the form of plant biomass on a given amount of land. Partitioning is crucial to increasing the energy contents of plant biomass and to deep-soil deposition of carbon. Beyond these factors, minimizing the input of nutrients and water per unit biomass produced is essential for sustainability and to avoid the depletion of resources.

Increasing photosynthetic efficiency to maximize yield per unit area land

Current highly-bred crops capture less than 3% of the total incident solar energy available throughout a growing season in their biomass¹⁵ and this figure is substantially lower for non-conventional crops with potential use in carbon capture or as feedstocks for novel foods that could replace dairy and meats. To avoid increased land-use as a consequence of increased use of plant biomass for BECCS and other purposes, interception and conversion efficiencies must be increased to approach their theoretical limits. New-to-nature carbon assimilation pathways^{16–18} and extending the spectrum of usable light for photosynthesis beyond the current limits in land plants will



contribute to these goals, as well as more conventional breeding approached, assisted by novel breeding technologies, such as genome editing.

Increasing water-use and nutrient use efficiency

Negative carbon emission technologies such as large-scale capturing of carbon in plant biomass for bioenergy and carbon storage requires massive amounts of land and potentially also irrigation. Quantitative assessment of BECCS with respect to water-use effects has shown that wide-spread use of irrigated biomass plantations would lead to severe water stress on a global scale, probably more so than water stress as a consequence of climate change¹⁹. At the same time, substantial input of mineral fertilizers will be required. While nitrogen fertilizer can potentially be made at the expense of renewable energy sources, phosphate is mined from rock and reserves are finite²⁰, hence nutrient use efficiency is key. Future research goals should thus focus on water-use efficient modes of photosynthesis, such as crassulacean acid metabolism (CAM²¹), in particular inducible and reversible CAM that allows for more efficient C₃ photosynthesis under sufficient water supply while switching to CAM at low soil water potential²². Further, efficient use of soil mineral nutrients through sustenance of a favourable rhizosphere microbiome is an important target for future research. This also hinges on carbon partitioning and photosynthetic efficiency since the nourishment of a suitable microbiome will require root exudates, *i.e.*, carbon released by the plants into the rhizosphere as a currency for nutrient acquisition by microbes.

Deep-soil deposition of carbon

Soils store approximately five-times the amount carbon than our current atmosphere²³ but they are far from saturated with carbon and hence provide massive additional carbon storage capacity²⁴. Plants, via their roots, provide an interface between the soil and atmospheric carbon pools and can serve as carbon pumps that assimilate atmospheric CO₂ and deposit it in the rhizosphere, as soil organic carbon (SOC). A share of SOC, mostly via microbial respiration, is released back into the atmosphere, whereas some SOC remains stored, particularly in deeper soil layers²⁵. The proportion of alkyl C and the mean age of SOC increase with increase in soil depth²⁶, hence aliphatic carbon compounds that are recalcitrant to rapid microbial degradation and that are produced in deepreaching root systems provide a viable means for carbon sequestration²⁵. Consequently action points for future research programs will encompass the mechanisms determining the structure of the plant root system and the partitioning of photosynthetic carbon into aliphatic compounds, such as suberin, in the deep rhizosphere.

Increasing the energy content of plant biomass

The most effective way of BECCS is direct use all of plant stem biomass for bioenergy (*i.e.*, without conversion to biofuels), while capturing most of the CO₂ released in the process¹². Current scenarios and models for BECCS are based on a plant biomass energy contents close to that of carbohydrates, based on the assumption that most of the above-ground biomass consists of ligno-cellulosic structures. Increasing the amount of highly reduced carbon in plant biomass by increasing the amount of oil, suberin, and lignin stored in stems would rise the energy contents of plant biomass by a factor of 1.5 and hence reduce the land-use by 50% per unit of plant biomass energy produced. This demands research directed at unraveling the mechanisms underpinning the partitioning of photosynthates between carbohydrates and fats/oils. Further future research goals include the



replacement of structural carbohydrates with more highly reduced structural components, such as lignin, and the replacement of carbohydrate stores, such as starch, with oil bodies throughout all above-ground organs.

Purpose-bred crops for plant products that can replace dairy, meat, and poultry

A large share of arable land is used for the production of feed for livestock and only a relatively minor part of this land is used for growing crops that directly enter the human food chain. Hence reducing human dependence on livestock-derived products would massively reduce agricultural land use and hence free land for restoration of biodiversity and for climate change mitigation by BECCS. However, changing consumer habits will require time, most likely in the order of generations. Achieving more rapid progress will require plant-based products that replace livestock-derived products, such as meat and dairy, without compromise in taste, texture, nutritional quality, and price point. Making such products at scale and acceptable cost will likely require novel, purpose-bred crops with properties suitable for livestock replacement that meet the expectations of traditional meat and dairy consumers. Large-scale elimination of livestock from the human food chain will massively reduce the amount of arable land used for food production and hence free land for maintaining biodiversity and climate change mitigation.

References

- 1. Ourbak, T. & Tubiana, L. Changing the game: the Paris Agreement and the role of scientific communities. *Clim Policy* 1–6 (2017) doi:10.1080/14693062.2017.1348331.
- 2. Matthews, H. D. & Caldeira, K. Stabilizing climate requires near-zero emissions. *Geophys Res Lett* 35, (2008).
- 3. Solomon, S., Plattner, G.-K., Knutti, R. & Friedlingstein, P. Irreversible climate change due to carbon dioxide emissions. *Proc National Acad Sci* **106**, 1704–1709 (2009).
- 4. Carvalhais, N. *et al.* Global covariation of carbon turnover times with climate in terrestrial ecosystems. *Nature* **514**, 213–217 (2014).
- 5. Bar-On, Y. M., Phillips, R. & Milo, R. The biomass distribution on Earth. *Proc National Acad Sci* **115**, 201711842 (2018).
- 6. Erb, K.-H. *et al.* Unexpectedly large impact of forest management and grazing on global vegetation biomass. *Nature* **553**, 73–76 (2018).
- 7. Quéré, C. L. *et al.* Global Carbon Budget 2018. *Earth Syst Sci Data* **10**, 2141–2194 (2018).
- Fajardy, M., Köberle, A., Dowell, N. M. & Fantuzzi, A. BECCS deployment: a reality check. 1–16 http://www.imperial.ac.uk/grantham/publications/briefing-papers/beccs-deployment-a-reality-check.php (2019).
- 9. Searchinger, T. D., Wirsenius, S., Beringer, T. & Dumas, P. Assessing the efficiency of changes in land use for mitigating climate change. *Nature* **564**, 249–253 (2018).
- 10. Leifeld, J. & Menichetti, L. The underappreciated potential of peatlands in global climate change mitigation strategies. *Nat Commun* **9**, 1071 (2018).
- 11. Smith, L. J. & Torn, M. S. Ecological limits to terrestrial biological carbon dioxide removal. *Climatic Change* **118**, 89–103 (2013).
- 12. Harper, A. B. *et al.* Land-use emissions play a critical role in land-based mitigation for Paris climate targets. *Nat Commun* **9**, 2938 (2018).
- 13. Schmidt-Traub, G., Obersteiner, M. & Mosnier, A. Fix the broken food system in three steps. *undefined* 181–183 (2019).
- 14. Monteith, J. L. Climate and the Efficiency of Crop Production in Britain [and Discussion]. *Philosophical Transactions Royal Soc B Biological Sci* **281**, 277–294 (1977).
- 15. Long, S. P., Marshall-Colon, A. & Zhu, X.-G. Meeting the Global Food Demand of the Future by Engineering Crop Photosynthesis and Yield Potential. *Cell* **161**, 56–66 (2015).
- 16. Scheffen, M. *et al.* A new-to-nature carboxylation module to improve natural and synthetic CO₂ fixation. *Nat Catal* 1–11 (2021) doi:10.1038/s41929-020-00557-y.



- 17. Trudeau, D. L. *et al.* Design and in vitro realization of carbon-conserving photorespiration. *Proc National Acad Sci* **115**, E11455–E11464 (2018).
- 18. Weber, A. P. M. & Bar-Even, A. Update: Improving the Efficiency of Photosynthetic Carbon Reactions. *Plant Physiol* **179**, 803–812 (2019).
- 19. Stenzel, F. *et al.* Irrigation of biomass plantations may globally increase water stress more than climate change. *Nat Commun* **12**, 1512 (2021).
- 20. Abelson, P. H. A Potential Phosphate Crisis. *Science* 283, 2015–2015 (1999).
- 21. Winter, K., Holtum, J. A. M. & Smith, J. A. C. Crassulacean acid metabolism: a continuous or discrete trait? *New Phytol* **208**, n/a-n/a (2015).
- 22. Brilhaus, D., Bräutigam, A., Mettler-Altmann, T., Winter, K. & Weber, A. P. M. Reversible Burst of Transcriptional Changes during Induction of Crassulacean Acid Metabolism in *Talinum triangulare*. *Plant Physiol* **170**, 102–122 (2016).
- 23. Schuur, E. A. G. et al. Climate change and the permafrost carbon feedback. Nature 520, 171–179 (2015).
- 24. Kell, D. B. Large-scale sequestration of atmospheric carbon via plant roots in natural and agricultural ecosystems: why and how. *Philosophical Transactions Royal Soc B Biological Sci* **367**, 1589–1597 (2012).
- 25. Schweitzer, H. *et al.* Innovating carbon-capture biotechnologies through ecosystem-inspired solutions. *One Earth* **4**, 49–59 (2021).
- 26. Lorenz, K., Lal, R., Preston, C. M. & Nierop, K. G. J. Strengthening the soil organic carbon pool by increasing contributions from recalcitrant aliphatic bio(macro)molecules. *Geoderma* **142**, 1–10 (2007).