CropBooster-P

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1 EXECUTIVE SUMMARY

**CropBooster-P, its implications and the '*Green deal'*

The CropBooster-P project is the response to the H2020 call "*Future proofing our plants*" and aims to draft a roadmap to develop the future crops for European agriculture. CropBooster-P explores options to future-proof our crops by conventional breeding and/or by more advanced methods, focusing on increasing crop yield with a concomitant increase in quality and sustainability. Quality relates to nutritional quality (e.g. proteins, fatty acids, vitamins, etc.) and/or industrial quality (e.g. fibres, extractability, conversion of biomass, digestibility, etc.) whereas Sustainability relates to abiotic stress tolerance (e.g. heat, freezing, minerals, water management including drought, salinity, etc.) and resource use efficiency (e.g. water use, nitrogen use, etc.). Overall, a more sustainable agriculture implies the production of more efficient crops to reduce water and fertilizers use and increase soil fertility through higher organic matter storage. Reduced fertilizer applications will significantly contribute to the 'zero-pollution point of the *Green Deal'* as recently proposed by the Commission. Also reductions in CO_2 emission due to a decreased use of fertilizers and decreased NH_4 emission due to an improved nitrogen use efficiency will result in the overall reduction of greenhouse gas emissions. As such, the CropBooster-P project fully aligns with the goals, ambitions and objectives of the '*Green Deal'* policy by supporting the transition towards more sustainable and socially just ways of producing, consuming and trading, while preserving and restoring our ecosystems. In addition, future-proofed crops will be essential in a sustainable primary production system, "*from farm to fork*", which is inclusive, safe and healthy to ensure food and nutritional security for all. These crops will also allow to unlock the full potential of both land-based and aquatic production systems to deliver sustainably, high quality biomass for both food, feed and non-food, bio-based purposes. Since CropBooster-P aims to increase the yield of improved products, it may in the longer-term also contribute to increase the incomes of farmers. In addition, consumers will gain a better perception of farming practices and agriculture at large. In the future, however, also improving and optimizing agricultural practices like, for instance, soil and water management, pest and disease control, and application of fertilizers will be essential to establish the envisaged future primary production system. Whilst fully acknowledging that achieving the desired productivity will need to include all aspects of the agricultural system, including e.g. also agronomy, phytopathology and plant micro-organisms interactions, the focus of CropBooster-P focuses only on the genetic basis for crop improvement delivered through conventional breeding and/or integrated with biotechnological methods. CropBooster-P focusses on traits related to increasing yield potential, yield stability under highly fluctuating environment, resource use efficiency and nutritional quality, and sustainability. Due to resources available to the project other important traits such as biotic interactions and organoleptic quality (consumer adoption) are out-of-scope of the CropBooster-P project. Such areas as these, will in the future be investigated in parallel projects. A tight coordination between these projects and CropBooster-P may be further developed in any future phase of the CropBooster-P project.

Conclusions and outcomes of CropBooster-P Work Package 1 (WP1)

To assess societal developments and potential impact of climate changes by 2050, a Scenario building analysis was carried out within the CropBooster-P project taking into account a multitude of current trends and eight key uncertainties. In this Scenario building analysis, four contrasting and extreme scenarios were developed which are individually implausible, but not impossible, and depict future socio-economic developments: '*Plantovation*', '*Your Food. Your Health. Your Choice*', '*Foodmergency*' and '*REJECTech*'. The real future will probably not be one of these four extreme scenarios, but a yet unknown mixture of these four. Accordingly, they

delineate together the future space of all possible scenarios and were used to assess the options to improve crop species. To identify future key options to improve crop productivity and quality, experts created and assembled a database including over 800 scientific and technical manuscripts highlighting the potential to improve a variety of traits in a wide variety of plant species using different state-of-the-art technologies. In this way, the database does not only capture information related to the plant traits, but also the technologies, genes and methods for crop improvement. Based on the database and the broad expertise represented in the CropBooster-P project, 20 key options were selected and used as examples of how crop yield could be improved while maintaining or improving nutritional quality in a sustainable manner. These key options include, among others, increasing photosynthesis, organ growth, nitrogen use efficiency, seed filling, protein and amino acid composition, micronutrient quality and improving the digestibility and conversion of plant biomass. Based on the database, we also identified some key options for future research that are common to yield, nutritional quality and sustainability, such as the further development of modelling approaches and improving methods for breeding and genome editing, and the largely underexplored potential of aquatic and certain underutilized species. All the key options described as enablers to improve yield, nutritional quality and/or sustainability refer to "transferable traits" e.g. traits that can be transferred between plant(s) (species) using biotechnological methods, encompassing modern plant breeding approaches like marker assisted selection/breeding (MAS/MAB) or genome assisted breeding, advanced breeding technologies and novel DNA mutagenesis technologies like CRISPR/Cas, gene transfer technologies (GMO), and synthetic biology approaches, allowing the transfer of complete novel metabolic and/or genetic pathways. Summarizing, all genetic- and biology-driven knowledge-based technologies allowing crop productivity improvement.

Which of the identified options to improve our crops that is actually used will, however, largely depend on the extent to which society will accept and allow their use. Therefore, a first analysis was done involving Stakeholder consultations to interrogate the implications of the different scenarios on Europe's options to future-proof its crop plants. This preliminary analysis showed that the four developed scenarios mainly differ in the extent to which application of biotechnological methods is accepted by society, and to the extent societal need/urgency requires the execution of each of these scenarios. Besides, the possible future scenarios also have major implications for agriculture, consumers and Europe. The '*REJECTech*' scenario is the most extreme scenario around the topic of society rejecting the use of technology in agriculture and would have major implications on our options to produce food. In a '*REJECTech*' scenario, we would mainly be dependent on empirical mutagenesis approaches, including marker assisted selection/breeding and TILLING populations. These are, however, time-consuming and limited and almost certainly will not meet our crop productivity demands in time. In addition, if gene transfer technologies (GMO) and synthetic approaches would not be accepted by the public at large, as technologies that could be used to improve crop productivity would be markedly reduced. Accordingly, under a '*REJECTech*' scenario, but also in a '*Foodmergency*' scenario, food choices are proposed to be limited and likely to be highly dependent on import. The '*Foodmergency*' and '*Your Food. Your Health. Your Choice*' scenarios differ from each other as under the '*Foodmergency*' scenario the production of calories is likely to occur at the expense of the environment, whereas in a '*Your Food. Your Health. Your Choice*' scenario, which is the most consumer-driven scenario, environmental concern-based choices might be dominant. Both a '*Your Food. Your Health. Your Choice*' and a '*Plantovation*' scenario are likely to co-occur with the emergence of a flourishing Bio-economy, with major applications of large scale ICT and AI technology in agriculture.

Finally, as part of the first year of CropBooster-P, the potential of existing off-the-shelf modelling approaches was demonstrated using a case study. Using mechanistic and thoroughly understood models of photosynthesis, parameters were adjusted that correspond to small simple adjustments expected to be readily achievable using current approaches. The potential effects of these modest changes were determined for both C3 and C4 crops simulated at 66 sites across

Europe. The largest effects were achieved by increasing all parameters in concert: biomass increases of more than 30% were predicted under recent historical climate conditions. Even with modest improvements of single photosynthetic traits, simulated average wheat yield increases across Europe under future climate scenarios were up to 35%. These results demonstrate the potential of improving photosynthesis to increase plant biomass. However, it is important to recognise that the most promising candidate approaches for improving photosynthesis (e.g., carbon concentrating mechanisms, photorespiratory bypass, or faster dynamic adjustment to changing light conditions) are expected to be still more powerful than the single-factor changes in Rubisco performance and electron transport that we were able to simulate. In future, development and distribution of more sophisticated models that incorporate a wider range of mechanisms and processes, that incorporate recent innovations, in particular dynamic changes in light availability and radiation distribution within crop canopies, could enable better predictions about how the most powerful approaches for increasing photosynthesis will function at crop scales.

Future CropBooster-P perspectives

During the first year of the CropBooster-P project we developed possible future scenarios, defined key options to improve crop yield, nutritional quality and sustainability and demonstrated by modelling approaches that tweaking individual traits will improve crop biomass, using photosynthesis as a case study. Taking together, we therefore have set the option space for considering (a) which crops and plant species should be in focus, (b) what technical possibilities will be available to adapt future plants & which ones should be considered, and (c) what crop traits need to be engineered into plants to meet the needs of future society. These first analyses will be followed by additional, more in depth analyses over the next 2 years to further develop, present and discuss these findings in a series of Stakeholder consultations involving consumers, farmers, industry, policy-makers and academia. The outcomes will be analysed for their ability to support the desired outcomes of the '*Green Deal*' and Farm-to-Fork strategies prior to being translated into recommendations. The final conclusions will be presented in the form of a Roadmap stipulating the best options forward that Europe has to sustainably future-proof its crops, while securing the broadest societal support possible for the transition towards the desired future agricultural production systems.

2 INTRODUCTION

Food security, growing crop yields and population growth are the greatest challenges facing humankind. We will need to prepare for feeding 9.7 billion people in a sustainable way by 2050, whilst transitioning from a fossil economy towards a bio-economy in order to mitigate the effects of global climate change. This will require a doubling of global crop productivity to produce enough plant biomass to both achieve food and nutrition security, as well as to meet the demands of a future bioeconomy. Projections from the current rates of crop yield increases suggest we will fall 40 – 70% short of this future demand. To make the challenge still more demanding the increase in crop production must be achieved while maintaining nutritional quality and will require crops that combine sustainability, efficient use of scarce resources (e.g. water and minerals) and cultivation schemes and practices that preserve Earth's biodiversity. The crops must also have good yield stability with a high resilience to adverse climate and volatile weather conditions and the environmental impact of agriculture will have to be minimized to, among others, increase carbon smartness ('*Green Deal*').

Europe has diverse climates and multiple regions that are vulnerable to climate change. Rising environmental constraints progressively limit agricultural productivity especially in the Mediterranean region. Conventional terrestrial agriculture will need plants that are adapted to the changing climate, and we must add the potential productivity of the marine system as a source for food and non-food biomass. To mitigate the effects of climate change, we will also need to use soil and wood carbon pools to sequester atmospheric carbon dioxide, something to which agriculture could make an important contribution. The future requirements for our crops are diverse and demanding, yet the outcomes will be valuable in terms of food and nutritional security, transitioning to a non-fossil carbon economy, sustainability and managing climate change. These value contributions will give a new purpose to the rural economy of Europe. So Europe is seeking a long-term strategy for future proofing its crops to realise these goals.

To meet these aspirational demands, our current crop plants will need to be re-designed and thus mapping out how they can be "future proofed" is urgently needed. Progress could be mired by the complexity of a multitude of possible crops and genetic changes, combined with multiple environmental change, policy and societal challenges. CropBooster-P is a breeding program that aims at addressing this by identifying opportunities to adapt and boost productivity in a backgound of environmental and societal changes. Our objective is to undertake a comprehensive evaluation of the most promising practical approaches to be enacted from 2021 onwards to achieve a sustainable food supply into the future. The technical goal of CropBooster-P is to produce a plan for the future proofing of Europe's crops, sustainably increasing their yield and adapting them for the future climates of Europe. This plan will include a set of technical options for doing this and a management plan for the implementation of these options, with the aim of providing innovative breeding starting points needed for the production of new crop cultivars. This will be done with the cooperation and engagement of the plant breeding sector.

Whilst fully acknowledging that achieving the desired productivity will need to include all aspects of the agricultural system (e.g. agronomy) the focus of CropBooster-P is solely on the genetic basis for crop improvement delivered through conventional breeding integrated with biotechnological methods. CropBooster-P includes traits related to increasing yield potential, yield stability under abiotic stress, resource use efficiency and nutritional quality, and sustainability. To create a critical scale effort with increased chances of success, CropBooster-P is excluding improvement of equally important traits such as biotic interactions and organoleptic quality.

The CropBooster-P programme is achieving its goals via a set of work packages that collectively identify the most important terrestrial, aquatic and/or underutilized crops for Europe's future. By identifying the best options for improving the yield of these crops while enhancing nutritional quality, testing these options against environmental, economic and other technical criteria to refine the best technical choices, and subjecting these options to public scrutiny. This is being achieved via a process of analysis, consultation and reviews to ensure there is as broader input from experts, stakeholders and public as possible. To support this process, we have carried out a scenario planning exercise in which we have developed four contrasting learning scenarios considering a multitude of trends and eight key uncertainties. These learning scenarios depict complementary future socio-economic developments and the insight into desirable and undesirable implications will assist the development of recommendations within CropBooster-P towards a desirable outcome in line with the '*Green Deal*' target.

CropBooster-P will thus devise a strategy for future proofing of Europe's plants and deliver an inclusive research programme to implement the better options in view of four contrasting future scenarios This will be a key resource for the forerunner of a programme to put this strategy into effect. This future programme will be a European contribution to future global food and nutrition security. It will embody the ambitions of the FOOD2030 Policy Agenda and many of the United Nations sustainable development goals. A sustainable increase in crop yields with guaranteed nutritional value will, we believe, be an agreeable value proposition to the public. The economic benefits of such a boost to yields at lower input cost and better land use efficiency would see the rural economy serving as engine of an expanding environmentally friendly, non-fossil manufacturing and energy economy. It would produce food, feed and feedstock in a sustainable, resource-use (e.g. water and nutrient use) efficient way; food-first, cascading and circular.

This report is the initial step in the CropBooster-P actions which covers the development of learning scenarios of future worlds and the assessment of the state of the art of in terms of increasing plant yield, nutritional quality, and sustainability. The report is a review of the literature and the technologies available to experts and key stakeholders. Also included is a modelling section demonstrating the potential impact of a key trait. The report includes 20 key options for traits that have already been successfully selected/manipulated and could contribute to 'future proofing our crops'. The utility of each example has been evaluated against 4 future scenarios. WP1 will serve as a foundation for the following WPs. Accordingly, it should be both a scientifically sound overview of the state-of-the-art (serving WP4), as well as an input document to engage into a discussion with particular stakeholders (WP2) and the general public (WP3).

DEFINITION BOX

To sustainably meet our future food requirements, we need to produce more food on the same or even reduced (i.e. as a result of urban development or sea level elevation etc.) land area with increased resource-use efficiency and do so prepared for climate change. This food must also be produced while ensuring environmental protection. The need to increase crop yield does not arise only from food and feed demands. The future will see an expansion for competing uses for agricultural products, for example, as feedstocks for the Bioeconomy or the production of biofuels. These competing uses for agricultural productivity are themselves driven by a desire to limit climate change and transition to a more sustainable base for industry and energy supplies.

*CropBooster-P prepares a Roadmap to develop the required superior crops for the future using a plant-centered breeding approach. Accordingly, improving crop management or agricultural practices are considered out-of-scope. The CropBooster-P project focusses on three main areas: breeding for increasing **yield**, breeding for increasing **nutritional quality** and breeding for increasing **sustainability**. In the project, the following definitions are used:*

***Yield** refers in first instance to the total amount of crop biomass produced per area per year. Increasing yield thus encompasses all breeding options to develop plants, both terrestrial and aquatic, that produce more biomass per unit of area (bigger plants) and/or that produce more biomass per unit of time (faster growth). This definition of yield is valid especially for plants that are used for non-food applications and that in general are harvested as total plant biomass. For food purposes an additional constraint for yield applies, as the overall yield increase of a plant mainly should be due to the increase of edible (harvestable) plant parts. So methods to increase, for instance sugar, protein and metabolite concentrations in specific plant parts and/or organs also are regarded as options to increase plant yield.*

*For food crops, quality concerns the **nutritional quality** of the edible parts of the plant. Increasing quality thus entails all breeding options that results in an increase in, for instances, protein content, mineral content, fatty acid content, etc. In our definition, organoleptic (taste, smell, mouth-feeling) properties are out-of-scope as these quality aspects are not considered as crucial to safeguard future food security and as the project does not deal with improving nutrient supply, yet they are recognized as key to enable diet shift. For non-food purposes, quality concerns those plant characteristics that determine the specific application for which the crop is used, for instance fibre digestibility for cattle or fibre quality for industry, oil content or THC concentration.*

***Sustainability** relates to two aspects that both are associated with harnessing our crops to the imminent effects of climate change: abiotic stress resistance, resilience and/or acclimation and resource use efficiency In one aspect thus, increasing sustainability encompasses all breeding options to increase the resistance of our crops against abiotic stresses like heat, water management (such as drought, salinity, flooding, etc.), freezing, etc. Due to capacity reasons, biotic stress resistance is out-of-scope of the CropBooster-P project. In the second aspect, increasing sustainability relates to breeding for better resource uptake and use efficiency of our crops, like increasing water, phosphorus, nitrogen, minerals, etc. In the context of the CropBooster-P project, Sustainability refers therefore only to environmental sustainability, whereas societal and economic sustainability are not taking into account, at least not in the scope of WP1.*

***Biotechnological methods** encompass modern plant breeding approaches like marker assisted selection/breeding (MAS/MAB) or genome assisted breeding, advanced breeding technologies and novel DNA mutagenesis technologies like CRISPR/Cas, gene transfer technologies (GMO),*

and synthetic biology approaches, a variant of gene transfer allowing the transfer of complete novel metabolic and/or genetic pathways. More broadly, biotechnological methods therefore refer to all biology-driven and knowledge-based technologies.

3 WHAT IS THE OPTION SPACE TO IMPROVE CROP PRODUCTIVITY? – Scenario Analysis

The purpose of the scenario analysis was to provide novel perspectives for the work of other work packages and to serve as a means of communication to facilitate a common understanding of possible future challenges both within the CropBooster-P workstreams and also with external stakeholders.

3.1 Scenario Analysis - Approach

Scenario analysis unfolded in two phases: (1) preparation and (2) scenario building and impact assessment. The first step that laid the groundwork for scenario building included developing a common understanding of the relevant areas of interest and deciding on the scope of scenarios (e.g., time frame and crop types). In the second step of preparation we performed trend research to improve our understanding of direction in which future worlds could develop. Therefore, we carefully selected the most relevant ones and explored a potential impact of 45 different technological, economic and societal trends. We then identified key uncertainties that would play out differently for each scenario to make sure that diverse future worlds are represented. Eventually, we arrived at four learning scenarios that were introduced to external stakeholders to jointly develop them further and identify potential impacts. By creating "prototypes" for various scenario aspects (e.g., the typical dinner of a French middle-class family, the required training of a farmer in 2050.) we filled scenarios with life and made them more tangible. To support co-creation with external actors we discussed the potential impact of the scenarios for CropBooster-P in dedicated subgroups with a focus on one of three topics: sustainability, yield, or nutritional quality. An extended version of the different steps of the Scenario building, can be found in *Annex 1*.

3.2 Outcome of Scenario Analysis

The outcome of the scenario analysis exercise are four learning scenarios for agriculture in the EU in 2050:
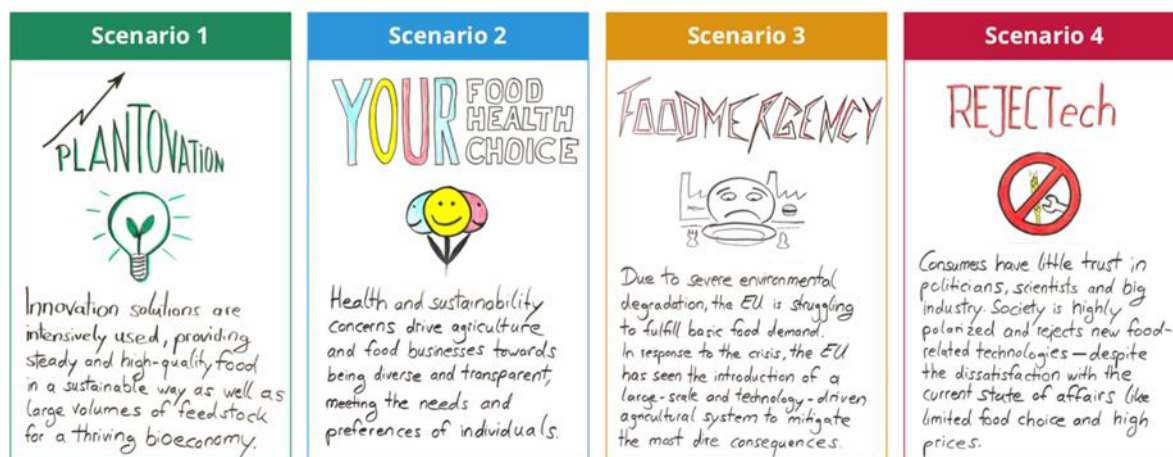
3.2.1 'PLANTOVATION': A SUCCESS STORY OF INNOVATION IN AGRICULTURE

Innovation solutions are intensively used, providing steady and high-quality food in a sustainable way as well as large volumes of feedstock for a thriving bioeconomy.

From today to 2050 — how the scenario may become reality:

- A technology revolution is already under way today:
    - Artificial intelligence beats the best Go players and creates artworks people can't distinguish from those made by humans
    - New gene editing technologies make things possible that seemed like science fiction just a while ago
- At the same time, we need to fix our CO_2 problem — and biomass provides a feasible, relatively cheap solution
- In the coming years, more and more entrepreneurs will be applying revolutionary technologies of the next generation to grow crops for food and non-food applications
- The reservations against certain plant technologies visible today quickly fade as startups celebrate breakthroughs and spectacular results — pleasing both consumers and investors
- The increasing flow of venture capital into this domain eventually leads to big, multinational "agritech" companies
- As a result, the next generation of multi-billionaires will not be in software — they will be in "agritech"

3.2.2 'YOUR FOOD. YOUR HEALTH. YOUR CHOICE': HOW CONSUMERS BECAME KING

Health and sustainability concerns drive agriculture & food businesses towards being diverse and transparent, meeting the needs and preferences of individuals.

From today to 2050 — how the scenario may become reality:

- Already today, one can buy a Coke with one's name printed on it or mass customize muesli to satisfy individual preferences
- Data as a resource and the ongoing digitalization will continue to enable new business models and societal opportunities
- By gathering and analyzing data, health will move from curing to preventing disease. Personalised medicine will take off in the coming years increasing focus and efficacy — which is urgently needed to contain civilization conditions like diabetes that are otherwise spreading quickly
- At the same time, the actual impact of diet on our health will become more and more transparent, leading to a convergence of medicine and nutrition
- Visionary entrepreneurs will be quick to seize the emerging opportunities and give consumers what they want: their food, their health, their choice

3.2.3 'FOODMERGENCY': A CAUTIONARY TALE ABOUT FOOD SECURITY

The EU is struggling to fulfill basic food demand due to severe environmental degradation. In response to this, the EU has seen the introduction of a large-scale and technology-driven agricultural system to mitigate the most direct consequences.

From today to 2050 — how the scenario may become reality:

- The warnings from scientists against climate change and its potential impact on agriculture are loud and clear
- Unfortunately, the pessimistic views turn out to be correct: the EU — like many other countries around the world — faces a series of severe environmental crises. As a result, the EU experiences dramatic food security challenges
- Globally, the situation is even grimmer in some cases. The global economy suffers and international food trade collapses due to global shortages
- As food security can no longer be taken for granted, the EU and national governments take radical steps to increase the production of food crops: this includes regulation that favors yield-oriented biotech and drastic market interventions to increase European production capacities for food
- Over the course of just a few years before 2050, a government-directed, large-scale agricultural system is created
- Society backs these policies because they seem to be without alternative. However, the widespread use of advanced biotech, instances of public mismanagement and personal restrictions maybe seen critically

3.2.4 'REJECTECH': WHEN SCIENCE LOST THE PEOPLE

Consumers have little trust in politicians, scientists and big industry. Society is highly polarised and rejects new food-related technologies – despite dissatisfaction with the current state of affairs like limited food choice and high prices.

From today to 2050 — how the scenario may become reality:

- Skepticism, fake news, and heated discussions in the (social) media have become a global phenomenon. This development also affects agricultural topics as the use of glyphosate illustrates. Europe becomes particularly precautionary
- As the possibilities from technologies like gene editing, A.I. and robotics increase, so does the probability of failed experiments
- A steady stream of food scandals and examples of scientific misconduct also do its share to further erode European society's trust in science, industry as well as in the institutions that are supposed to supervise them
- Over the years, sustainability becomes synonymous with the absence of advanced biotech. Eventually, the EU enacts strict regulation to please skeptical voters and large agri companies lose their license to operate
- The rejection of technology has its price, however: growing challenges from climate change and low productivity in agriculture result in supply problems and increasing prices. As agriculture develops significantly slower in the EU than abroad, trade imbalances arise and force EU agriculture to find alternative value adding activities

Note: Storylines are not predictions but rather possible yet uncertain paths of development.

See also:
Annex 1: CropBooster-P Scenario Analysis documentation (A PDF version illustrating the Scenario Building workshops).

The scenario analysis set the option space for considering (a) which crops and plant species should be in focus, (b) what technical possibilities will be available to adapt future plants & which

ones should be considered, and (c) what the crop traits are that need to be engineered into plants to meet the needs of future society.

3.3 Scenario implications for increasing crop productivity and quality

3.3.1 FOCUS ON YIELD

In the different scenarios yield is to be addressed in multiple crops, including scenario specific crops for different use purposes and under different regulatory conditions. Consequently, preparatory work for yield improvement should offer a broad range of biological starting points and be achievable through different technical approaches.

SCENARIO 1: PLANTOVation	Major yield improvements for a flourishing B2B environment will drive welfare and wellbeing of society and Europe; multi-purpose crops for bulk production and specialties will dominate a circular bioeconomy; yield improvement should seamlessly work in conjunction with acquisition of new product functionalities. IP and value share are core success factors.
SCENARIO 2: YOUR FOOD HEALTH CHOICE	Heterogeneous food preferences will be requiring smaller scale production chains. The yield of an increasing range of crop varieties cultivated with agricultural practices agreeable to the end consumer will be critical to keep costs of EU food production within a realistic range. The ecological impact may be positive and part of the value equation. Key is that consumer choice translates into proper pricing and value share across the EU ag chain.
SCENARIO 3: FOODMERGENCY	The prime scope is to accomplish a sufficient level of global food production, which may include diet shifts. To achieve yield under volatile and new weather conditions, it will be necessary to upgrade all biological processes linked to energy management and abiotic stress handling. R&D costs will be high, as will be the cost of cultivation that will require optimised agricultural practices for planting, rotation, nutrient input, etc. Few crops will qualify for this. This immediately poses sustainability issues.
SCENARIO 4: REJECTech	The inability to tackle crop yield within Europe with the same approaches as outside Europe implies that options for damage control need to be considered at policy level. In the background, yield improvement needs to be achieved through exploring novel knowledge-based breeding workflows that do not rely on GMO or gene editing, yet deliver a competitive annual yield gain.

3.3.2 FOCUS ON NUTRITIONAL QUALITY

Nutritional quality plays different roles across the scenarios and offers both commodity and specialty crop differentiation opportunities.

SCENARIO 1: PLANTOVation	To meet the (global middle-class) consumer demand for sustainable and supplement-free products, advanced breeding approaches for high quality food and feed are economically justifiable. Both commodity and specialty crops will undergo rounds of improvements balancing with improvements made for co-product and biorefinery strategies.
SCENARIO 2:	Customer demand drives the development in food and feed. To meet expectations on nutritional quality, taste and other sensory qualities, the

YOUR FOOD HEALTH CHOICE	core causative biological processes in crops and livestock need to be understood and converted to advanced breeding approaches. As consumer demand also includes a heterogeneous choice, a multitude of commodity and specialty crops need to be upgraded.
SCENARIO 3: FOOD EMERGENCY	The challenge to deliver food in sufficient quantity leads to nutritional quality not being a primary target. A lobby for diet shift may affect the ratio feed: food production thus reducing the pressure on land use. To incentivize consumers shift diet, timely availability of food with improved nutritional quality, taste and other sensory features could be key. This requires know-how similar as in scenario 2, yet with a key requirement that such improvement doesn't bring along any penalty in yield and abiotic stress handling.
SCENARIO 4: REJECTech	In the absence of competitive yield and volume-based off-farm prices, farmers may seek differentiation in quality including nutritional quality, taste and other sensory features of interest to the consumer. The know-how requirement will be similar to that in scenario 2, but as in scenario 3 improvements may not jeopardize yield. Moreover, it must be possible to develop the improvements through workflows not using GMO or gene editing methods.

3.3.3 FOCUS ON SUSTAINABILITY

Sustainability in agriculture is core across all scenarios. However, whereas sustainability opens up many opportunities in scenarios 1 and 2, it is rather a necessity due to environmental crises in scenario 3 in particular. Moreover, the meaning of the concept of sustainability and corresponding agricultural practices will differ significantly between scenarios. Sustainability requirements for crops include optimised production of biomass and harvestable product, suitability for co-product strategies, minimal requirements for land use, disease and pest treatment, nutrient input as well as tolerance to weather fluctuations, soil-friendly cropping needs and good rotation compatibility.

SCENARIO 1: PLANTOVATION	This scenario envisages the largest growth of productivity. Embracement of the bio-economy is foreseen. From a know-how perspective, this scenario puts the highest urgency on incorporating and testing different sustainability features. From a know-how development perspective, it implies that the biology underpinning these different features should be tackled in parallel. This may require international cooperation.
SCENARIO 2: YOUR FOOD HEALTH CHOICE	Customer demand for healthy and sustainable food produced in a transparent manner will open markets where the sector needs to prove its capability of meeting the specific emphasis points of customers related to sustainability. This may imply "chemical free" production procedures, no tilling, limited nutrient (N) supply. Know-how development should therefore focus on addressing these topics first, and have it seamlessly incorporated with the elevated requirements for nutritional quality and yield in the different crops.
SCENARIO 3: FOOD EMERGENCY	Society and governments across the globe are expected to agree to any proposal from the Ag value chain that would help stabilize or revert the situation. As in scenario 1, a broad repertoire of sustainability options needs to be investigated in parallel, but with some key differences. It is likely to that disease and pest management as well as nutrient supply are tackled by integrated crop–chemical approaches. Furthermore, improvement on those sustainability traits should not create any yield penalty.

SCENARIO 4: REJECTech	In the absence of competitive yield and volume-based off-farm prices, farmers may seek differentiation towards end customers by being a leader in sustainable agricultural practices. This would require know-how to create "sustainability traits" that do not rely on GMO and gene editing technology.

By design, the four scenarios are plausible but unlikely. Hence, reality in the year 2050 will probably include aspects from each of the learning scenarios as well as some unforeseen outcomes. The set of scenarios covers a broad range of outcomes related to major uncertainties about EU agriculture. As the scenario planning was performed as a starting point, it is recommended to proceed with further impact analysis to reach an even broader understanding of the diversity of possible future(s). This will provide new perspectives that make CropBooster-P's project results more robust and facilitate a more proactive stance towards future threats and opportunities.

Within Work Package 1 of CropBooster-P, the four scenarios frame the option space regarding three key topics: (1) yield, (2) nutritional quality, and (3) sustainability. In the context of the overall project, this initial Scenario Analysis provides the reference point for a multidimensional assessment including the economic, social and environmental impact (Work Package 2), societal needs and expectations (Work Package 3), international cooperation (Work Package 4), and finally strategy development (Work Package 5).

Scenario project provides the starting point for further exploration and for taking specific measures towards more desirable future outcomes. In order to maximize the value generated by the Scenario Analysis, we recommend the following steps:

1. Detail the scenarios: additional value can be captured from the scenarios by further developing some particular interesting aspects. This may include additional research on important trends and uncertainties.
2. Prepare for the future: Develop a CropBooster-P roadm stap balancing opportunity, need and risk by appreciating the spread and commonalities of desired outcomes under different scenarios.
3. Identify early warning signals: Before any of the scenarios fully materializes, there will be weak signals that can be picked up if one looks for them. By identifying these signals now and incorporating them into the roadmap, CropBooster-P can minimize risks, increase chances of success and deliver on time.
4. Create the future: Don't just wait and see how the future unfolds: take specific measures today to prevent undesirable outcomes or scenarios from happening, communicate and discuss them with relevant stakeholders.

4 HOW TO IMPROVE CROP PRODUCTIVITY? – KEY OPTIONS

4.1 Data Collection - Approach

In order to compile a toolbox of transferable strategies, methods and technologies for a sustainable improvement of crop plants, it is necessary to assess available state-of-the-art data, techniques and published information in terms of increasing plant yield, nutritional quality and sustainability. To achieve this goal, a survey- based data collection strategy was initiated. The focus of the data collection had the following pre-defined considerations:

- ✓ The four WP1.1 output scenarios were used as guides or frameworks within which to design relevant questions to include in the survey.
- ✓ The survey entrants focused on capturing current information (latest advances in a field, high impact publications, keystone publications).
- ✓ Studies highlighting transferable and heritable traits and technologies as well as technologies with economic potential were prioritised.
- ✓ A special focus was made to capture existing knowledge on studies made in underutilised terrestrial and aquatic crops.

The existing literature of relevance to the project is vast, repetitive across species and traits. Therefore, there was a need to prioritise targeted data capture that spanned the relevant genes, traits and technologies of interest with respect to crop improvement and have a prospective view. To achieve this goal, a panel of 68 experts belonging to 20 participant institutions was selected by the WP leaders and WP1 core team based on their scientific expertise and high visibility in their respective fields ensuring a broad representation of species, research fields, approaches and technologies. Each expert was asked to identify the 10-15 most relevant papers including few outstanding reviews in their field of expertise. These restrictions were imposed to limit biases and collect the most relevant data.

Data collection proceeded in the form of several surveys that fed into one core database. This presented the advantage of capturing data in real time, across countries and fields of expertise in a sequential, logical, easy-to-follow manner. The resultant database could then be queried for specific information based on area of interest. The database was built in two phases (1) defining priority crops and (2) design of a survey asking the questions of interest.

4.1.1 SPECIES SELECTION

In order to ascertain coverage of all categories of crops in the database, priority/ specimen crops to be focused on during the project were defined, with a few selected species representing each crop category. This selection was done by the WP1 core team and qualified by the WP1.2-1,4 partners, keeping in mind those species which are either model species or relevant crops for europe, well studied, application oriented and relevant to the scope of the CropBooster-P project.

The outcome of the crop species selection process was the selection of 52 species sorted under 2 categories: 21 in the **need to have** and 31 in the potentially beneficial to have (**Figure 1**). The **need to have** were covered extensively in the database, while those listed in the **nice to have** had a more general coverage.

Algae	Fucus, **Laminaria, Porphyra, Ulva**
Forage grasses	**Ryegrass, Alfafa,** Clover, Sugarcane, Miscanthus
Grain staples	**Wheat,** Barley, Rice, **Maize**, Sorghum, Rye, Oats, Durum wheat, Millet
N2 fixers	Field bean, **Soybean,** Lupin, **Pea,** Clover
Oilseed	**Sunflower**, Soybean, **Rapeseed**, olive, maize
Vegetables	**Tomato**, leafy vegetables (spinach), **Lettuce**, Brassicas, Pea, Carrots, Parsnip, Grapes
Fibres and lignocellulose	Hemp, **Poplar,** Willow, **Miscanthus,** Switchgrass, Douglas Sitka, Eucalyptus, Spruce
Root staples	**Potato, Sugarbeet**, Onion
Model Plants	**Arabidopsis, Tobacco,** Rice, Spinach, Maize
Others (fruit)	**Grape, Pome,** Citrus, Olive, Strawberry, Raspberry
	Need to have/nice to have

Figure 1 List of species selected. Selected species were sorted in need to have (underlined and bold) and nice to have (black) categories. Species are uniformly distributed in ten classes (bold blue).

The priority crops defined served as guidelines for the database collection. However, the data collection was not limited to these crops and provision was made to add crop species the survey taker felt was missing in this representation.

4.1.2 SURVEY DESIGN

The survey (**Annex 2**) was spit into three sections:

✓ *Section 1* contains data that is common to all three subtasks including: publication title, authors names, separation of reviews from research publications, year of publication, abstract or summary of article, bibliographic references, gene or trait involved, biological pathway, methods or technologies used to identify and characterise genes and traits, crop category, species, geographical region, orthologues, transferability potential, relevance to subtask and whether the gene/ trait under consideration affected other subtasks. The general survey also included an option for comments or relevant free text information from the authors. The information gathered here was essential in identifying themes or key options in improving crop plants (See section 4.3)

✓ *Section 2* was defined within each subtask and comprised 5 levels of specification (**Figure 2**). The subtask itself was defined as level 1 (Yield, Nutrition or Sustainability), the broad categories within each subtask were placed in level 2 (yield, nutrition or sustainability determinants). Within each of these levels, categories and types were defined and, subcategories and subtypes were further defined (levels 3 and 4) in order to have and extensive coverage. The last level recorded physical and environmental factors that affected the entry (gene/ trait). A detailed list of survey entries for Nutritional Quality is presented on the CropBooster-P website (https://www.cropbooster-p.eu/). The same subcategories and subtypes (levels 3 and 4) were used for data collection for Yield and Sustainability and are also presented on the CropBooster-P website (https://www.cropbooster-p.eu/).

Level 1	Level 2	Level 3	Level 4	Level 5
nutritional quality	Minerals	Macronutrient	Nitrogen Phosphorous potassium Calcium Sulfur Magnesium Carbon Oxygen Macronutrient- Hydrogen	Stress- heat Stress-cold Stress- high humidity Stress-flood Stress- drought Stress-salinity Stress-toxicity Stress-other Stress- nutrient overload Stress- Nutrient deficiency
		Micronutrient	Iron Calcium Magnesium Chloride Potassium Sulphur Manganese Zinc Iodine Selenium	Stress- soil toxins Stress- soil composition Stress- pH Fertilizer- form Fertilizer-quality Biostimulants Geographical factors Bioavailability Microbes- in rhizosphere Microbes-fertilizer use efficiency- nitrogen fixation Sulphur nutrition Pathogen toxins Intra species cultivar- specific variation Uptake and allocation to edible organs Membrane transporters Efflux proteins Organic molecule synthesis Stress- transposable elements
			Other	

Figure 2 Section 2- Example from the construction of levels specific to nutritional quality. For the final version of the survey generated, please see the survey key in *Annex 2*.

- ✓ *Section 3:* The final section aimed at specifying the relevance of each entry to the scenarios from the output of WP1.1. This enabled viewing each entry in a broader context, as well as identifying the common trends and commonalities between multiple survey entries, the traits identified, and the technologies specified within the survey entries.

In all sections, free text boxes have been added in order to enable experts highlighting and explaining the importance and the relevance of the selected reference.

Database access: the database was designed to be openly accessible, with the survey still running online so that continuous updates to the database can be made as research progresses.

The survey can be accessed at the URL https://forms.gle/B7XAW8L6iY3Fgfsp7.

See also:
Annex 2: Key to the survey (A PDF version of all the questions incorporated in the survey).
CropBooster-P website (https://www.cropbooster-p.eu/): List of database entries for Nutritional Quality and for Yield and Sustainability. Curated version from survey data extracted on 15.12.2019.

4.2 Outcome of Data Collection

The survey collected 878 entries, which were then manually checked for duplicate entries. The final number of individual entries were 799, from which 27% of the responses entered corresponded to reviews and 73% original papers. The distribution the references per year showed that the survey captured the 'state of the art' with respect to recent literature with 43% of the entries published in the last 3 years and up to 61% in the last 5 years (**Figure 3**).
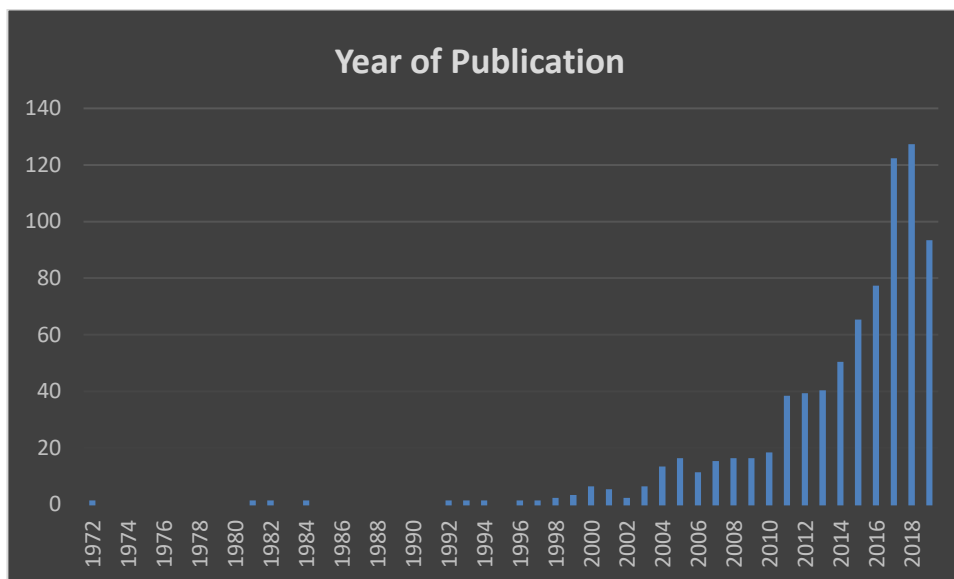
Figure 3 Distribution of the number of publications per year of publication collected in the survey. Figure based on survey data extracted on 15.12.2019.

Regarding crops, the majority of references cover the "need to have" species. All species categories defined previously are well represented with proportion ranging from 2% of the entries for Algae up to 28% for grain staples (**Figure 4**). It is worth to note that the model plants category only represent 22% of the entries. The **algae**, **fibers** and **root staples** are less represented than expected indicating a deficit in publications for the species falling into these categories.
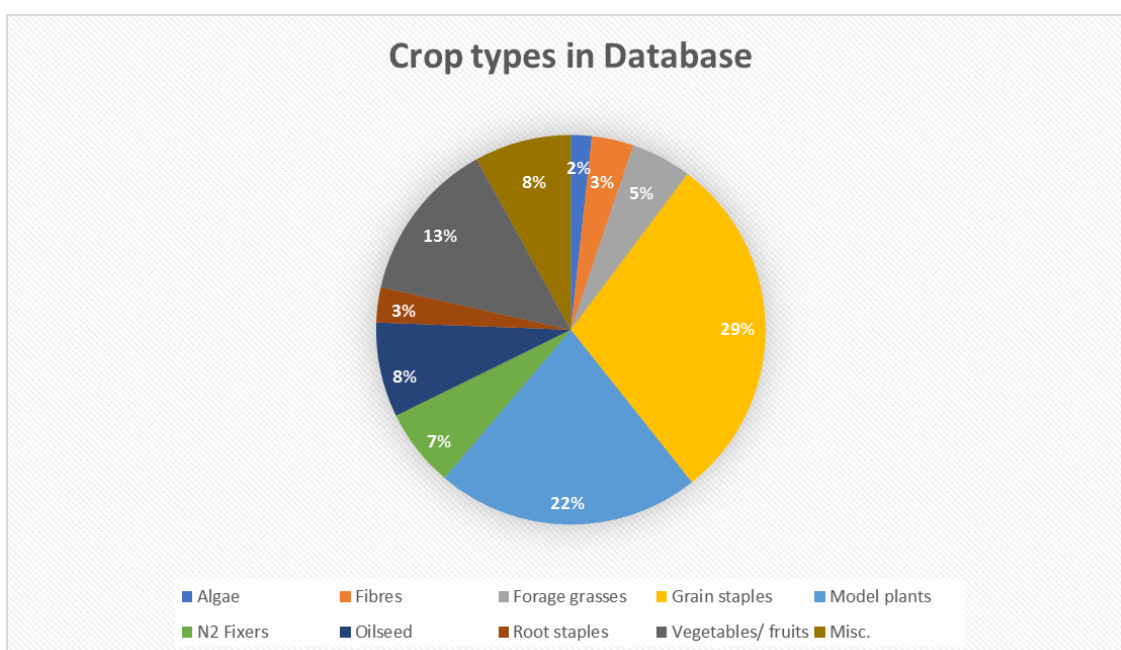
Figure 4 Distribution of publications collected in the survey sorted by the crop types. Figure based on survey data extracted on 15.12.2019.

When considering the entries per trait, 372 references fall in the Yield class whereas the nutrient class and sustainability contains 307 and 128 references respectively (**Figure 5**). However, the apparent under representation of reference in the sustainability field can be explained by the fact that the large majority of the traits/genes identified by experts in yield section have also been proposed to have a positive impact on sustainability and vice versa. This is confirmed when considering the "Does this gene/ trait involve or affect other subtasks?" field in the survey.
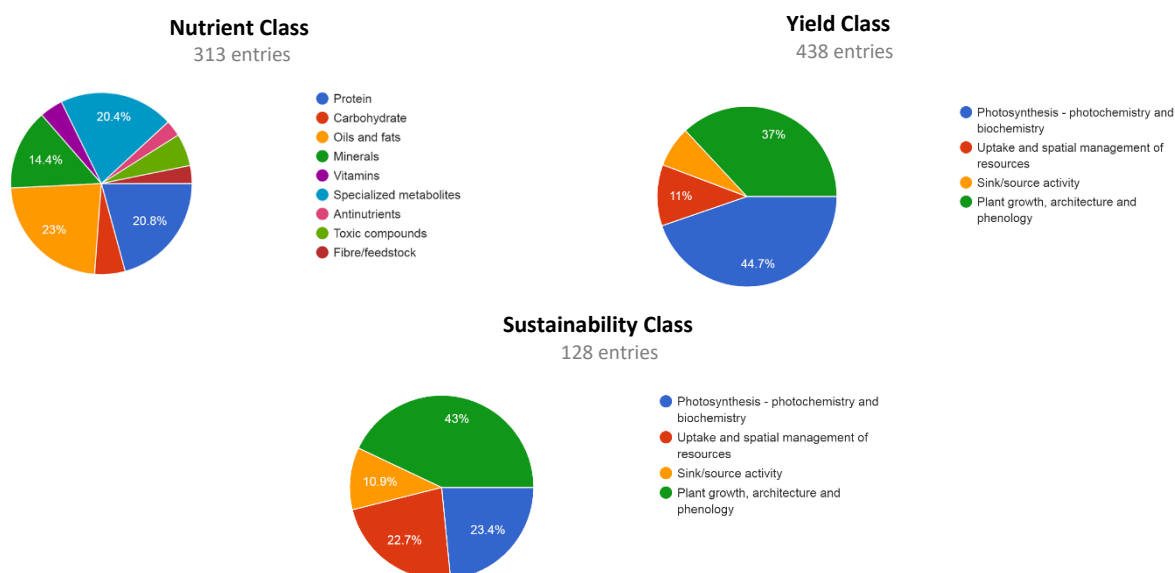
Figure 5 Distribution of publications collected in the survey sorted by traits. Figure based on survey data extracted on 15.12.2019.

Up to 50% of the references in the Yield class were associated to photosynthesis whereas 34% were associated to plant growth, architecture and phenology. References in this last group are almost equally distributed between roots and shoot development. Interestingly, 11% of the references attributed to yield involved water and nutrient uptake. When considering sustainability, the proportion of entries falling in the plant growth, architecture and phenology represents 43%, with most being associated with root development and root architecture. Similarly, 23% of the references belong to resource uptake and management with a large proportion relating to root uptake capacity. With regard to nutritional quality, 23% of all entries were associated to oils and fats, 21% to protein quality and 19% to specialized metabolites, all representing areas where recent advances have been made in terms of improving nutritional quality.

Taken together these results illustrate that the database is well balanced and represent an unprecedented expert resource to further and deepen investigation with regard to the most recent advances in improving crop quality. The flexibility of the database provides further resource to extract specific information, as well as view useful correlations across species and traits. Throughout the 3-year CropBooster-P project, it is aimed to further exploit the database in the following WPs and therefore also considered essential to keep the database updated. Accordingly, the database should be seen as a 'living' literature repository that is continuously fed to collect all state-or-the-art literature in the fields of Yield, Nutritional quality and Sustainability.

4.3 Key options to improve Yield, Nutritional quality and/or Sustainability

The database was used to identify key focus areas falling under Yield, Nutritional Quality and/or Sustainability or areas common to all three. Key experts were asked to compile the database information into a short summary for each of these target sections including a brief review of the most up to date literature and potential targets for improvement. The key options presented below cover several examples of the literature data that is included in the database. However, they only cover a subset of the information available in the database and numerous additional examples can be found.

4.3.1 IMPROVING METHODS FOR BREEDING AND GENOME EDITING

In order to apply any knowledge on loci, genes and gene regulatory networks that control plant traits, breeding technologies are required that can be used to either introgress natural or induced alleles into elite germplasm or modify the genome of the elite germplasm directly using site-directed mutagenesis.

Several technologies have been identified that require further development:

a) **Genomic selection.** Widely adopted by seed companies in recent years, genomic selection consists in elaborating a model that allows the prediction of agronomic performance of novel genetic material on the sole basis of its genotype. This model is based on a large data set previously acquired by genotyping and phenotyping a training set of (related) varieties (Millet et al., 2019). Genomic selection saves cost (less field experiments) and time (less generations) and allows more readily the simultaneous exploitation of minor quantitative trait loci (QTLs). The improvements needed for a more general use of the technology include the handling of epistasis between loci, the use of knowledge on gene function for more accurate predictions or the application to genetically distant material (Hickey et al., 2019; Voss-Fels, Cooper, & Hayes, 2019).

b) **Speed and fast breeding**. A game changer in (marker assisted) plant breeding is 'speed breeding'. Using highly controlled environments using growth chambers with tailored light regimes allows breeding rapid cycling of generations and shortening the classic breeding process significantly (Ghosh et al., 2018; Hickey et al., 2019). Speed breeding conditions will need to be optimized for each crop and variety. "Fast breeding" aims at sharply shortening the generation time of crops, for example by modification of the Flowering Time (FT) gene (van Nocker & Gardiner, 2014).

c) **Double hapoid (DH) breeding**. In maize, DH technology has been used for decades to speed up the generation of complete homozygous inbred lines that can be used in hybrid breeding. The gene responsible for haploid induction in maize has recently been mapped to MATRILINAL (MAT)/NOT LIKE DAD (NLD) (Gilles et al., 2017; Kelliher et al., 2017) and

has been used to make haploid inducers in other species such as rice and wheat (Liu et al., 2019; Yao et al., 2018). Further development of DH technology will aid to improve the efficiency of this technology and expansion in other crops (Chaikam, et al., 2019). Recently, DH technology has been also linked up with precision breeding (Kelliher et al., 2019; Wang et al., 2019).

d) **Accelerated domestication**. A particular application of precision breeding allows to domesticate in a single generation wild relatives of crop species with desirable agricultural traits through the simultaneous editing of previously identified domestication genes. Proof of concept has been provided in tomato (Li et al., 2018) and sufficient knowledge exists in at least a few other crop species (Zsögön et al., 2018).

e) **Precision breeding**. The development of CRISPR/Cas9 based gene editing technologies now allows to precisely change the genome of elite germplasm by a single nucleotide, mimicking natural alleles or introducing novel genetic variation in the population (Yin, et al., 2017). However, to efficiently apply precision breeding to all major crop species and to elite genotypes within the species, several technological hurdles need to be taken:

1) **Delivery.** The CRISPR/Cas9 system has to be delivered to a plant cell. If the delivery is as a DNA molecule, genetic transformation is needed, traditionally using *Agrobacterium tumefaciens*. Agrobacterium-mediated delivery is however genotype dependent and strain improvement might be needed for some crops (Altpeter et al., 2016; Anand et al., 2018; Zhang et al., 2019). Understanding better the steps of T-DNA integration in the genome might also allow the use of 'transient transformation' in which the T-DNA is expressed but not integrated (Veillet et al., 2019). Recently, alternative transient plant DNA delivery methods have been reported using nanoparticles (Demirer et al., 2019), which, however, need further development to deliver large CRISPR/Cas9 constructs. As the CRISPR/Cas9 system operates as a ribonucleoprotein complex (RNP), technologies have been developed for 'DNA-free' RNP delivery into explants or protoplasts (Liang et al., 2018; Svitashev, et al., 2016; Zhang et al., 2016). These are, however, dependent on the plant regeneration system available. Especially for the many clonally propagated crops, transient DNA or DNA-free technologies need to be further developed as the transgene cannot be crossed out.

2) **Regeneration.** After delivery, a new plant needs to be regenerated from the explant. This process is highly dependent on the plant species and the genotype. Both the optimization of the type and dose of the nutrients and plant hormones used in tissue culture, and the use of genetic tools such as BABY BOOM (BBM) have allowed to overcome regeneration recalcitrance in several species, including monocots (Jones et al., 2019; Lowe et al., 2016). However, the side effects of morphogenic genes, the ignorance of their mode of action and the dependency on the explant material and competent tissue is prompting more research in plant totipotency and regeneration (Gordon-Kamm et al., 2019).

3) **CRISPR technology.** CRISPR/Cas9 is presently harnessed for targeted mutagenesis, where the site but not the precise nature of the mutation (generally small insertions or deletions) is pre-determined. To fully exploit the potential of the technology and to make precise nucleotide changes, advanced CRISPR-based technology is needed such as base editing and prime editing (Anzalone et al., 2019). The further development of alternative technologies that allow homologous recombination in plants serves the same objective (Yin et al., 2017). In addition, the application of more versatile CRISPR systems, such as xCas9 or Cas9-NG, and alternative CRISPR systems, such as Cas12a/CPF1, need plant-specific adaptations (Malzahn et al., 2019).

f) **Gene drive and RNA targets**. Whereas genome editing delivers plants modified by a one-off action of CRISPR/Cas9 technology, other uses of the technology require the maintenance of the Cas9 and sgRNA transgenes in the plant genome. Proposed applications are gene drive, for example to constantly edit the genomes of weeds to render them susceptible to certain herbicides (Barrett et al., 2019), or RNA targeting Cas

variant, for example to efficiently cleave the genome RNA viruses (Wolter & Puchta, 2018). Proofs of concept are needed to test the feasibility and efficiency of such approaches.

4.3.2 IMPROVING NITROGEN UPTAKE AND USE

Plants require nitrogen (N) in bigger quantities than any of the other mineral elements. N in the form of amino acids is necessary for protein synthesis and great amount of N are thus used for the photosynthetic apparatus, particularly Rubisco, the primary carbon (C) fixing enzyme, and light-harvesting complexes (Zhu et al., 2008). In mature leaves of crop plants, Rubisco can account for up to one third of the total N and almost 2/3rd of the soluble proteins (Warren et al., 2000). N assimilation is integrated with photosynthesis, but also with photorespiration and respiration, in different tissues and organs, and at the intracellular and intercellular levels (Nunes-Nesi et al, 2010). Photosynthesis and subsequent glycolysis and Krebs cycle provide the C skeletons and energy required for the synthesis of amino acids. Therefore, even though interventions in primary processes such as photosynthetic efficiency do not require extra N to achieve greater productivity, they do need to be integrated and consider possible impacts on N nutrition.

Interplay between photosynthesis and N assimilation

In non-legume plants and under our temperate climatic conditions, nitrate is the main N source and is taken up from the soil solution by root cells. However, nitrate concentration in the soil solution dramatically fluctuates in both time and space, which restrains N acquisition by roots and constitutes a major limitation to photosynthesis and plant biomass production. Therefore, for optimising the acquisition of this crucial resource, plants must constantly acclimate to the changes in external nitrate availability by modifying their physiology and development. These responses to N supply involve the plasticity of both root structure (modulation of the exploration of the soil) and root function (modulation of intake capacity of the roots) but are also dependent on the N demand of the plant. The capacities of the roots to acquire N also depend on carbon fixation by photosynthesis. Sugars or other signals produced by photosynthesis in the shoots are transported down to the roots to stimulate nitrate uptake. Indeed, a specific regulatory pathway involved in the systemic regulation of the expression of nitrate and other macronutrient transporters by the C status and in particularly involving the oxidative pentose phosphate pathway has been revealed (Lejay et al., 2008). In addition, root C availability is a major parameter regulating root architecture and development (Müller et al., 1998; Freixes et al., 2002). Although the stimulatory effect of photosynthesis on root nitrate uptake has been intensively documented on the short term (several hours or days), many reports surprisingly indicate that the increase in photosynthetic activity associated with long-term growth of C3 plants under elevated atmospheric CO2 concentration results in a lowered N concentration in leaves and seeds, possibly due to impaired root nitrate uptake (Bloom et al., 2010).These multiple interconnections between N assimilation and C metabolism are of major importance for crop production and indeed ecophysiological studies demonstrated that carbon and nitrogen intakes are the major limiting variables of models describing plant biomass production (Foulkes et al., 2009).

Nitrogen use efficiency

Yield increases after the second world war - the so called "Green revolution " - were enabled by the massive use of N fertilizer produced by the energy intensive Haber-Bosch process. The global rise in N fertilizer consumption (increased from 11.3 Tg N year−1 in 1961 to 107.6 Tg N year−1 in 2013; Lu and Tian, 2017), together with the enhanced cropping of legumes that establish symbiotic interactions with N2-fixing bacteria, has expanded the pool of reactive N in the

environment (Fowler et al., 2013), with significant environmental consequences (Swarbreck et al., 2019). A main problem is the fact that the recovery of N fertilizers by crop plants is low, with in some cases only 30-50% of the applied N being taken up by the crop (Sylvester-Bradley and Kindred, 2009). The remainder is partly utilised by the subsequent crop but also partly lost from the agro-ecosystem, and fertilizer run-off into aquatic systems may lead to eutrophication (Johnson et al., 2007). Indeed, the environmental impact of N is multiple, including problems of excess N (losses of N contribute to eutrophication of aquatic ecosystems, acidification of soils, global warming, decrease in global species diversity, and more) and limiting N (depleting soil resources, endangering the income of farmers, and threatening food security). In Europe a recent estimation of social costs indicates that current measures to prevent an excess of 50 mg/L nitrate are beneficial for society and that a stricter nitrate limit and additional measures might well be justified (van Grinsven et al., 2010).

Today again we are challenged to meet expanding food demands. The global use of N in agricultural production is projected to increase 2.5 fold to reach 249 million tons annually by the year 2050 (Tilmann et al, 2011). This is barely sustainable for the environment and climate crises urges for a strong reduction of N deposition. Several possibilities can be investigated and might be used in combination to reach this crucial but ambitious goal: modified agricultural practices, use of the root microbiota for optimised N supply including N2 fixing symbiosis and producing crops with increased NUE.

Approaches to increased NUE and C assimilation

Nitrate uptake is a key component determining NUE. Indeed, as nitrate is not taken up efficiently overexpression of nitrate transporters was one very early strategy to improve NUE. However, up to now it was successful mainly in rice. This is probably due to the fact that rice growing in paddy soil is mainly using ammonium and thus nitrate uptake had not been optimised by breeding and in addition might be less tightly regulated by feedback mechanisms. Indeed, in most other cereal and model species nitrate uptake is regulated by the internal N status and these feedback mechanisms probably counteract the potential benefits of overexpression of transporter activities. In rice the overexpression of the low affinity nitrate transporter OsNPF6.5 (NRT1.1B) driven either by the CaMV 35S or the native promoter increased grain yield and NUE (Hu et al., 2015). Interestingly the beneficial effect of root microbiota on NUE in rice was impacted by NRT1.1 (Zhang et al., 2018). Overexpression of the high affinity nitrate transporter OsNRT2.1 increased NUE when expression was driven by the OsNAR2.1 promoter, limiting expression to the expression pattern of the nitrate transporter associated protein NAR2.1 that is required for the activity of NRT2 transporters (Chen et al., 2016). The overexpression of one isoform of a further high affinity transporter OsNRT2.3b increased nitrate but also ammonium uptake and improved grain yield as well as NUE by 40%. Interestingly, plants overexpressing this short form of OsNRT2.3 with a truncated pH domain increased total N, P, and Fe uptake by influencing pH homeostasis and thus benefiting as well C metabolism (Fan et al., 2016). This mechanism may exist mainly in plants growing under anaerobic soil conditions.

Attempts to increase NUE by boosting N assimilation while overexpressing nitrate reductase failed, however the overexpression of the chloroplastic isoform of glutamine synthetase (a key enzyme in assimilating ammonia in organic compounds) led to increased grain yield in wheat under both high-and low-N supply (Hu et al., 2018; for review James et al., 2018).

Several aspects probably explain the limitations of the strategy to overexpress individual transporters or enzyme involved in N assimilation. First, the global expression pattern might be of tremendous importance and in particular, posttranslational downregulation of nitrate transporters or enzymes activity may compensate for increased gene expression. Secondly, it might be necessary to tune an entire pathway or even the interaction of several pathways such as N assimilation and C assimilation in order to boost such interconnected metabolic pathways

and increase yield and NUE. Thus, a promising strategy is the modification of the regulation of those metabolic networks and the engineering of receptors, transcription factors (TF) or other regulatory players. For example, the overexpression of the Dof1 TF resulted in greater yield under low-N conditions in Arabidopsis (Yanagisawa et al., 2004) and in greater yield in wheat (Peña et al., 2017). A similar effect was obtained by the overexpression of the TF AtNLP7 driven by the CaMV 35S promoter, that improved plant growth by enhancing N and C assimilation in Arabidopsis and tobacco (Yu et al., 2016). Indeed, it is possible that the overexpression of OsNRT1.1B was particularly successful, as this protein has an important role in regulating nitrate signalling in addition to the transport of nitrate.

Nitrate signalling not only regulates multiple metabolic pathways but also several developmental processes. Amongst them, seed dormancy and flowering time are directly regulated by nitrate as a signal. This needs to be taken into account when engineering nitrate signalling as impaired dormancy or early flowering could impact strongly yield.

4.3.3 IMPROVING SEED FILLING

Seeds typically accumulate large amount of reserves, which are used to support germination and the early stages of the development of the seedling. The majority of these reserves are normally accumulated either in a specialized tissue named the endosperm (e.g. in cereal seeds) or in the cotyledons (e.g. in legumes). In addition, depending on the crop, the major energy and carbon storage compounds are represented by either carbohydrates or oils, while protein content (in percentage of the total seed weight) can be very variable (typically between 10 and 40 %). Seed filling is therefore a complex process, which involves different pathways and regulators, also depending of the type and location of the reserves. Particularly in annual crops, a major role in seed filling is played by nutrient remobilization between vegetative and reproductive organs. An optimal balance between biosynthetic activities in vegetative organs and translocation toward reproductive organs is essential to maximize accumulation of macro- and micronutrients in seeds. In many plant species the process of seed filling is therefore intimately linked to senescence, which allows the redistribution of nutrients from the source organs to the sink organs.

For seed crops, efficient seed filling is a key factor controlling yield. Optimising source-sink ration and promoting efficient translocation of assimilates to seeds, and integrating photosynthesis with sink processes should remain one of the main targets for breeding (Paul et al., 2017). Notwithstanding the complexity of the system, some important control points have been identified and the regulated senescence of source tissues has been shown to be crucial for the accumulation of major seed reserves. This work has also highlighted the advantages and limitations of model plants, where the balance between vegetative and reproductive tissues may be quite different that in major crops. Seed filling is likely to be particularly relevant to scenarios 1, 2 and 3. However, in scenario 2 relevance would be mainly due to the impact on seed composition, rather than to the impact on yield.

Regulation of senescence

Cytokinins are major regulators of development being implicated in a variety of processes including senescence. Various attempts have been made to regulate cytokinin levels in order to delay senescence and increase total biomass and seed yield. An autoregulatory mechanism, by which the expression of a rate limiting enzyme in cytokinin biosynthesis, isopentenyl transferase (IPT), is placed under a senescence specific promoter has been widely exploited in crop plants (Guo & Gan, 2014). This way, delayed senescence due to IPT expression leads to down-regulation of the transgene, avoiding accumulation of cytokinins and pleiotropic effects. This approach, using the SAG12 promoter (SAG12 encodes a senescence specific, vacuolar cysteine

proteinase) was first applied in tobacco and then in a variety of model and crop plants, including rice. Leaf senescence in transgenic tobacco plants was retarded, and no developmental abnormalities were observed. Retarded senescence allowed tobacco plants to produce 80% more flowers than the WT, leading to a more than 40% increase in both seed yield and biomass accumulation. In rice, an 8% increase in yield compared to WT was observed under field conditions. In a different approach (Abreu & Munne-Bosch, 2009), a delay in senescence was obtained by lowering salicylic acid (SA) in *Arabidopsis thaliana*. Salicylic acid accelerates the progression of leaf senescence and a reduction in its levels can be achieved either by the expression of bacterial SA hydroxylase in NahG transgenic lines or by mutation of sid2, the gene encoding isochorismate synthase. Reduction in SA levels leads to a marked increase in biomass and seed production, indicating that manipulation of SA level could be exploited to increase seed yield in crop plants.

Nitrogen remobilization to the grain

Coordination between senescence and grain filling is particularly crucial for nitrogen which is mainly stored as proteins that must be degraded to allow nitrogen remobilization toward developing seeds. The chloroplasts, which can contain more than 75% of the total nitrogen of the leaf (50% in the form of RuBisCO), are degraded at the early stages of senescence, while the mitochondria and the nucleus remain functional till later stages to support energy production and allow changes in gene expression that accompany the senescence process.

A time-series analysis of the translocation of nitrogen provided a comprehensive description of nitrogen remobilization between tissues in peas (Gallardo et al., 2019). During the time-span between flowering and beginning of seed filling, nitrogen taken up during the vegetative period is remobilised from leaves below the first flowering node and from roots toward upper leaves and pod walls. In a second phase nitrogen is remobilised from stems and leaves to seeds, pod wall, and roots. In a third phase, nitrogen is remobilised from all tissues to seeds. At the transcriptional level, this work highlights the role of genes involved in senescence and indeed regulation of senescence in vegetative tissues appears to be a major factor regulation nitrogen accumulation in seeds.

These results work should be put in relation with seminal work relating senescence and protein content in wheat. While the stay-green phenotype has been frequently selected to obtain varieties able to sustain high yields under post-anthesis drought conditions, these varieties are not associated with higher yields in all conditions, since the active degradation of chlorophyll and thus of this of photosynthesis-related proteins, including Rubisco, is required for nitrogen remobilization to developing seeds.

In wheat, high grain protein content (GPC) is a breeding target due to the positive relation between GPC and bread and pasta quality. The Gpc-B1 allele for high GPC was originally identified in wild emmer wheat *Triticum turgidum* ssp. *Dicoccoides* accession FA15-3, hereafter referred to as (DIC). The Gpc-B1 gene mapped on chromosome arm 6BS and is linked to consistent GPC increases (on average 14 g/kg) in both tetraploid and hexaploid wheat. Most importantly, differences in senescence were observed between tetraploid recombinant substitution lines segregating for the Gpc-B1 locus. Plants with the DIC allele at Gpc-B1 underwent earlier senescence with respect to the plants bearing the Langdon (LDN) allele being 3–4 days more advanced in their senescence process than the corresponding recurrent parent. Accelerated senescence increases mobilization of nitrogen and minerals to developing grains. Positional cloning identified a single gene encoding a NAC domain protein, which is mutated and non-functional in Langdon and modern durum varieties (Uauy et al., 2006). When considering the possibility of translating these effects in cultivated varieties, it should be considered that the effect of the DIC allele on total kernel weight and on total protein (i.e. total protein in 1000 seeds) was not consistent and was dependent on the variety.

Oil/Lipid metabolism

Many crop plants accumulate oils as main energy storage in seeds. This occurs also in the model plant Arabidopsis, where massive amounts of fatty acids are stored in the seeds. The synthesis of fatty acids occurs in seed plastids and relies on sucrose translocated from source tissues and produced by photosynthesis. After cleavage into glucose and fructose, sucrose is processed into pyruvate via glycolysis, and to Acetyl-CoA, which is utilised for fatty acid biosynthesis. Fatty acids are the incorporated into triacylglycerols at the level of the endoplasmic reticulum, and accumulated in oil bodies. Autophagic processes have been implicated in oil accumulation in seeds. ATG5 or ATG7 are two key components of the authophagic pathway and plants in which their expression has been abolished or enhanced show a moderate decrease and increase of fatty acid content, respectively, compared wild-type plants. While the effect of ATG5/ATG overexpression on oil content is minor, it adds to the concurrent effect on seed size, with an overall increase in oil yield of 20-50% compared with the WT (Minina et al., 2018). It can be hypothesised that more efficient mobilization of sucrose to developing seeds is the main driver for enhanced oil accumulation in ATG5/ATG7 overexpressing plants.

Carbohydrate metabolism

Several studies have shown that yield improvements are likely to require coupling of an increase in photosynthesis with the engineering of regulatory processes to enable toleration of higher sugar levels (reviewed in (Paul et al., 2017)). The importance of sink strength is exemplified by NAM RNAi wheat plants with delayed senescence and higher photosynthetic rate, which accumulate assimilates in the stem rather that in the grains (Borrill et al., 2015). In this respect, modulation of the levels of trehalose 6-phosphate (T6P) is a promising strategy in order to promote photo-assimilate partitioning to seeds. Trehalose is a nonreducing glucose disaccharide that is produced from T6P by the action of trehalose-6-phosphate phosphatase (TPP). The increased accumulation of trehalose was initially associated with improved growth under drought conditions (Pilon-Smits et al., 1998). T6P is a signal of sucrose status and a powerful regulator of gene expression for growth and development through the protein kinase SnRK1. Low levels of T6P are thought to increase sink strength by acting as a starvation signal, and thus stimulating sucrose flux toward the sink. Thus, a decrease in the levels of T6P in the phloem vasculature that supplies the developing grain has been proposed to increase sucrose import into the grain. The strategy has been shown to be effective in maize expressing rice a TPP under the control of a MADS6 promoter, which restricts expression to reproductive tissues. This resulted in increased yield both in the presence or absence of drought during the flowering period (Nuccio et al., 2015). More recently an alternative approach to increase T6P levels, i.e. the application of a plant-permeable analogues of T6P (Griffiths et al., 2016), has been shown to enhance starch accumulation in wheat kernels linked to inhibition of SnRK1.

Another possible strategy to increase carbohydrate accumulation in seeds is the engineering of SWEET4 proteins. SWEET4 genes encode hexose transporters involved in the uptake by the developing seeds of hexoses generated by cell wall invertase (Sosso et al., 2015). These genes were target for selection during domestication and modulation of their expression and/or activity can potentially affect carbohydrate transport into developing seeds.

4.3.4 THE POTENTIAL OF TREHALOSE-6-PHOSPHATE SIGNALLING

Part of the grand challenge to improve crops yields is to combine yield potential with resilience to abiotic stresses such as drought, the major non-biotic environmental factor that limits crops yields. In cereals, amongst a number of approaches that could be taken, one has been to target carbon allocation within the crop in favour of harvested ears and grain. A very direct way to alter carbon allocation is through modification of genes involved in the process. Knowledge of the

genes involved in carbon allocation has depended on fundamental research over a number of years on sugar signalling i.e. to understand how plants sense and respond to carbon availability to regulate carbon allocation. This is rather like glucose homeostatic mechanisms in mammals, except in plants sugar homeostasis uniquely involves the sugar produced in photosynthesis, sucrose. It has been found that trehalose 6-phosphate (T6P) is a signal of sucrose availability acting like a plant sugar fuel gauge (Paul et al., 2018). Early promise of modifying sugar signalling through T6P was shown in model species which delivered positive changes in growth and development and photosynthesis (Paul et al., 2001; Pellny et al., 2004). It was subsequently shown that T6P inhibits the feast-famine protein kinase, SnRK1 (Zhang et al., 2009) as the underpinning mechanistic basis for linking sucrose supply with physiological processes.

A strategy was developed to target the T6P/ SnRK1 regulatory mechanism in maize to alter carbon allocation towards female reproductive structures (Nuccio et al., 2015). This in itself could benefit yield potential which depends on sucrose supply, but also resilience to drought. Recently it was shown (Boyer & Westgate, 2004) that supplying sucrose to drought-stressed developing maize cobs reduced kernel abortion. Reproductive development has a high demand for both carbon and water and any restriction on water supply during this period can strongly limit yields. Expression of a trehalose phosphate phosphatase (TPP) driven by a MADS6 promoter active in female reproductive structures particularly in the phloem vasculature decreased T6P within developing cobs resulting in a shift in sucrose and amino acids from cob pith towards developing kernels (Oszvald et al., 2018) The altered allocation of resources could be explained through upregulation of gene expression of SWEET sucrose efflux transporters regulated by T6P/ SnRK1. The yield improvements compared to wild type resulting from this genetic modification were 9% to 49% under non-drought or mild drought and 31% to 123% under more severe drought conditions. Consistent results were obtained in field trials at different sites and years. Interestingly, modification of T6P in the maize cob resulted in an increase in photosynthesis. This shows optimisation of source-sink as a promising way to improve photosynthesis (Oszvald et al., 2018). There are very few examples where a transgenic modification of an intrinsic plant process has produced a consistent and clear yield increase in the field in a food security crop. There is only one example where genetic modification has improved performance in water-limited environments to the point of commercial release (Nuccio et al., 2018)

The T6P/SnRK1 mechanism is amenable to improvement in other food security crops sorghum, wheat and rice. In sweet and grain sorghum with contrasting sugar-accumulating phenotypes (Li et al., 2019) differential T6P signal due to changes in TPPs was associated with divergent sucrose, starch and cell wall metabolism and accumulation of different end products. This may lead to marker-assisted selection of TPP and other genes involved in T6P signalling for optimisation of the accumulation of different end products in crops. In wheat, the unlikely take-up of GM crops and hexaploid genome, only recently fully sequenced, means that targeted genetic intervention of the pathway make take longer than for other crops. However, a chemical method has shown the potential of the pathway for yield improvement. Chemical modification of T6P enables uptake by the crop and subsequent photo release of T6P in planta. When applied as a spray to wheat 10 days after anthesis grain size increase up to 20% (Griffiths et al., 2016). Importantly for genetic approaches the T6P chemical technology can be used as a chemical genetic screen to select for most promising genetic variation in source and sink processes in crops. Interestingly a TPP gene is associated with grain size in wheat (Zhang et al., 2017) confirming results from chemical T6P. In rice increased expression of a TPP gene selected through a QTL enables improved germination of rice under flooding through better mobilisation of starch reserves (Kretzschmar et al., 2015); salt stress tolerance in rice is associated with a trehalose phosphate synthase gene (Vishal et al., 2019).

It will be interesting and important for further improvements in crop yield and resilience to understand how the T6P pathway has been modified through breeding and what further changes can be made. Several T6P pathway genes are amongst those associated with domestication

improvement in maize (Hufford et al., 2012). Interventions that modify T6P through genetic modification in maize (Nuccio et al., 2015) and chemical application in wheat (Griffiths et al., 2016) and natural variation in wheat and rice show the potential of the pathway for further yield improvement and that the T6P pathway is not yet optimised in crops. Changing T6P appropriately can relieve current limiting factors for yield formation. The T6P pathway could be involved in the determination of both grain set as well as grain size, as sugar supply is a factor determining initiation of female reproductive primordia (Fischer, 2007) as well as maintenance of grain numbers once set (Nuccio et al., 2015). In other crops the potential of T6P to regulate synthesis and partitioning between sucrose, starch, cell wall and oil and interaction with abiotic stresses means that the T6P pathway is a dominant control point for crop traits and will likely feature strongly in crop improvement programme in a variety of ways.

4.3.5 THE POTENTIAL OF AQUATIC SPECIES

Seaweeds, or macroalgae, have presently two major uses: as a source of human food and as raw material for extracting colloids used as thickeners. Minor uses include fertilizers, feed, bioenergy, agrichemicals, cosmetics, nutraceuticals, and pharmaceuticals. Around 30 million tons of seaweed is produced per year with a value ~8,000 million euros. Growth of the sector is around 7% per year. Almost all of the production (95%) is through aquaculture and is located in Asia, with China being the most important producer followed by Indonesia, the Philippines, South Korea, and Japan (Ferdouse et al., 2018). Around 10,000 species of macroalgae are known, 500 have been used by humans, but only around 200 are cultivated in aquaculture. In addition, five genera represent more than 95% of the production and value: the brown algae *Saccharina (kombu)* and *Undaria (wakame)*, and the red algae *Pyropia (nori), Kappaphycus/Eucheuma* and *Gracilaria* (Chopin & Sawhney, 2009). Green seaweeds are less used, but genera like *Ulva* and *Caulerpa* are cultivated.

Direct human consumption

Seaweeds have been used as a food source for at least 4,500 years (Tseng, 1981) and are increasingly consumed as "sea vegetables". They are typically sold dry and are rehydrated before consumption. Seaweeds are rich in minerals, such as magnesium and iron, and especially iodine, fibers, and for some species, such as *nori*, in protein. Presently, no species is known to be toxic, although high levels of arsenic is a problem for some species. In addition, some species are good sources of vitamins A, K, and B12. Content of lipids and digestible carbohydrates are typically low. Algae are mostly consumed in Asia, especially in China, Korea and Japan. Seaweeds are used as a vegetable, a source of umami and thickener of broth in the Asian cuisine. Consumption in Europe is limited but increasing and the potential for increased use is high. Over 80% of the commercially available algae are used for human consumption (Chopin & Sawhney, 2009).

Industrial use

The cell walls of red and brown macroalgae are typically rich in charged, sulfated or carboxylated polysaccharides such as agars (agar-agar), carrageenans and alginates that have the capacity to form gels or increase viscosity of solutions. Seaweed-derived products represent 40% of hydrocolloids used in the food industry. Agars are typically extracted from different species of the red algae *Gracilaria spp.* and are mainly used in food. Carrageenans are extracted from other red algal species of *Chondrus*, *Eucheuma,* and *Kappaphycus* and are used as thickeners in dairy products. Alginates are extracted from brown algal species such as *Laminaria, Ascophyllum,* and *Saccharina*. They are principally used as thickeners, gelling agents and emulsifiers in the food industry. Furthermore, hydrocolloids are used in research, medicine and biotechnology. A part of the algal production is also used in agriculture as fertilizers to improve soil characteristics, as growth stimulators and inducers of defense mechanisms for plants.

Algae for industrial use is partially from aquaculture, e.g. most of the red algae; however, a part of the algae used for production of hydrocolloids and algae that are used in agriculture derive from the collection of wild algae, *e.g.* brown algae such as *Laminaria spp*. in Norway and France.

Aquaculture

Aquaculture of seaweeds takes place in over 50 countries. With an annual growth rate of 11.2% (2005-2016), seaweed is the most rapidly expanding aquaculture sector (Ferdouse et al., 2018). The algae are usually cultivated attached to ropes tied to floating structures on the surface of near shore waters or attached to the sea bottom. The brown algae and nori are typically seeded to ropes and nets and grown to maturity. The red algae *Gracilaria*, *Eucheuma,* and *Kappaphycus* are often grown on ropes through vegetative propagation from fragments.

Aquaculture of seaweeds have some advantages compared terrestrial production. Present production is at sea meaning that there is no competition for cultivable surface with terrestrial plants. There is, however, some competition for available plots from other aquaculture activities such as fish and shellfish culture. However, increased interest for integrated multi-tropic aquaculture has been introduced for algal cultivation to increase nutrient availability for the algae and reduce the environmental foot print of animal aquaculture. If off-shore cultivations of seaweeds can be realized the potential area for cultivation is also virtually unlimited.

Differences in stressor importance for subtidal and intertidal species can be used as a way of improving productivity in seaweed aquaculture. For instance, daily exposure to air in cultures of the red alga *nori* (*Porphyra* and *Pyropia* species) is used to control grazers, diseases, and epiphytes. The intertidal *nori* is more stress tolerant compared to subtidal epiphytes. The culture of the large brown algal kelps (*Laminaria, Undaria* and *Saccharina* species) is essentially immersed. The depth can be used to control, to an extent, light intensity, nutrient availability and temperature; since light and temperature typically decrease with depth while nutrient concentrations increase.

Costs for seaweed aquaculture is lower compared to fish aquaculture and can thus represent an interesting alternative or additional occupation for coastal communities. Seaweed cultivation does not require freshwater and removes nutrients from the surrounding seawater, potentially improving local water quality. The absence of competition with terrestrial plants make algal aquaculture an especially interesting candidate for increased food production. However, presently, there is an increased interest in on-shore algal cultivation to increase control and quality of the produced algae.

It should be noted that even though strain selection has been used in algal aquaculture, for example in *Saccharina japonica* to increase tolerance to unfavorable conditions, the potential for strain improvement through classical breeding is very high (Zhao et al 2016). One important parameter that is limiting this progress is the lack of complete life cycle control for some species. For example, *Gelidium* species that are used as a source of high quality agarose are typically harvested from wild populations rather than grown in aquaculture. In addition, with increasing knowledge in seaweed physiology and genetics new strains could also potentially be created using molecular biological tools (e.g. CRISPR/Cas9 and molecular breeding techniques).

Abiotic constraints

Seaweeds are found naturally in two relatively distinct environments: the intertidal zone and the subtidal zone. Organisms growing in the subtidal zone will, by definition, grow covered with seawater all the time and organisms growing in the intertidal zone will be more or less exposed to the atmosphere during low tide. Because of the changing tidal amplitudes with location, time of the lunar cycle and time of the year, intertidal seaweeds can be exposed to the atmosphere

either for hours every day or for a short duration twice a year. Examples of species that live high up in the intertidal and are emersed for hours every day are *Ulva*, *Fucus* and *Porphyra* species and examples of species that are rarely emersed are the big kelps such as *Laminaria* spp. and *Saccharina* spp. (Hurd et al., 2014).

In the subtidal environment the algae are constantly protected and constrained by the presence of seawater. This means that they are affected by the buffering capacity of the seawater implicating slower changes in temperature, salinity, dissolved inorganic carbon, pH, etc. This also protects the algae from meteorological phenomena such as rain and wind. The constraints include lower light, due to the shielding by seawater, higher competition and grazing. Algae that grow deeper down and thus at lower light intensities typically adapt to the lower light by increased pigments and larger antennae sizes to optimize photosynthesis (Ramus et al., 1976a,b).

Seaweeds growing in the intertidal will be subjected to both seawater and the atmosphere. This means that intertidal seaweeds during low tide are subjected to high light intensity, due to the absence of covering seawater, desiccation, caused by evaporation, osmotic stress, due to rain and freezing during the winter in temperate regions. As a consequence, intertidal seaweeds are typically more resistant to physical stressors compared to subtidal species. One part if this resistance is thought to be the reduced production of and the increased resistance to reactive oxygen species of high intertidal species. Seaweeds growing higher up in the intertidal tend to have higher concentrations of antioxidants, such as vitamins, and higher activity of reactive oxygen scavenging enzymes such as catalase and superoxide dismutase (Collén & Davison, 1999). In addition, since algae take up nutrients from the seawater, uptake is reduced during emersion, potentially leading to nutrient constraints. However, due to the harsher environment, typically the biological stressors, such as competitors and grazers, are less important in the intertidal zone. This means that, in general, intertidal seaweeds are more tolerant to abiotic stressors and subtidal species more tolerant to biotic stressors.

Nutrients

Similar to terrestrial agriculture, nutrients are essential for algae aquaculture. Nutrient conditions vary considerably with location due to efflux from shore, but availability is also strongly influenced by water movements due to currents. For a species like *nori,* nitrogen concentration should be above 15 µM to achieve the highest quality (Hurd et al., 2014). Low levels of nutrients cause loss of pigmentation in *nori* culture, which results in a reduction of photosynthesis and lower value since coloration is a quality criterion. Furthermore, low nutrients will reduce the levels of free amino acids reducing the taste and value of the algae. Increased nutrients have been achieved by nutrient addition, however, increased environmental concern coupled with legislation has made fertilization of algae more complex. Integrated multi-tropic aquaculture has been introduced to increase nutrient availability for algal cultivation. This can include for example the co-cultivation of fish, mussels and algae were the algae are fertilized by the fish and mussel cultivation and thus reduce the environmental impact as well as improving the algal aquaculture.

In seaweeds there is typically a correlation between increased rates of nutrient uptake with increased surface to volume ratios, typically with higher uptake rates in annual fast growing species. This is compensated in more compact species with a larger capacity for storage of nutrients (Raven & Taylor, 2003).

4.3.6 IMPROVING PHOTOSYNTHESIS

The primary determinant of a crop's biomass is the cumulative photosynthesis, less respiratory 'losses', over the growing season (Ort et al., 2015). Despite its importance, photosynthesis has a quantum efficiency well below its theoretical maximum with around 2 percent of the energy from sunlight being converted into plant growth. Light conversion efficiency is therefore a prime target when aiming to increase crop yield by raising the photosynthetic performance (Long, et al., 2015; Zelitch, 1982). Moreover, there is compelling evidence from free air CO_2 enrichment experiments that, provided other constraints do not become limiting, increasing photosynthesis does increase crop yields (Ainsworth & Long, 2005; Long et al., 2006). Despite its promise increasing the efficiency by which energy in the form of light is converted to biomass through the highly complex process of photosynthesis is, however, still a largely unexploited route to increase yields and has not yet been a target of direct selection.

Numerous potential targets have been identified that could be manipulated to increase crop yield through improvement of photosynthesis. Due to the complexity of the photosynthetic process in plants, these are varied, encompassing quite diverse metabolic and physical processes at the cell, leaf and canopy scale. Here we give a few examples of the approaches and progress that has been made which are related to subsequent sections on modelling of crop yield. We urge the reader to link these to the related case studies further in the document which integrate with abiotic stress, crop resource capture and use and quality.

Optimising light capture and the efficiency of light conversion to plant biomass

The light reactions of photosynthesis concern the capture of light energy by chlorophyll and associated pigments, oxidation of water, and electron transport through the photosynthetic apparatus in the chloroplast membrane resulting in generation of NADPH and ATP (Long et al., 2015). To improve photosynthetic performance in land plants, earlier studies mainly focused on the modification of single or small sets of genes involved in biosynthetic or regulatory pathways that had been identified as bottlenecks in processes related to CO_2 assimilation. Recent approaches in crop plants aim to optimize the photosynthetic light reactions and their associated regulatory mechanisms to increase yield and plant biomass.

In silico simulations of photosynthesis in crop-canopy-like conditions of shade and sun revealed that making light adaptation (physiological responses) faster should increase photosynthesis. In crops in the field the light available for photosynthesis fluctuates continuously, as clouds cross the sun, as the wind moves the leaves, and as the movement of the sun causes shadows to move across leaves. If it were perfectly efficient, photosynthesis would respond immediately to these rapid fluctuations in light, but this is not the case. Slow adjustment of photosynthesis to the new light conditions results in a loss of potential carbon uptake.

Manipulation to increase faster light adaptation of crops would not only increase yield but also be predicted to have a significant environmental impact because it would increase resource use efficiency (i.e., increase the amount of CO_2 assimilated both per unit of water transpired by plants and per unit of nitrogen incorporated into plant leaves).

I. Improving photosynthesis by faster recovery from photoprotection

The photosynthetic apparatus is often capturing more light energy than it can utilize and is therefore saturated. If chlorophyll molecules remain in an excited state, the excitation energy can be transferred to oxygen, producing singlet oxygen and subsequently a range of oxidising radicals. These in turn can damage the photosynthetic apparatus (Aro et al., 1993; Long et al., 1994). Plants protect the photosynthetic apparatus against excess radiation by changes within

the apparatus induced through a process involving the de-epoxidation of the xanthophyll pigment violaxanthin to zeaxanthin and rearrangements within the antenna systems. This result in dissipation of absorbed excess energy harmlessly as heat (Ahn et al., 2008; Havaux & Niyogi, 1999; Muller, et al., 2001). In a crop canopy, photosynthetic tissue can pass rapidly from saturating light to low light due to clouds and leaf self-shading. In this scenario photosynthetic tissue is transferred from light saturation to light limitation, and dissipation, i.e. the protection, of absorbed light energy as heat will lower the efficiency of photosynthesis. Modelling of the dynamics of these light fluctuations shows that this could cost up to 20-30% of the potential CO_2 assimilation (Zhu et al., 2004). It has been shown that bioengineering of an accelerated response to natural shading events in Nicotiana (tobacco), results in increased leaf carbon dioxide uptake and plant dry matter productivity by about 15% in fluctuating light (Kromdijk et al., 2016). Two genes involved the reversible conversion of zeaxanthin to violaxanthin and one gene involved in the rearrangements of the antenna systems were overexpressed in the better performing Nicotiana plants. The photoprotective mechanism that has been altered in this work is common to all flowering plants and crops, the findings provide proof of concept for a route to obtaining a sustainable increase in productivity for food crops and a much-needed yield jump.

An important lesson learned from these proof-of-concept studies is the complexity of the trait at the field and canopy level and the evidence that the expression of several genes around a specific trait might need to be co-regulated in order to yield benefits. In the case of photoinhibition where solely upregulating one central genetic component, PSBS, in rice led to a reduction of CO_2 assimilation at the leaf level (Hubbart et al., 2012). However when grown to full canopy size in fluctuating light, rice plants with higher PSBS had higher radiation use efficiency and yield (Hubbart et al., 2018) showing the important role of photoprotection in enhancing productivity.

II.   Improving photosynthesis by faster light induction of the Calvin cycle

There is similarly, a delay in achieving maximum photosynthetic efficiency when leaves are transferred from the shade to sun. This delay is associated with the activation of the central carbon fixing enzyme Rubisco, and has been identified in species including spinach, tobacco and soya bean (Hammond et al., 1998; Soleh et al., 2016; Woodrow & Mott, 1989). In wheat, measurements of dynamic limitations affecting CO_2 assimilation combined with a realistic model of a field crop light environment indicated that the slow adjustment of photosynthetic biochemistry during shade to sun transitions reduces photosynthesis by 21% compared with a scenario of perfect efficiency (Taylor & Long, 2017). Over-expression of an Rca from maize, the gene coding for Rubisco activase, in transgenic rice resulted in a slightly increased speed of induction at 25°C (Yamori et al., 2012). There is also evidence from wheat diversity panels, for significant genetic variation in the speed at which the rate of carboxylation by Rubisco, V_{cmax}, increases following shade-sun transitions (Salter et al., 2019). The reports provide evidence for a route to obtaining a sustainable increase in productivity in all food crops.

Measurement of variation in the speed of induction in photosynthesis on shade-sun transition within the 42 parental lines of the nested association mapping (NAM) of soybean showed considerable genotypic variation, although less so in relaxation on non-photochemical quenching (NPQ). Integration of this variation into a ray-tracing canopy model of soybean showed that utilization of this genetic variation could increase canopy photosynthesis by 20% (Soleh et al., 2017).

III.   Increasing electron transport rates leads to yield gains in biomass

The cytochrome b6f complex connects electron transport from photosystem II to photosystem I and it has been shown that the cyt b6f complex is a potential limiting step in the electron transport chain. This suggests that, by increasing the activity of this complex, it may be possible to increase the rate of photosynthesis. In a recent study (Simkin et al., 2017), transgenic

Arabidopsis (Arabidopsis thaliana) plants overexpressing the Rieske FeS protein (PetC), a component of the cytochrome b6f (cyt b6f) complex, were generated. Increasing the levels of this protein resulted in concomitant increases in the levels of cyt f (PetA) and cyt b6 (PetB), core proteins of the cyt b6f complex. Interestingly, this led to an increase in the levels of proteins in both the photosystem I and photosystem II complexes. Importantly, overexpression of the Rieske FeS protein resulted in substantial and significant impacts on the quantum efficiency of PSI and PSII, electron transport, biomass generation, and seed yield in Arabidopsis plants. These results demonstrate the potential for manipulating electron transport processes to increase crop productivity.

IV.   Adjustment of antenna pigment composition

Another approach suggested as beneficial from modelling is to reduce the antenna size of the photosystems in upper canopy leaves. The antennae contain the chlorophyll molecules that capture light energy and feed it to the photosystem centers (PSI and PSII) that drive electron transport. These antennae are under many environmental conditions too large, trapping more light energy than they may use. This may have been beneficial in the wild, where an individual when trapping more light in its upper leaves denies light to competing plants underneath, even if it cannot itself use the light. However, in a crop monoculture, it is a disadvantage as utilising the light-harvesting capacity of leaves in the entire canopy may give increased productivity. Therefore, reducing antenna size could save resources and allow more light to reach lower leaves (Ort et al., 2011). Chlorophyll-a-oxidase has been reported to be related to antenna size (Masuda et al., 2003) and can thus be a target for manipulation to increase light capture efficiency and assimilation. This approach does not require alterations in canopy architecture which could be combined to further enhance the distribution of light transmission.

V.   Decreasing photorespiratory costs

Net carbon capture is catalysed by Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase). However, Rubisco is flawed and some of the enzymes characteristics severely compromise photosynthetic productivity. Between 20-50% of the time Rubisco reacts with oxygen molecules instead of fixing CO_2. Rubisco catalyses a wasteful reaction with oxygen instead of fixing CO_2. Photorespiration is the process plants use to handle the toxic products of the reaction with O_2 and to restore carbon to the Calvin Benson cycle. However, photorespiration is wasteful releases some of previously fixed CO_2 and NH_3 and consumes energy. Regional scale models show that photorespiration currently decreases US soybean and wheat yields by 36% and 20%, respectively, and that a 5% decrease in the losses due to photorespiration would be worth approximately $500 million annually in the United States alone (Walker et al., 2016). The negative impact of photorespiration will increase as the planet warms because as it gets warmer Rubisco has more difficulty selecting CO_2 from O_2, causing more photorespiration.

Since the discovery of photorespiration it has been seen as an important target for crop improvement (Zelitch & Day, 1973). One strategy is to decrease the costs associated with photorespiration by introducing novel metabolic pathways to recycle the products of Rubisco oxygenation more efficiently; several alternate pathways have been proposed and have already achieved some success (Peterhansel et al., 2013). Introducing a photorespiratory bypass to Arabidopsis thaliana to recapture the photorespired CO_2 within the chloroplast increased rates of photosynthesis and biomass production in both chamber and greenhouse experiments (Kebeish et al., 2007; Maier et al., 2012). Computer simulations of these alternative pathways highlighted the importance of not only optimising gene expression to achieve maximum flux through the alternative pathway but also decreasing flux through the current pathway in order to maximize the benefits for crop plants under field conditions (Xin et al., 2015).

This approach have since been pursued in economically important agricultural model crop Tobacco where they evaluated 3 pathways (South et al., 2019). Over two years of replicated field trails, they demonstrated that the best performing engineered plants produced about 40 percent more biomass than the controls. Promising 7-27% grain yield increases have also been reported following introducing the alternative photorespiratory pathway rice (Shen et al., 2019) where the photorespiratory rate was suppressed by 18%-31% compared to normal, the photosynthetic rate increased by 15%-22%. The biomass of engineered rice plants was 14%-35%. Similar promising results have been reported for the oilseed crop Camelina sativa (Dalal et al., 2015) suggesting this approach will be used to improve the yields of a wide range of C3 crops (South et al., 2018).

VI.   C4 and CO2 concentrating mechanisms

Alternatively, the inefficiency of rubisco can be greatly decreased by increasing the concentration of CO_2 around the enzyme. Some photosynthetic algae, bacteria, and plants have evolved mechanisms to decrease the oxygenation reaction of Rubisco by carbon-concentrating mechanisms (CCMs). One of nature's answers was C4 photosynthesis. In C4 photosynthesis the biochemistry is segregated into specialized cell types: in the mesophyll cells, which are in contact with the intercellular air spaces, gaseous CO_2 is initially fixed by PEPC into C4 acids. The C4 acids are then transported to deeper, gas-tight, bundle sheath cells where decarboxylation occurs releasing CO_2 which is then recaptured by Rubisco. Because C4 photosynthesis increases the CO_2 concentration within the bundle sheath 10-fold, the wasteful 'oxygenase' activity of Rubisco is almost entirely suppressed (Carmo-Silva et al., 2008). C4 photosynthesis has evolved independently more than 60 times (Sage et al., 2012), providing a widespread and effective solution that overcomes the oxygenase activity of Rubisco and C4 photosynthesis is thus more efficient than C3 photosynthesis. In addition, C4 species generally also have greater water use efficiency, better nitrogen use efficiency and higher-temperature tolerance than C3.

This is certainly an ambitious goal, requiring anatomical and biochemical changes which is high risk but the potential benefits are enormous. Many of the necessary building blocks are already available within the C3 crops and recent developments in computation, and biotechnology make success a more realistic proposition and is being pursued within the C4 rice project (https://c4rice.com/). Substantial progress has been made in developing a toobox has already been made (Ermakova et al., 2019), C4 photosynthesis requires that both bundle sheaf and mesophyll cells not only contain the correct components but that they are also expressed and regulated correctly to be functional which will require additional iterative tuning cycles (Ermakova et al., 2019).

An alternative approach that does not require the anatomical changes is to exploit the cyanobacterial carbon concentrating mechanisms into the chloroplasts of C3 crops in which Rubisco is packaged inside carboxysomes (Price et al., 2013). Modelling has suggested this could increase the crop yield by over 30% (McGrath & Long, 2014). Substantial progress has been made in expressing both alpha and beta forms of the carboxysomes in plants (Lin et al., 2014), but to be effective the rest of the cyanobacterial system must also be present. This should be a straightforward engineering project.

Summarizing, from current success stories on improving photosynthesis it became clear that the adjustment of regulatory processes around the photosynthetic light reactions provides great potential for its optimization. Future considerations might also include the simple elimination of regulatory processes that allow plants adapt to variable light conditions that are dispensable when crops are grown. In principle, deleting all but the absolutely essential regulatory mechanisms should make more energy available for biomass production and increasing yield. The here discussed approaches offer the prospect of a renewal of the Green Revolution, which

is urgently required to meet the continuously increasing demand for superior high-yield crop varieties for human sustenance and industrial applications in the future.

4.3.7 INCREASING ORGAN GROWTH AND DEVELOPMENT

A key option to improve plant yield is to improve both intrinsic plant growth and/or plant growth resilience to abiotic stresses. Plant growth is controlled by complex, highly interconnected networks of regulators integrating many different signals (light, sugars, water availability, minerals, etc.) and translating these inputs in the activity of the cell cycle and/or cell expansion machinery. Despite the undeniable importance of roots for plant growth and development, leaves contribute most to vegetative plant biomass. Leaves are a direct source of food, feed, bio-materials and bio-energy and the primary organs for photosynthesis, serving as major carbon and energy producing factories allowing plants to sustain their growth, to complete their life cycle and to form other organs of agricultural importance, such as seeds and fruits (Barber, 2009; Demura & Ye, 2010; Tsukaya, 2013; Zhu, et al., 2010). These features render leaves the predominant organs contributing to plant yield, making leaf size and shape control a highly important field of study.

Distinct aspects of leaf development and the underlying molecular networks are being revealed with an increasing pace (Gonzalez et al., 2012; Hepworth & Lenhard, 2014; Nelissen et al., 2016; Powell & Lenhard, 2012; Vercruysse et al., 2019). In addition, an increasing number of studies indicate that the cellular and molecular mechanisms governing leaf growth in eudicots and monocots are largely conserved (Liu et al., 2009; Nelissen et al., 2016; Peterson et al., 2010; Raissig et al., 2017). Leaf development is a multifactorial and dynamic process including at least six major cellular mechanisms that contribute to leaf size and/or leaf shape determination: (1) the number of cells recruited to the organ primordium (Efroni et al., 2010; Kalve et al., 2014; Reinhardt et al., 2000), (2) the rate and (3) duration of cell division (Andriankaja et al., 2012; Donnelly et al., 1999; Gonzalez et al., 2012), (4) the rate and (5) duration of cell expansion and (6) the extent of meristemoid division, the re-iterative asymmetric division of stomatal precursor cells (Bergmann & Sack, 2007; Geisler et al., 2000; Larkin et al., 1997). Impinging on one of these processes often results in an alteration in cell number and/or cell size, affecting final leaf size and/or leaf shape (Gonzalez et al., 2012; Nelissen et al., 2016; Vercruysse et al., 2019). Over the last years, various regulators of leaf size, shape and differentiation, their targets and/or interacting proteins and the interactions between these genes were described, though mainly focusing on model crops (Beemster et al., 2005; Gonzalez et al., 2012; Hepworth & Lenhard, 2014; Nelissen et al., 2016; Tsukaya, 2013). Leaf growth regulatory genes encode proteins of diverse functional classes involved in the regulation of a single or multiple cellular processes (Gonzalez et al., 2010; Hepworth & Lenhard, 2014; Krizek, 2009). An example of a leaf growth regulatory module with highly conserved functions in numerous eudicot species is the PEAPOD (PPD)-KINASE-INDUCIBLE DOMAIN INTERACTING (KIX) module, interacting transcriptional regulators that repress meristemoid cell division (Baekelandt et al., 2018; Gonzalez et al., 2015; Vercruysse et al., 2019; White, 2006). Upon down-regulation of the PPD/KIX complex or up-regulation of STERILE APETALA (SAP), mediating post-translational degradation of the PPD/KIX complex, meristemoid cell division is significantly increased resulting in shoot biomass increases of up to ~50% in a broad range of species, including *Medicago truncatula* (medicago)*, Vigna mungo* (black gram) and poplar (Ge et al., 2016; Li et al., 2018; Naito et al., 2017; Wang et al., 2016; Yordanov et al., 2017). In *Glycine max* (soybean), down-regulation of the *PPD* orthologues was even shown to also significantly increase seed size, weight and amino acid content (Ge et al., 2016; Kanazashi et al., 2018; Naito et al., 2017). The strong effects on distinct plant organs in various plant species exemplifies that impinging on key leaf growth regulatory pathways such as the PPD-pathway offers great potential to increase plant yield.

Leaf cells have the unique capability to loosen or tighten their primary walls rapidly, in less than a minute in some cases, revealing that the molecular processes underlying irreversible wall expansion are dynamically controlled. A short list of potential molecular actors is now well established although for most of them, their exact role on cell wall extensibility remains to be precised. On the top of this list are the cell wall protein expansins known for a long time to play a crucial role on wall loosening (Cosgrove, 2000a, 2000b), but also on integrating various developmental, genetic and environmental sources of growth variation (Muller et al., 2007). Pectin materials are also identified as key component of cell wall mechanics and therefore growth control (Phyo et al., 2017). Finally, the most recent discoveries point towards the role of wall sensor pathways such as FERONIA (Li et al., 2016) and THESEUS (Hematy et al., 2007). How these molecular actors coordinate the response to environmental stresses is of crucial importance. Indeed, any growth modification in plant leaves is matched (and possibly controlled by) changes in cell wall properties in both the short and the long term (Cosgrove, 2018). Particularly relevant will be a better understanding of their link to the water fluxes towards the growing cells which ultimately must match the growth in volume of any organ (Touati et al., 2015).

Besides extending the knowledge on individual cellular mechanisms and regulators, it will also be crucial to analyze how genes, at the network level, cooperate to exert specific functions and to reveal the connections between the different networks. Understanding the instructor networks that govern organ size under normal, as well as stress conditions will have a major impact on agriculture, both by further enabling our ability to direct plant breeding and by enhancing the success rate in designing higher yielding crops. Despite the numerous biological insights, however, we are still far from reaching the point where, even in model species like *Arabidopsis thaliana* (Arabidopsis), we have a complete view of all the growth regulators, how these are organized in gene regulatory networks and the interconnections between these networks. Next to classical forward and reverse genetics, mapping of quantitative trait loci (QTL) and genome-wide association studies (GWAS) are currently the preferred means to identify genetic variants influencing organ growth. More recently, CRISPR/Cas technology became a game-changing technology that is revolutionizing our ability to modify favorable traits in crops. The impact of CRISPR/Cas is likely to be tremendous and almost on a daily basis, novel applications are reported, such as gene activation, chromatin remodeling and multiplex genome editing. To improve specific plant traits such as organ growth, both coding sequences and *cis*-regulatory elements (~short DNA motifs bound by transcription factors) could be targeted with a CRISPR/Cas genome editing approach (Fauser et al., 2014; Feng et al., 2013; Swinnen et al., 2016). Targeting the coding sequences of growth regulatory genes may, however, result in pleiotropic effects that are unprosperous for farming purposes. In contrast, targeting and inducing sequence variation in *cis*-regulatory elements involved in e.g. transcriptional regulation, may allow to modify a specific aspect of the gene expression profile, such as the developmental timing and/or tissue specificity, and may therefore be of substantial interest for biomass and food production on earth (Swinnen et al., 2016). Also the further development of systems biology approaches will render endless possibilities for improving organ growth. Co-regulated gene sets could for instance be used as input to identify *cis*-regulatory elements mediating condition-dependent transcriptional activity during development, in different organs, cell types, or in response to various signals. Such knowledge could ultimately be used to assembly synthetic chromosomes (or sections thereof) and/or to generate minimal plant genomes as a chassis for various applications, such as improving organ growth.

To be able to probe the role of the newly identified players or growth regulatory networks in organ growth and development, also detailed phenotyping will require further development, both in the context of growth chambers (cellular and leaf level), greenhouse (whole plant level) and field (canopy). In parallel, also the development and application of novel bioinformatics methods will be a requisite, allowing comparative sequence and expression analysis, network biology, the identification of conserved non-coding sequences and data integration (Van de Velde et al.,

2014; Van de Velde et al., 2016). Ultimately, mathematical modeling may enable us to fully grasp the complexity of the organ growth machinery.

To summarize, increasing organ growth offers huge potential for increasing plant yield. In the future, the aims for organ growth research are (1) to expand the existing growth regulatory networks and study their interconnectivity, (2) to engineer the regulatory networks to identify favorable gene combinations, (3) to visualize the gene regulatory networks in a spatial and temporal context, (4) to change the conformation of the network in such a manner that e.g. the timing of cell division during growth is extended, and (5) to probe the networks under agronomical conditions to facilitate the transfer of the knowledge on these networks to value for society. However, the impact of manipulations must be optimized for the performance of the whole canopy and not just individual organs.

4.3.8 INCREASING LEAF LONGEVITY, CARBON AND NUTRIENT REMOBILIZATION AND PARTITIONING

Providing N fertilizers to crops is essential for plant growth, efficient photosynthesis and then plant yield. However, N fertilizers are responsible of a large part of the GHG emissions. To increase the performances of plants without increasing the use of fertilizer it is interesting to get insights on the nutrient recycling and remobilisation processes that plant use to save nutrients. It is also important to understand how nutrient partitioning is regulated and modified according to developmental stages and environmental constraints. The studies of the impact of the recycling pathways on the plant lifespan, nutrient partitioning, seed production and the seed filling is essential to improve significantly nitrogen use efficiency under reduced fertilizer conditions for healthy planet.

Controlling leaf longevity for better nutrient use efficiency

Nutrient recycling mainly occurs in senescent plant organs. Then it is important to study the molecular basis of leaf senescence, and at first the regulation of leaf senescence. Leaf senescence is controlled by both endogenous and exogenous factors and governed by several transcription factors that mainly belong to the NAC and WRKY families. Amongst them, the NAMB1 NAC factor was demonstrated in wheat to control positively leaf senescence, protein, Fe and Zn contents in grains, but negatively grain yield (Uauy et al. 2006; Waters et al. 2009). Other important leaf senescence regulatory genes have been identified in several plant species. One of them is ATAF1 that negatively regulated GLK1, which is important for chloroplast maintenance, and positively regulates ORE1 which is another important TF involved in senescence onset (Garapati et al. 2015; Rauf et al. 2013). The *ataf1* mutants present better leaf longevity thus prolonged photosynthesis and better resistance to several environmental stresses. Interestingly ATAF1 is regulated by SNRK1 that with the TOR kinase control plant growth in response to the C and N status of plant tissues. The impact of the regulatory genes of the leaf senescence program on photosynthesis, nutrient partitioning, nutrient translocation and grain productions needs further investigations to understand how all these gene interact together to control leaf longevity, chloroplast maintenance and then plant growth and nutrient recycling all along the plant lifespan.

Modifying autophagy for chloroplast quality control during senescence and response to stresses

Mechanisms involved in chloroplast maintenance, quality control and at the reverse chloroplast degradation are then very important for photosynthesis and carbon fixation, and also for nitrogen management as chloroplast is the main proteinaceous N resource in plants. It was

recently demonstrated that autophagy that is regulated by both TOR and ATAF1 is very important for leaf longevity and organelle (especially chloroplast) quality control. There are several publications that demonstrated that autophagy is essential for leaf longevity, efficient nutrient remobilization and plant yields (Chen et al., 2019; Yu et al., 2019). All the ATG genes involved in the autophagy core machinery are important for macro- and micro-nutrient (Fe, Zn, Mg) recycling processes (Pottier et al., 2018). In addition, genes regulating and fine tuning autophagy activity in response to environmental stresses are potential interesting targets.

TOR and SNRK1 in plant growth and nutrient management

The antagonist TOR (Target of Rapamycin) and SnRK1 (Snf1-related kinase 1) are activated by nutrient and starvation or stress, respectively (Dobrenel et al., 2016). They play paramount roles in the regulation of nutrient use and storage through the control of autophagy, starch and lipid synthesis. TOR generally inhibits autophagy but also controls general root and shoot as well as the switch to autotrophic growth.

Partitioning of carbon and nitrogen between sources and sinks

An important component of plant productivity and nutritional value of harvested parts in plants is the partitioning of organic carbon (C) and nitrogen (N) compounds among the various organs of the plant. It is mediated by the vascular system and driven by a series of transport steps including export from sites of primary assimilation, transport into and out of the phloem and xylem, and transport into the various sink organs. Manipulating C and N partitioning to enhance yield of harvested organs has been at the basis of crop domestication and it remains a major avenue for increasing yield, stress resilience and nutritional value of seeds (Yadav et al., 2015). Research on the biochemistry, molecular and cellular biology, and physiology of C and N partitioning has now matured to an extent that strategic manipulation of these transport systems through biotechnology are being attempted to improve movement from source to sink tissues in general, but also to target partitioning to specific organs.

Boosting export by the leaf would be necessary if photosynthesis was enhanced. Main actors of the apoplastic loading are sucrose (SWEET and SUT) transporters. For instance, *AtSUC1*, *AtSUC2*, and *ZmSUT1* (a *Zea mays* gene from a different *SUT* subfamily) rescued phloem loading in the *Atsuc2-4* mutant (Dasgupta et al., 2014). At the other end of the pathway, apoplastic unloading (as it occurs in seeds, seprated from the maternal tissue) need to be enhanced. Over-expression of sugar transporter *AtSTP13* in *Arabidopsis* seedlings increases Glc uptake by the seeds, and increased biomass in the plant (Schofield et al., 2009). Conversely, in tomato, RNAi-mediated knockdown of the high affinity hexose transporter gene *LeHT* led to a massive decrease in fruit hexose accumulation (McCurdy et al., 2010). Similarly to attemps to boost sucrose loading and unloading, several attemps are made to manipulate N partitioning. For instance, (Zhang et al., 2015) combined "push" and "pull" strategy for enhancing N transport to developing pea seedling by overexpressing PsAAP1 (Amino Acid Permease 1) with Arabidopsis AtAAP1 promoter. This area of research clearly deserve future attention because of the many feedbacks and pleiotropic effects of the genes identified along the various pathways. Increased yield may not always be expected from increased sink activities. Indeed, a recent successful example showed that inhibiting expression (suing CRISPR technology) of the rice amino acid transporter *OsAAP3* increased grain yield due to a formation of larger numbers of tillers as a result of increased bud outgrowth (Lu et al., 2018).

Remobilization of organic nitrogen during leaf senescence

It is widely accepted that glutamine and asparagine that have two nitrogen atoms per molecule are the preferential source to sink amino acid forms in plants. Glutamine synthetases, asparagine synthetases and glutamate synthases are then important enzymes for source to sink mobilization

(Martin et al., 2006; Lothier et al., 2011; Quraishi et al., 2011). Amino acid transporters located in the vacuole or plasma membranes of source cells and in the phloem are also important. The amino acid permease 2 (AAP2) plays a role in the source sink nitrogen remobilisation (Zhang et al., 2010). The *aap2* mutants exhibit delayed leaf senescence, more branched inflorescences and higher seed yield. AAP8 expressed in phloem of the source leaves is important for N remobilisation to the seeds (Santiago & Tegeder, 2016). In the aap8 mutants the decrease of amino acid phloem loading leads to lower silique and seed numbers. The UmamiT11, UmamiT28, UmamiT29 transporters are highly up regulated in senescing leaves and play a role in seed filling (Mueller et al., 2015). The loss-of-function mutants accumulate high levels of free amino acids in fruits and produce smaller seeds. Similarly, the UmamiT18 was shown to modulate amino acid contents in the siliques (Ladwig et al., 2012).

Remobilization of inorganic nitrogen from senescing organs

It is likely that inorganic nitrogen remobilisation during leaf senescence also exists and depends of the size of the inorganic N pools stored in the leaves before senescence onset. Plants that have received large or sufficient amounts of nitrate have nitrate reserves stored in their vacuole to remobilize, which is not the case of nitrate-limited plants. The fact that some nitrate, ammonium and urea transporters are induced during leaf senescence suggests that they participate to the remobilisation of inorganic nitrogen from senescing tissues (Kojima et al. 2007; Bohner et al., 2015). The studies of the *Nrt1.7* and *NRT2.5* nitrate transporters strongly suggest that they contribute to the remobilisation of nitrate from the source leaves to the sink organs (Fan et al., 2009; Wu et al., 2014; Lezhneva et al., 2014). Urea synthetised from amino acid and nucleic acid catabolism is remobilised from old leaves involving the DUR3 urea transporter in Arabidopsis senescing leaves (Bohner et al., 2015). The Nrt1.6, Nrt1.5 and AMT1.5 transporters are up-regulated during leaf senescence and they could also participate to nitrate and ammonium remobilisation (Tegeder & Masclaux-Daubresse, 2018 for a review). They could also play other roles during leaf senescence, like Nrt1.5 that was shown to modulate phosphate levels in senescing leaves.

4.3.9 ALTERING PHENOLOGY (IN THE CONTEXT OF HEAT STRESS)

Staple crop production will be affected (and is affected yet) by climate changes causing more frequent heat and frost stress in the future (Barlow et al., 2015; Wheeler et al., 2000). As a key factor to adapt the crop life cycle to abiotic stress, phenology is an important topic for many research programs. In this regard, wheat and maize are sharing a common interest but cannot be treated in the same way regarding the genetics earliness. In maize, the adaptation of flowering time has proved to be an efficient lever for increasing yield under climate change (Parent et al., 2018). Although this approach requires an appropriate use of the genetic variability, the genetic determinism of maize phenology seems to be controlled by many small effect loci and, so, could not be seen as a good example of a single-gene (or small number of genes) trait (Buckler et al., 2009). Wheat phenology offers the possibility of acting on a small number of loci and so, gives the opportunity for researchers and breeders to directly use this genetic information to enhance breeding programs (Fischer, 2011). If we focus on heat stress during the grain filling period, earlier flowering is clearly one option to improve stress avoidance (Gouache et al., 2012).

Genetic components of wheat phenology regarding flowering

The genetic determinism of wheat phenology (number of days between the sowing and heading or flowering time) is now quite well known thanks to numerous studies carried during the last 30 years (see for example Trevaskis, 2010; Fischer 2011 or Bogard et al., 2014). In brief, wheat phenology could be defined by 3 components (Rousset et al., 2011; Le Gouis et al., 2012):

- _Vernalization_ (the requirement of exposure to cold temperature inducing the transition between the vegetative and the reproductive phase) is mainly governed by the Vrn gene family (for example: Vrn-A1, Vrn-A2 and Vrn-B3 on homoeologous chromosomes 4, 5 and 7).
- _Photoperiod_ (the sensitivity to the inductive effect of long days on the transition between the vegetative and the reproductive phase) is mainly governed by the Ppd-1 genes family located on homoeologous chromosomes 2 (for example: Ppd-D1, Ppd-B1 and Ppd-A1).
- _Earliness per se_ (the remaining variability independent of vernalization requirement and photoperiod sensitivity) is the less known component with only one locus mapped as a Mendelian factor located on chromosome 1D (Eps-D1).

These knowledges can obviously be used in breeding program (MAS and/or genomic selection) but a recent advance in climate change adaptation is the joined use of crop-models and genomic prediction to define cultivar ideotypes (Bogard et al., 2014; Gouache et al., 2017; Bogard et al. submitted). Compared to the classical crop-modelling approach to define ideotypes, the use of marker-based crop model parameters taking into account the genetic structures of phenology in the available germplasms avoids the risk to define "pure in silico" ideotypes that may be difficult to obtain by breeding due to potential genetic limitations (linkage, pleiotropy).

Trade-off with photosynthesis efficiency and other stress

Earlier flowering in wheat is one of the ways to avoid heat stress during grain filling. Nevertheless, this approach could also have a counter-effect on yield:
- By reducing the duration of the radiation interception, directly linked to crop biomass accumulation (Monteith, 1972). To avoid this bias and build a trade-off, phenology based ideotype studies must also include biomass and yield modelling.
- By impairing agronomic levers against abiotic stress like pest or weed control (Dedryver and Tanguy 1984, Vandersteen et al. 2011) if a change in sowing date is used instead of modifying wheat phenology _per se_.

Finally, modifying wheat phenology is not the only genetic pathway to reduce the negative effect of heat stress. Heat tolerance is another main field of investigation which is worth to reinforce in the future.

4.3.10   IMPROVING THE MICRONUTRIENT QUALITY

Plant food crops are produced and consumed worldwide, as fresh or industrially processed. Staple crops, fruits and vegetables are important sources of primary (proteins, carbohydrates, and fatty acids) or secondary metabolites, such as vitamins, fibers, minerals and antioxidants. Hence, the nutritional quality of plant food is also associated with minor components which are able to exert important functions in the prevention of human pathologies.

Micronutrients are essential for human nutrition; playing large role in the prevention and treatment of conditions or diseases, as well as the optimisation of physiological functioning. Both vitamins and minerals are considered as micronutrients. Deficiencies of micronutrients are widely prevalent in both developing and developed countries. Biofortification is a process to increase the micronutrient content of seeds, tubers, leafy vegetables or food crops and provides a means to increase micronutrient content of diets of the rural poor; the group most at risk of deficiencies (Hotz & McClafferty, 2007).

Improving vitamin content

Vitamins are available either in water-soluble or fat-soluble forms. Water-soluble vitamins include the vitamin C and the B-complex vitamins, including vitamin B6 and B12. They are easily lost through bodily fluids and so must be replaced daily. In comparison, fat-soluble vitamins (including vitamins A, D, E and K) are not lost as easily and so lower amounts are required. Vitamin deficiencies constitute major micronutrient deficiencies leading to economic losses as well as adverse affects on human health.

Vitamin A

Vitamin A exists in several forms called retinoids. Human can synthesize retinal from the abundant provitamin A carotenoids present in fruits and vegetables such as oranges (*Citrus aurantium*), broccoli (*Brassica oleracea*), spinach (*Spinacia oleracea*), carrot (*Daucus carota*), squash (*Cucurbita maxima*), sweet potato (*Ipomoea batatas*), and pumpkin (*Cucurbita maxima*) (Harrison, 2005). Provitamin A is low in wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and potato (*Solanum tuberosum*), and non-detectable in rice (*Oryza sativa*), millet (*Panicum miliaceum*), and sorghum (*Sorghum bicolor*). Both conventional and biotechnologicall approaches have been used to enhance Provitamin A content in crops. The gene PSY from plants or CRTB in bacteria encodes phytoene synthase and is the common target for carotenoid biofortification. The *Narcissus pseudonarcissus* PSY or *Zea mays PSY1* gene was introduced into rice with the *Pantoea ananatis* phytoene desaturase (*CrtI*) gene to increase vitamin A seed content (Ye et al., 2000; Paine et al., 2005). Similar approaches using both genes or each gene in isolation has been applied to canola (*Brassica napus*), flax (*Linum usitatissimum*), potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), maize (*Zea mays*), soybean (*Glycine max*) and wheat (*Triticum aestivum*) (Jiang et al., 2017). Other approaches include gene silencing of *StLCY-e* or *StCH-ε* in potato leading to suppression of LYCE and HYDB, respectively (Diretto et al., 2007; Van Eck et al., 2007).

Vitamin B

Folates, Vitamin B_9, are synthesised *de novo* in bacteria, fungi and plants and play important role as one carbon donors in all organisms. Usually folates are abundant in dark-green leafy vegetables but have low abundance in barley, corn lettuce, potato, rice, sweet potato, tomato and wheat. Two approaches have been used for biofortification of folates. Firstly, the overexpression of dihydrofolate synthetase (DHFS; FOLE in bacteria) used within corn (Naqvi et al., 2009). Or secondly, the overexpression of CTPCHI in tomato, lettuce and Mexican common bean (Nunes, Kalkmann & Aragon, 2009). VB_6 metablic engineering has been conducted in cassava (*Manihot esculenta*) using the simultaneous overexpression of the *Arabidopsis PDX1.1* and *PDX2* genes (Li et al., 2015).

Vitamin C

Vitamin C biofortification has been carried out in lettuce, maize, potato, tomato and strawberry by overexpressing genes involved in its biosynthesis (e.g. GuLO in lettuce), or silencing the genes involved in ascorbate recycling (e.g. MDHAR or DHAR in tomato, maize and potato) (Jain & Nessler, 2000; Naqvi et al., 2009; Gest et al., 2013).

Vitamin E

Plants are the primary source of dietary Vitamin E, producing tocopherol and tocotrienol derivates. Mostly, approaches to enhance vitamin E are to increase the activity of the enzymes in each step of the synthesis, including p-hydroxyphenylpyruvate dioxygenase (HPPD), homogentisate phytyltransferase (HPT1/VTE2), homogentisate geranylgeranyl transferase

(HGGT), homogentisate solanesyltransferase (HST), 2-methyl-6-phytyl-benzoquinol methyltransferase (MBPQ-MT/VTE3), tocopherol cyclase (TC/VTE1) and γ-tocopherol methyltransferase (γ-TMT/VTE4) (Jiang et al., 2016). Both single gene and multigene approaches have been adopted.

Improving mineral content

Minerals are available in two forms, macrominerals and microminerals, depending upon the amount that are required. Macrominerals include calcium, magnesium, phosphorous, sodium and potassium whereas microminerals include iron, copper, iodine, zinc and fluoride. Transgenic approaches to biofortification rely on improving mobilization from the soil, uptake from the rhizosphere, translocation to the shoot and accumulation of mineral elements in bioavailable forms in edible tissues. Most efforts have focused on increasing the content of iron and zinc.

Iron and Zinc

Iron uptake can be improved in non-graminaceous plants by overexpressing genes encoding Fe(III) reductases, and in graminaceous plants by increasing the synthesis and exudation of phytosiderophores (Connolly et al., 2003; Takahashi et al., 2001). Pea (*brz* and *dgl*) and *Arabidopsis* (*frd3*, also known as *man1*) mutants with constitutive Fe(III) reductase activity accumulate not only Fe but also Zn, Ca, Mg, Cu and Mn in shoots. Overexpression of a nicotianamine synthase (*AtNAS1*) also resulted in an increase in shoot Fe, Zn and Mn concentrations in transgenic tobacco whilst the over-expression of an Arabidopsis Zn^{2+} transporter (*AtZIP1*) in roots of barley (*Hordeum vulgare*) increased seed Fe and Zn concentrations. Other transgenic strategies to increase bioavailable Fe and Zn in edible portions have focused on increasing the concentrations of metal-binding proteins, such as ferritin and lactoferritin, increasing promoter compounds, such as ascorbic acid, b-carotene and cysteine-containing peptides, and reducing antinutrients, such as phytate and tannins, in edible portions (Holm et al., 2002; Lonnerdal, 2001). Ramesh, Choimes & Schachtman (2004) overexpressed Zn transporter *AtZIP1* of *Arabidopsis* in barley under an ubiquitin promoter. The transgenic lines produced smaller seeds with high Zn concentration. Manipulating the expression of genes regulating CAX transporters has been proposed as an approach to increase Zn concentrations in the edible tissues of transgenic plants.

Improving mineral content in the seed

Staple crops are poor in minerals, leading to widespread mineral nutrition deficiencies. While important progress has been made in the area of mineral uptake from soils, our understanding of the mechanisms that regulate the distribution of minerals within the plant and their accumulation in the seed is still limited. It is therefore unclear how the transport of minerals toward seeds could be improved (low mineral availability due to anti-nutritional factors is not treated here). In general, it appears that over-accumulation of minerals in leaves does not necessarily result in increased mineral content in the seeds. A comprehensive study performed in Arabidopsis shows that the continuous uptake and distribution to source tissues is at least as important as remobilization for the accumulation of mineral nutrients in seeds (Waters and Grusak, 2008).

Alternative approaches to improve micronutrient content

Wild germplasm of crops has been found to harbour sufficient variability for improvement in mineral content which could be used for improvement in modern day varieties. In rice, a fourfold difference was found in grain Fe and Zn content in some aromatic lines as compared to popular cultivars. In wheat, landraces and wild relatives were found to contain threefold higher grain iron and zinc content than the popular cultivars. Wild relatives have been used to transfer genes

for biotic and abiotic stress tolerance and yield and quality improvement in cultivated varieties, and likewise, these can also be used to transfer useful variability for grain iron and zinc content using conventional and modern breeding approaches. G × E interactions in wheat cultivars for iron, zinc and magnesium concentrations have been explored and it is reported that genotypes have a higher effect than environment on accumulation.

Agronomic strategies to enhance mineral content of cereal crops involve application of micronutrient fertilizers to the plants in readily phytoavailable state, correcting soil alkalinity, adopting crop rotation practices or introducing beneficial soil microorganisms. The most attractive agronomic strategy of biofortification is foliar application of mineral fertilizers to the plants in readily phytoavailable state. However, iron fertilization has met with limited success in biofortification because the applied Fe^{2+} gets rapidly oxidised to Fe^{3+} state, which is not absorbed by the plants. Agronomic biofortification has met with limited popularity in cereals because of the recurring expenditure and need for careful time-dependent applications of fertilizers.

4.3.11   IMPROVING PROTEIN/AMINO ACID COMPOSITION

Grain protein concentration (GPC) is a major quality criterion in bread wheat directly related to the market requirements of the bread industry in several countries. GPC (ie grain N concentration) is also one of the components of the Nitrogen Utilization Efficiency (Sylvester et al., 2009). So, enhancing GPC while maintaining grain yield has been a major target for numerous breeding and agronomic programs during the last twenty years (Cormier et al. 2016; Cohan et al., 2019). Achieving this objective is quite challenging due to the grain yield - GPC negative relationship (Monaghan et al., 2001) and the search for cultivars showing a positive deviation from this relation (Grain Protein Deviation) is now a priority in several countries (Oury & Godin, 2007; Bogard et al., 2013).

The *NAM-B1* gene

The *Gpc-B1* gene, located on chromosome 6B lately renamed *NAM-B1* because it codes for the *Non Apical Meristem Protein* belonging to the *NAC* transcription family, has proved to be highly associated to high GPC (Uauy et al., 2006a; Uauy et al., 2006b). Its functional allele accelerates wheat senescence and increases nitrogen remobilization from the leaves to the grain (Uauy et al., 2006a; Waters et al., 2009). These two metabolic pathways have been known to act on Nitrogen Utilization Efficiency and GPC for a long time (Gregersen et al., 2008; Masclaux-Daubresse et al., 2008). Nevertheless, the functional allele of *NAM-B1* is rarely present in modern bread or durum wheat cultivars (Hagenblad et al., 2012; Tabbita et al., 2017), a loss probably more due to modern crop improvement than to domestication per se (Asplund et al., 2010). Protocols and recommendation for *NAM-B1* introgression through marker-assisted selection have been made publicly available at https://maswheat.ucdavis.edu/protocols/HGPC/index.htm and facilitated the introgression of the functional *NAM-B1* allele in different genetic backgrounds (Brevis & Dubcovsky 2010, Kumar et al. 2011). According to Tabbita et al. (2017), different commercial breeding programs used *NAM-B1* and 18 commercial varieties carrying this allele have been released so far.

The *NAM-A1* gene

Alongside *NAM-B1*, bread wheat has other *NAM* genes, among which the homologous *NAM-A1* (on chromosome 6A) displays the same function as *NAM-B1* (Avni et al., 2014). Its functional allele *NAM-A1a* shows the same effect on GPC than the functional allele of *NAM-B1* and has been found more often in bread wheat elite germplasm, mainly in high baking quality cultivars (Cormier et al., 2015). Although *NAM-A1a* could have the same counter effect on yield, this

effect is strongly impacted by the interaction with the trial environment (Cormier et al., 2015). As an example, in Australia where growing conditions favor a shorter grain filling period avoiding heat and water stress, Alhabbar et al., (2018) recently demonstrated that *NAM-A1a* could be linked to higher yields. As a conclusion, it appears clearly that the use of *NAM-A1a* allele in elite material could be a real opportunity for improving GPC without reducing grain yield considering the local specificities of the senescence period regarding late stress (abiotic and biotic).

4.3.12   REDUCING ANTINUTRIENTS AND TOXIC METABOLITES

Antinutritional Factors (ANF) are compounds primarily produced by plants for their own defence against pest and predators, and which interfere with the absorption of nutrients reducing their intake, digestions and utilization and may produce other adverse effects. Together with ANF plants may also produce toxic metabolites (TM) which severely affect biological functions of the organisms that ingest them. All these compounds are commonly accumulated in edible parts of the plants such as seeds, leaves, roots and fruits and are frequently s plant family-specific. Besides being a problem in cultivated crops, presence of ANF is particularly relevant in wild and underutilised crops, such as some minor legumes like *Canavalia*, *Mucuna*, which possess a potentially rich nutritional value that is lowered by the presence of ANF. ANF exert biological activities that may have different impacts depending if the crop that accumulate them is used for human or animal consumption. For example, α-amylase inhibitor limits starch digestion and thus may limit energy assimilation in animals, however in human nutrition its presence in the diet has the potential to reduce the glycaemic index of the food and play a role in weight control as it limits calories derived from starch assimilation. Similarly, saponins (compounds characterised by the presence of triterpene or steroid aglycone moiety coupled with polar sugar molecules) are associated with a range of dietary effects from bitterness to bloat, however possible hypocholesterolaemic and anticarcinogenic activity have been reported.

ANF may be of protein origin or of non-protein origin and, traditionally, their presence in plant based food has been managed by adequate food processing such as heating, extrusion, fermentation, etc. However, strategies based on the genetic modification of the trait are sought. In some cases, breeding approaches have already been applied and shown to successfully reduce or even eliminate the presence of ANF.

Proteinaceous Antinutritional Factors

ANF of protein origin are thermolabile and comprise lectins, protease inhibitors (Kunitz type trypsin inhibitors-KTI and Bowman Birk type trypsin inhibitors BBI) and α-amylase inhibitors and are found particularly abundant in the seeds of legume crops. Mutations that reduce/abolish the accumulation/activity of these compounds have been described in soybean (Schmidt et al., 2015), common bean (Sparvoli et al., 2016) and pea (Clemente et al., 2015) and are the results of screening natural biodiversity (common bean) or mutant populations (common bean, soybean, pea). Livingston et al. (2007) used a biotechnological approach based on the ectopic expression of a mutated BBI gene to reduce the amount of BBI in soybean seeds. The prevalent amount of the mRNA from the mutated BBI gene was considered to out-compete the mRNA form the endogenous BBI resulting in a global reduction of BBI activity. At the light of these studies the best strategy to reduce the content of the proteinaceous ANF is by the identification or generation of mutants, for example by the application of CRIPSR-Cas gene editing technologies, or RNAi. These approaches can be easily transferred to proteinaceous ANF in other crops. An example is the detoxification of ricin in the seeds of castor bean through RNAi (Sousa et al., 2017). Ricin is a type2 ribosome-inactivating protein (RIP) made up by two chains: a catalytically-active A chain (ricin toxin A or RTA) joined by a single disulfide bond to a B chain (RTB) that is a galactose- and N-acetylgalactosamine-specific lectin. This protein is a well know

highly toxic protein due to the ribosome-inactivating activity of the A chain, that makes impossible the use of oil cakes after oil extraction as and edible source for animal feeding.

Non-proteinaceous anti-nutritional factors

Non-proteinaceous anti-nutritional factors (NP-ANF) include among others, phytate, oxalate, raffinose family oligosaccharides (RFOs) and tannins, all being prone to interfere with nutrient absorption. In crops, NP-ANF may act as osmolytes (RFOs), serve as storage or signalling molecules (phytate), or exhibit a variety of functions such as for oxalates involved in calcium regulation, plant protection, tissue support, and even light gathering and reflection. A major challenge to breeding and engineering strategies aiming at the removal of NP-ANF is therefore to consider the multiple roles that NP-ANF and associated pathways fulfilled in producing plants. Briefly, in the case of phytate (*myo*-inositolexa*kis*phospahte), its accumulation has been successfully reduced in many grain cereals and legume crops (maize, barley, rice, wheat, soybean, common bean) through screening of mutagenised populations and identification of low phytic acid (*lpa*) mutants. Characterization of these mutants contributed to deciphering phytate biosynthesis and transport genes (reviewed in Sparvoli et al., 2015). *lpa* mutants often display altered seed metabolite composition and/or negative pleiotropic effects, such as decreased tolerance to abiotic stresses, reduced germination, stunt growth (Stevenson-Paulik et al., 2005; Tong et al., 2017; Meis et al., 2003; Pilu et al., 2005), although good agronomic performance could be recovered through breeding. Alternatively, an engineering approach that used seed specific promoters has also been shown to successfully overcome the negative pleiotropic effects, as demonstrated in maize (Shi et al., 2007). A second example is that of RFOs which humans and monogastric animals are unable to digest, thereby reducing dietary net energy contribution and the intestine absorption capacity. On the other side, these compounds have been considered as prebiotics, stimulating the growth of beneficial microorganisms such as *Bifidobacterium* and *Lactobacillus* species. Similarly, condensed tannins bind to proteins, forming insoluble complexes that render proteins resistant to degradation in monogastric animals and humans. However, they may provide benefits to human health, particularly therapeutic effects against cardiovascular dysfunction, and are also beneficial in forage-based agricultural systems when incorporated into animal feed for ruminants. Thus, precaution must be taken when considering strategies to reduce the amount of NP-ANF in plant food.

Toxic metabolites in edible plant tissues

Many edible plant parts contain, in their raw state, wide varieties of antinutrients which are potentially toxic. These include for example, cyanogenic glycosides, saponins, or quinolizidine alkaloids that are produced as part of plants' secondary metabolism and are associated with resistance to abiotic/biotic stresses. Biotechnology approaches can be employed to down-regulate or even eliminate the genes involved in the metabolic pathways for the production, accumulation, and/or activation of these toxins in plants. For example, the solanine content of potato has already been reduced substantially using RNAi and genome editing approaches (Itkin et al., 2013; Sawai et al., 2014), and efforts are underway to reduce the level of the other major potato glycoalkaloid, chaconine. A more recent understanding of the transcriptional machinery controlling saponin biosynthetic pathways (Cardenas et al., 2016) may also offer additional targets for engineering programs in order to reduce levels of these compounds in edible tissues. In the case of glucosinolates which can act antinutrients for animal feed, mutations in several transporters strongly have been shown to reduce glucosinolate levels in seeds of different *Brassica* species (Nour-Eldin et al., 2017), an approach which is transferable to other oilseed crops, such as *Camelina sativa*. Similarly, current research in the protein-rich seeds of Lupin indicates that toxic quinolizidine alkaloids that may accumulate upon stress in selected non-bitter varieties, are also specifically imported from other plant tissues into seeds, opening the possibility of also using transport engineering to generate stress-resistant varieties that produce quinolizidine alkaloid-free seeds (Otterbach et al., 2019). Concerning cyanogenic glycosides,

Cassava is the only staple food that is potentially lethal due its cyanogenic glycoside content, if not adequately processed. Two strategies have been developed to reduce cyanogen toxicity in this crop. One of these is the generation of cyanogen-free cassava plants using conventional breeding; the other one consists in the development of transgenic cassava plants either deficient in the biosynthesis of cyanogenic glycosides (Jørgensen et al., 2005) or in which the broken-down of these cyanogenic glycosides is achieved by accelerated cyanogenesis (Narayanan et al., 2011).

Conclusions and future perspectives

Reduction/removal of ANF and toxic metabolites is one of the 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"). Strategies based on exploitation of the biodiversity and conventional breeding have been shown to be applicable in some cases, especially when the trait has a simple genetic basis (ex. removal of toxic proteins). However, as many of the AFN and toxic metabolites play a role in plants' adaptation to the environment (ie resistance to abiotic/biotic stresses) and/or derive from complex metabolic pathways, there is an urgent need of developing strategies able to overcome unwanted pleiotropic effects. For example, one promising approach is to abolish the accumulation of the toxic metabolites by inactivation of its specific transporter, as successfully shown for phytic acid or glucosinolates. Finally, increasing knowledge on gene functions will favour the use new breeding technologies (based on CRISPR-Cas) to target specific genes and speed up the improvement process.

4.3.13 SPECIALIZED METABOLITES – POLYPHENOLS

Plant secondary metabolites are generally divided in different classes; among them are polyphenols, carotenoids, terpens and others. Polyphenols form a wide group of plant secondary metabolites (more than 6000 different compounds have been so reported from the plant kingdom) which include different sub-groups i.e. flavonoids, anthocyanins and phenolic acids (**Figure 6**). These phytochemicals are generally biosynthesised in plant tissues in "sub-optimal" amounts for human needs. Therefore, one of the goals of research projects committed to crops nutritional improvement is related to the enrichment in the levels of these bioactive compounds in edible tissues/organs. To date, this goal has been mainly addressed by breeding varieties, or the identification of species/varieties naturally rich in a specific class of biocompounds. More recently, metabolic engineering and genome editing techniques promised to cut the long times so far required to transfer useful genetic traits into cultivated varieties.
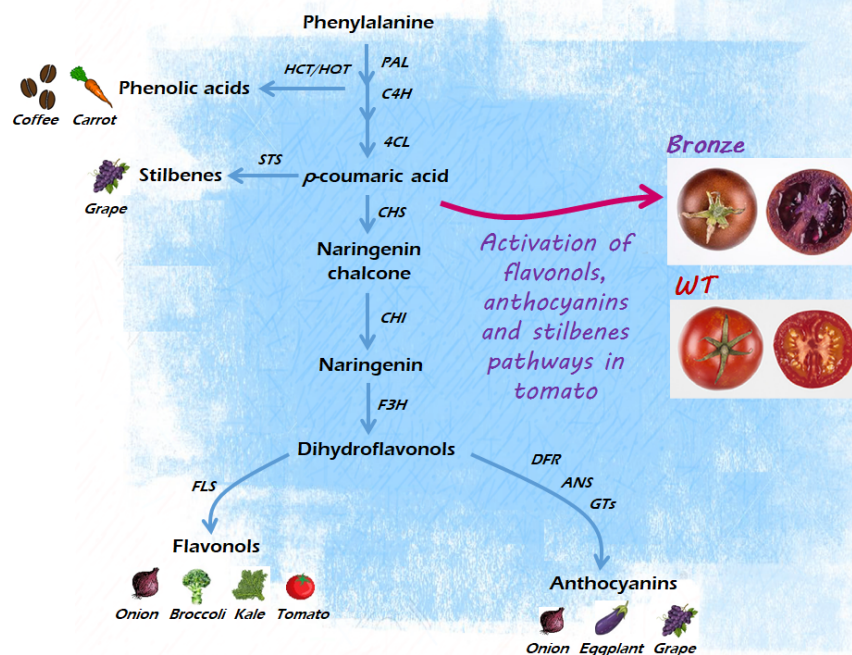
Figure 6 The polyphenols pathway and an example (the Bronze tomato) of new high polyphenols crops obtained by a metabolic engineering approach.

Metabolic engineering approach for nutritional quality improvement

One of the metabolic engineering strategies to enhance the nutritional value consists in the re-directing of the metabolic flux, by acting on regulatory or structural genes. In the first case, transcription factors (TFs), regulating the expression of structural genes along biosynthetic pathways, can be useful and powerful tolls for the enhancement in the levels of specialized metabolites.

For example, MYB and bHLH transcription factors have been reported to regulate the anthocyanin production in different species (Scarano et al., 2018). Following some studies regarding the ectopic expression of transcription factors from plant models, MYB and bHLH members have been described as regulators of anthocyanin biosynthesis, such as SlANT1 and SlAN2 in tomato. The over-expression of two TFs AmRosea1 and AmDelila was able to induce for the first time the synthesis of high levels of anthocyanins in tomato fruit (Butelli et al., 2008). An example of the use of structural genes to redirect the metabolic flux along a pathway is related to the over-expression of a grape stilbene synthase gene into tomato, which led to the synthesis and accumulation of measurable amount of resveratrol, a phytochemical not present naturally in this fruit. Using a combination of both the strategies we were able to develop a new tomato line (which was named Bronze for the typical color of the skin which accumulated high levels of flavonols (i.e. quercetin), anthocyanins and stilbenes (i.e. resveratrol) (**Figure 6**). Supplementation of 1% lyophilised bronze in the diet had a positive effect on the symptoms of intestinal inflammation in a mouse model (Scarano et al., 2017).

These examples can help to (re)consider the use of metabolic engineering approach for the nutritional improvement of important crops without drastic changes in the plant growth or final yield.

Novel strategies to modify/engineer genetic traits for the nutritional quality

To date, the metabolic engineering for the improvement of the nutritional quality has been achieved essentially by using transgenesis strategies. New advances are now rapidly developing based on gene targeting technologies. For example, the CRISPR/Cas9-mediated gene editing is a new biotechnological tool to introduce new genetic traits into a genome and therefore to improve the quality of crops and fruits. Gene editing provides an alternative to transgenesis, with the important advantage of site-specific, precise modifications of target genes (Cermak et al., 2015). One example is the knock-out of structural genes of polyphenol biosynthetic pathway to re-direct the metabolic fluxes (e.g. knocking-out the chalcone synthase gene that can enhance the hydroxicinnamic acids production). Another example is the over-expression of positive regulators, such as the SlANT1 gene in tomato, which has been reported to increase the accumulation of anthocyanins (Cermak et al., 2015).

The potential of the engineering of structural and regulatory genes in plants is very high, considering the possibility to use new advanced biotechnological tools (i.e. genome editing in plants) and the perspective of novel approaches in food design. The examples about the engineering of genes involved in the anthocyanins and flavonoids (CHS, ANT1) give an idea of how exploit genetic traits for crops nutritional quality improvement. This is important in the perspective of promoting high-quality foods and assuring food nutritional security both in low-income and industrialized countries.

4.3.14   BENEFICIAL NUTRITIONAL QUALITY IN UNDERUTILISED CROP SPECIES

In 2010 the FAO (FAO, 2010) stated with regards to neglected and underutilised crop species that "such crops often have important nutritional, taste and other properties, or can grow in environments where other crops fail". But the report also highlighted lasting constraints hindering their broader utilization "lack of priority given by local and national governments, inadequate financial support, lack of trained personnel, insufficient seed or planting material, lack of consumer demand and legal restrictions". Aside the social and policy related hurdles such crop traits fostering the broader economic success like access to high quality germplasm, improved yields and nutritional traits are essentially relevant in further breeding efforts. Nutritional improvement of agricultural crops relates to protein and amino acids, oils and fatty acids, carbohydrates, vitamins and related compounds, functional secondary compounds and minerals availability.

Though well-known and widely used as leafy vegetable (two main species) or cereal (four main species) throughout the globe, Amaranth belongs to such crops which lack a break through despite considerable breeding efforts (Williams & Haq, 2002) as well as it is frequently harvested from veld (Alercia, 2013; Rastogi & Shukla, 2013). The nutritional value of Amaranth can be superior to several commodity crops or vegetables as it harbours the broad range of essential compounds for healthy nutrition (Rastogi & Shukla, 2013). This becomes even more important as Amaranth species are considered drought tolerant if sufficient water is supplied in the early season. Moreover, drought stress may cause increased levels of bioactive compounds, phenolic acids, flavonoids and antioxidants (Sarker & Oba, 2018). The broad variety of Amaranth species bear a considerable genetic resource for further breeding in relation to nutrition and adapting agricultural systems to climate change (Rastogi & Shukla, 2013).

Europe is heavily depending on imports of protein crops mainly for feed use but also for the production of novel protein food. The dominating crop is soybean. Since EU is promoting alternative protein crops i.e. legumes such as lupins became more investigated and cropped after a decline in the 20th century. Lupin cultivation in Europe is largely insufficient to supply the food industry, and further up the value chain the development of lupin-based protein-rich foods

is limited (Lucas et al., 2015). Despite a high protein content in lupin seeds (~44%) the broader use is challenged due to allergens and antinutritive compounds (in e.g. unprocessed feed) and/or biotic, abiotic stress tolerance and unstable yields. Hence, the breeding efforts are essentially targeting yield stabilization, stress resistance and seed quality and late maturing (Abraham et al., 2019). Recent advances in genome sequence information of the genus lupins backed further breeding efforts based on the use of molecular markers and/or advanced breeding techniques (Hane et al., 2017, Książkiewicz et al., 2017).

The utilisation of medicinal plants can be an alternative cropping option especially in horticulture systems. Medicinal plants harbour a rich pool of secondary metabolites with various applications. Nevertheless, the broader use may be hampered by actual yields or accessibility. Therefore, breeding efforts may focus on increased and stable production of such compounds and novel derivatives. Essentially the specific metabolic pathways and their regulation are target for investigation. E.g. phenylpropanoids like rosmarinic acid are one group of such medicinal active compounds. Gene sequences encoding relevant enzymes for the pathway of rosmarinic acid synthesis were frequently cloned into bacteria targeting increased yield of protein, sequence analysis and analysis of gene functions (Sander & Petersen, 2011; Tuan et al., 2012). Such insight in relevant metabolic pathways, and knowledge of gene sequences and functions allows the target use of modern breeding techniques to optimise pathways in medicinal plants. E.g. such detailed information on gene sequences and functions was used to increase the production of rosmarinic acid in *Salvia miltiorrhiza*, well known in Chinese medicine, by specifically editing the rosmarinic acid synthase gene *SmRAS* using the CRISPR/Cas-9 system for genome edting (Zhou et al., 2018).

Beside terrestrial cropping aquatic production systems based on algae may provide key compounds for human nutrition among which are lipids and fatty acids, carotenoids, proteins, minerals and others. Prominent research is conducted with microalgae that allow mass production in bioreactors. Contrastingly, the targeted breeding of seaweeds is more challenging. Seaweeds for human consumption are mainly produced in South-east Asia (Buschmann et al., 2017). Nevertheless, there is ongoing research to explore productions in Europe (Azevedo et al., 2019, Marinho et al., 2019). The kelb *Saccharina latissima* was grown in Danish waters to investigate the content of antioxidants over seasons (Marinho et al., 2019). The study revealed "marked seasonal variations, and high biological variability" in the antioxidant content. In addition, the amount of antioxidants was less than know from other European alga like *Fucus*. The findings were in line with the general observation reported from Asia that the uniformity and stability of a seaweed varieties may change over time and production site (Hwang et al., 2019). While breeding and research programs focussed on increasing yields the quality aspect becomes more prominent as well as molecular analyses are integrated to assist breeding (Buschmann et al., 2017, Hwang et al., 2019).

Currently underutilised crop species may gain importance when adapting agriculture to changing climate condition while keeping the overall nutritional quality of agricultural production for human consumption (e.g. Amaranth). Herbs with specific health benefits may open new (niche) markets for farmers though the production of specific medicinal compounds may be shifted to more productive systems e.g. based on cloned microorganisms. Current protein sources may be enriched or substituted by alternatives e.g. lupins or even seaweeds. Underutilised crops/plants show different lack of breeding efforts and supporting research, what partly is due to the current economic value and lack of funding but also from intrinsic biological hurdles (accessible biological variability, propagation cycles, sensitivity to environmental conditions etc.) that slow breeding progress (e.g. in seaweeds). Molecular breeding techniques (from QTL analyses to genome editing) become more prominent to accelerate the utilisation of such crops/plants.

4.3.15 IMPROVING DIGESTIBILITY AND CONVERSION OF PLANT BIOMASS

The '*biorefinery*' concept covers, at its basic level, the exploitation of agro-industrial waste- and side-streams as means to improve resource exploitation and reduce carbon footprint. The more ambitious instantiation of the biorefinery concept deals with the multipurpose plant that is not only bred for the yield and quality of say the grain to be used for human consumption but also bred for the properties of the vegetative parts that through bioprocessing shall find uses as feed, liquid fuel or biomaterials. Bioprocessing comprises both fermentation and enzyme technology, i.e. systems of environmentally friendly processes.

It is well recognized that burning fossil fuels and deforestation are major contributors to climate change, and that plant biomass can serve as a renewable and potentially carbon-neutral raw material for the production of bioenergy and a variety of other bio-based products. Indeed, lignocellulosic biomass mainly consists of the cell wall polysaccharides cellulose (~50%), hemicellulose (~25%), and lignin (~20%), and all three fractions can be valorised in the bio-economy. In a biorefinery, the cellulose and hemicellulose can be converted by enzymatic treatment into primary sugars that can be further fermented into bio-ethanol and a wide range of other molecules of value for society, e.g. building blocks for bioplastics, detergents, etc. and lignin itself may find uses as for example fuel for ship engines and matrix material in biocomposites.

Because lignin is the main recalcitrance factor in the conversion of biomass to fermentable sugars (Chen & Dixon, 2007; Van Acker et al., 2013), engineering plants to deposit a lower amount of lignin allows achieving biomass that is easier to process into fermentable sugars. However, lignin is not the only limiting factor. For each biopolymer in the plant cell wall at least one enzyme that cleaves it is known, yet technology for cell wall disassembly without resorting to harsh chemicals or high temperatures is not known, even for plant material consisting mostly of primary walls (i.e. walls low in lignin). Hornification refers to the induced tight association of cellulose microfibrils (Diniz et al., 2004) and is known to contribute to recalcitrance with regard to saccharification (Luo & Zhu, 2011). Direct cellulose microfibril interactions were shown to constitute an important load-bearing factor even in primary walls (Scheller & Ulvskov, 2010). Hornification is thus not just an artifact. These are not the only "higher-order structures" to consider in the plant cell wall. Covalent bonds between lignin and polysaccharides, lignin and proteoglycans that feature tyrosine mediated cross-linking (extensin) are important as are the unknown mechanisms responsible for the extremely tightly bound residual galactose that is observed even in the purest cellulose preparations. Microorganisms that subsist on lignocellulosic biomass know how to disassemble these higher order structures. A first general goal is to better understand higher-order structures of plant cell walls (both primary and secondary) and to map the ensembles of proteins that microorganisms secrete to open up the biomass structure for digestion. The enzymes discovered this way will readily find uses for bioprocessing and thus reduce the need harsh pretreatment regimes and use of environmentally unfriendly chemicals. The second general goal is to tailor biorefinery crops to their particular end use. The design objectives depend on the objective. If nanocellulose for advanced materials is the target, then gentle disassembly that does not damage the microfibrils is desired. If saccharification is the goal then high yield of hexoses for minimal energy input is desired. Polymer replacement*,* i.e. downregulation of pentose rich polymers combined with ectopic deposition of a hexose-rich polysaccharide that fulfil the same function in the wall come into play. However, technology readiness level is higher when it comes to developing biorefinery crops that deposit less lignin without affecting the overall growth and development of the plant. Because lignin is also increasingly considered as a valuable biomass fraction that can be depolymerized by pyrolysis or catalysis to aromatic building blocks for the chemical industry (Van den Bosch et al., 2015), a technically related objective is to engineer biorefinery crops to deposit more lignin. Because the biorefinery uses chemical treatments to depolymerize lignin, a further promising strategy is

to alter the lignin composition such that the altered chemical reactivity to these biomass pretreatments improves the efficiency of lignin breakdown. In addition to lignin and cell wall polysaccharides, plant biomass also contains thousands of molecules of which the structures, and hence the properties (e.g. insecticidal, growth promoting, etc.) have remained unknown because they are difficult to purify in sufficient amounts to allows for structural elucidation by NMR. The identification of molecules from biomass crops, alongside with their biosynthetic pathways, opens perspectives for the valorisation of these molecules.

1. Cell wall architecture and its enzymatic disassembly

Cell wall composition is well understood and our understanding of the biosynthesis of the individual polysaccharides are growing slowly but steadily but how the biopolymers are deposited and what the resulting architecture (which, as opposed to 'composition' refers to higher order structures in the wall) looks like is very poorly understood. Uncovering this will translate into strategies to breed for cell wall properties in multipurpose or biorefinery crops. Mapping the architecture of plant cell walls may involve techniques of cell biology, physical techniques like neutron scattering and a wide range of bioimaging and spectroscopy techniques. However, it will undoubtedly be fruitful to study architecture in conjunction with studying the enzyme repertoires that microorganisms use to take lignocellulosic biomass apart. We probably do not need more cellulases nor pectinases but genomic high-through-put technologies will allow us to target the bonds in the wall architecture that we do not fully understand. New classes of enzymes discovered this way will greatly expand the toolbox available for bioprocessing.

2. Regulation of secondary cell wall formation

While the biosynthetic pathways leading to cellulose and lignin are relatively well-known, the mechanisms regulating the biosynthesis and deposition of the three main polymers (cellulose, hemicellulose and lignin) are far from being understood. Besides transcriptional regulation, secondary cell wall formation is controlled by miRNA targeting and chromatin remodeling (McCahill & Hazen, 2019). It is important not to rely only on model plants since for instance alternative splicing is a key mechanism to regulate secondary cell walls in trees but not in Arabidopsis (Camargo et al., 2019). Similarly, the epigenetic control of secondary cell wall formation emerged recently in eucalyptus, sorghum respectively (Soler et al., 2017; Turco et al., 2017) but not yet in Arabidopsis. A better understanding of the regulatory mechanisms through system biology approaches in wood will allow modeling of the responses induced by genetic engineering and will be instrumental for genomic selection of trees for instance. Also discovering specific regulators of each polymer's biosynthesis would enable to modulate them independently.

3. Altering the amount and structure of lignin

When improving plant cell walls, it all comes down to identifying the genes that are involved in the biosynthesis of the major cell wall polymers, and altering their expression levels in target crops such as poplar and maize (Chanoca et al., 2019). Whereas many genes involved in cell wall biosynthesis have already been elucidated, the specific roles of many others still need to be uncovered. For example, it is become clear that the lignin structure and lignin biosynthesis pathway in grasses are different from those in dicot species. Bioinformatics analyses learns that lignin biosynthesis genes in grasses typically reside in large gene families, but the roles of most gene family members are still unknown. We do not yet know enough about the lignin biosynthetic pathway in grasses yet. The function of these genes needs to be unravelled through reverse genetics: knock-outs, but also altered enzyme activities engineered by CRISPR/Cas, and overexpression of the biosynthetic genes. In addition, we need to investigate which combinations of mutations in the lignin biosynthesis pathway act synergistically to reduce or increase lignin

content, while avoiding negative effects on plant yield. Furthermore, the altered expression of transcription factor genes, or of genes of the shikimate pathway may affect lignin deposition.

4. Altering the structure of lignin by engineering

In addition to engineering lignin structure by genes of the host plant itself (modification of *p*-hydroxyphenyl (H)/guaiacyl (G)/ syringyl (S)/ cinnamaldehydes/ferulates (Ralph et al., 2019), it is as well possible to engineer easily degradable lignin polymers by using genes from other taxa in a synthetic biology approach (Oyarce et al., 2019; Vanholme et al., 2012; Wilkerson et al., 2014). In this approach, the host plant is transformed with (a) heterologous gene(s) that encode(s) (a) biosynthetic enzyme(s) that is (are) able to make a monolignol substitute. When transported to the cell wall and incorporated into the lignin polymer, this alternative monomer will generate a bond that is more susceptible to the biomass pretreatment used to degrade the lignin polymer. This is a largely unexplored field with numerous possibilities for specific applications.

5. Climate change and lignocellulosic biomass

Climate change with increasing temperatures, frequent episodes of drought or frost do not only decrease lignocellulosic biomass production but also affect its properties, by increasing/decreasing lignin content or modifying its composition for instance. These changes in turn affect biomass end-uses. Many examples of secondary cell wall remodeling in responses to environmental cues have been reported but how these environmental signals are integrated to modify secondary cell walls is largely unknown. Recently some structural or regulatory genes involved in secondary cell wall formation were shown to be responsive to stresses and moreover to confer stress resistance (i.e. drought and salinity stresses resistance when overexpressed in poplar for instance (Guo et al., 2017; Xu et al., 2017). It is likely that more examples will emerge in the near future as it was shown in Arabidopsis that several abiotic and nutritional stresses can co-opt the secondary cell wall regulatory network (Taylor-Teeples et al., 2015). Dissecting the mechanisms and the genes underlying stress tolerance at the level of secondary cell walls is an important challenge to address given the threat of climate changes on crops and forest trees.

6. Understanding and overcoming the yield penalty

Engineering plants to reduce lignin amount or alter its structure is often associated with a yield penalty. Several hypotheses exist that explain the molecular basis of the yield penalty (Muro-Villanueva et al., 2019), but the mechanisms that lead to dwarfism remain not fully understood.

7. Systematic identification of secondary metabolite structures in biomass crops

A main bottleneck in gene discovery studies, especially those involved in biosynthetic pathways, is that the identity of most (mostly secondary) metabolites is unknown. We can only identify the role of genes in metabolic pathways (e.g. phenylpropanoid pathway) if we know the identity of the differentially accumulating compounds in the corresponding mutants. Metabolite and pathway discovery are important from a fundamental biology point of view, but also open-up perspective for valorization of plant metabolites. This means that a large international concerted effort is needed to systematically identify the structures of unknown metabolites by mass spectrometry-based approaches, purification and NMR.

8. Translational research in biorefinery crops

When plants are cultivated in a greenhouse, their phenotypes and characteristics largely differ from those of plants grown in the field. This difference is due to seasonal effects, along with

biotic and abiotic interactions. Therefore, field trials are an essential step in the translation from basic research to applications. In the next phase, the biomass from the field-grown biomass crops needs to be investigated for altered biomass processing efficiencies. This is ideally done first at bench scale, subsequently at pilot biorefinery scale.

9. Strategies for DNA-free genome editing

Because of the high potential of CRISPR-based genome engineering for improving the processing efficiency of perennial biomass crops, efficient strategies for DNA-free genome-editing will be needed to speed up the translation from the lab to the field.

4.3.16 METABOLIC ENGINEERING IN OILSEEDS TO PRODUCE HEALTH-PROMOTING LONG CHAIN POLYUNSATURATED FATTY ACIDS

The omega-3 (n-3) long chain polyunsaturated fatty acids (LC-PUFA), eicosapentaenoic (EPA; 20:5 n-3) and docosahexaenoic (DHA; 22:6 n-3) are accepted as being essential components of a healthy balanced diet. EPA and DHA are predominantly produced by microalgae, moving through the marine food web into fish where they typically enter the human diet. Evidence based recommendations for the inclusion of EPA and DHA in the diet have been made by multiple health agencies and governments, however, it has become clear that the supply of marine EPA and DHA from capture fisheries cannot meet demand. With an ever-growing population, aquaculture has increasingly expanded to close the supply gap, however, while fish account for much of the EPA and DHA in human diets, they produce very little themselves. Consequently, farmed fish need a diet that contains high levels of EPA and DHA. Aquaculture is therefore both the major source of EPA and DHA to the human population, but also a significant consumer (Tocher et al., 2019). To address this issue the industry is seeking a sustainable source of EPA and DHA.

Plants have the capacity to produce a huge diversity of fatty acids, but none produce EPA and DHA. Therefore, the only solution is to use genetic modification and metabolically engineer the pathways for EPA and DHA synthesis into oilseeds. Plant lipid metabolism is an area of intensive research, including many examples of transgenic events in which oil composition has been modified. Moreover, significant progress has been made towards the predictive manipulation of metabolism and the reconstitution of desired non-native traits in oilseeds such as *Camelina sativa* (Camelina) (Napier et al., 2019). Camelina is an oilseed crop of the Brassicaceae and is recognised as a new crop for oil production due to its adaptability to diverse climates, low input requirements, short crop cycle and disease resistance. For researchers, Camelina is an excellent platform for the production of tailored oils because it is oil composition (high in polyunsaturated fatty acids α-linolenic acid (18:3) and linoleic acid (18:2), the substrates for omega-3 LC-PUFA synthesis and low in erucic acid (22:1), the ease with which it can be transformed via *Agrobacterium* floral vacuum infiltration and its genetic similarity to the well-studied model plant Arabidopsis (Haslam et al., 2016). Collectively this makes the manipulation of oilseed metabolism in Camelina tractable.

Seeds contain storage lipids predominantly in the form of triacylglycerol (TAG), a glycerol backbone onto which three fatty acids are sequentially esterified. The synthesis and assembly of TAG in plants is complex, involving a metabolic network of fatty acid fluxes through alternative pathways to produce different lipid compositions, in a species-specific manner (Bates, 2016). The successful production of non-native EPA and DHA from native fatty acids in seeds requires the introduction and coordinated expression of multiple transgenes. The genes necessary for EPA and DHA synthesis were sourced from marine microorganisms. The conversion of C18 fatty acids to C20+ EPA and DHA commences with the introduction of a double bond at the Δ6 position,

followed by C2 chain elongation and a second desaturation at the Δ5 position in the C20 acyl chain, generating EPA and arachidonic acid (20:4, n-6); the synthesis of DHA then requires a further two steps – Δ5 elongation and Δ4 desaturation (Haslam et al., 2016). Reconfiguring fatty acid synthesis in this way requires not only the seed-specific expression of multiple (more than five) transgenes but also co-ordination with the developmentally regulated programme of seed TAG assembly and storage. Fatty acids are exchanged between multiple lipids pools (acyl-CoA, phosphatidylcholine and diacylglycerol) en-route to assembly in TAG as seed lipid droplets. Therefore, endogenous acyltransferase activities are utilised for the successful high-level accumulation of EPA and DHA, rather than biosynthetic intermediates. Latterly research has identified, tissue-specific variation within seeds for the accumulation of high levels of non-native fatty acids in TAG. Most notable has been the asymmetric accumulation of EPA and DHA in the seed embryonic axis (Usher et al., 2017). A greater understanding of this metabolic compartmentation will provide future strategies for improved integration of new activities with endogenous metabolism; paving the way for predictable seed lipid engineering.

Following the successful demonstration of EPA and DHA synthesis in Camelina (Ruiz-Lopez et al., 2014), further evaluation of the omega-3 LC-PUFA trait was undertaken and demonstrated (by comparison with replicated glasshouse experiments) the feasibility of producing omega-3 LC-PUFAs in the field. Replicated field trials (undertaken at Rothamsted Research each year from 2014 to date) have demonstrated the stability of the omega-3 LC-PUFA trait (Usher et al., 2015). Furthermore, peer-reviewed studies in Atlantic Salmon and Sea Bream (Betancor et al., 2018; Betancor et al., 2016) have demonstrated that the oils sourced from genetically engineered Camelina can directly can directly replace all the added oil in feeds without negative impacts on fish growth or health. Suggesting that EPA and DHA containing oil, sourced from genetically engineered Camelina, can be a practical alternative to fish oil. In this way farmed fish, with sustainable levels of EPA and DHA, can contribute to a balanced healthy diet.

Genetically modified Camelina, synthesising EPA and DHA in seed oil, has the capacity to support human health via its inclusion in fish feed. However, it is also possible that tailored Camelina oil containing EPA and DHA can be used as a direct intervention in human diets. In the UK, adults and especially children have low levels of fish consumption, such that dietary recommendations are often not met. Latterly, a recent peer-reviewed study has demonstrated that EPA and DHA consumed as oil from transgenic Camelina are incorporated after a meal into blood lipids at least as well as when consumed as fish oil, suggesting that engineered Camelina seed oil was equivalent to blended fish oil and was a suitable dietary source of EPA and DHA in for humans (West et al., 2019). Engineered Camelina oil has the capacity to provide a direct benefit to human health, however further work is required to understand mechanisms of uptake and bioavailability.

In summary, our studies have demonstrated how it is possible to re-configure the seed lipid synthesis and assembly pathways of the oilseed Camelina for the effective production of non-native fatty acids EPA and DHA. To achieve the predicable accumulation of novel target fatty acids has required the development of new understanding, specifically the localised seed-specific expression of multiple transgenes and surrounding interactions with endogenous lipid acyl-exchange pathways. Furthermore, the stability of omega-3 LC-PUFA trait has been shown in glasshouse and field trials over multiple seasons. Most importantly, the utility of the tailored terrestrial oil and its efficacy as a replacement for marine-derived fish oil has been demonstrated. Collectively, the metabolic engineering of Camelina is a clear example of the application of fundamental basic science to the challenge of food production. The work has resulted in the development of a new crop prototype and continues to have impact in agriculture, aquaculture, biotechnology and human health. Work continues now to show how our omega-3 LC-PUFA Camelina can contribute to the challenge of global food security and human nutrition.

4.3.17 IMPROVING WATER MANAGEMENT

In view of global change and strong water demand from agriculture, water deficit is now recognised as the abiotic stress that affects the most crop productivity. Drought has major societal and economic implications for global food security, that are exacerbated in the context of climate change. Europe already faces declining water availability and higher variability of rainfalls, both in space and time, which translate into higher risks of water stress on crops and significantly impact European agriculture. Indeed, drought not only alters plant productivity but has also a significant impact on cell wall composition affecting digestibility by cattle or microorganisms for biofuel production. While controlled water deficit may improve fruit quality through higher concentration of flavor or antioxidant compounds, drought often decreases crop nutritional quality. Thus, understanding the overall modes of plant response to water availability is of upmost agronomic importance.

Improving water uptake and transport by roots

Water is taken up by roots and transported through xylem vessels up to the leaves where it is evaporated through stomata. These different steps have been subjected to intensive research as targets for drought tolerance. One of the most promising targets to improve drought tolerance is root. Concerning Root System Architecture (RSA), studies in controlled and field conditions have suggested that reduction of shoot born roots number (Sebastian et al., 2016) and deep rooting are important parameter for foraging for water when deep water is available (Uga et al., 2011; Uga et al., 2013; Gao & Lynch 2016; Kulkarni et al., 2017). However, Ogura et al. (2019), observed that Arabidopsis accession with shallow rooting system are more tolerant to drought suggesting that a more horizontally distributed root system might be an adaptation to efficiently capture water in the short period of time in environments with sparse rainfall. A proliferative root system was also proposed to extract more water and nutrients under stress condition in legumes (Ye et al., 2018) or trees (Ramirez-Valiente et al., 2018). Recent studies have also identified that roots can direct their growth or preferentially position lateral roots towards higher water availability respectively hydrotropic response (Dietrich et al., 2017) and hydropaterning (Orsa-Puente et al., 2018). Finally, Rosales et al., (2019) showed that roots have a differential adaptive response to water availability depending of their rank and age. Yet, much of the physiological and genetic components that act on RSA are as yet largely unknown even in model plants.

Water uptake is also critically determined by the intrinsic water transport capacity of the roots, i.e. their hydraulics. If the water channel proteins named aquaporins that facilitate water diffusion across cell membranes have been well characterised (Maurel et al., 2015) and have been identified as targets for drought tolerance many other components of root hydraulics are to be discovered. For instance, the crucial role of lignified and suberised barriers differentiated in the walls of exo- and endodermal cells (Barberon et al., 2016) remained largely underexplored. Similarly, quantitative genetic approaches in the model plant Arabidopsis unexpectedly identified HCR1, a raf-like MAP3K protein kinase (Shahzad et al., 2016) or XND1 a XYLEM NAC DOMAIN 1 transcription factor (Tang et al., 2018) as important regulators of root hydraulics, with sharp roles under water excess (HCR1) or deficit (XND1). These examples illustrate the power of natural variation approaches for dissecting and possibly improving root hydraulic performance under agricultural conditions.

Regulation of water losses

When subjected to water limitation, plants primarily maintain their water status by regulating transpiration through stomata movements. Stomata serve dual and often conflicting roles, facilitating carbon dioxide influx into the plant leaf for photosynthesis and regulating water efflux

via transpiration. Accordingly, stomata have been the center of interest for drought tolerance (reviewed by Lawson et al., 2014). Many studies exploiting natural diversity or transcription factors over expression have shown that reduction of stomata density in Arabidopsis, rice, wheat or tobacco significantly enhances drought tolerance by reducing water loss with under certain conditions limited effect on growth (Yu et al., 2013; Kulkarni et al., 2017; Liu et al., 2019). Besides number, regulation of stomata opening is also of particular interest. Several signaling pathways have been targeted including reactive oxygen species (ROS) (Hu et al., 2017) and more recently the chloroplast retrograde nucleotide phosphatase signaling pathway (Zao et al., 2019). But the most studied and engineered relies on the phyto hormone Abscissic acid (ABA). ABA has been identified to mediate rapid closure of stomata to maintain the leaf water status. Accordingly, manipulation of ABA production or sensitivity significantly promotes drought tolerance not only in model plants and crops but also in woody plants (reviewed by Lawson et al., 2014; Yang et al., 2016; Pedrosa et al., 2017; He et al., 2018; Miao et al., 2018; Guo et al., 2019; Yang et al., 2019).

However, ABA not only control stomata opening but also long-term growth and metabolic adjustments for tolerating periods of water deficit (Cutler et al, 2010; Zhao et al., 2016). Besides it role in regulating plant water status ABA has been shown to regulate root and shoot development, plant pathogen interactions either directly or in close interaction with other phytohormones including auxin, cytokinins, ethylene, Jasmonic Acid or Salicilic Acid but also metabolism, nutrient sensing and growth through the TOR kinase signaling pathway (reviewed by Yoshida et al., 2019). As the molecular and cellular processes that underlie plant responses to ABA, and their integration in complex regulatory loops acting at the whole plant level, remain however poorly understood, manipulation of ABA production or sensing in crops has often detrimental effects. However, progresses in deciphering the complex network of ABA perception and signaling in Arabidopsis that includes ABA receptors, kinases, transcription factors and stabilising PP2C protein complexes (Zhao et al., 2016) open new perspectives for improving drought tolerance in crops. Accordingly, targeted manipulation of Arabidopsis ABA receptors combined up to 40% increased WUE with high growth rates. In these plants, water productivity was associated with maintenance of net carbon assimilation by compensatory increases of leaf CO_2 gradients, thereby sustaining biomass acquisition (Yang et al., 2016; Yang et al., 2019). Similar results were obtained in rice were where targeted mutations in ABA receptors identified combinatorial mutants that control stomatal movement but reduced detrimental effects on seed dormancy or plant growth (Miao et al., 2018). Recently, Cutler's group identified and engineered small molecules that bind to abscisic acid (ABA) receptors and locally and specifically trigger signaling pathways improving plant tolerance to water stress with limited side effects (Mosquna et al., 2011, Okamoto et al., 2018). These examples pave the way for future and promising improvements of local and targeted manipulation of ABA signaling pathways. Similarly, an in deep understanding of the functioning and regulation of the guard cells that are surrounding stomatal pores identified ion fluxes as putative targets for engineering. Accordingly, expression of a synthetic light-gated K+ channel in guard cells in Arabidopsis accelerated both stomatal opening under light exposure and closing after irradiation and increased significantly biomass in fluctuating light without cost in water use by the plant (Papanatsiou et al., 2019). Similar strategy can be considered for forcing stomata closure at night that have been identified as an interesting approach to reduce transpiration rate at night to limit water use without altering growth (Coupel-Ledru et al., 2016). These examples demonstrate the potential of enhancing stomatal kinetics to improve water use efficiency without penalty in carbon fixation.

Optimising drought avoidance, development and water use efficiency.

Several other traits have been investigated to enhance drought tolerance. For instance, if molecular and cellular mechanisms underlying the early perception of water deficit by roots and downstream signaling processes are still largely unknown, some candidate genes such as OSCA1 and downstream signaling events involving ROS and calcium are now emerging (Hamilton et al.,

2015; Yuan et al., 2014; Martiniere et al., 2019). While these studies deal with local responses to water deficit, the capacity of roots to adapt to heterogeneous water resources or changing water demands of shoots or rapid change in salinity of soil solution. Other traits such as shoot architecture have been found promising. Indeed, a more erected port turned out to significantly reduced transpiration rate but also meristem temperature preventing an earlier cessation of development. Similarly breeding for phenological traits such as earliness have been of particular interest to reduce exposition to drought period in wheat (Legouis et al., 2012; Kulkarni et al., 2017) or woody plants (Allard et al., 2016) for instance or flowering time in maize (see phenology examples for further details). Besides phenology many research articles related to a wide range of species showed that crops harboring delayed leaf senescence have a marked increase in grain yield and biomass production, improvement in horticultural performance, and enhanced tolerance to drought stress without any abnormalities in growth and development (reviewed by Guo and Gan 2014 and see longevity, nutrient remobilization and partitioning example for further details). Besides annual crops, forest trees or Grapevine are long-lived organisms subject to repeated environmental constraints throughout their long lifetimes. They are facing specific threats such as drought-induced xylem blockages (e.g., embolisms) resulting in a decline in sap flow can be observed under severe and/or repeated drought. Understanding and manipulating xylem properties is of major interest for plant survival (reviewed by Brodersen et al., 2019). At the molecular level, epigenetic has recently emerged as a powerful set of mechanisms regulating various developmental processes, plant growth and responses to environmental variations. Such epigenetic mechanisms, which may remain stable along tree life, constitute a source of potentially improving adaptation of the plants in situations in which naturally occurring mutations are very rare (reviewed by Dia Sow et al., 2018). Interestingly, Ma et al. (2019) also identified in rice, a chromatin regulatory process through histones ubiquitination/de-ubiquitination that modulates plant capacity to cope with drought stress. Characterising the underlying mechanisms both in trees and annual crops is of particular interest to identify new breeding targets and improve acclimation and reduce the impact of repeated drought stresses.

Identifying and exploiting the genetic diversity of plant drought tolerance

Besides these hypothesis driven approaches, large number of breeding programs for drought tolerant crops have been initiated. Accordingly genetic programs based on biomass production or yield or nutritional quality under drought have identified thousands of quantitative trait loci (QTL) in almost all crops including cereals (Tuberosa et al., 2006; Ruta et al., 2010, Budak et al., 2015, Li et al., 2016) legumes (Ye et al., 2018), oleaginous crops (Masalia et al., 2018) fruits (Albert et al., 2017), forage and biomass producers (Shinozuka et al., 2012; Van der Weidje et al.,2017) or woody plants (Allard et al., 2016; Tandonnet et al., 2018). Even if difficult to manipulate, marker-assisted selection helped breeders to manipulate and combine these QTL to improve drought related traits. However, the genetic bases of the molecular, cellular and developmental responses underlying these QTLs remains mostly uncharacterised as they involve many gene functions regulated by water availability. Recent development of genomics based approaches will provide access to agronomically desirable alleles present in QTLs and analysis of sequence data and gene products should facilitate the identification and cloning of genes at target QTLs thus enabling breeders to improve the drought tolerance and yield of crops under water limited conditions more effectively. These new tools represent an extraordinary resource for improving drought tolerance but harnessing the full potential of these technologies will require a multidisciplinary approach and an integrated knowledge of the molecular phenotypic and physiological processes influencing tolerance to drought.

4.3.18   IMPROVING PHOSPHORUS USE EFFICIENCY

Phosphorus (P) is an essential plant macronutrient with major impact on crop productivity. With the increasing demand for food (+50% for 2050) as a result of a growing population, the demand for P fertilizer is steadily increasing. Given the fact that phosphate (Pi) rock (the source of P fertilizer) is a finite natural resource, the P availability has become a worldwide concern with respect to the pressing problem of food security. Besides, many cations naturally associated with phosphate are toxic metals (such as cadmium). This will force industry to look for lower quality sources of phosphates naturally contaminated by toxic metals (such as cadmium). To limit such problem, the EC has imposed increasingly strict standards for the coming years on the level of toxic metal content associated with Pi allowed. Consequently, due to purification cost the use of Pi fertilisers will become more and more costly (Benredjem & Delimi, 2009; Benredjem et al., 2016). Another argument in favour of reducing the use of phosphate fertilizer is that the excess of Pi promoted run off in waters leading to algal bloom of cyanobacteria and subsequently to rivers and lakes eutrophication. Therefore, a better management of this resource is requested, together with developing of an efficient recycling process of Pi from different sources such as waste water or urine by chemical methods (Talboys et al., 2016) or through phytoextraction (Bawiec, 2018). As far as increasing food production is concerned, developing plants with enhanced P-use efficiency is key to meet the challenge of modern agriculture.

Improving plant assimilation of Pi remains a key and challenging research area, both in science and agronomy. For decades, varietal selection relied on fertilizers to find plants that made the best use of the high quantities applied. We have seen that this situation has changed: it will be necessary to produce, if possible better, with very limited quantities of Pi.

Different strategies can be proposed to meet this challenge:

Improving Pi uptake

Modification of root architecture

In soil, the P distribution is heterogeneous, and it concentrates usually in the shallow soil layers. To cope with such situation, plants promotes topsoil foraging, thereby conferring an advantage for P acquisition. Such plasticity of the root is an important adaptive trait genetically determined. It varies between and within plant species. Some genes involved have been identified (Ticconi et al., 2004; Reymond et al., 2006; Svistoonoff et al., 2007; Ticconi et al., 2009; Balzergue et al., 2017). It includes a cell wall-targeted ferroxidase (LPR1), a P5-type ATPase (PDR2), and the transcription factor SENSITIVE TO PROTON RHIZOTOXICITY (STOP1) and its target ALUMINUM ACTIVATED MALATE TRANSPORTER 1 (ALMT1). Quantitative genetics and physiological analysis revealed the existence of natural variation for such trait in Arabidopsis, soybean, lupine, beans… offering opportunity to develop novel selection scheme. The above-outlined importance of root traits and plant growth for Pi uptake was used in crop/cereal to identify strong QTL and causal genes. The rice gene PHOSPHORUS STARVATION TOLERANCE 1 (OsPSTOL1) provides a good example. Indeed, OsPSTOL1 (initially named Pup1-specific protein kinase gene) has been identified as the causal gene within a major QTL enhancing plant growth in highly Pi-deficient soil (Wissuwa & Ae, 2001; Gamuyao et al., 2012). The QTL Phosphate uptake 1 (Pup1) was initially identified based on a small diversity study of 30 rice genotypes, showing that two rice land races, Dular and Kasalath, had the highest Pi content (Wissuwa & Ae, 2001). The protein kinase Pstol1 absent from phosphorus-starvation-intolerant modern varieties illustrate such potentiality (Gamuyao et al., 2012). Its overexpression in such varieties significantly enhances grain yield in phosphorus-deficient soil. Beside transgenic approach, classical selection can also be performed to identify such traits in traditional lines and use introgression for its transfer. With recent advances in molecular genetics approaches chances are very high that we can unravel

the genetic architecture of P efficiency in a large crops recombinant inbred line population, and therefore establish links between the genetics and physiology of P efficiency, such as associations between root morphology, P content and crops grain yield on soils with low-P availability. A last strategy relies on a few specific species, which developed specific anatomic adaption such as cluster roots (found for example in some Proteaceae, Leguminosae…) to cope with soils exhibiting low Pi content. This could provide a niche market for a few food-value crops (such as Lupinus, Macadamia…) or horticultural crops (Banksia, Grevillea…). These observations lead to agronomical strategies of intercropping non efficient and efficient crops together, so that the accompanying crop will benefit from the presence of the efficient crop (Latati et al., 2014). Nevertheless, such strategy has had very limited success in the fields!

Using mycorrhizal symbiosis

Another strategy is to exploit the mycorrhizal symbiosis between plants and specific soil fungi (80% of vascular plants present such association). In such case, the fungus will extract and provide Pi to the plant and will receive carbohydrates in exchange (Wipf et al., 2019). Several agronomical companies provide mycorrhizal fungus solutions or soil enriched with mycorrhizal fungi to stimulate plant growth. However, such strategies are far to be universal: their output depends of several parameters such as the nature of the crops, its mycorrhiza partner and the local soil environment. As such, they are currently difficult to control out of the laboratories and require additional studies (Hinsinger et al., 2011). The use of bacteria to facilitate plant nutrition remains confined in the laboratory, indeed the passage into the field remains very hazardous due to competitions with soil microflora and putative ecological consequences.

Improving Pi transporter efficiency

Pi is taken up the roots. Knowing that plant take up only 20% of applied phosphate, developing plant with improved Pi use efficiency is needed. In roots, Pi uptake is achieved through a suite of high-affinity transporters of the PHT1 family and loaded into xylem using PHO1 transporter (Poirier & Bucher, 2002; Nussaume et al., 2011). All these genes exhibited multiple layers of regulation. One of the main one relies on the PHR1 (PHOSPHATE RESPONSE 1 (Rubio et al., 2001)) master transcription gene family and its regulators (such as the SPX family (Puga et al., 2014; Wang et al., 2014; Wild et al., 2016)). The second one acts at posttranscriptional level involving key elements such as PHF1 (Phosphate facilitator 1) a protein facilitating the targeting of PHT1 transporters through the endoplasmic reticulum (Gonzalez et al., 2005; Bayle et al., 2011); miRNA (miR399), and its target the ubiquitin-conjugating E2 enzyme (PHO2), a key player in the regulation of Pi uptake and translocation transporter protein abundance (Aung et al., 2006; Bari et al., 2006). A third ones involved modification of the affinity of the transporters in response to low Pi whose precise mechanisms have yet to be discovered (Ayadi et al., 2015). Our understanding remains very fragmented and requires further analysis to master these pathways, which are essential for developing plants adapted to low Pi. These signaling cascade triggers a wide variety of mechanisms to limit the use of Pi by activating many metabolic shunts. In addition, it is interesting to note the existence of crosstalk between the metabolism of phosphate and other ions such as nitrogen, iron or zinc. Taking into account multiple interactions, even if it is complex, should make it possible to multiply the number of solutions. This is well illustrated by new pathways recently discovered activated by plant to boost Pi accumulation in zinc-dependent (Pal et al., 2017; Kisko et al., 2018).

Improving Pi accessibility

A large field of research describes how specific plants perform better regarding phosphorus extraction from the soil. This can be based on their ability to acidify the soil by releasing protons or citrate and malate to solubilize bound phosphate (Bieleski, 1973; Lu et al., 2012; Liang et al., 2013).

Optimising nutrient uptake

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 plants to be economically profitable. In this technological era boosting soil-less cultures, a full grasp of the plant ability to extract nutrients from solutions, including Pi, becomes a central aspect of biomass production. Making sure that plants will assimilate provided nutrients is a major economic and technological challenge. In this context, basic research on Pi uptake remains crucial.

4.3.19 IMPROVING SALT STRESS TOLERANCE

World agricultural lands suffer drastically and are even destructed by soil salinity. Beside of naturally developed salinity in soil due to long term continuous discharge of saline groundwater (primary salinity), soil salinization is also related to man activities (secondary salinity). Thus, irrigation with saline groundwater can cause an increase of salts in the root zone, particularly if leaching by rainfall or by applied irrigation is inefficient. Irrigation water leaves soil to the atmosphere through plant transpiration and evaporation, leaving dissolved salts in the soil, and increasing soil salinization. Pumping fresh water in the coastal aquifers provokes salt-water intrusion decreasing the water quality. Coastal agriculture is also threatened by salinity problems due to sea level rise, ingress of sea water through waves, through wind transport of salt spray or storms. FAO estimated that 20% of irrigated lands were salt-affected soils worldwide. In the European Union, mainly the Mediterranean countries (France, Greece, Italy), but also Bulgaria, Czech Republic, Germany, Hungary, Portugal, Romania, and Slovenia are concerned by salt-affected area (Toth et al., 2008). Each year, it is estimated that 10 million ha of agricultural lands are destroyed worldwide by soil salinization (Pimentel et al., 2004). Climate change, excessive use of groundwater, increasing use of poor-quality irrigation water, massive irrigation in dry climate, and poor leaching can accelerate the rate of soil salinization.

Successful genetic approach for salt stress tolerance in crops

Saline soils affect plant growth and development, due to the presence in excess of soluble salts (Na^+) in soils. Visual symptoms of salt injury are white leaf tip followed by tip burning, leaf browning and death, reduced plant growth, poor root growth, sterility and reduced seed production. Plant species exhibit a large array of tolerance to salt stress. For instance, in the group of cereals, barley (*Hordeum vulgare*) is the most tolerant, bread wheat (*Triticum aestivum*) is moderately tolerant, followed by durum wheat (*Triticum turgidum* ssp. *durum*) which is less tolerant, and rice (*Oryza sativa*) which is the most sensitive (Munns and Tester 2008). However, due to the genetic diversity in each crop species, salt tolerance variability could be found, for instance in maize and sorghum (Niu et al., 2012). A prototypical experimental approach for the identification of genes involved into the salt tolerance has been performed in rice *sativa* species, thanks to its high genetic diversity. Thus, several Quantitative Trait Loci have been identified in rice for salinity tolerance, with a majority of them sitting on chromosome 1, for instance related to Na^+ uptake into the plants (Flowers et al., 2000), ion (K^+ and Na^+) uptake (Koyama et al., 2001), or a Na^+ transporter

Detrimental effects of Na^+ ion on plant growth and development

A high salt concentration in soil solution increases its osmotic pressure, interfering with plant nutrition of water and nutrients. Hence, a two-phase model has been proposed, with an early response to the increase in external osmotic pressure, and a slower response due to the

accumulation of Na^+ in the shoot (Munns & Tester, 2008). The damages due to the osmotic effect of salinity induce metabolic changes similar to those caused by water stress. Osmotic stress has an immediate effect on the rate of shoot growth. Moreover, Na^+ exhibits specific ion toxicity and impairs nutrition of essential nutrients such as K^+. At the early phase, salt stress impairs photosynthesis, by reducing stomatal conductivity and transpiration in relation with the hormone abscisic acid (Fricke et al., 2004). The reduction of photosynthesis stimulates reactive oxygen species (ROS) production and the expression of enzymes involved in the oxidative stress to prevent damage to photosystems, lipids, proteins, and nucleic acids. However, one of the members of ROS family, H_2O_2, exhibits also a cell signalling role in salt tolerance (Ma et al., 2012). Thus, a balance between ROS production, removal by oxidative stress enzymes and sufficient quantity required for cell signalling has to be coordinated. Photosynthesis is also impaired by the specific Na^+ toxicity due to its close physicochemical properties with K^+ (i.e. hydration energy and hydration shell features). Na^+ competes with K^+ at binding sites in key metabolic processes in the cytoplasm (enzymatic reactions, protein synthesis), albeit Na^+ cannot totally replace K^+ as a coordinating ion (Nieves-Cordones et al., 2016).

Excess of Na^+ also impairs polarization of plant cell membrane potential, a phenomenon responsible for the entry and exit of solutes. Thus, polarization of cell membrane potential is strongly related to K^+ concentrations at both sides of the plasma membrane (Nieves-Cordones et al., 2016). Unfortunately, Na^+ competes with K^+ for uptake at the plasma membrane root cell, since both ions are transported by several transport systems (non-selective cation channels (NSCC) and high-affinity transporters (HKT)). Moreover, massive Na^+ uptake provokes a significant membrane depolarization, leading to a dramatic increase of K^+ leakage through depolarization-activated outward-rectifying (KOR) K^+ channels (Nieves-Cordones et al., 2016; Shabala, 2009).

Salt tolerance mechanisms in crops

As a consequence of all the above mentioned processes affected by salt stress, plants have developed several biochemical and molecular mechanisms to withstand the detrimental effects of salinity that can be grouped into three types (Munns & Tester, 2008). Firstly, oxidative stress encountered upon salt stress has to be managed at the cellular level by mechanisms of protection and damage repair. Secondly, the response to osmotic stress by maintaining water homeostasis involves compatible solute biosynthesis and aquaporins (water channels). Thirdly, these mechanisms involve the function and regulation of transport systems of Na^+ and/or K^+.

In maize seedlings, salt stress induced oxidative stress mainly in roots and mature leaves, and less in young leaves (AbdElgawad et al., 2016). Thus, H_2O_2 content and markers of oxidative damage of cell membranes (electrolyte leakage and lipid peroxidation) all showed an increase. Furthermore, antioxidants molecules (polyphenols, flavonoids, ascorbate…) and activities of enzymatic antioxidants (catalase, superoxide dismutase, peroxidase, etc.) increased significantly. Universal antioxidant defense mechanisms were activated in the whole plant, however it was suggested to develop more efficient metabolic engineering mechanisms specific to different organs and ages to cope with specific stress conditions (AbdElgawad et al., 2016).

Compounds that accumulate in the cytosol to balance the osmotic pressure most commonly are sucrose, proline, and glycine betaine. For instance, proline was accumulated as a non-toxic and protective osmolyte under salt stress in bread wheat, lentil, or rice (Lutts et al., 1996; Mattioni et al., 1997; Misra & Saxena, 2009). Engineering compatible solute accumulation in crops has been extensively performed with several successes in improving adaptation to salt stress. However, this has been done in very restricted conditions, or limited developmental stages and several other drawbacks rendered this approach marginal (Shabala, 2007). Otherwise, genetic manipulation of aquaporin expression to engineer salt stress tolerance did not lead to conclusive results. For instance, transgenic rice plants expressing at high level the plasma-membrane

aquaporin isoform OsPIP1;1 revealed to be more sensitive to salt stress than transgenic seedlings with middle to low levels of transgene expression that exhibited tolerance to salt (Liu et al., 2013). Salt stress induces a reduction of root hydraulic conductivity in several crop species, such as barley (Horie et al., 2011) ; this shutdown of water transport is believed to minimize water loss. Hence, the role of aquaporins in salt stress response has to be considered, however, their precise role needs to be clarified.

The discovery of the function of Na^+ transporter and K^+/Na^+ homeostasis supporting *Saltol* QTL stresses on the importance of the transport systems of these two ions (Ren et al., 2005). It is well accepted that the maintenance of a high cytosolic K^+/Na^+ ratio is optimal for salt stress tolerance. For this purpose, plants have developed several strategies that allow them (1) to limit the uptake of Na^+ into the root, (2) to increase its extrusion outside of root cells, (3) its sequestration into the vacuole, and (4) its transport and distribution to the leaves. The most important transporters contributing to Na^+ and K^+ homeostasis are describes hereafter. The Na^+/H^+ antiporter salt overly sensitive (SOS1), the only Na^+ efflux protein at the plasma membrane of plants characterised so far, has a role in extruding the excess of Na^+ ions from root epidermal cells (Martínez-Atienza et al., 2007). Once in the cytosol, Na^+ ions are compartmentalised into vacuole by means of the activity of Na^+/H^+ antiporter type NHX1 (Fukuda et al., 2004). Preferential expression of Na^+-selective influx transporter of the HKT1 family in root xylem parenchyma and in cells adjacent to phloem vessels in leaves leads to two interpretations. HKT1 could be involved in the unloading Na^+ from the root xylem, retaining this ion in root cells and preventing its accumulation in shoot. Also, HKT1 allows the recirculation of Na^+ from shoot to shoot by loading Na^+ into the phloem in the shoot (Ren et al., 2005). The involvement of K^+ transport systems in K^+/Na^+ homeostasis has to be stressed also. For instance, in rice AKT1, HAK1, and HAK5 are involved in the uptake of K^+ and play a role in salt tolerance (Chen et al., 2015; Li et al., 2014; Yang et al., 2014).

Future prospects for alleviation of salinity in crops

Plants beneficially interact with soil microorganisms (especially rhizosphere bacteria and mycorrhizal fungi), providing better nutrient and water nutrition and growth. Moreover increasing studies have documented on salt-tolerant microorganisms improving tolerance to salt stress in crops (Dodd & Pérez-Alfocea, 2012). Beneficial effects include improvement of nutrient (K^+) and water uptake, growth promotion, and alteration of plant hormonal status and metabolism, but the mechanisms are not well-understood, which needs further investigation. Thus, engineering interactions between crops and beneficial microorganisms could be advantageous for salt tolerance.

Considerable progresses in the understanding of the genetic and molecular bases of salt tolerance has been made in the last decades in several crop species such as rice, wheat, barley and tomato (Morton et al., 2019). This has conducted to breakthroughs, notably in salt sensitive species such as rice, like the identification of a major locus for Na^+/K^+ homeostasis in response to salt (*Saltol*) which contains OsHKT1;5 (Ren et al., 2005) and several salt responsive transcription factors in landraces. The *Saltol* locus is being integrated through genotyping platform along with other loci involved in productivity and grain quality in breeding lines and new cultivars. Further understanding can results from the exploration of tolerance mechanisms in glycophytes and wild relatives that may include halophytes. Mechanisms are indeed diverse in ranging from anatomical adaptations (reinforced root apoplastic barriers, leaf salt glands...), spatial compartmentation (such as Na^+ accumulation in leaf sheath in cereals) to Na^+ vacuolar compartmentation and efficient Na^+ exclusion / recirculation through the action of transporters.

For alleviating salinity stress effect in plants, two main strategies can be implemented and combined. The first one is based on genomics and breeding while the second relies on genetic engineering and/or the fast evolving genome editing technologies. The first approach is based

to the pangenome information becoming available in major crops (Golicz et al., 2016), notably when a large natural variation on salt tolerance/avoidance exist. Phenotyping of large collections will allow to identify useful natural allele variation at loci underlying architectural/anatomical structural/functional traits important for adaptation to salinity-prone environments. Key progresses have been made in the experimental design of phenotyping experiments that can be automated (Al-Tamimi et al., 2016) and in the imposition of salinity stress and in analysing relevant physiological data using appropriate indices (Negrão et al., 2016). Gene discovery trough genome-wide association studies in sequenced/densely genotyped diversity panels and high resolution multi-parental populations has to be pursued to identify and characterise new genes involved in salt tolerance mechanisms. Presence absence variation (PAV) has to be specifically investigated through the sequencing of landraces, since often unique adaptation genes are found only in their "dispensable" genome (striking examples exist in plants for salinity tolerance, submergence tolerance and phosphorus uptake efficiency, etc.) (Gabur et al., 2019). Superior alleles at these loci have to be first validated by genetic engineering/genome editing and then integrated into marker-assisted selection pipelines using conventional breeding. However, barrier to introgression in elite cultivars may sometimes result from structural divergence in the region where the useful locus reside and need to be recombined. This may notably be prevalent in the case of PAV. In this case site directed nuclease-mediated and homology dependent repair-assisted insertion of the missing gene in its actual genetic context might be the most straightforward approach. The second approach relies on genetic engineering and genome editing for the swift release of improved, salt tolerant plant materials. Genetic engineering may still be an attractive solution. As a striking example, overexpression of the type I H^+PPase genes in several plant species (Arabidopsis, tobacco, cotton, alfalfa and maize) has invariably conducted to enhanced salt and drought tolerance (Gaxiola et al., 2012). Ectopic expression/down regulation of transporters in specific tissues can be achieved through the use of tissue specific/inducible promoters. More practicable is the conversion of alleles through CRISPR/Cas9 directed inactivation or base editing ((Zhang et al., 2019) for a review). Gene/base editing may be used to alter simultaneously the sequence of several genes known to be important for salt tolerance, imposing beneficial variations identified in the natural diversity. Furthermore, gene editing coupled with homology-dependent repair may be used to alter domains in the best characterised transporters to decrease their affinity for Na^+ or Cl^-. Engineering spatial compartmentation and anatomical adaptations will require a better understanding of developmental processes underlying these traits. Importing the salt tolerance architecture from halophyte species into glycophytes is also an attractive perspective (Mishra & Tanna, 2017; Volkov, 2015) but this will require a prior deeper understanding of the genetic and molecular control of the salt tolerance/avoidance bases in these wild species, on which few information is nowadays available.

4.3.20   IMPROVING HEAT STRESS TOLERANCE

Increased day time and nocturnal temperatures are occurring on a global level and are often negatively correlated with productivity and yield (Jagadish et al., 2015; Ray et al., 2015). Interestingly yield progress is correlated with night time temperature in many cases (Welch et al., 2010) and frequently coincides with other stress factors such as water deficit. Heat stress is characterised by temperatures at which optimal plant functioning and homeostasis are impaired, leading to stunted growth, yield, quality and productivity. Multiple physiological, biochemical, and molecular processes determine rates of growth and productivity and hence heat stress can occur via different routes according to phenology and yield components. One of the clearest mechanisms by which heat stress determines yield is via impairment of key physiological processes within reproductive organs leading to sterility. Such phenomena indicate the importance of developmental stage when assessing the impact of heat stress on productivity.

It is well accepted that the cumulative fixation of CO_2 via photosynthesis is a principal determinant to this end. Heat stress can have significant implications for photosynthesis, for example electron transport systems and CO_2 reduction pathways are hugely sensitive to high temperatures. This is a concern for future food security targets, since rising temperatures in key crop production zones are likely to impact crop photosynthesis and therefore yield. Indeed, according to scenarios based on projected greenhouse gas emissions, temperatures are forecasted to increase in rice growing areas by as much as 4°C during the growing season, which could limit cereal yields by as much as 12%. Accordingly, this necessitates an understanding of how photosynthesis responds to elevated temperatures in rice as a foundation for developing varieties that are resilient to climate change.

Due to their ectothermic nature, crops display plastic developmental responses to temperature according to the phyllochron principle. This reflects alterations to underlying biochemical responses, such as enzyme activation states and hormonal regulation, that in-part dictate the response to temperature of the traits in question (Scranton & Amarasekare, 2017). In general, progressions through developmental stages of crop life cycles is hastened by increasing temperatures up to an optimum, which varies substantially across species, reflecting the importance of breeding climate-specific varieties (Hatfield et al., 2011). The morphology of leaves is also sensitive to temperatures above the optimum, thereby impacting light interception and fixation of CO_2 via photosynthesis (Gray & Brady, 2016). Below-ground responses to heat stress are also an important aspect of plant developmental responses to temperature. These responses are linked to above-ground responses thanks to the dependency of soil temperature on air temperature. When temperatures exceed species-specific optimum temperatures, development will be impeded. It is important to note that ceiling temperatures are usually developmentally specific, where vegetative development is more tolerant of higher temperature than reproductive development (Hatfield et al 2014).

With respect to reproductive development, and as described previously, elevated temperatures accelerate flowering time and reduce grain filling periods. If it is assumed that diurnal rates of carbon fixation via photosynthesis are unchanged, then yield will reduce to an extent that is proportional to the number of days early flowering time thanks to the lack of photosynthates that can be translocated to reproductive sinks and it will also reduce proportionally to the number of reduced grain filling days, as such phenological responses to temperature to this end are particularly damaging to crop productivity (Hatfield et al., 2011).

Photosynthesis is highly sensitive to heat stress periods and this can manifest at several levels. First in terms of metabolic efficiency the carboxylase activity of Rubisco is curtailed by an enhancement of the oxygenase reaction and a reduction in the activity or Rubisco activase which is a target for improvement (Crafts-Brandner et al., 2009) . This also leads to an increase in the rate of photorespiration which further reduces the CO_2 assimilation capacity. Variation in thermal tolerance of the Rubisco activase enzyme has been used as a means to improve photosynthesis at higher temperatures. Recent success has been achieved in manipulation of the rate of photorespiration to enhance yield (South et al., 2019).

As temperatures rise, rates of cellular respiration rise along with developmental rate, consuming storage carbohydrates more rapidly. At high temperatures, beyond the optimum and with increasingly unstable cellular conditions in the light the generation of reactive oxygen species can be high, disrupting membrane transport and metabolism and leading to increased oxidative stress and photoinhibition. Here the role of lipids becomes important: in wheat it has been shown that in some cases at least the reduction in photosynthetic rate at high temperatures is due to lipid desaturation, oxidation and damage to organelles (Djanaguiraman et al., 2018). Genotypes of wheat expressing heat shock proteins can tolerate heat stress better since they protect proteins and lipids from heat stress damage. Selection for heat tolerance in wheat has been

achieved in wheat by targeting improved photosynthetic efficiency selected by chlorophyll fluorescence (Sharma et al., 2015).

High temperatures can also have numerous detrimental impacts on plant fertility, leading to sterility and also lowered grain quality in terms of protein content. These impacts are typically manifested through alterations to the timing of reproductive developmental stages or through damage to reproductive structures (Gray & Brady, 2016). The expression of genes involved in the signalling pathways that elicit floral transitioning are often dependent on temperature cues in order to time flowering appropriately according to the prevailing environmental conditions such to maximise fitness. Consequently, either consistently high temperatures or temperatures that fluctuate between species specific-optimum temperatures and elevated temperatures are likely to induce early flowering which can be problematic since they force reproduction to occur before sufficient resources have been and allocated toward developing seeds, thus grain filling is reduced (Zinn et al., 2010). Furthermore, if elevated temperatures persist during the grain filling period, they can induce grain senescence, which in turn reduces grain weight even further.

The most substantial high temperature driven impacts to crop productivity are often resultant from the sensitivity of pollen (male gametophyte) to temperature (Zinn et al., 2010; Peet et al., 1998) asynchrony between male and female reproductive development (Herrero, 2003; Hedhly et al., 2008), the size and number of both carpels and stamens pollen viability and dispersal (Sato et al., 2002).

5 WHERE TO GROW OUR FUTURE CROPS? - Modelling

Where there is a reliable mechanistic understanding of biological processes, computational analysis can predict outcomes in novel scenarios where empirical models would rely on extrapolation. Mechanistic modelling is therefore a potentially very useful tool for understanding crop futures. Despite this, even photosynthesis, which is particularly well understood from a mechanistic standpoint and for which powerful models are available, is usually represented empirically in crop simulations.

5.1 Modelling - Approach

The aim of this exercise was to show that crop models incorporating mechanistic understanding of photosynthesis can be an effective data-led tool to identify how crop improvements will look in the future. We took a conservative approach to potential improvements that could be made to photosynthesis, predicting impacts of relatively simple, thoroughly understood, and achievable modifications (***Modelling Box***; Wu et al., 2019). Our results therefore represent a very realistic, lower limit of potential improvements to photosynthesis. Much larger gains are predicted from more complex, and considerably more difficult to model, examples of metabolic engineering. For example, engineering of C3 crops to include carbon concentrating mechanisms (Sheehy et al., 2015; Covshoff et al., 2012; Yin et al., 2017; McGrath et al., 2014); photorespiratory bypass mechanisms (Peterhansel et al., 2013; South et al., 2019; Shen et al, 2019); or faster adjustment of photosynthetic biochemistry under dynamic light (Kromdijk et al., 2016; Taylor et al., 2017).

To demonstrate that current generation crop models incorporating photosynthesis make useful predictions about relatively simple improvements to photosynthesis in both current (i.e., real and measured) and future (i.e., scenario-based, modelled) climates, we used two models that incorporate leaf-level photosynthesis. GECROS and DCaPST describe photosynthetic responses to light via electron transport, responses to $[CO_2]$ in terms of Rubisco and Calvin-cycle biochemistry, and feedback with crop water balance mediated by stomatal control of transpiration. The potential impacts of strategies directed at yield were addressed using meterological inputs for 66 sites spanning Europe. GECROS (Yin et al., 2017) modelled a broad array of changes to key limiting processes in both C3 (wheat, potato, and rice) and C4 (maize) crops under historical climate and $[CO_2]$ but without water limitation. DCaPST (Wu et al., 2019), with its more detailed representation of edaphic factors and management opportunities driven by the APSIM crop simulator, was used to evaluate a subset of photosynthetic improvements in winter wheat (Asseng et al., 2000; AHDB, 2018), testing their robustness to meteorology predicted from future climate scenarios developed by CMIP5 (ISIMIP2b datasets: isimip.org). Location specific soil parameters were obtained from ISRIC SoilGrids (soilgrids.org; Hengl et al., 2014), and SoilHydroGrids (Tóth et al., 2017).

MODELLING BOX

Modelling photosynthetic improvement in crop canopies

Representations of photosynthesis in both GECROS and DCaPST are embedded/linked with crop simulators (e.g., GECROS relational diagram below) and are underpinned by the relatively simple, but very effective model of Farquhar, von Caemmerer, & Berry (1980; the FvCB model) and its modification for C4 photosynthesis (von Caemmerer, & Furbank, 1999). These are connected to process modelling for diffusion of CO_2 and water, as well as the de Pury, & Farquhar (1997) sun-shade simplification of canopy radiation.

Changes to several parameters were evaluated:
- V_{cmax}: maximum rate of CO_2 + RuBP reaction catalysed by Rubisco - primarily affects rates of CO_2 uptake at ambient and sub-ambient CO_2
- J_{max}: maximum rate of CO_2 uptake that could be driven by electron transport rate and Calvin cycle turnover – particularly important at higher CO_2 and when less light is reaching the chloroplasts
- $S_{C/O}$: specificity with which Rubisco catalyses CO_2 uptake, rather than the alternative, O_2 uptake, that initiates photorespiration – affects photosynthesis under all conditions
- κ_{2LL}: rate of light-limited electron transport – affects photosynthesis at low light
- g_m: conductance controlling rates of CO_2 movement through the leaf to the site of CO_2 uptake in the chloroplast

And, for the C4 crop maize, in addition to V_{cmax}, J_{max}, and $S_{C/O}$:
- g_{bs}: conductance controlling the rate at which CO_2 concentrated around Rubisco in the bundle sheath compartment can be lost from the bundle sheath
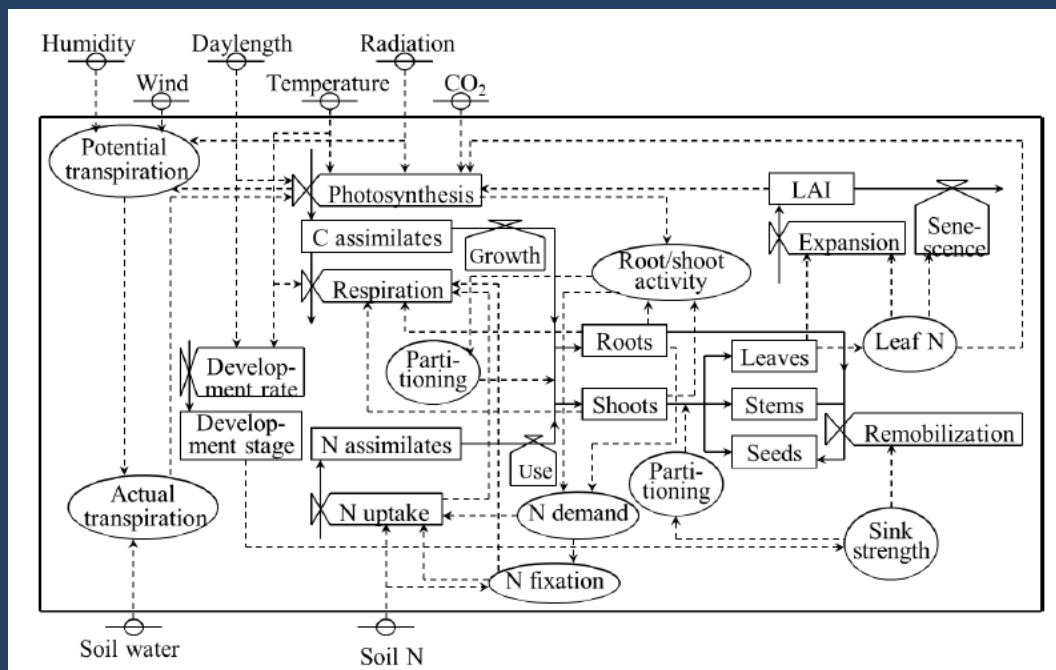

Figure 7 Relational diagram describing physiological processes and their interactions as affected by environmental variables, quantified in the GECROS model.

5.2 Outcome of Modelling

5.2.1 THE CROP MODEL GECROS

The impact of individual small (20%), and a combination of those individual small increases in photosynthetic parameters on the crop yield components of wheat, potato and maize are shown in table (**Table 1**).

The photosynthetic properties modelled largely act independently of each other ($S_{C/O}$ and g_m have overlap with other modifications: $S_{C/O}$ enables Rubisco to use available CO_2 more effectively, and g_m allows more CO_2 to reach the chloroplast). Individual 20% increases to the photosynthetic parameters generally gave only small to moderate increases in photosynthesis (+0.1-7.8%), biomass (+0.1-11.5%), and the efficiency with which intercepted radiation was converted to biomass (ε_c, +0.1-10.9%); however, their combination resulted in non-additive increases of 11.6-32.8% in total biomass depending on the crop. The scale of effect on total biomass was greatest for wheat, and less for potato and maize.

Table 1 GECROS estimates of percentage increase in photosynthesis, biomass, and radiation use efficiency (ε_c = biomass/intercepted radiation) in response to 20% increases in photosynthetic parameters, averaged across 66 sites in Europe

	Wheat (C3)			Potato (C3)			Maize (C4)		
	whole season canopy photosynthesis	above ground biomass	ε_c	whole season canopy photosynthesis	above ground biomass	ε_c	whole season canopy photosynthesis	above ground biomass	ε_c
V_{cmax}	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2
J_{max}	6.4	10.1	8.7	5.2	8.2	7.9	7.3	8.1	6.3
κ_{2LL}	7.8	11.5	10.9	4.5	6.5	7.0	-	-	-
g_m	1.1	1.5	1.5	0.7	0.9	1.1	-	-	-
$S_{C/O}$	3.5	5.2	4.9	2.3	3.4	3.7	1.4	1.5	1.3
g_{bs}	-	-	-	-	-	-	1.1	1.1	1.0
All	22.6	32.8	30.1	16.0	23.5	23.2	9.9	11.6	8.7

5.2.2 THE CROP MODEL DCAPST

The baseline DCaPST model without improvement to photosynthesis strongly predicted historical yield trial data from the UK (**Figure 8**), and a subset of the potential improvements explored using GECROS were simulated (20% increases in V_{cmax}, J_{max}, and g_m). When similar improvements were modelled under historical conditions, there was strong agreement between percentage increases in yield from DCaPST and biomass outcomes from GECROS (**Table 2**). For future scenarios, CO_2 fertilisation meant that wheat yields were predicted to increase by 15-23% under both scenarios even without photosynthetic improvement (**Table 3**). Consistent with this, the impact of photosynthetic improvement was predicted to decrease marginally (<1%) in future, but traits that provided important increases in yield under historical conditions were

expected to continue to be useful in future despite higher [CO_2] (**Table 2**). The combined effects of CO_2 fertilisation and photosynthetic improvements resulted in yield increases of up to ~35% in future climate scenarios compared with the historical baseline.

Figure 8 Mechanistic modelling of photosynthesis as part of the DCaPST model produces reliable estimates of winter wheat yield, compared with AHDB historical yield trial data from fungicide treated plots spaning UK latitudinal and rainfall (West-East) gradients.
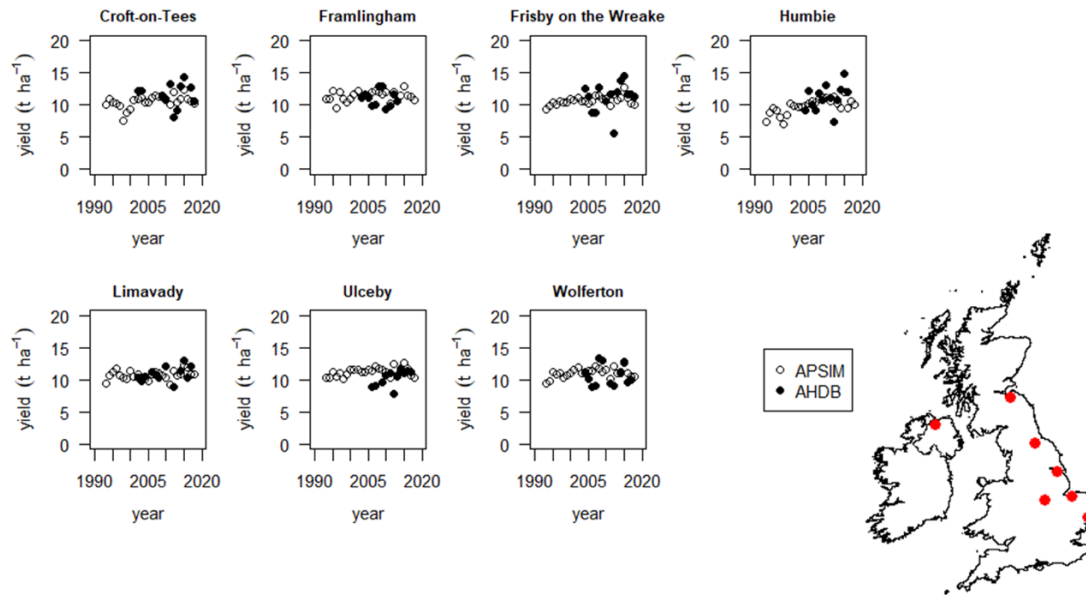
Table 2 Maximum values, across four climate forcings and 66 sites in Europe, for average predicted yield gains from alternative photosynthetic improvement strategies.

	% increase in yield relative to baseline		
	1981-2005	RCP 2.6 2041-2060	RCP 8.5 2041-2060
+20% $V_{c,max}$	1.7	0.7	<0.1
+20% J_{max}	9.9	9.5	9.0
+20% g_m	1.1	0.8	0.5

Table 3 Mean±SD yield predictions (t ha^{-1}) for 66 locations within Europe, using the baseline DCaPST model across four ISIMIP2b climate forcings, for three scenarios: historical (1981-2005), compared with best- (RCP 2.6) and worst-case (RCP 8.5) scenarios for future warming (2041-2060).

	Historical 1981-2005	RCP 2.6 2041-2060	RCP 8.5 2041-2060
GFDL	9.8±1.73	10.8±1.84	11.6±1.93
HadGEM	9.8±1.78	11.6±1.79	12.2±1.92
IPSL	9.8±1.71	11.6±1.85	12.3±1.61
MIROC5	10.1±1.86	11.8±1.99	12.7±2.12
Grand mean	**9.9±1.76**	**11.4±1.89**	**12.2±1.93**

5.3 The importance of whole canopy modelling

Light availabilities can differ between 20- and 50-fold between the top and bottom within a closed plant canopy (Stadt et al., 1999). Interception depends on a number of different factors including leaf orientation and shape, the spatial arrangement of photosynthetic surfaces (i.e. uniform versus clumping), sun elevation, the finite width of the sun's disc and changes in spectral distribution of PPFD within the canopy (Nobel et al., 1993). This necessitates methods to account for the structural complexity of plant canopies and the spatial arrangement of plant material when assessing potential crop performance.

Plant architecture refers to the spatial organisation of plant organs (Barthelemy et al., 2007). The resultant structure impacts many processes within the plant including mechanical stability (Moulia et al., 2006; Niklas, 1994), productivity and yield (Khush, 1996; Sakamoto et al., 2004), disease and stress resistance (Coyne, 1980; Wolfe, 1985; Jung et al., 1996; Ando et al., 2007; Grumet et al., 2013) and photosynthesis (Song et al., 2013). Canopy architecture varies greatly both within and between species. The arrangement of plant material, both spatially and temporally, leads to a highly heterogeneous light environment. Canopies with the same leaf area index can have very different efficiencies in light capture due to the arrangement of plant matter (Baldocchi & Collineau, 1994; Valladares & Niinemets, 2007). The geometry of leaves, particularly their shape and size, is result of a trade-off between light harvesting and temperature regulation plus more efficient use of resources (Bonan, 2002). Differences in foliage relative to vertical and the azimuthal orientation of leaves can generate varying patterns in light interception in canopies with similar levels of clumping or LAI. When the sun is located overhead, vertical leaves absorb less PPFD than horizontal leaves, thus reducing interception of excessive solar irradiance at midday (Valladares & Niinemets, 2007). Horizontal leaf angles are beneficial in an understory environment where most light enters from low zenith angles (Muraoka et al., 1998). Therefore leaf dimension and angle are key factors in assessing plant strategies for optimising light acquisition. For example, it was shown that 30% of the difference in light capture by upper and lower canopy species within a tall-grass meadow can be explained by differences in leaf orientation (Anten, 1999).

3D plant modelling

In order to assess the impact of plant architecture on processes and, ultimately, plant productivity and yield, detailed quantification of such structures is necessary. This desire for the creation of complex, geometrically accurate three-dimensional models of plants has led to the development of a number of different techniques in order to capture plant structure. (e.g. Watanabe et al., 2005; Quan et al., 2006; Song et al., 2013; Pound et al., 2014; Gibbs et al., 2019). Applications of such models are diverse, including the study of photosynthesis for both single plants and whole canopy structures (e.g. Song et al., 2013).

There are many different methods by which we can quantify canopy structure; this includes both destructive and non-destructive methods (Wilson, 1963; Anderson, 1971; Ross, 1981; Campbell & Norman, 1989; Chen et al., 1997; Bréda, 2003; Jonckheere et al., 2004; Weiss et al., 2004). Destructive methods require identification of the key architectural features of the plant, defined by a number of different parameters such as leaf length, angle and number etc., taking averages across a number of plants, then reconstructing a representative canopy (Watanabe et al., 2005; Alarcon & Sassenrath, 2011; Song et al., 2013). Reconstructing the plant structure from data, *in silico*, are often time-consuming and tedious due to the rigorous measurements required (Fourcaud et al., 2008; Vos et al., 2010). Non-destructive methods can be broadly split into two categories with differing levels of accuracy. Low accuracy methods use approximations of plant 3D structure can be used in which leaf angle can be assumed to be constant (e.g. Pagès et al.,

2009) or follow an ellipsoidal or spherical distribution (Rakocevic et al, 2000; Farque et al, 2001). These assumptions are particularly relevant in crops that exhibit regular and coordinated development, such as rice and wheat (Evers et al., 2005; Pagès & Drouet, 2007; Zheng et al., 2008). This method plus the destructive method is known as a rule-based approach to modelling. However, for those crops which exhibit highly heterogeneous canopies, use of standard leaf angle distributions can lead to a 4-15% difference (depending on light conditions and number of photosynthetic parameters used) in calculated photosynthesis values compared to 3-D models with explicitly described leaf angles (Sarlikioti et al., 2011).

Alternatively, highly accurate methods rely of digitising a pre-existing structure, but using a set of images as a basis. This is known as the image-based approach. The image-based models are highly desirable as a method of plant phenotyping (Houle et al., 2010; Santos & Oliviera, 2012; White et al., 2012), with the information needed to calculate a number of plant traits including leaf areas and angles, plant height, etc. However, the complexity of plant architecture means that image-based approaches are often challenging. In particular, similarities between multiple small leaf segments, lack of texture for feature matching and the high amount of self-occlusion lead to difficulties during reconstruction (Gibbs et al., 2019; Pound et al., 2014). The models produced may also be of limited application. For example, the silhouette-based method produces a static model which cannot be used for modelling aspects such as plant or leaf movement and the point cloud data cannot be used for modelling photosynthesis; for this surface detail is required (see Pound et al., 2014).

Light modelling

Light is the most immediately heterogeneous environmental factor influencing plant growth and survival. Based on the position of a section of leaf within the canopy, the light environment will be highly variable throughout the day. This can be extremely difficult, if not impossible, to measure. Once detailed structure is known, the next stage of scaling up canopy processes requires modelling the light environment experienced by individual leaf elements. By modelling the radiative exchanges between plant organs, light models are able to estimate the radiative fluxes received by each organ (Chelle & Andrieu, 2007). Such models take into account the fate of a light ray incident upon a leaf surface; whether it is reflected, absorbed or transmitted, and integrate these local processes over the whole structure. The complexity of the integration will depend upon the accuracy of the structural description of the canopy. The 3D plant model obtained in processes described above can be used so that light interception can be calculated using spatial representation of vegetation components (Borel et al., 1991; Goel et al., 1991; Chelle et al., 1998; Evers et al., 2015). Advantages of this approach include fluxes for individual geometric elements and consideration of their size, position and orientation (Chelle & Andrieu, 2007). Thus these are able to provide more information on the interception of light at the organ scale (Chelle, 2005) at the cost of increased numerical complexity. Both ray tracing and projection represent mechanisms of calculating primary lighting on a surface.

Due to the differential fate of light rays, radiation absorbed by each plant organ may come either directly from the sky hemisphere, or indirectly after scattering (direct or diffuse light). The proportion of the light that is reflected or transmitted varies with wavelength and depends upon leaf type, state and age. Calculating primary lighting of a set of surfaces can be achieved through either a source- or recipient-based approach (Wang et al., 2008). In the source-based approach, sampling occurs by following the propagation of light from selected directions in the sky hemisphere, and determining the surface element hit by each ray. Whereas the recipient-based approach uses the inverse sense of light propagation is followed from specific surface elements, into the sky hemisphere (Liu & Chen 2003; Zhang & Zhao, 2007). Primary light is effectively described when the irradiance of a surface reflects the sum of the contribution of each individual source. Both ray tracing and projection represent mechanisms of calculating primary lighting on a surface (Chelle & Andrieu, 2007). Projection enables the surface element to be superimposed

onto discretised screen located above the canopy, normal to the direction of light. Whilst this method is efficient in terms of speed and accuracy, there is risk of low resolution, particularly for small structural elements. Ray tracing is a stochastic method that relies upon the Monte Carlo method (Kalos & Whitlock, 1986) to account for the fate of light rays. It consists of casting light rays from a given light source, and following their paths through a canopy (Vos et al., 2009). When a ray encounters an object, the subsequent path depends on the optical properties of the material (Sinoquet et al., 1998). The Monte Carlo method is general and requires few assumptions. It enables simulations for large sets of variables; simulations of almost any type of light source, canopy structure and optical properties and separates the contribution of the different orders of scattering to the radiative variables (Chelle & Andrieu, 2007). Variants on the method have also been implemented to achieve higher efficiency, such as Quasi-Monte Carlo ray tracing (Cieslak et al., 2008).

Radiative models have been used in two main types of study: (i) investigating how a given canopy intercepts light; and (ii) simulating plant-light interactions dynamically, through the use of virtual plant models accompanied with specific organ irradiances. Knowledge of how the canopy intercepts light enables scaling from leaf/organ photosynthesis to whole canopy photosynthesis. This requires an understanding of the distribution of photosynthetic capacity (Kull et al., 2002). The changes in light profile resulting from sun flecks and sun angle cause the proportion of canopy light absorbed by individual leaves to change on a time scale too rapid for acclimation of leaf photosynthetic capacities (de Pury & Farquhar, 1999). This requires further consideration for models of whole canopy photosynthesis.

Direct versus diffuse radiation

Both direct and diffused light are important components of incident radiation (Gutschick & Wiegel, 1988; Herbert, 1991; Cavazzoni et al., 2002; Brodersen et al., 2008; Sarlikioti et al., 2011; Matloobi, 2012). Whilst all canopy characteristics effect the distribution of direct light within the canopy, diffused light distribution is mainly affected by foliage arrangement and by leaf angle to only a minor degree (Cavazzoni et al., 2002; Cescatti & Zorer, 2003). The shape, size and arrangement (including proximity) of the leaves affect the transmission of diffused light into lower canopy layers thus can influence canopy photosynthesis under both low- and high-light conditions (Valladares & Pearcy, 1999; Valladares & Niinemets, 2007). This is clearly seen within forests where the orientation of leaves in tree crowns in the vicinities of forest gaps frequently respond to diffuse rather than direct light (Valladares & Niinemets, 2007; as seen in Ackerly & Bazzaz, 1995; Clearwater & Gould, 1995; King, 1998). Alterations in the transmission of diffused light caused by differing architectures or global climate change (i.e. amount of cloud cover) could maximise canopy photosynthesis through a more even distribution of light (Brodersen et al., 2008). Leaf clumping is another trait that influences the transmission of direct and diffused light through the canopy and is able to alter the transmission of each component differently. For example, leaf clumping in tree crowns in Norway Spruce is able to increase the average transmittance at the base of the canopy by 4.9% for direct radiation and up to 10.9% for diffused radiation (Cescatti et al., 1998).

Accounting for architecture in modelling approaches

Traditional theories regarding light attenuation through a canopy often do not hold, particularly for structurally complex canopies as they rely on two main assumptions; leaves are small and they are evenly dispersed throughout canopy structure (Ross, 1981b). However, homogeneity is rarely attainable in the field both in sole cropping and multiple cropping systems and departure from random leaf dispersion (i.e. through clumping) is common (Burgess, et al., 2017; Burgess et al., 2015). Furthermore, these traditional theories simplify both plant architecture and light interception. For accurate prediction of light interception and photosynthetic processes at the canopy level, models must take into account the heterogeneity of canopy structure (Vos et al.,

2007) and the differences in photosynthetic potential within a canopy. Predicting whole canopy photosynthesis requires two steps: first the calculation of the PPFD profile within the canopy and secondly, its relation to the distribution in photosynthetic capacity. Detailed descriptions of canopy architecture are integral to this due to the spatial and temporal differences in PPFD profiles between canopies.

6 DISCUSSION AND OUTLOOK

The Green Revolution started in the 1960s, constantly increasing the yields of most crops and thereby meeting the increasing demand of an ever-growing population. The recent years, however, showed a limited progression, or even a reduction, in yields whereas all projections highlight a strong increase in demand, a harshening of plant growth conditions and limited access to water and fertilizers. Agriculture of the next decades will have to satisfy demands for nutritious food, biomass and bio-sourced products for industry and food for animals. Accordingly, it is imperative to breed more productive and resilient crops.

In the first year of the CropBooster-P project, we performed a modelling exercise depending on a series of parameters that summarize key properties of the photosynthetic processes. The modelling case study demonstrated that even a modest improvement in photosynthesis can improve crop yield in Europe, despite the predicted temperature effects of climate change. The models predicted that even with these modest improvements of single photosynthetic traits, simulated average wheat yield increases across Europe increased up to 35% under future climate predictions. This increase is relatively similar in a wide range of environments ranging from the western to central and northern Europe. In addition, the modelling approach was not parametrized for maximal photosynthesis efficiency possible, and therefore the expected yield improvements could potentially be much higher than reported in the case study here. The model however, presents some limitations that are important to consider. In the future, these modelling outcomes will require validation by measurements on field grown crop plants. In addition, the photosynthesis models used here, but also other available models, are still static. In the future, dynamic models should not only include and update in real time trends associated with climate change, but also year-to-year variations, extreme events (heat waves, late frost, etc.) and the effect that rapid fluctuations in environmental factors, such as irradiance have on photosynthesis, and therefore on yield. The canopy architecture could also be integrated with approaches based on a more accurate canopy model, allowing a more accurate simulation of radiation within the canopy under different solar angles and meteorological conditions. Nonetheless, these preliminary analyses demonstrated that huge crop yield increases are possible and that there lies great potential to increase crop yield by improving individual plant traits, such as photosynthesis. This model serves as a proof-of-concept for the predictive improvement of specific crop traits. Further development of models in the future to better account for multiple stresses such as nutrients availability, genetic variability, physiological processes and adaptive responses, and their interconnected dependencies, are essential to develop to predict plant behavior in response to a changing environment, nutrient availability, and cultivation patterns based on changing food requirements even better. This may allow to identify the most relevant alleles/genes/traits to be combined in breeding or engineering schemes to 'future proof our crops'.

To assess societal, economical and political developments by 2050, CropBooster-P explored options to 'future proof our crops' by breeding and/or by biotechnology. With different stakeholders, four possible future learning scenarios were developed. These learning scenarios set the option space for considering (a) which crops and plant species should be in focus, (b) what technical possibilities will be available to adapt future plants and which ones should be considered, and (c) what crop traits will need to be engineered to meet the needs of future society. Four contrasting and extreme scenarios were developed which depict future socio-economic developments: '*Plantovation*', '*Your Food. Your Health. Your Choice*', '*Foodmergency*' and '*REJECTech*'. By design, each of these four scenarios is unlikely, but plausible and hence, reality in the year 2050 is likely to include a combination of aspects from each of the four learning scenarios. The set of scenarios covers a broad range of outcomes related to major uncertainties

within EU agriculture. The learning scenarios were initially developed in Work Package 1 (WP1) of the CropBooster-P project as a starting point to define the future space in which the options to improve crop species can be assessed.

Within WP1 of the CropBooster-P project, the four scenarios were explored in relation to crop improvement traits, focusing on three key topics: (1) yield, (2) nutritional quality, and (3) sustainability. To identify future key options to improve crop productivity and quality, a large panel of experts from all over Europe were selected on the basis of their expertise and they created and assembled a database including over 800 recent scientific and technical manuscripts highlighting the potential to improve a variety of traits in a wide variety of plant and crop species. This database also investigates the potential of different state-of-the-art technologies and their possible applications for the different species, orthologues and niche crops. Finally, the survey also collected annotations, suggestions and comments made by the experts. In this way, the database does not only capture all information related to the plant traits, but also the technologies, genes and methods for crop improvement. Based on the database and the broad expertise represented in the CropBooster-P expert panel, 20 key options were selected and used as examples of how crop yield could be increased, such as by improving photosynthesis or organ growth and development, carbon and nutrients partitioning and/or remobilisation and by improving water and nutrients (N, P…) uptake and/or use efficiency. Overall, it will be crucial to explore the diversity of genes or gene combinations identified in model plants and identifying the most effective in each species providing a solution for distinct socio-economical future scenarios. The key options also cover aspects that might be essential to increase yield, whilst maintaining or improving nutritional quality in a sustainable manner, for instance to deal with the enhancement of yields under suboptimal water and mineral availability or challenging environmental growth condition (high/low temperatures, high/low light intensity, drought, flooding, etc.).

When considering the key options to increase crop yield by improving water management and drought tolerance, a large number of QTLs, traits and genes spread over many plant functions or signaling pathways functioning in leaves can be targeted. Also roots appeared are crucial to improve yield, but also sustainability and yield stability. Roots are essential for the uptake of minerals and water, as well as for interactions with the surrounding soil. They are the first place where responses to external stimuli take place and these responses contribute to the plant it's acclimation and adaptation to various environmental conditions. Accordingly, the architecture of the root system is extremely complex, branching can be high and roots can have contrasting morphological (length, orientation, etc.) and anatomical (size of vessels, proportion of stele, etc.) features affecting their function. As roots are belowground, however, capturing their development and activity is complex and time consuming. Several root phenotyping facilities are available but there is a huge need to develop phenotyping technics and infrastructures to deepen our knowledge and move faster towards a functional root characterization.

To date, however, plant stresses are often investigated separately and under steady state. Whereas in field crops often multiple stresses occur simultaneously (e.g. drought is often associated to heat, nutrient shortage due to reduced solubility, increased soil impedance, etc.). Investigating individual stresses only reveals part of the adaptive response and does not give access to emerging properties from stress combinations. Similarly, the impact of environmental stresses on plant yield need to be investigated dynamically to not only capture the dynamics of stress tolerance, but also stress priming and the dynamics of stress recovery. In addition, plants have to be considered holistically to capture the extensive local and systemic physical, metabolic and signaling exchanges between plant tissues and organs. Accordingly, the development of high throughput or very high functional phenotyping facilities will be necessary to assess the complexity of the plant system.

Exploring genetic diversity is also of key significance, with respect regarding key options to amongst others improve nutrition. The need to explore wild germplasm of crops, which could contain potential sources of natural variation in nutrient content is of particular relevance here. These ancient traits, that have been lost due to centuries of breeding and selection, can prove to be important in terms of improving robustness and nutrient content. These changes can be made in a sequential manner, with an understanding of consumer demand and public health requirements in various breeding programs. An important factor to consider while modifying the nutrient content of the plant is the implication to the plant in a holistic manner, including the impact on other nutritive factors such as fiber content, digestibility, yield and stress tolerance, all of which can be impacted by effecting specific changes to the plant nutrient uptake profile by various methods. In this regard, reduction of antinutritive factors also merit careful consideration, as they often serve a protective defense or stress response role in the plant and selecting against these traits could result in reduced plant fitness and viability. Specific approaches for tissue specific reduction of antinutritive factors could prove to be an important approach in modulating these undesirable compounds in the edible plant parts, while maintaining the overall plant fitness. The pressure on crops today to produce more and more in terms of yield while maintaining quality levels is putting an increased pressure on the environment. This report attempts a preliminary exploration of crops such as amaranth lupins and seaweeds, which have the potential to act as alternative sources of nutrients, which is especially relevant in a changing climate, where the need for hardier crops arises.

Based on the database, also some key options for future research were identified that are common to yield, nutritional quality and sustainability, such as the further development of modelling approaches and improving methods for breeding and genome editing. Altogether, the references and the key options reported by the experts clearly indicate that there are many options that could be further explored to improve yield, nutritional quality and/or sustainability. In addition, they highlight that individual changes often give relatively small benefits but that there lies major potential in combining/stacking individual improvements. However, we need to be aware of possible trade-offs between traits. For instance, a dramatic increase in root development can be considered as a desired trait to improve soil foraging and increasing tolerance to unfavorable environments. In contrast, however, the root system can then become a huge carbon sink that can negatively impact shoot biomass accumulation and finally grain yield. Similarly, though increasing nutrient uptake appears very promising intuitively, the result can be sometimes disappointing. Attempts to increase nitrate uptake without considering improvement of assimilation often result in a reduction of plant N contents due to activation of a nitrate dependent feedback regulatory loop. These examples illustrate the fact that it is essential to have a better understanding of plant physiology and metabolism to evaluate at the whole plant level the impact of individual trait improvements. Obtaining such knowledge becomes crucial when pyramiding individual traits in an integrated research program. More general, the references and examples also indicate the potential of aquatic and certain underutilized species, though this is largely underexplored so far. Both lupins and seaweeds for instance offer major potential as alternative protein sources and may develop into competitive protein crops, especially relevant in a changing climate, where a demand for hardier crops arises. The nutritive systems based on algae may provide key compounds for human nutrition among which are lipids and fatty acids, carotenoids, proteins, minerals and others. Altogether, however, major additional breeding efforts will be required allowing aquatic species to become a competitive resource. Modern analyses and breeding techniques would therein be essentially supportive. Other crop species that offer major potential are medicinal plants as a source for novel medical compounds and Amaranth, though also largely underexplored so far.

Table 4 Potential implications of the future scenarios on traits and technologies, consumer choices and economy & society.

		PLANTOVATION	YOUR FOOD HEALTH CHOICE	FOODMERGENCY	REJECTech
Traits and Technologies	Directed Technology (GMO, CRISPR, gene editing ...)	Green	Orange	Green	Red
	Empirical Technology (NGS, TILLING, marker assisted breeding…)	Green	Green	Green	Striped red/yellow
	Genetic resources (ancestral genes..)	Green	Orange	Green	Striped red/yellow
	Land races	Green	Orange	Green	Green
	New/ alternative species	Green	Green	Green	Striped red/yellow
	Modelling	Green	Green	Green	Green
	Integrated Physiology and Metabolism	Green	Green	Orange	Striped red/yellow
Consumer choices	Focus on nutrients	Green	Green	Orange	Orange
	Sustainable agriculture	Green	Orange	Red	Orange
	Tailored diet options	Green	Green	Red	Orange
	Shelf life	Green	Green	Red	Striped red/yellow
	Variety	Green	Green	Red	Striped red/yellow
Economy& Society	Industrial farming	Green	Striped red/yellow	Green	Red
	Technological innovations	Green	Green	Green	Red
	Green packaging	Green	Green	Red	Orange
	Bioeconomic potential	Green	Orange	Red	Red
	Traditional agricultural practices	Green	Orange	Orange	Green

Legend:
- Green: High possibility, unrestricted implementation
- Orange: Medium possibility, implementation possible but time consuming
- Red: Not possible to achieve under present conditions/ no priority
- Striped red/yellow: Implementation may be difficult/conditional to time and available technologies

In WP1, we also aimed to synthetize the huge amount of data collected in the survey and the information retrieved by the experts on the different key options to improve yield, nutritional quality and sustainability in relation to the learning scenarios. To do so, the WP1 core team performed a preliminary analysis of the impact that the different scenarios might have on a rather small number of key areas, both scientific and societal. Our preliminary analysis is presented in **Table 4**, showing a glimpse of the effects that the different scenarios might have. It should be kept in mind, however, that this is a very preliminary analysis, developed only by the WP1 core team and thus should be much more refined by the work that will be done in WP2 and WP3. Overall, however, the table illustrates that the four learning scenarios created in WP1 indeed are contrasting scenarios with their specific impacts for the future, being with a wider or reduced amplitude (green, red, orange color). All the key options described as enablers to improve yield, nutritional quality and/or sustainability refer to 'transferable traits' e.g. traits that can be transferred between plant(species) using empirical and directed methods. In all scenarios, there is a high need to investigate wild germplasm of crops which may contain the necessary variability which could be used for improvement in modern day varieties. In common for all key options, biotechnological methods pave the way for rapid integration of desired traits and genes encoding desirable traits in a fraction of the time it takes conventional breeding. Especially if multiple targets need to be introduced in a crop, the use of biotech will accelerate this transfer. In addition, biotech will also offer the opportunity to transfer gene between species, allowing to make rapid and significant progresses by direct transfer of specific traits and/or modulation of signaling pathways. Genetic modification often involves additional regulatory, intellectual property and consumer acceptance issues; all of which have driven a high regulatory burden associated with GMOs and inhibit the transition of projects from research to commercial development and adoption. Compared to the other three scenarios, however, '*REJECTech*' is a scenario in which a large number of biotechnological methods used to increase crop yield are not available. In this scenario, the options are limited since there are only very few options available of which most will take so much time that they in practice will be very limited. Similarly, manipulation of dissected complex signaling pathways (using e.g. synthetic biology) will be almost impossible without the use of GMOs, whereas this approach appeared extremely promising.

'*Plantovation*'

General: In this scenario, options would be unlimited and solutions are widely used for a thriving bioeconomy for fodder and feed, improved digestibility. There are no restrictions on research and technology. In this scenario, the full range of conventional and biotechnological options can be exploited to meet the future needs. Key options and technologies can be implemented relatively quickly (3-5years), technologies will allow targeted gene transfer in a very short time frame, implementation of more complex traits can be made possible by 2050. The shorter turnover period will enable researchers to quickly determine what works and what does not and are able to detect and correct implications of such implemented changes on a whole plant and field level much faster. The prevalence of integrated modelling strategies would further allow for the predictive accuracy of future crops, allowing a very precise approach to crop improvement. Equal thrust on yield, nutritional quality and sustainability can be envisaged resulting in broad consumer choices including a sustainable agriculture, tailored diet options, long shelf lives and big varieties of products.

- **(1) Yield**: Several traits can be integrated into the same crop using a variety of technologies, allowing fast improvement of crop productivity. In this scenario, photosynthesis (fast light adaptation, photorespiration and light reaction, Rubisco, photosynthetic carbon metabolism) could be improved by both exploiting natural variation and introducing new genes and pathways. Gene editing might be used to improve yield by impinging on genes involved in for instance NUE, PUE or water use efficiency. Modeling approaches including canopy responses would allow forecast of crop

performance and allow for preventative measures to protect crops against impending environmental changes in the short term.

- **(1) Nutritional quality**: Faster and more readily achieved genetic modification to improve for instance micronutrient content. The potential of, among others, Amaranth and seaweeds could be exploited in Europe because of intense and timely improvement based on major breeding efforts, accessing and utilizing the biological variability in this and related genera. In addition, they may be used as a template for harnessing other crops. Variation in nutritional content across soy and maize germplasm is huge, and could easily exploited in breeding. Alternatively, GMOs might be generated to for instance express long chain poly-unsaturated fatty acids to improve nutritional quality.
- **(1) Sustainability**: Stress resistance and nutrient partitioning can be exploited allowing for earlier harvest, less environmental pressure, etc. GMO crops could for instance be produced to modify stomatal ABA sensitivity to improve drought tolerance. On the longer term, there might be the possibility to create 'C4 wheat', 'nodulating wheat' or the production of clustered roots in all species to improve P or Fe uptake.

➔ **Yield, Nutritional quality and Sustainability have equal importance (1)**. **These major modifications at different levels is only possible using 'biotechnology'.**

'Your Food. Your Health. Your Choice'

General: This scenario allows to focus on personalized approaches. Accordingly, there is probably a need for a greater variety of crops, with less dominance of the current major crops to meet specific needs of consumers. While the restrictions on the potential applications to technologies are few, they are informed and mainly driven by consumer choices, leading to an increased period (5-10 years) of safety regulation and rigorous testing before innovations would be made available. The high degree of personalization would further slow down the speed of turnover. Emphasis on nutrient quality and alternative sources of nutrition with sustained yield and high sustainability can be envisaged. Altogether, however, the distinct focus compared with a '*Plantovation'* scenario could result in a strongly decreased industrial farming, though also decreased traditional agricultural practices, and a strongly decreased bio-economic potential. In this scenario yield is of high importance, though the priority might go even more towards improving the variety with regard to Nutritional quality and Sustainability. Though likely niche production for most species, the sector may gain economic value and socio- economic scope.

- **(2) Yield**: Examples are increased shelf life and longevity demands, including increased organ growth and delaying senescence. Also increases in seed filling may become priority. Yield improvement of new crops may be required to address possible negative trade-offs previously observed between yield and nutritional value (e.g increased yields to higher CO_2 in C3 plants tend to lead to a decrease in protein and a mineral contents in seeds). T6P solutions to yield and resilience may be exploited leading to diverse healthy choices in food supply.
- **(1) Nutritional quality**: Increased demand for alternative nutrient sources, alternative crops, superfoods and crops with improved micronutrient content and specializes metabolites with nutritive roles (antioxidants, polyphenols, omega-3 in Camelina as a plant based source of essential fatty acids). Also biofortification of micronutrients might become of utmost importance in this scenario. To increase nutritional quality, biotechnological solutions could be used, such as GMOs expressing long change poly-unsaturated fatty acids. There might also be a high need to change lignification to improve digestibility and conversion of biomass. Also Pi metabolism may offer opportunities to produce particular compounds according to the consumer needs. Pi status of the plant promotes many biochemical modifications affecting among others phospholipids. They are an important source of phosphorus in human nutrition, providing

also specific compounds such as lecithin, which are broadly used by food industry. Limiting phosphate promotes instead the production of galacto- and sulfolipids. All these different compounds have specific agro-economical interest (food, medicine, biofuel, etc.). The omega-3 Camelina trait is an example of an innovative solution to improve nutrition, directly in diets or via animal feed (aquaculture). Moreover, it has a significant environmental impact since it reduces the demands on oceanic capture fisheries, which are already at their limits of productivity. Engineering health-promoting fatty acids in seed oil may also drive food production towards the needs of individuals, addressing the requirements of a '*Your food, your health, your choice'* scenario. Since the '*Your Food. Your Health. Your Choice*' scenario permit GMOs, genetic modification can be exploited in this scenario. Nonetheless, despite the major benefits that nutritional enhancement may provide, thus far it is proven extremely difficult for these crops to be adopted by agriculture (farmers and consumers), as for instance seen by the consumer rejection of Golden rice, enriched in Vitamin A (reviewed in Napier et al. 2019).

- **(1) Sustainability**: Key options might be exploited that aim to minimize environmental impact producing alternative crops with increased resilience to stress, NUE, water management, etc. An example is for instance the work reported by Yang et al. (2016 and 2019) or Miao et al. (2018), who locally and specifically manipulated the ABA signaling pathway have been able to significantly increase plant drought tolerance without any deleterious effect under optimal growth condition or grain yield.

➔ **Yield is of utmost importance (2), but in this scenario Sustainability and Nutritional quality might become even more important (1).**

'*Foodmergency'*

General: In this scenario, the focus will be on maximizing yield and yield resilience in the face of extreme weather events probably requiring uniform production of a limited number of the most productive varieties of a few productive species. Accordingly, any solution that feeds the people will be exploited, even at the expense of longer term sustainability, including environmental degradation and damage. Technologies are available but will be focused on Yield-driven innovations, causing an upsurge of microscale production platforms. Here, the priority is on calorie production, with lower emphasis on nutritional quality, putatively resulting in decreases in terms of diet options, tailored diet options and food variety. Also sustainability is no longer a priority in a starvation economy, putatively resulting in a decreased sustainability of the agriculture, but also green packaging and bio-economic potential. Engineering crops can be done either by introgression of QTLs or by GM approaches (e.g. gene editing, mutagenesis) could be a response to a food crisis with rapid adoption and application of new approaches to avoid food shortages.

- **(1) Yield**: Increasing yield will be the main priority, for instance by increasing organ growth and development, longevity, nutrient remobilization and partitioning, shorter turnover periods of high caloric value food. Alternatively, significant effort could be put on breeding for new crops or crops cultivated with traditional farming, e.g. specific breeding programs for underused and new crops that can survive more uncertain climate. Modeling approaches can be exploited to propose the best crops and best cultivars to a specific climate and land.
- **(2) Nutritional quality**: Not applicable other than the basic nutrient requirements the crops are able to produce, with due to the food pressure little to no emphasis on increasing nutritional quality.
- **(2) Sustainability**: Sustainability is likely to be no priority, resulting in negative impacts on salt stress, drought stress and other indices.

➔ **In this scenario, Nutritional quality and Sustainability are important (2), but Yield and biomass production are undoubtedly of the greatest importance (1).**

'REJECTech'

General: In this scenario, many biotechnological approaches (e.g. GMOs, mutagenesis and gene editing) would be rejected and therefore not be possible to be used. Accordingly, the focus will in this scenario be on the exploration of the genetic diversity in wild related species and introgression of traits through classical breeding. Conventional breeding programs come again to the foreground along with traditional agricultural practices that rely on traditional breeding techniques, marker-based selection, etc. Research driven by technology slows down significantly, resulting in much larger timeframes (30-50 years) to implement changes to improve crop productivity. Classical selection, however, largely limits the spread of traits of interest within the species where they have been identified and many of the improvements may therefore even not be possible. In addition, breeding programs would be to slow to develop a considerable share. Alternatively, we would need to invest in the development of underutilized species (e.g. Camelina (Nutritional quality), lupins (PUE), sorghum (drought) ....). These changes can, however, not be controlled as precisely as modern precision editing techniques, leading to a lot more variation, unpredictability and inconsistency of results, putatively strengthening further mistrust of consumers in science. Focus would remain on the locally grown crop types, with food security potentially declining. Focus can be envisaged to be primarily on yield, and next on nutritional quality and sustainability. Technological innovations will be limited, as well as the bio-economic potential.

- **(1) Yield**: In this scenario, GMO based approaches would be impossible to improve yield. Mainly traditional breeding approaches will be exploited for better yielding cultivars, e.g genetic crosses. For instance, the option space of T6P is limited as genetic selection using markers for the T6P pathway may be required to produce higher-yielding stress-resilient crops. The increase in potential yield may, however, be rather limited.
- **(2) Nutritional quality**: GMO based approaches would be impossible to improve nutritional quality. Wild species germplasm may be exploited/may be an option to adapt to *REJECTech* conditions (underutilized crop species).
- **(2) Sustainability**: With regard to sustainability, it is likely that in this scenario we will mainly return to traditional agricultural practices, including intercropping, legumes for nitrogen fixation, etc. For instance, Parent et al. (2018) and Millet et al. (2019) combining fine climatic, phenotypic analysis and modelling accurately predicted the performances of maize germplasms to improve yield in drought conditions.

➔ **In this scenario, increasing Yield and biomass production will be priority (1), whereas the options with regard to improving Nutritional quality and Sustainability (2) might be less priority, mainly due to time restrictions.**

As mentioned earlier, the learning scenarios were developed in Work Package 1 (WP1) of the CropBooster-P project as a starting point to define the future space in which the options to improve crop species can be assessed. In the subsequent phases of the CropBooster-P project, however, a more extensive impact analysis will be performed to reach a broader understanding of the diversity of possible future(s).

To maximize the value generated by the scenario analysis, the following steps could be taken:
1. *Detail the scenarios*: additional value can be captured from the scenarios by further elaborating on some particularly interesting aspects. This may include additional research on important trends and uncertainties.

2. *Prepare for the future*: Develop a CropBooster-P roadmap balancing opportunity, need and risk by appreciating the spread and commonalities of desired outcomes under different scenarios.
3. *Identify early warning signals*: Before any of the scenarios fully materializes, there will be weak signals that can be picked up if one looks for them. By identifying these signals now and incorporating them into the roadmap, CropBooster-P can minimize risks and increase chances of success.
4. *Create the future*: Don't just wait and see how the future unfolds, but take specific measures today to prevent undesirable outcomes or scenarios from happening, communicate and discuss them with relevant stakeholders.

This will provide new perspectives that make the results of the CropBooster-P project even more robust and facilitate a more proactive stance towards future threats and opportunities. Altogether, in the context of the overall project, this initial scenario analysis provided the reference point for a multidimensional assessment including the economic, social and environmental impact (Work Package 2), societal needs and expectations (Work Package 3), international cooperation (Work Package 4), and finally strategy development (Work Package 5).

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8 REFERENCES

AbdElgawad, H., Zinta, G., Hegab, M. M., Pandey, R., Asard, H., & Abuelsoud, W. (2016). High Salinity Induces Different Oxidative Stress and Antioxidant Responses in Maize Seedlings Organs. *Frontiers in Plant Science, 7*. doi:ARTN 27610.3389/fpls.2016.00276

Abraham, E. M., Ganopoulos, I., Madesis, P., Mavromatis, A., Mylona, P., Nianiou-Obeidat, I., . . . Vlachostergios, D. (2019). The Use of Lupin as a Source of Protein in Animal Feeding: Genomic Tools and Breeding Approaches. *International Journal of Molecular Sciences, 20*(4). doi:ARTN 85110.3390/ijms20040851

Abreu, M. E., & Munne-Bosch, S. (2009). Salicylic acid deficiency in NahG transgenic lines and sid2 mutants increases seed yield in the annual plant Arabidopsis thaliana. *Journal of Experimental Botany, 60*(4), 1261-1271. doi:10.1093/jxb/ern363

Ackerly, D. D., & Bazzaz, F. A. (1995). Seedling Crown Orientation and Interception of Diffuse-Radiation in Tropical Forest Gaps. *Ecology, 76*(4), 1134-1146. doi:Doi 10.2307/1940921

Agriculture and Horticulture Development Board. (2018). Wheat Growth Guide. Retrieved September 1, 2019, from https://ahdb.org.uk/wheatgg

Ahn, T. K., Avenson, T. J., Ballottari, M., Cheng, Y. C., Niyogi, K. K., Bassi, R., & Fleming, G. R. (2008). Architecture of a charge-transfer state regulating light harvesting in a plant antenna protein. *Science (New York, N.Y.), 320*(5877), 794-797. doi:10.1126/science.1154800

Ainsworth, E. A., & Long, S. P. (2005). What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New Phytologist, 165*(2), 351-371. doi:10.1111/j.1469-8137.2004.01224.x

Al-Tamimi, N., Brien, C., Oakey, H., Berger, B., Saade, S., Ho, Y. S., . . . Negrao, S. (2016). Salinity tolerance loci revealed in rice using high-throughput non-invasive phenotyping. *Nature Communications, 7*. doi:ARTN 1334210.1038/ncomms13342

Alarcon, V. J., & Sassenrath, G. F. (2011). Modeling cotton (Gossypium spp.) leaves and canopy using computer aided geometric design (CAGD). *Ecological Modelling, 222*(12), 1951-1963. doi:10.1016/j.ecolmodel.2011.03.037

Albert, E., Segura, V., Gricourt, J., Bonnefoi, J., Derivot, L., & Causse, M. (2016). Association mapping reveals the genetic architecture of tomato response to water deficit: focus on major fruit quality traits. *Journal of Experimental Botany, 67*(22), 6413-6430. doi:10.1093/jxb/erw411

Alercia, A. (2013) Nutritious underutilized species – Amaranth. Biodiversity International, Rome. Retrieved from https://www.bioversityinternational.org/e-library/publications/detail/nutritious-underutilized-species-amaranth/

Alhabbar, Z., Islam, S., Yang, R. C., Diepeveen, D., Anwar, M., Balotf, S., . . . Juhasz, A. (2018). Associations of NAM-A1 alleles with the onset of senescence and nitrogen use efficiency under Western Australian conditions. *Euphytica, 214*(10). doi:UNSP 18010.1007/s10681-018-2266-4

Allard, A., Bink, M. C. A. M., Martinez, S., Kelner, J. J., Legave, J. M., di Guardo, M., . . . Costes, E. (2016). Detecting QTLs and putative candidate genes involved in budbreak and flowering time in an apple multiparental population. *Journal of Experimental Botany, 67*(9), 2875-2888. doi:10.1093/jxb/erw130

Altpeter, F., Springer, N. M., Bartley, L. E., Blechl, A. E., Brutnell, T. P., Citovsky, V., . . . Stewart, C. N., Jr. (2016). Advancing Crop Transformation in the Era of Genome Editing. *The Plant cell, 28*(7), 1510-1520. doi:10.1105/tpc.16.00196

Anand, A., Bass, S. H., Wu, E., Wang, N., McBride, K. E., Annaluru, N., . . . Jones, T. J. (2018). An improved ternary vector system for Agrobacterium-mediated rapid maize transformation. *Plant Molecular Biology, 97*(1-2), 187-200. doi:10.1007/s11103-018-0732-y

Anderson, M. (1971). Radiation and Crop Structure. In *Plant Photosynthetic Production. Manual of Methods* (Z Sestak, pp. 412–466). The Hague, Netherlands.

Ando, K., Grumet, R., Terpstra, K., & Kelly, J. (2007). Manipulation of plant architecture to enhance crop disease control. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, *2*(026), 026. Retrieved from http://www.researchgate.net/publication/237102115_Manipulation_of_plant_architecture _to_enhance_crop_disease_control_Address_1_Graduate_Program_in_Plant_Breeding_an d_Genetics

Andriankaja, M., Dhondt, S., De Bodt, S., Vanhaeren, H., Coppens, F., De Milde, L., . . . Inzé, D. (2012). Exit from proliferation during leaf development in *Arabidopsis thaliana*: a not-so-gradual process. *Developmental Cell, 22*(1), 64-78. doi:10.1016/j.devcel.2011.11.011

Anten, N. P. R., & Hirose, T. (1999). Interspecific differences in above-ground growth patterns result in spatial and temporal partitioning of light among species in a tall-grass meadow. *Journal of Ecology, 87*(4), 583-597. doi:DOI 10.1046/j.1365-2745.1999.00365.x

Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., . . . Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature, 576*(7785), 149-157. doi:10.1038/s41586-019-1711-4

Arnaud, C., Clement, M., Thibaud, M. C., Javot, H., Chiarenza, S., Delannoy, E., . . . Nussaume, L. (2014). Identification of Phosphatin, a Drug Alleviating Phosphate Starvation Responses in Arabidopsis. *Plant Physiology, 166*(3), 1479-+. doi:10.1104/pp.114.248112

Aro, E. M., McCaffery, S., & Anderson, J. M. (1993). Photoinhibition and D1 Protein Degradation in Peas Acclimated to Different Growth Irradiances. *Plant Physiology, 103*(3), 835-843. doi:10.1104/pp.103.3.835

Asplund, L., Hagenblad, J., & Leino, M. W. (2010). Re-evaluating the history of the wheat domestication gene NAM-B1 using historical plant material. *Journal of Archaeological Science, 37*(9), 2303-2307. doi:10.1016/j.jas.2010.04.003

Asseng, S., van Keulen, H., & Stol, W. (2000). Performance and application of the APSIM Nwheat model in the Netherlands. *European Journal of Agronomy, 12*(1), 37-54. doi:Doi 10.1016/S1161-0301(99)00044-1

Aung, K., Lin, S. I., Wu, C. C., Huang, Y. T., Su, C. L., & Chiou, T. J. (2006). pho2, a phosphate overaccumulator, is caused by a nonsense mutation in a MicroRNA399 target gene. *Plant Physiology, 141*(3), 1000-1011. doi:10.1104/pp.106.078063

Avni, R., Zhao, R. R., Pearce, S., Jun, Y., Uauy, C., Tabbita, F., . . . Distelfeld, A. (2014). Functional characterization of GPC-1 genes in hexaploid wheat. *Planta, 239*(2), 313-324. doi:10.1007/s00425-013-1977-y

Ayadi, A., David, P., Arrighi, J. F., Chiarenza, S., Thibaud, M. C., Nussaume, L., & Marin, E. (2015). Reducing the Genetic Redundancy of Arabidopsis PHOSPHATE TRANSPORTER1 Transporters to Study Phosphate Uptake and Signaling. *Plant Physiology, 167*(4), 1511-1526. doi:10.1104/pp.114.252338

Azevedo, I. C., Duarte, P. M., Marinho, G. S., Neumann, F., & Sousa-Pinto, I. (2019). Growth of Saccharina latissima (Laminariales, Phaeophyceae) cultivated offshore under exposed conditions. *Phycologia, 58*(5), 504-515. doi:10.1080/00318884.2019.1625610

Baekelandt, A., Pauwels, L., Wang, Z., Li, N., De Milde, L., Natran, A., . . . Gonzalez, N. (2018). Arabidopsis leaf flatness is regulated by PPD2 and NINJA through repression of CYCLIN D3 genes. *Plant Physiology*. doi:10.1104/pp.18.00327

Baldocchi, D., & Collineau, S. (1994). The physical nature of solar radiation in heterogeneous canopies: spatial and temporal attributes. In M. Caldwell & R. Pearcy (Eds.), *Exploitation of Environmental Heterogeneity by Plants. Ecophysiology Processes- Above and Belowground* (pp. 21–71). San Diego, New York, Boston. London, Sydney, Toronto: Academic Press.

Balzergue, C., Dartevelle, T., Godon, C., Laugier, E., Meisrimler, C., Teulon, J. M., . . . Desnos, T. (2017). Low phosphate activates STOP1-ALMT1 to rapidly inhibit root cell elongation. *Nature Communications, 8*. doi:ARTN 1530010.1038/ncomms15300

Barber, J. (2009). Photosynthetic energy conversion: natural and artificial. *Chemical Society Reviews, 38*(1), 185-196. doi:10.1039/b802262n

Barberon, M., Vermeer, J. E. M., De Bellis, D., Wang, P., Naseer, S., Andersen, T. G., . . . Geldner, N. (2016). Adaptation of Root Function by Nutrient-Induced Plasticity of Endodermal Differentiation. *Cell, 164*(3), 447-459. doi:10.1016/j.cell.2015.12.021

Bari, R., Pant, B. D., Stitt, M., & Scheible, W. R. (2006). PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiology, 141*(3), 988-999. doi:10.1104/pp.106.079707

Barlow, K. M., Christy, B. P., O'Leary, G. J., Riffkin, P. A., & Nuttall, J. G. (2015). Simulating the impact of extreme heat and frost events on wheat crop production: A review. *Field Crops Research, 171*, 109-119. doi:10.1016/j.fcr.2014.11.010

Barrett, L. G., Legros, M., Kumaran, N., Glassop, D., Raghu, S., & Gardiner, D. M. (2019). Gene drives in plants: opportunities and challenges for weed control and engineered resilience. *Proceedings. Biological sciences, 286*(1911), 20191515. doi:10.1098/rspb.2019.1515

Barthelemy, D., & Caraglio, Y. (2007). Plant architecture: A dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. *Annals of Botany, 99*(3), 375-407. doi:10.1093/aob/mcl260

Bates, P. D. (2016). Understanding the control of acyl flux through the lipid metabolic network of plant oil biosynthesis. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids, 1861*(9), 1214-1225. doi:10.1016/j.bbalip.2016.03.021

Bawiec, A. (2019). Efficiency of nitrogen and phosphorus compounds removal in hydroponic wastewater treatment plant. *Environmental Technology, 40*(16), 2062-2072. doi:10.1080/09593330.2018.1436595

Bayle, V., Arrighi, J. F., Creff, A., Nespoulous, C., Vialaret, J., Rossignol, M., . . . Nussaume, L. (2011). Arabidopsis thaliana High-Affinity Phosphate Transporters Exhibit Multiple Levels of Posttranslational Regulation. *Plant Cell, 23*(4), 1523-1535. doi:10.1105/tpc.110.081067

Beemster, G. T., De Veylder, L., Vercruysse, S., West, G., Rombaut, D., Van Hummelen, P., . . . Vuylsteke, M. (2005). Genome-wide analysis of gene expression profiles associated with cell cycle transitions in growing organs of Arabidopsis. *Plant Physiol, 138*(2), 734-743. doi:10.1104/pp.104.053884

Benredjem, Z., & Delimi, R. (2009). Use of extracting agent for decadmiation of phosphate rock. *Proceedings of the Jmsm 2008 Conference, 2*(3), 1455-1460. doi:10.1016/j.phpro.2009.11.116

Benredjem, Z., Delimi, R., Khelalfa, A., Saaidia, S., & Mehellou, A. (2016). Coupling of electrodialysis and leaching processes for removing of cadmium from phosphate ore. *Separation Science and Technology, 51*(4), 718-726. doi:10.1080/01496395.2015.1119842

Bergmann, D. C., & Sack, F. D. (2007). Stomatal development. *Annual Review of Plant Biology, 58*, 163-181. doi:10.1146/annurev.arplant.58.032806.104023

Betancor, M. B., Li, K. S., Bucerzan, V. S., Sprague, M., Sayanova, O., Usher, S., . . . Olsen, R. E. (2018). Oil from transgenic Camelina sativa containing over 25 % n-3 long-chain PUFA as the major lipid source in feed for Atlantic salmon (Salmo salar). *British Journal of Nutrition, 119*(12), 1378-1392. doi:10.1017/S0007114518001125

Betancor, M. B., Sprague, M., Montero, D., Usher, S., Sayanova, O., Campbell, P. J., . . . Tocher, D. R. (2016). Replacement of Marine Fish Oil with de novo Omega-3 Oils from Transgenic Camelina sativa in Feeds for Gilthead Sea Bream (Sparus aurata L.). *Lipids, 51*(10), 1171-1191. doi:10.1007/s11745-016-4191-4

Bieleski, R. L. (1973). Phosphate Pools, Phosphate Transport, and Phosphate Availability. *Annual Review of Plant Physiology and Plant Molecular Biology, 24*, 225-252. doi:DOI 10.1146/annurev.pp.24.060173.001301

Bogard, M., Allard, V., Martre, P., Heumez, E., Snape, J. W., Orford, S., . . . Le Gouis, J. (2013). Identifying wheat genomic regions for improving grain protein concentration

independently of grain yield using multiple inter-related populations. *Molecular Breeding, 31*(3), 587-599. doi:10.1007/s11032-012-9817-5

Bogard M., Hourcade D., Piquemal B., Gouache D., Deswarte JC., Cohan JP. (submitted) Marker-based crop model assisted ideotype design to improve avoidance of abiotic stress in bread wheat. Submitted to *Journal of Experimental Botany*

Bogard, M., Ravel, C., Paux, E., Bordes, J., Balfourier, F., Chapman, S. C., . . . Allard, V. (2014). Predictions of heading date in bread wheat (Triticum aestivum L.) using QTL-based parameters of an ecophysiological model. *Journal of Experimental Botany, 65*(20), 5849-5865. doi:10.1093/jxb/eru328

Bohner, A., Kojima, S., Hajirezaei, M., Melzer, M., & von Wiren, N. (2015). Urea retranslocation from senescing Arabidopsis leaves is promoted by DUR3-mediated urea retrieval from leaf apoplast. *Plant Journal, 81*(3), 377-387. doi:10.1111/tpj.12740

Bonan, G. (2002). *Ecological Climatology: Concepts and Applications* (1st ed.). Cambridge: Cambridge University Press.

Bonnot, C., Pinson, B., Clement, M., Bernillon, S., Chiarenza, S., Kanno, S., . . . Desnos, T. (2016). A chemical genetic strategy identify the PHOSTIN, a synthetic molecule that triggers phosphate starvation responses in Arabidopsis thaliana. *New Phytologist, 209*(1), 161-176. doi:10.1111/nph.13591

Borel, C. C., Gerstl, S. A. W., & Powers, B. J. (1991). The Radiosity Method in Optical Remote-Sensing of Structured 3-D Surfaces. *Remote Sensing of Environment, 36*(1), 13-44. doi:Doi 10.1016/0034-4257(91)90028-5

Borrill, P., Fahy, B., Smith, A. M., & Uauy, C. (2015). Wheat Grain Filling Is Limited by Grain Filling Capacity rather than the Duration of Flag Leaf Photosynthesis: A Case Study Using NAM RNAi Plants. *PLoS ONE, 10*(8). doi:ARTN e013494710.1371/journal.pone.0134947

Botte, C. Y., Deligny, M., Roccia, A., Bonneau, A. L., Saidani, N., Hardre, H., . . . Marechal, E. (2011). Chemical inhibitors of monogalactosyldiacylglycerol synthases in Arabidopsis thaliana. *Nature Chemical Biology, 7*(11), 834-842. doi:10.1038/nchembio.658

Boyer, J. S., & Westgate, M. E. (2004). Grain yields with limited water. *Journal of Experimental Botany, 55*(407), 2385-2394. doi:10.1093/jxb/erh219

Breda, N. J. J. (2003). Ground-based measurements of leaf area index: a review of methods, instruments and current controversies. *Journal of Experimental Botany, 54*(392), 2403-2417. doi:10.1093/jxb/erg263

Brevis, J. C., & Dubcovsky, J. (2010). Effects of the Chromosome Region Including the Gpc-B1 Locus on Wheat Grain and Protein. *Crop Science, 50*(1), 93-104. doi:10.2135/cropsci2009.02.0057

Brodersen, C. R., Roddy, A. B., Wason, J. W., & McElrone, A. J. (2019). Functional Status of Xylem Through Time. *Annual Review of Plant Biology, Vol 70, 70*, 407-433. doi:10.1146/annurev-arplant-050718-100455

Brodersen, C. R., Vogelmann, T. C., Williams, W. E., & Gorton, H. L. (2008). A new paradigm in leaf-level photosynthesis: direct and diffuse lights are not equal. *Plant Cell and Environment, 31*(1), 159-164. doi:10.1111/j.1365-3040.2007.01751.x

Buckler, E. S., Holland, J. B., Bradbury, P. J., Acharya, C. B., Brown, P. J., Browne, C., . . . McMullen, M. D. (2009). The Genetic Architecture of Maize Flowering Time. *Science, 325*(5941), 714-718. doi:10.1126/science.1174276

Budak, H., Hussain, B., Khan, Z., Ozturk, N. Z., & Ullah, N. (2015). From Genetics to Functional Genomics: Improvement in Drought Signaling and Tolerance in Wheat. *Frontiers in Plant Science, 6*. doi:ARTN 101210.3389/fpls.2015.01012

Burgess, A. J., Retkute, R., Herman, T., & Murchie, E. H. (2017). Exploring Relationships between Canopy Architecture, Light Distribution, and Photosynthesis in Contrasting Rice Genotypes Using 3D Canopy Reconstruction. *Frontiers in Plant Science, 8*. doi:ARTN 73410.3389/fpls.2017.00734

Burgess, A. J., Retkute, R., Pound, M. P., Foulkes, J., Preston, S. P., Jensen, O. E., . . . Murchie, E. H. (2015). High-Resolution Three-Dimensional Structural Data Quantify the Impact of

Photoinhibition on Long-Term Carbon Gain in Wheat Canopies in the Field. *Plant Physiology, 169*(2), 1192-+. doi:10.1104/pp.15.00722

Burgess, A. J., Retkute, R., Pound, M. P., Mayes, S., & Murchie, E. H. (2017). Image-based 3D canopy reconstruction to determine potential productivity in complex multi-species crop systems. *Annals of Botany, 119*(4), 517-532. doi:10.1093/aob/mcw242

Buschmann, A. H., Camus, C., Infante, J., Neori, A., Israel, A., Hernandez-Gonzalez, M. C., . . . Critchley, A. T. (2017). Seaweed production: overview of the global state of exploitation, farming and emerging research activity. *European Journal of Phycology, 52*(4), 391-406. doi:10.1080/09670262.2017.1365175

Butelli, E., Titta, L., Giorgio, M., Mock, H. P., Matros, A., Peterek, S., . . . Martin, C. (2008). Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nature Biotechnology, 26*(11), 1301-1308. doi:10.1038/nbt.1506

Camargo, E. L. O., Ployet, R., Cassan-Wang, H., Mounet, F., & Grima-Pettenati, J. (2019). Digging in wood: New insights in the regulation of wood formation in tree species. *Molecular Physiology and Biotechnology of Trees, 89*, 201-233. doi:10.1016/bs.abr.2018.11.007

Campbell, G. S., & Norman, J. M. (1989). The description and measurement of plant canopy structure. In G. Russell, B. Marshall, & P. G. Jarvis (Eds.), *Plant Canopies Their Growth Form and Function* (pp. 1–19). Retrieved from http://books.google.com/books?hl=en&lr=&id=qDSEjj91qNkC&oi=fnd&pg=PA1&dq=The+description+and+measurement+of+plant+canopy+structure&ots=7PwH40VhlY&sig=TyN-_KSEVQqqqYaGthOO_B66Cfg

Cardenas, P. D., Sonawane, P. D., Pollier, J., Vanden Bossche, R., Dewangan, V., Weithorn, E., . . . Aharoni, A. (2016). GAME9 regulates the biosynthesis of steroidal alkaloids and upstream isoprenoids in the plant mevalonate pathway. *Nature Communications, 7*. doi:ARTN 1065410.1038/ncomms10654

Carmo-Silva, A. E., Powers, S. J., Keys, A. J., Arrabaca, M. C., & Parry, M. A. J. (2008). Photorespiration in C(4) grasses remains slow under drought conditions. *Plant Cell and Environment, 31*(7), 925-940. doi:10.1111/j.1365-3040.2008.01805.x

Cavazzoni, J., Volk, T., Tubiello, F., & Monje, O. (2002). Modelling the effect of diffuse light on canopy photosynthesis in controlled environments. *Proceedings of the 4th International Symposium on Models for Plant Growth and Control in Greenhouses: Modeling for the 21st Century - Agronomic and Greenhouse Crop Models*(593), 39-45. doi:DOI 10.17660/ActaHortic.2002.593.4

Cermak, T., Baltes, N. J., Cegan, R., Zhang, Y., & Voytas, D. F. (2015). High-frequency, precise modification of the tomato genome. *Genome Biology, 16*. doi:ARTN 23210.1186/s13059-015-0796-9

Cescatti, A. (1998). Effects of needle clumping in shoots and crowns on the radiative regime of a Norway spruce canopy. *Annales Des Sciences Forestieres, 55*(1-2), 89-102. doi:DOI 10.1051/forest:19980106

Cescatti, A., & Zorer, R. (2003). Structural acclimation and radiation regime of silver fir (Abies alba Mill.) shoots along a light gradient. *Plant Cell and Environment, 26*(3), 429-442. doi:DOI 10.1046/j.1365-3040.2003.00974.x

Chaikam, V., Molenaar, W., Melchinger, A. E., & Boddupalli, P. M. (2019). Doubled haploid technology for line development in maize: technical advances and prospects. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik, 132*(12), 3227-3243. doi:10.1007/s00122-019-03433-x

Chanoca, A., de Vries, L., & Boerjan, W. (2019). Lignin Engineering in Forest Trees. *Frontiers in Plant Science, 10*. doi:ARTN 91210.3389/fpls.2019.00912

Chelle, M. (2005). Phylloclimate or the climate perceived by individual plant organs: What is it? How to model it? What for? *New Phytologist, 166*(3), 781-790. doi:10.1111/j.1469-8137.2005.01350.x

Chelle, M., & Andrieu, B. (2007). Modelling the light environment of virtual crop canopies. *Functional-Structural Plant Modelling in Crop Production, 22*, 75-89. Retrieved from <Go to ISI>://WOS:000252961900007

Chelle, M., Andrieu, B., & Bouatouch, K. (1998). Nested radiosity for plant canopies. *Visual Computer, 14*(3), 109-125. doi:DOI 10.1007/s003710050127

Chen, F., & Dixon, R. A. (2007). Lignin modification improves fermentable sugar yields for biofuel production. *Nature Biotechnology, 25*(7), 759-761. doi:10.1038/nbt1316

Chen, G., Hu, Q. D., Luo, L., Yang, T. Y., Zhang, S., Hu, Y. B., . . . Xu, G. H. (2015). Rice potassium transporter OsHAK1 is essential for maintaining potassium-mediated growth and functions in salt tolerance over low and high potassium concentration ranges. *Plant Cell and Environment, 38*(12), 2747-2765. doi:10.1111/pce.12585

Chen, J. G., Zhang, Y., Tan, Y. W., Zhang, M., Zhu, L. L., Xu, G. H., & Fan, X. R. (2016). Agronomic nitrogen-use efficiency of rice can be increased by driving OsNRT2.1 expression with the OsNAR2.1 promoter. *Plant Biotechnology Journal, 14*(8), 1705-1715. doi:10.1111/pbi.12531

Chen, J. M., Rich, P. M., Gower, S. T., Norman, J. M., & Plummer, S. (1997). Leaf area index of boreal forests: Theory, techniques, and measurements. *Journal of Geophysical Research-Atmospheres, 102*(D24), 29429-29443. doi:Doi 10.1029/97jd01107

Chen, Q. W., Soulay, F., Saudemont, B., Elmayan, T., Marmagne, A., & Masclaux-Daubresse, C. (2019). Overexpression of ATG8 in Arabidopsis Stimulates Autophagic Activity and Increases Nitrogen Remobilization Efficiency and Grain Filling. *Plant and Cell Physiology, 60*(2), 343-352. doi:10.1093/pcp/pcy214

Chopin, T., & Sawhney, M. (2009). Seaweeds and their mariculture. Steele JH, Thorpe SA, Turekian KK, editors. *The encyclopedia of ocean sciences*.

Cieslak, M., Lemieux, C., Hanan, J., & Prusinkiewicz, P. (2008). Quasi-Monte Carlo simulation of the light environment of plants. *Functional Plant Biology, 35*(9-10), 837-849. doi:10.1071/Fp08082

Clearwater, M. J., & Gould, K. S. (1995). Leaf Orientation and Light Interception by Juvenile Pseudopanax-Crassifolius(Cunn) C-Koch in a Partially Shaded Forest Environment. *Oecologia, 104*(3), 363-371. doi:Doi 10.1007/Bf00328372

Clemente, A., Arques, M. C., Dalmais, M., Le Signor, C., Chinoy, C., Olias, R., . . . Domoney, C. (2015). Eliminating Anti-Nutritional Plant Food Proteins: The Case of Seed Protease Inhibitors in Pea (vol 10, e0134634, 2015). *PLoS ONE, 10*(9). doi:ARTN e013803910.1371/journal.pone.0138039

Cohan, J. P., Le Souder, C., Guicherd, C., Lorgeou, J., Du Cheyron, P., Bonnefoy, M., . . . Laurent, F. (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:UNSP 10758810.1016/j.fcr.2019.107588

Collen, J., & Davison, I. R. (1999). Reactive oxygen metabolism in intertidal Fucus spp. (Phaeophyceae). *Journal of Phycology, 35*(1), 62-69. doi:DOI 10.1046/j.1529-8817.1999.3510062.x

Connolly, E. L., Campbell, N. H., Grotz, N., Prichard, C. L., & Guerinot, M. L. (2003). Overexpression of the FRO2 ferric chelate reductase confers tolerance to growth on low iron and uncovers posttranscriptional control. *Plant Physiology, 133*(3), 1102-1110. doi:10.1104/pp.103.025122

Cormier, F., Foulkes, J., Hirel, B., Gouache, D., Moenne-Loccoz, Y., & Le Gouis, J. (2016). Breeding for increased nitrogen-use efficiency: a review for wheat (T.aestivum L.). *Plant Breeding, 135*(3), 255-278. doi:10.1111/pbr.12371

Cormier, F., Throude, M., Ravel, C., Le Gouis, J., Leveugle, M., Lafarge, S., . . . Praud, S. (2015). Detection of NAM-A1 Natural Variants in Bread Wheat Reveals Differences in Haplotype Distribution between a Worldwide Core Collection and European Elite Germplasm. *Agronomy-Basel, 5*(2), 143-151. doi:10.3390/agronomy5020143

Cosgrove, D. J. (2000a). Loosening of plant cell walls by expansins. *Nature, 407*(6802), 321-326. doi:10.1038/35030000

Cosgrove, D. J. (2000b). New genes and new biological roles for expansins. *Current Opinion in Plant Biology, 3*(1), 73-78. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10679451

Cosgrove, D. J. (2018). Diffuse Growth of Plant Cell Walls. *Plant Physiology, 176*(1), 16-27. doi:10.1104/pp.17.01541

Coupel-Ledru, A., Lebon, E., Christophe, A., Gallo, A., Gago, P., Pantin, F., . . . Simonneau, T. (2016). Reduced nighttime transpiration is a relevant breeding target for high water-use efficiency in grapevine. *Proceedings of the National Academy of Sciences of the United States of America, 113*(32), 8963-8968. doi:10.1073/pnas.1600826113

Covshoff, S., & Hibberd, J. M. (2012). Integrating C-4 photosynthesis into C-3 crops to increase yield potential. *Current Opinion in Biotechnology, 23*(2), 209-214. doi:10.1016/j.copbio.2011.12.011

Coyne, D. P. (1980). Modification of Plant Architecture and Crop Yield by Breeding. *Hortscience, 15*(3), 244-247. Retrieved from <Go to ISI>://WOS:A1980JZ83500006

Crafts-Brandner, S. J., & Salvucci, M. E. (2000). Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO2. *Proceedings of the National Academy of Sciences of the United States of America, 97*(24), 13430-13435. doi:DOI 10.1073/pnas.230451497

Cutler, S. R., Rodriguez, P. L., Finkelstein, R. R., & Abrams, S. R. (2010). Abscisic Acid: Emergence of a Core Signaling Network. *Annual Review of Plant Biology, Vol 61, 61*, 651-679. doi:10.1146/annurev-arplant-042809-112122

Dalal, J., Lopez, H., Vasani, N. B., Hu, Z. H., Swift, J. E., Yalamanchili, R., . . . Sederoff, H. W. (2015). A photorespiratory bypass increases plant growth and seed yield in biofuel crop Camelina sativa. *Biotechnology for Biofuels, 8*. doi:ARTN 17510.1186/s13068-015-0357-1

de Pury, D. G. G., & Farquhar, G. D. (1997). Simple scaling of photosynthesis from leaves to canopies without the errors of big-leaf models. *Plant Cell and Environment, 20*(5), 537-557. doi:DOI 10.1111/j.1365-3040.1997.00094.x

de Pury, D. G. G., & Farquhar, G. D. (1999). A commentary on the use of a sun/shade model to scale from the leaf to a canopy. *Agricultural and Forest Meteorology, 95*(4), 257-260. Retrieved from <Go to ISI>://WOS:000082008000004

Dedryver, C. A., & Tanguy, S. (1984). Biology of Cereal Aphids in the West of France .5. Effect of the Sowing Date of Winter-Wheat on the Infestation of Fields by Rhopalosiphum-Padi (L), Sitobion-Avenae (F) and Metopolophium-Dirhodum (Wlk) and on the Development of Aphid Populations during the Following Spring. *Agronomie, 4*(8), 711-719. doi:DOI 10.1051/agro:19840802

Deery, D., Jimenez-Berni, J., Jones, H., Sirault, X., & Furbank, R. T. (2014). Proximal Remote Sensing Buggies and Potential Applications for Field-Based Phenotyping. *Agronomy*, *5*, 349–379.

Demirer, G. S., Zhang, H., Matos, J. L., Goh, N. S., Cunningham, F. J., Sung, Y., . . . Landry, M. P. (2019). High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plants. *Nature nanotechnology, 14*(5), 456-464. doi:10.1038/s41565-019-0382-5

Demura, T., & Ye, Z. H. (2010). Regulation of plant biomass production. *Current Opinion in Plant Biology, 13*(3), 299-304. doi:10.1016/j.pbi.2010.03.002

Dietrich, D., Pang, L., Kobayashi, A., Fozard, J. A., Boudolf, V., Bhosale, R., . . . Bennett, M. J. (2017). Root hydrotropism is controlled via a cortex-specific growth mechanism. *Nature Plants, 3*(6). doi:ARTN 1705710.1038/nplants.2017.57

Diniz, J. M. B. F., Gil, M. H., & Castro, J. A. A. M. (2004). Hornification - its origin and interpretation in wood pulps. *Wood Science and Technology, 37*(6), 489-494. doi:10.1007/s00226-003-0216-2

Diretto, G., Welsch, R., Tavazza, R., Mourgues, F., Pizzichini, D., Beyer, P., & Giuliano, G. (2007). Silencing of beta-carotene hydroxylase increases total carotenoid and beta-carotene levels in potato tubers. *BMC Plant Biology, 7*. doi:Artn 1110.1186/1471-2229-7-11

Djanaguiraman, M., Boyle, D. L., Welti, R., Jagadish, S. V. K., & Prasad, P. V. V. (2018). Decreased photosynthetic rate under high temperature in wheat is due to lipid desaturation, oxidation, acylation, and damage of organelles. *BMC Plant Biology, 18*. doi:ARTN 5510.1186/s12870-018-1263-z

Dobrenel, T., Caldana, C., Hanson, J., Robaglia, C., Vincentz, M., Veit, B., & Meyer, C. (2016). TOR Signaling and Nutrient Sensing. *Annual Review of Plant Biology, Vol 67, 67*, 261-285. doi:10.1146/annurev-arplant-043014-114648

Dodd, I. C., & Perez-Alfocea, F. (2012). Microbial amelioration of crop salinity stress. *Journal of Experimental Botany, 63*(9), 3415-3428. doi:10.1093/jxb/ers033

Donnelly, P. M., Bonetta, D., Tsukaya, H., Dengler, R. E., & Dengler, N. G. (1999). Cell cycling and cell enlargement in developing leaves of *Arabidopsis*. *Developmental Biology, 215*(2), 407-419. doi:10.1006/dbio.1999.9443

Drouet, J. L., & Pages, L. (2007). GRAAL-CN: A model of GRowth, Architecture and ALlocation of Carbon and Nitrogen dynamics within whole plants formalised at the organ level. *Ecological Modelling, 206*(3-4), 231-249. doi:10.1016/j.ecolmodel.2007.03.036

Efroni, I., Eshed, Y., & Lifschitz, E. (2010). Morphogenesis of simple and compound leaves: a critical review. *Plant Cell, 22*(4), 1019-1032. doi:10.1105/tpc.109.073601

Ermakova, M., Danila, F. R., Furbank, R. T., & von Caemmerer, S. (2019). On the road to C4 rice: advances and perspectives. *The Plant journal : for cell and molecular biology*. doi:10.1111/tpj.14562

Evers, J. B., Vos, J., Fournier, C., Andrieu, B., Chelle, M., & Struik, P. C. (2005). Towards a generic architectural model of tillering in Gramineae, as exemplified by spring wheat (Triticum aestivum). *The New phytologist, 166*(3), 801-812. doi:10.1111/j.1469-8137.2005.01337.x

Fan, S. C., Lin, C. S., Hsu, P. K., Lin, S. H., & Tsay, Y. F. (2009). The Arabidopsis Nitrate Transporter NRT1.7, Expressed in Phloem, Is Responsible for Source-to-Sink Remobilization of Nitrate. *Plant Cell, 21*(9), 2750-2761. doi:10.1105/tpc.109.067603

Fan, X. R., Tang, Z., Tan, Y. W., Zhang, Y., Luo, B. B., Yang, M., . . . Xu, G. H. (2016). Overexpression of a pH-sensitive nitrate transporter in rice increases crop yields. *Proceedings of the National Academy of Sciences of the United States of America, 113*(26), 7118-7123. doi:10.1073/pnas.1525184113

FAO, Commission on Genetic Resources for Food and Agriculture (2010) The second report on the state of the world's plant genetic resources for food and agriculture. Ro*me*. Retrieved from http://www.fao.org/3/i1500e/i1500e.pdfFarque, L., Sinoquet, H., & Colin, F. (2001). Canopy structure and light interception in Quercus petraea seedlings in relation to light regime and plant density. *Tree Physiology, 21*(17), 1257-1267. doi:10.1093/treephys/21.17.1257

Farquhar, G. D., Caemmerer, S. V., & Berry, J. A. (1980). A Biochemical-Model of Photosynthetic Co2 Assimilation in Leaves of C-3 Species. *Planta, 149*(1), 78-90. doi:Doi 10.1007/Bf00386231

Fauser, F., Schiml, S., & Puchta, H. (2014). Both CRISPR/Cas-based nucleases and nickases can be used efficiently for genome engineering in Arabidopsis thaliana. *The Plant journal : for cell and molecular biology, 79*(2), 348-359. doi:10.1111/tpj.12554

Feng, Z., Zhang, B., Ding, W., Liu, X., Yang, D. L., Wei, P., . . . Zhu, J. K. (2013). Efficient genome editing in plants using a CRISPR/Cas system. *Cell Research, 23*(10), 1229-1232. doi:10.1038/cr.2013.114

Ferdouse, F., Holdt, S. L., Smith, R., Murua, P., & Yang, Z. (2018). The global status of seaweed production, trade and utilization. *Food and Agriculture Organization of the United Nations*.

Fischer, R. A. (2007). Understanding the physiological basis of yield potential in wheat. *Journal of Agricultural Science, 145*, 99-113. doi:10.1017/S0021859607006843

Fischer, R. A. (2011). Wheat physiology: a review of recent developments. *Crop & Pasture Science, 62*(2), 95-114. doi:10.1071/Cp10344

Flowers, T. J., Koyama, M. L., Flowers, S. A., Sudhakar, C., Singh, K. P., & Yeo, A. R. (2000). QTL: their place in engineering tolerance of rice to salinity. *Journal of Experimental Botany, 51*(342), 99-106. doi:DOI 10.1093/jexbot/51.342.99

Foulkes, M. J., Hawkesford, M. J., Barraclough, P. B., Holdsworth, M. J., Kerr, S., Kightley, S., & Shewry, P. R. (2009). Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. *Field Crops Research, 114*(3), 329-342. doi:10.1016/j.fcr.2009.09.005

Fourcaud, T., Zhang, X., Stokes, A., Lambers, H., & Korner, C. (2008). Plant growth modelling and applications: the increasing importance of plant architecture in growth models. *Annals of Botany, 101*(8), 1053-1063. doi:10.1093/aob/mcn050

Fowler, D., Pyle, J. A., Raven, J. A., & Sutton, M. A. (2013). The global nitrogen cycle in the twenty-first century: introduction. *Philosophical Transactions of the Royal Society B-Biological Sciences, 368*(1621). doi:ARTN 2013016510.1098/rstb.2013.0165

Freixes, S., Thibaud, M. C., Tardieu, F., & Muller, B. (2002). Root elongation and branching is related to local hexose concentration in Arabidopsis thaliana seedlings. *Plant Cell and Environment, 25*(10), 1357-1366. doi:DOI 10.1046/j.1365-3040.2002.00912.x

Fricke, W., Akhiyarova, G., Veselov, D., & Kudoyarova, G. (2004). Rapid and tissue-specific changes in ABA and in growth rate in response to salinity in barley leaves. *Journal of Experimental Botany, 55*(399), 1115-1123. doi:10.1093/jxb/erh117

Fukuda, A., Nakamura, A., Tagiri, A., Tanaka, H., Miyao, A., Hirochika, H., & Tanaka, Y. (2004). Function, intracellular localization and the importance in salt tolerance of a vacuolar Na+/H+ antiporter from rice. *Plant and Cell Physiology, 45*(2), 146-159. doi:DOI 10.1093/pcp/pch014

Gabur, I., Chawla, H. S., Snowdon, R. J., & Parkin, I. A. P. (2019). Connecting genome structural variation with complex traits in crop plants. *Theoretical and Applied Genetics, 132*(3), 733-750. doi:10.1007/s00122-018-3233-0

Gallardo, K., Besson, A., Klein, A., Le Signor, C., Aubert, G., Henriet, C., . . . Burstin, J. (2019). Transcriptional Reprogramming of Pea Leaves at Early Reproductive Stages. *Frontiers in Plant Science, 10*. doi:ARTN 101410.3389/fpls.2019.01014

Gamuyao, R., Chin, J. H., Pariasca-Tanaka, J., Pesaresi, P., Catausan, S., Dalid, C., . . . Heuer, S. (2012). The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. *Nature, 488*(7412), 535-+. doi:10.1038/nature11346

Gao, Y. Z., & Lynch, J. P. (2016). Reduced crown root number improves water acquisition under water deficit stress in maize (Zea mays L.). *Journal of Experimental Botany, 67*(15), 4545-4557. doi:10.1093/jxb/erw243

Garapati, P., Feil, R., Lunn, J. E., Van Dijck, P., Balazadeh, S., & Mueller-Roeber, B. (2015). Transcription Factor Arabidopsis Activating Factor1 Integrates Carbon Starvation Responses with Trehalose Metabolism. *Plant Physiology, 169*(1), 379-390. doi:10.1104/pp.15.00917

Gaxiola, R. A., Sanchez, C. A., Paez-Valencia, J., Ayre, B. G., & Elser, J. J. (2012). Genetic Manipulation of a "Vacuolar" H+-PPase: From Salt Tolerance to Yield Enhancement under Phosphorus-Deficient Soils. *Plant Physiology, 159*(1), 3-11. doi:10.1104/pp.112.195701

Ge, L., Yu, J., Wang, H., Luth, D., Bai, G., Wang, K., & Chen, R. (2016). Increasing seed size and quality by manipulating *BIG SEEDS1* in legume species. *Proceedings of the National Academy of Sciences of the United States of America, 113*(44), 12414-12419. doi:10.1073/pnas.1611763113

Geisler, M., Nadeau, J., & Sack, F. D. (2000). Oriented asymmetric divisions that generate the stomatal spacing pattern in Arabidopsis are disrupted by the *too many mouths* mutation. *Plant Cell, 12*(11), 2075-2086. doi:10.1105/tpc.12.11.2075

Gest, N., Garchery, C., Gautier, H., Jimenez, A., & Stevens, R. (2013). Light-dependent regulation of ascorbate in tomato by a monodehydroascorbate reductase localized in peroxisomes and the cytosol. *Plant Biotechnology Journal, 11*(3), 344-354. doi:10.1111/pbi.12020

Ghosh, S., Watson, A., Gonzalez-Navarro, O. E., Ramirez-Gonzalez, R. H., Yanes, L., Mendoza-Suarez, M., . . . Hickey, L. T. (2018). Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. *Nature Protocols, 13*(12), 2944-2963. doi:10.1038/s41596-018-0072-z

Gibbs, J. A., Pound, M., French, A., Wells, D., Murchie, E., & Pridmore, T. (2019). Active Vision and Surface Reconstruction for 3D Plant Shoot Modelling. *IEEE/ACM Transactions on Computational Biology and Bioinformatics*. doi:10.1109/TCBB.2019.2896908

Gilles, L. M., Khaled, A., Laffaire, J. B., Chaignon, S., Gendrot, G., Laplaige, J., . . . Widiez, T. (2017). Loss of pollen-specific phospholipase NOT LIKE DAD triggers gynogenesis in maize. *The EMBO journal, 36*(6), 707-717. doi:10.15252/embj.201796603

Goel, N. S., Rozehnal, I., & Thompson, R. L. (1991). A Computer-Graphics Based Model for Scattering from Objects of Arbitrary Shapes in the Optical-Region. *Remote Sensing of Environment, 36*(2), 73-104. doi:Doi 10.1016/0034-4257(91)90032-2

Golicz, A. A., Batley, J., & Edwards, D. (2016). Towards plant pangenomics. *Plant Biotechnology Journal, 14*(4), 1099-1105. doi:10.1111/pbi.12499

Gonzalez, E., Solano, R., Rubio, V., Leyva, A., & Paz-Ares, J. (2005). PHOSPHATE TRANSPORTER TRAFFIC FACILITATOR1 is a plant-specific SEC12-related protein that enables the endoplasmic reticulum exit of a high-affinity phosphate transporter in Arabidopsis. *Plant Cell, 17*(12), 3500-3512. doi:10.1105/tpc.105.036640

Gonzalez, N., De Bodt, S., Sulpice, R., Jikumaru, Y., Chae, E., Dhondt, S., . . . Inze, D. (2010). Increased leaf size: different means to an end. *Plant Physiology, 153*(3), 1261-1279. doi:10.1104/pp.110.156018

Gonzalez, N., Pauwels, L., Baekelandt, A., De Milde, L., Van Leene, J., Besbrugge, N., . . . Inzé, D. (2015). A repressor protein complex regulates leaf growth in Arabidopsis. *Plant Cell, 27*(8), 2273-2287. doi:10.1105/tpc.15.00006

Gonzalez, N., Vanhaeren, H., & Inze, D. (2012). Leaf size control: complex coordination of cell division and expansion. *Trends Plant Sci, 17*(6), 332-340. doi:10.1016/j.tplants.2012.02.003

Gordon-Kamm, B., Sardesai, N., Arling, M., Lowe, K., Hoerster, G., Betts, S., & Jones, A. T. (2019). Using Morphogenic Genes to Improve Recovery and Regeneration of Transgenic Plants. *Plants (Basel, Switzerland), 8*(2). doi:10.3390/plants8020038

Gouache, D., Bogard, M., Pegard, M., Thepot, S., Garcia, C., Hourcade, D., . . . Le Bris, X. (2017). Bridging the gap between ideotype and genotype: Challenges and prospects for modelling as exemplified by the case of adapting wheat (Triticum aestivum L.) phenology to climate change in France. *Field Crops Research, 202*, 108-121. doi:10.1016/j.fcr.2015.12.012

Gouache, D., Le Bris, X., Bogard, M., Deudon, O., Page, C., & Gate, P. (2012). Evaluating agronomic adaptation options to increasing heat stress under climate change during wheat grain filling in France. *European Journal of Agronomy, 39*, 62-70. doi:10.1016/j.eja.2012.01.009

Gray, S. B., & Brady, S. M. (2016). Plant developmental responses to climate change. *Developmental Biology, 419*(1), 64-77. doi:10.1016/j.ydbio.2016.07.023

Gregersen, P. L., Holm, P. B., & Krupinska, K. (2008). Leaf senescence and nutrient remobilisation in barley and wheat. *Plant Biology, 10*, 37-49. doi:10.1111/j.1438-8677.2008.00114.x

Griffiths, C. A., Sagar, R., Geng, Y., Primavesi, L. F., Patel, M. K., Passarelli, M. K., . . . Davis, B. G. (2016). Chemical intervention in plant sugar signalling increases yield and resilience. *Nature, 540*(7634), 574-+. doi:10.1038/nature20591

Grumet, R., Colle, M., Ando, K., Xie, D. S., Havenga, L., & Switzenberg, J. A. (2013). Modified plant architecture to enhance crop disease control: genetic control and possible value of upright fruit position in cucumber. *European Journal of Plant Pathology, 135*(3), 545-560. doi:10.1007/s10658-012-0087-1

Guo, H. Y., Wang, Y. C., Wang, L. Q., Hu, P., Wang, Y. M., Jia, Y. Y., . . . Yang, C. P. (2017). Expression of the MYB transcription factor gene BplMYB46 affects abiotic stress tolerance

and secondary cell wall deposition in Betula platyphylla. *Plant Biotechnology Journal, 15*(1), 107-121. doi:10.1111/pbi.12595

Guo, T. L., Wang, N., Xue, Y. C., Guan, Q. M., van Nocker, S., Liu, C. H., & Ma, F. W. (2019). Overexpression of the RNA binding protein MhYTP1 in transgenic apple enhances drought tolerance and WUE by improving ABA level under drought condition. *Plant Science, 280*, 397-407. doi:10.1016/j.plantsci.2018.11.018

Guo, Y. F., & Gan, S. S. (2014). Translational researches on leaf senescence for enhancing plant productivity and quality. *Journal of Experimental Botany, 65*(14), 3901-3913. doi:10.1093/jxb/eru248

Gutschick, V. P., & Wiegel, F. W. (1988). Optimizing the Canopy Photosynthetic Rate by Patterns of Investment in Specific Leaf Mass. *American Naturalist, 132*(1), 67-86. doi:Doi 10.1086/284838

Hagenblad, J., Asplund, L., Balfourier, F., Ravel, C., & Leino, M. W. (2012). Strong presence of the high grain protein content allele of NAM-B1 in Fennoscandian wheat. *Theoretical and Applied Genetics, 125*(8), 1677-1686. doi:10.1007/s00122-012-1943-2

Hamilton, E. S., Jensen, G. S., Maksaev, G., Katims, A., Sherp, A. M., & Haswell, E. S. (2015). Mechanosensitive channel MSL8 regulates osmotic forces during pollen hydration and germination. *Science, 350*(6259), 438-441. doi:10.1126/science.aac6014

Hammond, E. T., Andrews, T. J., Mott, K. A., & Woodrow, I. E. (1998). Regulation of Rubisco activation in antisense plants of tobacco containing reduced levels of Rubisco activase. *The Plant journal : for cell and molecular biology, 14*(1), 101-110. doi:10.1046/j.1365-313X.1998.00103.x

Hane, J. K., Ming, Y., Kamphuis, L. G., Nelson, M. N., Garg, G., Atkins, C. A., . . . Singh, K. B. (2017). A comprehensive draft genome sequence for lupin (Lupinus angustifolius), an emerging health food: insights into plant-microbe interactions and legume evolution. *Plant Biotechnology Journal, 15*(3), 318-330. doi:10.1111/pbi.12615

Harrison, E. H. (2005). Mechanisms of digestion and absorption of dietary vitamin A. *Annual Review of Nutrition, 25*, 87-103. doi:10.1146/annurev.nutr.25.050304.092614

Haslam, R. P., Sayanova, O., Kim, H. J., Cahoon, E. B., & Napier, J. A. (2016). Synthetic redesign of plant lipid metabolism. *Plant Journal, 87*(1), 76-86. doi:10.1111/tpj.13172

Hatfield, J. L., Boote, K. J., Kimball, B. A., Ziska, L. H., Izaurralde, R. C., Ort, D., . . . Wolfe, D. (2011). Climate Impacts on Agriculture: Implications for Crop Production. *Agronomy Journal, 103*(2), 351-370. doi:10.2134/agronj2010.0303

Havaux, M., & Niyogi, K. K. (1999). The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. *Proceedings of the National Academy of Sciences of the United States of America, 96*(15), 8762-8767. doi:DOI 10.1073/pnas.96.15.8762

He, R. R., Zhuang, Y., Cai, Y. M., Aguero, C. B., Liu, S. L., Wu, J., . . . Zhang, Y. L. (2018). Overexpression of 9-cis-Epoxycarotenoid Dioxygenase Cisgene in Grapevine Increases Drought Tolerance and Results in Pleiotropic Effects. *Frontiers in Plant Science, 9*. doi:ARTN 97010.3389/fpls.2018.00970

Hematy, K., Sado, P. E., Van Tuinen, A., Rochange, S., Desnos, T., Balzergue, S., . . . Hofte, H. (2007). A receptor-like kinase mediates the response of Arabidopsis cells to the inhibition of cellulose synthesis. *Current Biology, 17*(11), 922-931. doi:10.1016/j.cub.2007.05.018

Hengl, T., de Jesus, J. M., MacMillan, R. A., Batjes, N. H., & Heuvelink, G. B. M. (2014). SoilGrids1km - Global Soil Information Based on Automated Mapping (vol 9, e105992, 2014). *PLoS ONE, 9*(12). doi:ARTN e11478810.1371/journal.pone.0114788

Hepworth, J., & Lenhard, M. (2014). Regulation of plant lateral-organ growth by modulating cell number and size. *Curr Opin Plant Biol, 17*, 36-42. doi:10.1016/j.pbi.2013.11.005

Herbert, T. J. (1991). Variation in Interception of the Direct Solar Beam by Top Canopy Layers. *Ecology, 72*(1), 17-22. doi:Doi 10.2307/1938897

Hickey, L. T., A, N. H., Robinson, H., Jackson, S. A., Leal-Bertioli, S. C. M., Tester, M., . . . Wulff, B. B. H. (2019). Breeding crops to feed 10 billion. *Nature Biotechnology, 37*(7), 744-754. doi:10.1038/s41587-019-0152-9

Hinsinger, P., Betencourt, E., Bernard, L., Brauman, A., Plassard, C., Shen, J. B., . . . Zhang, F. S. (2011). P for Two, Sharing a Scarce Resource: Soil Phosphorus Acquisition in the Rhizosphere of Intercropped Species. *Plant Physiology, 156*(3), 1078-1086. doi:10.1104/pp.111.175331

Holm, P. B., Kristiansen, K. N., & Pedersen, H. B. (2002). Transgenic approaches in commonly consumed cereals to improve iron and zinc content and bioavailability. *Journal of Nutrition, 132*(3), 514s-516s. Retrieved from <Go to ISI>://WOS:000174189800034

Horie, T., Kaneko, T., Sugimoto, G., Sasano, S., Panda, S. K., Shibasaka, M., & Katsuhara, M. (2011). Mechanisms of Water Transport Mediated by PIP Aquaporins and Their Regulation Via Phosphorylation Events Under Salinity Stress in Barley Roots. *Plant and Cell Physiology, 52*(4), 663-675. doi:10.1093/pcp/pcr027

Hotz, C., & McClafferty, B. (2007). From harvest to health: Challenges for developing biofortified staple foods and determining their impact on micronutrient status. *Food and Nutrition Bulletin, 28*(2), S271-S279. doi:Doi 10.1177/15648265070282s206

Houle, D., Govindaraju, D. R., & Omholt, S. (2010). Phenomics: the next challenge. *Nature Reviews Genetics, 11*(12), 855-866. doi:10.1038/nrg2897

Hu, B., Wang, W., Ou, S. J., Tang, J. Y., Li, H., Che, R. H., . . . Chu, C. C. (2015). Variation in NRT1.1B contributes to nitrate-use divergence between rice subspecies. *Nature Genetics, 47*(7), 834-+. doi:10.1038/ng.3337

Hu, M. Y., Zhao, X. Q., Liu, Q., Hong, X., Zhang, W., Zhang, Y. J., . . . Tong, Y. P. (2018). Transgenic expression of plastidic glutamine synthetase increases nitrogen uptake and yield in wheat. *Plant Biotechnology Journal, 16*(11), 1858-1867. doi:10.1111/pbi.12921

Hu, Y., Wu, Q. Y., Peng, Z., Sprague, S. A., Wang, W., Park, J., . . . Park, S. (2017). Silencing of OsGRXS17 in rice improves drought stress tolerance by modulating ROS accumulation and stomatal closure. *Scientific Reports, 7*. doi:ARTN 1595010.1038/s41598-017-16230-7

Hubbart, S., Ajigboye, O. O., Horton, P., & Murchie, E. H. (2012). The photoprotective protein PsbS exerts control over CO2 assimilation rate in fluctuating light in rice. *Plant Journal, 71*(3), 402-412. doi:10.1111/j.1365-313X.2012.04995.x

Hubbart, S., Smillie, I. R. A., Heatley, M., Swarup, R., Foo, C. C., Zhao, L., & Murchie, E. H. (2018). Enhanced thylakoid photoprotection can increase yield and canopy radiation use efficiency in rice. *Communications Biology, 1*. doi:ARTN 2210.1038/s42003-018-0026-6

Hufford, M. B., Xu, X., van Heerwaarden, J., Pyhajarvi, T., Chia, J. M., Cartwright, R. A., . . . Ross-Ibarra, J. (2012). Comparative population genomics of maize domestication and improvement. *Nature Genetics, 44*(7), 808-U118. doi:10.1038/ng.2309

Hurd, C. L., Harrison, P. J., Bischof, K., & Lobban, C. S. (2014). Seaweed ecology and physiology. Cambridge University Press.

Hwang, E. K., Yotsukura, N., Pang, S. J., Su, L., & Shan, T. F. (2019). Seaweed breeding programs and progress in eastern Asian countries. *Phycologia, 58*(5), 484-495. doi:10.1080/00318884.2019.1639436

Itkin, M., Heinig, U., Tzfadia, O., Bhide, A. J., Shinde, B., Cardenas, P. D., . . . Aharoni, A. (2013). Biosynthesis of Antinutritional Alkaloids in Solanaceous Crops Is Mediated by Clustered Genes. *Science, 341*(6142), 175-179. doi:10.1126/science.1240230

Jagadish, S. V. K., Murty, M. V. R., & Quick, W. P. (2015). Rice responses to rising temperatures - challenges, perspectives and future directions. *Plant Cell and Environment, 38*(9), 1686-1698. doi:10.1111/pce.12430

Jain, A. K., & Nessler, C. L. (2000). Metabolic engineering of an alternative pathway for ascorbic acid biosynthesis in plants. *Molecular Breeding, 6*(1), 73-78. doi:Doi 10.1023/A:1009680818138

James, D., Borphukan, B., Fartyal, D., Achary, V.M.M., Reddy, M.K. (2018) Transgenic manipulation of glutamine synthetase: a target with untapped potential in various aspects of crop improvement. In Gosal, S.S. & Wani, S.H. (eds.). Biotechnology of crop improvement. pp 367-416. Springer International Publishing AG.

Jiang, L., Wang, W. X., Lian, T., & Zhang, C. Y. (2017). Manipulation of Metabolic Pathways to Develop Vitamin-Enriched Crops for Human Health. *Frontiers in Plant Science, 8*. doi:ARTN 93710.3389/fpls.2017.00937

Jonckheere, I., Fleck, S., Nackaerts, K., Muys, B., Coppin, P., Weiss, M., & Baret, F. (2004). Review of methods for in situ leaf area index determination - Part I. Theories, sensors and hemispherical photography. *Agricultural and Forest Meteorology, 121*(1-2), 19-35. doi:10.1016/j.agrformet.2003.08.027

Jones, T., Lowe, K., Hoerster, G., Anand, A., Wu, E., Wang, N., . . . Gordon-Kamm, W. (2019). Maize Transformation Using the Morphogenic Genes Baby Boom and Wuschel2. *Methods in molecular biology (Clifton, N.J.), 1864*, 81-93. doi:10.1007/978-1-4939-8778-8_6

Jung, G. W., Coyne, D. P., Skroch, P. W., Nienhuis, J., ArnaudSantana, E., Bokosi, J., . . . Kaeppler, S. M. (1996). Molecular markers associated with plant architecture and resistance to common blight, web blight, and rust in common beans. *Journal of the American Society for Horticultural Science, 121*(5), 794-803. doi:Doi 10.21273/Jashs.121.5.794

Kalos, M., & Whitlock, P. (1986). *Monte Carlo methods. Volume I: basics*. Chichester: Wiley.

Kalve, S., De Vos, D., & Beemster, G. T. (2014). Leaf development: a cellular perspective. *Front Plant Sci, 5*, 362. doi:10.3389/fpls.2014.00362

Kanazashi, Y., Hirose, A., Takahashi, I., Mikami, M., Endo, M., Hirose, S., . . . Yamada, T. (2018). Simultaneous site-directed mutagenesis of duplicated loci in soybean using a single guide RNA. *Plant Cell Reports, in press*. doi:10.1007/s00299-018-2251-3. (Accession No. 29333573)

Kebeish, R., Niessen, M., Thiruveedhi, K., Bari, R., Hirsch, H. J., Rosenkranz, R., . . . Peterhansel, C. (2007). Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in Arabidopsis thaliana. *Nature Biotechnology, 25*(5), 593-599. doi:10.1038/nbt1299

Kelliher, T., Starr, D., Richbourg, L., Chintamanani, S., Delzer, B., Nuccio, M. L., . . . Martin, B. (2017). MATRILINEAL, a sperm-specific phospholipase, triggers maize haploid induction. *Nature, 542*(7639), 105-109. doi:10.1038/nature20827

Kelliher, T., Starr, D., Su, X., Tang, G., Chen, Z., Carter, J., . . . Que, Q. (2019). One-step genome editing of elite crop germplasm during haploid induction. *Nature Biotechnology, 37*(3), 287-292. doi:10.1038/s41587-019-0038-x

Khush, G. S. (1996). Prospects of and approaches to increasing the genetic yield potential of rice. In R. Evenson, R. Herdt, & M. Hossain (Eds.), *Rice Research in Asia. Progress and Priorities* (pp. 59–71). Wallingford, UK: CABI.

King, D. A. (1998). Relationship between crown architecture and branch orientation in rain forest trees. *Annals of Botany, 82*(1), 1-7. doi:DOI 10.1006/anbo.1998.0638

Kisko, M., Bouain, N., Safi, A., Medici, A., Akkers, R. C., Secco, D., . . . Rouached, H. (2018). LPCAT1 controls phosphate homeostasis in a zinc-dependent manner. *eLife, 7*. doi:ARTN e3207710.7554/eLife.32077

Kojima, S., Bohner, A., Gassert, B., Yuan, L. X., & von Wiren, N. (2007). AtDUR3 represents the major transporter for high-affinity urea transport across the plasma membrane of nitrogen-deficient Arabidopsis roots. *Plant Journal, 52*(1), 30-40. doi:10.1111/j.1365-313X.2007.03223.x

Koyama, M. L., Levesley, A., Koebner, R. M. D., Flowers, T. J., & Yeo, A. R. (2001). Quantitative trait loci for component physiological traits determining salt tolerance in rice. *Plant Physiology, 125*(1), 406-422. doi:DOI 10.1104/pp.125.1.406

Kretzschmar, T., Pelayo, M. A. F., Trijatmiko, K. R., Gabunada, L. F. M., Alam, R., Jimenez, R., . . . Septiningsih, E. M. (2015). A trehalose-6-phosphate phosphatase enhances anaerobic germination tolerance in rice. *Nature Plants, 1*(9). doi:Artn 1512410.1038/Nplants.2015.124

Krizek, B. A. (2009). Making bigger plants: key regulators of final organ size. *Current Opinion in Plant Biology, 12*(1), 17-22. doi:10.1016/j.pbi.2008.09.006

Kromdijk, J., Glowacka, K., Leonelli, L., Gabilly, S. T., Iwai, M., Niyogi, K. K., & Long, S. P. (2016). Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science (New York, N.Y.), 354*(6314), 857-861. doi:10.1126/science.aai8878

Ksiazkiewicz, M., Nazzicari, N., Yang, H. A., Nelson, M. N., Renshaw, D., Rychel, S., . . . Annicchiarico, P. (2017). A high-density consensus linkage map of white lupin highlights synteny with narrow-leafed lupin and provides markers tagging key agronomic traits. *Scientific Reports, 7*. doi:ARTN 1533510.1038/s41598-017-15625-w

Kulkarni, M., Soolanayakanahally, R., Ogawa, S., Uga, Y., Selvaraj, M. G., & Kagale, S. (2017). Drought Response in Wheat: Key Genes and Regulatory Mechanisms Controlling Root System Architecture and Transpiration Efficiency. *Frontiers in Chemistry, 5*. doi:ARTN 10610.3389/fchem.2017.00106

Kull, O. (2002). Acclimation of photosynthesis in canopies: models and limitations. *Oecologia, 133*(3), 267-279. doi:10.1007/s00442-002-1042-1

Kumar, A., Li, C. S., & Portis, A. R. (2009). Arabidopsis thaliana expressing a thermostable chimeric Rubisco activase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures. *Photosynthesis Research, 100*(3), 143-153. doi:10.1007/s11120-009-9438-y

Kumar, J., Jaiswal, V., Kumar, A., Kumar, N., Mir, R. R., Kumar, S., . . . Gupta, P. K. (2011). Introgression of a major gene for high grain protein content in some Indian bread wheat cultivars. *Field Crops Research, 123*(3), 226-233. doi:10.1016/j.fcr.2011.05.013

Ladwig, F., Stahl, M., Ludewig, U., Hirner, A. A., Hammes, U. Z., Stadler, R., . . . Koch, W. (2012). Siliques Are Red1 from Arabidopsis Acts as a Bidirectional Amino Acid Transporter That Is Crucial for the Amino Acid Homeostasis of Siliques. *Plant Physiology, 158*(4), 1643-1655. doi:10.1104/pp.111.192583

Larkin, J. C., Marks, M. D., Nadeau, J., & Sack, F. (1997). Epidermal cell fate and patterning in leaves. *Plant Cell, 9*(7), 1109-1120. doi:10.1105/tpc.9.7.1109

Latati, M., Blavet, D., Alkama, N., Laoufi, H., Drevon, J. J., Gerard, F., . . . Ounane, S. M. (2014). The intercropping cowpea-maize improves soil phosphorus availability and maize yields in an alkaline soil. *Plant and Soil, 385*(1-2), 181-191. doi:10.1007/s11104-014-2214-6

Lawson, T., & Blatt, M. R. (2014). Stomatal Size, Speed, and Responsiveness Impact on Photosynthesis and Water Use Efficiency. *Plant Physiology, 164*(4), 1556-1570. doi:10.1104/pp.114.237107

Le Gouis, J., Bordes, J., Ravel, C., Heumez, E., Faure, S., Praud, S., . . . Rousset, M. (2012). Genome-wide association analysis to identify chromosomal regions determining components of earliness in wheat. *Theoretical and Applied Genetics, 124*(3), 597-611. doi:10.1007/s00122-011-1732-3

Lejay, L., Wirth, J., Pervent, M., Cross, J. M. F., Tillard, P., & Gojon, A. (2008). Oxidative pentose phosphate pathway-dependent sugar sensing as a mechanism for regulation of root ion transporters by photosynthesis. *Plant Physiology, 146*(4), 2036-2053. doi:10.1104/pp.107.114710

Lezhneva, L., Kiba, T., Feria-Bourrellier, A. B., Lafouge, F., Boutet-Mercey, S., Zoufan, P., . . . Krapp, A. (2014). The Arabidopsis nitrate transporter NRT2.5 plays a role in nitrate acquisition and remobilization in nitrogen-starved plants. *Plant Journal, 80*(2), 230-241. doi:10.1111/tpj.12626

Li, C., Wu, H. M., & Cheung, A. Y. (2016). FERONIA and Her Pals: Functions and Mechanisms. *Plant Physiology, 171*(4), 2379-2392. doi:10.1104/pp.16.00667

Li, C. H., Sun, B. C., Li, Y. X., Liu, C., Wu, X., Zhang, D. F., . . . Li, Y. (2016). Numerous genetic loci identified for drought tolerance in the maize nested association mapping populations. *BMC Genomics, 17*. doi:ARTN 89410.1186/s12864-016-3170-8

Li, J., Long, Y., Qi, G. N., Li, J., Xu, Z. J., Wu, W. H., & Wang, Y. (2014). The Os-AKT1 Channel Is Critical for K+ Uptake in Rice Roots and Is Modulated by the Rice CBL1-CIPK23 Complex. *Plant Cell, 26*(8), 3387-3402. doi:10.1105/tpc.114.123455

Li, K. T., Moulin, M., Mangel, N., Albersen, M., Verhoeven-Duif, N. M., Ma, Q., . . . Vanderschuren, H. (2015). Increased bioavailable vitamin B6 in field-grown transgenic cassava for dietary sufficiency. *Nature Biotechnology, 33*(10), 1029-1032. doi:10.1038/nbt.3318

Li, N., Liu, Z., Wang, Z., Ru, L., Gonzalez, N., Baekelandt, A., . . . Li, Y. (2018). STERILE APETALA modulates the stability of a repressor protein complex to control organ size in *Arabidopsis thaliana*. *PLoS Genetics, 14*(2), e1007218. doi:10.1371/journal.pgen.1007218

Li, T., Yang, X., Yu, Y., Si, X., Zhai, X., Zhang, H., . . . Xu, C. (2018). Domestication of wild tomato is accelerated by genome editing. *Nature Biotechnology*. doi:10.1038/nbt.4273

Li, Y., Wang, W. Q., Feng, Y. P., Tu, M., Wittich, P. E., Bate, N. J., & Messing, J. (2019). Transcriptome and metabolome reveal distinct carbon allocation patterns during internode sugar accumulation in different sorghum genotypes. *Plant Biotechnology Journal, 17*(2), 472-487. doi:10.1111/pbi.12991

Liang, C. Y., Pineros, M. A., Tian, J., Yao, Z. F., Sun, L. L., Liu, J. P., . . . Liao, H. (2013). Low pH, Aluminum, and Phosphorus Coordinately Regulate Malate Exudation through GmALMT1 to Improve Soybean Adaptation to Acid Soils. *Plant Physiology, 161*(3), 1347-1361. doi:10.1104/pp.112.208934

Liang, Z., Chen, K., Zhang, Y., Liu, J., Yin, K., Qiu, J. L., & Gao, C. (2018). Genome editing of bread wheat using biolistic delivery of CRISPR/Cas9 in vitro transcripts or ribonucleoproteins. *Nature Protocols, 13*(3), 413-430. doi:10.1038/nprot.2017.145

Lin, M. T., Occhialini, A., Andralojc, P. J., Devonshire, J., Hines, K. M., Parry, M. A. J., & Hanson, M. R. (2014). beta-Carboxysomal proteins assemble into highly organized structures in Nicotiana chloroplasts. *Plant Journal, 79*(1), 1-12. doi:10.1111/tpj.12536

Liu, C., Zhong, Y., Qi, X., Chen, M., Liu, Z., Chen, C., . . . Chen, S. (2019). Extension of the in vivo haploid induction system from diploid maize to hexaploid wheat. *Plant Biotechnology Journal*. doi:10.1111/pbi.13218

Liu, C. W., Fukumoto, T., Matsumoto, T., Gena, P., Frascaria, D., Kaneko, T., . . . Kitagawa, Y. (2013). Aquaporin OsPIP1;1 promotes rice salt resistance and seed germination. *Plant Physiology and Biochemistry, 63*, 151-158. doi:10.1016/j.plaphy.2012.11.018

Liu, Q., & Chen, L. (2003). A radiation transfer model to predict canopy radiation in thermal infrared band. *Journal of Remote Sensing*, *7*, 161–167.

Liu, S., Jia, F. L., Jiao, Z. Y., Wang, J. J., Xia, X. L., & Yin, W. L. (2019). Ectopic expression of secretory peptide PdEPF3 in Arabidopsis confers drought tolerance with reduced stomatal density. *Acta Societatis Botanicorum Poloniae, 88*(2). doi:ARTN 362710.5586/asbp.3627

Liu, T., Ohashi-Ito, K., & Bergmann, D. C. (2009). Orthologs of *Arabidopsis thaliana* stomatal bHLH genes and regulation of stomatal development in grasses. *Development, 136*(13), 2265-2276. doi:10.1242/dev.032938

Livingstone, D., Beilinson, V., Kalyaeva, M., Schmidt, M. A., Herman, E. M., & Nielsen, N. C. (2007). Reduction of protease inhibitor activity by expression of a mutant Bowman-Birk gene in soybean seed. *Plant Molecular Biology, 64*(4), 397-408. doi:10.1007/s11103-007-9163-x

Long, S. P., Ainsworth, E. A., Leakey, A. D. B., Nosberger, J., & Ort, D. R. (2006). Food for thought: Lower-than-expected crop yield stimulation with rising CO2 concentrations. *Science, 312*(5782), 1918-1921. doi:10.1126/science.1114722

Long, S. P., Humphries, S., & Falkowski, P. G. (1994). Photoinhibition of Photosynthesis in Nature. *Annual Review of Plant Physiology and Plant Molecular Biology, 45*, 633-662. doi:DOI 10.1146/annurev.pp.45.060194.003221

Long, S. P., Marshall-Colon, A., & Zhu, X. G. (2015). Meeting the Global Food Demand of the Future by Engineering Crop Photosynthesis and Yield Potential. *Cell, 161*(1), 56-66. doi:10.1016/j.cell.2015.03.019

Lonnerdal, B. (2003). Genetically modified plants for improved trace element nutrition. *Journal of Nutrition, 133*(5), 1490s-1493s. Retrieved from <Go to ISI>://WOS:000182828100016

Lothier, J., Gaufichon, L., Sormani, R., Lemaitre, T., Azzopardi, M., Morin, H., . . . Masclaux-Daubresse, C. (2011). The cytosolic glutamine synthetase GLN1;2 plays a role in the control of plant growth and ammonium homeostasis in Arabidopsis rosettes when nitrate supply is not limiting. *Journal of Experimental Botany, 62*(4), 1375-1390. doi:10.1093/jxb/erq299

Lowe, K., Wu, E., Wang, N., Hoerster, G., Hastings, C., Cho, M. J., . . . Gordon-Kamm, W. (2016). Morphogenic Regulators Baby boom and Wuschel Improve Monocot Transformation. *The Plant cell, 28*(9), 1998-2015. doi:10.1105/tpc.16.00124

Lu, C. Q., & Tian, H. Q. (2017). Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: shifted hot spots and nutrient imbalance. *Earth System Science Data, 9*(1), 181-192. doi:10.5194/essd-9-181-2017

Lu, J., Gao, X. R., Dong, Z. M., Yi, J., & An, L. J. (2012). Improved phosphorus acquisition by tobacco through transgenic expression of mitochondrial malate dehydrogenase from Penicillium oxalicum. *Plant Cell Reports, 31*(1), 49-56. doi:10.1007/s00299-011-1138-3

Lu, K., Wu, B. W., Wang, J., Zhu, W., Nie, H. P., Qian, J. J., . . . Fang, Z. M. (2018). Blocking amino acid transporter OsAAP3 improves grain yield by promoting outgrowth buds and increasing tiller number in rice. *Plant Biotechnology Journal, 16*(10), 1710-1722. doi:10.1111/pbi.12907

Lucas, M. M., Stoddard, F. L., Annicchiarico, P., Frias, J., Martinez-Villaluenga, C., Sussmann, D., . . . Pueyo, J. J. (2015). The future of lupin as a protein crop in Europe. *Frontiers in Plant Science, 6*. doi:ARTN 70510.3389/fpls.2015.00705

Luo, X. L., & Zhu, J. Y. (2011). Effects of drying-induced fiber hornification on enzymatic saccharification of lignocelluloses. *Enzyme and Microbial Technology, 48*(1), 92-99. doi:10.1016/j.enzmictec.2010.09.014

Lutts, S., Kinet, J. M., & Bouharmont, J. (1996). Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (Oryza sativa L) cultivars differing in salinity resistance. *Plant Growth Regulation, 19*(3), 207-218. doi:Doi 10.1007/Bf00037793

Ma, L. Y., Zhang, H., Sun, L. R., Jiao, Y. H., Zhang, G. Z., Miao, C., & Hao, F. S. (2012). NADPH oxidase AtrbohD and AtrbohF function in ROS-dependent regulation of Na+/K+ homeostasis in Arabidopsis under salt stress. *Journal of Experimental Botany, 63*(1), 305-317. doi:10.1093/jxb/err280

Ma, S. Q., Tang, N., Li, X., Xie, Y. J., Xiang, D. H., Fu, J., . . . Xiong, L. Z. (2019). Reversible Histone H2B Monoubiquitination Fine-Tunes Abscisic Acid Signaling and Drought Response in Rice. *Molecular Plant, 12*(2), 263-277. doi:10.1016/j.molp.2018.12.005

Maier, A., Fahnenstich, H., von Caemmerer, S., Engqvist, M. K. M., Weber, A. P. M., Flugge, U. I., & Maurino, V. G. (2012). Transgenic introduction of a glycolate oxidative cycle into A-thaliana chloroplasts leads to growth improvement. *Frontiers in Plant Science, 3*. doi:ARTN 3810.3389/fpls.2012.00038

Malzahn, A. A., Tang, X., Lee, K., Ren, Q. R., Sretenovic, S., Zhang, Y. X., . . . Qi, Y. P. (2019). Application of CRISPR-Cas12a temperature sensitivity for improved genome editing in rice, maize, and Arabidopsis. *BMC Biology, 17*. doi:ARTN 910.1186/s12915-019-0629-5

Marinho, G. S., Sorensen, A. D. M., Safafar, H., Pedersen, A. H., & Holdt, S. L. (2019). Antioxidant content and activity of the seaweed Saccharina latissima: a seasonal perspective. *Journal of Applied Phycology, 31*(2), 1343-1354. doi:10.1007/s10811-018-1650-8

Martin, A., Lee, J., Kichey, T., Gerentes, D., Zivy, M., Tatout, C., . . . Hirel, B. (2006). Two cytosolic glutamine synthetase isoforms of maize are specifically involved in the control of grain production. *Plant Cell, 18*(11), 3252-3274. doi:10.1105/tpc.106.042689

Martinez-Atienza, J., Jiang, X. Y., Garciadeblas, B., Mendoza, I., Zhu, J. K., Pardo, J. M., & Quintero, F. J. (2007). Conservation of the salt overly sensitive pathway in rice. *Plant Physiology, 143*(2), 1001-1012. doi:10.1104/pp.106.092635

Martiniere, A., Fiche, J. B., Smokvarska, M., Mari, S., Alcon, C., Dumont, X., . . . Maurel, C. (2019). Osmotic Stress Activates Two Reactive Oxygen Species Pathways with Distinct

Effects on Protein Nanodomains and Diffusion. *Plant Physiology, 179*(4), 1581-1593. doi:10.1104/pp.18.01065

Masalia, R. R., Temme, A. A., Torralba, N. D., & Burke, J. M. (2018). Multiple genomic regions influence root morphology and seedling growth in cultivated sunflower (Helianthus annuus L.) under wellwatered and water-limited conditions. *PLoS ONE, 13*(9). doi:ARTN e020427910.1371/journal.pone.0204279

Masclaux-Daubresse, C., Reisdorf-Cren, M., & Orsel, M. (2008). Leaf nitrogen remobilisation for plant development and grain filling. *Plant Biology, 10*, 23-36. doi:10.1111/j.1438-8677.2008.00097.x

Masuda, T., Tanaka, A., & Melis, A. (2003). Chlorophyll antenna size adjustments by irradiance in Dunaliella salina involve coordinate regulation of chlorophyll a oxygenase (CAO) and Lhcb gene expression. *Plant Molecular Biology, 51*(5), 757-771. doi:Doi 10.1023/A:1022545118212

Matloobi, M. (2012). Light Harvesting and Photosynthesis by the Canopy. In M. Najafpour (Ed.), *Advances in Photosynthesis- Fundamental Aspects* (p. Chapter 12).

Mattioni, C., Lacerenza, N. G., Troccoli, A., De Leonardis, A. M., & Di Fonzo, N. (1997). Water and salt stress-induced alterations in proline metabolism of Triticum durum seedlings. *Physiologia Plantarum, 101*(4), 787-792. Retrieved from <Go to ISI>://WOS:000071277900018

Maurel, C., Boursiac, Y., Luu, D. T., Santoni, V., Shahzad, Z., & Verdoucq, L. (2015). Aquaporins in Plants. *Physiological Reviews, 95*(4), 1321-1358. doi:10.1152/physrev.00008.2015

McCahill, I. W., & Hazen, S. P. (2019). Regulation of Cell Wall Thickening by a Medley of Mechanisms. *Trends in Plant Science, 24*(9), 853-866. doi:10.1016/j.tplants.2019.05.012

McGrath, J. M., & Long, S. P. (2014). Can the Cyanobacterial Carbon-Concentrating Mechanism Increase Photosynthesis in Crop Species? A Theoretical Analysis. *Plant Physiology, 164*(4), 2247-2261. doi:10.1104/pp.113.232611

Meis, S. J., Fehr, W. R., & Schnebly, S. R. (2003). Seed source effect on field emergence of soybean lines with reduced phytate and raffinose saccharides. *Crop Science, 43*(4), 1336-1339. doi:DOI 10.2135/cropsci2003.1336

Miao, X., Xu, R., Fan, B., Chen, J., Li, X., Mao, W. W., . . . Li, B. (2018). PD-L1 reverses depigmentation in Pmel-1 vitiligo mice by increasing the abundance of Tregs in the skin. *Scientific Reports, 8*. doi:ARTN 160510.1038/s41598-018-19407-w

Millet, E. J., Kruijer, W., Coupel-Ledru, A., Alvarez Prado, S., Cabrera-Bosquet, L., Lacube, S., . . . Tardieu, F. (2019). Genomic prediction of maize yield across European environmental conditions. *Nature Genetics, 51*(6), 952-956. doi:10.1038/s41588-019-0414-y

Minina, E. A., Moschou, P. N., Vetukuri, R. R., Sanchez-Vera, V., Cardoso, C., Liu, Q. S., . . . Bozhkov, P. V. (2018). Transcriptional stimulation of rate-limiting components of the autophagic pathway improves plant fitness. *Journal of Experimental Botany, 69*(6), 1415-1432. doi:10.1093/jxb/ery010

Mishra, A., & Tanna, B. (2017). Halophytes: Potential Resources for Salt Stress Tolerance Genes and Promoters. *Frontiers in Plant Science, 8*. doi:ARTN 82910.3389/fpls.2017.00829

Misra, N., & Saxena, P. (2009). Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Science, 177*(3), 181-189. doi:10.1016/j.plantsci.2009.05.007

Monaghan, J. M., Snape, J. W., Chojecki, A. J. S., & Kettlewell, P. S. (2001). The use of grain protein deviation for identifying wheat cultivars with high grain protein concentration and yield. *Euphytica, 122*(2), 309-317. doi:Doi 10.1023/A:1012961703208

Monteith, J. L. (1972). Solar-Radiation and Productivity in Tropical Ecosystems. *Journal of Applied Ecology, 9*(3), 747-766. doi:Doi 10.2307/2401901

Morton, M. J. L., Awlia, M., Al-Tamimi, N., Saade, S., Pailles, Y., Negrao, S., & Tester, M. (2019). Salt stress under the scalpel - dissecting the genetics of salt tolerance. *Plant Journal, 97*(1), 148-163. doi:10.1111/tpj.14189

Mosquna, A., Peterson, F. C., Park, S. Y., Lozano-Juste, J., Volkman, B. F., & Cutler, S. R. (2011). Potent and selective activation of abscisic acid receptors in vivo by mutational

stabilization of their agonist-bound conformation. *Proceedings of the National Academy of Sciences of the United States of America, 108*(51), 20838-20843. doi:10.1073/pnas.1112838108

Moulia, B., Coutand, C., & Lenne, C. (2006). Posture control and skeletal mechanical acclimation in terrestrial plants: Implications for mechanical modeling of plant architecture. *American Journal of Botany, 93*(10), 1477-1489. doi:DOI 10.3732/ajb.93.10.1477

Muller, A., Guan, C. H., Galweiler, L., Tanzler, P., Huijser, P., Marchant, A., . . . Palme, K. (1998). AtPIN2 defines a locus of Arabidopsis for root gravitropism control. *EMBO Journal, 17*(23), 6903-6911. doi:DOI 10.1093/emboj/17.23.6903

Muller, B., Bourdais, G., Reidy, B., Bencivenni, C., Massonneau, A., Condamine, P., . . . Tardieu, F. (2007). Association of specific expansins with growth in Maize leaves is maintained under environmental, genetic, and developmental sources of variation. *Plant Physiology, 143*(1), 278-290. doi:DOI 10.1104/pp.106.087494

Muller, B., Fastner, A., Karmann, J., Mansch, V., Hoffmann, T., Schwab, W., . . . Hammes, U. Z. (2015). Amino Acid Export in Developing Arabidopsis Seeds Depends on UmamiT Facilitators. *Current Biology, 25*(23), 3126-3131. doi:10.1016/j.cub.2015.10.038

Muller, P., Li, X. P., & Niyogi, K. K. (2001). Non-photochemical quenching. A response to excess light energy. *Plant Physiology, 125*(4), 1558-1566. doi:DOI 10.1104/pp.125.4.1558

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology, 59*, 651-681. doi:10.1146/annurev.arplant.59.032607.092911

Muraoka, H., Takenaka, A., Tang, Y., Koizumi, H., & Washitani, I. (1998). Flexible leaf orientations of Arisaema heterophyllum maximize light capture in a forest understorey and avoid excess irradiance at a deforested site. *Annals of Botany, 82*(3), 297-307. doi:DOI 10.1006/anbo.1998.0682

Muro-Villanueva, F., Mao, X. Y., & Chapple, C. (2019). Linking phenylpropanoid metabolism, lignin deposition, and plant growth inhibition. *Current Opinion in Biotechnology, 56*, 202-208. doi:10.1016/j.copbio.2018.12.008

Naito, K., Takahashi, Y., Chaitieng, B., Hirano, K., Kaga, A., Takagi, K., . . . Tomooka, N. (2017). Multiple organ gigantism caused by mutation in *VmPPD* gene in blackgram (*Vigna mungo*). *Breeding Science, 67*(2), 151-158. doi:10.1270/jsbbs.16184

Napier, J. A., Haslam, R. P., Tsalavouta, M., & Sayanova, O. (2019). The challenges of delivering genetically modified crops with nutritional enhancement traits. *Nature Plants, 5*(6), 563-567. doi:10.1038/s41477-019-0430-z

Naqvi, S., Zhu, C. F., Farre, G., Ramessar, K., Bassie, L., Breitenbach, J., . . . Christou, P. (2009). Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. *Proceedings of the National Academy of Sciences of the United States of America, 106*(19), 7762-7767. doi:10.1073/pnas.0901412106

Negrao, S., Schmockel, S. M., & Tester, M. (2017). Evaluating physiological responses of plants to salinity stress. *Annals of Botany, 119*(1), 1-11. doi:10.1093/aob/mcw191

Nelissen, H., Gonzalez, N., & Inzé, D. (2016). Leaf growth in dicots and monocots: so different yet so alike. *Current Opinion in Plant Biology, 33*, 72-76. doi:10.1016/j.pbi.2016.06.009

Nieves-Cordones M, Al Shiblawi FR, Sentenac H (2016) Roles and Transport of Sodium and Potassium in Plants. In, Sigel A, Sigel H, Sigel RKO (eds) The Alkali Metal Ions, Their Role for Life. Springer International Publishing, Cham, pp 291-324

Niklas, P. J. (1994). Plant allometry: The scaling of form and process. In *Trends in Ecology & Evolution* (Vol. 10). https://doi.org/10.1016/S0169-5347(00)89015-1

Niu G, Xu W, Rodriguez D, Sun Y (2012) Growth and Physiological Responses of Maize and Sorghum Genotypes to Salt Stress. *ISRN Agronomy*, 2012, 12

Nobel, P., Forseth, I., & Long, S. (1993). Canopy Structure and light interception. In D. Hall, J. Scurlock, H. Bohlar-Nordenkampf, R. Leegood, & S. Long (Eds.), *Photosynthesis and Production in a Changing Environment: a field and laboratory manual* (pp. 79–90).

Nour-Eldin, H. H., Madsen, S. R., Engelen, S., Jorgensen, M. E., Olsen, C. E., Andersen, J. S., . . . Halkier, B. A. (2017). Reduction of antinutritional glucosinolates in Brassica oilseeds

by mutation of genes encoding transporters. *Nature Biotechnology, 35*(4), 377-+. doi:10.1038/nbt.3823

Nuccio, M. L., Paul, M., Bate, N. J., Cohn, J., & Cutler, S. R. (2018). Where are the drought tolerant crops? An assessment of more than two decades of plant biotechnology effort in crop improvement. *Plant Science, 273*, 110-119. doi:10.1016/j.plantsci.2018.01.020

Nuccio, M. L., Wu, J., Mowers, R., Zhou, H. P., Meghji, M., Primavesi, L. F., . . . Lagrimini, L. M. (2015). Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions. *Nature Biotechnology, 33*(8), 862-+. doi:10.1038/nbt.3277

Nunes-Nesi, A., Fernie, A. R., & Stitt, M. (2010). Metabolic and Signaling Aspects Underpinning the Regulation of Plant Carbon Nitrogen Interactions. *Molecular Plant, 3*(6), 973-996. doi:10.1093/mp/ssq049

Nunes, A. C. S., Kalkmann, D. C., & Aragao, F. J. L. (2009). Folate biofortification of lettuce by expression of a codon optimized chicken GTP cyclohydrolase I gene. *Transgenic Research, 18*(5), 661-667. doi:10.1007/s11248-009-9256-1

Nussaume, L., Kanno, S., Javot, H., Marin, E., Pochon, N., Ayadi, A., . . . Thibaud, M. C. (2011). Phosphate import in plants: focus on the PHT1 transporters. *Frontiers in Plant Science, 2*. doi:ARTN 8310.3389/fpls.2011.00083

Ogura, T., Goeschl, C., Filiault, D., Mirea, M., Slovak, R., Wolhrab, B., . . . Busch, W. (2019). Root System Depth in Arabidopsis Is Shaped by EXOCYST70A3 via the Dynamic Modulation of Auxin Transport. *Cell, 178*(2), 400-+. doi:10.1016/j.cell.2019.06.021

Okamoto M, Cutler SR. 2018. Chemical Control of ABA Receptors to Enable Plant Protection Against Water Stress. Methods Mol Biol. 2018;1795:127-141

Orosa-Puente, B., Leftley, N., von Wangenheim, D., Banda, J., Srivastava, A. K., Hill, K., . . . Bennett, M. J. (2018). Root branching toward water involves posttranslational modification of transcription factor ARF7. *Science, 362*(6421), 1407-+. doi:10.1126/science.aau3956

Ort, D. R., Merchant, S. S., Alric, J., Barkan, A., Blankenship, R. E., Bock, R., . . . Zhu, X. G. (2015). Redesigning photosynthesis to sustainably meet global food and bioenergy demand. *Proceedings of the National Academy of Sciences of the United States of America, 112*(28), 8529-8536. doi:10.1073/pnas.1424031112

Ort, D. R., Zhu, X. G., & Melis, A. (2011). Optimizing Antenna Size to Maximize Photosynthetic Efficiency. *Plant Physiology, 155*(1), 79-85. doi:10.1104/pp.110.165886

Oszvald, M., Primavesi, L. F., Griffiths, C. A., Cohn, J., Basu, S. S., Nuccio, M. L., & Paul, M. J. (2018). Trehalose 6-Phosphate Regulates Photosynthesis and Assimilate Partitioning in Reproductive Tissue. *Plant Physiology, 176*(4), 2623-2638. doi:10.1104/pp.17.01673

Oury, F. X., & Godin, C. (2007). Yield and grain protein concentration in bread wheat: how to use the negative relationship between the two characters to identify favourable genotypes? *Euphytica, 157*(1-2), 45-57. doi:10.1007/s10681-007-9395-5

Oyarce, P., De Meester, B., Fonseca, F., de Vries, L., Goeminne, G., Pallidis, A., . . . Boerjan, W. (2019). Introducing curcumin biosynthesis in Arabidopsis enhances lignocellulosic biomass processing. *Nature Plants, 5*(2), 225-+. doi:10.1038/s41477-018-0350-3

Pages, L., Vercambre, G., Gautier, H., Najla, S., Grasselly, D., & Genard, M. (2009). Tomato plant architecture as affected by salinity: Descriptive analysis and integration in a 3-D simulation model. *Botany, 87*(10), 893-904. doi:10.1139/B09-061

Paine, J. A., Shipton, C. A., Chaggar, S., Howells, R. M., Kennedy, M. J., Vernon, G., . . . Drake, R. (2005). Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology, 23*(4), 482-487. doi:10.1038/nbt1082

Pal, S., Kisko, M., Dubos, C., Lacombe, B., Berthomieu, P., Krouk, G., & Rouached, H. (2017). TransDetect Identifies a New Regulatory Module Controlling Phosphate Accumulation. *Plant Physiology, 175*(2), 916-926. doi:10.1104/pp.17.00568

Papanatsiou, M., Petersen, J., Henderson, L., Wang, Y., Christie, J. M., & Blatt, M. R. (2019). Optogenetic manipulation of stomatal kinetics improves carbon assimilation, water use, and growth. *Science, 363*(6434), 1456-+. doi:10.1126/science.aaw0046

Parent, B., Leclere, M., Lacube, S., Semenov, M. A., Welcker, C., Martre, P., & Tardieu, F. (2018). Maize yields over Europe may increase in spite of climate change, with an appropriate use of the genetic variability of flowering time. *Proceedings of the National Academy of Sciences of the United States of America, 115*(42), 10642-10647. doi:10.1073/pnas.1720716115

Paul, M., Pellny, T., & Goddijn, O. (2001). Enhancing photosynthesis with sugar signals. *Trends in Plant Science, 6*(5), 197-200. doi:Doi 10.1016/S1360-1385(01)01920-3

Paul, M. J., Gonzalez-Uriarte, A., Griffiths, C. A., & Hassani-Pak, K. (2018). The Role of Trehalose 6-Phosphate in Crop Yield and Resilience. *Plant Physiology, 177*(1), 12-23. doi:10.1104/pp.17.01634

Pedrosa, A. M., Cidade, L. C., Martins, C. P. S., Macedo, A. F., Neves, D. M., Gomes, F. P., . . . Costa, M. G. C. (2017). Effect of overexpression of citrus 9-cis-epoxycarotenoid dioxygenase 3 (CsNCED3) on the physiological response to drought stress in transgenic tobacco. *Genetics and Molecular Research, 16*(1). doi:ARTN 1601929210.4238/gmr16019292

Pellny, T. K., Ghannoum, O., Conroy, J. P., Schluepmann, H., Smeekens, S., Andralojc, J., . . . Paul, M. J. (2004). Genetic modification of photosynthesis with E. coli genes for trehalose synthesis. *Plant Biotechnology Journal, 2*(1), 71-82. doi:10.1111/j.1467-7652.2004.00053.x

Pena, P. A., Quach, T., Sato, S., Ge, Z. X., Nersesian, N., Dweikat, I. M., . . . Clemente, T. (2017). Molecular and phenotypic characterization of transgenic wheat and sorghum events expressing the barley alanine aminotransferase. *Planta, 246*(6), 1097-1107. doi:10.1007/s00425-017-2753-1

Peterhansel, C., Krause, K., Braun, H. P., Espie, G. S., Fernie, A. R., Hanson, D. T., . . . Sage, R. F. (2013). Engineering photorespiration: current state and future possibilities. *Plant Biology, 15*(4), 754-758. doi:10.1111/j.1438-8677.2012.00681.x

Peterson, K. M., Rychel, A. L., & Torii, K. U. (2010). Out of the mouths of plants: the molecular basis of the evolution and diversity of stomatal development. *Plant Cell, 22*(2), 296-306. doi:10.1105/tpc.109.072777

Phyo, P., Wang, T., Kiemle, S. N., O'Neill, H., Pingali, S. V., Hong, M., & Cosgrove, D. J. (2017). Gradients in Wall Mechanics and Polysaccharides along Growing Inflorescence Stems. *Plant Physiology, 175*(4), 1593-1607. doi:10.1104/pp.17.01270

Pilon-Smits, E. A. H., Terry, N., Sears, T., Kim, H., Zayed, A., Hwang, S. B., . . . Goddijn, O. J. M. (1998). Trehalose-producing transgenic tobacco plants show improved growth performance under drought stress. *Journal of Plant Physiology, 152*(4-5), 525-532. doi:Doi 10.1016/S0176-1617(98)80273-3

Pilu, R., Landoni, M., Cassani, E., Doria, E., & Nielsen, E. (2005). The maize lpa241 mutation causes a remarkable variability of expression and some pleiotropic effects. *Crop Science, 45*(5), 2096-2105. doi:10.2135/cropsci2004.0651

Pimentel, D., Berger, B., Filiberto, D., Newton, M., Wolfe, B., Karabinakis, E., . . . Nandagopal, S. (2004). Water resources: Agricultural and environmental issues. *Bioscience, 54*(10), 909-918. doi:Doi 10.1641/0006-3568(2004)054[0909:Wraaei]2.0.Co;2

Poirier, Y., & Bucher, M. (2002). Phosphate transport and homeostasis in Arabidopsis. *Arabidopsis Book, 1*, e0024. doi:10.1199/tab.0024

Pottier, M., Dumont, J., Masclaux-Daubresse, C., & Thomine, S. (2019). Autophagy is essential for optimal translocation of iron to seeds in Arabidopsis. *Journal of Experimental Botany, 70*(3), 859-869. doi:10.1093/jxb/ery388

Pound, M. P., French, A. P., Murchie, E. H., & Pridmore, T. P. (2014). Automated Recovery of Three-Dimensional Models of Plant Shoots from Multiple Color Images. *Plant Physiology, 166*(4), 1688-U1801. doi:10.1104/pp.114.248971

Powell, A. E., & Lenhard, M. (2012). Control of organ size in plants. *Current biology : CB, 22*(9), R360-367. doi:10.1016/j.cub.2012.02.010

Price, G. D., Pengelly, J. J. L., Forster, B., Du, J. H., Whitney, S. M., von Caemmerer, S., . . . Evans, J. R. (2013). The cyanobacterial CCM as a source of genes for improving

photosynthetic CO2 fixation in crop species. *Journal of Experimental Botany, 64*(3), 753-768. doi:10.1093/jxb/ers257

Puga, M. I., Mateos, I., Charukesi, R., Wang, Z., Franco-Zorrilla, J. M., de Lorenzo, L., . . . Paz-Ares, J. (2014). SPX1 is a phosphate-dependent inhibitor of Phosphate Starvation Response 1 in Arabidopsis. *Proc Natl Acad Sci U S A, 111*(41), 14947-14952. doi:10.1073/pnas.1404654111

Quan, L., Tan, P., Zeng, G., Yuan, L., Wang, J. D., & Kang, S. B. (2006). Image-based plant modeling. *Acm Transactions on Graphics, 25*(3), 599-604. doi:Doi 10.1145/1141911.1141929

Raissig, M. T., Matos, J. L., Anleu Gil, M. X., Kornfeld, A., Bettadapur, A., Abrash, E., . . . Bergmann, D. C. (2017). Mobile MUTE specifies subsidiary cells to build physiologically improved grass stomata. *Science (New York, N.Y.), 355*(6330), 1215-1218. doi:10.1126/science.aal3254

Rakocevic, M., Sinoquet, H., Christophe, A., & Varlet-Grancher, C. (2000). Assessing the geometric structure of a white clover (Trifolium repens L.) canopy using 3-D digitising. *Annals of Botany, 86*(3), 519-526. doi:10.1006/anbo.2000.1209

Ralph, J., Lapierre, C., & Boerjan, W. (2019). Lignin structure and its engineering. *Current Opinion in Biotechnology, 56*, 240-249. doi:10.1016/j.copbio.2019.02.019

Ramesh, S. A., Choimes, S., & Schachtman, D. P. (2004). Over-expression of an Arabidopsis zinc transporter in Hordeum vulgare increases short-term zinc uptake after zinc deprivation and seed zinc content. *Plant Molecular Biology, 54*(3), 373-385. doi:Doi 10.1023/B:Plan.0000036370.70912.34

Ramirez-Valiente, J. A., Deacon, N. J., Etterson, J., Center, A., Sparks, J. P., Sparks, K. L., . . . Cavender-Bares, J. (2018). Natural selection and neutral evolutionary processes contribute to genetic divergence in leaf traits across a precipitation gradient in the tropical oak Quercus oleoides. *Molecular Ecology, 27*(9), 2176-2192. doi:10.1111/mec.14566

Ramus, J., Beale, S. I., & Mauzerall, D. (1976). Correlation of Changes in Pigment Content with Photosynthetic Capacity of Seaweeds as a Function of Water Depth. *Marine Biology, 37*(3), 231-238. doi:Doi 10.1007/Bf00387608

Ramus, J., Beale, S. I., Mauzerall, D., & Howard, K. L. (1976). Changes in Photosynthetic Pigment Concentration in Seaweeds as a Function of Water Depth. *Marine Biology, 37*(3), 223-229. doi:Doi 10.1007/Bf00387607

Rastogi, A., & Shukla, S. (2013). Amaranth: A New Millennium Crop of Nutraceutical Values. *Critical Reviews in Food Science and Nutrition, 53*(2), 109-125. doi:10.1080/10408398.2010.517876

Rauf, M., Arif, M., Dortay, H., Matallana-Ramirez, L. P., Waters, M. T., Nam, H. G., . . . Balazadeh, S. (2013). ORE1 balances leaf senescence against maintenance by antagonizing G2-like-mediated transcription. *EMBO Reports, 14*(4), 382-388. doi:10.1038/embor.2013.24

Raven, J. A., & Taylor, R. (2003). Macroalgal growth in nutrient-enriched estuaries: a biogeochemical and evolutionary perspective. *Water, Air and Soil Pollution: Focus*, **3**, 7-26.

Ray, D. K., Gerber, J. S., MacDonald, G. K., & West, P. C. (2015). Climate variation explains a third of global crop yield variability. *Nature Communications, 6*. doi:ARTN 598910.1038/ncomms6989

Reinhardt, D., Mandel, T., & Kuhlemeier, C. (2000). Auxin regulates the initiation and radial position of plant lateral organs. *Plant Cell, 12*(4), 507-518. doi:DOI 10.1105/tpc.12.4.507

Ren, Z. H., Gao, J. P., Li, L. G., Cai, X. L., Huang, W., Chao, D. Y., . . . Lin, H. X. (2005). A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nature Genetics, 37*(10), 1141-1146. doi:10.1038/ng1643

Reymond, M., Svistoonoff, S., Loudet, O., Nussaume, L., & Desnos, T. (2006). Identification of QTL controlling root growth response to phosphate starvation in Arabidopsis thaliana. *Plant Cell and Environment, 29*(1), 115-125. doi:10.1111/j.1365-3040.2005.01405.x

Rosales, M. A., Maurel, C., & Nacry, P. (2019). Abscisic Acid Coordinates Dose-Dependent Developmental and Hydraulic Responses of Roots to Water Deficit. *Plant Physiology, 180*(4), 2198-2211. doi:10.1104/pp.18.01546

Ross, J. (1981a). *The Radiation regime and architecture of plant stands*. The Hague, Netherlands.

Ross, J. (1981b). The Radiation regime and architecture of plant stands. In *The Hague*. The Hague, Netherlands: Dr Junk.

Rousset, M., Bonnin, I., Remoue, C., Falque, M., Rhone, B., Veyrieras, J. B., . . . Goldringer, I. (2011). Deciphering the genetics of flowering time by an association study on candidate genes in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics, 123*(6), 907-926. doi:10.1007/s00122-011-1636-2

Rubio, V., Linhares, F., Solano, R., Martin, A. C., Iglesias, J., Leyva, A., & Paz-Ares, J. (2001). A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes & Development, 15*(16), 2122-2133. doi:DOI 10.1101/gad.204401

Ruiz-Lopez, N., Haslam, R. P., Napier, J. A., & Sayanova, O. (2014). Successful high-level accumulation of fish oil omega-3 long-chain polyunsaturated fatty acids in a transgenic oilseed crop. *Plant Journal, 77*(2), 198-208. doi:10.1111/tpj.12378

Ruta, N., Liedgens, M., Fracheboud, Y., Stamp, P., & Hund, A. (2010). QTLs for the elongation of axile and lateral roots of maize in response to low water potential. *Theoretical and Applied Genetics, 120*(3), 621-631. doi:10.1007/s00122-009-1180-5

Sage, R. F., Sage, T. L., & Kocacinar, F. (2012). Photorespiration and the evolution of C4 photosynthesis. *Annual Review of Plant Biology, 63*, 19-47. doi:10.1146/annurev-arplant-042811-105511

Sakamoto, T., & Matsuoka, M. (2004). Generating high-yielding varieties by genetic manipulation of plant architecture. *Current Opinion in Biotechnology, 15*(2), 144-147. doi:10.1016/j.copbio.2004.02.003

Salter, W. T., Merchant, A. M., Richards, R. A., Trethowan, R., & Buckley, T. N. (2019). Rate of photosynthetic induction in fluctuating light varies widely among genotypes of wheat. *Journal of Experimental Botany, 70*(10), 2787-2796. doi:10.1093/jxb/erz100

Sander, M., & Petersen, M. (2011). Distinct substrate specificities and unusual substrate flexibilities of two hydroxycinnamoyltransferases, rosmarinic acid synthase and hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl-transferase, from Coleus blumei Benth. *Planta, 233*(6), 1157-1171. doi:10.1007/s00425-011-1367-2

Santiago, J. P., & Tegeder, M. (2016). Connecting Source with Sink: The Role of Arabidopsis AAP8 in Phloem Loading of Amino Acids. *Plant Physiology, 171*(1), 508-521. doi:10.1104/pp.16.00244

Santos, T., & Oliviera, A. (2012). Image-based 3D digitizing for plant architecture analysis and phenotyping. *Workshop on Industry Applications (WGARI) in SIBGRAPI 2012 (XXV Conference on Graphics, Patterns and Images)*. Ouro Preto, MG, Brazil.

Sarker, U., & Oba, S. (2018). Drought stress enhances nutritional and bioactive compounds, phenolic acids and antioxidant capacity of Amaranthus leafy vegetable. *BMC Plant Biology, 18*. doi:ARTN 25810.1186/s12870-018-1484-1

Sarlikioti, V., de Visser, P. H. B., & Marcelis, L. F. M. (2011). Exploring the spatial distribution of light interception and photosynthesis of canopies by means of a functional-structural plant model. *Annals of Botany, 107*(5), 875-883. doi:10.1093/aob/mcr006

Sato, S., Peet, M. M., & Thomas, J. F. (2002). Determining critical pre- and post-anthesis periods and physiological processes in Lycopersicon esculentum Mill. exposed to moderately elevated temperatures. *Journal of Experimental Botany, 53*(371), 1187-1195. doi:DOI 10.1093/jexbot/53.371.1187

Sawai, S., Ohyama, K., Yasumoto, S., Seki, H., Sakuma, T., Yamamoto, T., . . . Umemoto, N. (2014). 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*(9), 3763-3774. doi:10.1105/tpc.114.130096

Scarano, A., Butelli, E., De Santis, S., Cavalcanti, E., Hill, L., De Angelis, M., . . . Santino, A. (2018). Combined Dietary Anthocyanins, Flavonols, and Stilbenoids Alleviate Inflammatory Bowel Disease Symptoms in Mice. *Frontiers in Nutrition, 4*. doi:UNSP 7510.3389/fnut.2017.00075

Scarano, A., Chieppa, M., & Santino, A. (2018). Looking at Flavonoid Biodiversity in Horticultural Crops: A Colored Mine with Nutritional Benefits. *Plants-Basel, 7*(4). doi:ARTN 9810.3390/plants7040098

Scheller, H. V., & Ulvskov, P. (2010). Hemicelluloses. *Annual Review of Plant Biology, Vol 61, 61*, 263-289. doi:10.1146/annurev-arplant-042809-112315

Schmidt, M. A., Hymowitz, T., & Herman, E. M. (2015). Breeding and characterization of soybean Triple Null; a stack of recessive alleles of Kunitz Trypsin Inhibitor, Soybean Agglutinin, and P34 allergen nulls. *Plant Breeding, 134*(3), 310-315. doi:10.1111/pbr.12265

Scranton, K., & Amarasekare, P. (2017). Predicting phenological shifts in a changing climate. *Proceedings of the National Academy of Sciences of the United States of America, 114*(50), 13212-13217. doi:10.1073/pnas.1711221114

Sebastian, J., Yee, M. C., Viana, W. G., Rellan-Alvarez, R., Feldman, M., Priest, H. D., . . . Dinneny, J. R. (2016). Grasses suppress shoot-borne roots to conserve water during drought. *Proceedings of the National Academy of Sciences of the United States of America, 113*(31), 8861-8866. doi:10.1073/pnas.1604021113

Shabala, S. (2009). Salinity and programmed cell death: unravelling mechanisms for ion specific signalling. *Journal of Experimental Botany, 60*(3), 709-711. doi:10.1093/jxb/erp013

Shahzad, Z., Canut, M., Tournaire-Roux, C., Martiniere, A., Boursiac, Y., Loudet, O., & Maurel, C. (2016). A Potassium-Dependent Oxygen Sensing Pathway Regulates Plant Root Hydraulics. *Cell, 167*(1), 87-+. doi:10.1016/j.cell.2016.08.068

Sharma, D. K., Andersen, S. B., Ottosen, C. O., & Rosenqvist, E. (2015). Wheat cultivars selected for high F-v/F-m under heat stress maintain high photosynthesis, total chlorophyll, stomatal conductance, transpiration and dry matter. *Physiologia Plantarum, 153*(2), 284-298. doi:10.1111/ppl.12245

Sheehy, J. E., & Mitchell, P. L. (2015). Calculating maximum theoretical yield in rice. *Field Crops Research, 182*, 68-75. doi:10.1016/j.fcr.2015.05.013

Shen, B. R., Wang, L. M., Lin, X. L., Yao, Z., Xu, H. W., Zhu, C. H., . . . Peng, X. X. (2019). Engineering a New Chloroplastic Photorespiratory Bypass to Increase Photosynthetic Efficiency and Productivity in Rice. *Molecular Plant, 12*(2), 199-214. doi:10.1016/j.molp.2018.11.013

Shi, J. R., Wang, H. Y., Schellin, K., Li, B. L., Faller, M., Stoop, J. M., . . . Glassman, K. (2007). Embryo-specific silencing of a transporter reduces phytic acid content of maize and soybean seeds. *Nature Biotechnology, 25*(8), 930-937. doi:10.1038/nbt1322

Shinozuka, H., Cogan, N. O. I., Spangenberg, G. C., & Forster, J. W. (2012). Quantitative Trait Locus (QTL) meta-analysis and comparative genomics for candidate gene prediction in perennial ryegrass (Lolium perenne L.). *BMC Genetics, 13*. doi:Artn 10110.1186/1471-2156-13-101

Simkin, A. J., McAusland, L., Lawson, T., & Raines, C. A. (2017). Overexpression of the RieskeFeS Protein Increases Electron Transport Rates and Biomass Yield. *Plant Physiology, 175*(1), 134-145. doi:10.1104/pp.17.00622

Sinoquet, H., Thanisawanyangkura, S., Mabrouk, H., & Kasemsap, P. (1998). Characterization of the light environment in canopies using 3D digitising and image processing. *Annals of Botany, 82*(2), 203-212. doi:DOI 10.1006/anbo.1998.0665

Soleh, M. A., Tanaka, Y., Kim, S. Y., Huber, S. C., Sakoda, K., & Shiraiwa, T. (2017). Identification of large variation in the photosynthetic induction response among 37 soybean [Glycine max (L.) Merr.] genotypes that is not correlated with steady-state photosynthetic capacity. *Photosynthesis Research, 131*(3), 305-315. doi:10.1007/s11120-016-0323-1

Soleh, M. A., Tanaka, Y., Nomoto, Y., Iwahashi, Y., Nakashima, K., Fukuda, Y., . . . Shiraiwa, T. (2016). Factors underlying genotypic differences in the induction of photosynthesis in

soybean [Glycine max (L.) Merr.]. *Plant Cell and Environment, 39*(3), 685-693. doi:10.1111/pce.12674

Soler, M., Plasencia, A., Larbat, R., Pouzet, C., Jauneau, A., Rivas, S., . . . Grima-Pettenati, J. (2017). The Eucalyptus linker histone variant EgH1.3 cooperates with the transcription factor EgMYB1 to control lignin biosynthesis during wood formation. *New Phytologist, 213*(1), 287-299. doi:10.1111/nph.14129

Song, Q. F., Zhang, G. L., & Zhu, X. G. (2013). Optimal crop canopy architecture to maximise canopy photosynthetic CO2 uptake under elevated CO2 - a theoretical study using a mechanistic model of canopy photosynthesis. *Functional Plant Biology, 40*(2), 109-124. doi:10.1071/Fp12056

Sosso, D., Luo, D. P., Li, Q. B., Sasse, J., Yang, J. L., Gendrot, G., . . . Frommer, W. B. (2015). Seed filling in domesticated maize and rice depends on SWEET-mediated hexose transport. *Nature Genetics, 47*(12), 1489-+. doi:10.1038/ng.3422

Sousa, N. L., Cabral, G. B., Vieira, P. M., Baldoni, A. B., & Aragao, F. J. L. (2017). Bio-detoxification of ricin in castor bean (Ricinus communis L.) seeds. *Scientific Reports, 7*. doi:ARTN 1538510.1038/s41598-017-15636-7

South, P. F., Cavanagh, A. P., Liu, H. W., & Ort, D. R. (2019). Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. *Science, 363*(6422), 45-+. doi:ARTN eaat907710.1126/science.aat9077

South, P. F., Cavanagh, A. P., Lopez-Calcagno, P. E., Raines, C. A., & Ort, D. R. (2018). Optimizing photorespiration for improved crop productivity. *Journal of Integrative Plant Biology, 60*(12), 1217-1230. doi:10.1111/jipb.12709

Sow, M. D., Allona, I., Ambroise, C., Conde, D., Fichot, R., Gribkova, S., . . . Maury, S. (2018). Epigenetics in Forest Trees: State of the Art and Potential Implications for Breeding and Management in a Context of Climate Change. *Plant Epigenetics Coming of Age for Breeding Applications, 88*, 387-453. doi:10.1016/bs.abr.2018.09.003

Sparvoli, F., & Cominelli, E. (2015). Seed Biofortification and Phytic Acid Reduction: A Conflict of Interest for the Plant? *Plants (Basel, Switzerland), 4*(4), 728-755. doi:10.3390/plants4040728

Sparvoli, F., Laureati, M., Pilu, R., Pagliarini, E., Toschi, I., Giuberti, G., . . . Bollini, R. (2016). Exploitation of Common Bean Flours with Low Antinutrient Content for Making Nutritionally Enhanced Biscuits. *Frontiers in Plant Science, 7*. doi:ARTN 92810.3389/fpls.2016.00928

Stadt, K. J., Gendron, F., Lieffers, V. J., Messier, C., & Comeau, P. G. (1999). Predicting and managing light in the understory of boreal forests. *Canadian Journal of Forest Research, 29*(6), 796-811. doi:DOI 10.1139/cjfr-29-6-796

Stevenson-Paulik, J., Bastidas, R. J., Chiou, S. T., Frye, R. A., & York, J. D. (2005). Generation of phytate-free seeds in Arabidopsis through disruption of inositol polyphosphate kinases. *Proceedings of the National Academy of Sciences of the United States of America, 102*(35), 12612-12617. doi:10.1073/pnas.0504172102

Svistoonoff, S., Creff, A., Reymond, M., Sigoillot-Claude, C., Ricaud, L., Blanchet, A., . . . Desnos, T. (2007). Root tip contact with low-phosphate media reprograms plant root architecture. *Nature Genetics, 39*(6), 792-796. doi:10.1038/ng2041

Svitashev, S., Schwartz, C., Lenderts, B., Young, J. K., & Mark Cigan, A. (2016). Genome editing in maize directed by CRISPR-Cas9 ribonucleoprotein complexes. *Nature Communications, 7*, 13274. doi:10.1038/ncomms13274

Swarbreck, S. M., Wang, M., Wang, Y., Kindred, D., Sylvester-Bradley, R., Shi, W. M., . . . Griffiths, H. (2019). A Roadmap for Lowering Crop Nitrogen Requirement. *Trends in Plant Science, 24*(10), 892-904. doi:10.1016/j.tplants.2019.06.006

Swinnen, G., Goossens, A., & Pauwels, L. (2016). Lessons from Domestication: Targeting Cis-Regulatory Elements for Crop Improvement. *Trends in Plant Science, 21*(6), 506-515. doi:10.1016/j.tplants.2016.01.014

Sylvester-Bradley, R., & Kindred, D. R. (2009). Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency. *Journal of Experimental Botany, 60*(7), 1939-1951. doi:10.1093/jxb/erp116

Tabbita, F., Pearce, S., & Barneix, A. J. (2017). Breeding for increased grain protein and micronutrient content in wheat: Ten years of the GPC-B1 gene. *Journal of Cereal Science, 73*, 183-191. doi:10.1016/j.jcs.2017.01.003

Takahashi, M., Nakanishi, H., Kawasaki, S., Nishizawa, N. K., & Mori, S. (2001). Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. *Nature Biotechnology, 19*(5), 466-469. doi:Doi 10.1038/88143

Talboys, P. J., Heppell, J., Roose, T., Healey, J. R., Jones, D. L., & Withers, P. J. A. (2016). Struvite: a slow-release fertiliser for sustainable phosphorus management? *Plant and Soil, 401*(1-2), 109-123. doi:10.1007/s11104-015-2747-3

Tandonnet, J. P., Marguerit, E., Cookson, S. J., & Ollat, N. (2018). Genetic architecture of aerial and root traits in field-grown grafted grapevines is largely independent. *Theoretical and Applied Genetics, 131*(4), 903-915. doi:10.1007/s00122-017-3046-6

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*. doi:ARTN 388410.1038/s41467-018-06430-8

Taylor-Teeples, M., Lin, L., de Lucas, M., Turco, G., Toal, T. W., Gaudinier, A., . . . Brady, S. M. (2015). An Arabidopsis gene regulatory network for secondary cell wall synthesis. *Nature, 517*(7536), 571-U307. doi:10.1038/nature14099

Taylor, S. H., & Long, S. P. (2017). Slow induction of photosynthesis on shade to sun transitions in wheat may cost at least 21% of productivity. *Philosophical Transactions of the Royal Society B-Biological Sciences, 372*(1730). doi:ARTN 2016054310.1098/rstb.2016.0543

Tegeder, M. (2012). Transporters for amino acids in plant cells: some functions and many unknowns. *Current Opinion in Plant Biology, 15*(3), 315-321. doi:10.1016/j.pbi.2012.02.001

Tegeder, M., & Masclaux-Daubresse, C. (2018). Source and sink mechanisms of nitrogen transport and use. *New Phytologist, 217*(1), 35-53. doi:10.1111/nph.14876

Ticconi, C. A., Delatorre, C. A., Lahner, B., Salt, D. E., & Abel, S. (2004). Arabidopsis pdr2 reveals a phosphate-sensitive checkpoint in root development. *Plant Journal, 37*(6), 801-814. doi:10.1111/j.1365-313X.2004.02005.x

Ticconi, C. A., Lucero, R. D., Sakhonwasee, S., Adamson, A. W., Creff, A., Nussaume, L., . . . Abel, S. (2009). ER-resident proteins PDR2 and LPR1 mediate the developmental response of root meristems to phosphate availability. *Proceedings of the National Academy of Sciences of the United States of America, 106*(33), 14174-14179. doi:10.1073/pnas.0901778106

Tilman, D., Balzer, C., Hill, J., & Befort, B. L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences of the United States of America, 108*(50), 20260-20264. doi:10.1073/pnas.1116437108

Tocher, D. R., Betancor, M. B., Sprague, M., Olsen, R. E., & Napier, J. A. (2019). Omega-3 Long-Chain Polyunsaturated Fatty Acids, EPA and DHA: Bridging the Gap between Supply and Demand. *Nutrients, 11*(1). doi:ARTN 8910.3390/nu11010089

Tong, C., Chen, Y. L., Tan, Y. Y., Liu, L., Waters, D. L. E., Rose, T. J., . . . Bao, J. S. (2017). Analysis of Lysophospholipid Content in Low Phytate Rice Mutants. *Journal of Agricultural and Food Chemistry, 65*(26), 5435-5441. doi:10.1021/acs.jafc.7b01576

Toth, B., Weynants, M., Pasztor, L., & Hengl, T. (2017). 3D soil hydraulic database of Europe at 250 m resolution. *Hydrological Processes, 31*(14), 2662-2666. doi:10.1002/hyp.11203

Toth G., Adhikari K., Varallyay G., Toth T., Bodis K., Stolbovoy V. (2008) Updated map of salt affected soils in the European Union. In: Toth G, Montanarella, L. and Rusco, E. (ed) Threats to Soil Quality in Europe. Office for Official Publications of the European Communities, Luxembourg, pp 65-77

Touati, M., Knipfer, T., Visnovitz, T., Kameli, A., & Fricke, W. (2015). Limitation of Cell Elongation in Barley (Hordeum vulgare L.) Leaves Through Mechanical and Tissue-Hydraulic Properties. *Plant and Cell Physiology, 56*(7), 1364-1373. doi:10.1093/pcp/pcv055

Trevaskis, B. (2010). The central role of the VERNALIZATION1 gene in the vernalization response of cereals. *Functional Plant Biology, 37*(6), 479-487. doi:10.1071/Fp10056

Tseng, C. K. (1981). Commercial cultivation [Algae]. Botanical monographs (USA).

Tsukaya, H. (2013). Leaf development. *The arabidopsis book, 11*, e0163. doi:10.1199/tab.0163

Tuan, P. A., Park, W. T., Xu, H., Park, N. I., & Park, S. U. (2012). Accumulation of Tilianin and Rosmarinic Acid and Expression of Phenylpropanoid Biosynthetic Genes in Agastache rugosa. *Journal of Agricultural and Food Chemistry, 60*(23), 5945-5951. doi:10.1021/jf300833m

Tuberosa, R., & Salvi, S. (2006). Genomics-based approaches to improve drought tolerance of crops. *Trends in Plant Science, 11*(8), 405-412. doi:10.1016/j.tplants.2006.06.003

Turco, G. M., Kajala, K., Kunde-Ramamoorthy, G., Ngan, C. Y., Olson, A., Deshphande, S., . . . Brady, S. M. (2017). DNA methylation and gene expression regulation associated with vascularization in Sorghum bicolor. *New Phytologist, 214*(3), 1213-1229. doi:10.1111/nph.14448

Uauy, C., Brevis, J. C., & Dubcovsky, J. (2006). The high grain protein content gene Gpc-B1 accelerates senescence and has pleiotropic effects on protein content in wheat. *Journal of Experimental Botany, 57*(11), 2785-2794. doi:10.1093/jxb/erl047

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., & Dubcovsky, J. (2006). A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science, 314*(5803), 1298-1301. doi:10.1126/science.1133649

Uga, Y., Okuno, K., & Yano, M. (2011). Dro1, a major QTL involved in deep rooting of rice under upland field conditions. *Journal of Experimental Botany, 62*(8), 2485-2494. doi:10.1093/jxb/erq429

Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., . . . Yano, M. (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature Genetics, 45*(9), 1097-+. doi:10.1038/ng.2725

Usher, S., Han, L. H., Haslam, R. P., Michaelson, L. V., Sturtevant, D., Aziz, M., . . . Napier, J. A. (2017). Tailoring seed oil composition in the real world: optimising omega-3 long chain polyunsaturated fatty acid accumulation in transgenic Camelina sativa. *Scientific Reports, 7*. doi:ARTN 657010.1038/s41598-017-06838-0

Usher, S., Haslam, R. P., Ruiz-Lopez, N., Sayanova, O., & Napier, J. A. (2015). Field trial evaluation of the accumulation of omega-3 long chain polyunsaturated fatty acids in transgenic Camelina sativa: Making fish oil substitutes in plants. *Metab Eng Commun, 2*, 93-98. doi:10.1016/j.meteno.2015.04.002

Valladares, F., & Niinemets, U. (2007). The Architecture of Plant Crowns: From Design Rules to Light Capture and Performance. In *Functional plant Ecology* (pp. 101–150). https://doi.org/10.1029/2001JD001242

Valladares, F., & Pearcy, R. W. (1999). The geometry of light interception by shoots of Heteromeles arbutifolia: morphological and physiological consequences for individual leaves. *Oecologia, 121*(2), 171-182. doi:DOI 10.1007/s004420050919

Van Acker, R., Vanholme, R., Storme, V., Mortimer, J. C., Dupree, P., & Boerjan, W. (2013). Lignin biosynthesis perturbations affect secondary cell wall composition and saccharification yield in Arabidopsis thaliana. *Biotechnology for Biofuels, 6*. doi:Artn 4610.1186/1754-6834-6-46

Van de Velde, J., Heyndrickx, K. S., & Vandepoele, K. (2014). Inference of transcriptional networks in Arabidopsis through conserved noncoding sequence analysis. *The Plant cell, 26*(7), 2729-2745. doi:10.1105/tpc.114.127001

Van de Velde, J., Van Bel, M., Vaneechoutte, D., & Vandepoele, K. (2016). A Collection of Conserved Noncoding Sequences to Study Gene Regulation in Flowering Plants. *Plant Physiology, 171*(4), 2586-2598. doi:10.1104/pp.16.00821

Van den Bosch, S., Schutyser, W., Vanholme, R., Driessen, T., Koelewijn, S. F., Renders, T., . . . Sels, B. F. (2015). Reductive lignocellulose fractionation into soluble lignin-derived phenolic monomers and dimers and processable carbohydrate pulps. *Energy & Environmental Science, 8*(6), 1748-1763. doi:10.1039/c5ee00204d

Van der Weijde, T., Huxley, L. M., Hawkins, S., Sembiring, E. H., Farrar, K., Dolstra, O., . . . Trindade, L. M. (2017). Impact of drought stress on growth and quality of miscanthus for biofuel production. *Global Change Biology Bioenergy, 9*(4), 770-782. doi:10.1111/gcbb.12382

Van Eck, J., Conlin, B., Garvin, D. F., Mason, H., Navarre, D. A., & Brown, C. R. (2007). Enhancing beta-carotene content in potato by RNAi-mediated silencing of the beta-carotene hydroxylase gene. *American Journal of Potato Research, 84*(4), 331-342. doi:Doi 10.1007/Bf02986245

van Grinsven, H. J. M., Rabl, A., & de Kok, T. M. (2010). Estimation of incidence and social cost of colon cancer due to nitrate in drinking water in the EU: a tentative cost-benefit assessment. *Environmental Health, 9*. doi:Artn 5810.1186/1476-069x-9-58

van Nocker, S., & Gardiner, S. E. (2014). Breeding better cultivars, faster: applications of new technologies for the rapid deployment of superior horticultural tree crops. *Horticulture Research, 1*, 14022. doi:10.1038/hortres.2014.22

Vandersteen, J., Jaunard, D., Mahy, G., Bizoux, J. P., Monty, A., De Proft, M., . . . Bodson, B. (2011). Dynamic of black-grass populations depending on the sowing time of winter wheat. *Communications in Agricultural and Applied Biological Sciences, 76*(3), 485-490. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22696956

Vanholme, R., Morreel, K., Darrah, C., Oyarce, P., Grabber, J. H., Ralph, J., & Boerjan, W. (2012). Metabolic engineering of novel lignin in biomass crops. *New Phytologist, 196*(4), 978-1000. doi:10.1111/j.1469-8137.2012.04337.x

Veillet, F., Perrot, L., Chauvin, L., Kermarrec, M. P., Guyon-Debast, A., Chauvin, J. E., . . . Mazier, M. (2019). Transgene-Free Genome Editing in Tomato and Potato Plants Using Agrobacterium-Mediated Delivery of a CRISPR/Cas9 Cytidine Base Editor. *International Journal of Molecular Sciences, 20*(2). doi:10.3390/ijms20020402

Vercruysse, J., Baekelandt, A., Gonzalez, N., & Inze, D. (2019). Molecular networks regulating the cell division during leaf growth in Arabidopsis. *J Exp Bot*. doi:10.1093/jxb/erz522

Vishal, B., Krishnamurthy, P., Ramamoorthy, R., & Kumar, P. P. (2019). OsTPS8 controls yield-related traits and confers salt stress tolerance in rice by enhancing suberin deposition. *New Phytologist, 221*(3), 1369-1386. doi:10.1111/nph.15464

Volkov, V. (2015). Salinity tolerance in plants. Quantitative approach to ion transport starting from halophytes and stepping to genetic and protein engineering for manipulating ion fluxes. *Frontiers in Plant Science, 6*. doi:ARTN 87310.3389/fpls.2015.00873

von Caemmerer, S., & Furbank, R.T. (1999). Modeling C4 photosynthesis. *In: Sage R.F., Monson R.K., eds. C4 Plant Biology, Toronto, Academic Press,* 173–211.

Vos, J., Chelle, M., DeJong, T., Fournier, C., Struik, P. C., & Yin, X. Y. (2007). Functional-structural plant models in crop production. *Functional-Structural Plant Modelling in Crop Production, 22*, 265-268. Retrieved from <Go to ISI>://WOS:000252961900023

Vos, J., Evers, J. B., Buck-Sorlin, G. H., Bruno, A., Michael, C., & De Visser, P. H. (2009). Functional-structural plant modelling: A new paradigm in crop science. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology, 153a*(2), S223-S223. doi:10.1016/j.cbpa.2009.04.552

Voss-Fels, K. P., Cooper, M., & Hayes, B. J. (2019). Accelerating crop genetic gains with genomic selection. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik, 132*(3), 669-686. doi:10.1007/s00122-018-3270-8

Walker, B. J., VanLoocke, A., Bernacchi, C. J., & Ort, D. R. (2016). The Costs of Photorespiration to Food Production Now and in the Future. *Annual Review of Plant Biology, Vol 67, 67*, 107-+. doi:10.1146/annurev-arplant-043015-111709

Wang, B., Zhu, L., Zhao, B., Zhao, Y., Xie, Y., Zheng, Z., . . . Wang, H. (2019). Development of a Haploid-Inducer Mediated Genome Editing System for Accelerating Maize Breeding. *Molecular Plant, 12*(4), 597-602. doi:10.1016/j.molp.2019.03.006

Wang, H. P., Zhao, K., & Song, F. B. (2008). Approach to modeling and virtual-reality-based simulation for plant canopy lighting. *Chinese Geographical Science, 18*(4), 374-381. doi:10.1007/s11769-008-0374-x

Wang, Z., Li, N., Jiang, S., Gonzalez, N., Huang, X., Wang, Y., . . . Li, Y. (2016). SCF^{SAP} controls organ size by targeting PPD proteins for degradation in *Arabidopsis thaliana*. *Nature Communications, 7*, 11192. doi:10.1038/ncomms11192

Wang, Z. Y., Ruan, W. Y., Shi, J., Zhang, L., Xiang, D., Yang, C., . . . Wu, P. (2014). Rice SPX1 and SPX2 inhibit phosphate starvation responses through interacting with PHR2 in a phosphate-dependent manner. *Proceedings of the National Academy of Sciences of the United States of America, 111*(41), 14953-14958. doi:10.1073/pnas.1404680111

Watanabe, T., Hanan, J. S., Room, P. M., Hasegawa, T., Nakagawa, H., & Takahashi, W. (2005). Rice morphogenesis and plant architecture: Measurement, specification and the reconstruction of structural development by 3D architectural modelling. *Annals of Botany, 95*(7), 1131-1143. doi:DOI 10.1093/aob/mci136

Waters, B. M., Uauy, C., Dubcovsky, J., & Grusak, M. A. (2009). Wheat (Triticum aestivum) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. *Journal of Experimental Botany, 60*(15), 4263-4274. doi:10.1093/jxb/erp257

Weiss, M., Baret, F., Smith, G. J., Jonckheere, I., & Coppin, P. (2004). Review of methods for in situ leaf area index (LAI) determination Part II. Estimation of LAI, errors and sampling. *Agricultural and Forest Meteorology, 121*(1-2), 37-53. doi:10.1016/j.agrformet.2003.08.001

Welch, J. R., Vincent, J. R., Auffhammer, M., Moya, P. F., Dobermann, A., & Dawe, D. (2010). Rice yields in tropical/subtropical Asia exhibit large but opposing sensitivities to minimum and maximum temperatures. *Proceedings of the National Academy of Sciences of the United States of America, 107*(33), 14562-14567. doi:10.1073/pnas.1001222107

West, A. L., Miles, E. A., Lillycrop, K. A., Han, L. H., Sayanova, O., Napier, J. A., . . . Burdge, G. C. (2019). Postprandial incorporation of EPA and DHA from transgenic Camelina sativa oil into blood lipids is equivalent to that from fish oil in healthy humans. *British Journal of Nutrition, 121*(11), 1235-1246. doi:Pii S000711451900082510.1017/S0007114519000825

Wheeler, T. R., Craufurd, P. Q., Ellis, R. H., Porter, J. R., & Prasad, P. V. V. (2000). Temperature variability and the yield of annual crops. *Agriculture Ecosystems & Environment, 82*(1-3), 159-167. doi:Doi 10.1016/S0167-8809(00)00224-3

White, D. W. R. (2006). *PEAPOD* regulates lamina size and curvature in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America, 103*(35), 13238-13243. doi:10.1073/pnas.0604349103

White, J. W., Andrade-Sanchez, P., Gore, M. A., Bronson, K. F., Coffelt, T. A., Conley, M. M., . . . Wang, G. Y. (2012). Field-based phenomics for plant genetics research. *Field Crops Research, 133*, 101-112. doi:10.1016/j.fcr.2012.04.003

Wild, R., Gerasimaite, R., Jung, J. Y., Truffault, V., Pavlovic, I., Schmidt, A., . . . Mayer, A. (2016). Control of eukaryotic phosphate homeostasis by inositol polyphosphate sensor domains. *Science, 352*(6288), 986-990. doi:10.1126/science.aad9858

Wilkerson, C. G., Mansfield, S. D., Lu, F., Withers, S., Park, J. Y., Karlen, S. D., . . . Ralph, J. (2014). Monolignol Ferulate Transferase Introduces Chemically Labile Linkages into the Lignin Backbone. *Science, 344*(6179), 90-93. doi:10.1126/science.1250161

Williams TJ, Haq N (2002) Global research on underutilized crops: an assessment of current activities and proposals for enhanced cooperation International Centre for Underutilised Crops, University of Southampton

Wilson, J. (1963). Estimation of foliage denseness and foliage angle by inclined point quadrats. *Australian Journal of Botany*, *11*(1), 95. https://doi.org/10.1071/BT9630095

Wipf, D., Krajinski, F., van Tuinen, D., Recorbet, G., & Courty, P. E. (2019). Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. *New Phytologist, 223*(3), 1127-1142. doi:10.1111/nph.15775

Wissuwa, M., & Ae, N. (2001). Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. *Plant Breeding, 120*(1), 43-48. doi:DOI 10.1046/j.1439-0523.2001.00561.x

Wolfe, M. S. (1985). The Current Status and Prospects of Multiline Cultivars and Variety Mixtures for Disease Resistance. *Annual Review of Phytopathology, 23*, 251-273. doi:DOI 10.1146/annurev.py.23.090185.001343

Wolter, F., & Puchta, H. (2018). The CRISPR/Cas revolution reaches the RNA world: Cas13, a new Swiss Army knife for plant biologists. *The Plant journal : for cell and molecular biology, 94*(5), 767-775. doi:10.1111/tpj.13899

Woodrow, I. E., & Mott, K. A. (1989). Rate Limitation of Non-Steady-State Photosynthesis by Ribulose-1,5-Bisphosphate Carboxylase in Spinach. *Australian Journal of Plant Physiology, 16*(6), 487-500. doi:Doi 10.1071/Pp9890487

Wu, A., Hammer, G. L., Doherty, A., von Caemmerer, S., & Farquhar, G. D. (2019). Quantifying impacts of enhancing photosynthesis on crop yield. *Nature Plants, 5*(4), 380-388. doi:10.1038/s41477-019-0398-8

Wu, T., Qin, Z. W., Fan, L. X., Xue, C. B., Zhou, X. Y., Xin, M., & Du, Y. L. (2014). Involvement of CsNRT1.7 in nitrate recycling during senescence in cucumber. *Journal of Plant Nutrition and Soil Science, 177*(5), 714-721. doi:10.1002/jpln.201300665

Xin, C. P., Tholen, D., Devloo, V., & Zhu, X. G. (2015). The Benefits of Photorespiratory Bypasses: How Can They Work? *Plant Physiology, 167*(2), 574-585. doi:10.1104/pp.114.248013

Xu, C. Z., Fu, X. K., Liu, R., Guo, L., Ran, L. Y., Li, C. F., . . . Luo, K. M. (2017). PtoMYB170 positively regulates lignin deposition during wood formation in poplar and confers drought tolerance in transgenic Arabidopsis. *Tree Physiology, 37*(12), 1713-1726. doi:10.1093/treephys/tpx093

Yamori, W., Masumoto, C., Fukayama, H., & Makino, A. (2012). Rubisco activase is a key regulator of non-steady-state photosynthesis at any leaf temperature and, to a lesser extent, of steady-state photosynthesis at high temperature. *Plant Journal, 71*(6), 871-880. doi:10.1111/j.1365-313X.2012.05041.x

Yanagisawa, S., Akiyama, A., Kisaka, H., Uchimiya, H., & Miwa, T. (2004). Metabolic engineering with Dof1 transcription factor in plants: Improved nitrogen assimilation and growth under low-nitrogen conditions. *Proceedings of the National Academy of Sciences of the United States of America, 101*(20), 7833-7838. doi:10.1073/pnas.0402267101

Yang, T. Y., Zhang, S., Hu, Y. B., Wu, F. C., Hu, Q. D., Chen, G., . . . Xu, G. H. (2014). The Role of a Potassium Transporter OsHAK5 in Potassium Acquisition and Transport from Roots to Shoots in Rice at Low Potassium Supply Levels. *Plant Physiology, 166*(2), 945-U757. doi:10.1104/pp.114.246520

Yang, Z. Y., Liu, J. H., Poree, F., Schaeufele, R., Helmke, H., Frackenpohl, J., . . . Grill, E. (2019). Abscisic Acid Receptors and Coreceptors Modulate Plant Water Use Efficiency and Water Productivity. *Plant Physiology, 180*(2), 1066-1080. doi:10.1104/pp.18.01238

Yang, Z. Y., Liu, J. H., Tischer, S. V., Christmann, A., Windisch, W., Schnyder, H., & Grill, E. (2016). Leveraging abscisic acid receptors for efficient water use in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America, 113*(24), 6791-6796. doi:10.1073/pnas.1601954113

Yao, L., Zhang, Y., Liu, C., Liu, Y., Wang, Y., Liang, D., . . . Kelliher, T. (2018). OsMATL mutation induces haploid seed formation in indica rice. *Nature Plants, 4*(8), 530-533. doi:10.1038/s41477-018-0193-y

Yarish, C., Chopin, T., Sahoo, D. B., Fei, X. G., He, P., Chung, I., . . . Ask, E. (2005). Recent Developments in the Large Scale Open Water Mariculture of Seaweeds and Their Utilization. *Phycologia, 44*(4), 113-113. Retrieved from <Go to ISI>://WOS:000205501500273

Ye, H., Roorkiwal, M., Valliyodan, B., Zhou, L. J., Chen, P. Y., Varshney, R. K., & Nguyen, H. T. (2018). Genetic diversity of root system architecture in response to drought stress in grain legumes. *Journal of Experimental Botany, 69*(13), 3267-3277. doi:10.1093/jxb/ery082

Ye, X. D., Al-Babili, S., Kloti, A., Zhang, J., Lucca, P., Beyer, P., & Potrykus, I. (2000). Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science, 287*(5451), 303-305. doi:DOI 10.1126/science.287.5451.303

Yin, K., Gao, C., & Qiu, J. L. (2017). Progress and prospects in plant genome editing. *Nature Plants, 3*, 17107. doi:10.1038/nplants.2017.107

Yin, X. Y., & Struik, P. C. (2017). Can increased leaf photosynthesis be converted into higher crop mass production? A simulation study for rice using the crop model GECROS. *Journal of Experimental Botany, 68*(9), 2345-2360. doi:10.1093/jxb/erx085

Yordanov, Y. S., Ma, C., Yordanova, E., Meilan, R., Strauss, S. H., & Busov, V. B. (2017). *BIG LEAF* is a regulator of organ size and adventitious root formation in poplar. *PLoS ONE, 12*(7), e0180527. doi:10.1371/journal.pone.0180527

Yoshida, T., Christmann, A., Yamaguchi-Shinozaki, K., Grill, E., & Fernie, A. R. (2019). Revisiting the Basal Role of ABA - Roles Outside of Stress. *Trends in Plant Science, 24*(7), 625-635. doi:10.1016/j.tplants.2019.04.008

Yu, J. L., Zhen, X. X., Li, X., Li, N., & Xu, F. (2019). Increased Autophagy of Rice Can Increase Yield and Nitrogen Use Efficiency (NUE). *Frontiers in Plant Science, 10*. doi:ARTN 584 10.3389/fpls.2019.00584

Yu, L. H., Chen, X., Wang, Z., Wang, S. M., Wang, Y. P., Zhu, Q. S., . . . Xiang, C. B. (2013). Arabidopsis Enhanced Drought Tolerance1/HOMEODOMAIN GLABROUS11 Confers Drought Tolerance in Transgenic Rice without Yield Penalty. *Plant Physiology, 162*(3), 1378-1391. doi:10.1104/pp.113.217596

Yu, L. H., Wu, J., Tang, H., Yuan, Y., Wang, S. M., Wang, Y. P., . . . Xiang, C. B. (2016). Overexpression of Arabidopsis NLP7 improves plant growth under both nitrogen-limiting and -sufficient conditions by enhancing nitrogen and carbon assimilation. *Scientific Reports, 6*. doi:ARTN 27795 10.1038/srep27795

Yuan, F., Yang, H. M., Xue, Y., Kong, D. D., Ye, R., Li, C. J., . . . Pei, Z. M. (2014). OSCA1 mediates osmotic-stress-evoked Ca2+ increases vital for osmosensing in Arabidopsis. *Nature, 514*(7522), 367-+. doi:10.1038/nature13593

Zelitch, I. (1982). The Close Relationship between Net Photosynthesis and Crop Yield. *Bioscience, 32*(10), 796-802. doi:Doi 10.2307/1308973

Zelitch, I., & Day, P. R. (1973). The effect on net photosynthesis of pedigree selection for low and high rates of photorespiration in tobacco. *Plant Physiology, 52*(1), 33-37. doi:10.1104/pp.52.1.33

Zhang, J. Y., Liu, Y. X., Zhang, N., Hu, B., Jin, T., Xu, H. R., . . . Bai, Y. (2019). NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. *Nature Biotechnology, 37*(6), 676-+. doi:10.1038/s41587-019-0104-4

Zhang, L. Z., Tan, Q. M., Lee, R., Trethewy, A., Lee, Y. H., & Tegeder, M. (2010). Altered Xylem-Phloem Transfer of Amino Acids Affects Metabolism and Leads to Increased Seed Yield and Oil Content in Arabidopsis. *Plant Cell, 22*(11), 3603-3620. doi:10.1105/tpc.110.073833

Zhang, N., & Zhao, Y. (2007). Effects of plant crown shape on reflectance of grassland. *Journal of Remote Sensing*, *11*, 9–19.

Zhang, P. F., He, Z. H., Tian, X. L., Gao, F. M., Xu, D. G., Liu, J. D., . . . Cao, S. H. (2017). Cloning of TaTPP-6AL1 associated with grain weight in bread wheat and development of functional marker. *Molecular Breeding, 37*(6). doi:ARTN 78 10.1007/s11032-017-0676-y

Zhang, Q., Zhang, Y., Lu, M. H., Chai, Y. P., Jiang, Y. Y., Zhou, Y., . . . Chen, Q. J. (2019). A Novel Ternary Vector System United with Morphogenic Genes Enhances CRISPR/Cas Delivery in Maize. *Plant Physiology, 181*(4), 1441-1448. doi:10.1104/pp.19.00767

Zhang, Y., Liang, Z., Zong, Y., Wang, Y., Liu, J., Chen, K., . . . Gao, C. (2016). Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. *Nature Communications, 7*, 12617. doi:10.1038/ncomms12617

Zhang, Y. H., Primavesi, L. F., Jhurreea, D., Andralojc, P. J., Mitchell, R. A. C., Powers, S. J., . . . Paul, M. J. (2009). Inhibition of SNF1-Related Protein Kinase1 Activity and Regulation of Metabolic Pathways by Trehalose-6-Phosphate. *Plant Physiology, 149*(4), 1860-1871. doi:10.1104/pp.108.133934

Zhang, Y. X., Malzahn, A. A., Sretenovic, S., & Qi, Y. P. (2019). The emerging and uncultivated potential of CRISPR technology in plant science. *Nature Plants, 5*(8), 778-794. doi:10.1038/s41477-019-0461-5

Zhao, C. C., Wang, Y. Y., Chan, K. X., Marchant, D. B., Franks, P. J., Randall, D., . . . Chen, Z. H. (2019). Evolution of chloroplast retrograde signaling facilitates green plant adaptation to land. *Proceedings of the National Academy of Sciences of the United States of America, 116*(11), 5015-5020. doi:10.1073/pnas.1812092116

Zhao, X. B., Pang, S. J., Liu, F., Shan, T. F., Li, J., Gao, S. Q., & Kim, H. G. (2016). Intraspecific crossing of Saccharina japonica using distantly related unialgal gametophytes benefits kelp farming by improving blade quality and productivity at Sanggou Bay, China. *Journal of Applied Phycology, 28*(1), 449-455. doi:10.1007/s10811-015-0597-2

Zhao, Y., Chan, Z. L., Gao, J. H., Xing, L., Cao, M. J., Yu, C. M., . . . Zhu, J. K. (2016). ABA receptor PYL9 promotes drought resistance and leaf senescence. *Proceedings of the National Academy of Sciences of the United States of America, 113*(7), 1949-1954. doi:10.1073/pnas.1522840113

Zheng, B. Y., Shi, L. J., Ma, Y. T., Deng, Q. Y., Li, B. G., & Guo, Y. (2008). Comparison of architecture among different cultivars of hybrid rice using a spatial light model based on 3-D digitising. *Functional Plant Biology, 35*(9-10), 900-910. doi:10.1071/Fp08060

Zhou, Z., Tan, H. X., Li, Q., Chen, J. F., Gao, S. H., Wang, Y., . . . Zhang, L. (2018). CRISPR/Cas9-mediated efficient targeted mutagenesis of RAS in Salvia miltiorrhiza. *Phytochemistry, 148*, 63-70. doi:10.1016/j.phytochem.2018.01.015

Zhu, X. G., Long, S. P., & Ort, D. R. (2008). What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? *Current Opinion in Biotechnology, 19*(2), 153-159. doi:DOI 10.1016/j.copbio.2008.02.004

Zhu, X. G., Long, S. P., & Ort, D. R. (2010). Improving Photosynthetic Efficiency for Greater Yield. *Annual Review of Plant Biology, Vol 61, 61*, 235-261. doi:10.1146/annurev-arplant-042809-112206

Zhu, X. G., Ort, D. R., Whitmarsh, J., & Long, S. P. (2004). The slow reversibility of photosystem II thermal energy dissipation on transfer from high to low light may cause large losses in carbon gain by crop canopies: a theoretical analysis. *Journal of Experimental Botany, 55*(400), 1167-1175. doi:10.1093/jxb/erh141

Zinn, K. E., Tunc-Ozdemir, M., & Harper, J. F. (2010). Temperature stress and plant sexual reproduction: uncovering the weakest links. *Journal of Experimental Botany, 61*(7), 1959-1968. doi:10.1093/jxb/erq053

Zsogon, A., Cermak, T., Naves, E. R., Notini, M. M., Edel, K. H., Weinl, S., . . . Peres, L. E. P. (2018). De novo domestication of wild tomato using genome editing. *Nature Biotechnology*. doi:10.1038/nbt.4272