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CropBooster-P

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1 Introduction to the CropBooster Program

1.1 CropBooster-P and the CropBooster Program

CropBooster: a programme to improve the sustainability, productivity, and nutritional value of tomorrow's future-proofed crops.

The “Preparatory action to Boost Global Crop Yield for Food & Nutrition Security and fueling a Bioeconomy” project **CropBooster-P,** is a Coordination and Support Action (CSA) to devise the CropBooster plan to future-proof Europe's crops. CropBooster proposes a Europe wide research programme with the ambition to increase the productivity and sustainability of Europe's agriculture by improving the crops we grow. This forms the basis to develop a high-yielding, efficient, resilient, climate-smart sustainable agriculture that will deliver sovereign food security whilst protecting the environment as an integral part of Europe’s Sustainable Food Systems. The required crop improvements will be achieved using breeding technologies that are acceptable in Europe and build on an unprecedented collaboration (Figure 1.1).

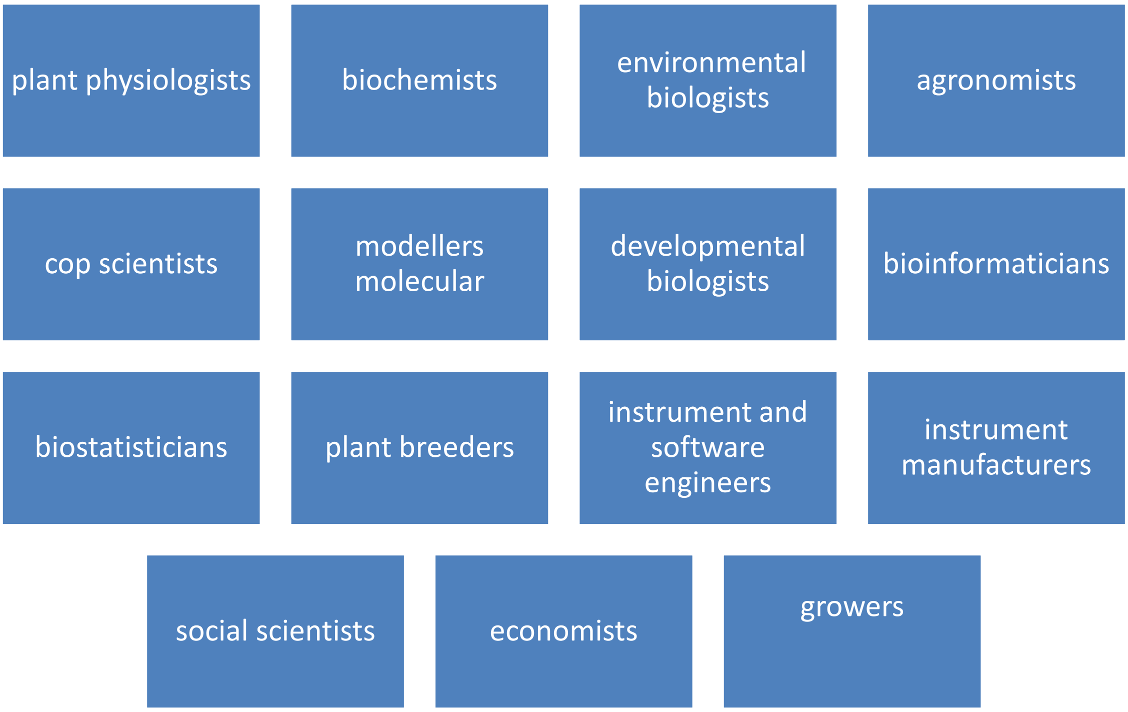

Figure 1.1. CropBooster is adopting a highly multidisciplinary approach.

CropBooster proposes a unique programme in agricultural research that will produce the genetic discoveries and innovations needed by the breeding industry to create the next generation of crops designed for the new challenges of a changing climate. This plan has been codesigned by the community of European plant scientists and stakeholders to take the world leading science that we are developing, building on the advance of recent decades to drive a new **Green Revolution for Europe**. CropBooster will focus on improving our crop plants and will not be a programme to improve the agricultural systems in which these crops will be grown but will work alongside systems improvement and aligned to the EC’s priorities. A number of Partnerships designed to improve agri-food production systems are already established or in the process of being established including the Partnership for Agro-Ecology, the European Partnership for Safe and Sustainable Food Systems and the European Partnership for a Circular bio-based Europe. Within this constellation of programmes CropBooster will be the only programme specifically focusing on crop-improvement, and how future-proofing crops is a key enabling approach.

We see our programme as being an essential component of the EU agri-food research network, complementing the other programmes by providing them with the improved crops they will need.

We must all work together

1.2 Foreword: Impacts and adaptation of arable crops to climatic and atmospheric change.

EU imports of plant products between 2001 and 2021 have grown faster than exports. By 2021 imports net of exports were exceeded €20M double that of 2013 (EUROSTAT, 2922). This has two key implications. First, this increasing demand on world markets will raise the price of primary foodstuffs and feeds worldwide, affecting the ability of the world's poorest to access sufficient food. Since 2014 the proportion of the world population that is food insufficient has been rising, reaching 1 in 8 by 2021. Secondly, while the EU strives to achieve agricultural sustainability in its arable agriculture, its net growth in imports results in it relying increasingly on crops likely produced at lower standards of sustainability, than those aimed at within the EU. The EU is also falling behind in yield improvements in the major crops. For example, average yields of maize across the EU between 2006 and 2010 were 7.2 t ha^{-1} compared to 6.8 t ha^{-1} in Argentina and 9.6 t ha^{-1} in the USA. For the period 2016-2020 this number was essentially unchanged in the EU at 7.3 t ha^{-1} but increased by 8% to 7.3 t ha^{-1} in Argentina and 13% to 10.8 t ha^{-1} in the USA (FAOSTAT, 2022a). Soybean is one of the largest grain imports to the EU. EU production was 2.3Mt in 1990, and increased a mere 0.7Mt by 2020, while imports rose from 13 Mt to 18 Mt, so widening the gap by over 4 Mt and increasing the external EU land footprint with environmental impact in some the most vulnerable and important areas of biodiversity (Boerema *et al.*, 2016, FAOSTAT, 2022a, FAOSTAT, 2022b). EU food imports also cost massive amounts of virtual water, I.e. water used in other countries to produce that food, often unsustainably (Serrano *et al.*, 2016). Can we expect this trend of decline in EU arable production and increased dependence on imported plant foodstuffs to continue? Will this be exacerbated by climate change or could opportunities be taken to make the EU more self-sufficient in arable crops despite climate change? Three key aspects of the impacts and opportunities for adaptation to climatic and atmospheric change are considered here: 1) The direct effect of rising CO_2; 2) temperature; and 3) drought.

1) Rising [CO_2] Today the global average [CO_2] is approaching 420 ppm (µmol mol^{-1}), almost double the average of the last 25M years (IPCC, 2021a) and the concentration in which the ancestors of our major crops evolved. Half of that increase has occurred in just the last 50 years. This is far too little time for evolution or breeder selection to act. Analysis has shown that the key process of photosynthesis is not adapted to this change and that with very limited natural variability could only be adapted by bioengineering. C3 photosynthesis is limited by [CO_2] and so increases could be expected to increase yield in the absence of other changes, such as temperature and drought. In controlled and sheltered environments large increases in yield have been shown by elevating [CO_2] to anticipated mid- to late-century concentrations. However, when the major C3 field crops (rice, wheat and soybean) were grown under more realistic open air elevations of [CO_2] using Free-Air CO_2 Enrichment (FACE) technology, only about half of the increase seen in greenhouses was achieved in yields of the world's most important food and feed crops: rice, wheat and soybean, while no increase was observed in maize, except under drought (Long *et al.*, 2006, Ainsworth & Long, 2021). When photosynthesis exceeds the capacity of the plant to utilize additional carbohydrate, soluble sugars feedback on gene expression to lower investment in the photosynthetic apparatus and in turn photosynthetic rate(Drake *et al.*, 1997). However, more recent FACE studies have shown that the most new rice and soybean cultivars, with high yield potential, can take full advantage of the increase in photosynthesis resulting from anticipated [CO_2] for mid-Century. This suggests that targeted breeding could increase yields of all the major C3 crops under rising [CO_2](Ainsworth & Long, 2021). FACE experiments have shown other, and unexpected, challenges of rising [CO_2]. The Japanese Beetle is a major pest of soybean. Under FACE damage by this and other beetle larvae, was almost doubled. Feeding these beetles soybean leaves grown under elevation [CO_2] in FACE, versus control leaves, increased their longevity and fecundity sufficiently to double their rate of population growth. Subsequent analysis showed this was due to down-regulation of a plant protease inhibitor. Similar changes in population growth were observed in the soybean aphid and the Western Corm Rootworm (Ainsworth & Long, 2021). The latter is expected to have little effect on maize yields in the Americas, where most of the crop carries multiple transgenes for BT, however if replicated in European fields, this effect could be devastating in the absence of resistance. In addition to changes in secondary metabolism, in non-legumes growth in elevated [CO_2] results in decreased grain nitrogen and protein contents, and decreases in some minerals, notably Fe, Zn and Ca (Myers *et al.*, 2014). However, the percentage reductions are much smaller than the variation within germplasm, indicating that this is an effect that could be readily mitigated by breeding to restore these contents (Ainsworth & Long, 2021). A near universal effect of increased [CO_2] is a decrease in stomatal conductance and so transpiratory water loss from the leaf. This has led to the assumption that crops will use less water and be more drought tolerant under elevated [CO_2]. Open-air studies of crops grown from emergence to harvest under elevated [CO_2] using FACE show a less promising picture. Where soybean experienced moist soil following emergence and higher shoot water status under elevated [CO_2], root development was less than in plants grown in current [CO_2], with the result that when later season mild drought occurred production was decreased more in the elevated [CO_2] than control plants (Gray *et al.*, 2016). However, in maize under well-watered conditions there was no increase in yield with elevated [CO_2], which can be attributed to the fact that its C4 photosynthesis is already CO_2 saturated in the current atmosphere. Under drought, yield was increased due to partial stomatal closure in response to elevated [CO_2] lowering water loss and so conserving more of the limited soil moisture (Leakey *et al.*, 2006, Manderscheid *et al.*, 2014).

2) Temperature. While the only direct effect of rising [CO_2] on plants is at the level of photosynthesis, temperature affects all plant processes. As a result the mechanisms of how rising temperatures and heat waves will affect crop production are more poorly understood (Prasad *et al.*, 2006, Bita & Gerats, 2013, Gourdji *et al.*, 2013). However, while [CO_2] increase vary little across the global surface, temperature shows large spatial and inter-annual variation, which coupled with yield records allows extraction of temperature effects on crop yields. Such analyses have shown that for the US cornbelt for each 1°C increase in growing season temperature maize yields would be lowered 1.31 ± 0.09 t ha^{-1} and soybean by 0.38 ± 0.03 t ha^{-1} (Lobell & Asner, 2003). Across the approximately 40 Mha put down to each of these crops in the corn-belt each year the 2 °C temperature rise scenario would cost 52 Mt of maize and 15 Mt of soy, an effect likely replicated across other major growing areas for these crops. It might appear that yields could be restored by moving growing areas further north and south. In N. America and Eurasia, the high yields are achieved on deep organic prairie and steppe soils, in most cases the soils further north are poorer and more erodible. A detailed analysis of inter-annual and spatial variation in wheat yields in Australia, indicated that a 2 °C increase would halve yields (Asseng *et al.*, 2011). In agreement with this loss, an open air experiment, using infra-red heaters to give a precise elevation of the crop surface temperature resulted in a grain yield loss of 29% in spring planted wheat with elevation of daytime temperature of 1.3 °C in Arizona (Ottman *et al.*, 2012). Of course at the northern edge of crop growing regions in the northern hemisphere we could expect such temperature increases to raise yields. How do higher temperatures cause crop yield losses? Exact mechanisms are not always known, but increased temperature will cause increased dark respiratory and photorespiratory losses of carbon, inhibit carboxylation in photosynthesis, and in particular affect flower fertility(Bita & Gerats, 2013, Gourdji *et al.*, 2013). Two factors impact photosynthesis at the level of the primary carboxylase of C3 photosynthesis, Rubisco. First, its specificity for CO_2 relative to oxygen declines with temperature, such that the ratio of wasteful photorespiration to photosynthesis increases with temperature (Long *et al.*, 2004, Walker *et al.*, 2016). Secondly, the activation of Rubisco declines with temperature, because its activating chaperone, Rubisco activase (Rca) shows a strong loss of activity at high temperatures (Crafts-Brandner & Salvucci, 2000, Perdomo *et al.*, 2017). There is however, emerging bioengineering advances that could partially counter these effects. First, photorespiratory by-passes have been successfully engineered into crops to decrease energy losses to the process and increase yield, with the greatest benefit at high temperatures (South *et al.*, 2019, Cavanagh *et al.*, 2022). Secondly, more temperature tolerant forms of Rca have been identified, and their transgenic up-regulation shown to increase temperature tolerance by 2-3 °C (Degen *et al.*, 2020, Degen *et al.*, 2021). The future will combine elevation of both [CO_2] and temperature. Combining FACE and open air heating technologies, it was found that a 2.7 °C increase resulted in a 19-31% loss of soybean yield under both ambient and elevated [CO_2]. Metabolic modeling indicated that a key limitation to photosynthesis when elevated temperature and [CO_2] were combined, was regeneration of the CO_2 acceptor molecule in photosynthesis, RubP. By transgenically increasing capacity for RubP regeneration in soybean, photosynthesis was restored and the yield loss eliminated (Kohler *et al.*, 2017). However, photosynthesis is not the only process affected. A 3 day elevation of temperature by 6 C, during soybean pod development lowered yield by 10% (Siebers *et al.*, 2015). Temperature increases of 2-3 °C can have a strong effect on reproductive growth affecting pollen development, pollen tube growth, and seed development (Prasad *et al.*, 2006, Zinn *et al.*, 2010, Siebers *et al.*, 2015). Stomata which allow CO_2

into the leaf at the expense of water are also present in floral parts, even when these are not photosynthetic (Sinha *et al.*, 2022). Here they can serve to cool the flower to counteract warming, opening the possibility to engineer increases stomatal numbers, size or opening, linked to floral specific gene promoters. Because the water holding capacity of air increases exponentially with temperature, an uncertain proportion of the effects of increased temperature on yield is the consequential increase in plant to air water vapor pressure deficit.

3) Water While global precipitation is expected to increase under global change, this is more than offset by increased potential evapotranspiration driven by rising temperatures. Further altered precipitation and less stable weather patterns may result in increased incidence and prolongation of droughts (IPCC, 2021b). This is compounded by the fact that where increases in yield are being achieved through improved genetics and agronomy, the amount of water required to produce a tonne of plant biomass (water use efficiency – WUE) has not improved (Ort & Long, 2014). Further, rising VPD which will drive increased transpiration and further lower WUE (Lobell *et al.*, 2014). Taking these factors into account, for the current year-on-year improvements in maize yields achieved in the corn-belt to continue, an ever increasing proportion will need to be irrigated (Ort & Long, 2014)– unless WUE can be improved. Globally, much use of irrigation water is unsustainable (Ringler, 2022), meaning that to meet future demands yields will need to increase while using less water overall. Two traits must be separated in considering water use and drought. Drought tolerance is the ability of a plant to survive a period of no or insufficient water, and then continue growth when and if water returns. Water use efficiency (WUE) is how much water is used in making a unit of crop biomass. Of course increased WUE in a crop will conserve more soil moisture, and so indirectly make a crop more drought tolerant. Decreased transpiration in vegetative growth will conserve soil moisture for reproductive growth, which is often more sensitive to water shortage (Sinclair, 2018). Despite much effort in gene discovery (Ingram & Bartels, 1996, Zhu, 2002), little improvement in drought tolerance of our major crops has been achieved. Constitutive expression of cold shock protein B (CSPB) from *Bacillus subtilis* in maize (DroughtGard™) may improve yield in dry conditions, although this is not always observed (Mason *et al.*, 2018). Stay-green (SG), that is ability to maintain green leaves, when they would normally senesce in response to drought, can also protect yield in response to transient or late droughts (Tuinstra *et al.*, 1998, You *et al.*, 2007). Modeling has indicated several factors that could improve crop water use efficiency (Drewry *et al.*, 2014). More vertical leaves in crop canopies allow more light to leaves in the lower and more humid lower canopy, where less water will be lost per unit CO_2 assimilated, due to a lower VPD. In warm climates lower chlorophyll contents will result in cooler leaves and less evaporation for little loss in photosynthesis. As noted above C4 crop photosynthesis is already CO_2 saturated in the present atmosphere, such that decreased stomatal conductance would lower transpiration, without affecting photosynthesis (Pignon & Long, 2020). As $[CO_2]$ rises the cost of decreasing stomatal conductance on photosynthesis is small relative to the gain in water use efficiency. A transgenic reduction in the ratio of stomatal conductance to photosynthesis resulted in a 1% loss of CO_2 uptake compared to a 15% reduction in transpiration in field tobacco (Glowacka *et al.*, 2018).

Conclusion The eminent wheat physiologist and breeder, Lloyd Evans, noted half a century ago that it was hard to see how future food demand could be met without bioengineering of the major crops (Evans, 1997). We can no longer ponder and model potential futures for another half century, practical work in making future proofed crops needs to be >90% of activity. There also needs to be focus on crops. There is unlikely to be sufficient intellectual, even if financial, resource to address every crop. Two thirds of human calories, directly or indirectly, come from four crops: maize, rice, soybean and wheat. Placing effort in these is therefore the most likely course to provide adequate food for the future, however ideal it might be to pursue more diversity. The genomic, diversity, and bioengineering resources are greater for these crops than any others. As noted above some of the impacts of global change could be dealt with through conventional or marker-assisted breeding, but most future proofing can only be addressed by bioengineering.

1.3 Introduction to the Societal Challenges Facing Future Crops and the Potential Solutions

1.3.1 Agriculture in a changing World

The priorities for Europe's agriculture have changed tremendously and repeatedly during the last 100 years as we moved from a traditional agriculture through the increasingly intensive high-tech agriculture of the post-WWII period. This drive to increase yields in the post-war period was motivated by food shortages within Europe and increasing demand from outside Europe, which led to the global Green Revolution. The post-war European agricultural revolution, along with the Green Revolution has met the need for food and largely reduced the risk of famine due to crop failures. In Europe we are now facing a future in which we will need to ‘recast’ our agriculture to meet future demands for food, feed and industrial feedstocks and reduce dependency on fossil carbon and high inputs. That agriculture will need to be highly productive but also stable, sustainable, and resilient to global climate change. Add to this the uncertainty in Europe's future security arising from the current Ukraine crisis and the consequences of the Covid-19 pandemic. At the height of the pandemic there was enormous disruption to global trade, transport, and production, this all impacted on food security and availability. Shipping costs rose sharply and food prices were also increased. The future looks very challenging. Europe's agriculture must be ready for these changes and CropBooster will be part of this adaptation.

To give sense of the scale of the changes we face, here are three quotations providing a snapshot of two moments, one back in 2008, and two in 2022:

First, from 2008:

> “ *Well, we can always buy the food from somewhere.*

a caricature by Hilary Benn (the British Minister of Agriculture in 2008) of the view of some in the UK Government up to 2008[1].

Second, from 2022:

> “ *Of all things, eating matters most, and food is the*
> *[greatest] necessity of the people*

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[1] Fourth Report of Session 2008–09; Securing food supplies up to 2050: the challenges faced by the UK; House of Commons Environment, Food and Rural Affairs Committee; https://publications.parliament.uk/pa/cm200809/cmselect/cmenvfru/213/213i.pdf

President Xi Jinping is quoted from a speech given in March 2022 at the fifth session of the 13th National Committee of the Chinese People's Political Consultative Conference. He emphasized that China should be well-prepared, remain vigilant on food security, and adhere to the principle of self-sufficiency based on domestic grain production, guaranteed capacity, moderate food imports and technological support according to a report in China Daily[2]

Thirdly, a headline from the British *Daily Mail* newspaper on 15th March 2022[3]

> “ *British farmers must dig for victory once again: Our island is blessed with good soil and plentiful rainfall – which is why, as food prices soar, we should remember the history lesson that self-sufficiency is vital*

These quotations illustrate how much the World has changed from the late 20th century/early 21st century to now. Europe has moved from a period that extended from the late 1960s to 2007 when food security was easy and seen to be little more than a responsibility of the free market, into first the food crisis of 2007–2008, and more recently the threat to food security posed by the Russia-Ukraine conflict. In between 2008 and 2022 the COVID crisis demonstrated how fragile global supply chains had become. In addition, climate change is increasingly impacting on agriculture. The 2008 food crisis and changing climate have led to a period of considerable reflection about what the future for agriculture should be. CropBooster-P was born in that time.

The 2007-2008 food crisis reminded the developed World that food security was not something that should take for granted. Analyses about the future priorities of agriculture identified crop yields and the options for yield improvement as priorities. This led to growing scientific interest in how yields, in particular, could be increased, and the importance of improved photosynthesis as part of the pathway to bigger yields was widely recognized. This led to new international programmes such as RIPE (https://ripe.illinois.edu) and the C4 rice project (https://c4rice.com/). Better photosynthesis is not useful as a goal on its own - it is the assimilated carbon dioxide that makes photosynthesis useful. This extra biological carbon creates essential options for plant breeding. It can be used to increase yield, but it can also be used to build bigger and better roots for resilience, store carbon to enable a harvest in a climatic shock, sequester atmospheric carbon dioxide, create more non-food biomass that can be used as an industrial feedstock, and so forth without losing yield.

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[2] https://global.chinadaily.com.cn/a/202203/10/WS622933a1a310cdd39bc8b8d6.html

[3] https://www.dailymail.co.uk/news/article-10612915/British-farmers-dig-victory-writes-JAMIE-BLACKETT.html

1.4 The Sustainability Question

It is widely recognised that modern high-yielding agriculture of the kind widely practiced in the developed World must become more sustainable (see below). High-yield, high-input western agriculture - the source of much of our food - was seen to be polluting via nitrogen and phosphorous run-off and in the case of nitrogenous fertilisers greenhouse gas emissions associated with their production and use. On average about 0.6% of the nitrogenous fertiliser used in EU agriculture is lost as N_2O, a powerful greenhouse gas (GHG). Currently about 1.2% of global CO_2 emissions are due to the Haber-Bosch process used to make NH_3 (see below). Intensive agriculture was also seen to be overly demanding of water (globally about 70% of freshwater is used by agriculture), and damaging to soil quality particularly due to the net loss of soil carbon, soil erosion and more general deterioration of soil quality. Additionally, agriculture is seen to be harmful to global biodiversity, through both the erosion of natural ecosystems to provide the land for agriculture, and the need to use pesticides to combat pests and disease-causing organisms. Taking land into cultivation is also associated with GHG emissions due to the oxidation of soil organic carbon.

This substantial list led to measures in the EU to reduce nitrogenous fertiliser inputs and the development of the Green Deal, with its emphasis on an agriculture working with lower inputs and a goal of 20% organic production by 2030. But this policy has been projected to result in reductions in agricultural productivity of 1–11%, depending on the extent of implementation of the policy. The Green Deal has the potential to be a force for positive change in society. At the food and agricultural level changes supporting a move towards lower inputs and prioritising environment must be balanced against needs to compensate the lower production that is predicted for current crops. This needs to be compensated by other major shifts such as, a change in dietary habits that leads to a global reduction in demand for primary productivity; substantial increases in crop resource use efficiency; or by an increase in agricultural land area (probably at the expense of natural systems). Indeed, without better crops the Green Deal risks an increase in food prices and reduction in yields. The increase in food prices will also affect Europe, the Ukraine - Russia conflict has shown how many poor people there are in Europe and how vulnerable they are to food price increases. There appears to be a growing tension between the policy objective to increase sustainability and decrease the environmental impact of agriculture, and the need for high-yielding agriculture requiring high inputs to produce affordable food. The better, more efficient crops developed through CROPBOOSTER will have the potential to offer a long-term solution.

1.5 Global Breadbasket Instability: A Wake Up Call

It is not currently possible to consider food security without commenting on the impact that the Ukraine-Russia conflict is likely to have on the World. This conflict is testing the World's readiness to deal with a shortage of food and fertiliser, and increased prices for commodity crops, like wheat, sunflowers or maize. The food problems of 2022 are not due to the war in the Ukraine alone. India has had extreme heat, which has reduced yields and led the Indian Government imposing a selective ban on wheat exports. Other countries are doing likewise disrupting the global market as a mechanism for food-security. Even before the Ukraine war this global market was under strain due to COVID, increasing energy costs (fuel for farms, transport and fertiliser manufacturing), and the effects of La Nina (from 2020). In China, floods in 2021 have damaged the winter wheat crop, in contrast the wheat growing areas of the US are experiencing a drought that is expected to reduce yields by 20% relative to 2021. Europe has also had a dry spring. The Russian invasion of Ukraine has turned a bad start to 2022 into a catastrophic one.

Together Russia and the Ukraine account for about 30% of the global exported wheat and barley, 50% of that for sunflower oil and seeds, and 17% for maize. (FAO 169/3). They export about 12% of the calories traded globally. As a result of the combined effects of the war in the Ukraine and sanctions imposed on Russia in response, wheat prices in Chicago (which serves as a trading datum) have risen by 40% since the invasion. The port of Odessa, currently inaccessible due to hostile naval activity, is the route through which 98% of the Ukraine's grain exports passes and talks are under way to persuade Russia to allow exports to occur, by no means certain. If the grain currently in storage in the Ukraine is not exported via Odessa, then this year's (2022) harvest, may be left.

In addition to the impact on food supplies the Ukraine conflict has created uncertainties in supplies of oil and natural gas and therefore the price of fuels (important for farming), in and industrial processes (including fertilizer manufacture) that depend on natural gas as a feedstock. The Ukraine war has made this situation worse; Russia and the Ukraine were big exporters of nitrogenous fertilisers, Russia is also an exporter of two other important fertilisers, potassium (2nd in the world) and phosphate (3rd in the world). From a low in early 2020 (the kick-off of COVID), prices of nitrogen containing fertilisers have risen by a factor of 4,. Recent price increases have been in the range of 30% (https://blogs.worldbank.org/opendata/fertilizer-prices-expected-remain-higher-longer). Fertilisers are a high up-front cost for farmers. The pandemics and political socio-economic crises demonstrate the risks to farming and production, globally, there are now diminished supplies of some key agricultural products (wheat, barley, maize, sunflower oil) and a reduction in fertiliser supplies.

Recent events have been a critical reminder of how fragile our food supply system is. The focus now must be on the question of how can we keep our people fed in a way that can be afforded not only economically but sustainably. Globally, food price increases are likely to lead to political instability in poor countries and renewed migration pressure on Europe and political and social tensions. Failures in food security may have local causes but they can have global consequences; even if Europe can manage the coming problems of restricted food supplies and increased fertiliser prices, the consequences of other countries failing to manage their food security will impact on Europe. Even within the EU-27, poverty has not gone away; about 22% of our population is considered to be at risk of poverty and social exclusion (https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Living_conditions_in_Europe_-_poverty_and_social_exclusion#Key_findings) so food prices will have an impact upon Europeans.

CropBooster-P was set up with the aim of producing a plan to future proof our crops - improve the crop genotypes we use in our agriculture systems so that these genotypes give our crop production systems the chance of meeting our needs. Many of problems highlighted by the Ukraine crisis are targets for improvement by CROPBOOSTER - the programme to laid out by CropBooster-P. The crisis in the Ukraine was not, however, the reason for CropBooster-P; this had its origin in an even bigger set of global problems whose likely impact will have been illuminated by the Ukraine. These problems can be summarised as: population growth coupled to economic growth, global warming and climate change and agriculture, the continued need for agricultural sustainability, and the need to move the chemical industry and manufacturing to a more sustainable bio-based model.

1.6 Multiple Societal Challenges Require Future Proofed Crops

There are multiple societal Challenges at a global scale that are creating needs for modern robust and resilient **Future Proofed Crops**. These Challenges include:

1.6.1 1. Population growth

The World population is expected to reach 9.5 - 10 billion by 2050, and this population is expected to become richer, with increased food consumption. Taking both the increase in population together with the increased affluence it has been estimated that by 2050 global food production would need to be increased by 100 - 110% relative to a 2005 baseline. Meeting this increase alone would be a challenge given the low current rates of yield increase for our major crops. The expected global shortfalls are estimated to be 18% for maize, 43% for rice, 43% for wheat and 31% for soya, given stagnating rates of yield improvement and assuming the land area for cultivation does not increase. Given the importance of food, it is expected that if improving the yields of major crop plants (eg maize, rice, wheat and soya) is not accelerated then demand for crops must be met by increasing the land area under cultivation, and this will be at the expense of natural systems.

A major uncertainty in these predictions is whether the demand for primary production (field crops) for either human or animal consumption will increase as expected. If Europeans transition to a more plant-based diet, as recommended by the 2019 Intergovernmental Panel on Climate Change policy recommendations[4] the problem is dramatically reduced, but we cannot be sure if this will happen in on a global scale or even in Europe. Dietary shifts to a more meat- based diet with increasing affluence in the Global South will create much bigger problem. These are global issues that Europe cannot isolate itself from - even if Europeans do the right thing, we cannot be sure others will. **Future agriculture will need to be higher yielding to balance feeding a growing global population and preserving the biodiversity of the planet, already under threat due to climate change.**

1.6.2 2. Climate change

Climate change is predicted to have complex, crop dependent- specific effects on agriculture. This climate change is due to increases in atmospheric concentration of greenhouse gases, primarily carbon dioxide, methane and dinitrogen monoxide (nitrous oxide). An increase in carbon dioxide levels, once it is substantial enough, will increase photosynthesis and increase yields of crops like rice, wheat, and soya; the increase in crop plants like maize that use the C4 photosynthetic mechanism is less. So more carbon dioxide, more yield - surely a good thing? Indeed that should be the case, but it requires significantly higher

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[4] IPCC, 2019: Summary for Policymakers. In: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems [P.R. Shukla, et al., (eds.)]. In press.

CO_2 levels to be reached, and if increasing carbon dioxide levels (and other greenhouse gases) in the atmosphere increases temperatures, that can have a negative effect on photosynthesis, if that makes a warm season a hot season, but not if it makes a cold season a warm season. Climate change is also changing rainfall amounts and seasonality. These changes in rainfall need to be taken along with temperature changes - a change to warmer wetter springs could be good for crops like wheat that grow early in the year, but a change to hotter drier summers would be bad for crops like maize that grow in the summer. The future climate (including that of Europe) is expected to become more extreme, with more floods and droughts, more heat waves, higher night-time temperatures and cold snaps. However, the distribution of vulnerability and opportunity will be uneven and (currently) unpredictable. Future crops need to be developed for this still uncertain future, making use of climate models to predict the possible climates for Europe can guide understanding of the properties crops will need, if they are to flourish in this changed world. There will of course be adaptation in which crops are grown where, but the crops themselves will also need to be made more resilient - better able to deal with abiotic stress and extreme weather events so that farmers can have greater yield stability from year to year irrespective of the fluctuations in weather. **This needs new climate-smart, resilient crops.**

1.6.3 3. Agricultural Sustainability

Sustainability implies carrying out an activity in a way that does not diminish the ability to continue the activity into the future. This seductively simple proposition brings with it two problems – i) how is the ability to carry out an activity defined, and ii) at what scale is the judgement of sustainability made? It is systems that are sustainable and systems are globally interconnected, so ultimately sustainability must be at the planetary level. Sustaining agricultural yields (and the supply of nutritious diets) should also permit sustainability of environments and not degrade or pollute them. Such a large-scale approach is complex and hard to quantify. In practice sustainability is regulated and assessed at a more definable and quantifiable local level. In the case of agriculture that means the field, or the farm, and possibly the catchment level. An implication of this is that World Sustainability might well be elusive. In particular, sustainability must work economically, as well as with an environmental dimension.

Agriculture is an essential industry because it provides our food, and increasingly feedstock for biobased industries. To meet these production needs in the face of global challenges agriculture needs to become sustainable and is likely to play a role in transforming other industries to become more sustainable. Food production is just one part of the supply chain and must be demand led (economics) but agriculture also has the greatest influence over the sustainability of land use. Increasingly agriculture is also needed as a supplier to biobased industries helping society transition away from the fossil carbon economy by providing chemical feedstocks, fibres, building materials, energy and more. Agriculture can also help deal with GHGs by sequestering carbon dioxide (see below).

From the perspective of farmers, sustainability includes the following:

- **Environmental sustainability**: reducing the impact of agricultural practices on the environment such as soil degradation and promoting the ability of farmers to maintain and enhance the ecosystem services essential to production.

- **Micro-economic sustainability**: maintaining and improving the economic performance of their business so they can be certain of sufficient income to give them a reasonable quality of life and develop their business.
- **Macro-economic sustainability**: maintaining and improving the economic performance of the value chain within their marketplace, this value chain connects farmers with consumers, and farmers need to grow the products the consumer wants to buy.

In the context of 2022, all these aspects of sustainability are strongly interdependent and cannot be seen in isolation when attempting to build new methods for improving sustainability.

Ultimately CROPBOOSTER is a programme to improve our crop plants so they will perform better in a future sustainable agrifood and biobased economies. Target traits to improve will include resource use efficiency of water and fertilizer. Plants with increased food biomass can reduce pressure on our remaining wild spaces, and plants with increased non-food biomass will allow us to transition to a bioeconomy. Increased root biomass - a factor in improved resource use efficiency - can make possible increased soil organic carbon sequestration, using plants to harvest more carbon dioxide from the air and essentially bury it for the long-term in the soil. Increased carbon for roots enables greater resilience and minimises losses from soil degradation. These sustainability related improvements are within the scope of CropBooster but the pressure to increase agricultural productivity, particularly harvestable food biomass will also be important. **This will require higher primary productivity.**

1.6.4 Plants and the non-food bioeconomy

Plants (including trees and algae) produce an energy rich, chemically diverse biomass that can be an energy source and a potential source of organic feedstocks that could allow the chemical industry to transition away from fossil carbon, such as oil, natural gas and coal. Until the post WWII expansion of the crude oil-based chemical industry, many organic chemical feedstocks were made by fermenting plant biomass. The transition to a sustainable bioeconomy is increasing demands to use the non-food aboveground agricultural biomass as a feedstock for the chemical industry. The future scale of this demand is currently not possible to accurately predict, nor the efficiency with which plant biomass can be converted to chemical feedstocks. Currently, 10% of all grains are used to make biofuel, and 18% of vegetable oil is used to make biodiesel. This use of what is potentially food, as an energy source, may not be continued if there is a short fall in the availability of crops for food. Transitioning away from first generation to second generation biofuels (ie those made from non-food biomass) would avoid this conflict. It is to be hoped that better energy sources for transport will allow the complex organic molecules produced by biological sources to be used for more creative applications. Whatever the demand made upon agricultural production for feedstocks, an increase in yield is expected, not just of the food components, but of the non-food components. This is especially the case if we want to use agriculture to sequester atmospheric carbon dioxide by adding more organic carbon to soil, with the bonus that the increase in soil organic carbon will result in a soil with better agricultural properties in terms of nutrient retention or buffering, water holding capacity and mechanical properties.

In addition to their use as chemical feedstocks, plants have for millenia been used as a source of fibres and wood, with the latter being important as a building material, for furniture and for paper, which itself has many diverse applications. The movement to a more sustainable base for manufacturing is likely to result in an increase in demand for wood and plant-derived fibres, and unless the yield of trees and plants that producing plants is increased this would result in more competition with crop plants and land dedicated to natural ecosystems. Forests can be dual purpose, however, furnishing both wood and waste biomass from forests that are also nature reserves. **CropBooster will develop “blueprints” for improved plants to be translated to the crop breeding sector to breed plants that will deliver the biomass, wood and fibres for the bioeconomy of the future.**

1.6.5 Plants and carbon dioxide sequestration

Agriculture currently accounts for about 10% of all EU greenhouse gas emissions, with about 4% of that coming from agricultural soils (N_2O, CO_2). Soils naturally contain organic carbon but in agricultural soils this pool of carbon has been depleted. The '4 per 1000'[5] initiative launched at COP-21 aims to restore the lost soil carbon pools and by doing so reduce atmospheric carbon dioxide levels. If the amount of soil carbon in the top 30 - 40 cm of cropland were to be increased by 0.4% per year (hence the name '4 per 1000') this would sequester 0.9 - 1.85 Gt C yr-1 - current GHG emissions are about 13 Gt C equivalents yr^{-1}, so this sequestration is not trivial and would help shift agriculture from a GHG source to a sink. A challenge for this strategy is to improve the properties of plants so they add captured carbon effectively to the slowly turning-over soil carbon pool. Soil management practices (eg no-tillage agriculture) have a major role to play in allowing a build-up of soil carbon, but improved crop plants can contribute. Much work has been carried out (and publicised) on soil management practices and sequestered carbon. It is also true that increased root biomass via alteration of partitioning within the plant (regardless of soil management) will have multiple and beneficial effects on both crop function, resilience, soil quality and the ability of the system as a whole to sequester carbon below ground. Without an increase in plant primary production, however, any increase in soil carbon must at the expense of other uses for photosynthetically captured carbon. The allocation of carbon to harvestable biomass or yield in most crops has already been maximised, implying that any increase to soil carbon will be at the expense of yield. Increasing soil carbon therefore should be achieved by increasing photosynthesis. **One of the goals of CropBooster is to ensure that more carbon is captured for allocation to roots where appropriate and that this be done with no yield penalty**.

1.7 Plant Resource Use Efficiency

Part of the magic of plants is their ability to take simple inorganic substrates, like carbon dioxide, water and fertiliser and use these, with the addition of energy from sunlight, to build organic life. This property of plants and algae means that they drive most of the life in the Biosphere. One of the key phenomena of the biosphere are ecosystems, and a foundation concept in ecosystems functioning are webs of energy flow

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[5] Soils for Food Security and Climate see https://4p1000.org/?lang=en

and nutrient cycles, but nutrient cycles are a problem in modern agriculture and create challenges for agricultural sustainability. Before industrialisation agriculture was a local activity and not much was exported from what were essentially small scale, local food producing systems. Nutrients, like nitrogen, or phosphorous, would be harvested as part of the food biomass and returned to the fields as human and animal waste (urine, faeces etc). A closed loop for nutrients. Modern agriculture is very different to this model; from farms we export food, comprised in part of carbon, nitrogen and phosphorous and there is no explicit recycling model. The exported carbon ends up as carbon dioxide and will be refixed by plants, and while nitrogen (an essential part of proteins) can be recycled as a gas this can only be refixed in agriculture (ie converted to a form useful in biology) by certain bacteria that are symbionts in the legume family or by some free-living soil bacteria. Phosphorous has no recycling path except via sewage sludge. To replace the nutrients we remove in our food we must add them back either as fertiliser or via nitrogen fixation. This process of adding nutrients to the crop is essential for yields. Nutrients can be added via manure - the option in organic agriculture, and also for other farming systems. In the EU 95% of animal manure is already used for this purpose. Beyond the capacity of manures, mineral nutrients must be added via 'chemical' fertilisers, largely N, P and K. These have been mentioned above. To increase the environmental sustainability of agriculture and our food security we need to make more efficient use of these fertilisers and improving the nutrient capture properties of plants can play a critical part in this.

There is an urgent need to improve the efficiency of plants to take up and use nutrients. Although this has been a goal of crop breeding for many years, it has not been critical while fertilisers remained readily available and cheap. This is now changing and the pressure will be to generate higher yield with less fertiliser under farm conditions. There is strong reason to believe that this can be done by an improvement of plant traits alongside improved practices. Exciting breakthroughs may occur in the fields of nitrogen fixation, the ultimate goal being the generation of universal N fixing crops. Already there are companies successfully exploiting the use of N fixing bacteria as a ‘biostimulant’.

Nitrogen is a particularly critical nutrient. The world uses 100 Mt N in nitrogenous fertilisers every year and about 95% of this is produced by the Haber-Bosch process. Only 17 Mt of N is, however, consumed by humans - the rest is lost from the agricultural system and becomes a pollution problem. Even at the plant level nitrogen use efficiency is poor; for example, the global nitrogen use efficiency of wheat has declined from 80% in 1960 (just before the Green Revolution) to 30% in 2000. It is inefficiencies of this kind we need to reduce, and better plants will play a role in this. While this loss of nitrogenous fertiliser from the system is a problem, we should also recognise two important things; first Haber-Bosch nitrogen is responsible for feeding 48% of the World's population (a 2008 estimate), and second, while the average loss of nitrogen from the agricultural system is an issue, some farmers use nitrogen very efficiently and produce high yields. We can learn from these farmers and Agoecology principles to create synergies between farm practice and the increasesd NUE performance in crops driven by genetic improvements.

Water Use Efficiency (WUE) and optimal responses to drought, such as drought recovery is one of the most complex problems to solve and makes our crops most vulnerable to climate change. This problem is geographically complex and variable across Europe with Northern countries having more rainfall and southern countries have less but rainfall is erratic and patterns are shifting with climate change. This creates additional stresses for crops due to localized flooding and water logging. Studies of natural variation are already demonstrating greater WUE and resilience to water logging traits amongst plants. This represents rich potential genetic resources for crop improvement. Major crops such as wheat, maize and barley are not usually irrigated, so water inputs cannot assume to be manageable for such crops and so yield becomes more attuned to genetic improvement of capture, efficiency of use and of resilience/ this problem has a distinct solution compared to say nitrogen use efficiency where inputs can be regulated. ,. The solutions are likely to involve innovative plant science, utilising outputs from both root and shoot biology. Typically root and shoot science has been separate: CropBooster aims to produce a programme that unites the two. We can also learn and work alongside countries outside Europe which already experience higher and temperatures and water stresses.

1.7.1 Nutritional and post-harvest value of crop and food

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1.7.2 Yield and sustainability gaps

There is more than one yield for crops. In trials of crop plants, they are grown as well as possible, with optimal nutrition, irrigation, and management of pests and diseases. Under these conditions crops manage their best field achievable yields - their yield potential. In practice, under real world conditions on farms agriculture yield potential is not reached, instead farm yields are delivered (See Section 4). CropBooster proposes to understand the basis for the difference between farm yield and yield potential to direct crop improvement and future agricultural practices. The ambition is to improve the performance of crop plants on the farm, and yield is part of this performance. Some of this “potential” to “farm” yield differences can be attributed to lower inputs on the farm versus the trial, but it is clear that some farmers have higher yields than others, just as some farmers make more efficient use of fertilisers than others (ie they have a higher farm environmental sustainability). CropBooster needs to learn from the best farmers and agronomists how this exceptional performance is achieved, once we know this we can try to breed crops that have this “built-in”, so farm yields will be higher with fewer inputs. This will combine state of the art farming practice with new high performing and resilient crops.

A summary of what the CropBooster Program will improve by producing science based “blueprints” for better crop plants

- **More efficient use of resources or inputs in agriculture (in the case of CropBooster these are water, nutrients (especially water, N and P)**
- **Increases in crop yields to feed the growing global population, which will also be more affluent, and is expected to have an increased per capita food demand; responding to estimates that global food and fodder production may need to increase by up to 110%**
- **The faster transition to a more sustainable non-fossil carbon bioeconomy to meet increasing demand biobased materials and products from non-food agricultural biomass using novel feedstock crops designed to better support**
- **Soil quality or ‘health’**
- **The adaptation of our crops to climate change, which will affect different parts of Europe differently and which in addition to changes in average climate will be accompanied by increasing weather extremes**
- **Crop resilience in the face climate change and increases in extreme of weather**
- **The mitigation of greenhouse gases. For plant husbandry in Europe the significant gases are carbon dioxide and dinitrogen monoxide ($N2O$; traditionally called nitrous oxide); carbon dioxide, fixed by photosynthesis, can be sequestered below ground as soil organic carbon, while dinitrogen monoxide is released from soil as part of the nitrogen cycle so there is a connection to nitrogen uptake efficiency and the soil microbiome - we can influence both carbon sequestration and dinitrogen monoxide release**
- **The adaptation of C3 photosynthesis to increasing atmospheric carbon dioxide mole fraction, which will also improve nitrogen use efficiency or water use efficiency; increasing atmospheric carbon dioxide will in any case result in increased in agricultural productivity, all other things being equal**
- **The nutritional value and other objective quality parameters of crops**
- **The need to reserve space for natural ecosystems and therefore to increase yields and sustainability without any expansion of the area of croplands.**

2 Codesign by Scientific Focus Groups

2.1 Rationale

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

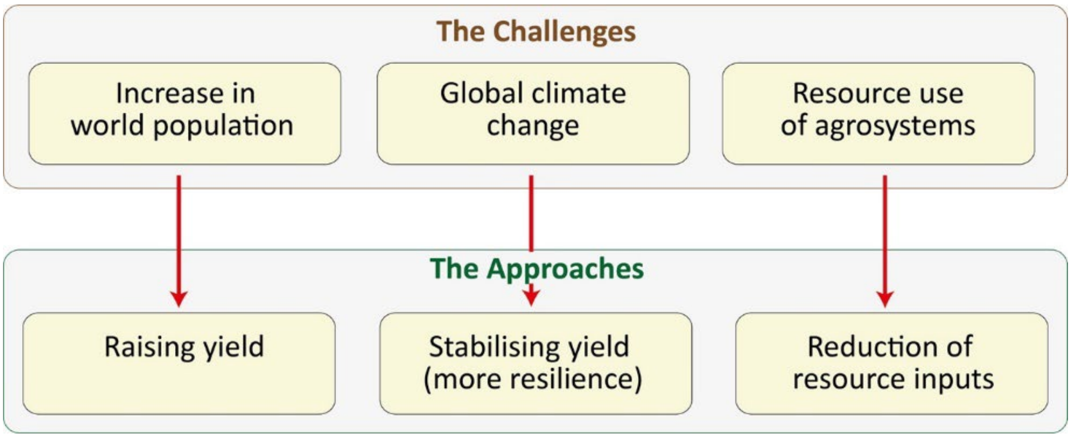

***Figure 2.1**: Major challenges for agriculture and general approaches to cope with them*

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

Thirdly, the current food system has become unsustainable, and there is an urgent need for substantial changes as stressed by the EU's Green Deal and its Farm-to-Fork Strategy. Mineral fertilizers and pesticides are heavily used in agriculture posing risks for human health, but, more importantly, they harm the environment as well as biodiversity. If the reductions in inputs are not to be mirrored at the yield level, the resource use efficiencies of our current crops have to be improved. Alternatively, novel (orphan) crops that are better adapted to the changing environments than the current ones have to be identified, trialed and, if found suitable, introduced into farming. Presently, European diets are relatively meat-rich necessitating that large amounts of primary plant production for the feeding of livestock. There are policy recommendations to increase plant-based protein as part of a healthy diet, this requires that the cultivation of crops have to be intensified that are rich in food constituents such as proteins, vitamin or micronutrients which hitherto are mainly provided by meat consumption.

Coping with these challenges and providing solutions requires an integrative and interdisciplinary approach that has to deal with three pressing issues of crop production, namely yield, nutritional quality, and sustainability. For each of these three topics we have, therefore, set up expert panels whose aim was to look at the three issues from various angles.

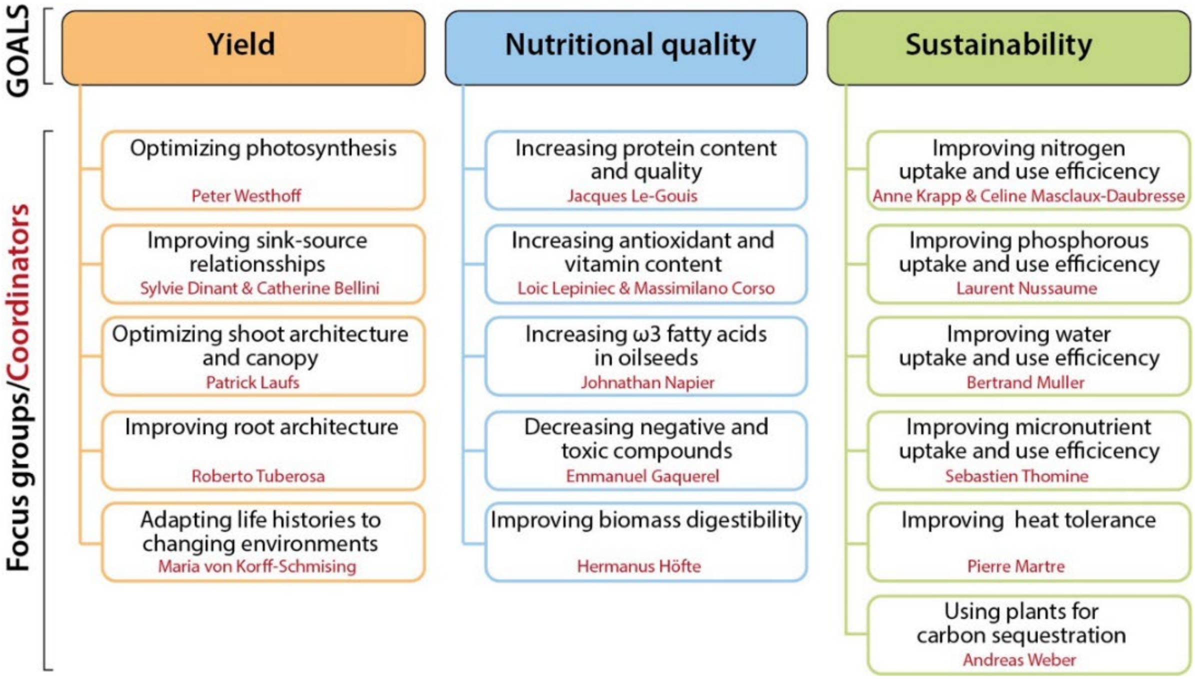

Figure 2.2*: Goals, topics and coordinators of focus groups.*

Each focus group had to review the state of knowledge of their field, to identify the future challenges in the research field that should be addressed with high priority, and finally to outline action points for a future research programme. The implications of this approach (“Focus Groups”) for structuring the European plant research landscape are obvious. The coordinators of the 15 “Focus Groups”, established contacts with an average of 9 experts per “Focus Group”, 46 experts related to “Yield”, 37 experts for “Nutritional Quality” and 51 experts for “Sustainability”. Altogether, this approach involved more than 130 experts, from 70 institutes or universities and 15 countries (https://www.cropbooster-p.eu/data/upload/files/d4-2-report-22-11-2021-v1.pdf).

2.2 Results Elaborated by the Focus Groups and in Workshop Session (June 8/9, 2021)

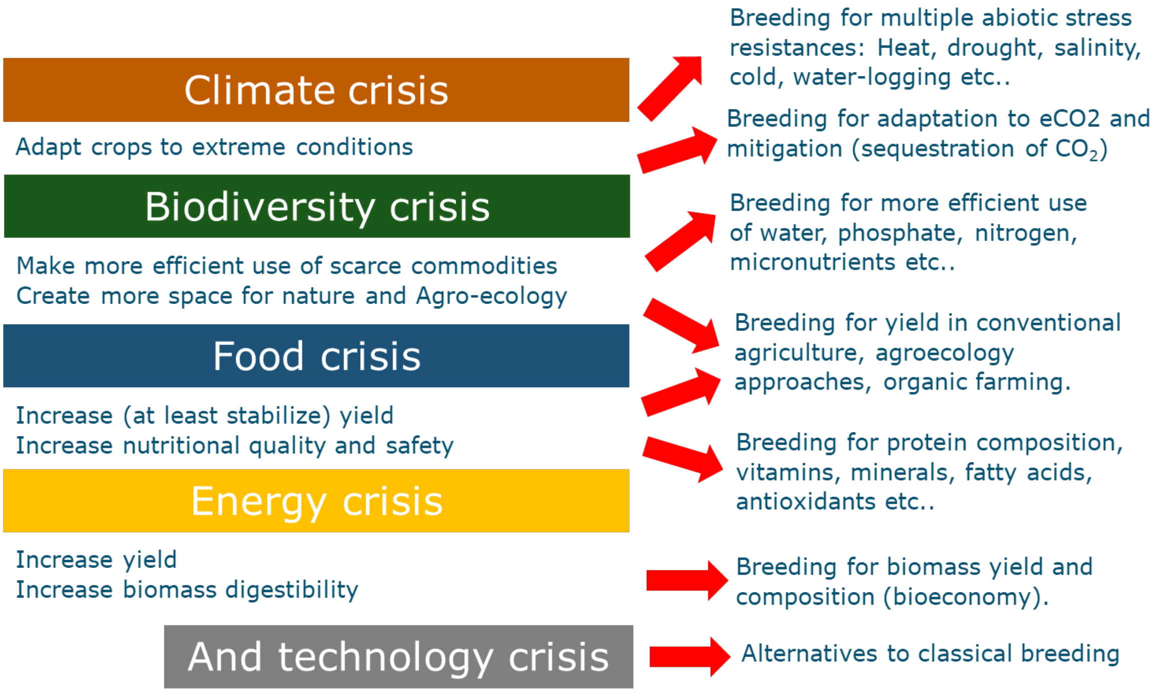

Figure 2.3: *Suggestion of topics for the research agenda. In summary, the reports of the 16 "Focus Groups" and the presentation and discussion of these reports during an Online-Workshop on June 08/09, 2021, led 4 to the recommendation of the following high priority topics for a future EU research agenda in the field of plant sciences, all under the headline "Better Crops for Tomorrow's Needs" (https://www.cropbooster-p.eu/data/upload/files/d4-2-report-22-11-2021-v1.pdf).*

2.2.1 Yield

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

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

Photosynthesis is a multidimensional process. The core photosynthetic reactions taking place in the plant's green cells are evolutionary conserved. In land plants these cells are predominantly located in the leaves which represent the organ of photosynthesis. The inner anatomy of a leaf and its form, therefore, strongly influence its overall photosynthetic output. Atmospheric carbon dioxide enters the leaf through the stomates, while stomates release the plant's water by evaporation. The availability of water, extracted from the soil by the roots, therefore, affect the degree of stomata opening and, hence, the rate of photosynthesis. Roots are also key players in the uptake of nutrients. Since the photosynthetic apparatus represents a heavy investment of resources, nutrient availability and uptake is central for photosynthetic performance.

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

The photosynthetic output of a field also depends on the length of the growing season which can be defined by water and radiation availability as well as temperature . Increasing the cultivation time of a field to cover as much of the potential growing season as possible could for instance be achieved by the use of perennial crop species or of successive crop plantings.

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

Action Points for Yield

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

2.2.2 Nutritional Quality

Healthy diets are a key goal of the EU's Green Deal implying that the eating habits of Europe's citizens have to change substantially. The consumption of meat and other animal-derived food has to be complemented with sustainable farming and an increase in consumption of plant-based products. This change is needed as part of the change to healthier more sustainable diets for a growing population. Future food systems need need to align better with available resources and reduce environmental costs, for instance by the release of green-house gases.

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

Action points for Nutritional Quality

- **Increase nutrient yield and quality per area arable land used.**
- **Put a focus on how global climate change impacts on the micronutrient and vitamin content of plant-based foods.**
- **Understand the mechanistic interrelationship between yield and resource-use efficiency, on the one hand, and content and composition of essential nutrients in crops, on the other hand; transfer this know-how to plant breeding and agricultural practices.**
- **Intensify breeding for organic agriculture.**

2.2.3 Sustainability

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

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

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

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

The work led to the following action points for designing future research programs.

Action Points for Sustainability

- **Exploitation of natural diversity in the adaptation of plants to varying resource availability (water, macro- and micro-nutrients), and abiotic/biotic stresses; in this context the exploitation of genetic variability existing and accessible in gene bank collections should be supported and strengthened.**
- **Further development of tools for precision phenotyping, especially under conditions of practical agricultural plant production, also allowing the analysis of root development and function.**
- **Investigating the impact of soil parameters on plant performance, with a focus on understanding functional processes regulating the interaction of plants with their soil environment.**
- **Development of know-how in agroecology: Addressing the question what is the impact of agricultural plant production (different types of crops, different types of agricultural practices) on ecosystems of farmland; investigating the development of living communities under various agricultural/horticultural conditions, and its dependence on environmental factors**
- **Test/development of agricultural and forestry practices for using crop plants and trees in carbon sequestration concepts; this should aim at identifying features of plants that need to be optimized to improve the efficiency of such processes?**

Overall, stakeholder interactions enhanced the stakeholder co-design approach. The interactions confirmed and enforced the CropBooster consortium thinking about the important toic areas, For example stakeholders highly valued Sustainability, and then in particular drought resistance, heat resistance, water used efficiency. With respect to Yield, stakeholders agreed that improving photosynthesis is currently the best approach. In terms of Quality, stakeholders valued increasing protein quality most highly, driven by a desire to move towards a more plant-based diet. This clearly linked to sustainability and climate change mitigation.

3 The Principle of What CropBooster Will Do: What is the Model for Improving Crop Plants?

The CropBooster focus is on increasing yield whilst maintaining or improving nutritional quality in a sustainable way. Yield, Nutritional Quality and Sustainability are all complex traits that have numerous underlying component traits which frequently interact. These interactions can have both positive and negative impact on yield, nutritional quality and sustainability.

A fundamental understanding of these traits allows the identification of processes that have the greatest potential to improve crops. Coupling this understanding with computational models enables us to evaluate in silico different approaches and their probable success in different crops and environments. Whilst in some cases the objectives could be achieved by breeding in many cases requiring major modifications will only be possible using New Plant Breeding Techniques (NPBTs).

The science community already have a good understanding of some component traits. For example, there is good evidence that improving photosynthesis can increase the yield of crops provided that other constraints do not become limiting. The CropBooster –P project and the wider plant sciences community have already identified targets like accelerating the response to shading and decreasing the cost of Photorespiration that have already been exploited both *In Silico* and in both model and in crop plants to increase biomass.

In other cases understanding is less mature and possible approaches have yet to be tested in models or in the field. In a few cases further research is necessary to improve the fundamental understanding of the component traits to avenues of exploitation can be identified.

3.1 The Goal of CropBooster.

The goal of CropBooster is to create the genetic innovations or discoveries needed to future-proof our crop plants: to sustainably increase crop-yields while maintaining the nutritional value of these mainstream crops. The CropBooster Program will deliver the blueprints for future crops that the breeding industry can use to develop novel elite breeding material. These future crops should be adapted to the future climate and be resilient to the more extreme weather expected in the future. Yield should not only higher and sustainable, but durable. They should be the kind of crops that farmers want to plant – the CropBooster programme must take account of the needs and perspectives of farmers, and we must work with farmers, agronomists and breeders to ensure that outputs are suitable. CropBooster must also connect with the food value-chain to ensure that ideas for improvement will produce crops that are useful for a new range of applications arising from the expansion of the non-food bioeconomy. This reflects the increasing need to use agriculture to produce the feedstocks for the chemical industry, wood, fibres etc. Agriculture in the future will be expected to contribute more broadly to our manufacturing base as well as being the means for society to have food and nutritional security. These goals will require yet another revolution in agriculture, with changes in production and the means of production that will depend on both the crop cultivars being grown, and the way those cultivars will be grown. CropBooster will be the programme that creates or discovers genetic innovation in key selected crops and the programme will work alongside those other projects that will improve how crops are grown - this symbiosis must be very close, just as it must be with all of the other partners in the new agriculture.

> *“* ***The CropBooster mission is to discover the genetic innovation needed to future-proof modern crops, this is not a commercial plant breeding programme.*** *CropBooster will be work with the European plant breeding sector and the value chain to provide them with both new knowledge, and innovations. This will help accelerate the development of future elite crop lines. These future crops are required by society for our future sustainable agriculture and to ensure food security and reliable feedstocks and products for the biobased economy.*

3.2 The Technical Sections of this Document

We will explain in detail how CropBooster will work:

1. The crops CropBooster currently propose to focus on (section 4) - this is inevitably a provisional list as we cannot be certain how the spectrum of Europe’s crops will be affected by climate change and demand-led changes to agriculture
2. The current state of yield and sustainability gaps in Europe (section 5) and how climate change might affect these - this is a summary of the state of the art of actual crop productivity in Europe for a range of crops and how actual farm yields, and trends of yields, of crops differ from the yield potential, and some observations about the sustainability of this production (as nitrogen use efficiency and the availability of phosphate in Europe’s soils).
3. The major traits CropBooster will target for improvement (section 6); these are divided into traits (largely below ground traits) that contribute to the environmental sustainability of crop plants, traits linked to the crop growth and the canopy, traits linked to primary production; traits linked to nutritional value and traits linked to post-harvest value for fodder and non-food applications.
4. How Crop Booster’s science will improve these traits (section 7); this section describes the options and available tools needed to actually identify the genes associated with traits to be improved - establishing this link to genes is essential to improve a trait. In this analysis the CropBooster consortium assume that conventional Genetic Modification (GM) will continue to be not practically useable in Europe while Gene Editing might become so. Conventional marker assisted breeding and genomic selection will continue to be available. In this section the current options for plant breeding are described including, the use of natural variation as a source of genetic discovery and the tools for identifying the genetic loci and genes that give rise to variation in a trait, and how to phenotype (ie measure) plants to qualify their traits.
5. How CropBooster can use the genetic discoveries to be made to improve our crop plants (section 7).

6. An overview of seaweeds as Blue Economy crops is assessed and research areas outlined (Section 8)
7. The acceptance of new crop breeding technologies and proposed crop improvement strategies to be pursued in CropBooster, and an evaluation societal aspects including socioeconomic and environmental factors be undertaken for leading innovations (Section 9).

3.3 The Choice of Crop Plants

The choice of crop plants we chose to prioritise were filtered from a more extensive of species drawn up in a meeting of most of the CropBooster-P consortium. The aim in drawing up this shortlist was to produce a more manageable list of species that covered a range of different crop types important in Europe. This package of crops was further discussed with Euroseeds, a partner in the CropBooster consortium and took on board their suggestions with one exception - we retained poplar as a representative of woody plants. The aim of making this diverse selection was to try an develop within CropBooster expertise in different types of crops from different taxonomic groups with different market applications. In this way CropBooster could develop a broad-based toolkit for trait improvement that could be relatively easily transferred to other crops not foregrounded in our list.

3.4 The Choice of Traits

An important point to make is that we know more about plant development, biochemistry and physiology than ever, and in how plant properties, or traits influence their performance in the field in the various ways that are important to us. The better we understand plant traits and resolve them into more specific traits the better we understand how to improve plant performance in the ways we want to. The traits we chose to focus on where the result of a filtering process beginning with workpackage 1 and ending with workpackage 5 - the work package responsible for this document. The trait list produced by work package 4, which was based on the results of work pages 1 - 3, was particularly important in guiding our trait selection. We have included the summary of deliverable 4.2 of work package 4 as a preface to this deliverable, and the work package leaders of work packages 1 and 4 are on the authoring team for this deliverable. The traits we chose encompass those that seem to be critical on the plant side for environmental sustainability as they underlie nutrient and water use efficiency (currently we do not cover micronutrient uptake, but this could be easily added) so root focussed traits. These sustainability related roots traits we combine with other root traits (architecture, carbon sequestration and soil carbon etc) as it seems natural to view these different root related traits as part of package of traits. Above ground, yield and its resilience or durability are vital properties we wish to improve. Yield and canopy development are included in a super-trait in which we do not include photosynthesis: this may be considered contrary to most other approaches which tend to view photosynthesis as being closely coupled to yield. Photosynthesis is certainly the engine of plant productivity, but it is so for both above and below ground biomass. Photosynthesis for us is a flexible tool; increased or more durable (ie stress tolerant) photosynthesis could be used to increase yield or below ground biomass, or both. In addition to quantity of yield we need to pay attention to the nutritional quality of yield. We chose nutritional quality over a broader view of quality which includes organoleptic assessments of quality because organoleptic quality is complex and often culturally dependent. Nutritional quality is more easily definable. In nutrition we focus on secondary metabolites, fatty acids and other secondary metabolites. We at this point are including micronutrient metals, such as Zn2+ or Fe2+, but they could be easily added to our traits list. Our final trait is value for post-harvest use and use as fodder, though this could become a much more important trait in the future as a market for using plant biomass to chemical feedstocks develops. Currently how this conversion will be done has not been fully developed so knowing the best properties for that biomass is unknown. Our current approach assumes that the biomass would be converted to fuel (ethanol).

In our choice of traits we will focus on traits related to productivity and sustainability, their resilience and durability, their tolerance to abiotic stress, and their more general adaptation to soils, agricultural systems and future climates of Europe. We at this stage have not included biotic stress. This was a deliberate and choice that we feel is justified by the extensive and excellent research already being done on improving plant resistance or tolerance to biotic stress. This is in contrast to the targeted improvement of sustainability and more specific aspects of production (eg photosynthesis). These have so far not been as systematically or effectively targeted as has biotic stress. We have therefore chosen to emphasise these less favoured traits in order to bring their improvement up to that found for biotic stress.

3.4.1 Refining the choice of traits

The choice of traits we will target are based on the accumulated wisdom of about 50 years of scientific investigation into which traits limit plant performance in the various ways that performance can be assessed. They are also complex traits which can easily be subdivided into sub-traits, not all of which will be equally important. Europe is also not an environmentally homogeneous environment - soils and climate differs tremendously across the EU-27, nor are all agricultural systems the same, so we must expect that the (sub)traits that most limit crop performance will differ across Europe. CropBooster will therefore need to analyse which traits are limiting in the different agri-environmental regions of Europe. This will require further agri-ecophysiological investigation of our crop plants across Europe - this work must be done in the field and will require new instruments, better measurement techniques and procedures, better crop models with which to understand the significance of the results at the level of crop production. The collaboration of physiologists, biochemists, soil scientists, agronomists, engineers, physicists, biomathematicians, software developers will be needed to do this research. We will need a new generation of instruments that are cheap enough to be left in the field to measure the plant and soil activity, and we see great scope here for working with other programmes (EMPHASIS, SUSCROP) to apply these measurements more widely. This research will also help us better resolve traits into subtraits. The better we understand the subtraits that are most important the more targeted and specific will be our trait improvement.

3.4.2 How do we improve these traits?

Improving the traits of a plant can be done via two routes, possibly used together - modifying the way the plant is grown or changing the genetics of the plant to make it do what you want in the environment you have. CropBooster will focus on the second option, though it will work with other programmes (eg

SUSCROP) that are trying to improve the cultivation process. Improving a trait genetically requires that we breed for that trait and there are a range of plant breeding options available globally. These are summarised in section 6, Options for plant breeding. In the EU. Genetic change of plants within the EU can be achieved using conventional breeding techniques and for crops marker assisted breeding and increasingly genomic selection are the key techniques. The use of novel plant breeding techniques, genetic modification and gene editing, is limited because of regulatory barriers, though it is possible that gene editing may be allowed to some extent in the EU in the future. CropBooster is compatible with conventional breeding techniques and gene editing.

3.5 How Do We Identify Genes or Genomic Regions that Control a Trait?

There is no single answer to this question, which will depend on a combination of predominantly biological insights and techniques. Our approach to trait improvement will largely depend on making use of the natural variation for traits that exists in the wild relatives, landraces, and heirloom varieties etc of our crop plants. The elite lines upon our agriculture is based are the result of incredible progress in plant breeding, but that breeding has narrowed the genetic base of our crop plants (see section 6). We can mine the wild relatives (etc) of our crop plants for their genetic diversity which can use to improve the traits of crop plants to make them more future-proof. We are essentially going to use genotypes that we have abandoned or bypassed in the past development of our agriculture to improve our future crop plants. This will depend on us being able to identify the genes that control the traits we want to improve.

Our knowledge of how genes influence traits can be divided into three classes and for all of these we are depending on the accumulation of biological wisdom and technical creativity. In some case we know the gene that significantly influences a trait, we may even know how variation in that gene maps on to differences in the trait. An example of this is the chloroplast gene that codes for the large subunit of rubisco - this subunit has major effects on the properties of the enzyme rubisco, and these properties are an important subtrait of photosynthesis. As we know the connection between the gene and the trait it is easy to systematically explore how variation in the gene correlates with variation in the enzyme, and then to select the ‘best’ genes and use these for breeding. There are, however, not many of these simple cases. In some cases we know that there are mutants that encapsulate perfectly the trait we want even though the underlying genetic basis may not be perfectly understood. An example of this is leaf greening mutants; in some cases we do know the genetic basis for this phenotype, but not always. If we have a clear mutant which has the desired phenotype then it is a relatively simple task to map, or localise, the causal gene on the genome of the plant and even to fully identify the gene. The mutant alone could be used for breeding, but better localisation, and even full identification of the gene, makes breeding for this phenotype easier and more efficient. In some other cases there is evidence from plant molecular biology that a gene appears to be pivotal in controlling a trait but further research is needed to confirm this. In this case we just need a little more research, and usually translation of laboratory results from a model species to field research on a crop plant (in the correct genetic background) to prove or disprove the value of these laboratory-derived insights into the value of gene as a tool for trait improvement.

While these examples show how close we can be to knowing which gene or combination of genes could be valuable in trait improvements, these are not the most common situation. In many situations owe know there is variation for a trait within the wild relatives (etc) of crop plants but the genes underlying this variation are unknown. We now have the tools to identify the location of these causal genes on the genome of our crop species, and even to identify the genes in question and to use this knowledge to breed for better crops. These tools depend on a combination of three technologies that have expanded tremendously in the last 20 - 30 years; genomics, phenotyping, and biostatistics. The approach depends on correlating genetic diversity with phenotypic diversity. Identifying and fingerprinting that diversity depends on genomics. Improvements in genomics and sequencing technology mean that is now cheap to sequence a genome and thus to identify variation and to catalogue that variation at the base-pair level. Somewhere within this genomic variation lies the genetic differences that determine the differences in the phenotype of individuals of that species. If we assemble or create by specific breeding techniques, populations of genotypes (mapping populations) whose genetic variation has been catalogued and which that contain diversity for the trait we are interested in then by comparing genotypic variation (variation in the genes) with phenotypic variation (variation in the individuals) we can identify genomic regions whose variation correlates with variation in a phenotype. We localise the genes or combination of genes that underly variation in the phenotype, or variation in a trait. Usually the first correlations will only show approximately the locations of the causal genes underlying variation in the phenotype but that may already be good enough for breeding. But further localisation the causal genes even to the level of individual genes may be possible if required. This is transformational' technology the allows the genes underlying variation in a trait or phenotype to be efficiently localised and so provide the genetic data needed for breeding. For use in conventional breeding the actual genes do not need to be identified, though knowing the causal genes and their variants is helpful in better understanding the genetic algorithm of the trait. For gene editing then it is necessary to identify the specific gene, its alleles and their sequences. The details of mapping populations and genetic diversity, phenotyping, breeding techniques, and biostatistics are explained in more detail in section 6.

Once the genes have been identified they can be used in breeding but the role of CropBooster cannot stop there. CropBooster will hand over its genetic discoveries to the plant breeding sector who can use them to produce new and better cultivars. The plan of CropBooster is to involve the plant breeders, agronomists and farmers in the decision steps leading the research aimed at identifying the genes, and once the breeders have taken over the discoveries CropBooster will remain available to monitor the performance of the lines containing the genetic discoveries, just as it will be available once these cultivars are made available to farmers. In this way we will create a feedback process to correct the selections of traits and phenotyping techniques made by CropBooster.

4 The Primary Target Species

Our future crops will need to match both established and emerging challenges at the European and global levels. Crops must keep sustaining global food security, while satisfying the growing needs for increased nutritional quality to provide for improved and healthier diets. In meeting these needs, our crops must also allow for sustainable use of resources, especially those becoming increasingly scarce due to increased consumption linked to the growing global population, and use for livestock. According to FAO figures an estimated 33 % of cropland globally is used for livestock feed production[6]. These pressures combined with climate change are causing environmental problems for agriculture including: reduced soil fertility due to the intensification of agriculture; environmental problems associated with scarcity of fertilizer manufacture or extraction and the costs of these fertilisers; changes of land use and the loss of biodiversity that arises from the loss of natural spaces; water scarcity and poor water quality affecting an increasing portion of cultivated lands worldwide; and the impacts of environmental perturbations due to climate change (especially warming). Lately, the economic impacts and trade disruption due to diseases (e.g. the Covid pandemics) and wars (e.g. the Ukraine war) have further increased pressure on agriculture and associated value chains. These recent socioeconomic crises have created an expectation that the EU will increase measures to ensure self-sufficiency at the EU level and strengthen the independence of Europe in terms of its food supply, resources and fertilizers.

Based on these considerations, a program aiming at developing future crops should include the following crops. We have taken into consideration the advancement of genetic resources:

1. **C3 cereals: wheat and barley:** These are the staple foods for Europe and will continue to be used as the main component of diets for humans, farm animals and birds. The genetics of these two cereal species is well-known through international research efforts (e.g. https://wheatgenome.org/). Their breeding has been carried out since the beginning of agriculture and this is has led to continuous development of cultivated varieties that have improved certain qualities. Whilst these have mostly satisfied breeders' and users' needs (for example yield or the rheological properties of flours), this has been achieved at the expense of other traits of fundamental importance (resistance to biotic and abiotic stresses, low gluten content, etc.). This production-oriented breeding has eroded natural variability of the genetic base of these main cereals and other key crops. However, the availability of cultivated crops' ancestors (wild cereal species or relict varieties) alongside access to new plant breeding technologies (eg, genome editing, genomic selection, directed evolution, the exploitation of epigenetic traits) may allow the (re)introduction of many useful (often lost) traits into our future cereal crops. This has the promise to increase the environmental sustainability of agriculture over expanding areas of Europe and worldwide. Future-proofing wheat and barley would improve water and other resource use efficiency and improve soil organic carbon, for example via optimization of the root architecture ideotype, or of the vegetative cycles of the crops, but do so with the maintenance of yield.
2. **C4 grains: maize and sorghum:** C4 crops have superior photosynthetic performance and greater resource use efficiencies and yields that other European crop species, which is why the introduction of C4 traits in other crop species has been attempted (e.g. the 3to4 project funded by the European Commission, and the international C4 Rice programme). Maize is a staple food worldwide and an important component of the feed for many agricultural animal and bird species.

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[6] https://www.fao.org/3/ar591e/ar591e.pdf last accessed June 25th, 2022

Maize has not been the main component of diets in Europe, but has the potential to become more widely cultivated in Europe due to its high yields under temperate and (sub)tropical environments. The genetic background of maize has also been widely explored and the same considerations highlighted for C3 cereals (see above) also apply to this important crop. Sorghum is an expanding crop which is used predominantly as an animal feed because it has antinutritional factors that prevent it's used in human diets. Sorghum's main strength lies with the superior water use efficiency which makes it a promising crop for those expanding areas (including, for example, the entire southern part of Europe) where low water availability is a permanent or recurrent problem, or which are prone to desertification.

3. **Root crops: sugar beet and potato:** Root crops include some of the most important food and cash crops in Europe. They are demanding in terms of use of soil resources, and their monoculture often causes soil fatigue or phytosanitary problems. Note that because a lot of research is already undertaken on phytosanitary issues CropBooster is not proposing to deal with biotic stresses. Genetic improvement of crops and adoption of suitable agricultural practices has the potential to mitigate the soil issues. Potato has become one of the staple foods for Europe because their carbohydrates (complex sugars) can contribute a large calorific component of the human diets from a small area of land. Potato yield failures due to disease have recurrently caused economic crises, and even famines, in some cases then driving emigrations of starving populations, for example the Irish potato famine of 1845-1849 being one of the most conspicuous examples of this. As a major crop plant potato is unusual in that it is vegetatively propagated, but this clonal propagation of potato is associated with inbreeding depression and consequent yield reductions. Unlike potato, sugar beet is not consumed directly by humans but is the main cash crop for sugar production in temperate zones of the world. Despite current ambitions to reduce the consumption of simple and complex sugars as part of efforts to control the growing weight and obesity problem in populations worldwide, these two crops remain important for European farmers. The improvement of their cultivation raises interesting problems both for genotyping and phenotyping. Both crops have their harvestable biomass below ground, so the assessment of yield and the phytosanitary status of the tuber and taproot during growth is difficult, and production of cultivars with higher resource use efficiency and lower sensitivity to infections will be challenging. Despite the concerns associated with excessive sugar and carbohydrate consumption these crops will remain important in Europe, and re-evaluating European policies to bolster sovereign food security, (which will include adequate sugar sources) in a world plagued by increasing economic crises is becoming an urgent task.
4. **Horticultural crops (protected and open-field cultivation): tomato:** Among the many horticultural crops cultivated in Europe, CropBooster will focus attention on tomato, as this very important food and cash crop also yields important secondary metabolites (pigments, antioxidants) with dietary value, and has potential as a bio factory for bioeconomy products. As with many other widely cultivated crops, the intensive breeding that has led to the current highly productive tomato varieties has often neglected or involuntarily selected against secondary metabolites of high nutritional value, and metabolites (often labelled as antinutrients), that help the plants protect

themselves against pests, pathogens and abiotic stresses. Re-thinking breeding strategies is imperative to sustainably produce many of our food and cash crops, and tomato is an excellent of this. In the future tomato cultivars should be created for field cultivation that are able to maintain high yields even under climate constraints, that are resilient or tolerant of abiotic stresses, and which have an expanded capacity to produce compounds of high nutritional value.

5. **Silvicultural crops: poplar:** Among silvicultural plants, poplar attracts the greatest interest in the EU as a fast-growing plantations tree for the production of wood and also providing many ecosystem services. It matches well with ambitions to make sustainable use of resources. The poplar genome has largely been explored and provides a knowledge base to build on. Poplar species also have the capacity to hybridise, this been exploited in the past and can be expected to produce further interesting advances in the properties of this crop. CropBooster will aim to develop highly resilient and resource efficient trees, with high production standards even in marginal lands that are unsuited for intensive agriculture, or when used for phytoremediation in environments where soil or water pollution prevents food production. Remarkably, however, poplars often contribute to air pollution due to their high emissions of highly reactive hydrocarbons. Reducing emissions of poplar volatiles is another important future goal to further exploit the ecosystem services of this plant, this may also further improve the performance of poplars in terms of high growth and yield in low-stress conditions.

6. **Oilseed crops: sunflower and oilseed rape:** Oilseed crops are primarily grown for edible oil, which has growing importance given the increasing global consumption of vegetable oil as a healthy alternative to animal fats, and instead of margarine (made from hydrogenated vegetable oil). Moreover, oilseeds are attracting attention due to an increase in their demand as livestock feeds (especially the meal fraction after the oil is removed, this used as an important high-protein animal feed product), in pharmaceuticals, biofuels, and other oleochemical industrial uses. There is interest in developing for human use the protein-rich cake left after oil extraction. Oilseed crops are generally highly efficient in the use of resources, and the area under cultivation and intensification of their production has increased in recent decades. Future-proofing oilseed plants envisages enhancing both the nutritional value of the oil as well as its quality for industrial purposes. Methods for doing this by means of genetic modification of the plant lipid biosynthetic pathway have received much attention, but for easy access to the EU market these improvements must currently be done using non-GMO approaches, possibly making use of GE technologies, if that becomes more readily acceptable in the EU. Sunflower and oilseed rape are likely to be the oil seed crops that will expand most in the EU. Sunflower is well suited to Europe's soils and climate, and represents one of the most productive crops in terms of biomass and seeds. Oilseed rape is not only an oilseed crop but is an important protein crop, about 50% of the seed is protein and improving its quality would allow its use for human consumption.

7. **7. Nitrogen fixing crops: pea, soybean, lupin,** and **small legumes** for feed and **clover, vetch, and alfalfa** for fodder. Nitrogen is an essential nutrient for plant growth and development, in the form of amino nitrogen ($-NH_2$) it is an essential part of amino acids and proteins, as well as being essential for nucleic acids. There is plenty of nitrogen in the atmosphere (about 80% of the air is

nitrogen (N_2)), but plants cannot use N_2 as a nitrogen source. Instead, plants depend upon soil nitrogen, mainly in the form of ammonia and nitrate. Intensive agriculture needs to replace the soil nitrogen taken up by crops. This replacement is mainly done by applying industrially produced nitrogenous fertilizers. However, excess or careless application of nitrogenous fertilizers has led to ecological problems due to the leaching of excess nitrogenous fertilisers into the water table and water course and consequent pollution and eutrophication of water bodies. In addition more dinitrogen monoxide (nitrous oxide, N_2O), a potent greenhouse gas, is evolved from agricultural soils as a consequence of nitrogenous fertilizer use. Biological nitrogen fixation, on the other hand, is a natural means of providing amino nitrogen for plants, in the correct form and amount. Nitrogen fixing microorganisms (especially bacteria) are symbionts of many herbaceous and tree species worldwide. Their action is particularly effective as symbionts of legumes, which have been long used as the main source of vegetable protein. Legumes also provide nitrogen-rich green-manure or fertilizer in horticulture, or as a nitrogen-fixing crop in agricultural rotations. Protein-rich plant diets have been proposed as a cornerstone of new and healthier diets and as an alternative to a meat consumption. Animal proteins cannot be sustainably produced in sufficient quantities to satisfy growing population needs and are associated with high GHG emissions. Diets high in animal protein are also associated with negative side-effects and health risks including cancers. CropBooster has selected case-study species whose importance is either established or expected to increase in Europe as protein sources for human and livestock diets, and for restoring nitrogen levels in heavily exploited soils. Some crops which are extremely productive (soybean) or yielding novel proteins (lupin) are novel to Europe, but they are likely to emerge as relevant for agricultural and agro-industrial purposes. Other crops are already grown for food (pea) and feed (pea, alfalfa, clover), as well as for green manure applications. In all cases, future-proofing legumes should reduce the content of the antinutritional factors that have often limited the use of these plants in human and animal diets, despite them being good protein sources.

5 European Crop Yields as a Function of Genetics, Environment and Management.

5.1 Overview of Europe’s Crop Production

This section describes the trends in yields of the main crops in Europe, including the geographical differences. In western and southern Europe yields of important crops tend to stagnate or even decrease, while those in eastern parts of the continent increase, leading to convergence of yields across the continent. Actual yields of farmers’ fields are the result of interactions between crop genetics (G), environment (E - climate and soils) and crop management (M). The yield gap of a specific crop is defined as the difference between farmers’ actual yield and the so-called potential yield that can be achieved with an adapted cultivar (G) in defined local climate and soil conditions (E) assuming perfect management, i.e. absence of yield limiting (water, depending on the possibility of irrigation, and nutrients) and reducing (weeds, pests and diseases) factors. Yield gap closure is achieved through improvement of management. In general, north-western Europe shows the smallest relative yield gaps (around 20-30 % for the most prominent crop, area-wise, rainfed wheat), suggesting that there is little room for yield improvement in terms of management, while the relative yield gaps are generally higher in eastern and south-western Europe (e.g. with relative yield gaps of 50% or more for rainfed wheat). Causes of yield gaps can be attributed to inefficient use of resources (e.g. nitrogen), a lack of resources or to a lack of available or adopted technology. While there are indications for some countries there is still genetic progress in (potential) yields, this may not be reflected in higher on-farm yields because of climate change and/or environmental or economic factors affecting input use and crop management. Analyses reveal there is substantial scope to improve resource use efficiency (output-input ratios) and a need to reduce the environmental impact (input minus output, e.g. the surplus of nitrogen). Climate change interacts with yields and resource use efficiency, through effects on temperature, precipitation, CO2 concentration and increased incidence of extreme events. Using adaptation measures, average positive effects of climate change may apply to northern countries, while negative effects still dominate in southern Europe.

5.1.1 Europe’s main arable crops

To understand the current situation of agricultural production in Europe, we first provide some statistics to assess the dynamics and geographical differences across the continent. A first impression is obtained by considering the crop production and harvested area in Europe (excluding the Russian federation) in two years such as 1992 and 2020, using FAOSTAT data aggregated by crop group (cereals, oil crops, pulses, roots and tubers, sugar crops and vegetables - Table 1). These groups roughly correspond to those used in the latest EUROSTAT report on the key figures of the European food chain (Commission and Eurostat 2021), but FAOSTAT data allows to go further back in time than Eurostat.

Some crop groups showed an increased total production in the European area in 2020 compared to 1992, while the area under production decreased. The most striking example is cereals (+ 30.9 % production, - 5.8 % harvested area). Oil crops showed an increase in production, along with a relatively smaller, but still substantial, increase in the harvested area (+ 152.8 % production, + 71.5 % harvested area). Pulses, roots and tubers and sugar crops showed a decrease in both production and area (pulses: - 37.9 % production, - 22.2 % harvested area; roots and tubers: - 27.8 % production, - 49.0 % harvested area; sugar crops: - 23.3 % production, - 56 % harvested area).

Today (average 2018–2020), the production of cereals is dominated (44 % of the volume) by wheat. Wheat, maize and barley together account for almost 90 % of the cereal production. For oil crops, a similar share of 87 % of production is composed of sunflower seed (with roughly half of it coming from Ukraine only), rapeseed and olives. For pulses 88 % of the production comes from peas, beans and pulses. Almost all the production of roots and tubers comes from potatoes, and almost all that of sugar crops from sugar beet. During the period 2000 – 2020, production of common wheat and spelt has increased, while production of rye, durum wheat and potatoes has shown a consistent downward trend over time, and production of dry pulses has been highly fluctuating (European Commission and Eurostat 2021). Fischer, Byerlee et al. (2014) noted a decrease of both area and yield of protein-rich crops in humid Europe, with consumption being negatively related to income. More recently, Manners, Varela-Ortega et al. (2020) indicated that protein rich crops have experienced a decline in both production and consumption in Europe, followed by a reversal recently, perhaps in the context of the protein transition and the need of diversification.

Currently, the majority of the cereals (in terms of production volume) in Europe are grown in France, Germany, Poland, Romania and Ukraine, where the extensive arable land areas and the temperate weather conditions allow for relatively high total production. Considering the average of the most recent three years of FAOSTAT data available, Europe accounts for 12.2 % of world cereal production and about 12.7 % of Europe's production is exported.

Table 5.1. *Production and harvested areas in Europe (excluding Russian Federation) as averages of the periods 1992–1994 and 2018–2020. Source: FAOSTAT (2022).*

	production (million t)		**area harvested (million ha)**		**yield (t ha^{-1})**	
Crop	**1992-1994**	**2018-2020**	**1992-1994**	**2018-2020**	**1992-1994**	**2018-2020**
Cereals	**308.08**	**403.39**	**81.19**	**76.51**	**3.79**	**5.27**
Wheat	137.43	176.95	32.20	33.92	4.27	5.22
Maize	56.46	115.18	12.50	15.74	4.52	7.32
Barley	76.15	71.66	22.31	15.62	3.41	4.59
Triticale	4.37	12.58	1.26	3.21	3.47	3.92
Oats	11.67	9.66	4.45	3.16	2.62	3.06
Rye	14.26	9.13	5.50	2.56	2.59	3.56
Grain, mixed	3.74	3.09	1.51	1.07	2.47	2.88
Rice, paddy	2.28	2.93	0.41	0.44	5.59	6.72
Sorghum	0.75	1.19	0.17	0.24	4.55	5.01
Other	0.97	1.01	0.88	0.56	1.10	1.82
Oilcrops	**27.66**	**69.91**	**15.98**	**27.41**	**1.73**	**2.55**
Sunflower seed	8.59	25.39	6.60	11.08	1.30	2.29
Rapeseed	8.17	21.91	3.33	7.68	2.45	2.85

	production (million t)		area harvested (million ha)		yield (t ha^{-1})	
Olives	7.71	13.86	4.34	5.13	1.78	2.70
Soybeans	1.43	7.24	0.68	2.77	2.10	2.62
Seed cotton	1.16	1.10	0.40	0.36	2.88	3.08
Oilseeds nes	0.17	0.21	0.05	0.24	3.38	0.88
Linseed	0.34	0.16	0.48	0.11	0.71	1.38
Other	0.09	0.03	0.10	0.05	0.91	0.71
Pulses	**10.16**	**6.30**	**3.87**	**3.01**	**2.62**	**2.09**
Peas, dry	8.03	2.84	2.59	1.22	3.10	2.34
Broad beans, horse beans, dry	0.51	1.60	0.24	0.60	2.13	2.66
Pulses nes	0.65	1.10	0.26	0.75	2.48	1.47
Beans, dry	0.48	0.37	0.39	0.20	1.24	1.90
Lupins	0.08	0.26	0.06	0.19	1.34	1.38
Other	0.41	0.12	0.34	0.06	1.23	2.06
Roots and Tubers	**118.08**	**85.23**	**6.71**	**3.42**	**17.60**	**24.90**
Potatoes	117.93	85.22	6.70	3.42	17.61	24.90
Other	0.15	0.01	0.01	0.00	14.32	23.97
Sugar Crops	**176.60**	**135.42**	**4.69**	**2.07**	**37.62**	**65.49**
Sugar beet	176.44	135.42	4.69	2.07	37.60	65.49
Sugar cane	0.16	0.00	0.00	0.00	80.01	
Total area (major crops)			112.44	112.42		

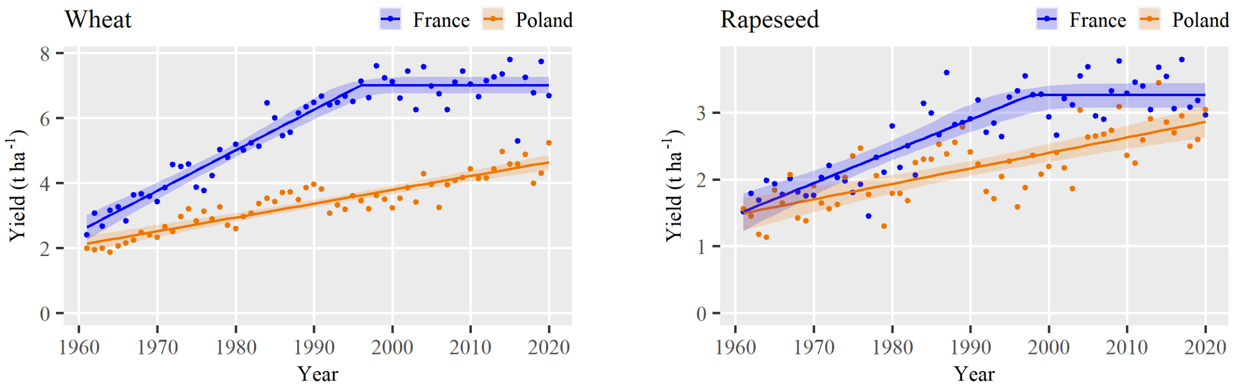
5.2 Trends of Crop Yields in the World and in Europe

Globally, a steep increase in productivity of grain crops, such as maize, wheat and rice has been achieved since the end of the Second World War (Grassini, Eskridge et al. 2013, Liang-Bing, Kai-Yuan et al. 2021). This increase was the result of the so-called Green Revolution, that allowed the exploitation of the genetic improvements through improved crop management with irrigation, synthetic N fertilizers obtained through the Haber-Bosch process and the spread of mechanization (Borlaug 2000). Fischer, Byerlee et al. (2014) noted that globally the increase in grain yield (arithmetic average) of rice, maize and wheat was 53 kg ha-1 year-1 between 1960 and 2010 (a slightly lower value of 52 kg ha-1 year-1 is obtained from FAOSTAT by extending the time period to 2020). Between 1961 and 2008 the global yield of major staple crops increased linearly, with + 84, 40, 27 and 31 kg ha-1 year-1 respectively for maize, rice, wheat, and soybean (Ray, Mueller et al. 2013). Similar values were obtained by Fischer, Byerlee et al. (2014) considering two more recent decades (from 1990 to 2010): + 80, 43, 30 and 25 kg ha-1 year-1 Extending this estimation to include the most recent decade (1990 – 2020), a lower rate of increase can be noted for maize and rice (+ 75 and 41 kg ha-1 year-1, respectively), while a higher increase rate emerges for wheat and soybean (+ 38 and 28 kg ha-1 year-1, respectively). Overall, the rate of increase of maize yield seems to be reducing over time (though still very high in absolute terms), and that of wheat seems to be rising. Soybean shows the greatest fluctuations, depending on the considered period. Western countries (Europe and US) experienced this yield increase earlier and in a stronger way, but experienced also an earlier stagnation of farm yield, starting from the 1990s (Le Gouis, Oury et al. 2020).

Considering crop yields in Europe, historical trends of main crops (barley, maize, rapeseed, rice, sorghum, soybean, wheat) showed a linear increase until the mid '90s, when stagnation occurred in Western Europe (Ray, West et al. 2019). Grassini, Eskridge et al. (2013) also noted that, in general, many crops increased yield fast after the Second World War, but are plateauing more recently. Yield plateaus were found for wheat in Western Europe (France, Germany, The Netherlands, Denmark) and maize in Southern Europe (France and Italy). On the other hand, In Eastern Europe, a trend of continuous increase is observed for wheat (Le Gouis, Oury et al. 2020). According to Fischer, Byerlee et al. (2014), sugar yield of sugar beet increased by 0.31 t ha-1 year-1 across Europe between 2001 and 2010. Yields of rapeseed, that finds favourable growing conditions in northwestern Europe, have also been strongly increasing, reaching an average yield of the last 20 years of 3.6 t ha-1 in Germany (FAOSTAT).

Differences of crop yields and yield trends across Europe can be illustrated by comparing a country in the western EU27, such as France, with one in the eastern part of the EU27, Poland. The trends of the major (highest production) crops within each crop group can be visualized to highlight these differences (Figure 5.1) and identify some general aspects. Generally, in France crop yields are higher than in Poland, but yields in France reached a plateau or have been decreasing during or after the 1990s. The increase in wheat, rapeseed and peas yield came to a halt starting from the late 90s, with peas even showing a decreasing trend after that. The increase in potato and sugar beet yield in France also levelled off starting from the second decade of 2000. However, the seemingly decreasing trend for the recent years shows high uncertainty in the estimations after the break points, suggesting that the situation will need to be closely monitored in the next years to determine yield trends with more confidence. In comparison to France, Poland is characterized by consistently lower yields, but the increasing trend is continuing. Hence, yields of France and Poland are converging.

This section aims to analyse and quantify the progress in yield (Section 5) and resource use efficiency (Section 6) of some main crops in Europe, and to disentangle the effects of genetics (G), environment (E) and management (M). To this end, some key concepts are first introduced and defined.



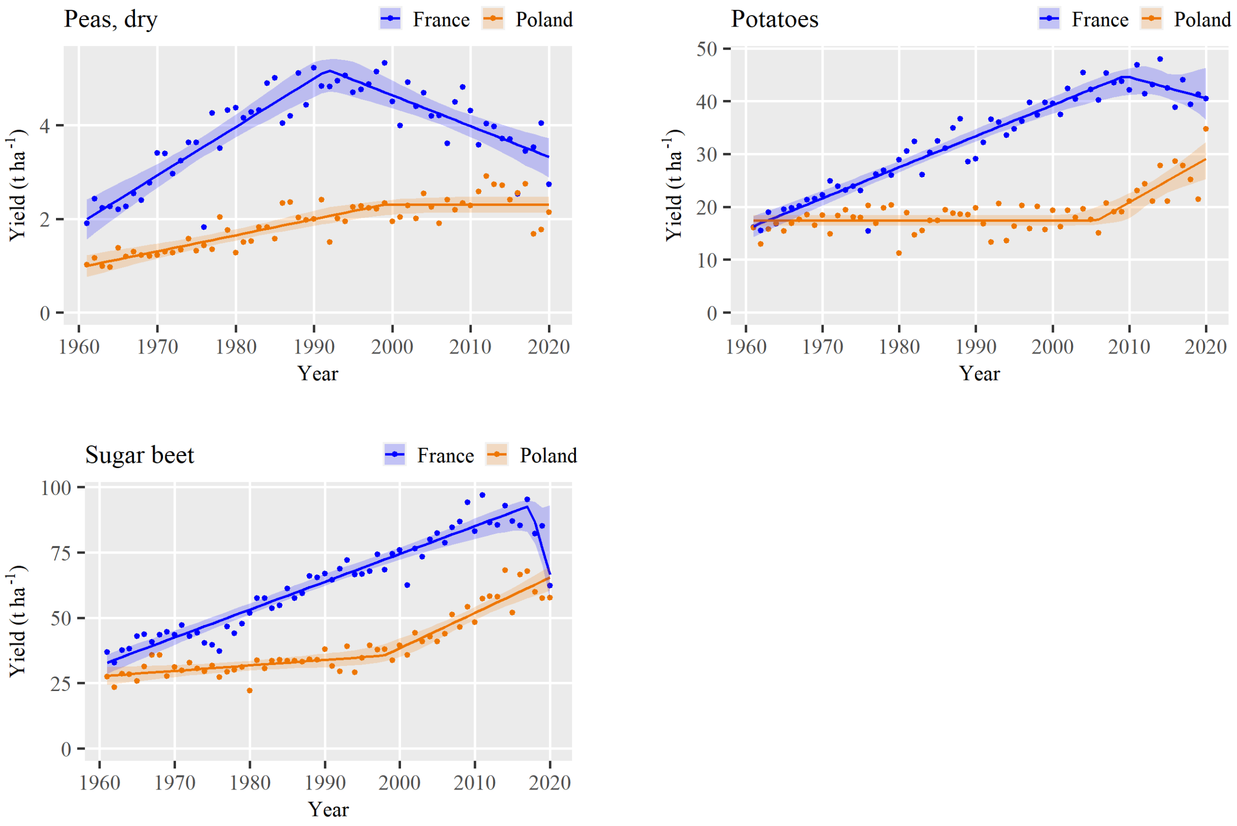


Figure 5.1. *. Crop yield trends (1980 – 2020) of the major crops per crop group (source: (FAOSTAT 2022)), in France (Western EU) and Poland (Eastern EU). Several regression models were tested to explain yield trends: linear, linear – upper plateau, linear – lower plateau, broken stick, quadratic plateau, exponential, plateau – exponential, asymptotic. Regression lines of the best fitting models (lowest AIC) are reported, with bootstrapped 95 % confidence intervals.*

5.3 Benchmarking Concepts

In assessing crop yields and land productivity (e.g. yield per ha) as well as efficiency and environmental impact of crop production (e.g. yield per kg of nitrogen input) the notion of benchmarking is very relevant (van Grinsven, van Eerdt et al. 2019). Benchmarking requires meaningful and well defined indicators, such as yield per ha, yield per kg of nitrogen input, etc., and a means of estimating whether indicator values are good or less good compared to theoretical or empirical maximum or optimum values. In crop production this is generally done in at least three distinct ways: i) comparison against experimental values derived under optimum conditions; ii) comparison of best or highest values achieved by peers; iii) comparison against simulated theoretical maximum or optimum values.

5.3.1 Yield gaps

An obvious and well-known example is the estimation of so-called yield gaps (van Ittersum, Cassman et al. 2013) (Fiure. 5.2). The yield gap of a specific crop is generally defined as the difference between farmers' actual yield (Ya) and the so-called potential yield that can be achieved with an adapted cultivar (Genetics – G) in defined local climate and soil conditions (Environment – E) assuming absence of yield limiting (water, depending on the possibility of irrigation, and nutrients) and reducing (weeds, pests and diseases) factors. Such potential yields assume perfect crop management of an adapted cultivar and can be defined for both irrigated (Yp, assuming no water limitation) and rainfed (Yw, water is generally limiting) conditions. In other words, the yield gap indicates the scope for yield increase through improved crop management (M) in given climate and soil conditions. Generally maximum yields of farmers (at least the average across a geographical unit) is at most 75-85% of Yp or Yw (often labelled the exploitable yield – van Ittersum, Cassman et al. (2013)), because of diminishing returns to inputs with economic and environmental consequences. Actual farm yields are thus the result of G x E x M interactions

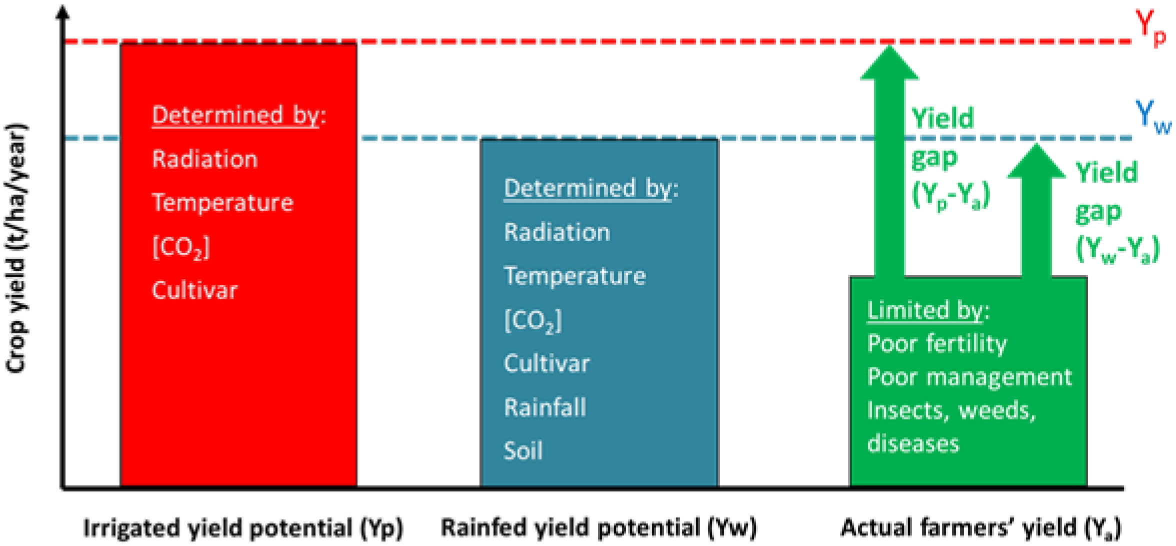


Figure 5.2. *Concepts of potential and actual yields and of yield gap (modified from van Ittersum and Rabbinge (1997)).*

Recently, the notion of yield gap has been extended with the genetic yield gap. The notion of a 'genetic yield gap' (*Yig*) of a crop species is defined as the gap between the 'genetic yield potential' (*Yi; i* stands for ideotype) of a crop species under irrigated (*Yip*) or rainfed (*Yiw*) conditions, optimized in a target environment, *i.e*. an optimal cultivar or crop ideotype, and the potential yield of the current local cultivar in that environment (*Yp* or *Yw*) (Senapati and Semenov 2019, Senapati and Semenov 2020). An ideotype is defined here as an optimal combination of cultivar traits that represents an ideally adapted crop cultivar which delivers the highest yield in a target environment. The 'genetic yield potential' of a crop species represents the yield that could be achievable in a target environment (E) through genetic improvements (G). Note that climate change (part of E) affects potential yields and yield gaps.

Considering the G x E x M interactions, researchers sometimes identify and disentangle the genetic progress in yield from those related to environmental and management factors. To tackle this issue, the distinction between a rainfed and irrigated crop needs to be considered, and it should be kept in mind that within the same country or administrative unit, rainfed and irrigated fields of the same crop can co-exist.

5.3.2 Yield gap decomposition

Yield gap analysis quantifies the difference between actual and potential yields, but does not necessarily explain why actual farmers' yields are lower than the potential. Identification of the causes of yield gaps requires a form of yield gap decomposition into its biophysical causes. A form of yield gap decomposition was formalised and applied by Silva and colleagues (Silva, Reidsma et al. 2017, Silva, Reidsma et al. 2021). They decomposed the yield gap into efficiency, resource and technology yield gaps (Figure 6.3). Efficiency yield gap is defined as how far the yield on a specific field or farm is from the production frontier for a given input level and hence how much additional output (yield) could be achieved given observed levels of input(s). Basically, this refers to the degree to which inputs have been used efficiently, for instance associated to accurate application in time and space. The resource yield gap is defined as the difference between what the highest farm yields (or a percentile of highest farm yields) are in a given geographical location and the technical efficient yield for a given level of inputs. It indicates the degree of lack of inputs to achieve higher yields. Finally, the technology yield gap is the difference between the potential yield level (*Yp* or *Yw*, depending on whether irrigation is possible) and the highest farm yields. This refers to the absence in the sample of farms of the necessary technology (e.g. type of crop protection, precision management or crop rotation) to achieve the potential yield. Van Dijk, Morley et al. (2020) also added socio-economic causes of yield gaps, which often underly the biophysical ones, including price ratios of inputs and outputs, access to knowledge and inputs and legislation. Note that such yield gap decomposition requires individual farm data from a defined geographical unit (characterised by soil and climate) on input use and yields, and preferably also explanatory variables including management and farm characteristics.

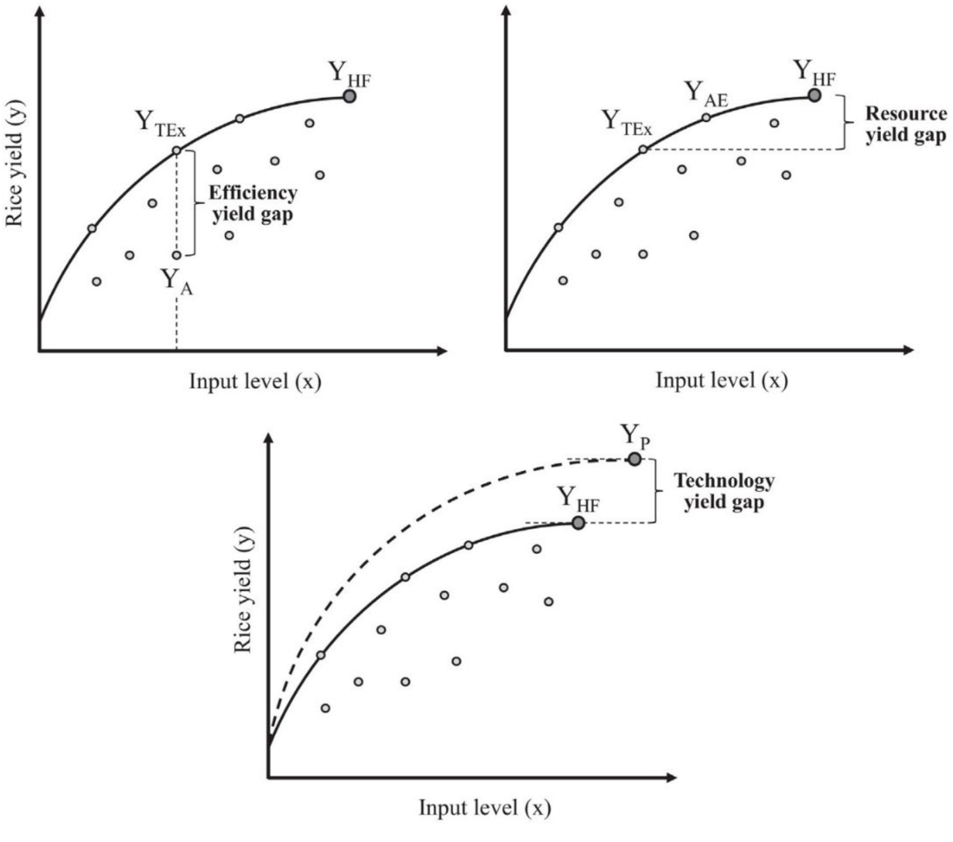


Figure 5.3. *Graphical representation of efficiency, resource and technology yield gap, from Silva, Reidsma et al. (2017).* Y_{HF}, Y_{TEx} *and* Y_a *are abbreviations for highest farmer's yield, technical efficient yield at a specific input level and actual yield of each individual farm, respectively.*

5.4 Resource Use Efficiency

Benchmarking often focuses on yields but increasingly, benchmarking of the resource use and environmental performance is becoming equally important. High yields at the expense of poor resource use efficiency (low ratios between yield and inputs) or high environmental impact (low resource use efficiency and/or high resource use or resource emissions per unit area) are undesirable. Examples of indicators and approaches on this topic are the water productivity indicator and the work on nitrogen use efficiency and nitrogen surplus, as for instance operationalized by the EU nitrogen expert panel (EU Nitrogen Expert Panel, 2015).

Water productivity is defined as the ratio between the yield and the amount of water use by the crop (Global Water Partnership 2000). Like yield levels, a potential and actual water productivity can be distinguished. These potentials can be derived from empirical data from a set of farmers' fields, and then drawing boundary lines, or from the use of crop growth models (French and Schultz 1984, Zwart and Bastiaanssen 2004, Grassini, Yang et al. 2009, Edreira, Guilpart et al. 2018). Comparison of potential with actual water productivities allows for computation of water productivity gaps (Edreira, Guilpart et al. 2018).

For nitrogen (or other macro nutrients such as phosphorus and potassium) the indicators of nitrogen use efficiency and nitrogen surplus are useful. The EU nitrogen expert panel has brought together these two indicators plus those on N output and N input in one framework (Quemada, Lassaletta et al. 2020). The nitrogen use efficiency is defined as the ratio of N output (N in the harvested product) over N input (all N entering the field or farm through deposition, biological N-fixation, manure or mineral fertilisers). N surplus is defined as the difference between N input and N output and a proxy for risks of N losses to the environment. The benchmarking of this indicator is so far mostly empirical by comparing fields, farms or regions and drawing boundary lines (e.g. Silva, Van Ittersum et al. (2021)); it is much more challenging to give theoretical upper limits for these indicators. Losses of nitrogen in the cycle are inevitable and therefore an NUE=1 may not be desirable as it would probably imply mining of soil N and hence soil organic matter. The EU N expert panel proposed a desirable range of NUE values of 0.5 to 0.9 but the best value will be highly associated with the permissible N surplus, as a relatively high NUE may still be associated with high N surplus if the N input levels are high.

There have been attempts to validate different production functions to the use of agricultural inputs, including the laws of Liebig, Liebscher and Mitscherlich (De Wit 1992, van Noordwijk and Brussaard 2014, van Grinsven, Ebanyat et al. 2022). All these functions reflect the phenomenon of decreasing marginal returns with increasing amounts of nutrients (De Wit 1994). Liebig's law of the minimum implies that yield is proportional to the availability of the most limiting production factor. Liebscher's law of the optimum captures that the production factor in minimum supply contributes more to yield, the closer the other factors are to their technical optimum. Mitscherlich's law, a special case of Liebscher's law, has recently been supported by experimental evidence on cereals (Van Grinsven et al., 2022): it implies that cereals require an absolute amount of crop-available N (Nav) to meet a certain relative yield (Y/Ymax), irrespective of the level of Ymax. To realize this, the agronomic management other than nitrogen management, e.g., plant density, crop protection against weeds, pests and diseases, must be optimal.

5.5 Yield Gap and Yield Trends in Europe

A systematic approach is employed to provide insights in the yield gap throughout all important food producing countries in the world in the Global Yield Gap Atlas (GYGA) platform (https://www.yieldgap.org/). It uses a common protocol using model simulations and local data. A recent work by Schils, Olesen et al. (2018) summarizes the GYGA analysis for Europe and provides insights on the grain yield gap of widely cultivated rainfed cereals (wheat, barley and maize), and on irrigated maize. An example of the information provided by the platform is shown for wheat in Figure 5.4. In general, north-western Europe demonstrated the smallest relative yield gaps for rainfed cereals (around 30%), suggesting that there is little room for improvement in terms of management, while the relative yield gaps are generally higher in eastern and south-western Europe (e.g. with relative yield gaps of 50% or more for rainfed wheat) (Figure 5.4). The yield gap was observed to decrease with increasing country gross domestic product (GDP), indicating a positive relationship between socioeconomic development and yield gap closure.

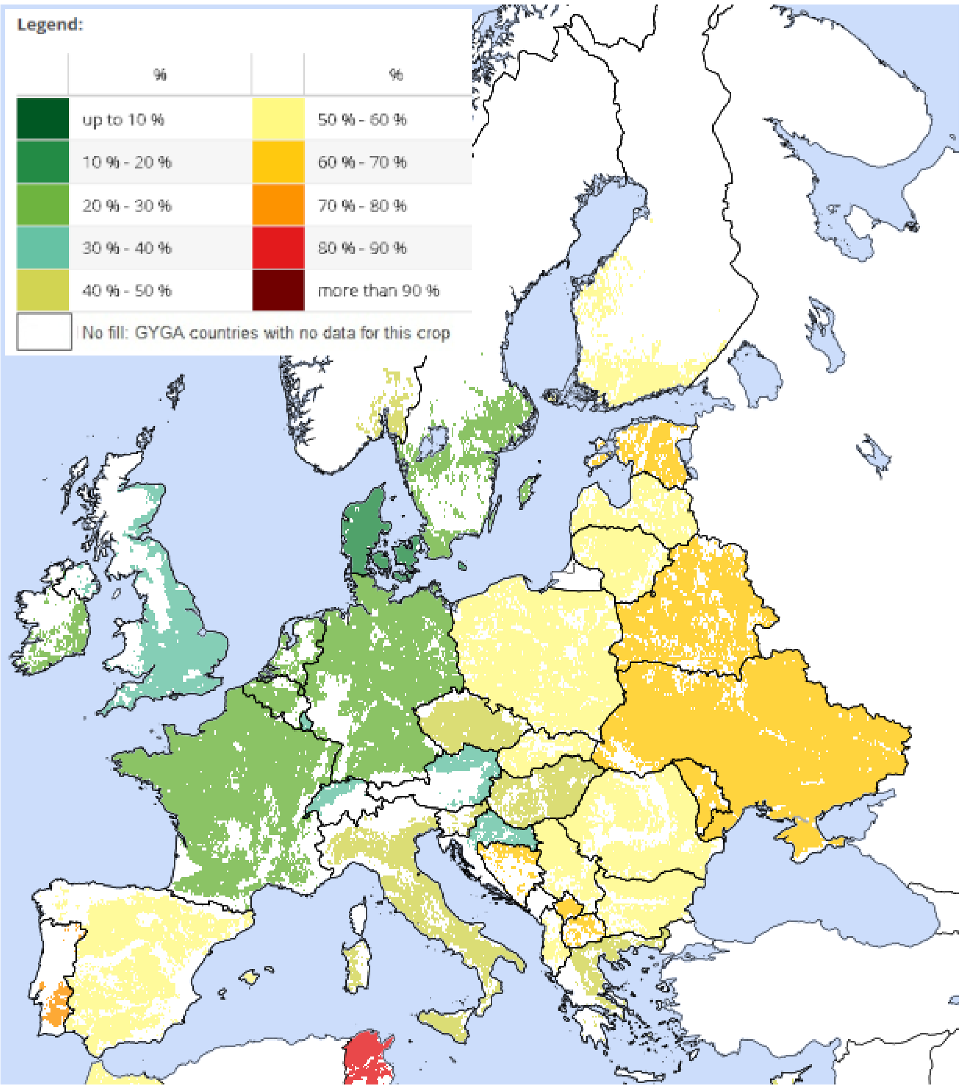


Figure 5.4. *Relative yield gap of rainfed wheat by country. The SPAM crop mask is applied to highlight the wheat cultivated areas. Source: GYGA (https://www.yieldgap.org/) and Schils et al.*

*(2018). Relative yield gap is calculated as: (1 – Ya / Yw) * 100%.*

Further insights can be provided by highlighting differences between European countries along the west-east and the north-south gradients. Examples are provided in Figures 5.5 and 5.6, using countries that cultivate the same crops. Using again the France-Poland comparison, Figure 5 shows recent estimations of the yield gaps of the major rainfed crops cultivated in both countries (barley, maize and wheat). The potential yield (Yw) of these crops is similar in both countries, with barley and wheat potential yield being on average 12 % and 4 % higher in France than in Poland, and the maize potential yield being 16% higher in Poland. However, the actual farm yield (using most recent data available from FAOSTAT, i.e. up to 2020) is consistently (much) higher in France than in Poland (+70 % for barley, +32 % for maize and +57% for wheat). This leads to a lower relative yield gap in France (23%, 12% and 28% for barley, maize and wheat, respectively), than in Poland (49%, 44% and 52%, for the same crops), highlighting the differences between western and eastern parts of Europe. Figure 6 shows recent estimations of the yield gaps of major irrigated crops (maize and soybean) cultivated in both France and Italy. The potential is similar in both countries, but while the maize actual yield is similar in the two countries, Italy reaches higher soybean actual yields than France (+54%). This contributes to a smaller relative yield gap for the two irrigated crops in Italy: the relative yield gap of maize is 28% in France and 24% in Italy, of soybean 42% in France and 19% in Italy.

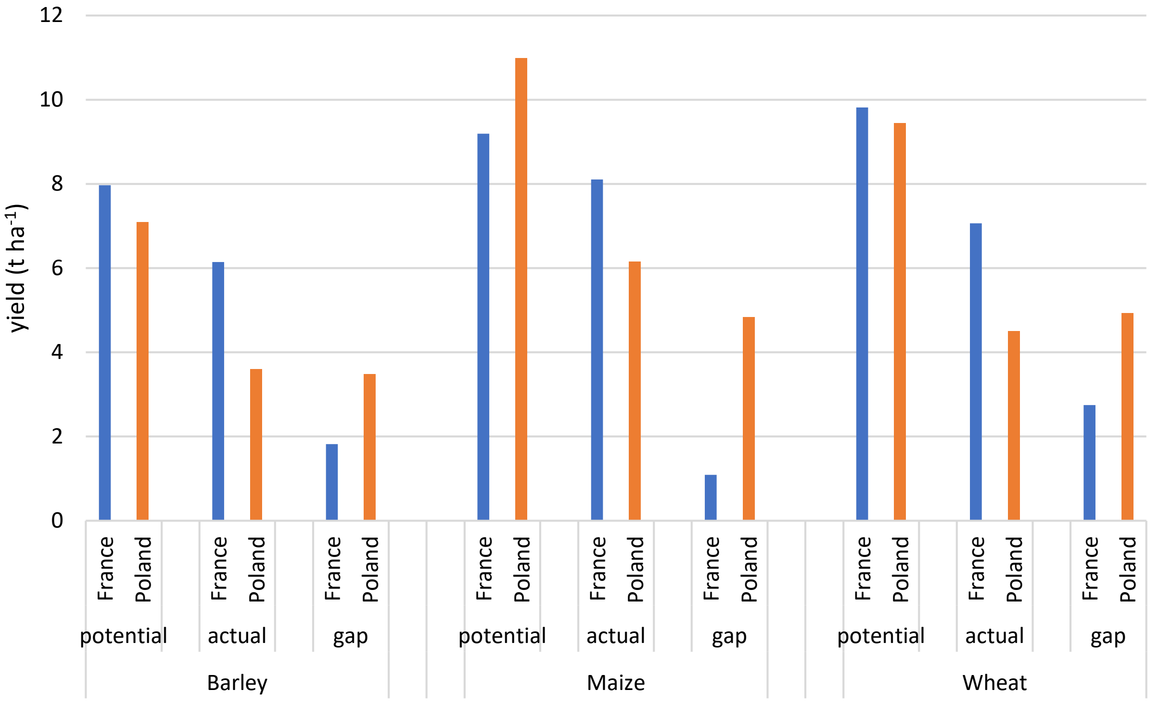


Figure 5.5. *Yield and yield gap of major rainfed cereals in France and Poland. Source: GYGA (2022). Actual yield was obtained from FAOSTAT (up to the year 2020), except for maize in France (GYGA).*

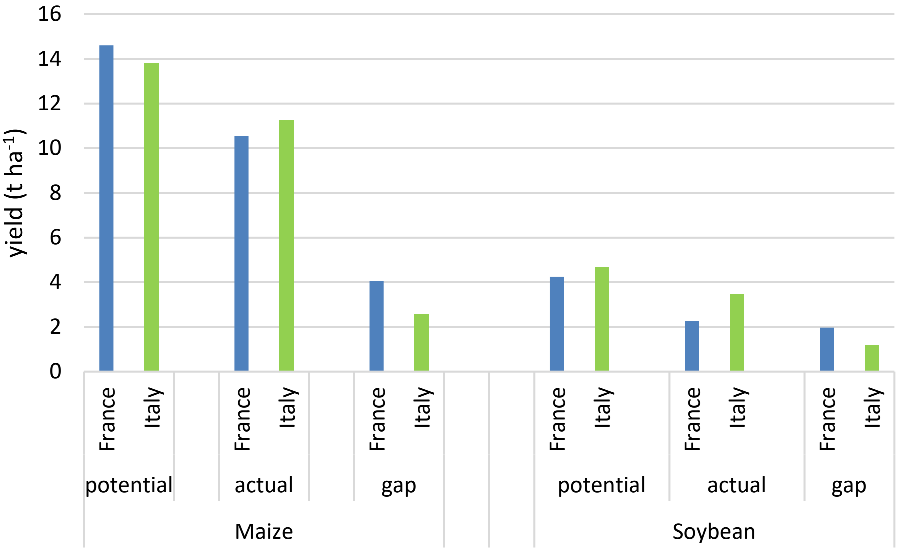


Figure 5.6 *Yield and yield gap of major irrigated grain crops in France and Italy. Source: GYGA (2022). Actual yield was obtained from FAOSTAT (up to the year 2020), except for maize in France (GYGA).*

Other works offer useful insights on cereals, quantifying the genetic and non-genetic yield trends of specific crops. Considering maize, the trend of genetic improvement was related to an improved leaf architecture (with increased LAI and radiation use efficiency) (Taube, Vogeler et al. 2020), and by a more efficient reallocation of N during the grain filling period (Mueller, Messina et al. 2019). From 1991 to 2016, rainfed forage maize (virtually all aboveground parts are harvested) in the Netherlands increased its genetic potential by 173 kg ha^{-1} year^{-1}, going from 13.2 to 22.9 t ha^{-1} in varietal experiments with optimal management, due to the consistent adoption of newer varieties (Schils, Van den Berg et al. 2020). Non-genetic improvement was quantified at 65 kg ha^{-1}, indicating that the yield gap has been widening, since the genetic progress is faster than the increase of farm yield (Schils, Van den Berg et al. 2020). High-yielding EU countries for rainfed maize grain are France, Austria, Germany and Belgium (actual yield close to or exceeding 9 t ha^{-1}), while in Eastern Europe (e.g. Romania and Moldova) yields are 6 t ha^{-1} or less. However, in recent years the progress of farm yields is slowing down in Western Europe, this is different to Eastern Europe. Spain and Italy, instead, provide irrigation to maize; in both countries the progress of maize yield has slowed considerably, perhaps also caused by a decrease in potential yield due to a warming trend in these regions (Supit, van Diepen et al. 2010, Fischer, Byerlee et al. 2014). James (2011) also argued that the slowing pattern could depend on the banning of genetically modified (GM) maize in Europe, with the exception of Spain, where the yield has been increasing faster because of the adoption of that technology. Schils, Olesen et al. (2018) noted that while the relative yield gaps of rainfed maize clearly increase going from west to east, the relative yield gaps of irrigated maize show no clear trend, but a great variability across bordering countries (e.g. with Portugal and Albania having a much greater relative yield gap than Spain and Greece). Whether or not irrigation is applied also constitutes a major agronomic management trait that influences the interaction of the crop with the environment on an annual basis: year-to-year variability of rainfed grain maize in Europe can be partially attributed to drought stress, that accounts for 24 % of the variability (Webber, Ewert et al. 2018), while drought stress only marginally influences annual variability of irrigated maize (Schils, Olesen et al. 2018). In general, the interannual yield variability of a rainfed crop is higher than the variability of the same, but irrigated crop, within the same agri-environmental area (Grassini et al., 2015; Van Ittersum et al., 2013).

Considering wheat, Brisson, Gate et al. (2010) analyzed the trend of wheat yield in France between 1950 and 2008, estimating a genetic progress of 115 kg ha-1 year-1, with a statistically significant plateau of farm yield commencing in 1997 (that can also be noticed in Figure 6.1). The authors considered the slowing in yield gap closure, an effect of temperature and drought stress, of the replacement of legumes as preceding crops, and of reduced fertilization. As noted by Fischer, Byerlee et al. (2014), it should be considered that the last two aspects are mainly influenced by policy measures and economic developments. For wheat, the yield gap seems to be somewhat widening in western Europe due to several factors slowing the farm yield progress, such as the adverse effects of greater extreme events due to climate change, stricter environmental regulations and changes in crop rotations while the genetic progress is still continuing (as reported for France by Brisson, Gate et al. (2010). Genetic improvement is associated mainly with increased grain number, grain weight and total dry matter, and in some cases increase in radiation use efficiency, leaf photosynthesis and stomatal conductance (Fischer, Byerlee et al. 2014).

Other studies at a more local scale focus mainly on the Netherlands, considering the common crops of the Dutch arable farming system (Silva, Reidsma et al. 2017, Silva, Reidsma et al. 2021). Silva, Reidsma et al. (2021) estimated a yield gap of 30 % for rainfed wheat and of 34 % for barley. (Silva, Reidsma et al. 2017) also estimated the yield gap of ware potatoes (29 %), starch potatoes (40 %), and sugar beet (26 %). Rijk, van Ittersum et al. (2013) and the later update by the same authors (Rijk et al., 2019) compared the genetic progress (estimated from variety trials) to farm yield evolution in the Netherlands. The authors demonstrated that wheat had a linear genetic yield potential improvement between 1978 and 2016, while the progress of farm yield had a concave shape, indicating a widening yield gap. As for spring barley, the genetic progress accelerated (convex curve), but this was not fully translated in an equally fast increase of farm yield (linear pattern). A slowly growing yield gap was also found for starch potatoes, i.e. the increase in actual farm yields was less than the estimated genetic progress of yield potential. Sugar beet showed a stable or even narrowing yield gap depending on the pathogen resistances of different varieties. Furthermore, Silva, Reidsma et al. (2017) and Silva, Reidsma et al. (2021) decomposed the yield gap of Dutch arable farms into efficiency, resource and technology yield gaps. These two studies pointed out that the resource yield gap was the lowest (< 10% of potential yield for all the crops), due to the high availability and use of inputs in this part of Europe. The efficiency yield gap ranged between 9 % and 18 % of potential yield, depending on the crop. It is mainly caused by suboptimal timing of the management operations, due to unfavorable weather for trafficability. The technology yield gap (ranging between 7 % and 31 %) was related to narrow crop rotations and water limitations for irrigation (which was generally lower in clay soils, that have a higher water retention capacity).

5.6 Resource Use Efficiency and Environmental Sustainability

Sustainable intensification seeks to increase agricultural production and associated economic returns per unit time and land without negative impacts on soil and water resources or the integrity of associated non-agricultural ecosystems (Pretty and Bharucha 2014). This requires a balance between high yields, high resource use efficiency and acceptable (local and global) emissions to the environment.

Historically, intensification has been the dominant pathway in many parts of the world: producing more on existing agricultural land associated with greater input use. Indeed, the use of inputs in Europe increased over time with GDP, leading to an increase in yield, but each country is experiencing a “turning point” that indicates a shift in environmental awareness under more advanced socioeconomic conditions (Zhang, Davidson et al. 2015). This evolution is clearly captured in so-called Environmental Kuznets Curves (EKC), that indicate the existence of an inverted U shape relationship between per capita income and pollution or environmental impact (Dinda 2004). This relationship holds for many economic sectors, and also for agriculture and the N and P inputs. With the economic growth of European countries, the attention towards environmental issues has increased, leading to the progressive introduction of environmental regulations, especially from the 1990s onwards. This likely contributed to a different weighting of the three dimensions yield gap closure, resource use efficiency and emissions to the environment. Certainly in Western Europe this may have contributed to some stagnation of yields and improved environmental performance.

5.6.1 Nitrogen Use

In the context of the N use, the framework proposed by the EUNEP (2015; 2016) to analyse the association between nitrogen output (yield), NUE and N surplus (defined above) is useful (Figure. 5.7). The framework allows to take a snapshot of the use of N resources at field and farm level, considering N inputs (N from fertilization, deposition and seed materials) and N outputs (from exported yield). NUE > 0.9 indicate a risk of soil mining, while NUE < 0.5 indicates inefficient N use. The area in-between those two NUE levels identifies a safe operating space for improving the use of N. The addition of a target for minimum productivity and of a desired maximum Ns (80 kg N ha^{-1} $year^{-1}$, as proposed by EUNEP), helps to further focus the scope for improvement. This template was recently adopted by Quemada Saenz-Badillos, Lassaletta Coto et al. (2019) to analyse European farms. The authors used ten years of data from six countries across five environmental zones: the Atlantic central (France, Ireland, and The Netherlands), Atlantic North (Denmark, Germany), Continental (Germany), Mediterranean North (Spain) and Mediterranean South (Italy). Considering arable farms, the median NUE was 0.6, while it was much lower for dairy (0.19) and pig farms (0.43). The values of dairy and pig farms were even lower when externalization of the feed resources was considered. The median N surplus of arable land in the analysis of Quemada et al. (2020) was 68 kg N ha^{-1} and the median N outputs 100 kg N ha^{-1}. Considering geographical differences, some farms in Spain and Germany achieved the highest NUE (0.8, close to the 0.9 limit proposed by the EUNEP to identify risk of soil N mining). Danish farms, on the other hand, had a relatively low NUE and high N surpluses (58 kg N ha^{-1}, on average) because of the prevalent use of organic fertilizers and the greater share of organic farms (28%). This might indicate both greater environmental losses and an increased retention of N by a build-up of soil organic matter. Similar results were found in the work by (Hutchings, Sorensen et al. 2020), that estimated an average NUE of 0.7 in arable lands in Europe, with lower NUE in the north than in the south of Europe. This is mostly attributed to the different importance of mineralization and NH_3 emissions (higher in the south) and leaching (higher in the north).

Other studies on NUE are more tuned to local farming systems: an example is provided by (Silva, Van Ittersum et al. 2021), who estimated the N balance of crops of Dutch arable farms following the EUNEP framework. Average NUE of major Dutch crops (potatoes, winter wheat, spring barley, spring onions and sugar beet) was within or above the 0.5 – 0.9 range proposed by EUNEP. At the same time, all the crops had an average N surplus (N input – N output) below the recommended threshold of EUNEP, but about 40 % of ware potato, starch potato and winter wheat fields had a surplus above the threshold, indicating the necessity and scope for further improvement in their N management. The relatively high NUE and high Ns for most crops are the result of high N outputs (yields) combined with high N application rates. Moreover, high NUE and low N surplus were mostly associated with lower N application rates and with the use of mineral fertilisers instead of organic fertilisers.

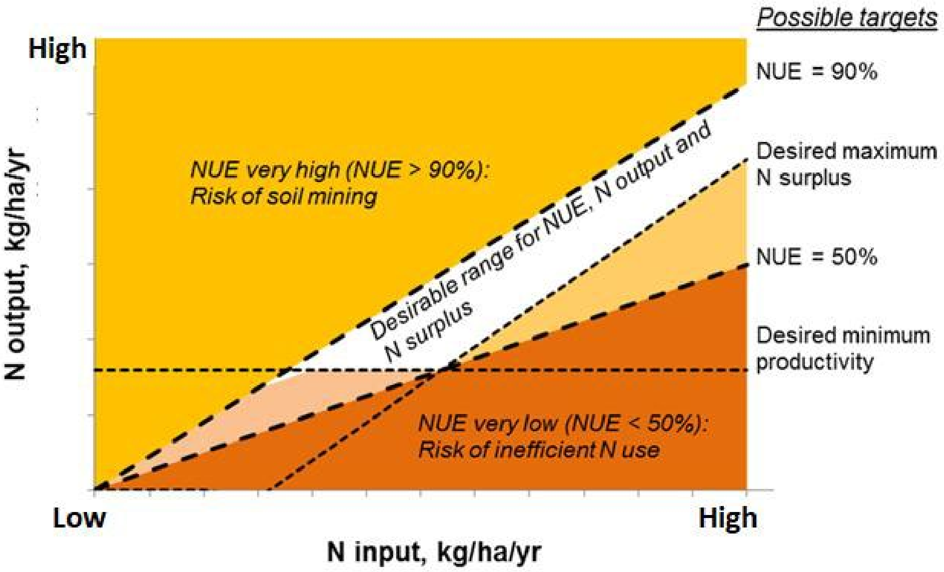


Figure 5.7. *Nitrogen Use Efficiency framework (EUNEP, 2016).*

Another useful indicator of the use of N by crops is the N uptake gap, i.e. the difference between the minimum N uptake of a crop for a given yield target and the actual N uptake of the crop (ten Berge, Hijbeek et al. 2019). Schils, Olesen et al. (2018) found the N uptake gaps for a target yield of 80% of the yield potential of cereals (which is about the maximum yield that farmers could achieve) to be highly variable across regions in Europe. Considering rainfed cereals, N uptake gaps were estimated to vary between 0 and 150 kg N ha^{-1} for wheat and between 0 and 100 kg N ha^{-1} for maize. Average values over Europe were 87, 77 and 43 kg N ha^{-1} for wheat, barley and maize, respectively, and were much higher for the eastern part of Europe than for the western part. However, the authors pointed out that an increase in the N inputs should be pursued only with care, as it may lead to an increase in N surplus and pollution (leaching, N2O and ammonia emissions). It must also be noted that N uptake gaps may not necessarily point at an absolute lack of nitrogen in the system, but rather at other growth limiting or reducing factors that hinder the uptake of N from the soil which is actually available (e.g. pests and diseases, soil compaction, lack of other nutrients). Agronomic measures can be identified to reduce, for example, ammonia emissions (UNECE 2015), such as fertilizer management with rapid incorporation and/or injection in the soil, soil management like cover crops, mulching and reduced tillage. **New and consistent methods that allow a better quantification of nutrient balances and yield response are still to be actively researched.**

5.7 Climate Change

The quantification of the effects of climate change (part of E) on crop yield is paramount to correctly estimate genetic and non-genetic components of the yield progress, and yield gap avoiding confounding effects. In particular, it should be noted that adaptation strategies can be adopted in the agricultural sector as a response to the effect of climate change. The most recent report of the IPCC (Intergovernmental Panel on Climate Change, Pörtner, Roberts et al. 2022) highlights adaptations to climate change that are currently ongoing in the agricultural sector. Considering the past progresses on farm yield, the report indicates that, globally, the yields of major crops have increased 2.5 – 3 – fold since the 1960s. This is largely due to genetic progress, advances in agricultural practices, and adaptation to climate change that lessened its adverse effects or exploited positive effects. However, discrepancies between regions of the world have been detected and are expected under future projections. In northern Europe, climate change had positive effects on wheat yield, while in southern Europe climate warming has negatively impacted yields of almost all major crops, contributing to yield stagnation (Moore and Lobell 2015, Agnolucci and De Lipsis 2020, Bras, Seixas et al. 2021, Pörtner, Roberts et al. 2022). Ray, West et al. (2019) highlighted that the effects of climate change slowed down the post war trend of yield increases in western and southern Europe. Bras, Seixas et al. (2021) reported that crop losses due to drought and heatwaves have tripled over the last five decades in Europe.

Supit, van Diepen et al. (2010) estimated the effects of climate change, in terms of changes in temperature and radiation, on potential yields in Europe over the period 1976 – 2005, without accounting for adaptation of crop management and the increase of CO2 levels. For wheat and barley, the simulated yield potential decreased mainly in eastern Europe and northern/central Italy. The decreases were between 0.04 and 0.09 t ha^{-1} $year^{-1}$ for wheat and 0.03 and 0.05 t ha^{-1} $year^{-1}$ for barley. On the other hand, in Belgium the potential yield increased by 0.05 t ha^{-1} year-1 and 0.03 t ha^{-1} $year^{-1}$ for wheat and barley, respectively. Rapeseed potential yield decreased in most European countries (0.03 – 0.05 t ha^{-1} $year^{-1}$), with a non-linear increase in Denmark only. Potato potential yield also showed an average decrease (0.01 – 0.08 t ha^{-1} $year^{-1}$), with the exception of the north of the UK. The potential yield of pulses decreased in the coastal regions of Spain and Portugal, in central Europe and northern Italy, by 0.02 – 0.03 t ha^{-1} $year^{-1}$, while it increased mainly in Belgium and UK (+ 0.02 and + 0.03 t ha^{-1} $year^{-1}$, respectively). In general it is suggested that in the future the higher temperatures will continue to shorten the growing season in warmer countries, anticipating flowering and, therefore, leaving less radiation available for grain filling.

Aside from the effects on potential yield, the management (M) adaptation strategies adopted at farm level to maintain or increase actual yield must be considered. The shifting of sowing and harvesting dates to exploit warmer temperatures in northern Europe (Rijk, van Ittersum et al. 2013, Taube, Vogeler et al. 2020), especially in autumn, had a positive effect on some crops like maize. To date, wheat yields have been higher or increasing over time in northwestern and central-western Europe, potentially due to less intense heat and drought stresses at flowering compared to Southern Europe. Researchers and breeders focused their efforts on the selection of genetic traits that could increase yield by exploiting the favourable weather conditions, such as a longer grain filling period (Senapati and Semenov 2020). On the other hand, wheat has been more constrained by heat and drought stresses in Southwestern and Eastern Europe. Within the available varieties, there is currently little possibility to select for yield-enhancing traits. Heat and drought resistance or resilience will need to be addressed first, by selecting for stress resistance at flowering, optimal phenology and canopy structure and increased root water uptake and root elongation (Senapati and Semenov 2020). Aside from the effect on temperatures, climate change led to an increase in CO2 concentrations. This effect, per se, is beneficial to plant growth, constituting the so-called “CO2 fertilization”. However, the beneficial effect of CO2 fertilization is offset by the other changes in weather (Supit, van Diepen et al. 2010, Wilcox and Makowski 2014). Other than the average effect on temperatures and CO2, climate change also increased extreme weather events, leading to greater interannual yield variability and more unpredictable changes (Le Gouis, Oury et al. 2020). In addition, climatic anomalies were increasingly experienced at a larger scale, threatening food security through multiple stresses (Zampieri, Ceglar et al. 2017).

Recently, Zhao, Bindi et al. (2022) analyzed and summarized the impact of climate change and adaptation on crop production focusing on the entire Europe. Since the 80s, the increase in temperatures has prolonged the frost-free period all across Europe, delaying the end of the growing season of most crops by 8 days and anticipating the start of the growing season by 3 days (Jeong, Ho et al. 2011). The number of frost-free days increased relatively more in northern Europe, where farming could better adapt to climate change through the selection of new cultivars, the advancement of sowing dates in milder winters. In addition to that, the recent warming has permitted the expansion of crop areas to higher latitudes (Ceglar, Zampieri et al. 2019). In northern Europe (Peltonen-Sainio 2012) and Germany (Bonecke, Breitsameter et al. 2020), the warming trend has offered the opportunity for exploiting a greater growing season length and, therefore, to produce more thanks to a greater radiation intercepted. At the same time, though, cereals in these areas are increasingly experiencing excessive precipitation and elevated temperatures during grain filling, and dry spells in the last part of the growing season, that diminish the benefits of an extending growing season (Peltonen-Sainio 2012, Bonecke, Breitsameter et al. 2020).

6 Promising Traits to Target for Future-Proof Crops

6.1 Introduction to Promising Traits

This section describes the target traits – **the KEY TRAITS** – where research is most needed to generate breakthroughs aimed at growing more productive, more resilient, and more sustainable crops in future environments.

Traits control yield of plants and therefore limit productivity. Enhancing plant productivity has been the main goal of the entire farming value-chain so far, and yield-controlling traits have often been well investigated at the physiological level, with research often identifying and sub-traits (etc), which are themselves often further explored in detail. This is especially the case of the components of primary production. Photosynthesis, the process driving plant productivity, is a major tool to control and mitigate climate change and pollution, is highly conserved and relatively inefficient. Low efficiency may be the secret ingredient of photosynthesis stability, but marginal improvements of CO_2 and light harvesting by photosynthesis can drive large positive effects both on food security and the control of greenhouse gases. Extensive knowledge of photosynthesis has allowed it to be dissected into many sub-traits, improving any of which may lead to considerable enhancement of productivity, as well as to more efficient use of resources.

While photosynthesis is the cardinal process to be improved in future-proofed crops, higher yields will not be achieved if primary production is curbed by whole plant and whole crop limitations. A trait that will be highlighted in this research plan as a promising area for developing future-proofed crops is plant architecture at canopy and root system level. Canopies must integrate productivity in terms of primary production at leaf level. Recent research highlighted the importance of canopy structure in setting limitations of productivity, for example light interception in spatially and temporally dynamic (fluctuating) environments. Since plants are central for the maintenance and restoration of soil quality, fertility and overall "health", the multiple interactions between roots and soil has received rising interest and may drive important results in terms of productivity. If the two main components of photosynthesis (CO_2 and light) are 'air-borne', all other essential components for ensuring plant survival, growth, and production are soil-borne, from water to mineral nutrients. The root system of plants, and its many sub-traits will be therefore highlighted as a major area for future research.

While primary production mainly relies on photosynthetic carbon acquisition as its fundamental trait supporting quantitative biomass production, the harvested, most valuable plant products s are often fruits, or seeds or other storage organs (eg potatoes, sugar beet), though fibres, wood, secondary product, cut flowers etc are also economically valuable products of agriculture. There is a close relationship between photosynthesis and biomass production, the same is not generally the case when the yield is not (only) dependent on biomass accumulation, relying instead on additional transport and modification of photosynthates.

A second group of traits that need attention when future-proofing plants is sustainability of production. This includes traits that improve resource use efficiency and resilience to stresses. In many cases, these again involve components (sub-traits) that also set photosynthesis and plant architecture. For example, stomatal closure and canopy and root architecture are instrumental in controlling water uptake and use efficiency, especially in conditions of drought stress. Nutrient use efficiency will also rely on root characteristics, as well as on the biochemical plant capacity to use the nutrients. In other cases, sub-traits involve secondary metabolites that are induced as protective compounds after the onset of abiotic or biotic stress conditions, or even in response to priming to stress conditions. It is remarkable that synthesis of such useful classes of metabolites are dependent on stress induction.

Finally, the third group of traits that must be characterized in future-proofed plants are the quality of food and non-food productions. These traits may be very different (sometimes alternative) to those characterizing yield in terms of biomass production. The main trait here is the nutritional value of food and feed productions. The nutritional value of productions includes numerous sub-traits; of paramount importance will be the protein value of food, given the rising importance of proteins and their simpler elements (amino acids, peptides) in diets, and the compelling necessity to replace animal proteins when feeding a growing population. Equally valuable will be the amount and quality of secondary metabolites with beneficial dietary properties. These involves many classes of compounds (from carotenoids to polyphenols, to flavonoids) that generally share strong antioxidant properties for both plants and plant eaters, and are also sources of vitamins and micronutrients, preserving cellular integrity and contributing to fight inflammatory responses. Primary and secondary metabolites will also characterize the growing interest towards non-food productions, primarily contributing to bio-economy uses. Here, together with biomass traits that warrant sufficient quality and quantity of productions (e.g. yield of plant parts that do not have a primary agronomic use, such as straws in the wheat industry), it is also important to include traits such as the production of specific molecules of industrial importance (e.g. in pharmaceutics, cosmetics, bio-constructions) or the capacity to efficiently recycle agricultural and food wastes.

Independent of their importance for productivity, sustainability and resilience of future-proofed plant's traits need to be investigated both at genotypic and phenotypic level. For example, the discovery of QTLs or causal genes underpinning natural variation for the traits, or in support of genomic selection, or as targets for gene-editing will greatly advance our capacity to make use of the genetic variability, or to restore or boost genetic backgrounds associated to useful traits. Analysing the scope for variation for a trait not only within genotypes of agronomic importance, but also on wild relatives and forgotten cultivars that can be crossed with our crops, (and within the plant kingdom as a whole), will allow investigations into the limits of plant adaptability and functionality, while reconstituting agrobiodiversity. Investigating the impacts of changing environments and farming practices on genetically-defined crops (plant phenotypes being the results of such interactions) will be the other pillar of trait exploration and analysis. Interdisciplinary expertise is clearly needed to produce informed options for genotyping and phenotyping future-proofed crop traits. CropBooster has the ambition to gather such a wealth of competences, optimizing the possibility to explore the biological potential for control of traits, and to successfully future-proof crops, under whatever scenario of regulatory framework in the EU, and under the multiple climatic changes that may negatively (or positively) impact Europe in the future. To this purpose, a modelling exercise will also be implemented to deliver a suitable model describing the impact of future climate on traits and sub-traits.

A detailed analysis of each trait, and associated sub-traits, follows.

6.2 Environmental Sustainability Linked Traits

6.2.1 Key rooting traits in major crops for resilience and for yield potential

Root architecture and anatomy is critical to crop production especially in environments where key resources of water and nutrients are limiting. A better matched and a responsive root anatomy enables greater tolerance to drought, greater water and nutrient use efficiency and higher abiotic stress resilience. They also enable better tolerance to soil degradation. There is additional unrealised potential for the adaptation of agricultural root systems to help stabilise soil systems and sequester greater amounts of carbon below ground through a combination of breeding and soil management.

Variability in root traits of field-grown plants is controlled by a plethora of quantitative trait loci (QTLs) and their interactions with the environment and management practices (GxExM) that are starting to be revealed by quantitative genetics approaches. Several functional-structural models of RSA have been developed over the last two decades allowing one to simulate how various root traits and processes influence water and nutrient uptake (listed in https://www.quantitative-plant.org/, reviewed in Postma et al). Here we discuss root traits generally with little reference to species.

Understanding root responses to soil stresses is vital to develop novel crop varieties [Lynch, 2019]. For example, roots experiencing water deficit have been observed to increase their angle to reach deeper soil profiles [Uga et al, 2013]. Water deficit in upper soil profiles also suppresses branching and growth of crown roots [Sebastien et al, 2016; Gao & Lynch, 2016]. It is possible to generate new ideotypes of root architecture that are efficient in their use of resources and able to exploit the soil profile more efficiently. For example, the root surface area ideotype featuring ‘few but long laterals’ is proposed to ensure resources are redirected to extend the root system into deeper soil profiles (Lynch 2019). Developmental plasticity offers breeders opportunities to create crops with ‘customised’ root system architecture (RSA) better adapted to forage for heterogenous soil resources [Hodge, 2004].

Broadly speaking there are two classes of soil resources, mobile (deep) and immobile (shallow). For example, nitrate (NO3–) is highly mobile and leaches into deeper soil layers. To aid N capture breeders could exploit steeper root angle in brace and crown roots [Trachsel et al, 2013], elongation of lateral and seminal roots [Gioia et al, 2015], reduced root length density near the soil surface and reduced numbers of axial roots [Zhan & Lynch, 2016]. Such plastic traits serve to increase exploration of deeper soil layers where N is more abundant. In contrast, phosphate (P) is immobile and concentrated in topsoil. To improve P capture, breeders could select crops with increased numbers and lengths of roots in patches of high P Flavel et al, 2014], shallower root angle [Rubio et al, 2003], increased numbers and lengths of root hairs [Bates & Lynch, 2001]. The ‘steep deep and cheap’ ideotype is often cited for improved water and N capture is suited for efficient exploration of soils at depth. This consists of a range of traits that confer steep root angles (e.g. the gene DRO1), a reduced metabolic cost (respiration) of root production by limiting axial roots and inducing aerenchyma formation and promoting the ability of roots to penetrate denser sub soil layers (Pandey et al 2020). The lack of oxygen at soil depth is another challenge that may be helped by the generation of aerenchyma.

There will commonly be a need to balance deep and shallow exploitation and intermediate root types may be better suited where both top and sub soil foraging is possible at minimal cost to the plant. In this context, the availability of greater photosynthate for 'building' root systems will theoretically enable greater resource uptake. Deciding on appropriate root traits for increased yield is not easy and depends on the soil type and the particular limitation to yield whether it is water, nutrients or both. Degraded and compacted soils present specific problems. However the range of traits, DTLS and genes now available suggests that we have the means to breed for specific root architectures as long as we understand the soils and the systems in question.

The root systems that are needed in high input and low input systems may be very different. The traits covered in this section largely consider those that are useful where resources are limiting or at risk of leaching and runoff which has environmental implications. As covered in the 'yield resilience' section the root mass allocated in yield potential systems can be surprisingly small. This may be due to the lack of limitation of resources and favourable soil conditions. The low root mass may contribute to the high yield potentials in question and to trade-offs between root mass and yield potential are an important question, yet to be met. The advantages of a large and deep root system even in yield potential systems are greater carbon sequestration and potentially greater resource use efficiency.

6.2.2 Roots, soils and their role in sustainability of agricultural systems

Future crop phenotypes will need to adjust to future soils which will most likely be harder, drier and poorer in quality in response to both the changing climatic and prevalent agronomic practice. The principles of Conservation/Regenerative Agriculture, now gathering pace in their adoption globally, are i) reduce soil disturbance, ii) utilise benefits of roots (cover crops or cash crops in rotation) and iii) return amendments/organic material to the soil. Future root phenotypes and the breeding efforts associated with these need to be directed at maximising growth and yields under these changing environments. Soil degradation is wide-spread globally including across European soils and the trend is going the wrong way. A major issue in this regard is that significant damage can be done to soils in short time periods (e.g. hours/days) by compaction, pollution or erosion for example, however the remediation and recovery options are usually long term (e.g. weeks/months/years) and in some cases soils are never returned to their former state. Practices that conserve and improve soil health are urgently needed. Many farmers are familiar with such practices, and employ various combinations, but impacts on yield/economics are major barriers to larger scale uptake. For example, many farmers cite a reduction in yield as a reason for not adopting zero tillage, anecdotal evidence suggests this is likely during the first few years post-conversion but unlikely in the longer term as the soil fauna is given time, undisturbed, to develop a new structure that can function in a similar way to ploughed soils, and that financial losses could be offset by reduced fuel and machinery costs, but evidence and government support for this is lacking. Either way, if the current systems of over-mechanisation continue or a reduction in cultivation is adopted, the impact of future root systems will be to grow in dense soil conditions.

Root traits have not been fully exploited when considering adaptation to different agricultural soils to improve yield. Figure 6.1 provides a demonstration of root ideotypes to have emerged from recent research. Conventional thinking considers that roots are unable to penetrate compacted soils due to the mechanical force required. However, Pandey et al (2021) recently discovered plant roots employ the gaseous hormone ethylene to sense soil compaction. Compacted soil restricts diffusion of ethylene out of roots, triggering growth inhibition and radial swelling. Remarkably, roots of mutants insensitive to ethylene are able to penetrate compacted soil. Breeders could build on this recent discovery by selecting crops with reduced sensitivity to the plant hormone ethylene.

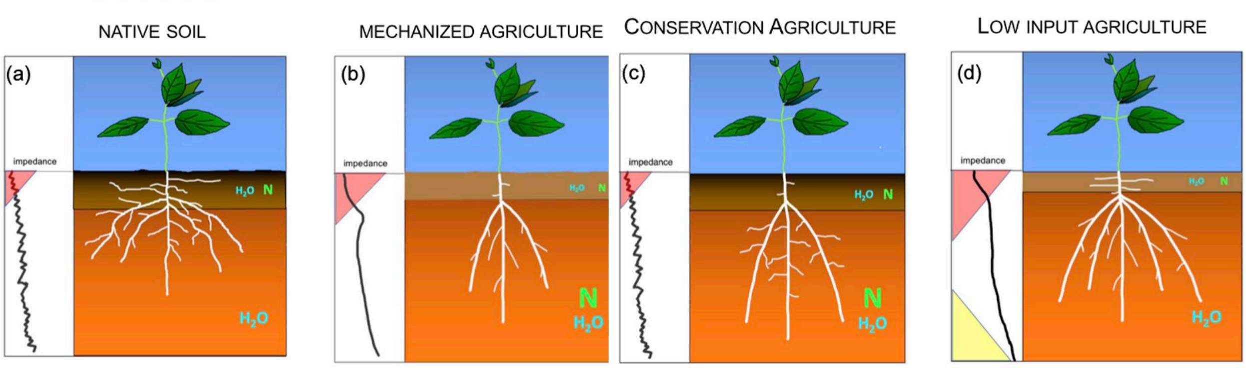


Figure 6.1. From Lynch et al (2021). A Conceptual scheme of four soil scenarios, their impedance profiles (shown in the left portion of each panel as increasing impedance from left to right) and hypothetical root phenotypes adapted to them, as described in the text. (a) Native soil, (b) Soils under conventional tillage, (c) Conservation Agriculture, (d) Soils under low-input agriculture.

It is generally regarding that deeper rooting is a key priority for future crops due to the potential benefits associated with nutrient and water availability. Under contemporary agricultural systems, the impacts of machinery on soil compaction are predominantly manifest in the upper 30-50 cm of soil, however the soil below 50 cm depth is also usually at a high bulk density (typically >1.5 g cm^{-3}) due to the overburden pressure of the soil above. Under zero tillage, bulk density can easily exceed 1.5 g cm^{-3} especially in the first 3 years since conversion. Thus future tolerance to high bulk density soils is a key root trait that needs to be bred for. Atkinson *et al* showed there is a dynamic range (that is likely to be soil texture specific) in which the root angle of wheat is regulated by soil bulk density, at low densities (e.g. 1.2 g cm^{-3}) root angles do not change when roots interact with or cross soil macropores/biopores however above 1.5 g cm^{-3} wheat root change their angle of growth and seek to colonise the pore space as a means to facilitate easier growth. This demonstrates the importance of soil micropores, and in particular biopores as these can be strongly influenced by management e.g. through selection of cash/cover crops or via increased earthworm populations associated with reduced/zero till. Recently Hu et al. (2021)) extended this work to explore the proliferation of roots into dense subsoils with a specific focus on assessing the extent of biopore colonisation. They found, for wheat plants, that root growth below 50 cm was almost exclusively limited to biopores (e.g. legacy root and earthworm channels) but conversely no significant genotypic variation. This demonstrates the crucial importance of including environmental conditions in future breeding efforts. However, when it comes to soil this presents a considerable challenge due to its significant spatial and temporal heterogeneity. For example, in the UK alone there are over 700 different soil types that all vary considerably in texture (e.g. particle size), structure (e.g aggregation), organic matter content, pH, stoniness etc. From a mechanical perspective, focusing on sand and clay based soils accounts for the majority of soil types (e.g. in the UK sandy and clay loam soils account for around 70% of all soil types). In the future, it seems like our soils will be harder either through reduced cultivation or reduced moisture content or both. Efforts to reduce and minimise compaction are likely to gather pace and an increase in the development of a faunally driven structure (also known as bio-tillage) are likely. Other benefits of this are greater diversity in the emerging soil microbiome which then confer benefits for crop development such as those through mycorrhizal association (e.g. Neal et al Scientific Reports 2020). Practices such as inter and cover cropping are viable options for increasing root channels at depth in soil that will aid cash crops so root systems that are better able to explore and exploit deeper located soil resources are preferable. Breeding for plant traits that can successfully adapt to these environmental conditions is a major challenge but one with high potential for impact.

Carbon sequestration

Present agricultural systems are generally not carbon – rich compared to natural systems. Nonetheless crop plants occupy a unique position in terms of the potential of mitigation of climate change because of their ability to fix and sequester CO_2. The potential for carbon fixation in agricultural systems is higher than currently achieved (as recommended in WP4 focus groups). This will require a re-imagining of the purpose and the role of crops as more than just food and energy providers but as also generating a substantial ecosystem service at the below ground level. Sequestering more carbon in the soil has several clear benefits assuming that trade-offs can be avoided. In low input systems this increases possibilities for resource capture, especially at greater rooting depth. Increase in soil organic carbon creates more resilient soils with higher resistance to erosion and compaction. Additional benefits of greater root mass (at depth) include water conservation, outlined in the focus group of WP4, and reduced run off of fertilisers, notably N. It will therefore mean reduced use of fossil fuels for irrigation and fertiliser production. Most crops have roots that do not run further than a depth of one meter but extending this to two metres substantially increases potential storage. A substantial amount of carbon moved to the Rhizosphere. Root mass decays slowly in soil and even more so at depth where much less is released from microbial respiration. and the amount of carbon sequestration that can be achieved by depositing at depth is substantial. Estimates suggest 50 t ha^{-1} that could be sequestered in croplands (Kell 2012).

The amounts of carbon that can be sequestered depend greatly on the crop species, the soil type and management system. Clay soils are found to be most suitable for root carbon sequestration . Compact soils present a problem unless traits for rooting in such soils can be introduced. Perennial grasslands are highly suitable and can generate very large root biomass. Cereals, especially wheat, and legumes have relatively high root masses for crops (Matthew et al 2017). It is most suitable for low disturbance agriculture such as low till or no till.

The traits discussed above include those that would be appropriate for directing a greater proportion of carbon to root biomass at depth. It should not be done at the expense of yield potential. As pointed out elsewhere, yield potential often involved de-investing in roots in order to divert carbon to the shoot.

However it is also true that non-structural carbohydrates can remain in wheat plants after harvest, implying a sub optimal partitioning to grain or a surplus. If photosynthesis can be boosted and partitioning optimised such that harvest index is not compromised further, then increased root biomass is a realistic goal. It would need to be seen as a concerted strategy to extend the function of arable crops. With these caveats we advise that increased root mass be a feature of the future crops of Europe to aid resource use efficiency, fight climate change and preserve soils.

We can highlight several target traits such as optimised metabolic efficiency through both anatomy and architecture; improved penetration of compact soils; Enhanced root mass at depth with no cost to yield potential; Improved responses to Flooding / excess water:

There are substantial challenges that face root and soil research but these should be fruitful if successfully overcome. The study of RSA and root anatomy under excess soil moisture (waterlogging) is one of the most understudied and impactful climate stress: excess early season moisture is often statistically linked to poor yields and can interact strongly with late season drought by limiting soil exploration early in season. We need to evaluate the effects of new RSA and root anatomy ideotypes on yield, quality and GHG emission of crops cultivated under conventional and organic farming as well as for various uses (e.g. food, feed, ecosystem services). We still require Cloning of root mutants and major QTLs governing RSA and root anatomy plasticity in response to flooding/excess water on early season root establishment and growth. Responses of roots and soil to elevated atmospheric CO2 is not sufficiently understood. Genetic improvement to various Soil stresses (drought, Nitrogen, Pi, temperature, salinity, aluminium/pH, compaction) remains a target. Similarly, the effects of various management practices on RSA and root anatomy are not well understood.

Recommendations for Future below ground Related Research Activities

Research Priorities

High-throughput phenotyping of root traits in

- Controlled conditions (e.g., aeroponics, semi-hydroponics, hydroponics, soil mesocosms, “novel” artificial substrates)
- Experimental fields with rain shelters and/or Free-Air Carbon dioxide Enrichment (FACE) facility
- Field conditions (e.g., canopy temperature, robot for brace root, soil cores, anatomy using LAT)
- Large rhizotrons (e.g., ‘Deep Frontiers’ project in Denmark) Imaging
- Non-invasive imaging in 3/4D soil conditions (e.g., ‘Hounsfield CT Facility’ in Nottingham)
- Automated image analysis based on artificial intelligence (AI)
- Development of user-friendly image analysis software

6.3 Resource Use Efficiency: Nitrogen, Phosphorus and Water

6.3.1 Nitrogen Use Efficiency

The relative rate of global yield increase in cereal yields is declining and is currently about 1.1% yr^{-1} (Hall and Richards, 2013). Although there is scope for raising harvest index towards the theoretical maximum of ca. 0.64 (Foulkes et al., 2011), future increases in grain yield will increasingly depend on raising above-ground biomass production. Increasing biomass of the crop implies an additional requirement for N capture to support photosynthesis. Increased fertilizer inputs, however, will have economic and environmental impacts. Nitrogen (N) fertilizer represents a major cost for the grower and has environmental impacts through N leaching causing eutrophication of freshwater and marine ecosystems and N_2O (a greenhouse gas) emission associated with denitrification by soil bacteria.

N is an essential component found in many secondary and signalling compounds such as proteins, hormones and vitamins. It is also a basic element of genetic material DNA and RNA. In cereal plants, tillering and stem elongation are the critical growth stages because N limitation at these stages affects ear growth, grains per unit area and final yield. For higher yield, all crops need an optimum green canopy for photosynthesis that depends on healthy chlorophyll in plant cells. The key photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) typically constitutes up to 30% of the total N in leaves (Lawlor, 2002), and, under field conditions, leaf photosynthetic rate is highly correlated with RuBisCo content. Therefore, N plays a critical role in final biomass and grain yield production in crop plants. Crop NUE results from the combination between how effectively plants capture the N (N-uptake efficiency, NUpE) and how the plants use the N (N-utilization efficiency, NUtE). NUpE is calculated by dividing the amount of above-ground N content at harvest by available N in the soil, and NUtE is calculated as the dry matter in grain divided by N in above-ground plant biomass at harvest (Moll et al. 2002).In addition to allowing an increase in biomass production without any additional or with a lowered fertilizer input, optimized NUE by plants is also required in the context of climate change for both securing the nutritional quality of crops and favouring carbon storage in soils. Indeed, the elevation of atmospheric CO_2 concentration leads to reduced concentrations of nutrients in most organs of C3 plants (Loladze 2014). This is especially true for the protein content of cereal grains (Högy and Fangmeier 2008), and may have dramatic consequences in the future for food quality and human health (Smith and Myers 2018). For yet unknown physiological reasons, this may result from a negative impact of elevated CO_2 on NUpE (Bloom et al 2010). Furthermore, higher C/N ratio of the plant biomass in response to elevated CO_2 will lead to a strong alteration of the biogeochemical processes determining the stability of the soil organic matter, which may prevent C storage in the soil (Bertrand et al. 2019). However, enhancement of NUE has limits. Indeed, unbalanced N distribution into agro-systems at the expense of soil microflora due to excessive N harvest index of crops can be detrimental, by hampering N incorporation into stable soil organic matter that is required for long term increase of terrestrial C sink (Terrer et al. 2021). Thus, improving NUE is necessary for increasing crop yields with an environmental-friendly use of fertilizers, but this must be reasoned in a broader sustainability context, by taking also into account the nutritional quality of crops, their interactions with soil microorganisms (Dellagi et al. 2020; Xie et al. 2022; Wang et al. 2020) and their potential role in mitigating greenhouse gas emissions.

Traits for NUE Improvement

While some improvement in agronomic NUE can be achieved through slow-release fertilizers (Li et al., 2018), bio-fertilizers (Quevedo-Amaya et al., 2020) and crop management practices (Santiago-Arenas et al., 2020), the improvement of intrinsic plant NUE has to be tackled through genetic improvement (Foulkes et al., 2009, Hawkesford, 2014). However, to date, NUE has not been the target of dedicated breeding improvement, but has been improved through indirect selection for yield, in environments targeted by breeding programs. This indirect selection for yield serves as a benchmark for any alternative approach (Sadras and Richards, 2014).

Genetic variation has reported promising traits to increase NUE in cereals including deeper roots for increased N uptake (Foulkes et al., 2011; Cormier et al., 2016), nitrate assimilation (Cormier et al., 2016), leaf photosynthetic rate (Gaju et al., 2016; Carmo-Silva et al., 2017), leaf chlorophyll content (Sharma et al., 2021; Thind and Gupta, 2010; Mathukia et al., 2014; Jia et al., 2020) stay-green traits related with improved post-anthesis N remobilization (Gaju et al., 2011; Derkx et al., 2012; Hawkesford, 2014; Lee and Masclaux-Daubresse 2020) and/or increased post-anthesis N uptake (Bogard et al., 2011; Gaju et al., 2014). Although the analysis of all these traits is smooth in laboratory, their study in the field is far more complex and requires to adapt methodologies to monitor N flux, root architecture and N metabolite analyses.

In addition, traditionally, crop breeding is carried out under non-limiting N supply, and plant breeders select cultivars that perform well under optimum N supply conditions. Given that genotype and N supply do interact, to obtain reliable information about which genotypes will yield well under low N supply may require both breeding and testing at low levels of N input (Brancourt-Hulmel et al., 2005), along with improved understanding of the physiological traits that determine higher NUE. Facing new environmental constraints due to climate change, resilience of the good yield and improved NUE genotypes to drought, heat, flooding and elevated CO_2, also requires breeding and understanding. In addition, efforts must also be dedicated to other crops than cereals.

As NUE is a complex, polygenic trait, molecular breeding for N-efficient varieties is considered the most effective method to raise NUE in wheat (Cormier et al., 2016). However, this strategy depends on the availability of reliable and accurate molecular markers linked to N-efficient genes for marker-assisted and genomic selection (Han et al., 2015). The identification of N-efficient gene markers to be considered relies on the physiological studies performed on N and C metabolisms, leaf senescence processes (Chardon et al. 2012; Li et al. 2017; Lee and Masclaux-Daubresse 2020; The et al. 2021) and on metaQTL analyses (Sandhu et al. 2021; Coque 2008; Uauy 2006). Improving root system size and architecture is also a promising track. Larger, deeper and more branched root systems may have three key advantages in connection with the NUE, yield and sustainability issues. First, using root system architecture as a breeding target is a yet under-investigated strategy for improving NUE or adaptation to abiotic stress, and many relevant root-related traits and associated genes have been highlighted in this context (Kell 2011, Lynch 2015, Ogura et al. 2019, Lombardi et al. 2021, Jia et al. 2022). Second, enhanced C demand for root growth may prevent sink limitation of photosynthesis, therefore stimulating whole plant biomass production (Dingkuhn et al. 2020). Third, optimizing root system growth, development and composition is a pre-requisite for increased transfer and sequestration of the atmospheric C in the soil (Kell 2012, Poirier et al. 2018).

Nevertheless, molecular breeding for N-efficient varieties is still a formidable task, since NUE genes are highly influenced by environmental conditions such as rainfall pattern and soil N availability (Cormier et al., 2013; Lammerts Van Bueren and Struik, 2017), and the genericity/transfer of gene markers belonging between dicots and monocots, C3 and C4 plants, legume and non-legume plants still require deeper knowledge. A large volume of high-quality phenotypic data is needed to dissect NUE traits into measurable components for the development of accurate molecular markers (Araus and Cairns, 2014; Nadeem et al., 2018). Thus, molecular breeding for N-efficient varieties requires the deployment of effective phenotyping methods (Araus et al., 2018) , currently a bottleneck limiting the genetic improvement of NUE traits (Nguyen and Kant, 2018). In addition, effective screening methods for identifying N-efficient germplasm are required that perform consistently in the greenhouse and field conditions to accelerate breeding outcomes (Nguyen and Kant, 2018). Efforts on gene markers have to be done to facilitate transfer of knowledge between crops.

Finding, testing and using candidate genes for NUE

There is variation in key NUE traits amongst modern genotypes in cereals as described above, however a much greater potential for variation may exist in a wider germplasm base (Hawkesford and Griffiths, 2019). In landraces and relatives of cereals, whilst biomass may be high, yields and harvest index are often low so the traditional measures of NUE are less useful. Traits such as total N uptake, N-remobilization and biomass potential, however, are still valuable underpinning traits as part of a crop NUE ideotype. It is beyond the scope of this research plan to review the many traditional QTL and association mapping studies for improving NUE reported in cereals in recent years. Fortunately, comprehensive reviews have recently been published (Sandhu et al, 2021; Teng et al., 2022; Fiaz et al., 2022). In addition, there have been several attempts to find genes involved in N-response through transcriptomic network analyses and functional genomics (Pathak et al., 2020; Du et al. 2020; Araus et al. 2016; Bi et al. 2007).

Key genes for NUE revealed by molecular physiology and functional genomics on model plants and crops, such as maize and rice, are numerous. Physiology of N transport highlighted candidate genes for N-uptake and source to sink N-transfer (Tegeder and Masclaux-Daubresse, 2018; Fan et al. 2017; The et al. 2021). Studies on senescence-related N-recycling mechanisms revealed candidates for N-remobilization (Havé et al. 2017). Studies dedicated to NAC transcription factors in wheat, barley, rice and many other plant species offer candidate genes for N-remobilization and tolerance to abiotic stresses (Singh 2021; Lee and Masclaux-Daubresse, 2021; He et al. 2015; Uauy et al. 2006; Xu et al. 2022). Regulators integrating N-response, such as the NLP or NRT1.1 proteins, are also of interest as they can modulate N metabolism and plant growth at different levels, and show polymorphism correlated with natural genetic variation of NUE (Guan et al. 2017; Bouguyon et al. 2015, Hu et al. 2015).

From those studies, single gene and gene stacking transgenic strategies have been successfully used to test improvement of N-uptake, assimilation or remobilization in wheat and rice (Mandal et al., 2018; Raghuram and Sharma, 2019; Sinha et al., 2020; Chen et al., 2020; Lee et al. 2020; Uauy et al. 2006).

NUE research efforts have been mainly focused on cereals and model plants, and knowledge on the molecular mechanisms and the genetic variation in other plant species, as vegetables and forage plants, deserve attention. Legumes have been mainly studied for their symbiotic capacities, but bottlenecks in other traits related to N recycling and remobilization when root senesce and N fixation decrease, is poorly documented. Studies on forage plants have focused on growth and N contents, but the physiological mechanisms and their regulation under fluctuating environment and depending on N-regimes need further investigation.

For all the plant crops cited above, only limited studies have been performed, in lab and even less in the field, to explore in deep the phenotypes related to the candidate genes associated with NUE (Karunarathne et al. 2020; He et al., 2020). Nevertheless, considering the data currently available, combining GWAS and GS with marker-assisted selection (MAS) may be one approach to accelerate the breeding efficiency to develop the lines with better performance for GY and NUE under diverse N management and environmental conditions (Uauy et al. 2006; Kaul et al. 2019). Using and improving new technologies, such as genome editing, would speed the transfer of favourable alleles into crops and the possibility to characterize deeper phenotypes associated to candidate genes.

In future work, it will be necessary to develop new germplasm incorporating wider genetic resources and diversity for improvement of some traits, e.g. rooting traits, N-uptake, N-remobilization, and N allocation to the seeds (cereals, legumes) or to any other organ of interest (tubers, fruits, taproot). It will be necessary to test in the field the candidate genes identified from physiology and gene networks, and to continue efforts in functional genomics and forward genetics to detect new candidate genes adapted to climate change predictions. It will be necessary to develop methods to facilitate candidate genes transfer from model plants to crop and from crop to crop. There will be a requirement for the further development of high-throughput phenotyping screens, allowing better characterization of NUE traits, e.g. N fluxes and allocation within the plants and under various environments, to underpin breeding of high-NUE genotypes well adapted or resilient to the climate change scenarios. Specific traits for cereals, legumes, vegetable NUE need to be taken into account to define ideotypes and phenotyping strategies. The available genomics technologies (marker technology, arrays, mutagenesis systems etc) are largely in place and there is a requirement now for their exploitation in different crops, vegetable, legume and forage species to develop markers to assist breeding to improve NUE in both the short-term (5-10 years) and medium-term timeframes.

Recommendations for Future Nitrogen Related Research Activities

Research Priorities

- **Definition of useful mapping populations/panels (information on traits for mapping populations/panels through phenotyping)**
- **CE 'smart-screens' for phenotyping genetic diversity under various environmental conditions (development of precise phenotyping methodologies well correlated to field expression of traits and suitable to test climate effects; hydroponic systems for PUE phenotyping; experimental designs to monitor N-fluxes (using markers and isotopes) within the plant and quantify bottlenecks in uptake, storage and remobilization; facilities to compare different climates)**
- **Crop physiology: modelling (development of modelling frameworks to design virtual crop ideotypes with improved NUE and PUE)**
- **Confirmation of lab results on candidate genes in the field (consider NBT in the field for research purpose)**
- **Crop physiology and development trade-offs (trade-off between N-uptake and N-recycling in different crop species, identification of master genes (for leaf senescence and N regulations, N assimilation, N transport, N recycling))**
- **Develop molecular tools to enable smooth transfer of NUE master genes between crop families**
- **Crop physiology and resilience to low N and climate change (NUE genes playing a role in plant tolerance to N-limitation, drought, eCO2, high temperature …; Gene association, GWAS, molecular physiology under contrasted environments)**
- **Checkpoint levels controlling N-metabolism and N-allocation (transcription factors, Post-translational-modifications, epigenetics).**
- **Multiscale analyses in a dynamic way, taking into account plant growth and development (N-fluxes, N-allocation and multiomics in different organs along plant life cycle, identification of transitions and checkpoints in inorganic/organic N management)**
- **Comparison of NUE strategies (legume/non-legumes/C3-C4/cereals-dicots in response to climate change and N regimes; trade-off between N-uptake and N-recycling for NUE)**
- **Gene discovery: genomics, informatics and reverse genetics (mapping and gene expression related to N-uptake, N-remobilization, leaf senescence, root architecture to highlight important genes. Multi-disciplinary approaches, namely transcriptomics, proteomics, metabolomics, fluxomics with appropriate bioinformatics support.)**
- **Development of formulation of soil- or foliar-based fertilizers that improve the efficiency of uptake and strategies for their use.**
- **Development of plant-microbe associations for NUE and PUE (identification of genotypes, germplasm, molecular physiology and GWAS)**
- **Development of Crop-Legume associations (beneficial genotype associations, agroecology)**
- **Root architecture for NUE and C sequestration (GWAS, molecular physiology, gene discovery…)**

- **NUE and GPC in cereals (common regulators and main genes)**

6.3.2 Phosphorus Use Efficiency

Phosphorus - Improving phosphorus uptake and use efficiency

Phosphorus (P) is a major macronutrient limiting plant growth and yield that is considered a limited strategical resource (Jiao et al., 2012). There is disagreement on when a global shortage of extractable P might occur, but it is expected to at least take place within next 100 to 150 years (Gilbert, 2009). It requires special mention in terms of future research plans for crop improvement due to its physical and biological availability and problems regarding its measurement, overapplication leading to environmental damage and finally in agricultural phosphorus deficiency.

Symptoms of P deficiency are not easy to detect at an early stage and breeders lack easy tools for rationalizing quantification. Plants acquire P exclusively in the form of inorganic phosphate (Pi) from the soil, either directly through the roots or indirectly through the mycorrhizal fungi associated with them. Optimal acquisition of Pi involves specific adaptations of root architecture (e.g., top soil foraging, modification of root hairs, cluster roots), coordinated Pi uptake and distribution in various tissues through complex regulatory mechanisms implicating multigenic families of transporters, and secretion of phosphatase and organic acids to recover organic Pi or solubilize mineral Pi.

Despite its relative abundance, P remains very unevenly distributed in soils across the world. It is estimated that one third of total cultivated soils are lacking available P for optimal plant growth (MacDonald et al., 2011; Alewell et al., 2020). The reasons for this are multiple. Firstly, Pi has very poor mobility leading to the majority of the Pi applied from fertilizers being recovered by microorganisms at the expense of the crops. Secondly, Pi forms insoluble complexes with many soil cations or chelates with clays, resulting in reduced bioavailability for roots which is strongly regulated by pH. In acidic and deeply altered soils most Pi is found bound to clay minerals and oxy(hydr)oxides of Fe and Al. In such conditions, secretion of organic acids by the plant and/or microorganisms is an important mechanism to enhance Pi availability.

The complexity of Pi interactions with other ions means that measuring Pi bioavailability in soil is problematic and in turn makes precision agriculture difficult. It is assumed that no more than 20% of the Pi fertilizers applied are recovered by plants, leading to over application and severe environmental damage (algal blooms, metal pollution) in rivers and lakes. Moreover the vast majority of the mined P-rich rock contains high levels of toxic metals such as cadmium, chromium, mercury, leads, polonium, uranium or thorium, and some of these metals are found in applied Pi fertilizers. This already impacts the trophic chain and led the EC to reinforce the legislation on the amount of cadmium present in Pi fertilizers (https://ec.europa.eu/growth/content/cadmium-fertilisers_is).

The estimation of P availability in agricultural soils and hence the need for P application is also complicated because of the role of so-called legacy soil P. This legacy soil P has been built up due to historical application of P and low recoveries of this applied P. Most of this surplus P will stay in soil P pools but crops in different parts of the world will benefit from this legacy soil P, hence making it feasible that zero or even slightly negative P balances in many places may be sufficient to achieve high crop yields. However, **further studies are needed to better assess present P use and future requirements of specific crops and locations**. The situation on grassland is known to be very different from that for arable crops. Research has indicated that global P inputs in grasslands should increase drastically from 2005 to 2050 to accommodate the projected improvement of grass production for livestock feeding, while maintaining soil P status. Here, it should be considered that Europe contributes most to the cumulative global mineral P fertilizer applications in grasslands (about 80 % of the total); most grasslands across the world are not fertilised.

The Role of the Soil Microbiome

Improvement in efficiency of phosphorus use and limitation of environmental impact must account for the complex interactions between plants and soil mediated by the microbiome. In Europe the soil organic P (Po) constitutes 25% to 50% of total extractable P in arable and pasture soils, respectively. Po therefore warrants attention as a quantitatively important soil P resource. To be used by plants (and microorganisms), the Pi group must be released by phosphatases secreted into the external medium. The release of these enzymes is up-regulated at very low levels of Pi in solution and microorganisms that produce these enzymes are also the primary users of the Pi released, thus decreasing the bioavailability of Pi for plants (Pistocchi et al., 2018).

Microorganisms also release organic acids that contribute to the solubilization of mineral Pi. Fertilization of tropical ferralsol with rock P was found to be as efficient as the more soluble Pi-based fertilizer (triple superphosphate) to enhance the yield of soybean (Ndungu-Magiroi et al., 2015). Sparingly soluble Pi from rock P stimulated the populations of native Phosphate Solubilizing Bacteria (PSB) presenting a way of minimizing the utilization of mineral P fertilizers. In addition to microbial communities living in the rhizosphere, about 80% of plant species establish a symbiosis with mycorrhizal fungi that promote benefits for plant Pi nutrition. Mycorrhizal fungi considerably increase the volume of soil that can be exploited by the root, resulting in a better Pi acquisition (Briat et al., 2015; Wipf et al., 2019; Briat et al., 2020). Hyphae can also recruit bacterial communities able to solubilize mineral P and/or mineralize organic P (Briat et al., 2020). Given the importance of mycorrhizal symbiosis, several products are now commercially available. Recent arbuscular mycorrhizal fungi (AMF) inoculant benchmarks (Basiru et al., 2020) found that most of the products (84%) provide plant nutrient benefits either using soil application or seed coating. This last technique has great potential for increasing inoculation efficiency in large- scale production. Other commercial products are also based on addition of micro-organisms, such as non-mycorrhizal fungi and/or bacteria expected to help Pi solubilization. However it is currently difficult to control bacterial populations out of the laboratories due to competitions with soil microflora and putative ecological consequences (Hinsinger et al., 2011). Field experiments conducted so far with this type of product showed relative little benefits unlike controlled environments where microbiomes can be manipulated at will. Application in this case may be realistic at the medium term (5-10 years)

Genetic improvement of plant P responses

Our understanding of plants' adaptation to Pi deficiency is now extensive including the discovery of partially independent Pi signaling pathways which have strong interaction with other nutrients. One of these pathways, regulated by local extracellular Pi, controls root tip growth and metal homeostasis. The

Fe:Pi ratio determines the level of reactive oxygen species (ROS), increased levels of which leads to callose deposition, impairing symplastic movement necessary for meristem maintenance and primary root growth. Another pathway is systemically regulated by the overall plant Pi status and controls the remaining Pi starvation responses, primarily dependent on intracellular Pi sensing. In total more than 20 Pi signalling components have been identified, including sensing via Pi-containing metabolites (Pi-rich inositol pyrophosphates) (Zhu et al., 2019). They promote the association of repressors (SPX proteins) with the master transcription factors (PHR1) regulating the Pi starvation responses (Wang et al., 2014;Puga et al., 2017).

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

Future challenges in phosphorus nutrition to be addressed with high priority

Elucidating Pi signaling and transport

Despite crucial discoveries, our understanding of Pi signalling in plants remains fragmentary compared to other signalling pathways. The exact nature of the signal pathways and the complexity of interconnection with other pathways (such as carbon, nitrogen, metals) need clearly to be resolved. These studies remain challenging as many Pi signalling genes are arranged in multi gene families whose activity can be regulated in very distinct ways. In addition, the dynamic Pi transport from soil to roots and between the various tissues involves a multitude of Pi importers and exporters. It is important to rationalize our search for gene candidates for translational biology targets. To develop precision agriculture and spare Pi as a crucial strategic resource, we clearly need the physiological knowledge of Pi transport and adaptation to Pi deficiency.

The role of microbiomes in plant Pi acquisition

The potential of microbial inoculants is not yet firmly established in the field with soil conditions likely to be important in determining effectiveness. Knowledge on how inoculated microbes are able to develop in the rhizosphere or the roots in the field as a function of (i) the crop species and (ii) the soil conditions, especially Pi availability and pH is needed. Regarding the crop species, it is likely that crop type (cereal, brassica, legume) and root characteristics will be important with greater effectiveness in legumes and tuberculated species. The use of PSB microbial inoculants alone should be studied. Geochemical characteristics of soil should be taken into account when applying bio-inoculants based on phosphate-solubilizing organisms especially considering inhibitory effects of acid soils. Hence, we are still lacking key knowledge.

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

There is a need to distinguish Pi uptake efficiency, e.g. traits that lead to greater acquisition of Pi from the environment, from phosphate use efficiency (PUE) that encompass traits that not only improve Pi acquisition, but more importantly improve its overall utilization to increase production of the harvestable products (typically seeds) under fixed amount of bioavailable Pi. Important aspects of PUE include: (1) Pi recovery in soils; (2) improved soil exploration by roots, (3) Pi uptake, (4) physiological use of Pi for growth and (5) yield production. Analysis of PUE traits has been performed in many species to compare variety such as for Coffea (Neto et al., 2016) or to map QTL in

However, the identification of the causal genes responsible for these traits has not progressed. So far the only exception being the gene PSTOL1, encoding a protein kinase, involved a root architecture in rice (Chin et al., 2011; Gamuyao et al., 2012). PSTOL1 expression has been shown to enhance top soil foraging in other species, such as sorghum, demonstrating interest of translational biology for these approaches (Hufnagel et al., 2014). It is clear that we should increase the quest of targets for PUE traits, identify the underlining causal genes and use potentially them as markers for selecting P efficient crops. Performing analysis of PUE in plant species with different PUE characteristics may help to identify bottlenecks and relevant genes in different genetic backgrounds. Interactions with other nutrients needs to be included in such studies.

In field studies long term bio availability in soils is needed and it would be very fruitful to systematically combine systematically PUE traits quest with standard measurements of P (such as the Olsen test) in soils. Species within Leguminosae*, Proteaceae, Casuarinaceae, Myricaceae, Eleagnaceae*, and *Betulaceae* often develop adaptative traits such as cluster roots (specific root adaptation improving Pi recovery in the environment). However few plants in those families have economical interest (ex *Lupinus*) for farmers but they may provide an opportunity to limit the use of fertilizers within the framework of a rational agricultural policy or provide interest for culture in Pi depleted soil.

Development of improved fertilizers to improve the efficiency of P nutrition

Some practical solutions relying on improved P fertilization of soils should be considered such as coating of fertilizers (to increase progressive release period in the soil), improving Pi bioavailability (by identifying process to recover mineral or organic Pi) and precision agriculture (reduce Pi by providing micro dosage of fertilizer.

Improved recycling of organic P could decrease the need for mineral P fertilization and reinforce the bio-economy. Application of organic sources have an uncertain effect. As stated above, phosphatases will be mainly released by microbial populations in case of P deficiency and the released Pi will be mostly taken up by these microbial populations. Therefore, improving the competitive advantage of the plant roots towards newly released Pi from organic sources is a possible approach.

Interactions with other elements needs to be considered in any solution. For example, nitrogen fertilization, using either ammonium or nitrate-based fertilizer, could drive pH changes in the rhizosphere. Indeed, ammonium will induce an acidification while nitrate will induce an alkalinization. Thus, depending on the soil context, mineral Pi solubilization could be strongly enhanced at neutral pH in soil high in cations such as Ca or Mg, just by providing ammonium as fertilizers. In contrast, acidic soils containing mineral P associated with Fe and Al, nitrate fertilization should be favoured. These approaches need development.

Hydroponics within horticulture provides opportunities to precisely regulate P uptake and control release and may avoid the unpredictable impact of soils. This is limited to specific high-value crops that are economically profitable.

Recommendations for a future research program

Research Priorities

- **Elucidating Pi signalling and transport.** Pi signalling in plants remains fragmentary compared to other signalling pathways. The exact nature of the signal pathways and the complexity of interconnection with other pathways (such as carbon, nitrogen, metals) need clearly to be resolved
- **The role of microbiomes in plant Pi acquisition.** Understanding how inoculated microbes can develop in the rhizosphere or the roots in the field as a function of (i) the crop species and (ii) the soil conditions, especially Pi availability and pH is needed. The use of PSB microbial inoculants alone should be studied.
- **Identify Phosphate Use Efficiency (PUE) traits** or select plants adapted to Pi depleted soil -to distinguish Pi uptake efficiency, e.g. traits that lead to greater acquisition of Pi from the environment, from phosphate use efficiency (PUE) that encompass traits that not only improve Pi acquisition, but more importantly improve its overall utilization to increase production of the harvestable products (typically seeds) under fixed amount of bioavailable Pi. Important aspects of PUE include: (1) Pi recovery in soils; (2) improved soil exploration by roots, (3) Pi uptake, (4) physiological use of Pi for growth and (5) yield production.
- **Increase the quest of targets for PUE traits**, identify the underlining causal genes and use potentially them as markers for selecting P efficient crops.
- **Develop and test novel solutions** acting on modification of soil parameters or novel fertilizers.
- **Deciphering indicators to monitor reliably bio-available Pi** - crucial to limit and/or rationalize Pi fertilizer management. Computer models predicting changes in lignocellulosic biomass are essential for predicting climate changes and P nutrition is an important feature (Kvakic et al., 2020). However, they cannot yet take into account at broad scale the problems of phosphate bioavailability.

6.3.3 Water uptake and water use efficiency

Cultivars that are more efficient in terms of water use are urgently needed to complement efficient crop management practices (Condon, 2020). Water Use Efficiency (*WUE)* has been identified as a breeding target with a common use by agronomists and farmers. This WUE refers to the ratio of grain yield to the amount of water transpired, and sometimes to the ratio of yield to total water use (Passioura and Angus, 2010). Efficient water use has been further considered at lower levels of plant organisation and shorter timescales (Figure 6.2): transpiration efficiency (*TE)* was defined at plant level as aerial biomass/water transpired, while instantaneous *WUE* designed the ratio of photosynthesis (A_N) to transpiration rates (E) at leaf or plant level, largely dependent on radiation and vapour pressure deficit (*VPD*), and intrinsic *WUE* (WUE_i) was defined as the ratio of A_N to stomatal conductance (g_s). This very active field of research at the different levels and timescales opens multiple avenues to breed for crops with higher *WUE*.

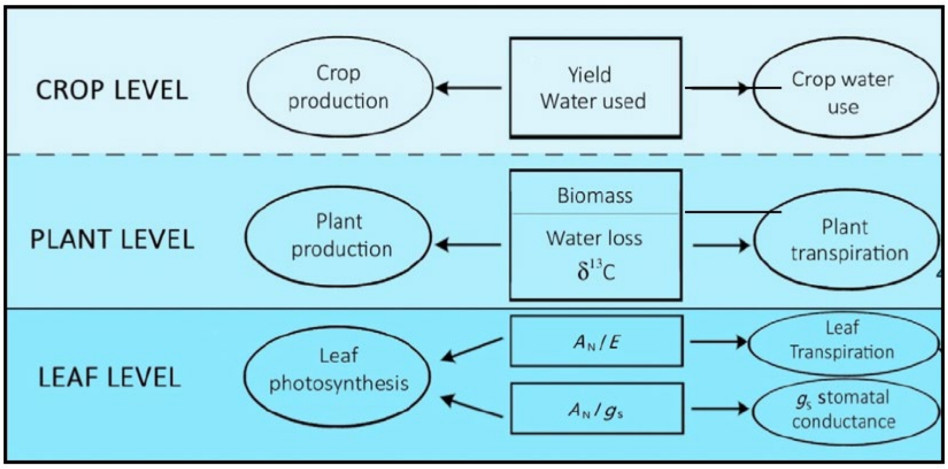


Figure 6.2. *The different dimensions of water use efficiency ((Medrano et al., 2015)*

Coupling between growth or An (Photosynthesis)and E (Transpiration rate)

The linkage between diffusive limitation to water loss and CO2 uptake is common to all plant lineages (Brodribb et al., 2020) because adaptive processes and evolution have locked together the regulation of water and carbon fluxes in vascular plants via stomata (for CO_2 capture) and leaf area (for CO_2 and light capture). Progress in WUE must therefore work on uncoupling assimilation from transpiration. Another key aspect of *WUE* is that it increases under drought, primarily because stomatal conductance, and thus water loss, declines more than carbon fixation (Edwards et al., 2012). Therefore, high WUE is often associated with conservative behaviour (low transpiration, low assimilation) and thus low yield (Rebetzke et al 2002). In contrast, fast organ expansion is generally accompanied by efficient root water uptake and high water use with turgid leaves and open stomata (Tardieu et al., 2015). Therefore, high WUE may not be intrinsically a favourable trait to target. It will very much depend on the scenario the crops are exposed to (Tardieu 2012, 2022).

Margins of progress in WUE have been identified

Beyond the physiological and structural links between transpiration and photosynthesis, there exist margins of progress towards higher WUE for a given photosynthesis, *i.e.* by decoupling transpiration and carbon fluxes. Indeed, transgenics with differences in A_N do not always couple with g_s (von Caemmerer et al., 2004; Lawson et al., 2008). Isotopic analysis of ^{13}C in plant tissue has been used to reveal genotypic variation in *TE*, and to develop new cultivars with large *TE* (Rebetzke et al., 2002). More recently, quantitative genetic approaches applied to water uptake and *WUE*-related traits have identified multiple genetic loci/SNPs (Dhanapal et al., 2015; Des Marais et al., 2016; Wang et al., 2018; Arab et al., 2020; Ferguson et al., 2020). In terms of mechanisms, searching for traits underlying WUE independently of photosynthesis identified with genetic variation for instance for night time stomatal opening and cuticular permeability (Duursma et al., 2019; Schoppach et al., 2020). Overall, according to modelling exercises, decreasing specifically gs should broadly increase yields, with greater benefits in low yielding arid conditions and this effect should be enhanced under eCO_2 conditions (Leakey et al 2019).

WUE at the plant cycle timescale

WUE can be managed considering the whole crop's cycle. For instance, plants growing fast at early stages of development save water by covering soil and weeds, escape terminal drought (and high *VPD*) situations and result in higher *WUE* (pradoa, 2020). At the other end of the cycle, stay-green traits can result in improved WUE under drought conditions (Górny and Garczyñski, 2002)

WUE at the daily timescale

Night-time transpiration (whether due to leaky stomata or other pathways) can result in significant water loss and reduction of WUE (Schoppach et al., 2020). Interestingly, there is ample genetic variability for this trait (Coupel-Ledru et al., 2016) even though potential functions and tradeoffs of nocturnal conductance have not been fully explored. Moreover, WUE is usually higher in the morning due to lower evaporative demand in the morning than in the afternoon. Therefore, water saving strategy can be to reduce nighttime transpiration and limit transpiration in the afternoon (Nelson et al., 2018; Tamang and Sadok, 2018)

WUE and stomata

A straightforward way to alter WUE is to act directly on stomata. In C_4 species, natural variation in g_s could explain substantially more variation in *iWUE* than A_N (Leakey et al., 2019). This paved the way for improvement in *iWUE* by reducing g_s through reduced stomatal density. As an example, *EPF1* has been targeted to improve WUE in barley (Hughes et al., 2017), rice (Caine et al., 2019; Mohammed et al., 2019), wheat (Dunn et al., 2019)and poplar (Wang et al., 2016) with little apparent influence on regulation of canopy temperature Molecular mechanisms controlling stomatal morphology and patterning have been elucidated in *Arabidopsis thaliana*(Chater et al., 2017) offering multiple ways to manipulate stomatal density. However, evidence suggests that key stomatal genes can be divergent between the lineages, notably in grasses (Raissig et al., 2016; Abrash et al., 2018). Moreover, reduction in stomata size or density, or increase in stomatal responsiveness (through manipulation of ABA biosynthesis or ABA receptors) generally increase *WUE* although at the expense of A_n (Lawson and Vialet-Chabrand, 2019).

Globally, mechanistic modelling suggests that enhancing intrinsic WUE (*iWUE*) by reducing stomatal conductance (g_s) while maintaining rates of net CO_2 assimilation (A_N) can increase biomass production across a broad range of environmental conditions (Truong et al., 2017; Leakey et al., 2019). However, the optimum relationship A-gs for iWUE often does not work (e.g Leakey et al., 2019, different species, or Medrano et al., 2015, different canopy positions).

Stomatal dynamics ie. speed of responses of stomata to differentially stimuli such as light or VPD differentially have great influence on short term WUE. For instance, smaller stomata facilitate faster response to environmental cues (Lawson and Blatt, 2014). In line with this, manipulation of stomatal kinetics improves carbon assimilation, water use and growth (Papanatsiou et al 2019).

WUE and canopy architecture

Another pathway for improving WUE is to act on plant architecture which has differential impacts on transpiration and photosynthesis (Medrano et al., 2015). For instance, shaded leaves waste water without benefit for A_N (Albasha et al., 2019). Another lever can be the distribution of N within the canopy with great impact on photosynthesis, much less on transpiration. Optimal use of water requires coordinated, within-plant variations in A_N and g_s with a key role of N redistribution (partly and indirectly controlled by local climate) for the former, and a key role of local climate (more directly) for the latter (Leuning et al 1995).

WUE and leaf anatomy

Optimal use of water requires coordinated development of paths for water and CO2. Beyond stomatal conductance, the so-called **mesophyll conductance** has attracted great attention since acting on it is supposed to have no effect on transpiration. Indeed, improving the mesophyll to stomatal conductance ratio may result in improved WUE (Flexas et al., 2013). Potentially, this could be addressed by improving the tortuosity in the mesophyll (Lundgren et al., 2019), cell wall thickness, cell wall composition and/or Sc/S, i.e. the chloroplast distribution (Flexas et al., in press), as well as the presence of trichomes and/or sunken stomata, which affect the boundary layer conductance (Galdon-Armero et al., 2018). Similarly, sub-stomatal anatomy influences microscale evaporative conditions without altering stomatal aperture and diffusion of CO_2 (Buckley et al., 2017).

Another relevant target is carbonic anhydrases which uncouple CO_2 from H_2O diffusion in leaves (Momayyezi et al., 2020)

WUE, water uptake and water supply to growing tissues

The overall hydraulics of the plant is known to play a great role in the process. Several quantitative trait genes linking root hydraulics to water availability and in a broader sense environmental signalling have been characterized (Shahzad et al., 2016; Tang et al., 2018). For instance, aquaporins upward growing zones (Ehlert et al., 2009) and/or large contact surface with maturing xylem (Martre et al., 2000) are favourable to volumetric growth.

What technologies are needed to unleash progress on WUE

HT Phenotyping is key to identify genotypes with favourable behaviour, either in a forward genetic approach or to validate candidate genes in a reverse genetic approach. The most obvious technologies for capturing transpiration contrasts at high throughput are thermal imaging and chlorophyll fluorescence (McAusland et al., 2013), including under fluctuating environments (Vialet-Chabrand and Lawson, 2019). This can be translated at high throughput in platforms combining transpiration and leaf area measurements (Vadez et al., 2015; Alvarez-Prado et al., 2017). More recently, optical topometry and machine learning were used for stomata patterning and leaf gas exchange (Xie et al., 2020)

Genetics technologies are now commonplace on WUE. Genome wide analyses helped to disentangle the link between WUE related traits (Coupel-Ledru et al., 2016), (Maurel and Nacry, 2020). New alleles can be searched for into exotic germplasm (Ferguson et al., 2020). Finally, using transgenics will open new roads eg for stomata kinetics to study coupling between A and Gs (Papanatsiou et al., 2019)

Modeling is key in the portfolio since models coupling soil and root hydraulics help to identify major resistances (Carminati and Javaux, 2020). Then models are essential to predict the integrated role of variations in WUE. For instance, hydraulic based models have been developed allowing the evaluation of the value of traits on WUE in different G x E x M scenarios (Albasha et al., 2019). Furthermore, photosynthesis and transpiration traits can be put in perspective of plant strategies into which growth is balanced with other functions such as defense (Karabourniotis et al., 2014).

Gaps in our knowledge

The very active domain of WUE needs a concerted effort to tackle unsolved question that blur the targets to tackle. These efforts are needed at different levels:

At the cell / leaf level, much effort is needed to understand both the coupling and the uncoupling between A and gs as well as to better understand the mechanisms behind stomata dynamics.

At the plant / canopy level, further models development are needed to predict the best suite of conductances (g_a, g_s, g_m) and their properties ($g_s$dynamics) under predicted climate scenarios. Increasing WUE may not always be beneficial (closing stomata results in warmer leaves, can it be detrimental under hot climates) and we need tools to predict when a given physiological set-up is most favourable (Tardieu 2022)

The link between the plant's hydraulic and the soil hydraulics is another black box in our knowledge. Indeed, the plant resistances can be by far exceeded by soil resistances, the latter being also influenced by microbial activity in the rhizosphere.

How to make the best use of available / manipulated genetic variation for breeding WUE?

A burning question is of course is there enough genetic variation in the current elite gene pool used by breeders to improve WUE in current and future climate/management conditions? Another is how to stack (genetically) most suitable traits related to stomata / mesophyll conductance / photosynthesis, and tissue (root) hydraulics to optimize WUE?

Avenues for application of research results

The current research on WUE is mature enough to start providing guides and targets to breeders. Crop model-based simulation could provide scenario-based ideotypes for WUE. Further, genetic exploration could identify pathways to build such ideotypes. For instance, water efficient ideotypes could combine improved gm/gs, low tortuosity (high porosity) in the mesophyll and thin cell wall thickness. Screening (for WUE and/or traits underlying WUE) already take place on existing large breeding / pre-breeding populations. In addition to this material, non-transgenic targets (tilling) obtained from current understanding of the regulation of gs and/or A and their links would be of high interest.

Recommendations for future research

Research Priorities

- **Further dissect complex mechanisms** accounting for coupling / uncoupling conductance and assimilation
- **Screen (ie phenotype) large genetic diversity panels** in platforms and in the field (possibly including genetic resources, mutants, GM) for WUE and allelic diversity
- **Manipulate epidermal/stomatal patterning and dynamics** to optimize g_s, g_m to current or future CO2 concentrations
- **Pyramid interesting traits to overcome negative impacts of higher WUE**
- **Improve models**, eg. incorporating steady-state / dynamic processes (gs...)
- **Develop systems-view of water use**, considering trade-offs between potentially opposite objectives such as high WUE / yield penalty including optimization, risk analysis (based on probability of drought occurrence), stabilizing farmer’s economic yield.
- **Co-select new germplasm with innovative management practices** for highest benefits in terms of water use

6.4 Yield, Yield Stress Tolerance and Yield Resilience

The principles underlying yield formation are given here, with an emphasis on yield potential in cereal crops. A holistic and conceptual summary of yield limitations and possible routes to enhancing yield with an emphasis on co–stimulation of source, sink and roots. Yield resilience to environmental perturbations is proposed as a combination of direct cell and tissue level tolerance traits to disruption (by high temperature for example) in sensitive tissue at specific developmental stages and the stabilisation of photosynthesis, respiration and growth across multiple tissue types enhancing primary productivity including allocation of biomass to roots.

Crop yield in terms of productivity (dry weight) per unit land area has been the predominant target of breeders selecting in most cases under high-yielding conditions. Re-prioritisation towards improving resilience to stresses is needed but must not come at a cost to crop yield potential and must even incorporate an increase in current annual yield gain (Ray et al 2012). This section will briefly summarise the components of crop yield, and how they might be improved and made more resilient to difficult environmental conditions. This is a complex topic and so we take a generalised overview approach.

It is important to recognise the different yield gaps that exist. Yield potential (YP) refers to the maximum yield per unit land area that is achievable in an environment in which the cultivar is well adapted, free of disease and resources are non-limiting (often on a research station but not necessarily so). Attainable farm yield (AY) refers to yield under good management (considering technological and economic constraints) and agronomy environment. Actual farm yield is usually below this. Whilst closing these different yield gaps requires different approaches, it has been found yield potential improvement brought about yield gains were not only in environments where other gaps have been closed (Foulkes et al 2022, Aisawi et al 2015), but also when the crop is grown under relatively low-yielding conditions (Cattivelli et al., 2008; Ferrante et al., 2017; Richards, 2000). Indeed, wheats selected in CIMMYT for their high yield potential were released in drought environments. Whilst it is not certain that a variety, or cultivar with high yield potential would perform well under any resource poor conditions, selection has identified high yielding material adapted to poorer conditions by starting with germplasm with good yield potential (Voss-Fels et al. 2019).

Yield can be usefully described as the product of intercepted radiation (usually photosynthetically active radiation or PAR), the 'conversion' of that radiation to biomass via photosynthesis and the partition of biomass to the harvestable part of the plant:

$$\text{Yield} = \Sigma \text{PARi} \times \text{FPARi} \times \text{RUE} \times \text{HI} \qquad \text{[Equation 1]}$$

(PARi = incident radiation; FPARi = the fraction of incident that is intercepted; RUE = the radiation use efficiency in grams of dry weight per unit intercepted radiation and HI= harvest index, the proportion of plant dry weight in the harvestable part).

Eqn 1 Provides a deceptively simple means of understanding the physical processes conferring yield and have provided stimulus to research into primary processes such as photosynthesis due to the understanding that RUE operates some way below theoretical maxima (Zhu et al 2010). With the assumption that light interception and HI are optimised (this is by no means certain), improving RUE via photosynthesis under optimal conditions is a promising avenue for yield potential improvement (the photosynthetic components of RUE are covered elsewhere in this document) and this continues to be the case. However, Eqn 1 is insufficient for understanding the physiological and developmental processes that coordinate to determine final yield. Taking wheat as an example it is important to understand that final yield depends upon the optimal formation of reproductive organs, the sink. Indeed, in wheat the number of reproductive sinks has thus far been the most important factor driving yield potential (Miralles and Slafer 2007).

Moving forward it is essential to ensure that high photosynthetic capacity (source) is matched to sufficient reproductive sink size in order to achieve significant yield gains. Photosynthesis in earlier phases of growth is important to construct an efficient canopy and to determine the size and number of sinks but also to generate a rich store of photosynthate in the plant as a whole. In cereals this is stem and sheath localised carbohydrate (fructans for wheat and starch for rice) and amino acids which may later be remobilised during grain filling to augment carbon and nitrogen content of grains.

It is useful, therefore, to consider that crops have a 'yield construction' phase when photosynthetic 'source' is used to build sufficient reproductive structures and then (likely post anthesis) a 'yield realization' phase when source strength is used to fill the grains determining actual yield. Both phases depend on high photosynthesis to greater or lesser extents and optimal coordination between the source and the sink determines final yield (Murchie et al 2022). In general, the two are in balance such that an enhancement of one can induce an enhancement of the other, within developmental limitations.

Experiments that have manipulated source or sink have clearly shown control acting in both directions (Zhu et al 2004). The internal factors that regulate the feed forward and feedback processes are reasonably well understood with some of the molecular players known (White et al 2016). Metabolic control of source activity occurs in the leaf, whereby the accumulation of hexose sugars repress the export from the chloroplast and the expression of photosynthesis (Smith and Stitt 2007). It has been proposed that processes such as the glucose sensor hexokinase, the TOR protein kinase signalling pathway, the protein kinase SnRK1 and the regulatory metabolite Trehalose 6 phosphate all act to regulate source sink activity and thereby influence plant growth (Smeekens et al 2010, Paul et al 2020).

6.4.1 Yield resilience

Predicted shifts in climate patterns will further cause crops to be subjected to both severe heat and cold events including in the most productive regions of the planet at diverse latitudes. Crop models show that decline in yields of major crops is expected even within a decade but there is much variation-some regions will see gains and some a decline. There is therefore an uneven distribution of vulnerability with geopolitical events compounding further, as we have seen in 2022. In considering resilience high temperature will be used as an example.

Temperature is central to the regulation of plant development, phenology and yield and can be a major source of abiotic stress. Climate models predict increases in global temperatures between 1- 6^{o}C by 2100, and these increases being more pronounced during the night than during the day (Jägermeyr et al. 2021). The next 20 years is the most relevant period of time for plant breeders where an increase in temperature of c. 0.5-2.0^{o}C needs to be considered. Temperature, however, is a complex factor. The underlying average rise is important but the reduction in yield is caused by variations in temperature at key developmental stages (such as heat waves around reproductive phases) and in combination with other limiting conditions such as drought. Much of the work in this area has focussed on traits measurable during the day. However, yields in many species are strongly predicted by minimum (night time) temperatures rather than maximum (daytime) temperatures. Even under well-watered conditions; the higher the minimum temperature, the lower the yield. High nocturnal temperatures drive a faster plant developmental rate and are associated with shorter cropping durations, lowered day photosynthesis, and reduced yield (see below).

In general, high temperatures negatively affect yield through multiple mechanisms including decreases in fertility, disruption of development and growth, inactivation of photosynthesis, increased water deficit and metabolic perturbation (oxidative stress, mitochondrial respiration and photorespiration) (Ferguson et al 2020). ‘Tissue-level’ tolerance to high temperatures has received much attention. Recent work has highlighted the promise of producing Rubisco that is functional at high temperatures through thermotolerant Rubisco activase (Degen et al 2021). Lipid composition and protein stability will be also important traits (Posch et al 2019). Here we consider resilience as a set of traits operating across the whole plant and within the yield forming components as described above.

High temperatures that are beyond the optimal range of metabolic processes have multiple consequences for yield which have origins in phenological and metabolic perturbation. The impact of high temperatures depends upon the period of onset, duration and intensity. High temperatures impair seedling emergence and establishment and accelerates vegetative development. The process of flowering (anthesis) in many species is highly sensitive to temperatures in the range 35-40°C and can cause infertility by pollen sterility and embryo abortion among other mechanisms (Jagadish 2019). In wheat the most critical mechanism may be increased floret mortality and the failure to progress in development under high temperatures which markedly reduces the number of fertile florets at anthesis (reducing grain number and yield). A reduction in grain set at high temperatures is also important. A good example of something that was enormously successful in providing resilience to drought at flowering in maize was reducing the anthesis-silking interval, a purely developmental attribute completely independent of any changes in C economy of the crop. Adjustments in phenology indeed have provided (and may perhaps continue providing) improvements in resilience to different stresses.

The impairment of photosynthesis during high temperature periods has well–studied consequences for the yield forming processes described above. Photosynthesis is commonly lowered during heat stress by impairment to photosystem II electron transport, deactivation of Rubisco activase, the ratio of photorespiration to photosynthesis and direct damage to components (in extreme cases). Chronically lower photosynthesis will reduce the RUE value and lower yield, compounded by an accelerated development. An important target should be the stabilisation of photosynthesis in foliar and non-foliar structures in crop plants to maintain carbon supply (Ferguson et al 2020).

The rapid formation of a canopy and the development of reproductive structures (sink formation) are energy–intensive processes and depend on a ready supply of carbohydrate. Source limitations are especially important during early vegetative and reproductive development. Virtually every process in the formation, transport and usage of source photosynthate are affected by heat stress (Asseng et al 2017). In this sense, recent work that hypothesises a role for signalling of sucrose availability may be important. The compound trehalose-6-phosphate is a candidate since it can both indicate sucrose availability and regulate its distribution and use in the plant (Griffiths et al 2016). There is some work showing that manipulation of such signalling in organ specific ways can benefit whole plant thermotolerance (Lyu et al 2018, Nuccio et al 2015).

Intermediate storage sinks in crop plants such as the stem/sheath organs in cereals have multiple roles. They typically start to accumulate before flowering and probably increase sink strength generally. They provide a reserve of carbon and other nutrients that can be utilised for yield and for sink formation. In the case of wheat, they provide a buffer of energy that is available for reproductive development when suboptimal conditions limit concurrent photosynthesis (Tahir et al 2005). Such stem reserves are important for yield performance of cereal crops post-anthesis. They can increase resilience of reproductive development and yield during periods of stress such as heat and drought, that reduce photosynthesis during the grain-filling phase. It follows that the capacity of the stem to accumulate water soluble carbohydrates (WSCs) has been associated with yield in wheat, and quantitative trait loci (QTL) have been identified (Snape et al., 2007).

Increasing night time (minimum) temperatures correlate with yield stagnation and decline (Lobell et al 2007). The reasons for this may be the increased rate of respiration which responds to enhanced metabolic demands at higher temperatures by up regulation. This has the effect of more rapidly consuming carbohydrates and accelerating plant development. This was impressively demonstrated in rice where high nocturnal temperatures consumed stem storage starch faster, weakening the relationship between remobilisation and grain weight formation (Xu et al 2021). This is an excellent demonstration that enhanced carbon for stem storage and / or optimised temperature sensitivity of respiration that results in stem carbohydrate depletion would directly confer yield resilience.

6.4.2 The role of roots in yield and resilience

Roots have had a neglected role in breeding for yield, relative to above ground, in part due to the difficulty of analysis and phenotyping. Obviously, they have a critical role in yield formation, in resilience and in the sustainability of agriculture but specific selection for root traits is not common despite the fact that root system architecture (RSA) is important for acquiring limited soil resources (Ober et al 2020) and genes for specific RSA traits are now known (Maqbool et al 2022). It is easier to identify root traits that are of a benefit in resource poor environments e.g. to go deep for water acquisition during drought (steep, cheap and deep), to proliferate in specific layers for phosphate, to strengthen the root plate to minimise lodging and to penetrate compacted soils for better water extraction (Ober et al 2020).

Thus roots provide opportunities to enhance resilience through the enhanced scavenging of resources. The techniques available for root phenotyping within pre breeding systems are now improved (see Ober et al 2020 for a review). An overall enhanced root biomass has the means to mitigate above ground temperature stress by maintaining water supply for transpirational cooling (Lopes and Reynolds 2010). QTLs for cool canopies were associated with proliferation for rooting at depth under drought, or near the surface during hot and irrigated conditions in wheat (Pinto and Reynolds 2015) providing support for the role of a large root system to buffer environmental fluctuations. Recent work has shown that roots can be adapted to have a lower respiratory load by the constitutive formation of aerenchyma. It is likely that stomatal morphology and vascular morphology will be important. Recent work has shown that lowered stomatal density permits high photosynthetic capacity with increased water use efficiency and sufficient canopy cooling (Caine et al 2018).

It is more difficult to identify root traits that are associated with high yield potential. There is evidence that modern cultivars of wheat have smaller root systems than older landraces (Waines et al 2007). We know relatively little of root traits needed for high yield potential. Key traits like RUE do not account for root mass in their measurement so we are not aware of any trade-off or conflict in partitioning to achieve yield potential. Any increase in root mass should not occur at the expense of yield in optimal conditions. This is important considering the high respiratory cost of roots. Assuming that there was no competition, the role of roots in sequestering soil carbon is a promising one. As well as roles in temperature and drought tolerance this has multiple benefits including the improvement of soil organic carbon, especially in deeper sub soils. The roots of cereals are less suitable for this, being fibrous. However, the ancestors of rice, wheat and sorghum generate rhizomes which are axillary shoots and would be more suitable for sub surface carbon sequestration (Hays et al 2022).

6.4.3 Recommendations for Future Yield Related Research Activities

Here we have emphasised the coordination of diverse plant processes in the formation of yield. The complexity of directing the coordination of metabolic and developmental events to achieve higher yield potential is a challenge.

Crop based models have helped to address these challenges but frequently they do not accommodate biological nuances and trade-offs within the plant. The source, sink and related components have recently been described in the form of a 'Wiring Diagram' for wheat (Reynolds et al 2022). This research tool provides a means of linking inter-related physiological processes, describing their relationship and linking to the genes or underlying QTLs . It is not a model or a static diagram but a framework for understanding current knowledge of the physiological traits determining yield potential that could be extended to other species.

6.4.4 Yield potential

Radiation use efficiency and component processes of photosynthesis and respiration operate below theoretical maximum and can be improved through targeting Rubisco, photorespiration, photoprotection, canopy architecture, electron transport (other parts of this document). However concurrent sink capacity needs to be improved. Enhancing photosynthesis in both leaf and non–leaf parts of the plant is likely to be needed.

Sink size remains a limiting factor in some crop species and can be improved by altering partitioning during reproductive development. Using the example of wheat (where sink size is determined by the number of grains and their individual potential size) the period of stem elongation is critical and is when grain number is determined. Partitioning between internodes and fruiting efficiency, the number of grains per unit spike dry weight is a key trait to enhance grain number (Rivera-Amado et al 2019).

Modification of life history traits to affect resource allocation is a promising principle that can be applied across species but will need to take into account the biology and yield components in each case. WP4 concluded that the genetic networks controlling life cycle transitions, the mechanisms controlling resource allocation and underlying trade-offs between different life history traits are still not well resolved. Furthermore, we still know relatively little about the conservation of regulatory networks controlling key life history traits across species and more specifically between crop species and their wild relatives.

6.4.5 Yield resilience

Our holistic approach to yield resilience operates across the crop life cycle. It is important to distinguish 'direct' effects of a stress (such as impairment of reproductive development in a heat wave or a stress event during mid grain filling) from the role of inadequate production or partitioning of fixed carbon which is, at its core, an energy- based limitation. Improvement of whole plant carbon dynamics remains a challenge but a viable one. It is difficult because of the complexity of source-sink relationships and their genetics (see WP4 report). There are many examples of where tolerance to abiotic stress and yield resilience can be conferred by changes in key regulatory genes (such as the DREB family of TFs) but a central question is whether this results in a reduction in yield potential. A good example of where this works is the SUB1 gene in rice which confers tolerance to flooding.

Component processes could be targeted: long distance signalling, sugar signalling and regulation of loading and unloading to enhance carbon allocation and improve yield. Breakthroughs have been made such as manipulation of sensing and signalling mechanisms based on T6P/SNRK1 signalling in maize, manipulation of unloading mechanisms from apoplasmic to symplasmic pathways by targeting protein partners of a SWEET sugar transporter in potato. The signalling that controls source sink dynamics and matches their relative activities is a major target. We need to be careful that mechanisms may not translate between species.

Stabilising photosynthesis across multiple tissue types such as leaf, stem, reproductive tissues will be important. Reduction in tissue damage and enhanced rapid recovery from periods of stress will be needed. Stabilising metabolic perturbations such as increased respiration during periods of high temperature in storage tissues will be critical.

Exploitation of root structure, architecture and function to enhance resource use efficiency and mitigation of water and temperature stress will be important. This is likely to be combined with improved stomatal morphology and dynamics.

6.5 Primary Production

6.5.1 Leaf Primary Production

The biomass of plants and other living organisms comprises of an array of molecules and ions ranging from the very complex (eg proteins, DNA) to the simple (eg potassium ions, phosphate ions). A typical plant cell contains several thousand organic chemical compounds along with small inorganic ions (eg PO_4^{3-}, NO^{3-}, K^+, Mg^{2+} etc). All of this chemical complexity is made by plants from simple starting materials obtained from the soil or, in the case of the carbon the air. The physiological mechanism that converts, or assimilates, inorganic carbon dioxide from the air into energy rich carbon-containing molecules is photosynthesis. This process uses energy obtained from absorbed light to drive the chemical conversion of carbon dioxide into carbohydrates, a process that requires an input of energy. By converting carbon dioxide into an energy rich organic form, photosynthesis drives life in the biosphere. Agriculture, similarly, depends on photosynthesis for its productivity; after water, plant biomass is largely carbon-containing molecules, predominantly carbohydrates but also proteins, lipids and other biochemicals. 45% of the dry weight of a plant is carbon, 45% is oxygen, 6% is hydrogen - the remainder being other elements. This carbon, and much of the oxygen, comes from the carbon dioxide assimilated by photosynthesis. If more plant is needed, be that stems, roots, seeds, fruits etcetera, then, more carbon will be needed and this carbon comes from photosynthesis. The fixation of that carbon by photosynthesis is a complex process that is strongly affected by the acclimation of the plant, by the impact the environment on plant growth and physiology, and by the genetics of the plant. Photosynthesis is organised over many scales ranging from the level of quantum mechanics to the level of the canopy. This complexity makes improving photosynthesis more challenging but offers many ways to improve it. Taking the natural diversity of photosynthesis, there are many models for radically rebuilding photosynthesis, though its complex interconnected nature would make this a brave undertaking. This approach would also depend on GM techniques, which currently remain problematic in the EU. Less radical approaches based on conventional breeding or gene editing (if that would become possible in the EU), however, still allow many routes to improvement based on a better exploitation of photosynthetic variation in the wild relatives and less highly domesticated genotypes of these plants. The more broadly we can apply gene editing the more flexibility there will be.

How does photosynthesis work? A summary of the process

Photosynthesis comprises a diverse group of physiological processes that make use of the energy of absorbed light, (as photons) to drive metabolism, enabling otherwise energy requiring (or endergonic) reactions to proceed. Oxygenic photosynthesis, named because during its operation this form of photosynthesis releases oxygen, is by far the most abundant type of photosynthesis and sustains most of the life in the biosphere. It is this kind of photosynthesis that takes place in cyanobacteria, algae and plants.

Oxygenic photosynthesis is made up of the following steps:

(i) Light absorption by photosynthetic pigments (especially chlorophylls, but also including carotenoids and in cyanobacteria phycobilins) that are organised into two photosystems (photosystems I and II (PSI and PSII));

(ii) The use of this absorbed energy to drive chemical reactions that result in the formation of energy-rich and metabolically useful compounds (these are reduced ferredoxin and NADPH, and ATP) via a photosynthetic electron transport chain;

(iii) The use of NADPH, ferredoxin or ATP to drive metabolism, which in plants and most other organisms is predominantly the fixation of carbon dioxide into an organic form, specifically carbohydrates in the form of sugar phosphates, which are a metabolically useful form of molecules. The biochemical pathway (actually a cycle) that fixes carbon dioxide into carbohydrates is the Calvin Benson cycle.

(iv) The supply of carbon dioxide. In the case of the leaves of most plants this is provided from the air around the leaves and reaches the photosynthetic cells in the leaf by a process of diffusion from the surrounding air; with carbon dioxide first entering the leaf via stomata, small pores on the surface of the leaf. The limitation to diffusion imposed by diffusion through the stomata is called the stomatal resistance. The stomata are highly regulated as they serve not only to allow carbon dioxide to enter the leaf but to prevent water vapour from the leaving the leaf, so they must maintain a balance between two priorities - the first being ensuring an adequate supply of carbon dioxide for photosynthesis and the second restricting the loss of water vapour from the leaf so as to prevent the leaf from drying out. Once the carbon dioxide has entered the leaf it must still diffuse to the point of carbon dioxide fixation, which is in the chloroplasts, and this diffusive pathway through the mesophyll includes movement through the air space in the leaf and the aqueous phase of the cell wall and the cell itself. The limitation imposed by diffusion in the mesophyll (air spaces and cell) is called the mesophyll resistance.

(v) The transport of photosynthetically formed carbohydrates away from the photosynthetic cells of the leaf and then the leaf as whole to the growing points of the leaf and storage organs - if the demand for carbohydrates by the growing points of the plant and storage organs is reduced then photosynthesis becomes limited or restricted via a feedback mechanism.

The Calvin Benson cycle (CBC) fixes about 95% of all the biologically fixed carbon dioxide in the biosphere and thus plays the paramount role in the global primary production of biomass. In plants and other eukaryotic photosynthetic organisms the CBC takes place in the chloroplast. To understand how photosynthesis can be improved we need to have some understanding of the operation of the cycle, which is commonly viewed as comprising of three stages:

- **Stage 1: The carboxylation phase:** the enzyme ribulose-1,5-carboxylase/oxygenase (Rubisco) catalyses a reaction between carbon dioxide and a 5-carbon sugar phosphate, ribulose-1,5-bisphosphate (RuBP) - this reaction produces two molecules of phosphoglycerate (PGA), a 3-carbon acid.
- **Stage 2: The reduction phase:** PGA is chemically reduced to a 3 carbon sugar phosphate (a triose phosphate) by NADPH, the reaction also requires ATP - this is the first carbohydrate formed in photosynthesis and some of this triose phosphate is exported out of the chloroplast to make sucrose, and then transported to the growing point of the plant and the storage organs.
- **Stage 3: The regeneration phase**: triose phosphate is converted back to the 5-carbon RuBP; used in the carboxylation phase. Some of the intermediates of the regeneration phase can be exported from the Calvin cycle to make starch in the chloroplast or metabolites needed for other biosynthetic pathways in the cell. The regeneration phase uses ATP.

This form of photosynthesis is called C3 photosynthesis because the first stable products of the carbon dioxide fixation process is phosphoglycerate, a 3-carbon acid. Despite its global impact, the Calvin cycle and C3 photosynthesis is widely viewed as being flawed because of the properties of Rubisco. This enzyme is slow, a typical plant Rubisco will turnover at most about 3 times per second (normal enzymes turnover at about 1000 times per second) but this requires a carbon dioxide concentration at Rubisco of about 1000 ppm, much lower than that found in leaves. Most plant Rubisco has a relatively poor affinity for carbon dioxide, and also catalyses another reaction between oxygen and RuBP at a significant rate under normal working conditions. This oxygenation reaction also uses RuBP, which is no longer available for carboxylation, and the products of oxygenation consume NADPH and ATP during their recycling to RuBP (but of course without any carbon dioxide fixation). During this recycling process one carbon dioxide molecule is released for every two oxygenation events and this release directly antagonises the process of carbon dioxide fixation. The oxygenation reaction catalysed by Rubisco has a major effect on the efficiency of photosynthesis; removing the oxygenation reaction will increase carbon dioxide fixation by about 50% in a leaf photosynthesising in air at about 20°-25°C. This represents a major loss of potential plant primary productivity and reduces the potential productivity of agriculture. In addition, the oxygenation reaction increases relative to carboxylation as temperatures increase. It is believed that one of reasons agriculture only really took off after the end of the last ice age was that previous atmospheric levels of carbon dioxide were so low that Rubisco functioned very poorly and plant growth was too slow to make agriculture economically feasible.

In addition to the problem caused by the oxygenation reaction, the poor affinity of Rubisco for carbon dioxide compared to current atmospheric carbon dioxide creates yet another problem for photosynthetic efficiency. The carbon dioxide supply in C3 photosynthesis is via diffusion transporting molecules from a high concentration to a low concentration. This implies that the carbon dioxide concentration in a chloroplast must be lower than that in the surrounding air - in most leaves the concentration drop is about 200 ppm (compare this to 400 ppm in the air). This means that Rubisco, which needs a concentration of about 1000 ppm to reach it maximum activity, is working at much less than its full potential and thus creating another problem for the efficiency of photosynthesis. These problems with Rubisco have made it a major target for strategies to improve crop productivity. Evolution has produced some workarounds for the Rubisco problem but it has not been able to produce a Rubisco that is fast, has a high affinity for carbon dioxide and does not catalyse the oxygenation reaction. All nature's solutions rely on using carbon dioxide concentrating mechanism - CCMs. These make use of pumps to increase the carbon dioxide concentration around Rubisco and so allow it to function under conditions where there is little oxygenation and Rubisco is working at near to its full capacity. The problem with all concentrating mechanisms is that they require energy and this energy has to come from photosynthesis, which has an impact on the efficiency of the process. In the case of land plants, the CCM is the C4 cycle (giving rise to the name C4 photosynthesis) which depends on cellular specialisation in the leaf between the bundle sheath and mesophyll cells combined with a biochemical pump to concentrate carbon dioxide in the bundle sheath cells. C4 plants include many fast growing tropical crops, such as maize, millet and sorghum, and the C4 cycle, because of its high affinity for carbon dioxide, allows photosynthesis to take place with much less loss of water from the leaves. Algae and cyanobacteria have different forms of CCM which rely on a physical pump. Considerable effort is being made to transform C3 plants with either the C4 mechanism (eg the C4 Rice project https://c4rice.com) or that of algal or cyanobacteria (eg the Ripe project https://ripe.illinois.edu). These approaches, however, depend on GM technology so any plant improved in this way would face regulatory barriers in the EU. The current situation in the EU requires alternative approaches to be considered. This can be based on exploring the diversity of Rubisco (i.e. with kinetic properties better matched to increasing atmospheric carbon dioxide levels) in the wild relatives of crop plants or a better rubisco whose genetic sequence can be transferred to crop plants by gene editing, if this would become practical for crop improvement in the EU. Some crop plants also have wild relatives that have a partial C4 photosynthesis (eg *Moricandia arvensis* in the brassicaceae) and it might be possible to cross these C3/C4 intermediates with (eg) C3 brassicas and thus improve their photosynthetic properties in that way.

Photosynthesis in the Field.

Laboratory measurements of leaf photosynthesis has revealed many details of how it functions and how it acclimates to different environments. This is particularly true for crop plants. Much less is known, however, about how photosynthesis performs in the field, particularly in the longer term, what limitations act on photosynthesis in the field and how it is integrated across whole plant. There is also less information on non-crop plant species, many of which might harbour important models and genetics for improved photosynthetic properties. Improving photosynthesis will need much more attention to performace in field conditions and in particular, understanding how activity of photosynthesis limits growth versus how often photosynthesis is being limited by poor growth, and how we can make the best of photosynthetic productivity in these two quite contrasting situations. Improving photosynthesis in plants with limiting growth will result in no benefit unless we can either improve growth or find some way of storing the primary production that is in excess of the needs of growth. When photosynthesis is limiting, we need to accelerate the process as far as growth allows, and once we hit that barrier increase growth or sink activity (e.g. fruit or seed number) yet again.

The Fluctuating Environment and Abiotic Stress

In the natural world the environment is constantly changing, and these changes occur over many time scales. Photosynthesis is strongly affected by changes in the environment, particularly (but not exclusively) light intensity, water supply and temperature. Fluctuations in the order of days or shorter can have negative effects on photosynthetic productivity and some environmental factors, like light and temperature, can change even at the sub-second level. As a result of these fluctuations, photosynthesis in the field is often not at steady state, but is in fact in a state of continuous adaptation. These adaptations in the short term (hours or less), are at the level of physiology while in the longer term,(hours, days or longer) are dependent on partially rebuilding or remodelling the photosynthetic machinery. Responses to fluctuations or changes in the environment are therefore complex and layered, extending over a range of biological responses. The faster photosynthesis responds the closer it will be to its optimum - this will increase three things:

- ✓ **Carbon dioxide fixation**
- ✓ **Water use efficiency**
- ✓ **Nutrient use efficiency**

Faster responses to the environment are therefore beneficial but these adaptive responses are poorly understood, especially the control and regulation of longer-term remodelling to longer term environmental changes. Understanding and accelerating the response to a changing environment has been underestimated in the search for crop improvement. Environmental change leads to challenges for plant adaptation, and if the fluctuation is great enough, the environment can become stressful to the plant. For photosynthesis the extremes of temperate or drought are the most important stress factors. Other environmental factors can also limit plant processes, though overall growth seems to be the most environmentally fragile process. Future changes to climate are expected, where Europe will become warmer, and in many places overall drier (potentially at agriculturally important times) but also in the extent and frequency of weather extremes. We need new resilient cultivars that will be able to withstand and recover quickly from the stress. Photosynthetic resilience and the basis of its variation is not well understood but there is variation for the extent that photosynthesis decreases in response to a stress and how quickly it recovers once the stress is removed. We can exploit this phenotypic variation to both improve the stress response and resilience of photosynthesis and, via genetics, resolve the physiological basis for stress tolerance and resilience.

Canopy light use efficiency is not the same as that for single leaf

In natural ecosystems plants grow as individuals and often in competition with other species. In the field, plants are rarely grown as individual leaves or even plants, but instead as populations, often of the same genotype. The unit of agricultural production is a canopy of plants that do not need to compete with other - we deal with the outsider species, like weeds, that would form a competitive challenge to our crops. Typically, domestication and breeding has minimized traits that waste plant resources through competition but instead optimize cooperation in a mono species community, such as reduced shade avoidance and upright leaves (for cereals). Because the plants are not competing, they can cooperate. There are advantages to removing the competitive properties of crop plants, but the heritage of our crop plants is one of competition. It is this canopy of non-competing plants that is the photosynthetic system. Most of our understanding of photosynthesis is at the leaf level or lower. An improvement to leaf photosynthesis will be an improvement to canopy photosynthesis but at the canopy level we can aim for improvements that would be counter-intuitive at the leaf level. An example of this is reducing leaf light absorption. At the leaf level this would reduce photosynthetic light-use efficiency on an incident light basis, but when applied at the canopy level lower leaf chlorophyll allows better light distribution through the canopy and increases canopy light use efficiency, and nitrogen, phosphate and water use efficiencies. It is important to note that the high leaf area serves photosynthesis, as well as storage of elements such as nitrogen that are needed for later translocation to the grain (in the case of cereals) to maintain harvest N content. Canopies also create light-flecks and shade flecks - regions of fluctuating light which on a windy day can change within milliseconds. Such phenomena probably act to distribute light more efficiently within the canopy. For efficient photosynthetic light, water, and nutrient efficiency we need to accelerate the rate of change of photosynthesis and stomatal opening to these rapid fluctuations in light intensity - something for which we know natural variation exists.

How can photosynthesis be improved - is it not already optimised?

Photosynthesis has been evolving for some 2.7 billion years. The basic motor of photosynthesis (Photosystems I and II, the photosynthetic electron transport chain, the Calvin cycle) is now genetically conserved as if it was optimised. Within the land plants the photosynthetic pigments show little variation (and plants are largely green as a consequence) and while this is not so of the algae and cyanobacteria, the useful pigment diversity of these groups could only be exploited using GM techniques. Increasing sophistication can be observed in leaf architecture and vasculature moving from lower plants through the gymnosperms to the angiosperms. These advances make high rates of photosynthesis possible by allowing high rates of carbon dioxide diffusive transport and high rates of water transport.

Photosynthesis may be optimised, but optimised for what? Photosynthesis in a forest tree may be optimised for the life and the environment of trees, the photosynthesis of fast-growing weeds (typical of the ancestors of crop plants) might be optimised for the life and environment of weed, but none of these are adapted to the environment of agriculture in which nutrients and water are well provided, and weeds, pests and herbivores are controlled. As a result of the domestication of plants suited for typical high yielding agriculture, we have created plants that thrive in environments that have no obvious natural analogue, especially in comparison to protected cultivation. Even crop plants from before the green revolution were grown in a highly managed and exceptional environment when compared to the wild relatives of our crop plants. Photosynthesis has not been deliberately optimised for agriculture, but being grown in an unusual environment, photosynthetic (and other) traits that we can use to improve crops plants can be found amongst the wild relatives, the landraces etc . Plants exist in nature that have higher photosynthetic rates, faster recoveries from stress, faster responses to fluctuations in irradiance etc showing a proof of concept that we could utilize in our crops. These are the genetic resources that can be used to make crop plants better, not only for photosynthesis, but for the other major traits that we want to improve.

Targets for change

Key targets for improvement should be seen as a tool-kit of options to shape a final combination of properties for a particular goal. These options are restricted to what might be possible using selection by conventional breeding options from crop plants and their wild relatives or by means of gene editing of alleles. More radical options (eg photorespiratory) that would be possible by means of genetic modification techniques have not been included in this list. Some of these options are applicable only to C3 species and some only to C4 species

Changes to leaf light absorption and photosynthetic light use efficiency

a) **decreasing non-photosynthetic, blue light absorbing pigments** (maybe only for greenhouse and similar crops - these blue light absorbing pigments may also serve in an anti-herbivore role and thus could be indispensable in the field)

b) **removing PSII protection** (**the qE mechanism) for greenhouse crops** - with increasing use of light-diffusing glass and active shading the benefits of qE are probably marginal

c) **increasing speed and extent of state transitions** - state transitions help balance the light-absorption properties of photosystems I and II and an imbalance in their activity represents a loss of photosynthetic light use efficiency

d) **Minimising or optimizing the onset of photoinhibition** (sometimes referred to as qI) which reduces quantum yield and adds a cost to repair of photosystems. This requires a balance against any minimization of qE.

Changes to the Calvin cycle and electron transport

e) **adapting or selecting Rubisco** for increased atmospheric CO_2 and climate change

f) **increasing RuBP** supply by accelerating the regeneration phase of the Calvin cycle

g) **increasing Pmax** (connected to points a and b) in the canopy to increase carbon dioxide fixation through the canopy - increasing Pmax will increase photosynthetic light-use efficiency at a sub-saturating irradiance so even if irradiance is not saturating a high Pmax will be beneficial

Responses to fluctuating light

h) **improving rates of stomatal opening and closing** in response to increases and decreases in irradiance - this will ensure a better carbon dioxide supply for photosynthesis and reduce unnecessary loss of water vapour from the leaves. This may require an increase in stomatal energy supply.

i) **improving rates of photosynthetic metabolic activation in response to irradiance** increases to make better use of fluctuating light.

j) **improving rates of qE relaxation** in response irradiance decreases to avoid unnecessary limitation of photosynthesis because of too much photo protection of PSII

k) **increasing speed and extent of state transitions** - state transitions help balance the light-absorption properties of photosystems I and II and an imbalance in their activity represents a loss of photosynthetic light use efficiency improving the properties of rubisco activase to improve the speed with which it activates rubisco and the speed at which it deactivates (recent work)

Leaf architecture

l) **increase mesophyll conductance** to increase the supply of carbon dioxide to photosynthesis

m) **increased stomatal aperture for greenhouse crops** - a high humidity and adequate water supply would allow stomatal resistance to be decreased and higher stomatal conductance we allow for a higher carbon dioxide concentration within the leaf and thus better photosynthesis

n) **Transferring C4 or C3/C4 intermediate metabolisms** from wild relatives to related C3 crop species

o) **Optimised venation and hydraulic conductance** and associated root capacity for water extraction.

Canopy architecture

p) **improving canopy light absorption profiles** during key growth phases (e.g. establishment in short duration cycles) to increase the canopy light, water and nutrient use efficiency and to influence the frequency and duration of light flecks for optimised dynamic photosynthesis.

q) **The use of stay-green mutations** to improve the contribution of leaves to total canopy production especially in short duration cycles

Stress and resilience

r) **increasing the resilience of photosynthesis to stress** - this is likely to involve improving not only photosynthesis, particularly the recovery of PSI and PSII from photo damage, but also the resilience of growth

s) **improving the properties of rubisco activase** to improve its high temperature tolerance

t) **Optimal carbohydrate storage strategies** to cope with stress e.g. lowered respiration at high temperatures.

The exotic

u) using artificial evolution to evolve new photosynthetic components

What is the quantitative relationship between photosynthesis and plant growth?

There are two simple equations that are commonly used to express how photosynthesis connects to plant growth. The first simply links the energy of the sunlight incident on a crop-plant canopy (St; units J m^{-2}) with the harvestable biomass (Yp; units kg m^{-2}) produced by that canopy via 4 parameters: εi is the efficiency of light interception by the canopy, εc is the efficiency of conversion of that energy to biomass, k is the energy content of biomass per unit weight J kg-1), and η is the fraction of the total plant biomass that is harvested

$$Y_p = S_t.\epsilon_i.\frac{\epsilon_c}{k}.\eta$$

[Equation 2]

The term εc is the same as radiation use efficiency discussed above and is a complex term that also includes other factors associated with physiological processes like respiration, but it is dominated by photosynthesis.

	achieved	theoretical
ε_i (interception)	0.89	0.9
ε_c (conversion)	0.032 (C3)	0.094
η (partitioning)	0.6	0.65

Table 6.1 the achieved and theoretical limits to the terms ε_i, ε_c, and η; data from Steve Long

Of the parameters that are subject to our control via breeding (and excluding k as plants dry matter is carbohydrate which they need to be erect) η and εi are close to or at their maximum, while εc, the term that is dominated by photosynthesis, is substantially below its theoretical maximum value.

Another model that usefully summarises the relationship between photosynthesis and plant growth is that of Blackman in 1919 which uses a parameter, the relative growth rate (RGR) to model or describe the biomass increase of a plant with time:

$$M_t = M_0 \cdot \exp^{RGR \times t}$$

[Equation 3]

Where M0 is the dry mass of the plant at time zero, Mt the dry biomass at some future point in time, t is the elapsed time, exp is the natural base, and RGR is the relative growth rate. This equation makes no reference to photosynthesis, RGR can, however, be expanded:

$$RGR = LMF \times SLA \times ULR$$

[Equation 4]

Where LMF is the leaf mass fraction (the fraction of plant mass that comprises the leaves) SLA is the specific leaf are (the area of leaf per unit dry mass of leaf) and ULR is the net assimilation rate (photosynthesis - respiration) per unit leaf area - all photosynthetic and respiration gas exchange (i.e. carbon dioxide uptake or release) is assumed to occur in the leaves in the simple equation that predates a more complete understanding of respiration and photosynthesis. So:

$$M_t = M_0 \cdot \exp^{(LMF \times SLA \times ULR) \times t}$$

[Equation 5]

Plant growth depends on photosynthesis via the ULR but also on how the plant is built (LMF and SLA) - it is possible to have an increase in photosynthesis (ULR) but if (eg) SLA decreases to the same extent the growth rate will not be changed. This complex relationship between photosynthesis and growth made it difficult in the past to demonstrate a simple dependency between photosynthesis and growth despite this seeming an intuitively obvious relationship. Nonetheless it is clear that better photosynthesis results in better plant growth. Greenhouse crops like tomato are grown at a carbon dioxide concentration of 800 - 1000 ppm, much higher to than in the atmosphere, to increase their photosynthesis (by outcompeting the oxygenation reaction that leads to photorespiration), resulting in increased productivity. Increasing the carbon dioxide around plants growing in the field, the so called FACE (free air carbon dioxide enrichment) environment, has the same result provided no other factor (eg drought or poor mineral nutrition) dominates growth. Pioneering research using GM techniques have also shown that increases in photosynthesis result in increases in plant growth and productivity. For these reasons improvements in photosynthesis is seen to be the last remaining major target for achieving major increases in plant productivity. Improved photosynthesis does not need to lead to more yield - if the ambition is a bigger root system, or more exudates, or more non-food biomass they can also be increased through increases in photosynthesis without there being any loss in food-yield. Better photosynthesis is therefore the gateway to options to improve what we do with our crops.

Canopy Architecture and Primary Production

Canopy and plant ‘architecture’ is defined here as the 3-dimensional structural arrangement of plant organs. Crop canopy architecture is a central component of yield: it defines the efficiency of absorption of radiation and its conversion into dry matter. The description of architecture is often associated with light since the optimal distribution of light is linked with other essential components such as photosynthesis, nitrogen and pigments. However, architecture has been influenced by a number of other unrelated processes through breeding and selection that may have acted independently or even against light use efficiency and there is compelling evidence that the architecture of canopies remains a promising target for further improvement (e.g. Murchie and Burgess 2022). Key aspects of canopy architecture are also dependent on environment, management and input of resources such as the influence of fertilisers on tillering in cereals.

Canopy architecture can be studied on different levels and scales: developmental biology and genetics at the cellular level that ultimately control the number, size and positioning of plant organs in space. Meanwhile plant and crop physiologists consider architecture as being fundamental in determining the efficiency of capture and conversion of radiation and the exchange of gases with the atmosphere. There is an overall understanding of how architecture determines yield, but details remain scant such as verification of the optimal vertical distribution of photosynthetic components (Walker et al 2018). This is partly due to the difficulty of measurement and phenotyping: most analyses are carried out at the canopy surface, not within the canopy. It was established decades ago that a substantial portion of yield arises from lower, shaded portions of the crop canopy. To confound this, there has been little connection between the molecular and whole canopy scales mentioned above, despite a surge in discovery of genes that underly architectural traits. There is now an urgent need to utilise advances in developmental biology alongside exploitation of genetic diversity to enable improvement in architecture to enhance yield potential.

Key proofs of concept now exist to guide improvement of yield through crop architecture. It is important to consider light interception which is largely a function of leaf area as related, to the efficiency of conversion (see equation 1 in the yield section). For example upright leaves especially in narrow leaved crops such as cereals have long been considered a beneficial trait due to their role in optimising light penetration and thus (a) maintaining upper leaves in a state of lowered light saturation and (b) increasing photosynthesis in lower leaves. Empirical evidence that upright canopies confer higher grain yields now exists for wheat (Richards et al 2019). However, these canopy types can present problems for weed competition and soil water evaporation leading to the concept of an optimised canopy with upright upper leaves and flatter lower leaves which are better able to intercept diffuse or direct radiation low in the canopy. To date these canopy types have not been utilised systematically in breeding (Murchie and Burgess 2022).

Patterns of light in the canopy are determined by architecture. The fluctuations of light that cause photosynthesis to rise and fall are the result of solar movement and wind. Improving photosynthesis responses to these dynamics may improve yield (Long et al 2022 and other sections in this report). However, the architecture of the canopy itself also determines the frequency and duration of light flecks (Durand et al 2021) where a higher frequency of flecks maintains photosynthetic induction state, This implies that canopies can be engineered to help photosynthesis better exploit fluctuating light. Recent work suggests these fluctuations in light may be on a faster scale than ever considered previously, with sub-second changes in light intensity recorded in field canopies (Durand et al., 2021). A conceptually similar proposal applies to wind induced movement where movement permits opportunities for more rapid transmission of photons to lower layers (Burgess et al 2016) if movement properties can be manipulated.

These dynamics have implications for photosynthesis whereby consideration of performance under ‘real’ conditions is inherently more complex than previous results under steady state.

Vertical distribution of photosynthetic activity is tightly linked to light distribution, and in turn, canopy architecture. Several lines of evidence suggest we need to be able to alter photosynthetic properties in a canopy position specific manner. Prioritising N concentration to the top of the canopy, where it can be better used in light saturated photosynthesis has been proposed. Self-shading is a crux of the potential return on investment in leaf manufacture. Breeder selection of dark green leaves (more likely to contain high N levels) means that modern canopies may be unnecessarily self-shading because light saturated photosynthesis is less chlorophyll–limited than a shaded leaf. Minimising chlorophyll content in upper leaves while maintaining nitrogen content (and Rubisco) may be a means to enhance lower layer canopy photosynthesis (Walker et al 2018, Salter et al 2019). Enhancing tolerance to photoinhibition in lower layers may also prevent costly PSII repair.

Traits and a way forward

Toolbox for architecture: **Identification of genes and QTLs for individual leaf angle, stem angle, canopy height, leaf density, leaf size , curvature and shape in multiple species is needed to create a genetic toolbox of architectural traits.** The same kind of toolbox should be generated for more subtle features of 3D spatial features such as clumping, light distribution, vertical distribution of morphologies. Example genes in the establishment of leaf angle are known and include those involved in hormone signalling: the Brassinosteroid pathway, auxin and absissic acid (Mantilla-Perez et al 2017).

Structural traits: lodging resistance and optimised movement properties for distribution of light, CO_2 and water. Little is known about genes underlying the former.

Smart canopies: The manipulation of leaf angle according to vertical canopy position and therefore independent of position on the stem is an important target trait in order that the above optimised 3D architecture can be applied (Mantilla Perez 2017). However, in many species the genetic regulation of such distribution is not known and so phenotyping combined with genetic resources is necessary.

Traits conferring optimised vertical distribution of pigments: e.g. lowered chlorophyll and increased concentration of Rubisco towards the top of the canopy are needed. Enhanced photosynthetic efficiency in lower parts of the canopy by reduced respiratory costs, lowered N content and enhanced light harvesting and optimised photoprotection.

Phenotypic data sharing: A sharing platform or database for phenotypic information is required. Data sharing and knowledge of best practice is really limited or completely lacking within phenotypic approaches and available large datasets- such as annotated images for deep learning- will contribute to more rapid advancements.

6.6 Nutritional and Post-Harvest Value for Food Use

6.6.1 Increasing protein content and quality

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

Grain protein content (i) is a major criterion for grading products and for exportation, (ii) it highly depends on nitrogen (N) availability, (iii) could be expected to compensate for the reduction in the use of exogenous gluten in baked goods and more broadly the predictable reduction in the use of bakery improvers. If there are limitations in the future due to restrictions of N fertilization; protein quality will have to be elaborated in more detail. For instance, the wheat protein composition should be finely tuned to obtain gluten structures and properties able to give the required end-use quality for processing.

Scientific background

The variation for processing and end-use quality is largely explained by the grain protein concentration and composition. Whatever the species, a trend is generally observed for a lower grain protein concentration for modern cultivars (eg Caballero-Rothar et al., 2019 for maize, Brancourt et al., 2003 for wheat). However, there is the possibility of improving the yield while maintaining seed protein content in grain legumes such as pea and soybean, and possibly maize, as no strong negative correlation between yield and seed protein content was observed (Burstin et al. 2007; Cober and Voldeng 2000; Tenorio et al., 2019). In cereals, the exploitation of the deviation from the negative relationship between grain yield and protein concentration (GPD, Monaghan et al 2001) is possible if both its genetic variability and stability are considered (Oury and Godin 2007). In addition, some regulators, as the TaNAM transcriptional factor in wheat (Uauy et al., 2006), gives the hope of being able to increase the protein content without negatively affecting yield (Tabbita et al., 2013).

The major loci and genes for protein composition, even for some regulatory genes involved in the protein and starch syntheses (globulins in legume seeds, glutenins and gliadins in wheat, modifiers of the opaque-2 mutation in maize), have been identified. Wheat storage proteins represent about 80% of the total grain protein. They mainly consist of polymeric glutenins and monomeric gliadins that are subdivided into several fractions according to their electrophoretic mobility. Glutenins are classified as high molecular weight (HMW) and low molecular weight glutenin subunits (LMW-GSs). HMW-GSs are encoded by the Glu-1 loci located on the long arms of the homoeologous chromosomes of group 1 (Payne 1987). Each locus comprises two tightly linked genes. LMW-GSs are encoded by multigene families located at the orthologous Glu-3 loci on the short arms of chromosomes of group 1. Finally, genes for gliadins occur on the short arms of chromosomes of group 6. The globulins account for up to 70% of total proteins in mature pea seeds. They are classified into the 7S (vicilins and convicilins) and 11S (legumins) multigene families, which differ in their amino acid composition and structure (Osborne, 1909; Shewry et al., 1995). Some regulatory genes involved protein synthesis were identified (e.g. ABI5 in pea seeds (Le Signor et al., 2017), SPA in wheat (Albani et al., 1997; Ravel et al., 2009)).

In wheat and pea, a large genetic variability exists both for protein content and protein composition. In pea, genomic regions controlling protein content and composition have been identified for marker-assisted breeding programmes (Bourgeois et al., 2011). In wheat, molecular markers have been developed since the 1990s, particularly for glutenins sequences (eg Liu et al., 2012; Iba et al., 2018; Ravel et al., 2020). Despite the difficulty to capture the complexity of these gene families and to interpret the results compared to electrophoretic mobility in sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), some of these markers were successfully used in breeding.

Proteins of cereals, like wheat and maize, are known to contain relatively high levels of sulfur-rich essential amino acids such as methionine (Gorissen et al., 2018). They are however relatively poor in lysine. This is the contrary for legumes, such as pea and soybean, that are richer in lysine but poorer in S-rich amino acids. Within a species, the genetic variability for the composition in amino-acids seems not very large. There are however differences such as observed for wheat (Anjun et al., 2005). For example, data standardized by the protein concentration of a set of varieties show variations of 20, 10 and 20% for lysine, threonine and isoleucine (Rodehutscord et al., 2016). In maize, the opaque-2 (o2)-a natural recessive mutation led to nearly double the lysine and tryptophan content in endosperm due to a decrease in the synthesis of zein proteins and increase in the other seed proteins rich in lysine and tryptophan. The mutation causes however inferior kernel phenotype. To circumvent this problem, plant breeders can restore kernel hardness via the introduction of Quantitative Trait Loci (QTLs) referred as o2 modifiers (Mo2s). Recently, differentially expressed endosperm genes that coincides with Mo2 QTLs have been identified and thus are potential candidates that can be used to restore a standard kernel (vitrous instead of opaque) phenotype (Li et al., 2020)

In grain legume seeds, the presence of several antinutritional factors (trypsin inhibitors, tannins, vicine & covincing for faba bean) has already been reduced (Page et al., 2003). However, further improvements remain to be done for human nutrition purposes, notably regarding the functionality and organoleptic properties of legume proteins.

The effects of low nutrient (N, S) availability on seed protein content and composition have been analyzed, notably in combination with drought, and candidate genes that could limit the negative impacts of these stresses on seed development were identified. Indeed, in addition to genetic factors already mentioned above, the protein content and composition is strongly affected by environmental factors influencing N availability and cultivar development time (see the review of Johannson et al., 2020). Both factors are impacted by environmental (temperature, precipitation, CO_2, etc.) and agronomic (soil properties, crop management practices (e. g N fertilisation management)) components. A number of candidate genes that could limit the negative impacts of these environmental factors on seed development have been identified (eg Bancel et al., 2019; Bonnnot et al., 2017; 2020; Bourgeois et al., 2009; Henriet et al., 2019; 2020; 2021).

Trends in research and technologies development

Due to the development of low input systems, intensive research is currently conducted to study the impact of sustainable field managements on protein content (eg Pearsons et al., 2022; Costanzo et al., 2021; Pelligrini et al., 2021).

Determining how domestication and modern breeding has affected grain quality is also subjected to intensive studies. This is particularly the case for cereals (eg Csarvari et al., 2021; Geisslitz et al., 2019; Horvat et al., 2021; Kulathunga et al., 2021; Serban et al., 2021) with the increase of wheat related disorders (celiac disease, gluten intolerance, etc.). This is also the case for legumes where compounds with negative effects in animal feed were eliminated by selection although they can have positive effects in human health (e.g. tannins which have antioxidant functions). In addition, the potential of forage species as a source of proteins in human nutrition is being considered (Julier et al., 2017).

In the past, the effect of individual protein fractions or gene alleles were analysed but more and more the effect of combinations of different alleles or protein fractions are considered: eg in wheat grain the relative percentages of glutenins/gliadins/albumins/globulins and the presence of different glutenins alleles combinations, and in legume seeds the percentages of 7S globulins/11S globulins/2S albumins.

It is recognized now that there is a need to understand effects at all levels: from gene expression to the phenotype and the biophysical effects on molecular interactions. Therefore, tools for multi-level data integration are developing (Vincent et al., 2015).

Translational genomics is proposed when relevant to take advantage of information coming from different species (Dai et al., 2021; Li and Yang, 2017; Palakurthi et al., 2021).

This is of course not only relevant for grain quality, but the genomes of all the major crops have been sequenced (eg Schnable et al., 2009 for maize, IWGSC et al., 2018 for bread wheat, Kreplak et al., 2019 for pea). Very good reference genomes exist and more and more sequences for other genotypes are now available to describe the pangenome of a given species. High density genotyping arrays and genotype by sequencing methodologies have been developed for Genome Wide Association Studies.

Low cost and high throughput proteomic tools are being used and they are particularly adapted to analyzing grain proteins related issues (eg Bancel et al., 2019; Bonnnot et al., 2017; 2020; Bourgeois et al., 2009; Henriet et al., 2019; 2020; 2021).

Ethylmethanesulfonate (EMS)-induced allelic variations (TILLING populations) have been developed in maize (Weil and Monde, 2007), wheat (Krasileva et al., 2017) and pea (Dalmais et al., 2008). Both techniques are used to improve nutritional quality traits. As some quality traits are determined by major genes these two approaches are thus very efficient. In a recent work, pea TILLING lines producing seeds that do not accumulate saponins, source of bitterness, have been identified (Vernoud et al. 2021).

Genome editing, that can target multicopy genes (as it is almost always the case for polyploid species like bread wheat) is mastered for most crops. For example, Zhang et al. (2018) analyzed gene edited wheat mutants that lacked one, two or all three homoeologous copies of the TaGW2 gene (Song et al., 2007). Besides affecting grain weight, width and length, TaGW2 mutations also modified grain protein content and two wheat end-use quality-related parameters, flour protein content and gluten strength, were considerably elevated in the mutants. The Crispr/Cas9 technique was also used to target the main α-gliadins immunodominant peptide responsible of the coeliac disease leading to a reduction immunoreactivity by 85% (Sanchez-Leon et al., 2018).

Ecophysiological modelling could be a relevant tool to understand the interplay between environmental and agronomic factors for the establishment of the seed or of the flour quality. Martre et al. (2006) developed for example a model of wheat grain N accumulation and partitioning between storage proteins. Based on the hypotheses that the gliadin and glutenin fractions are limited by N sources, that structural and metabolic proteins are sink-regulated and that N partitioning between gliadins and glutenins is constant during grain development and unmodified by growing conditions, this model simulates well grain protein fractions under a wide range of N fertilization regimes, temperatures, and water availability.

Future Challenges in the Field to be Addressed with High Priority

The quality of a production is a very complex and changing concept. It is necessary to better understand in each case (processing, end-use, nutrition health) what are the required criteria and possibly diversify and specialize the varieties.

It is important to stress that both a high quality (whatever the criterion) and a stable quality are required. In that context, the effect of global change is a major challenge for breeding. Future climatic scenarios include an increase in mean temperature (IPCC, 2021) that will accelerate development rates and shorter growth cycles and, even in the case of no decrease in the amount of rainfall, will lead to less available water due to increased evapotranspiration. Furthermore, predicted changes in a particular region includes increases in pluvial flooding in northern Europe and more frequent droughts in the Mediterranean area (IPCC, 2021). Some studies also reported that the elevated atmospheric CO_2 concentration could interfere with N metabolism resulting in lower nitrate assimilation by wheat plants (Bloom et al., 2010) that could alter the quality of the main crops (Ebi et al., 2021). In addition, economic and ecological constraints will favour the development of agricultural systems that use less inputs such as phytosanitary products and fertilizers. All these factors will probably increase the genotype by environmental interactions and the necessity to breed for cultivars more efficient and adapted to specific growing regions and agricultural systems.

The negative correlation between grain yield and grain protein concentration is well known in cereals. Genetic gains for grain yield have resulted in lower protein contents. In legumes, the very low or absence of correlation between seed yield and protein content suggests that protein content can be increased without lowering yield (Burstin et al. 2007). Depending on the species, there is a need to better understand this negative correlation and possibly identify genes that can alter it (notion of GPD in wheat). There are several candidate genes that need to be validated by breeding, such as that encoding glutamine synthetase and glutamate synthase (Nigro et al., 2020). For cereals, there is then a need to improve the efficiency of conversion of applied N to grain protein (see the NUE section). Other trade-offs may exist, for example the consequences on resistance to biotic and abiotic stresses (cold, drought, fusarium & bruchids in legumes) of the elimination by genetics of antinutritional / off-flavour factors.

In that context, one challenge is to know whether it is possible to maintain the processing and end-use quality levels when protein content is going down (because higher grain yields, less fertilization or less efficient N acquisition) by improving the protein quality and finely tuned the protein composition (Foulkes et al., 2009).

The quality of a product is estimated generally only on a few easy and rapid to measure criteria (e.g. grain protein concentration for wheat). Development of rapid tests are needed for baking quality so that breeding and trading for real baking quality and nutritional aspects for humans and animals can replace simpler tests.

One main societal challenge is probably around the possible acceptance of genetic engineering (GMO) and CRISPR modified crops (targeting protein quality) for commercial use in the long term if positive benefits on environment and health are demonstrated.

One challenge for cereals is the socioeconomic analyses along crop rotations regarding nutritional proteins. One question is whether it is necessary to have wheat grain with 12% protein when legumes can deliver much more. Studying societal acceptance of the consumption of legumes is needed to develop their production for human nutrition.

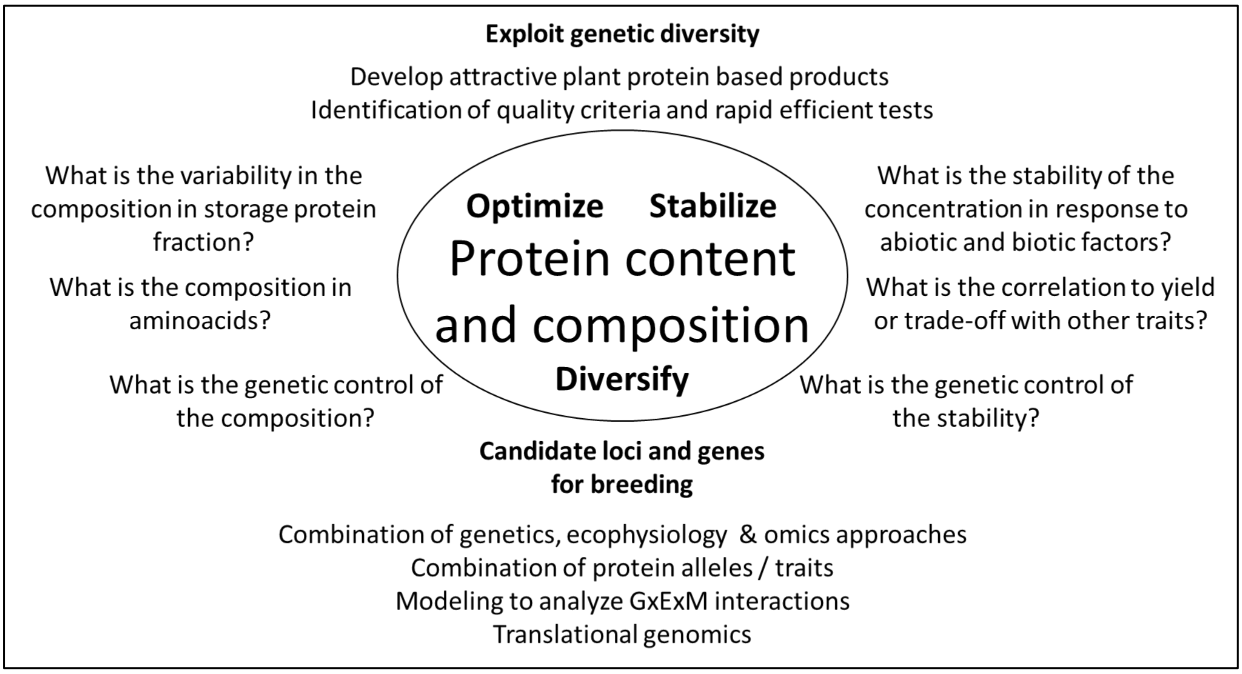


Figure 6.3: A schematic presentation of genetic levers to improve grain quality through optimizing, stabilizing and diversifying protein content and composition

6.6.2 *Fatty Acids*

There is now much evidence as to the health-beneficial properties of omega-3 long chain polyunsaturated fatty acids (abbreviated to omega-3 LC-PUFAs, and defined as fatty acids of 20+ carbons in length and containing three or more double bonds), based on epidemiological data and also controlled dietary interventions. Omega-3 LC-PUFAs (in particular, eicosapentaenoic acid, 20:5, n-3 and docosahexaenoic acid, 22:6, n-3; abbreviated to EPA and DHA, respectively) are known to reduce the risk of heart attack and cardiovascular disease, and also to be important in aspects of neonatal nutrition (in particular in aspects of the establishment of ocular vision), in conjunction with the omega-6 LC-PUFA arachidonic acid (20:4, n-6; abbreviated to ARA) . There is also a wide-held belief that current Western diets contain excessive levels of omega-6 fatty acids, and as a consequence, are underrepresented in levels of omega-3 fatty acids in general. This has lead to associations between this dietary trend and the wide-scale prevalence of obesity, type-2 diabetes and metabolic syndrome in Europe and North America. In response, a number of national advisory bodies and health professionals have advocated the increased consumption of omega-3 LC-PUFAs in the range of ~0.5g/day, not least of all as this represents a simple, efficacious dietary treatment with which to avert a potentially serious pandemic of these illnesses.

However, the current source of omega-3 LC-PUFAs is marine fish oils, and these are subject to a number of pressures. Firstly, it is widely accepted that natural fish stocks are (at best) at their maximum sustainable levels, mainly due to depletion via over-fishing. Secondly, environmental pollution of marine ecosystems has resulted in the accumulation of potential toxins such as heavy metals, dioxins and plasticizers in fish and their oils, limiting their utility in human nutrition. Most significantly, the expansion of aquaculture (fish farming) requires considerable fish oils for the optimal growth and nutrition of the farmed animals, consuming ~80% of marine fish oil. Thus, far from being a replacement for the diminishing natural reserves of marine fish, aquaculture adds to the problem on account of its non-sustainable requirements. These in part are due to the inherent metabolism of all animals (including fish) which are extremely inefficient in the in vivo synthesis of LC-PUFAs (both omega-3 and omega-6) from dietary precursors, which take the form of the so-called essential fatty acids (EFAs).

EFAs are strictly defined as linoleic acid (18:2, n-6; LA) and α-linolenic acid (18:3, n-3; ALA) which are almost never limiting in human diets but have trended in the last 100+ years to move from being skewed towards ALA (omega-3/n-3) towards LA (omega-6/n-6), predominantly as a societies have adopted diets rich in animal protein whilst simultaneously reducing their consumption of plant-based diets and also marine products. More recently, the stark manifestations of the impact of climate change and the realisation of the need to adopt a diet that support both the healthy individual and a healthy planet have led to the proposal of the “Planetary Plate” as part of the EAT-Lancet report (2019). This proposes a diet that is significantly reduced in the amount of animal products, replaced instead with plant protein. The only exception to this was a recommendation for increased consumption of fish, predominantly as a source of omega-3 LC-PUFAs (aka fish oils), since there is no terrestrial source of these fatty acids. However, recommendations to significantly increase the consumption of fish, especially as a means to elevate the levels of EPA and DHA, are problematic as analysis of marine reserves reveals a wide gap in what is currently available and what is required to ensure that all seven billion people on this planet get their fair share of these vital nutrients.

It is for this reason that CropBooster and others have considered how genetic improvement could help deliver to these objectives. Perhaps the most obvious target for improvement is the abundance of plant oils rich in the EFA omega-3 ALA. Currently most vegetable oils are rich in either omega-6 (LA) such as sunflower oil or dominated by monounsaturated fatty acids such as oleic acid (such as Canola and maize). Of the commodity crops, only linseed/flax have seed oils rich in ALA, and the global acreage is relatively modest. However, the genetic determinants for the levels of ALA are well-known, being the desaturase FAD3. Thus, conventional breeding approaches or gene editing could be used to attempt to elevate the levels of ALA.

At a higher level of technical complexity and also regulatory approval, major progress has been made in the last few years with the development of plants with the capacity to accumulate EPA and/or DHA. This can only be achieved by genetic modification (GM) through the introduction of an entire biosynthetic pathway from marine microalgae. By this approach, the oilseed crops *Brassica napus* (Canola) and *Camelina sativa* (Camelina) have been brought to an advanced stage of technology-readiness. In the case of Canola, recently granted deregulated status in the USA means that the crop is approved for commercial cultivation and can be grown at any scale. These crops provide a scalable solution to the ever-growing demand for omega-3 fish oils, either for direct human nutrition or for aquaculture (which is the current dominant need). To meet the challenges associated with the Planetary Plate and feeding the global population within planetary boundaries, aquaculture will continue to expand but now, thanks to advanced plant sciences and genetic technologies, it is possible to uncouple this production system from its reliance on marine ingredients, in doing so making it truly green and sustainable.

Recommendations for Future Nutritional Value Related Research Activities

Research priorities

- **In response to global change challenges, genetics and breeding, along better management strategies, offer opportunities to improve and stabilize grain quality while reducing the levels of inputs, the application of fertiliser (lower costs and environmental footprint), the use of pesticides and the energy required for processing.**
- **To address the increase of gluten related health-related disorders, genetics and breeding could also offer opportunities to propose new wheat varieties, which will solve the problem of sensitivities to gluten.**
- **Rapidly developing marker and genomic assisted breeding can be used to combine the best genetics for yield-protein content-protein quality.**
- **Phenomic selection can be developed to use near-infrared spectroscopy (NIRS) as a high-throughput, low cost and non-destructive tool to indirectly capture genetic variants and compute relationship matrices for predicting complex traits, may be an interesting alternative to genomics selection for quality traits. Many QTL were previously described but not often used in real breeding programs. The identification of major genes using genome sequences and development of statistical methods may change this.**
- **Speed breeding applications.**
- **The use of translational genomics to transfer knowledge of the regulation of seed protein content and quality from models to crops (e.g. M. truncatula or Arabidopsis to legume crops) or between crops (e.g. pea-faba bean-soybean-lentil…) should be increased.**
- **The development of predictive models to estimate grain N content, N translocated to the grain (grain N removal) and N balance in the soil taking into account environmental factors (temperature, water variability) and considering genetic parameters linked to the genotype is also increasing and should support future crop breeding.**
- **There is still the need to better understand the genetic basis of of protein content (N use efficiency / interaction with microbes), to breed varieties competitive for grain yield, with same or more protein content and that requires less fertilization. This includes the identification of the molecular determinants of components responsible for off-flavours of legume-derived ingredients, and of for gluten-related diseases.**
- **The greatest challenge may be to understand G x E interactions for seed protein content and composition (quality). This could include biophysical effects as well as effects on gene expression. This should include climate change and agroecological practices (e.g. the use of legumes in cereal cropping systems).**
- **Exploiting the genetic diversity (natural as well as induced by TILLING) to increase the quality through improved pre-breeding / breeding remains a challenge.**
- **Enhancing plant oils levels of ALA by exploiting the genetic determinants for ALA levels using conventional breeding approaches or gene editing, or potentially GM.**
- **Exploring GM options for using modified plants as “cell” factories to produce high value fatty acids in a sustainable way replacing less sustainable aquaculture sources.**

6.6.3 Post-Harvest for non-Food Crops

Improving the usefulness of plant biomass for fodder and as feedstock

Plant biomass is removed from the field not only for human consumption, but also for use as animal fodder and non-food applications. Crop development programmes also need to consider adjusting plants to improve the usefulness of plant biomass for fodder and as feedstock

The non-food applications of agricultural crops are very diverse - think of the number of crops cultivated for fibres for example. We cannot consider all of these non-food applications but areas of interest will include the use of biomass as a resource for the European biobased industries sector, cell bases and phytofactories for secondary metabolites and crops as a source of biofuels. The future Cropbooster programme would remain open to improving crops for other applications.

For example, the quality of lignocellulosic biomass s determined by the composition and structure of the cell wall polymers. The mail components of the cell wall are cellulose, hemicellulose, pectins, lignin and proteins. The composition and functionalities of each of these components as well as the interaction between them are key in determining cell wall properties and functionalities Among the main factors affecting lignocellulosic biomass quality are:

- Lignin content
- Lignin composition, in particular the content of the different monolignols and the S:G lignin ration.
- Degree of substitution of hemicellulosic polysaccharides
- Linkage between cell wall polymers, namely through ferulic acid and p-coumaric acid
- Cellulose crystallinity
- Pectin content and composition

Breeding goals

From a breeders perspective quality of biomass is defined as digestibility, which is a measure of feed quality, and as saccharification efficiency, which is a measure of how easily the cell walls can be broken down in a fermentation process leading to biofuels or other biobased products. The factors affecting lignocellulosic biomass quality listed above are correlated with digestibility and saccharification efficiency, so they are good indicators of these traits. Improving saccharification efficiency and digestibility are therefore ambitions for breeding. As the lignocellulosic component of a plant plays an essential role in supporting the plant, improving this component has to be achieved while maintaining the yield and architecture of the plant. Lignocellulosic biomass quality, and more specific saccharification efficiency and digestibility are very complex traits that are controlled by many genes and pathways. While GM helps us dissect these complex traits and understand the effect of individual genes, the application in breeding of new varieties, with the current available methods (which focus on modification of single genes) is limited. The use of other methods or tools such as GWAS to identify the genes underlying useful variation in biomass quality, and Genomic Selection as a breeding tool to simultaneously select for multiple genetic loci, are more promising for the development of new varieties of lignocellulosic crops.

Key Knowledge Gaps

What we know of the link between cell walls and biomass quality is to a large extent based on testing the effect of knocking down/out or over-expressing single (or few) genes. To address this a holistic evaluation of lignocellulosic biomass quality is needed.

The pleiotropic effects of cell wall quality on biomass yield, and specifically how we can improve lignocellulosic biomass quality while maintaining or improving plant yield is not yet well understood.

The effects of biotic and abiotic factors on biomass quality need to be better understood- this is particularly important given the breadth of climates in the EU and in forecasting (and mitigating) the effects of climate change on biomass quality.

How we can better utilise marginal land for biomass production needs further research. Recently reports on lignocellulosic crops, such as Miscanthus, have revealed that biomass quality is improved when grown on marginal lands, in particular under the effect of drought. Understanding the mechanisms and factors behind this improved quality has the potential to all breeding for varieties that can be cultivated viably on marginal lands.

Recommendations for Future Research Based on Knowledge Gaps

Research priorities

- **A holistic evaluation of lignocellulosic biomass quality.** This will require the evaluation of the combined effect of different genes/cell wall components and a better understanding which combinations of cell walls polymers and their respective modifications will lead to improved saccharification efficiency and digestibility.
- Research to develop a better understanding of the **effects of biotic and abiotic** factors on biomass quality
- Better understanding of **pleiotropic effects of cell wall quality on biomass yield,**
- New research to understand of the **effects of biotic and abiotic factors** on biomass quality
- Effects of **soil types, land management and drought on biomass crops** (including marginal lands).

7 How to Improve Traits?

The Mechanisms for Genetic Improvement and What is Required to Achieve Crop Breeding Ambitions

Plant breeding encompasses a broad set of methods used to introduce different desired traits in a plant variety or population. It involves identifying parent plants with desirable characteristics to create favourable combinations in the next generation. The process of selecting superior performing plants for food, feed and fibre products dates back more than 10,000 years and has been substantially refined in the last century Early farmers relied on extant genetic variation in wild plant populations and selected individual plants with desired traits. (Kaiser et al., 2020 https://doi.org/10.1016/j.tifs.2020.03.042).

Since the onset of human intervention in the evolution of land plants in the Neolithic era, there could be four major phases of plant breeding categorised (Ramstein et al, 2019):

- **Unconscious selection**, and later **conscious mass selection**, without any theoretical framework,
- **Artificial selection** in progenies from controlled crosses guided by knowledge of genetics,
- **Marker based assisted selection** based first on a few genetic markers and then on genome wide approaches (genomic selection) guided by knowledge of genetics and molecular genetics,
- **Targeted modifications by the introduction of transgenes or genome editing**, guided by knowledge of molecular genetics.

In addition to these approaches, induction of new genetic variation/diversity introduced by **random mutagenesis**, using either chemical or physical agents followed by phenotypic selection for specific traits, have played a role in many different crops (e.g., durum wheat, IAEA mutant database: https://www.iaea.org/resources/databases/mutant-varieties-database). While all of these approaches are still in use depending on crop species in question, state-of-the art breeding is increasingly making use of DNA sequencing and bioinformatic tools . Continuous innovation in plant breeding methods facilitates to exploit existing genetic diversity or create novel genetic diversity and to introduce traits of differing nature in the most efficient way and in the shortest time possible.

Note, phenomic selection is now also being used, which does not need genetic marker information, but detailed phenomic marker cues (https://academic-oup-com.ezproxy.library.wur.nl/g3journal/article/8/12/3961/6026883).

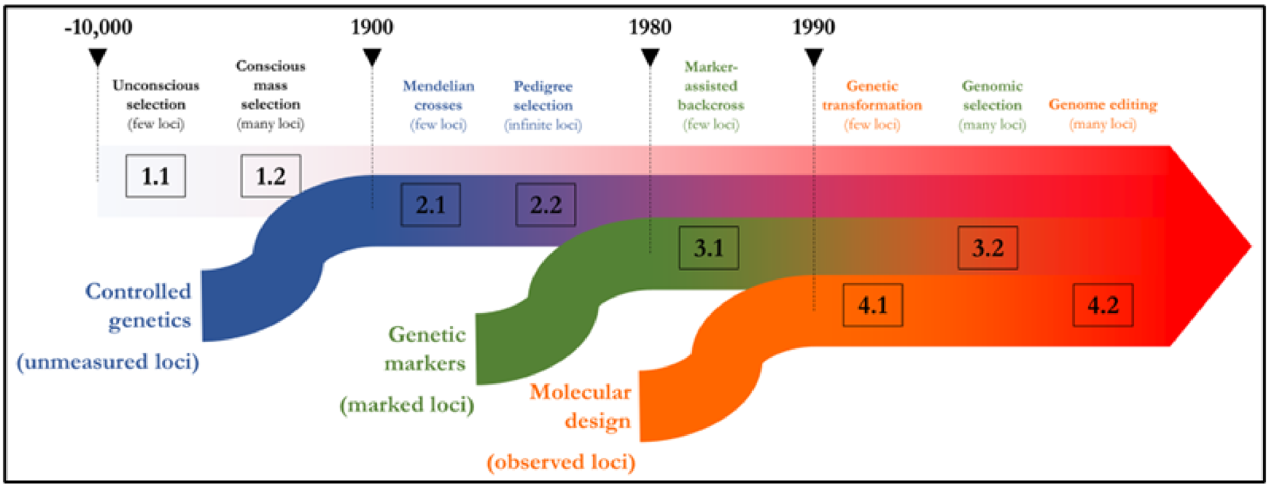


Figure. 7.1: Timeline of plant breeding phases (from Ramstein et al, 2019).[7]

7.1 Genomic selection

Genomic selection (GS) is becoming the preferred tool when the phenotype to be selected is under a complex genetic control, i.e. when the number of genes controlling it such that marker assisted selection is no longer the most efficient method to improve the trait. It exploits knowledge stemming from quantitative genetics, and avoids the identification of explicit associations between markers and quantitative trait loci (QTLs) discovered either by classical linkage mapping of bi-parental or more complex populations or by genome wide association studies (GWAS) of diversity panels. GS saturates the genome with a large number of molecular markers in such a way that all QTLs are in linkage disequilibrium with at least one marker. In commercial breeding, it is in the course of replacing marker assisted selection (MAS), which generally takes into account only a small number of genetic markers that are linked to a small number of loci which have strong effects. Genomic selection, on the other hand, by encompassing the entire genome, is able to simultaneously capture the effects of many tens of QTLs, including those with weak effects. It also reduces the cost of phenotyping by using predictive models; after calibrating a mathematical model on a diverse, intensively phenotyped training population to derive a predictive multivariate equation, it is possible to predict without further phenotypic evaluation the genetic combination necessary to obtain a plant variety adapted to particular biotic and abiotic environments. https://academic-oup-com.ezproxy.library.wur.nl/g3journal/article/8/12/3961/6026883 .

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[7] Ramstein, G.P., Jensen, S.E. & Buckler, E.S. Breaking the curse of dimensionality to identify causal variants in Breeding 4. Theor Appl Genet 132, 559–567 (2019). https://doi.org/10.1007/s00122-018-3267-3

7.1.1 Issues GS Research needs to focus on:

GS, even more than MAS, can be used as a black box approach that exploits anonymous molecular markers linked to desired traits without requiring any knowledge of the underlying genes, or their genomic location, or the physiological or metabolic nature of the trait. This implies that knowledge from quantitative genetics can be translated into novel plant varieties without the time and labour-intensive mapping and/or cloning of QTLs. Present limitations of GS are:

i. epistatic effects, i.e. the QTLs are not independent of each other but interact producing positive and negative effects on the trait,

ii. the lower prediction accuracy for traits with low heritability, i.e. traits difficult to assess precisely or strongly impacted by the environment,

iii. the limited power of the predictive models which decreases as the genetic distance between the training population and the breeding material increases and, in any case rarely exceeds 70%, and

iv. inherent limitations linked to the type of reproduction (autogamous/allogamous) and the level of polymorphisms within a given species.

7.1.2 Recommendations for Genomic Selection Related Research Activities

To overcome the limitations outlined above research is now needed to supply the models used in GS with a greater range of data. This will be supported by the following:

Research priorities

- **Access to high throughput phenotyping platforms, such as ground-based or airborne field devices, or phenotyping systems based in controlled environment rooms will be needed that can accurately reproduce natural environments**
- **High throughput genotyping at very high marker density will be required, e.g. low coverage whole genome resequencing data**
- **"omics" data (epigenomics, transcriptomics, proteomics, metabolomics, ionomics) for the target crops needs to collected and made available, and**
- **Accessibility to functional data (although these may be useful only if they improve the prediction accuracy)**
- **Open Science Open data approaches across the community will need to be refined to ensure the target dataset are FAIR (findable, accessible, interoperable, and reusable.**

Having a broader base of data will improve the prediction of agricultural performance of genotypes produced by the predictive models used in GS. By implication, this will require the integration of large heterogeneous datasets, this creates major statistical and computational challenges that still need to be resolved. The data used to train or calibrate the statistical models that connect the trait to genomic variation also needs to be specific for the trait and accurate. In the case of high-throughput phenotyping this means that the trait is well enough understood and can be measured accurately with the available high-throughput tools. "Omics" data similarly needs to be obtained from biological samples collected under conditions in which the impact of the trait is strong. This requires that the trait is sufficiently well understood so that these sampling conditions can be devised. Finally, sharing genomic and phenomic information on different panels would definitely increase the predictive accuracy of GS, emphasizing the important role of Open Science and Open Data. It may be challenging to include data from private companies in an open science fora, though this could be attractive where genotypes may be anonymized. This openness can be difficult to apply for breeding programs involving private companies, when competitive IP positions can be important. But the future programme should balance the *Open as Possible as Closed as Necessary* approach and also look at Open Innovation models to encourage collaborations.

7.2 Genome editing (GE)

Genome editing is becoming the preferred tool with which to exploit knowledge stemming from functional genomics, i.e. the role of gene products (mostly proteins, but also RNA) in the biological processes underlying an agronomically interesting trait. There are hundreds of proof-of-concept genotypes being developed (Dima et al., 2022 https://doi.org/10.1016/j.tplants.2022.05.002). This suggests that in the near future genome editing will become another important tool in the plant breeders toolbox , as long as it is not slowed down by regulatory constraints. In contrast to random classical mutagenesis, GE allows both the site in the genome and the nature of a mutation to be highly controlled. This specificity eliminates the formation of hundreds of unwanted mutations elsewhere in the genome (an effect of classical mutagenesis) or mutations not changing a target gene in the desired manner. It also makes it possible to obtain several mutations within a single gene or in multiple alleles as well as different genes of gene families e.g. in polyploid species with complex genomes. In contrast to classical transgenesis, which adds additional and often foreign genes to the genome, GE directly modifies existing genes in a specific way, this minimises the risk of the unpredictable effects that some fear in transgenics.

GE like transgenesis or classical mutagenesis (if used as a reverse genetics tool), requires upfront knowledge of the gene(s) to be mutated and the type of mutation(s) required to obtain an agronomically desired trait. This knowledge may stem from the species of interest but also from closely or even distantly related species. Using this knowledge, GE then allows the design and creation of novel alleles in the extant gene pool of a crop species. This genetic innovation can be used to enlarge the range of ideotypes/genetic variation available to the plant breeder. GE can also easily be multiplexed so dozens of genes contributing to one or several traits can be edited in one generation of plant development. This is a major advance over MAS and will allow for more rapid breeding. Despite the potential of GE compared to conventional and transgenic approaches there remain limitations to the technical scope of GE. Currently these limitations concern the sites accessible to GE in a genome, the mutations that can be obtained, and the species and genotypes to which GE can be applied due to lack of genome sequences and knowledge as well as enabling technologies like transformation or tissue culture technologies in certain species, specifically smaller and underutilized crops. However, continuous innovation in genome editing and related enabling technologies increasingly closes the gap to the ideal situation where any site in the genome can be mutated in any imaginable way in any plant species and any variety within the species.

7.2.1 Recommendations for GE Related Research Activities

Research priorities

- **To enlarge the number of sites that can be targeted by GE in a genome,** research efforts are needed to overcome the requirement of the so-called PAM sequence, i.e. the presence of the three bases (NGG) next to the target site in the case of the standard Cas9 enzyme and look to exploit the new Cas enzymes. Both the discovery of other RNA guided nucleases with different PAM sites and the engineering of the standard or new Cas9 enzyme towards a "PAM-less" version have resulted in progress towards this objective but are far from resolving it completely. The opening of chromatin in parallel to Cas9 action may be another requirement to be able to access any site in a genome**.**
- **To provide efficient and versatile GE tools such as base-editing and prime-editing** to complement existing classical single- or double-strand break induction and repair tools, and become available for routine use in plant breeding. This will facilitate allele conversion approaches, upon identification of rare, favourable alleles with only small sequence differences compared to more common reference alleles. This will tremendously accelerate introgression of favourable alleles in common breeding germplasm, avoiding the need for time-consuming backcross schemes.
- **New tools to determine mutation outcomes.** Bearing in mind that in 95% of the publications only the site but not the mutation itself has been predetermined, the development of adequate tools allowing researchers to predetermine the outcome of the mutation is one of the main occupations for the CRISPR-Cas community.
- **The range of crop species accessible for GE needs to be enlarged.** Some major crops, such as sunflower or many legumes, are presently impossible or very difficult to edit owing to the difficulty (or impossibility) of regenerating fertile plants from ***in vitro*** edited explants. Possible solutions include genome sequencing and gene discovery research, the optimization of transformation and tissue culture protocols including alternative delivery tools for genome editing, the temporary use of transgenes boosting tissue proliferation or meristem neo-formation, or the development of ***in planta*** technologies.
- **The use of haploid inducer editing (HI-editing).** This is a promising solution for the particular, but frequent case, where within a given crop species **GE** protocols work only with lab genotypes, and not elite varieties. In HI-editing (also referred to as trans editing), the mutation triggering CRISPR-Cas tool is introduced into the lab genotype and triggers mutations in the elite line without being introduced into its genome.

7.2.2 The combination of GS and GE in plant breeding

GS and GE have obvious complementarities. On the one hand, GS makes use of QTL data but does not require identification or a functional understanding of causal genes underlying the QTL. On the other hand, GE depends on the link between genetic variation and phenotypic variation being mechanistically understood. So, can these two technologies be used together for efficient plant breeding? In the absence of regulatory constraints, the following possible options for doing this can be envisaged.

Research priorities

- **The use of novel alleles produced by GE in GS:** It seems rather straightforward to introduce novel alleles into the assembly of new varieties by **GE**, and in particular novel alleles not available in the natural diversity of the species. However, a theoretical framework for the optimal timing of this introduction in GS schemes still needs to be established by computer simulations.
- **Accelerated domestication by GE:** There is presently a strong trend to enlarge the genetic basis in plant breeding by introducing interesting adaptive traits from wild relatives into elite lines of crop plants. This process is often hampered by a strong loss of agronomic performance after wild crosses. This is the result of introducing several negative characteristics from the wild relatives into the elite lines alongside the introduction of desired traits. If the genetic basis of the desired trait is known, the causal polymorphism can be edited into a crop genotype (which will lack, or nearly so, the negative characteristics of the wild relative) and this modified genotype can then be used to more efficiently integrate the new allele into elite genotypes. Alternatively, in species where key domestication genes are known and amenable to genome editing, the wild relative with its desirable traits can be converted into pre-breeding material suitable for further breeding in a single generation by multiplex genome editing of domestication genes. A proof-of-concept for this approach has been demonstrated using wild tomato.
- **Allele conversion by GE:** In GS, the assembly of dozens of favourable alleles into a single plant is a time-consuming process. In the cases where the causal polymorphisms are known, multiplexed conversion of unfavourable to favourable alleles by **GE** can greatly speed up the process. So far, there is no proof-of-concept for this theoretical approach.
- **Fast breeding**: Another aspect where **GE** could be efficient is for the reduction of generation time from seed to seed, notably for perennial crops. Examples exist for grapevine and apple.

7.3 GE-mediated plant breeding tools

The breeding tools available for a given plant species vary greatly due to the biological constraints, most notably at the reproduction phase. GE could be used to transfer certain breeding properties or characteristics from species where they naturally exist to others where they do not and so accelerate breeding efficiency. In addition, GE could be used to add completely new possibilities to the palette of tools presently available to breeders.

Research priorities

- **Doubled haploid (DH) technology:** Maize breeding has been profoundly accelerated by inbred-line creation through a unique ***in vivo*** system that creates haploid lines. These haploid lines are a short-cut to the production of a homozygous diploid line. More recently, **GE** has allowed this mechanism to be transferred from maize to wheat and rice. Further efforts are needed to transfer this property to dicots.
- **Fine tuning of gene expression:** Variation in quantitative traits is often due to cis-regulatory variation rather than coding variation, causing temporal or spatial changes in gene expression. As our knowledge about the regulatory code controlling gene expression increases thanks to new technologies such as single cell and spatial analysis of transcription and chromatin accessibility, it will become possible to identify single cis-regulatory elements and target them for editing to affect gene expression and consequently the phenotype. Gene editing can also be used as a tool for random mutagenesis of regulatory regions of specific genes to induce gene expression changes as has already been shown in tomato.
- **Abolition of self-incompatibility:** Self-incompatibility inhibits generation of inbred lines by recurrent selfing, which can be a serious constraint for efficient plant breeding. **GE** can be used to overcome this barrier and to facilitate inbred-line production or fruit production in self-incompatible crops, as recently shown in chicory**.**
- **Recombination rate:** In GS, the assembly of individuals carrying favourable combinations of alleles depends on random, and sometimes rare, recombination events that occur during meiosis. Depending on the stage of the breeding process both higher (mixing) and lower (fixing) recombination rates are desired. Approaches using **GE** to modulate the recombination rate as required now become feasible but proof-of-concept has yet to be provided.
- **Targeted recombination:** Transferring a desired gene allele from one cultivar to another also depends on recombination between their genomes, a process that occurs at meiosis. Recombination will, however, transfer not only the desired genes alleles but also alleles of other genes that are genetically linked with the desired gene. These other genes alleles may have undesirable effects on the target cultivar - a phenomenon called 'linkage drag'**.** Targeting meiotic recombination to specific sites in the genome would allow breaking of linkage drag between alleles for desired traits and those for genetically linked deleterious traits. This can possibly be achieved by GE in just the same way as structural rearrangements within or between chromosomes.

7.4 Regulation of GS and GE

Regulation of GS and GE Plant varieties, independent of breeding methods are subject to a comprehensive regulatory framework for EU-breeders, seed producers, processors et.al. (e.g. Protocols for DUS and VCU testing ; Seed Marketing Directives (….) and Official Controls; Plant Health Regulation) Certain plant products are in addition regulated under the regulatory framework for genetically modified organisms. These regulations are disproportionally expensive and time-consuming, in practice banning their use for cultivation.). This is a strong disincentive for any company to enter the EU market and reduces competitiveness and diversity of the EU seed sector and SMEs specifically (Jorasch, P., 2020) Potential, Challenges, and Threats for the Application of New Breeding Techniques by the Private Plant Breeding Sector in the EU; Front. Plant Sci., 2020 | https://doi.org/10.3389/fpls.2020.582011) In many other parts of the world, a differentiated regulatory approach has been introduced for plants obtained by GE. If the genetic change in the product of GE is similar to genetic changes in products of conventional plant breeding or spontaneous processes in nature, they are not subject to biotech regulations. The situation in Europe may evolve in coming years since the European Commission is carrying out a consultation process in the second half of 2022 that is part of its roadmap to develop a proportionate regulatory framework for plants obtained by targeted mutagenesis and cisgenesis. The outcome of this regulatory revision and the time of implementation will be decisive for the access and use of these new breeding tools in research and breeding for Europe. Genetic diversity and the domestication of plants.

Genetic diversity is the raw material that breeders use to develop new varieties with improved characteristics. Without genetic diversity, long-term genetic gain by breeding cannot be sustained, and breeding will fail to match the challenge posed by the climate crisis and meet the demand for crop productivity that is economically and environmentally sustainable. Agrobiodiversity is not only the biological diversity of those species associated with agriculture, but it is also the result of human ingenuity applied to the challenge of improvement of domesticated plants over about ten millennia. Genetic diversity refers to the diversity within the breeders gene pool (EFSA Journal 2012;10(2):2561 – section 2.1). It is therefore a reservoir of variation that can be accessed by breeders and researchers to provide valuable traits for crop improvement, including quality, yield and yield components, disease resistance, and adaptation and tolerance of local growing conditions.

Since the Neolithic revolution, mankind has selectively shaped the available biological diversity into something more desirable and productive than that offered by the wild ancestors of our domesticated plants and animals. The converse has also happened. The development of improved breeds has led to changes in human evolution and to social and behavioural changes, so there has been a co-evolution of mankind and the domesticated species.

Not all aspects of genetic diversity are desirable for modern farming systems. Negative traits that made the wild crop relatives and early domesticated varieties of plants less suitable for agricultural use, including shattering, lodging, seed dormancy, weediness, and low productivity, have been diminished, while the frequency and magnitude of desirable traits have been increased. These changes were firstly the result of unwitting selection by early farmers of better genotypes, followed later by more deliberate selection. While the resulting improvements to our crops made agriculture more productive, they also narrowed the genetic diversity remaining in the cultivated crop . This resulted in the formation of ‘selection bottlenecks’ in the genetic diversity of crops. This process culminated with the green revolution of the 20th Century, when advances in genetics, chemistry, and mechanization enabled a step change in crop yields worldwide. In addition, global agriculture is relying on high performing, but highly uniform allelic combinations. The reduction of genetic and crop diversity makes agriculture less resilient especially in the wake of new biotic and abiotic stresses deriving from climate change.

7.5 Genetic diversity and the future of crop plants

To further improve our crop plants and make them more resource efficient, nutritious, high yielding and climate-ready, modern breeding needs research methods that can identify and extract desirable genetic diversity from the breeders gene pools of our crop species. By tapping into this wealth of diversity, plant breeders will produce the step changes needed to address the challenges of 21st century agriculture. The exponential evolution of -omics technology in the past two decades, including genomics, metabolomics, and phenomics, has enabled fast and cost-effective screening and assessment of genetic agrobiodiversity. DNA sequencing is now a routine and cost-effective task for crop germplasm collections. By sequencing the DNA of landraces, wild relatives and varieties belonging to the same crop species, this allows mapping of the extent of variation that is available to breeding, and unravels the genetic information specific for each individual and that determine their traits. In this post-genomic era, the challenge becomes assigning a meaning to the sequences, and to do that requires identification of those genes and genetic factors that underly any desired phenotype. **This will be one of the key tasks of CropBooster.**

7.5.1 The genetic basis of traits important in crop plants: quantitative trait loci

Most traits of agronomic relevance are quantitative in nature, meaning that they are controlled by several different loci (or genes) on the genome. Following recent advances in molecular biology, the CropBooster consortium recognise there is no easy way to adapt most quantitative traits by adjusting any individual genetic factor. With few exceptions, there are no “silver bullet” genes that can revolutionise the target crop traits needed to improve: yield and yield components, photosynthesis, adaptation, these are all complex traits controlled by a large number of small-effect genetic factors called quantitative trait loci (QTL). This distribution of control of traits across a larger number of loci brings with it the advantage of protecting the traits against the effect of mutations, because single mutations will have only small effects on the trait. This makes traits, and therefore fitness, more stable. QTL are therefore the genomic locations that contribute to genetic control of trait determination, but mostly only to a small extent. They frequently interact with external environmental conditions including management practices. The improvement of complex traits therefore depends on two things:

i. the adaptation of QTL, and a large effect will depend on the manipulation of several QTL, and
ii. understanding of how the **E**nvironment interacts with **G**enotype to shape the final phenotype (the so-called **G** X **E** interaction).

Crop improvements can be achieved by conventional hybridisation techniques that basically depend on combining the genomes of two parents that each have desirable traits and looking for favourable combinations of the traits in their progeny. This is relatively practical for simple traits, like flower colour or disease resistance, but is much more difficult for complex traits due to the large number of genetic factors associated with each trait, and the small likelihood that all the desired variants of the genes will end up in one individual. If the QTL underlying the trait are known, or even better, if the specific genes are known, then the improvement of traits can be accelerated using molecular breeding approaches, including GE that depend on having very specific knowledge of the DNA sequences underlying the traits of interest. This requires unravelling the connection between the genome and the phenotype and identifying the QTL that give rise to a trait. Once the QTL underpinning variation in a trait are identified it is possible to use this information to breed for improvements in this trait. **In the EU, the regulatory restrictions (described above) currently means that using gene editing or other regulated GMO techniques to produce a marketable cultivar for agriculture and food production is highly unlikely.** There is a growing momentum for change relating to **GE**.

The process of identifying the QTL underlying a trait is referred as mapping, and the mapping and characterization of QTL relies on statistical methods that analyse the covariation of genomic diversity and phenotypic diversity in a group of genetically distinct individuals. By covariance we mean identifying variation in the genome that correlates with variation in the phenotype. Different methods for mapping exist, and the rapid developments in genomic data production capacity and big data analysis make QTL mapping a dynamic research area. Once QTL have been identified in a collection of individuals, genomics, phenomics, as well as metabolomics and proteomics, may be used to reconstruct the network of molecular mechanisms that connect genetic variation to the phenotypes and which are the targets of breeding. As the problems of measurement and data production are now less of a limiting factor, the key to fully characterizing the genetic basis of complex traits in any species, and thus advancing breeding is, once more, agrobiodiversity - the available pool of genetic diversity that can be analysed. The choice of genetic materials that will be analysed with the available toolbox of omics technologies determines the range and significance of QTL that will be detected. A selection of individuals that provide an incomplete or biased representation of primary and secondary allele pools will reduce the effectiveness of QTL mapping approaches and limit the scope of future discovery.

QTL mapping depends on having a genetically diverse collection of plants (a population) that also has variation in the trait of interest. By comparing variation for the trait with genetic variation the QTL can be discovered, so overall QTL mapping is a statistical association exercise, where something relatively simple - variation in a trait - is compared with the genetic differences (which are often huge) between the individuals that make up the population. There are two important elements in QTL mapping: first, phenotyping of the trait, which must be precise, repeatable, and accumulated on hundreds of individuals and, ideally, different environmental conditions, and second the mapping population itself. The following sections will describe the basic kinds of mapping populations.

7.5.2 Mapping populations

There are two main types of mapping population; those that are assembled from existing genotypes of the species, and those that are made up genotypes that have been custom-made for mapping. Each of these approaches have advantages and disadvantages.

Diversity panels as a route to QTL identification

Naturally occurring mutation and evolution gives rise to the gene pool of a species. Gene pools of this kind contain the genetic diversity that was the foundation of our crop plants during the domestication process and the later development of these species by breeders. Due to the genetic bottleneck mechanisms explained above, the less highly bred a genotype is the greater the genetic diversity it is likely to represent (diversity is a relative term). The more primitive the genotypes - the more they are like wild ancestors of the crop species - the greater will be the pool of diversity they represent. A collection of genotypes of this will therefore contain genetic diversity as well as variation for traits and such a collection is called a diversity panel. The discovery of gene-traits associations in diversity panels is normally done using a genome wide association study (GWAS). This approach is commonly used to map genes underlying variation in traits in human populations.

The advantage of a diversity panel is that it can include a wide range of phenotypic diversity, especially if it is made up of wild relatives or ancestors of crop plants, and through many millennia of crossing, the genome of the members of the panel has become very subdivided. It is these subdivisions that will be mapped in the first instance. The subdivisions (or haplotypes) normally contain just a small number of genes, which means that if an association between a haplotype and a phenotype is discovered it is relatively easy to identify the causal gene. The disadvantage of mapping using a diversity panel is that the complex relationship between a genome and a phenotype, taken alongside the intrinsic genetic diversity of the panel, means that associations between the genome and the phenotype can be weak, making it harder to reliably identify QTL. For example, it is necessary to take account the relatedness between members of a panel because related genotypes (for example from the same geographic region) can result in false associations simply because non-causal haplotypes may co-vary with causal haplotypes within this region.

Biparental mapping populations as a route to QTL identification.

Diversity panels represent genetic diversity in the form of genotypes that are the result of mutation and evolution, possibly including selection by farmers and breeders, and so are built of existing genotypes, which may be very ancient. An alternative container of genetic diversity is the biparental mapping population. These are custom made mapping populations that are the result of crossing between two or more parents followed by further crossings to create a population that contains genetic contributions from the two parents. There are several forms of biparental mapping population that differ in how the crossing is managed. While a diversity panel may theoretically represent all the genetic diversity of a species, a biparental population contains only the diversity of parents, and this may be limited. This however greatly simplifies the mapping system making biparental populations a key tool in crop genetics. A biparental population can be built using two parents with contrasting phenotypes, and the progeny will display the intermediate phenotypes (and sometimes greater diversity than that which extends between the parents) that can usually easily be mapped. Biparental populations are therefore good at allowing mapping of genes between parents with contrasting phenotypes. However, the small diversity represented by the two parents of choice can result in it being difficult to identify QTL for correlated traits. The capacity to characterize trait trade-offs and interactions between different genetic factors may also be reduced in biparental mapping populations. As a result the QTL identified may be specific to the genetic background of the selected parents and may have a different behaviour when introduced in different genetic backgrounds for breeding purposes. Another disadvantage of these populations is that because of the small number of crossing events separating the parents from the individuals in the population, the degree of genetic mixing between the parental genomes is small, so the haplotype blocks are big, and may contain many thousands of genes. Identifying the causal gene or genes in this block usually requires fine-mapping - further mapping involving further hybridisation (or making use of pre-made populations for fine-mapping if available), which is time consuming. The advantages of biparental populations and diversity panels are therefore complementary: diversity panels are genetically precise but statistically weak, while biparental populations are genetically imprecise but statistically strong.

7.5.3 The best of both worlds: Multiparent populations.

In recent years, a third way to do QTL mapping has emerged that combines the strengths of GWAS and QTL mapping using biparental lines, by increasing diversity in a crossing pedigree. Multiparental populations (MPP) have been now developed in major crops and model species by intercrossing up to 50 multiple parental lines, in a common crossing scheme resulting in a progeny segregating for multiple parental alleles. MPPs can be produced with different crossing schemes, most frequently multiparental advanced intercrosses (MAGIC) and nested association mapping (NAM) populations. When compared with classic biparental QTL mapping populations, MPP segregating progenies represent a larger portion of agrobiodiversity, due to the multiple parental lines, and higher reshuffling of that diversity due to multiple intercrossing generations. This in turn results in better abilities to map the QTL and higher precision in identifying their location on the target genome, without the downsides of GWAS mapping methods. Thanks to their closed crossing design, MPPs allow the layering of multiple -omics data that is then used to further refine the localization of genetic factors responsible for the trait of interesting. In this sense, MPP aim to be an everlasting platform for QTL mapping.

7.5.4 Data-driven methods; towards genomic selection

The availability of increasingly large -omics datasets makes possible the prediction of trait values from information at the DNA level. This **genomic selection** approach is now commonplace in crop breeding. It requires large collections of data characterizing genomic and trait diversity in collections of individuals, either diversity panels or crossing populations, grown, and measured in different environmental conditions and experimental setups. The larger the available pool of data and the more representative or relevant that data is, the more comprehensive and accurate will be the training of genomic selection models. Having formalized a relationship between genome-wide DNA diversity and trait values, genomic selection models can then be used to predict trait performance in collections for which the genomic information is known but the trait values are unknown, e.g., in progenies of crosses or in selected agrobiodiversity collections. It can also be applied to known genetic materials to forecast how they will grow in untested locations and varying environments, including climate change scenarios. Genomic selection derives what are called genomic-estimated breeding values, which are then used to select specific allelic combinations to support genetic gain (ie improvement by genetics). These models do not require QTL information, and fully capture the myriad of small-effect variants contributing to trait determination. Their use in breeding improves selection accuracy while reducing costs linked to phenotyping. They also provide a data-driven framework to characterize trait correlations and trade-offs, allowing to advance selection of genotypes in a holistic manner.

7.5.5 Means of genotyping

The genomics revolution, allowing the genotyping of hundreds of individuals with thousands of molecular markers, offers the technology supporting the mining of allelic diversity in crop collections. In the last two decades, advancement in sequencing technologies resulted in higher throughput and lower associated costs, enabling the characterization of complex genomes. Crop genomes are particularly elusive, since they are extremely large, feature extensive regions with repetitive DNA elements, widespread structural variation, and often result from polyploidization events. All these features make it more difficult to provide an efficient and faithful characterization of the diversity existing in crop collections and require cutting edge sequencing approaches to fully assess their potential. In recent years, genomic tools have seen the introduction of long read sequencing, that further reduced sequencing costs and increased the capacity to describe crop genomes. Concurrently, improvements of assembly algorithms and bioinformatic tools to deal with complex data has allowed whole genome sequencing to be widely used for *de novo* assemblies as well as re-sequencing of both model and non-model plant species. Availability of this information, followed by a comprehensive re-annotation of gene models can enhance the mapping of QTL. Draft genome sequences are currently available for numerous crops, supporting the characterization of natural allelic variation with increased efficiency. Genomics is an enabling technology that comes in many flavours; besides genomic sequencing *per se*, an array of different technologies and strategies for genotyping can be used to describe variation at the nucleotide level, most commonly identified with single nucleotide polymorphisms. Depending on the type of population used (that is, diversity panels or segregant populations) and depending on the type and amount of agrobiodiversity that is under study, researchers may choose among a vast array of genotyping technologies to find the one that suits the research the most. Once genomic data is used in genetic mapping, research can push the boundaries of our understanding of the molecular mechanisms determining traits as well as support breeding efforts aimed at the production of more efficient varieties.

7.5.6 Mapping Methods

Big data coming from genomics, phenomics, and other large-scale experiments requires appropriate tools to allow it to be analyzed to find the important biological signals. It is no surprise that bioinformatic tools evolved hand-in-hand with the availability of omics data. Plentiful approaches exist, each tailored to specific data types and applications. The optimization of those methods means that it is now possible to run most of the analyses on laptop computers, though this level of hardware becomes limiting it is possible to transfer the task to remote high-performance computers that are now widespread in the scientific community and industry.

In absolute terms, mapping of QTL and development of genomic selection models are both relatively straightforward statistical approaches based on regression of trait values against DNA diversity. They are based on the availability of genomic diversity information in a group of individuals (the population) through DNA markers, and trait variation in this same collection of individuals. DNA diversity is used as an independent variable, and trait variation is tested as the dependent variable to search for significant associations between the two. However, several factors needed to be taken account of to ensure that regression approaches are optimally suited to the data. These include the type of the genetic materials, the type and amount of molecular data available, and the target trait(s) of the analysis. When using a diversity population or a segregating population, different mapping approaches can be used. When using a diversity population Genome-wide association studies (GWAS) are performed using single-marker tests that each test the association of allelic variation and the trait. In the case of segregating populations, QTLs are mapped by co-inheritance with blocks of DNA that are reconstructed from the pedigree of crossed lines. The reconstruction of blocks of inheritance as well as the capacity of the GWAS to detect associations depend on the density of the markers and the amount of recombination: the greater these are, the better the definition of candidate variance. Different approaches can be used to correct the results of the mapping analysis for uneven genetic structure of the population that may result in inflation of the statistic and increased false positive rate. These methods include the incorporation of covariates as well as the computation of estimated kinship values across individuals. Mapping methods are typically implemented in open-source software packages that are continuously updated by the scientific community. There is room for further improvement and refinement of mapping methods, and promising avenues are represented by Bayesian statistical approaches as well as by deep learning methods leveraging the sheer availability of data. Artificial intelligence systems can be trained to recognize specific combinations of nucleotides and use this information to support the prediction of phenotypes. Regardless of the methods used, once QTL or association peaks are identified, different approaches can be used to narrow down the mapping intervals to the genetic elements that are causative to the trait. This depends on several features, including the structure of the population used for mapping. DNA sequencing and RNA sequencing data can be overlaid to QTL mapping data to guide the identification of candidate genes. Once that happens, and with the guidance of reference sequence information in the region of the QTL, it is possible to target candidates via biotech approaches including GE.

7.6 Natural Variation Driven Model-Assisted Crop Improvement

Exploiting natural variability for yield and related crop performance traits provides a means to address the societal challenges facing agriculture is being explored in multiple projects. The Case Study of photosynthesis in the ongoing H2020 CAPITALISE pilot study (https://www.capitalise.eu/) will be used to illustrate the potential of the modelling approaches as a basis for future CropBooster strategies for crop improvement. This approach entails first gathering of large, heterogeneous data for crop genetic resources to characterize their variability at the genomic, molecular, physiological, and performance level. Mathematical modelling approaches are in turn developed and applied to analyse the resulting data and to plan the next steps of crop improvement.

Mathematical modelling approaches for crop improvement can be categorized into: (1) statistical, (2) mechanistic, and (3) hybrid. This categorization reflects the type of problems addressed. For instance, statistical approaches aim to identify and model relationships between measured features and traits solely based on the measured data. Common representatives include the now classical machine learning approaches as well as modern deep learning techniques to solve regression and classification problems. In contrast, mechanistic approaches, as the name indicates, use the established knowledge of molecular mechanisms to identify the key determinants of studied traits. Mechanistic approaches can be classified based on the type of processes modelled (e.g., steady state vs. dynamic, stochastic vs. deterministic) and depend on system parameters that are either obtained from literature or are inferred based on the gathered data. What is common to both approaches is that they aim to predict yield-related traits for unseen individuals and/or environments that can guide the development of crop improvement strategies.

Crop improvement strategies rooted in statistical modelling rely on availability of genomic data and data about studied traits to first identify genetic basis of the studied traits. For instance, mapping approaches (e.g., genome-wide association) can pinpoint loci statistically associated with a given trait. As a result, they can propel the discovery of genetic and molecular mechanisms underlying the trait. In contrast, genomic and phenomic prediction approaches neglect the identification of loci controlling a trait and instead aim to identify individuals with desired traits based on purely data-driven models. In the context of CAPITALISE, for example, both of these strategies are being used to determine loci underlying photosynthetic efficiency as well as genotypes with improved performance with respect to photosynthesis-related traits. Since photosynthetic efficiency affects and is determined by multiple other traits, the intention is also to develop statistical approaches to predict multiple traits. As a result, novel use of heterogeneous data available from past projects can be collated and used as demonstrated by part of CAPITALISE. **The resulting approaches can be used in any setting that entails the prediction of multiple traits from genomics data**.

Improvement of a process (e.g., photosynthetic efficiency) with mechanistic models allows the identification of the bottlenecks of the underlying molecular and signalling pathways and to design strategies to overcome these bottlenecks. For instance, kinetic models of the Calvin-Benson cycle – the collection of metabolic reactions underpinning photosynthesis – have been used to determine reactions whose rate increase is predicted to increase photosynthetic rate. In addition, and in contrast with statistical modelling approaches, mechanistic models can be used to simulate unseen environments. However, the discovery of bottlenecks and simulations of different environments with the help of mechanistic models of cellular pathways requires knowledge about the parameters that denote key enzyme kinetic properties (e.g., turnover number) and can be used to describe the rate of the modelled biochemical reactions. To this end, data about the concentration of the modelled molecular components is needed to obtain crop- and individual-specific estimates of enzyme parameters. Nevertheless, the parameter values used in the existing models of photosynthesis are measured from in vitro assays that may differ in orders of magnitude compared to those from *in vivo* estimates. In the context of the CAPITALISE project the aim is to develop crop-specific models of photosynthesis, parameterized based on data gathered from experiments. Such an approach, applied with data from multiple crop individuals, will allow surveying the variability in parameter values and identify those that distinguish individuals with low and high photosynthetic efficiency. Yet, while this approach makes excellent use of the collected molecular and physiological data, it does not explore the potential of linking genomic data with enzymatic parameters for informed selection of better performing individuals, as done in the case of statistical models above.

Hybrid modelling approaches rely on combining statistical and mechanistic modelling techniques to make full use of the big, heterogeneous data gathered from experiments surveying natural variability. In addition, hybrid models have the potential to simulate both unseen individuals and environments – essential for selection of individuals with traits tailored for future climate conditions. In CAPITALISE, a recently introduced hybrid modelling approach called network genomic selection is being used. This combines genomic prediction with mechanistic models of metabolism with the aim of improving the accuracy of growth prediction.

The development of modelling approaches for crop improvement in the next five to ten years is expected to: (1) identification of master control loci for multiple traits and prioritizing of loci for experimental validation by developing network-based approaches, further propelling the application of genome-wide association, (2) integration of epistatic and environmental effects in genomic prediction models, improving the accuracy and widening the applicability of genomic and phenomic prediction approaches, (3) mechanistic models at the level of individual and micro-environment, aligning the modelling developments with precision agriculture, and (4) strengthening the hybrid integration of machine / deep learning models with mechanistic models of cellular processes (beyond metabolism) to predict yield-related traits. For instance, the latter can include genomic prediction of enzyme kinetic properties that can be integrated with large-scale models of crop metabolism to improve predictions of molecular traits (e.g., protein allocation) and growth, at the cost of requiring more data for model training.

7.7 Plant Phenotyping - what is it and why do we need it

CropBooster - aiming at identifying, designing, selecting future-proofed plant varieties - will actively rely on our capacity to capture phenotypes ie single or series of plants traits related to the structure, the function and the performance. This capacity must be developed on large numbers (classically hundreds) of plants to be compatible with genetic analyses. Because most traits targeted are affected by the plant’s environment, a similar effort has to be done to capture both the aerial and the underground (bio)physical environment. Finally, this information needs to be analysed, organized and stored through efficient workflows.

Traits can target the appropriate spatial scale depending on the purpose: the cell (eg cell wall composition), the organ (eg leaf orientation), the plant (ie plant growth rate), the canopy (ie canopy temperature). Analyses should also consider the many feedback and feedforward loops between the plant and its biotic and abiotic environment (eg leaf angle will influence canopy temperature and thus transpiration). Traits can be measured and evaluated at fixed points in time (eg plant height at onset of seed filling) or as time dependent phenomena (eg plant growth rate or senescence). Phenotypes may also be seen as multivariable traits (ie patterns) leveraging on high throughput omic techniques (proteome, ionome, metabolome, transcriptome, fluxome etc).

The impact the plant has on its environment (eg changes produced in the soil microbiome, root exudation etc.) is also part of the phenotype and need to be considered in the impact analysis. Importantly, the environment the plant is exposed to, having great influence on the phenotypes, may be to a greater or lesser degree subject to human management (eg sowing dates, planting density are major levers of risks management). Finally, as soon as integrated traits are considered, including interactions, feedback loops and time dependencies (yield is typically in this category), models are essentials to predict the outcome of a series of traits and help to foresee whether traits can be considered “favourable” in current and future varieties front of a range of climatic and management scenarios.

7.7.1 Criteria for efficient phenotyping systems

The following are important considerations in the design and use of high-throughput phenotyping systems:

(i) The phenotyping system should be carefully calibrated with appropriate standards and ground truth data..

(ii) The temporal sampling should match the rate of change of the phenotype. For instance, stomata reopening must be captured within minutes. Carbon stores are classically evaluated at end of day or end of night. Phyllochrones change within days.

(iii) The measurement technique should be as much as possible non-invasive and non-destructive so that phenotyping large population is doable and statistical issues related to considering independent plants for sampling are minimized

(iv) The phenotyping system must be high throughput to accommodate enough individuals to meet the demands of the (eg) genetic analysis: and be statistically powerful enough to account for low signal / noise ratio. An example is yield contrasts of eg 5% which can have massive impact on production in a region but be hard to capture with an under powered statistical design.

(v) The spatial scale of the phenotyping needs to be carefully considered. A classic example is photosynthesis at the cm² level that rarely scale up to C capture at the whole plant level because of self-shading, leaf orientation, light saturation of sunlit leaves etc. Models are essential for upscaling.

(vi) The activity around data must be carefully anticipated. There is a structured community developing ontologies, semantics, making workflows reusable in order to make the whole data process truly FAIR.

(vii) As any R&D activity, HT phenotyping is costly with a Return on Investment (ROI) very difficult to estimate. Nevertheless, cost per plant (or plot) should be reduced as much as possible to allow more data to be capture, allowing higher statistical power. This perspective becomes possible thanks to low-cost sensors, open-based computers and open access workflows.

A future crop improvement programme will require a major expansion of phenotyping systems within the EU, fulfilling these criteria, as planned within Cropbooster and associated programmes.

7.7.2 Technology challenges

HTP heavily rely on non-destructive imaging.

Phenotyping technologies range from the simple (eg RGB) to the highly sophisticated and expensive (eg x-ray, fluorescence or Raman spectroscopy). Many of the techniques that offer an analytical or physiological measurement (x-ray fluorescence, NIR spectral imaging, chlorophyll fluorescence imaging) have a pedigree that extends back to bench-top instruments and deep phenotyping; the use of these technologies is therefore supported by an extensive base of protocols and experience. There are at least two challenges with the use of instruments of this kind (i) integrating them into the robotic systems for data acquisition and (ii) making the most out of the data collected.

Beside non-destructive imaging, some phenotypes are manually measured on plant samples using established, conventional laboratory analytical techniques. In principle these manual methods could be automated, or translated to, or replaced by, robotic, high throughput field phenotyping systems. Working jointly with other programmes, such as ESFRI EMPHASIS, Cropbooster will be active in developing new automated phenotyping hardware. This will involve cooperation with engineers and technology providers (either in academia or in the private sector thanks to the proximity between technologies for phenotyping and for precision agriculture), plant biologists and agronomists, physicists, and chemists. Developers will both write the control software for the HT phenotyping devices but also the model-based and machine learning systems that are full part of the data translation methods to convert machine-level measurements into biological data.

Controlled conditions vs field conditions

High throughput, robotic phenotyping systems can be split between those built into controlled environment rooms, those built into greenhouses, and those that use open field plant cultivation. Of these, the controlled environment rooms offer the highest quality control of the growth environment, while field sites are representative of the growth conditions that plants will experience in agriculture, with greenhouses being in between these two cases. While controlled environment rooms rarely fully simulate field conditions, although the level of environmental control available in growth rooms is improving, especially since the advent of LED lighting, which allows both a higher irradiance, up to the level of natural sunlight, and better spectral control of that irradiance. Additionally, software that accurately simulates weather in 'real time' is now routinely available and can provide high resolution fluctuations. Realistic soil environments can also be increasingly reproduced using large plant growth containers. Controlled environment and greenhouse phenotyping system are usually where the more advanced, sophisticated phenotyping technologies are be found. In the early stages of their application to phenotyping, these more sophisticated techniques are often not rugged enough for field use, and they may also need a controlled measurement environment that is not available in the field. Phenotyping facilities with access to controlled environment rooms and high-tech phenotyping tools in the EU are currently centred on a relatively small, yet rapidly growing, number of institutions - high throughput phenotyping systems are increasingly seen as ‘must-have’ technology. The distribution of these facilities will need to be extended, monitored for functionality and complementarity, and supported for access policies and technical upgrades, and again Cropbooster expect to work with ESFRI EMPHASIS to achieve this.

Despite the convenience of working with controlled environment and greenhouse phenotyping systems, scientists need to transition more phenotyping technologies to the field. In the case of high-throughput techniques this will need robotic land-vehicles and drones, and adaptations to the high-throughput phenotyping tools currently restricted to controlled environment spaces to make them suitable for field use. One of the advantages of field phenotyping is that it is not so dependent on specialised phenotyping centres built around controlled environment or greenhouse systems. With effort, field sites can even impose heat and drought treatments with elevated CO_2. Field sites can be located where most useful, taking account of the diversity of soils and climates in Europe. These field sites can also be relatively cheap to maintain. Potentially, field phenotyping could develop into a technologically advanced sector dependent on robots and high-tech sensors and able to use farmer's fields as 'living laboratories'. This will require advances in engineering, instrumentation design and application, and the software and hardware tools needed to allow the control of phenotyping in the field. The plant breeding sector also carries out extensive trials throughout Europe and if a more open relationship could be established with this sector, we could not only phenotype their trials but make use of historical trials data obtained from known genotypes grown throughout Europe and beyond, under a wide range of climate, weather and soil conditions. High-throughput phenotyping, also generates huge amounts of data and metadata that needs to be curated and archived in ways that allows its easy access and use by a large community of interested users (FAIR principles). The information systems that allow easy access to data and its processing needs to be made available - this will require software development but also the training of the community of users.

The plant in the field - new plant and environmental monitoring systems and deep phenotyping in the field.

The high-throughput phenotyping described above this should be supplemented with low-cost plant and environmental monitoring and with better deep-phenotyping under field conditions. With cheap sensors, Cropbooster aims to monitor the field environment of partner farmers, and extend this to low cost options for plant monitoring to provide some basic record of plant functioning on a 24/7 basis. This could be done together with the "Living Labs" idea developed by SUSCROP. This kind of monitoring when done by qualified scientists is very useful in defining better the plant/environment interaction, but is very expensive when done by hand. Where possible expensive people should be replaced with something cheap that can work around the clock. There are options (e.g. https://www.photosynq.com, https://www.robopec.com/en/constructions/instrumentalization-and-measures/phenoman/) that could be used as a springboard for a new generation of capable but cost effective instruments for field monitoring of plants. In addition to field monitoring, there is a need to have more detailed measurements made of plant (leaf and canopy) photosynthesis made in the field. We have very sophisticated tools to the deep phenotyping of photosynthesis in the lab - these tools can thoroughly analyse key aspects of photosynthesis. These techniques need to be repackaged and adapted so they can be used in the field to better understand how photosynthesis functions in the field. For example it is important to know when photosynthesis limits plant growth or when it is limited by plant growth. This will require the development of new forms of instruments and Europe has SMEs that can make this kind of hardware and the firmware needed for it work to actually make measurements. Cropbooster can build on actions such as HE-CAPITALISE. This requires physics, engineering, coding, physiology, agronomy and farmers in highly multidisciplinary collaborations to work with plants in field. The more science learns from the plant in the field the better plants can be improved for the fields of the future.

Manipulating the environment in the field

In addition to benefiting from natural variation in field climates, there are options to manipulate the plant microclimate. For instance, heat can be imposed either short or long term, using IR panels directed at specific field plots in the field (White et al 2011). These can be used to target periods of the day and night and also specific developmental phases. They can also be combined with free air CO_2 enrichment (TFACE) and field-imposed drought stress using rain out shelters and irrigation. This kind of promising approach has yet to be used for high throughput phenotyping but will be important to develop. It is also noticeable that controlled environment chambers are increasingly capable in terms of mimicking and replicating field environments with precision and should play a more prominent role (McAusland et al 2020)

7.7.3 Phenotyping for Photosynthesis

Measuring carbon dioxide fixation: the gold standard for measuring photosynthesis

Carbon dioxide fixation is the paramount photosynthetic process so if we would like to phenotype photosynthesis this would seem to be the process that should be measured. The problem with measuring carbon dioxide fixation is that the equipment is expensive, the method is relatively time consuming and it currently has not been automated so it has to be done by hand. Carbon dioxide fixation measurement systems are often call gas analysis systems. To make a single measurement of photosynthesis in leaf at ambient irradiance with a portable gas analysis system takes in the order of 5 minutes. Measuring a small mapping population (eg a back-crossed inbred line) with 75 genotypes and 4 replicates per genotype would take 15.6 hours with a single gas analysis system. Measurement of complete responses of photosynthesis to a range of light intensities (also called a light response curve) or carbon dioxide concentration (also called an A/Ci) provides data that can be further parameterised and so provides a more complete description of photosynthesis than a single point measurement, but these will take longer, 1.5 - 2 hours each, or, at 2 h per run, - 600 hours for the 75 genotype, 4 replicate population. Measurements of carbons dioxide fixation are therefore commonly viewed as ‘slow-throughput’. Worse, photosynthesis responds strongly to changes in the environment, and with changes in plant size and age, and it even varies over the course of day, so ideally all plants in a population should be measured within 1 - 2 hours and without any change in the environment. There is, therefore, a mismatch between the properties of gas analysis measurements and the ideal measurement of photosynthesis for phenotyping, especially if working with plants in the field. Using controlled environment facilities allows the environment to be controlled, but the time required to make a measurement is still a problem, and controlled environment facilities are not the real world. When measurements in the field must be used one solution is to increase the number of gas analysis systems, which is very expensive as a portable gas analysis systems costs 40 000 - 65 000 €, and to correct the data made under varying conditions in the field to standard values.

Whatever promise the future might hold, currently, however, phenotyping field grown plants with large numbers of gas analysis systems and considerable labour and cost has only been done for short periods of time (days/weeks time range). To reduce the amount of field work and the labour of managing the analysers in the field measurements are sometimes made on cut-shoots where leaves or shoots are cut in the field and taken back to a measuring station where the analysers are kept and were the photosynthetic responses are measured. For cost and manpower reasons, however, these approaches are likely to remain limited until more automation is available. There already exist robots for harvesting cucumbers and other fruit in greenhouses and the image processing and dexterity required to identify a leaf and attach a leaf chamber to measure photosynthesis seems to be no greater than identifying a cucumber and harvesting it. Robots capable of comparable tasks are being developed for phenotyping purposes (e.g. Lincoln and Nottingham, UK) Robotic systems could be used in the field with less human intervention and their development for field or other use should be a high priority. To follow - The future may include Robotic gas exchange systems that can identify a leaf using computer vision, apply a cuvette and make a measurement of photosynthesis or respiration (and a small grab sample) using multiple arms and cuvettes would increase the throughput. Robots capable of comparable tasks are being developed at universities (e.g. Lincoln UK and also Nottingham).

The high-throughput alternative to phenotyping using measurements of carbon dioxide fixation

An alternative to using gas analysis is to use optical measurements of photosynthesis; much of the high-through technology for measuring photosynthesis is based on optical imaging techniques that permit us to take a high-resolution image of photosynthetic activity at the leaf level - even imaging individual chloroplasts in leaves is possible. These imaging techniques are in general rapid in use and they allow truly high-throughput measurement of plant and leaf photosynthesis - measuring 1 500 plants simultaneously with a 2 s protocol will become possible this summer as some new facilities in Wageningen come on-line. The imaging of photosynthesis depends on chlorophyll fluorescence, light which is emitted by the energy-rich excited state of chlorophyll a that is formed when photosynthetic systems (leaves, algae etc) absorb light. Fluorescence is light, so it can be imaged and recorded by specially adapted cameras. Chlorophyll fluorescence has been used intensively since the 1980s as tool for deep-phenotyping of photosynthesis alongside its use for the more routine monitoring of plant photosynthetic functioning and stress. It is used even in very applied situations such as glasshouse control systems to monitor photosynthesis and stress. There is, therefore, an extensive body of literature and experience underpinning its use not only for high-throughput phenotyping but also for more general applications. With a palette of only 5 basic chlorophyll fluorescence measurements, made under specified conditions, a large range of more physiologically meaningful parameters can be calculated that describe in detail the operation and control of photosystem II (PSII).

Two parameters have been shown to be especially valuable; one, the operational light-use efficiency of PSII (ΦPSII), is used as a proxy for the light-use efficiency of carbon dioxide fixation, while the dark-adapted Fv/Fm is widely used an index of stress. There are three main ways of applying fluorescence and other kinds of imaging. In moving camera systems the plants are stationary and the camera moves to the plants - this kind of system is best suited to working with small plants. This system is often found in controlled environment rooms. In fixed camera systems the fluorescent imaging camera is part an imaging station that is fixed and to which the plants are brought using a conveyor system - this kind of system is used more in greenhouse systems when working with older larger plants. Finally, there are multiple fixed-camera systems (currently (mid 2022) only two in the world) in which a whole population of plants is imaged by multiple cameras and all plants can therefore be imaged simultaneously. This kind of system is best suited to small plants in controlled environment systems. Using chlorophyll fluorescence, therefore, it is possible to high-throughput phenotype for photosynthesis using robust protocols and robust instrumentation. It is possible to phenotype primarily for photosynthesis but also to use photosynthesis to monitor the physiological status of the plant, especially the stress status of the plant. The ability to mass-measure photosynthesis is with us.

What else can chlorophyll fluorescence be used to phenotype? - sustainability and resilience.

Imaging of photosynthesis allows the production of a spatially resolved photosynthetic map. Differences within and between leaves can be measured and patterns of change quantified. Chlorophyll fluorescence is accurate and reproducible, day after day, so long term measurements are not only feasible but done routinely. It can also be applied to phenotype processes other than photosynthesis, such as tolerance to mineral deficits and thus nutrient use efficiencies. Thermal (or infra-red) imaging (ie sensitive measurements of leaf or plant temperature) allows stomatal resistance and thus water loss by the leaf to be estimated, and this image, when combined with images of ΦPSII (an image of photosynthesis) allows the water-use efficiency of photosynthesis to be calculated. Imaging systems that combine thermal imaging and chlorophyll fluorescence imaging in one camera system have been built with this application in mind. Plant level properties that connect to agricultural sustainability can therefore be imaged using chlorophyll fluorescence and done so on thousands of genotypes.

Damage or injury leaves due to heat or cold stress can be sensitively imaged and measured, as can damage by pests and diseases. Plant and photosynthetic responses to stress or changes in the environment can be followed accurately in time and variation in the response of genotypes during and after the stress or change recorded in incredible detail. Plant Resilience to stress can thus be measured using chlorophyll fluorescence - again on thousands of genotypes if required. Much in fact remains to made of the millions of fluorescence images made as part of high-through phenotyping campaigns in the few centres in the world that are equipped to do this, which emphasises the need for better archiving and curation and wider accessibility and links to programmes such as EMPHASIS which tries to produce a common format for data and metadata in phenotyping.

7.7.4 Things to do better with fluorescence!

The spatial distribution of photosynthesis, which can be imaged with fluorescence, within and between leaves has information we are only beginning to exploit. The distribution of photosynthetic parameters within leaves is likely to be correlated with different kinds of stress (eg end product limitation, nutrient deficiencies) that we want to know about. Fluorescence imaging depends on light and cameras so it is also easy to combine with other optical techniques, such as methods to image leaf chlorophyll content, leaf anthocyanin content (often an indicator of stress), imaging of fluorescent proteins used to track changes in gene expression or cellular metabolic status, normal RGB imaging etc. Images can be taken at night using near infra-red light so plant growth and leaf movement can be monitored 24/7. Despite all the energy that has been put into chlorophyll fluorescence techniques and technology, its development as a phenotyping tool for above-ground plant processes is not yet complete. For example, there need to be improvements in whole plant fluorescence imaging of larger and more complex plants (like a whole maize plant), while controlled changes in the gaseous composition of the atmosphere around the plant of leaf allows chlorophyll fluorescence to be used to measure a wider range of physiological responses connected to photosynthesis. The technique also needs to be adapted to work better in the field where plants are often constantly in motion making imaging of fluorescence more challenging. Solar induced chlorophyll fluorescence can be measured from satellites and is still under development, but it will certainly be useful for measuring the amount of solar irradiance that has been absorbed by the photosynthetic machinery of the canopy.

Photosynthesis: Going beyond chlorophyll fluorescence

Chlorophyll fluorescence is in the class of light-based biophysical techniques developed for, and used in, photosynthesis research but it is not alone. Other techniques based on small wavelength specific changes in leaf light absorption (light-induced absorbance changes) that occur as part of photosynthesis are used effectively to deep-phenotype photosynthesis and could be used to provide even more information about this already information rich process. Being light-based these measurement technologies can be easily integrated with chlorophyll fluorescence measurements to extend the range of parameters that can be extracted from leaves.

More challenging, but more important, would be measurement of metabolic state of the leaf and using these measurements to infer other photosynthetic parameters that define the plant phenotype, though once scientists enter metabolic phenotype in general research moves beyond a simple association with photosynthesis and begins to get a window onto plant metabolism. Metabolic phenotyping has been attempted using the technique of near-infra red reflectance spectroscopy, or spectral imaging, using wavelengths up to about 2500 nm. When combined with machine-learning or multivariate techniques these measurements have used to estimate high-level photosynthetic parameters of the kind that would normally be estimated from the responses of carbon dioxide fixation to carbon dioxide concentration (an A/Ci curve). These photosynthetic parameters (Vcmax and Jmax) are phenotypically very valuable descriptors of photosynthesis. While getting the carbon dioxide fixation data to estimate an A/Ci curve takes 1.5 - 2 hours the spectral imaging procedure takes only seconds (positioning the plant or moving the sensor takes longer than making the measurement). These correlations between NIR spectral features and physiological properties are valuable, but better still would be a more information-rich spectrum than that provided by NIR reflectance that also gave information about the biochemical composition of the leaf. Raman spectroscopy could be the source of this kind of spectrum that could be used via machine learning or multivariate analysis to assess the higher level physiological status of leaf or plant non-destructively and thus extend the measurement of the phenotype. This application is, however, still over the horizon and a more certain approach would be to use a destructive approach (all the phenotyping approaches described so far have been fundamentally non-destructive) and collect fast-frozen leaf samples for an ‘omics analysis (the transcriptome, the metabolome, the proteome, the lipidome etc). While sampling of this kind is destructive and currently done manually, it would via the measurement of a wide range of ‘omics data very strongly anchor the phenotype within the world of biochemistry. Potentially it would allow a much more complete description of the state of the organisms that would link easily into metabolic models that would be used to amplify the value of data. To be able to make use of this omic’s dimension would require automation of procedures and large decreases in the costs of some of the procedures - automation and quantity are key to this.

7.7.5 Phenotyping growth and 3D structure above ground

Phenotyping of growth rate is routinely performed in all phenotyping systems using a variety of approaches. In the field, multispectral imaging of the normalised difference vegetation index (NDVI) or even simple RGB imaging can be cheaply done manually or remotely (eg via drones or land-based robotic systems) to separate and quantify the green area of a field or experimental plot due to crops. The green leaf area is measured in nearly all imaging phenotyping systems, where it is often used as a proxy for dry weight, or the 'digital biomass'. Imaging of the three dimensional architecture of canopies is more demanding. In addition to creating a 3D canopy model by combining 2D images from RGB cameras the 3D architecture of canopies can be measured directly using LIDAR. The production of 3D canopy models creates great opportunities for phenotyping the above ground growth and architecture of plants, but some problems remain. For example, the measurement systems are all optical in nature and are vulnerable to the occlusion of one (eg) leaf by another, and LIDAR produces a point cloud which needs to be converted to a plant image comprising leaves, stems, inflorescences etc. Despite these problems, 3D imaging can, nonetheless, present opportunities for analysis of a new range of traits related to 3D canopy architecture. Automated 3D and imaging reconstruction of plant canopies by LIDAR or RGB is a rapidly expanding and exciting area of phenotyping. The combination of 3D imaging with imaging of functional traits by e.g. fluorescence or spectral reflectance should be a priority.

Specific phenotyping challenges for architecture improvement

Canopy architecture, like root architecture, has inherent issues with high throughput automated phenotyping in a manner that generates high resolution data for pre breeding or research. It is greatly preferrable if architecture can be measured in field grown canopies where emergent properties of canopies (which show different properties than isolated plants) are visible. These conditions create problems however. Some traits can be measured with ease such as canopy height, green area coverage. There is a plethora of manual equipment available for field sensing of standard canopy traits such as LAI, extinction coefficient and fractional interception including light sensors, light bars and ceptometers (Murchie et al. 2018). Whole canopy (surface) traits can be measured directly or via proxies: remote sensing using hyperspectral reflectance combines with statistical regression has also been used to predict 'high level' traits such as canopy radiation use efficiency (Robles-Zazueta et al. 2021). A new methodology is needed that enables automated measurements of sophisticated photosynthetic and physiological traits in leaves obscured below the canopy surface

The desired outcome is a high-quality 3D point clouds or full surface mesh representations, preferably from field grown plants, that can be used for in silico analysis. This is something that is made difficult by occlusion in dense canopy stands, self-shading and movement of plants in the wind. It is usually not possible to gain a complete canopy structure without post processing, making assumptions or removing plants and scanning individually. Nonetheless, RGB and Lidar techniques are capable of producing 3D clouds of the accessible portions of the canopy. These are used for 2 purposes: first to directly extract 'standard' architectural traits such as angle, curvature, size etc (see below). Secondly, for more in-depth canopy analysis. Whilst the former can be done manually, methods are needed to refine and improve the accuracy and throughput of the latter. For the simulation of light and photosynthesis, a point cloud alone is insufficient and thus a full surface-based model is required. Whilst some such methodologies exist, they nevertheless remain time consuming, potentially costly (in terms of both money and knowledge required) and contain several restrictions or imperfections. However, once surface representations are available, light simulations can be performed such as ray tracing, which have been used to model the impact of canopy architecture on productivity and yield (Song et al 2013).

Improved phenotyping approaches can be achieved through use of advanced image analysis techniques such as deep learning. Deep learning-based feature detection can be used to extract architectural features, but this is complicated by the need for large training datasets and the lack of unique features needed for matching. Neither of these post -processing steps are currently high throughput and will require technical development and computing power. It will also be necessary to develop the relationship between photosynthesis, yield components and architecture via more sophisticated modelling. Nonetheless, the combination of 3D imaging in combination with deep learning from field grown plants remains a significant and achievable objective.

7.7.6 Phenotyping for below ground traits

Phenotyping for plant root architectural traits can be carried out under controlled conditions (growth chambers and glasshouses) or in the field, but both are challenging due to the opaque nature of soil. The simplest approaches are to destructively extract roots, wash off the soil and quantify the extracted root system but this will disturb the spatial arrangement of the root system, losing valuable information especially relating to the 3D structure such as root angle. Under controlled conditions, artificial media with suitable optical characteristics can be used with transparent containers (plates, rhizotubes, rhizoboxes) but comparisons with growth in soil are difficult. Rhizotrons (soil containers with transparent windows) are commonly used in high-throughput phenotyping platforms and whilst allowing growth in soil, usually only give 2D information on the arrangement of the root system architecture. Field rhizotrons in the form of tubes allow access to root systems in agri but (as with soil cores) only provide localised information. . Non-destructive imaging of root traits in soil and soil rhizosphere interactions in soil is currently only available in controlled laboratory conditions, using either X-ray Computed Tomography (X-ray, gamma-ray or neutron CT), Magnetic Resonance Imaging (MRI), or Positron Emission Tomography (PET) technologies. X-ray CT is perhaps the most commonly used of these technologies due to the greater availability of lab-based commercial instruments (which has been driven by non-destructive testing requirements of the engineering/ manufacturing sector) whereas MRI and PET are more specialist research instruments. Tomographic imaging approaches generate 3D maps of radiation attenuation by the sample (e.g. a plant grown in soil). Image segmentation techniques are then used to classify constituent materials and allow measurement of root traits in 3D. Plants can be grown under a range of controlled abiotic and biotic stress conditions to assess adaptive root response traits. Furthermore, as crucial information can be collected on the microstructure of the soil at the same time – insights into the functional behaviour of the soil-plant-(microbe) is possible. However, challenges exist with low-throughput of the technique. While advances in X-ray detector panels of commercial CT scanners have resulted in ever faster data acquisition times with higher spatial and contrast resolutions, image segmentation is still a major bottleneck. Computer vision approaches, based on machine learning, artificial intelligence offer promising innovate solution is to alleviate the image analysis challenge. MRI has the advantage of identifying roots of plant grown in soil via measurement of water distributions. Therefore, image segmentation is less of a challenge and as the plant receive no exposure to ionising radiation there is undoubtably less risk of undesired exposure effects. PET imaging adds benefits of measuring dynamic functional traits of roots in soil such as water uptake and movement of macronutrients, trace elements, and signalling compounds (e.g. Schmidt et al. 2020). Geophysical techniques (Electrical Resistance Tomography (ERT), Electromagnetic Impedance (EMI) and Ground Penetrating Radar (GPR)) have been used to study root form and function but are all limited to larger root systems and provide proxies for root activity (e.g., water profiles in the case of ERT) rather than direct measurement of root traits.

7.7.7 Remote sensing: spectral reflectance and thermal imaging

Non-invasive and remote analysis of crop stands for structure and function is one of the most sought after approaches for field phenotyping. Without having to rely on slow manual labour it can permit rapid and frequent assessment without disruption to growth. The high frequency of measurement permits integration of results over time which reduces anomalies caused by day variation in weather. It lends itself to complete automation meaning that uniformity across multiple sites is more easily achieved. The small form factor of modern sensors (Hyperspectral and multi spectral cameras, thermal cameras, RGB cameras) means that they can be easily loaded onto a suitable platform, whether this is manual, ground vehicle (phenomobile), drone or aircraft and guided by satellite navigation with great precision. With technical skill, large datasets can be obtained in a short period of time over plots with thousands of genotypes. For field phenotyping especially, remote sensing combined with reliable ground truthing will be critical as a tool for pre breeding and discovery.

What data can be collected in this way? Spectral reflectance along with RGB have become common go – to measurement because of its cost and the fact that the data can be applied to a large range of plant properties from growth analysis, pigment composition, photosynthesis, water content, nutrient content, stress condition. It is also available on contact or handheld devices where the data is more reliable. Typically, devices will collect information on the reflected (and by default re-emitted) radiation from the ground across a specific spectral band, depending on the device, typically between 250-2500nm although it is common for devices to specify regions (400-700; 700-1100 and 1100-2500) A hyperspectral (high resolution of wavebands) and multispectral (specific wavebands only) collect spatial data as well as reflectance data.

Typically, spectral indices are developed which reliably relate to a specific trait. This can have a known underlying basis such as chlorophyll and pigment content (NDVI or PRI), water content, but can also be used to generate reliable correlations for which the spectral index does not have a known basis. This has included drought and salt tolerance, water relations, chemical composition (protein, nitrogen, carbohydrate), root properties and more. ‘High level ‘traits such as yield and radiation use efficiency have also been successfully predicted using hyperspectral indices. Hundreds of indices have been published and it is possible to apply data to all of these or target a smaller number depending on the requirement. Deep learning algorithms have been used to mine data for phenotyping for abiotic and biotic stress that can be applied to breeding. A more amenable approach generates models of likely relationships using existing indices and has been used for example to predict radiation use efficiency. Using a PLSR approach, Zazueta et al. (2020) showed that the use of remote sensing models based on hyperspectral imaging predicted RUE with up to 70% accuracy compared with ground truth data in wheat.

An case study is provided here by remote screening for nitrogen use efficiency (NUE) in wheat. The spectral reflectance index (NDVI), has been shown to have a significant correlation with genetic variation in grain yield. As an alternative to NDVI, canopy chlorophyll content index (CCCI) and near-infrared spectroscopy (NIRS) have also been proposed for more accurate measurement of N status. With the availability of these indices, measurements of crop N status could be made rapidly, allowing fast screening of large segregating populations, while breeding for improved NUE in crops. Besides spectral vegetation indices (SVIs), multivariate analysis such as partial least squares regression (PLSR) holds the advantage of including more spectral information than SVIs. While SVIs can be derived from both multi- and hyperspectral data, the potential improvements by multivariate analysis are restricted to hyperspectral data, which entails more expensive sensors. Recently, ground and aerial based RGB imaging has been successfully used to study NUE in wheat and maize under field conditions which offer scope to develop rapid field screening for deployment in plant breeding programs.

Various ground and aerial-based platforms are currently in use that carry multiple sensors and cameras, resulting in significant increases in the efficiency and throughput of field phenotypic data capture. Winter wheat acquired hyperspectral canopy measurements on 26 measurement dates during vegetative and reproductive growth, and 48 vegetation indices from the visible (VIS), red edge (RE) and near-infrared (NIR) spectrum were tested for assessing the influence of measurement stage and index selection. Results showed water band and RE indices should be preferred over NIR/VIS indices for DM traits and N-related traits, respectively, and milk ripeness was suggested as the most reliable stage. These platforms have been used to phenotype various agronomic traits in a range of crop species in the field and are of much potential value for NUE-related traits. Furthermore, automated image-based phenotyping methods in controlled environment conditions for rapid vegetative screening of wheat germplasm for NUE have recently been developed.

Despite its popularity and potential, hyperspectral indices require caution. Typically it is a passive process and affected by light and environment, generating data noise. Models may not be applicable on new varieties and new environments. For this reason, spectral reflectance should still be regarded as 'emerging' but its ease of deployment and wide application means that it will continue to play an important role.

Other measurements are useful either in combination with hyperspectral or on their own. sensing in the infra-red allows thermal properties to be evaluated. In the right environment, transpiration al cooling is a proxy for photosynthesis and water use efficiency and can be a reliable indicator of stomatal closure in response to drought, especially in warm environments. In the laboratory, thermal imaging has been successfully combined with chlorophyll fluorescence to predict structural properties. Combination of sensor data in this way is highly promising.

Remote sensing of chlorophyll fluorescence (sun induced fluorescence) has achieved much publicity for its supposed ability to predict photosynthetic rate. This should be viewed as separate to standard PAM fluorescence since the basis for analysis does not allow the separation of photochemical and non photochemical components and should be considered as under development.

LIDAR provides 3D canopy structural data to be combined with spectral reflectance or RGB data. Multispectral LIDAR devices are available off the shelf and provide limited functional analysis in 3D but this is a promising future area for phenotyping. The problems to be solved are canopy movement causing blurring of images.

7.7.8 Phenotyping - NUE

In recent years, a number of new high-throughput non-destructive sensing and imaging phenotyping techniques have been developed using various sensors and cameras making significant advances in phenotyping for N studies in crops (Fiorani and Schurr, 2013; Deery et al., 2014). For example, traits such as N content, chlorophyll content, photosynthetic parameters and leaf area in crop plants may be quantified through spectral reflectance indices. Different types of indices have been developed to measure N status, biomass, chlorophyll content and carotenoid content of plants (Balyan et al., 2016) on the basis of spectral regions such as the visible (400–700 nm), NIR (700–1000 nm) and shortwave infrared (1000–2500 nm) (Mulla, 2013; Fahlgren et al., 2015). By analysing the light portion reflected, detailed information about plant structure and crop N status can be revealed (Homolová et al., 2013). The spectral reflectance index (NDVI), has been shown to have a significant correlation with genetic variation in grain yield in wheat (Gaju et al., 2016; Babar et al., 2006). As an alternative to NDVI, canopy chlorophyll content index (CCCI) and near-infrared spectroscopy (NIRS) have also been proposed for more accurate measurement of N status (Haboudane et al., 2002). With the availability of these indices, measurements of crop N status could be made rapidly, allowing fast screening of large segregating populations, while breeding for improved NUE in crops. Besides spectral vegetation indices (SVIs), multivariate analysis such as partial least squares regression (PLSR) holds the advantage of including more spectral information than SVIs. While SVIs can be derived from both multi- and hyperspectral data, the potential improvements by multivariate analysis are restricted to hyperspectral data, which entails more expensive sensors. Using a PLSR approach, Zazueta et al. (2020) showed that the use of remote sensing models based on hyperspectral imaging predicted RUE with up to 70% accuracy compared with ground truth data in wheat. Recently, ground and aerial based RGB imaging has been successfully used to study NUE in wheat and maize under field conditions (Prey et al., 2018; Buchaillot et al., 2019; Kyratzis et al., 2017) which offer scope to develop rapid field screening for deployment in plant breeding programs.

Chlorophyll fluorescence is another rapid technique providing detailed information on the state of photosystem II (PSII) at a relatively low cost. Chlorophyll fluorescence is light re-emitted by chlorophyll molecules while returning from excited to non-excited states and is used as an indicator of photosynthetic energy conversion in plants (efficiency of PSII). In wheat, chlorophyll fluorescence has been successfully used to quantify the effect of N nutrition on net photosynthesis (Shangguan et al., 2000). Gaju et al. (2016) found a positive association of flag-leaf chlorophyll fluorescence with flag-leaf photosynthesis rate in 15 winter wheat genotypes. High-throughput phenotyping platforms have been developed to analyse plant growth on a large scale based on automated non-destructive analysis combining chlorophyll fluorescence and thermal and hyperspectral imaging (Humplík et al., 2015), e.g. an automated platform developed by LemnaTec GmbH.

Various ground and aerial-based platforms are currently in use that carry multiple sensors and cameras, resulting in significant increases in the efficiency and throughput of field phenotypic data capture (Shi et al., 2016; Virlet et al., 2017). Prey et al. (2020). Winter wheat acquired hyperspectral canopy measurements on 26 measurement dates during vegetative and reproductive growth, and 48 vegetation indices from the visible (VIS), red edge (RE) and near-infrared (NIR) spectrum were tested for assessing the influence of measurement stage and index selection. Results showed water band and RE indices should be preferred over NIR/VIS indices for DM traits and N-related traits, respectively, and milk ripeness was suggested as the most reliable stage. These platforms have been used to phenotype various agronomic traits in a range of crop species in the field and are of much potential value for NUE-related traits. Furthermore, automated image-based phenotyping methods in controlled environment conditions for rapid vegetative screening of wheat germplasm for NUE have recently been developed (Nguyen et al., 2019).

More challenging, due to the absence of non-destructive high-throughput methods to analyse isotope tracing in the field, is the phenotyping in the field N fluxes to determine bottlenecks in N-uptake, transient N-storage and N-remobilization at the different stages of crop development. The success of the 15N tracing approach in mapping N uptake and N mobilization QTLs in maize in the field by Coque et al. (2008) that permitted the identification of glutamine synthetase alleles as NUE master genes (Martin et al. 2006), strongly encourage to develop in field isotope labelling for further GWAS assays.

Methods to analyse root architecture in the field remain to be improved as root shape and development is not only crucial for nutrient acquisition but also for in the soil carbon sequestration and climate change mitigation.

7.7.9 Machine Learning and Deep Learning in plant phenotyping

Data mining and data processing tools are in a state of rapid development but machine learning and deep learning is increasingly used to predict traits parameters from large datasets such a those collected from remote sensing. Such approaches are often considered advanced statistics and there is some overlap but there is a variety of approaches and data mining of phenotypic data is becoming a field of its own. The resources required range from basic to high performance computing depending on the method and task. More researchers are using computationally accessible advanced statistics and machine learning techniques such as partial least squared regression.

Applications run through phenomics such as automated recognition and quantitation of plant organs (such as wheat ears) and the quantification of cell types such as stomata. Automated recognition and quantification requires a combination of the disciplines of computer vision, statistics and machine learning / deep learning making phenotyping a truly multi-disciplinary field.

Limitations come not with the accuracy of the algorithms in feature recognition (which can approach 100 % and be more reliable than human work) but rather with the training of the neural networks which initially requires a human ‘eye’. For example to train a network to recognise stomata of a typical species, measure the cell width and length and calculate maximum leaf conductance may take an expert several days to annotate sufficient images to provide sufficient accuracy. This may need to be repeated for multiple species and varieties. Once trained this approach can save months and even years of human work making high throughput possible where it was not previously.

7.7.10 Phenotyping secondary metabolites

Non-destructive, high-throughput techniques can also be used to phenotype secondary metabolites of importance for plant protection against stresses (carotenoids, anthocyanins, polyphenols) and interactions between plants and other organisms, driving attraction or repulsion of these hosts. This important field relies on relatively new optical and olfactive technologies, respectively. Secondary metabolites that are characterized by their color (eg flavonoids, carotenoids), which is related to their import antioxidant properties, can easily be characterized with optical measurements based on their light absorption/fluorescence properties. Aside from now classic measurements of chlorophylls, based on wavelengths with maximum sensitivity to chlorophyll content in the wide spectral range from 530 to 630 nm and near 700 nm (the normalized difference vegetation indices (NDVI)) (Gitelson et al. 2014)), hyperspectral cameras were instrumental in further developing these technologies, allowing to easily measure ratios of absorbed/re-emitted metrics for anthocyanins (331/581 nm) and carotenoids (331/631 nm) (Manjunath et al. 2016).

Measurements of volatile organic compounds (VOCs) of interest for plant interactions with other organisms, driving defense or sensitivity to biotic stresses, as well as for setting plant resistance/resilience to abiotic stresses and plant roles in determining atmospheric chemistry composition, and the presence of precursors of greenhouse gases and other pollutants (e.g. ozone) (Loreto and Schnitzler 2010) can be phenotyped using Proton Transfer Reaction- Time of Flight-Mass Spectrometry (PTR-TOF-MS) that allows ultrasensitive (ppm level) and ultra-fast (1 s for the entire VOC blend) in vivo measurements of the volatiles emitted by plants as well as by enemies or friends interacting with plants (Cappellin et al. 2016). This technique now allows immediate measurements of constitutive or stress-induced volatile isoprenoids, the green-leaf (C6) volatiles that are produced by wounding, VOCs reflecting flowering or senescence, as well as volatile or gaseous hormones (methyl jasmonate, methyl salicylate, ethylene) thus allowing a very complete interpretation of the status of plant interaction with other organisms, and of plant response to the environment, eventually leading to the use of measurements of VOCs to guide decision support systems for plant protection and farming practices (Brilli et al. 2019).

Many important secondary metabolites are neither naturally volatile nor light-absorbing in the UV-Vis spectral regions. Phenotyping the development of seeds and other plant tissues in which secondary metabolites may be contained (at least when consumed by people) is also important. The techniques for measuring the growth of leaves etc can be done using optical imaging and LIDAR techniques (see section on Phenotyping growth and 3D structure above ground). Measuring in vivo the content of secondary metabolites with no useful UV-Vis spectroscopic signature nor sufficient volatility to all them to be measured via the vapour phase will require the development of other spectroscopic techniques, such as Raman or NIR spectroscopy, used either in conjunction with either high-throughput robotic, non-destructive systems in the field or controlled environment, or in conjunction with automated, destructive, high-throughput, laboratory analytical systems.

7.7.11 Phenotyping protein content

As yet no fast and robotic high-throughput system to generally measure protein levels in plant parts has been developed. NIR reflectance imaging, and Raman, spectroscopy can be used to measure protein levels in leaves and this method may be extensible to the measurement of proteins in seeds. Raman spectroscopy would also seem likely to be useful for these measurements. The development of laboratory, robotic analytical systems will also be important for the detailed measurement of protein and amino acid contents of seeds and other plant parts

7.7.12 Priority Actions for Phenotyping Techniques to be Developed for a Future Research Program

The further development of high-throughput phenotyping devices to handle and monitor hundreds of individual pots and plants (small plants to flowering plants) in controlled conditions and in the field is necessary. These techniques are of key importance to track plant growth and qualitative traits (priority action “3. Elucidating the interaction between secondary metabolites (SM) and proteins or lipids to improve the quality of crop products for human health”) in responses to abiotic and biotic stresses (priority action “2. Characterizing the plasticity of specialized metabolome responses to abiotic and biotic stresses to improve crop resilience and adaptation to climate change”) and/or on a large number of genotypes / species (priority action “1. Discovery and characterization of SM diversity in crops and wild relatives”). To standardize the growing conditions in controlled growth chambers or greenhouses, pots should be frequently and automatically rotated (e.g. Tisné et al., 2013). Stress imposition, e.g. drought, high or low temperature, nutrient deficiency, should be also controlled automatically. These principles should be adopted as far as possible in future experimental work to improve the robustness of the experiments.

The Science community have multiple aspirations to support further research. These include:

- Continued advancement of the capability of affordable automated field phenotyping capable of the analysis of large numbers of genotypes for complex traits across multiple contrasting sites. These traits include growth, development, architecture, physiology and function. This includes the development of rapid remote sensing by various techniques (spectral reflectance, RGB, chlorophyll fluorescence etc) alongside ground truthing analysis.
- The continued advancement of sensor technology for remote and contact measurements in order to refine trait complexity e.g. photosynthesis, leaf anatomy, 3D structure, chemical composition, stress analysis.
- Continued development of automated methodologies such as robotics to carry sensors to plants.
- The ‘coming of age’ of root phenotyping in the field in order to understand how root function, development and physiology influences yield, sustainability and resilience
- The continued development of infrastructure for data collection, storage, curation and integration across countries and organisations and multiple traits.

- Deep learning / machine learning / AI to continue integration with computer vision and sensor output in order to develop automated trait recognition. This includes the use of such methods as 3D reconstruction of plant architecture from point cloud data
- Our understanding of the translation of phenotype from controlled environment to complex field environments to be refined.

Priorities and Challenges for Plant and Crop Phenotyping

- Discovery and characterization of SM diversity in crops and wild relatives
- Characterizing the plasticity of specialized plant metabolome responses to abiotic and biotic stresses to improve crop resilience and adaptation to climate change.
- Elucidating the interaction between SM and proteins or lipids to improve the quality of crop products for human health
- Interoperable information Systems for FAIR data sharing in the community need developing and refining

Connecting HT phenotyping data to models needs to be improved

- Further develop phenotyping systems in controlled conditions that allow plant growing and non-destructive monitoring (e.g. plant imaging, IR, X-ray fluorescence) beyond flowering and seed development/maturation.
- Improve the protocols to estimate and screen plant and seed specialized metabolite accumulation, both for beneficial (e.g. flavonols, anthocyanins) and antinutritional (e.g. saponins) compounds. Most of these methods are destructive, e.g. NIR spectrometry, but allowed the fast screening of a large number of samples for GWAS analyses.

8 The Blue Domain

Marine macroalgae, also referred to as seaweeds, or kelps for the largest brown algae, are a group of exceptionally diverse aquatic plants, encompassing three independent evolutionary lineages: the green, red and brown algae (Brodie et al 2017). Macroalgae can be found along nearly all coastlines around the globe and, in some cases, in the open ocean. They have traditionally been used for food and feed, sources of various chemicals and minerals, as well as fertilizers (McHugh 2003).

Macroalgae, especially species of the brown algal kelp order Laminariales and the green algae of the genus Ulva, belong to the fastest growing plants in the world. Annual production capacities of Saccharina spp; have been estimated from 170 to 340 tonnes fresh weight (FW)/ha at sea, with a corresponding dry weight (DW) of 26-33 tonnes/ha, and commercial yields for S. japonica in China is 25 tonnes DW/ha on a total surface of 40,000 ha (Zhang et al 2015). Land-based cultivation, integrated with animal production, of Ulva spp. has shown effective productivities of 400 to 940 tonnes FW/ha/year (Bolton et al 2009) equivalent to 40 to 90 tonnes DW/ha/year; smaller scale trials have reached even higher yields with 200 tonnes DW/ha/year (Mata et al 2010). By comparison, the average yield of soybean, corn and sugar cane in Europe is 2.3, 3.1 and 85.5 tonnes/ha, respectively (FAO). Therefore, developing the performances of algal crops related to these species should lead to a potential of development of large-scale biomass integrated biorefineries as explored between 2017 and 2021 in the EU project GENIALG (genialgproject.eu).

This article focuses on cultivated algae rather than wild harvest, even though this is locally an important economic activity in Europe.

8.1.1 Uses

The dominant use of seaweed, with over 80%, is as a food source and seaweeds have been eaten for at least 4,500 years (Chopin & Sawhney 2009, Tseng 1981). They are often consumed as “sea vegetables” and are typically sold dry and are rehydrated before consumption. Macroalgae are typically rich in fibers and low in digestible carbohydrates and lipids. Seaweeds are rich in minerals, such as magnesium and iron, and especially iodine, and for some species, such as nori and sea lettuce, in protein. In addition, some species are good sources of vitamins A, K, and B12. Algae are mostly consumed in Asia, especially in China, Korea and Japan where they are used as a vegetable, a source of umami and thickener of broth. Consumption in Europe is presently limited but increasing.

The cell walls of red and brown seaweeds are typically rich in polysaccharides, such as, agars, carrageenans and alginates, which are used as hydrocolloids and have the capacity to form gels or increase viscosity of solutions. Seaweed-derived products represent 40% of hydrocolloids used in the food industry. Algal thickeners are also used in research, medicine and biotechnology. Potential industrial uses of algae that are currently expanding in scope and range to newly emerging sectors such as bioactive molecules for nutraceuticals or pharmaceuticals (Holdt & Kraan 2011).

People living in many coastal regions have a long tradition of using seaweeds as fertilizers to improve soil characteristics and as a source of nutrients. In addition to this, seaweeds have also been identified, as a source of growth stimulators and inducers of defense mechanisms for plants (Craigie 2011).

8.1.2 Aquaculture

World seaweed production is primarily supported by aquaculture. In 1969, the 2.2 million tonnes of world seaweed production was evenly provided by wild collection and cultivation. After half of a century, while the wild production remained at 1.1 million tonnes, the cultivation production has increased to 34.7 million tonnes that accounted for 97 percent of the seaweed production in 2019 (Cai et al 2021). There is a strong regional imbalance in seaweed production. In 2019, seaweed production in Asia (99.1 percent from cultivation) contributed to 97.4 percent of the world production, and seven of the top ten seaweed producing countries were from Eastern or South-eastern Asia; however, the scale of terrestrial agriculture surpasses seaweed aquaculture by two orders of magnitude (FAO 2021a). Seaweed cultivation is concentrated on a relatively small number of species. FAO statistics record only 27 different seaweed species cultivated in 2019, even though seaweeds accounted for nearly 30 percent of world aquaculture production in terms of wet weight (FAO 2021d).

The aquaculture of seaweeds necessitates the control over the life cycle and thus also gives the possibility of controlled breeding and strain selection. However, despite the importance of algal aquaculture, plant breeding of algal species is presently limited and also has a limited history. Most attempts to develop kelp cultivation during the last twenty years in Europe have not led to the conservation of any valuable genetic material and only a few gametophytic clones are available in collections across Europe at CCAP (Oban, UK), RCC (Roscoff, France) or in laboratory collections. The life history of kelps facilitates the creation of purebred parent lines almost instantly: male and female kelp gametophytes are free-living, and can be propagated as haploid clones in unlimited manner (Westermeier et al 2010).

Today, breeding assisted by molecular markers is widely developed in both agronomic research and basic sciences dedicated to the characterization of genetic basis of phenotypes. In addition, within the IDEALG project (www.idealg.org)and in China, there is a growing body of studies that deal with the history of domestication and its consequences at the genome level in marine species. A numbers of molecular markers such as microsatellites and single nucleotide polymorphisms (SNPs), which are required to analyze the genomic bases of domestication process are now available for several species of brown and red algae (Valero et al 2011). Genotyping of both neutral and potentially selected loci (i.e. genes associated with fitness) are needed to understand the domestication processes. Genetic maps are also necessary to develop genetic approaches for these species, such as the mapping of single gene traits or QTLs, and hence to enable the development of breeding programs for these species. The construction of the maps will build on the recent experience with the construction of a genetic map for Ectocarpus (Heesch et al 2010) and for the kelps Saccharina japonica and S. latissima (Zhang et al 2015, Nehr et al unpublished), and will use microsatellite markers designed based either on deep EST sequencing data or on complete genome sequences. Most previous attempts to select seaweed strains were based on simple phenotypic traits such as growth rate, blade length, tolerance to temperature or the yields of principal, target compounds such as iodine in kelps or carrageenan or agar in Kappaphycus/Eucheuma and Gracilaria spp. A high density genetic map for S. latissima was used to implement breeding approaches in GENIALG (genialgproject.eu). Modern phenotyping approaches are being developed in agronomic research. It is now feasible to develop medium-throughput chemical phenotyping of different seaweed genotypes. It should be noted that when strain selection has been used in algal aquaculture, for example in the brown alga Saccharina japonica to increase tolerance to unfavorable conditions, the potential for strain improvement through classical breeding is very high (Zhao et al 2016). Thus, even though the production of biomass per hectare is high there is probably a large potential for improvement.

The unprecedented opportunities and advantages offered by omics (e.g., genotyping by sequencing) applied to modern breeding, biotechnology for translational research and biomass processing also offers important tools for seaweed research and breeding.

There are also a number of challenges facing seaweed aquaculture. One consideration is that since aquaculture of seaweeds, at least presently, is done in open farms often with species or genera that exist locally, there is the added problematic that the possibility of genetic flow from cultivated algae to their wild counterparts and spread of diseases is much more important (Brakel et al 2021) than in terrestrial systems, where gene flow between cultivated and local species is less likely.

One emerging problem in seaweed aquaculture is that with increasing production there is an increase of pathogens causing diseases (Ward et al 2020). These includes bacteria, viruses, fungi and oomycetes, but also problems with epiphytes, other organisms like algae growing on the cultivated species reducing photosynthesis and quality of the product. In order to understand better diseases in algae we need a better understanding of the disease causing species and their life cycle. One potentially important part of pathogen-host interaction in seaweed is the microorganisms surrounding the seaweeds thus the alga as a holobionts.

New solutions are also required in order to tackle the main challenges facing macroalgal cultivation in Europe and other continents that remain far beyond the leading Asian countries: scalability of seeding, cultivation and harvest; survivability of the crop and installations; energy use and ecosystem impact; predictability of yield and quality of harvested biomass; and cost effectiveness.

One area of promising research on seaweeds aquaculture is the integrated multi-trophic aquaculture, were algae are grown in conjunction with, for example fish and/or shellfish farms to take advantage of the nutriments, provide feed for higher trophic levels and achieve other ecosystem services provided by algal aquaculture such as mitigate eutrophication, ocean acidification, deoxygenation and restore ecosystems. This has the potential of increasing the economic value of seaweed aquaculture.

8.1.3 Present knowledge and future research.

The present scientific knowledge on the biology of seaweeds and their use in aquaculture are very limited compared towhat is known for terrestrial plants. It is thus important that an effort is put into research of basic biology of seaweed as well as a better understanding of traits important for aquaculture. One reason for the limited knowledge of macroalgae is the lack of model organisms among the seaweeds. This has partly changed because of the establishment of Ectocarpus as a model species for brown algae, but no obvious model species exist for red and green macroalgae. For Ectocarpus, following the genome sequencing numerous studies has established, for example traditional genetics, life cycle studies, mutant studies, and recently targeted CRISPR-Cas9-based gene knockouts (Badis et al 2021). This opens up the opportunity of gene editing for improving the understanding of seaweed biology. To improve our knowledge of species used in aquaculture, the development of red and green algal model, for example nori (Porphyra/Pyropia spp) and Ulva sp. as well as a transfer of knowledge from Ectocarpus to brown algal species used in aquaculture, would be very valuable.

There are a number of research challenges in the field to be addressed with high priority. The most relevant unsolved questions includes: Seaweed domestication, it is important to use a strong multidisciplinary approach to tackle the issues of seaweed domestication, such as, which genes and traits are important for domestication? Biobanking and germplasm storage, seaweeds do not have dry seeds that can easily be stored and other methods need to be used and perfected. Selective breeding, how do we efficiently do selective breeding in seaweeds using modern plant breeding methods? Understanding seaweed holobionts, the algae and their surrounding microorganisms as an entity are of crucial importance. Pathologies, with increasing large scale cultivation the problems with diseases are increasing, yet we know little about seaweed pathology. Seaweed nutritional and health benefits for humans, animal and plants is still largely unknown and need further study. We also need to know more of the ecosystem services of seaweed to mitigate eutrophication, ocean acidification, deoxygenation and restore ecosystems. In addition, even though the impact of seaweed farming on the ecosystems are probably limited increased knowledge on the effects of intensive farming is needed.

In order to attack the unsolved questions we need acquire and adapt many of the methods used in plant breeding for terrestrial plants. This includes more efficient methods for gene editing, high throughput phenotyping, imaging systems and effects of cultivation practices on crop performances and environmental issues

8.1.4 Economical and societal issues

There are a number societal and economic constraints and challenges for the development of an important European seaweed aquaculture. Even though interest and consumption of seaweed products increases in Europe the short-term demand is limited and the long-terms trends are unknown. There is also a limited availability of suitable farm sites for near shore aquaculture due to competition with other activities and local resistance. Thus, one important part is the need for outreach activities and engagement to educate and inform the public about the biology and potential seaweed ecosystem services and contributions to the economy.

From an economic point of view, there is in the short term a lack of trained aquaculturers. There is also clearly an important competition from Asia with their better knowledge and longer tradition with seaweeds aquaculture. There is also a lack of specific legislation for seaweed aquaculture (Lähteenmäki-Uutela et al 2021).

9 Economic, Environmental & Social Impacts and Feedbacks of CropBoosting

9.1 Motivation

Crop improvements have a great potential to help us deliver sustainable development goals and improve the prosperity, well-being and health of current and future generations. The potential is there to reduce fertiliser and pesticide use, improve the nutritional quality of crops, and enhance the quality and resilience of crop yields, whilst meeting the needs of growing populations and changing markets. However, our current understanding is lagging behind on the extent to which crop improvements can deliver on these potentials and robust analyses of the potential knock-on effects for societies, economies and the environment – both good and potentially negative - are lacking. How much can the adoption of these crop improvements help us sustainably produce healthy and affordable food? How much can specific plant trait improvements help deliver healthier soils and clean water resources? What impacts would adopting these technologies have on the global competitiveness of Europe's agri-food sector? These are crucial questions to future-proofing our crops that have not been answered with robust research to date.

Very few studies have attempted to analyse or quantify concurrently the economic, social and environmental impacts of crop improvements. The few studies that exist are limited to enhanced yields and assumed increased farm profits (economic impacts), as well as land conversion savings and GHG emissions associated with it (environmental impacts). More studies that systematically attempt to quantify the benefits, costs and risks of crop improvements are needed to form a fuller business case and guide the development and adoption of improved or new crops. Through systematic reviews and an extensive program of participative research, engaging stakeholders across the agri-food sector, CropBooster-P has highlighted that our knowledge and capacity to evaluate the economic, environmental and social impacts of crop improvements is very limited due to a lack of evidence and constitutes a major gap, which needs to be addressed with primary research. For more information on the research and evidence underlying this gap, please see D2.4.

CropBooster-P examined the attitudes and priorities of various stakeholders and the lay public regarding proposed research goals and breeding technologies. In terms of crop improvement, participants ranked sustainability higher than nutrient quality and pure yield enhancement. In addition, the use of new plant breeding technologies was expected to be (more) acceptable if they serve needs identified as high priority (D 3.1, D 3.2). Because communication frames attitudes toward complex scientific issues in different social groups (including the general public in Europe), their attitudes, expectations and means of communication were explored through questionnaires and interviews. The surveys underscored the importance of a dialogue between scientists/researchers and different stakeholders/societal groups to achieve acceptance and recognition. They also revealed significant differences among the preferred topics about which different groups communicate. The diversity was even more pronounced in the means and media used to communicate and obtain information. For details, see D3.3.

Anticipating changing societal priorities and facilitating feedback are considerable challenges in long-term research programs. Here, we provide a set of recommendations on how a research program could address this key issue, focusing on three areas: (1) impact assessment research, (2), a systems approach to intervention i.e., Intervention Logic and (3) monitoring the shifting priorities of society. The development of an Intervention Logic would ideally be combined with the development of learning scenarios to better appreciate how the impacts of a research program can vary and evolve over time and in response to changing internal and external factors. Identifying key indicators for future directions would ensure that long-term research programs remain responsive and anticipate societal needs and market demands by making provisions to continuously update implementation plans.

9.2 Proposed Approach

9.2.1 Impact assessment research

The urgency and complexity of the climate crisis, environmental sustainability issues, and the security issues facing our food and bio-economy sectors mean that we can no longer afford to take the traditional linear pipeline approach where the efficacy of plant breeding research is evaluated post innovation. Instead, we must continually shape the technical side of research through interdisciplinary evaluative research. Such research helps to prepare the ground for quicker uptake of solutions, providing robust science for which application and implementation is maximised and that can underpin financial decisions, business case development, farm practices, value chain innovation and policies, and maximise benefits.

We recommend that an interdisciplinary long-term research program that seeks to analyse, quantify and predict the impact of crop technologies and innovations is put in place, alongside and closely interacting with a plant innovation program. This will ensure that the positive benefits and unintended consequences of plant breeding are robustly analysed and considered alongside the fundamental science.

We therefore recommend that this program should comprise three elements:

1. Developing impact indicators: To evaluate the multiple social, economic and environmental impacts that new crop developments will have, a clear set of indicators must be developed. These indicators need to be transferable across crop types and traits and need to be meaningful for multiple farming systems, geographies and value chains. We recommend that these indicators are developed using a participative approach, engaging multiple experts across distinct disciplines and key stakeholders of the agri-food system and the bio-economy. One potential approach would be to use a capital framing, where natural, human, social and produced capitals in relation to crop systems are considered, and the indicators align to the stocks and flows of these capitals. Other approaches, such as Ethical Matrices that target the more intangible trade-offs and effects could also be employed, as they have been to other food related issues (e.g. Millar and Tompkins, 2007[8]

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[8] Millar, K., and Tomkins, S. (2007). Ethical analysis of the use of gm fish: emerging issues for aquaculture development. J. Agric. Environ. Ethics 20, 437–453. doi: 10.1007/s10806-007-9051-z

). Indicators on agri-environment would, where appropriate align with the Agri-environmental indicators of the Common Agricultural Policy.

2. **Developing and trialling mixed-methods approaches for estimating positive and negative impacts:** We anticipate that mixed-methods approaches will be required to capture the multi-dimensional impacts of plant innovations. The potential environmental impacts are diverse: affecting air, water, climate, soils and biodiversity in various ways depending on the agri-environmental context and application and on farm management practices. Combinations of modelling advances and applications, data analytics and strategic gap-filling through empirical and fundamental science will be required to estimate the impact of crop innovations on aspects such as soil health, water quality and flows, air quality, GHG emissions and biodiversity (for example in soils and at landscape scales). Incorporating the modification of plant traits into models that provide insights into environmental functioning and quality, as well as integrating models to explore potential biophysical feedbacks are significant scientific undertakings. With respect to socio-economic impacts, combinations of qualitative and quantitative primary research will be needed to more fully understand the potential impacts of crop innovations on aspects such as farm profitability, employment, value chain resilience, trade and commodity, and consumer markets. The social impacts also include changes to nutritional quality and health and cultural values of food and landscapes.
3. Developing impact evaluation frameworks and tools: As crop innovations move through a pipeline, we must ready the tools for evaluating impacts, such as assessing baseline environmental, social and economic indicators and ways to monitor the progress in a diversity of environments. Putting these tools in place prior and during the development of plant innovations is crucial for their ultimate success. The new mixed-method approaches (highlighted in point 2 above) should also be integrated and summarised in accessible tools that provide system-level insights into environmental, social and economic impacts that support decision-making. These would need to be tailored to a variety of contexts including policy and value-chain decision-making.

This work should be guided by the crops and traits in other parts of the broader program, and a variety of case studies should be undertaken representing key crops and contexts.

9.3 A systems approach to intervention

9.3.1 Intervention logic (IL)

Following the European Green Deal, the Farm to Fork Strategy and the Biodiversity Strategy and further legislative initiatives on sustainable food production, it is essential to ensure that plant science research projects consider the complex landscape in which they will conduct the research and clearly identify their desired impact in terms of future proofing agriculture. Constructing research programs this way can help predict how different actors might react, what actions could be triggered by the research, and determine how actors and actions can help drive the desired impact. With CropBooster-P, the cornerstones for such a large European plant research program have been developed. For successful implementation, a mechanism that helps steer the resultant research towards societies' potentially changing requirements must be put in place.

When driving transformational change, the development of an intervention logic (IL) (reference link to EC doc) can provide an overview of the socio-economic and environmental context of a challenge and how different solutions will impact different dimensions. An IL positions the implementation of the research activities in relation to its corresponding outcome (input -> activity -> output/results) and determines overall impact. An IL can serve both as a communication tool, facilitating discussions between different actors, and as an intervention logic analytical tool, identifying relationships and (inter)dependencies, and considering how different actors are expected to react, and what actions it will trigger, i.e. the cause and effect relationship. This format allows to identify objectives and expectations of the different actors early on, thereby determining indicators by which to evaluate the success of an intervention.

9.3.2 Learning scenarios

The development of learning scenarios that map borderlines for the evolution of the plant production systems and society as depicted in CropBooster-P by contrasting future worlds ('Plantovation', 'My Choice', 'Food Emergency' and 'REJECTech') can help to define possible future directions and identify early indicators (D1.8). Learning scenarios were built by extrapolating a multitude of trends and uncertainties to develop alternative future worlds within the limits of what may plausibly happen. These scenarios can be used to anticipate research and innovation needs to meet future market demands (Cornelissen et al., 2020 TIBS) and can help drive transformational change. It is unlikely that a single (extreme) scenario will happen, but the path to the future state will be "in between". Indicators of future direction can help predict the direction in which society might be heading, thereby increasing certainty about future needs and market demand. This can, for example, be done by feeding learning scenarios into mapping systems to predict outcomes and impacts over time and identify crucial check points. They might also reveal which indicators need to be tracked to inform and manage development pathways.

9.3.3 Systems approach: combining IL and scenarios

For maximum impact, a future research program should always take a systems approach, considering the interlinking and (inter)dependencies of different elements and actors in society. Developing research plans using IL will ensure that the projects are considered within the bigger picture and across multiple dimensions (socio-economic, environmental and political). The development of an IL would ideally be combined with the development of learning scenarios to better appreciate how the impacts of a research project can vary and evolve over time and in response to changing internal and external factors. Identifying key indicators for future directions would ensure that long-term research projects remain responsive and anticipate societal needs and market demand by making provisions to continuously update implementation plans.

9.3.4 Monitoring changing societal priorities

The success and impact of an extended plant science program to future proof our crops, as well as of individual projects therein will depend on broad societal approval, including the approval of farmers.

Societal relevance has to be guaranteed and midterm shifting priorities of society's needs and attitudes need to be anticipated and accounted for to enable responsive program management. Moreover, such a program will be affected by evolving regulations, such as the seed marketing legislations, the GMO or Nagoya/Digital Sequence Information and benefit-sharing protocol, intellectual property (IP) schemes, the Common Agricultural Policy, and the Sustainable Food System Policy, narrowing or widening options and providing long-term directions for breeding approaches and products. Monitoring these dynamics will facilitate a responsive implementation of the research program and application of its results.

Monitoring should be integrated as an overarching activity accompanying ongoing research. It should be tightly linked with communication efforts that enable responsiveness to and an informed involvement of the different actors and should not be performed as separate projects throughout the course of the program. Monitoring should allow for anticipatory management of the research program, allowing for timely but limited adjustment of activities. Extensive changes to a live program would be beyond the scope of its research mission.

In the course of CropBooster-P, "sustainability traits" were cited as prominent research goals by most stakeholders. Traits referring to food/feed quality and yield were also considered important, but with lower priority. In addition, classical breeding techniques were broadly accepted, while new plant breeding techniques such as genetic engineering and genome editing were contentious topics. Nevertheless, CropBooster-P showed that science communication enabling a deliberate reflection of such topics supported informed and problem-oriented decisions from stakeholders.

Key targets of monitoring activities in relation to a future research program are:

i. Changing societal, economic and political preferences and priorities with regard to the research fields and the applied methods in the program. (see D1.$$$, D2.$$$, D3.$$$ highlighting priorities of expert and lay groups)

ii. The development of new (scientific) solutions and challenges related to the program's research topics and methods (see D4.$$$, highlighting subjects, networks etc.)

iii. The regional structure of societal priorities, research challenges, etc. (see D$$$, D3.$$$, highlighting the regional diversity of communication means and topics)

iv. The evolution and impact of the legal landscape with regard to European and international regulations on seed marketing, GMO and NGTs, exchange of genetic resources (Nagoya) and Digital Sequence Information (DSI) and IP

v. Rescaling of the learning scenarios developed in CropBooster-P. (see D1.8; section 3.). The developed scenarios (see D1.8 section 3.2: 'Plantovation', 'My Choice', 'Food Emergency' and 'REJECTech') could be used as a starting point to assess how changes in societal attitudes might shape future agricultural production.

9.3.5 Suggested Research Approaches

For monitoring the key targets i), iii) and v) interaction approaches like focus groups, expert groups or citizens' juries will enable an intensive dialogue with stakeholders that provides first-hand insights in their perceptions and positions. Such formats allow for direct feedback from project scientists. Questionnaires and interviews make it possible to obtain a representative picture of opinions, even if clarification of complex issues is not easy to achieve.

For monitoring the activities of the key targets ii), iv), and v) desktop research can be used, in combination with expert interviews/panels and literature reviews .

9.3.6 Outcomes and outputs

The outcomes of this recommended research would include:

(i) A scientifically fostered systems approach, considering the interlinking and (inter)dependencies of different research elements and actors and how these affect the priorities of plant research programs.

(ii) Continuously adjusted learning scenarios to better appreciate how the impacts of a research project (social, economic and environmental) can vary and evolve over time.

(iii) New approaches and frameworks for evaluating and monitoring the broader impact of crop innovation at EU and global scale.

(iv) A set of indicators and a suite of compelling, scientifically-robust case studies on the economic, environmental, and social impacts of specific crop improvement options that help guide and motivate the development and adoption of these crops.

(v) New interdisciplinary communities across environmental, plant and social sciences, and economics that open up new opportunities for collaborative science.

10 Annexes

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6. 4 Phosphorus Use Efficiency

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6.7 Nutitional and Post-Harvest Value for Food Use

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7.7 Phenotyping- what is it?

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8. The Blue Domain

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