The Molecular and Physiological Basis of Nutrient Use Efficiency in Crops

Part I

Generic Aspects of Crop Nutrition

Chapter 1

An Overview of Nutrient Use Efficiency and Strategies for Crop Improvement

Malcolm J. Hawkesford

Abstract

Understanding the molecular basis of crop nutrient use efficiency is a prerequisite for genetic improvement aimed at maximizing yield and minimizing inputs. Plant breeding has been hugely successful at developing high yielding varieties, albeit often with high nutrient requirements. In addition, substantial progress has been made in improving nutrient use efficiency in terms of agronomic practice. Recent developments in genetic and genomic resources, combined with existing physiological and biochemical knowledge, should facilitate substantial further genetic improvements targeted at yield increase and efficient resource utilization. However, this is not a trivial task, given the complexity of the plant systems involved: The traits need to be defined and resolved into specific processes, and appropriate genetic targets need to be identified. This overview considers some issues relating to likely traits, the potential molecular basis of the traits, and potential routes for improvement.

Introduction

The major challenge facing plant biology is to improve crop production to feed an expanding world population. This is against a background of pressure on agricultural land use and climate change having negative impacts on growing conditions and limiting geographic regions for agriculture (Parry and Hawkesford, 2010). The adverse effects of agriculture, and specifically fertilizer use, include damage to the environment, a large carbon footprint for the manufacture and use of agrochemicals, and the utilization of non-renewable resources. One solution is to increase the area of land for agriculture, as well as increasing production while maintaining the current rate of inputs; however, this is predicted to have substantial negative impacts on the environment (Tilman et al., 2001; Tilman et al., 2002), is unsustainable in terms of phosphate use, and would have a huge economic footprint in terms of energy demands for nitrogenous fertilizer production. The challenge is to increase yield, decrease inputs, and improve resistance to

The Molecular and Physiological Basis of Nutrient Use Efficiency in Crops, First Edition.
Edited by Malcolm J. Hawkesford, Peter Barraclough.

abiotic and biotic stresses. Improving crop nutrient use efficiency ideally requires an understanding of the whole system, from the macro- (agro-ecosystem) to the molecular level. While acknowledging the critical contribution of agronomy to improving efficient nutrient use, particularly in classically inefficient systems, there is a point at which crop genetic improvement becomes essential for further improvement. This may be achieved by conventional breeding, as indeed has been the case to the present day with ever increasing yields, albeit often in parallel with increased nutrient demands, with marker-assisted breeding utilizing genetic information derived from basic plant science, and by the utilization of this same information to produce genetically modified crops.

Nutrients, along with light, temperature, and water, are critical determinants of crop production, but fertilizers are costly inputs and inappropriate overuse can have many ecologically damaging effects, making efficient use of fertilizers a major issue for agriculture. For example, excessive nitrogen use results in a major fraction of anthropogenic nitrous oxide and methane emissions, which contribute substantially to climate change, and inefficient nutrient uptake may result in pollution of inland and coastal waters by leaching and runoff. Worldwide, it has been estimated that nitrogen use efficiency (NUE) for cereal production is only 33% (nitrogen removed in grain as a percentage of that applied) (Raun and Johnson, 1999). Both agronomic practice and plant breeding have a responsibility to optimize efficient nutrient use, particularly nitrogen, in crop systems. Furthermore, crop improvements to anticipate changing patterns of rainfall and temperature must include an anticipation of nutritional demands influenced by changing cropping systems and crop ideotypes.

Optimal plant growth demands a balanced nutrient supply, with a deficiency of any individual essential nutrient having a detrimental effect on production (law of the minimum). Some nutrients are required at high levels (the macronutrients nitrogen, phosphorus, potassium, and sulfur; see Chapters 8–14), while others are only required at low levels (the micronutrients: iron, zinc, magnesium, etc., see Chapters 15, 16, and 17). In some cases, excess or luxury accumulation of nutrients in plant organs is an issue, negatively impacting on crop growth or quality for the consumer.

Agricultural production systems have a range of demands for nutrients; low input as compared with intensive highly managed systems will have different issues and the solutions will be specific for each system. Solutions for efficient fertilizer capture and conversion to biomass or yield in high-input agriculture will be quite different from targets in extensive, organic, or low-input agriculture. In recent years, the emphasis has alternated from a primary objective of improving yields (a target that has always been present in all but the most productive environments) to minimizing impacts on the environment (especially in intensively cropped systems), and back to yield in order to achieve global food security. In the 21st century, sustainable food production has become a major issue with a growing world population, negative impacts of climate change, and demands on land use.

To optimize progress on crop improvement, an understanding of nutrient use efficiency from the agronomic or agro-ecosystem level down to the molecular level (the genes involved and their regulation) is required. Substantial progress is being made on the functions and regulation of genes and proteins; molecular data are usually interpreted at the cellular level; however, it is essential that this understanding is placed at the organ, plant, and whole-crop levels. Targets for improvement need to take into account the different agricultural systems, crop physiology and yield components, and the demands of the consumer. Safe, sustainable, and secure food, feed, fiber, and fuel production will demand optimized genetic material including the trait of nutrient use efficiency.

Yield and fertilizers: the need for crop improvement

Meeting the yield potential

Plant breeding has resulted in considerable increases in yield for many crops (for example: maize [Evans and Fischer, 1999], wheat [Ortiz-Monasterio et al., 1997; Brancourt-Hulmel et al., 2003], sugar cane [Robinson et al., 2007]), with the principal target being resistance to biotic and abiotic stresses, as well as for yield itself. Yield is a good measure of nutrient use efficiency, especially as related to nitrogen (Barraclough et al., 2010): The definition of NUE is grain or total biomass (depending on crop) yield divided by available nutrient (see Table 1.1). Theoretical or best yields are seldom achieved in practice as production field conditions are seldom as ideal as breeding plots, which have optimum inputs and agronomy (Fig. 1.1A). This discrepancy is inevitable as the "yield gap" is usually reported on a national level and will encompass growth of varieties in a range of conditions and environments (Austin, 1999; Fischer and Edmeades, 2010). Including traits for resistance to as many stress factors as possible, for example, drought (Chapter 18) and salinity stress (Chapter 19) in breeding programs, will narrow this gap but is unlikely ever to close it. Breeding for resistance to stress, as well as yield, is a key target for crop improvement (Araus et al., 2008). A greater issue is the observed plateau of yield improvements, probably due to abiotic and biotic stress. Initial large improvements in wheat yields were brought about by introduction of dwarfing genes and the consequent improvement in (grain) harvest index (HI). However, in more recent years, there have been incrementally smaller improvements in theoretically achievable yields. In some cases, theoretical yields may not be achievable due to limiting fertilizer application. Theoretical, record, and average (wheat) yields are approximately 18, 16, 8 (in the United Kingdom), and 3 $\mathrm{t\,ha^{-1}}$ (worldwide), respectively.

Nutrient response curves

As already stated, most plant breeding is performed under "ideal" conditions, which usually include high fertilizer inputs. Nutrient use efficiency has seldom been a key target; however, yield and NUE are closely related at a given fertilizer input. Nutrient use efficiency is the product of both uptake and utilization efficiencies (see the next sections for fuller descriptions), and therefore selecting for yield effectively selects for the combination of these two very separate traits. Small improvements, or even negative trends in one trait (most likely acquisition or uptake), may be hidden by gains in the other (in this case, utilization efficiency). Therefore, selecting for yield alone may not select for optimal nutrient acquisition characteristics, especially at reduced inputs or in environments with specific nutrient deficiencies. It is obvious that maximum acquisition is determined by availability; however, the efficiency of scavenging mechanisms will have a substantial impact on acquisition, although this may not be sustainable in the long term in any single location if nutrients are being mined and not replaced. Traits that will contribute to efficient acquisition are mainly root-associated properties for which there are immense practical difficulties for assessment. A simple approach is to determine overall nutrient capture (nutrient uptake), which integrates the separate features of root architecture and function contributing to this trait (see below for details on the dissection of this trait),

Table 1.1. Some definitions of NUE, mostly used with respect to nitrogen

Abbreviation	Term	Formula	Unit	Notes
FUE	Fertilizer use efficiency	(NUp/N applied) × 100	%	% of applied fertilizer recovered by crop
NUE	Nutrient (usually nitrogen) use efficiency	NUpE × NUtE = yield/N available	kg kg^{-1}	NUE is closely related to yield at a given nutrient input
NUpE	Nutrient (usually nitrogen) uptake efficiency	NUp/Nav (soil + fertilizer)	kg kg^{-1}	Also NPE (physiological efficiency)
NUtE	Nutrient (usually nitrogen) utilization efficiency	Yield/NUp	kg kg^{-1}	Also NAPE (agrophysiological efficiency)
HI	Harvest index	Mass of harvested fraction (e.g., grain)/total aboveground biomass (e.g., grain + straw)	fraction	Measure of partitioning of yield to harvested fraction of biomass
NHI	Nutrient (usually nitrogen) harvest index	Fraction of nutrient harvested fraction (e.g., grain)/nutrient in total aboveground biomass (e.g., grain + straw)	fraction	Measure of partitioning of nutrient to harvested fraction of biomass
NAE	Nitrogenagronomic efficiency	Yield with fertilizer minus yield without nitrogen applied	kg kg^{-1}	Improvement in yield for fertilizer applied
ARE	Apparent recovery efficiency	NUp/nitrogen applied as fertilizer	kg kg^{-1}	

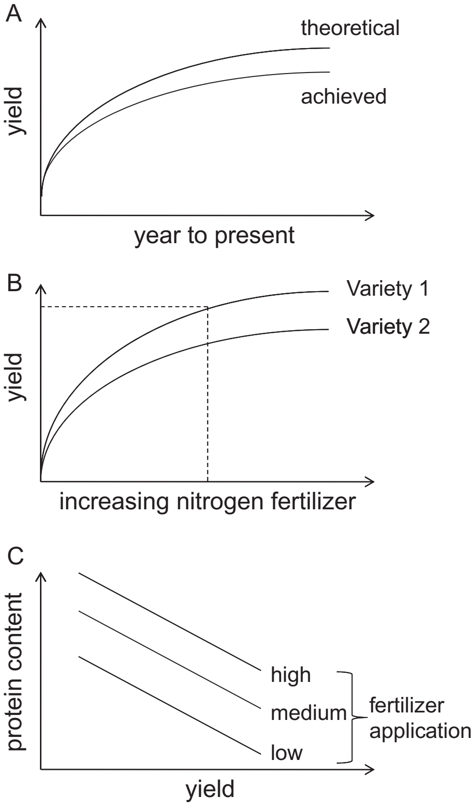

Fig. 1.1. Schematic yield trends and relationships with nutrient inputs. (A) Yield improvements in recent years (second half of the 20th century onward), showing theoretical yield improvements delivered by breeders under ideal growing conditions and those typically achieved on farms. (B) Possible theoretical nitrogen response curves for two varieties, where variety 1 outperforms variety 2 (increasing nitrogen inputs further often results in a down trend of this curve) at all nitrogen inputs. (C) The inverse relationship between yield and nutrient content, in this case nitrogen content expressed as protein content, at three fertilizer levels.

but this is not ideal as selection for the component traits is not achieved. There is little data to indicate whether best uptake performance as selected at high inputs equates to best performance at low inputs.

Increasing inputs of nitrogen fertilizer will, in the absence of other limiting nutrients or environmental constraints, result in increasing yield (Fig. 1.1B). Similarly, fertilization with any limiting nutrient will improve yield. The tendency for yields to reach a plateau as shown for nitrogen in Figure 1.1B are indicative of secondary limiting factors being present, which may be other nutrients, or may be due to constraints with conversion to harvestable biomass (limiting photosynthesis). A consequence of this plateauing is decreased NUE, specifically attributable to the nitrogen utilization efficiency component (see below).

Yield quality conundrum

In many crops, including grain crops, yield is determined by photosynthetic carbohydrate production and storage. As a consequence, as yield is increased, other nutritional components are often diluted as is seen for the protein content in grain (Fig. 1.1C; Monaghan et al., 2001). Similarly, reduced micronutrient concentrations in grain will occur as a result of dilution with starch. Outliers to the regression relationship between yield and quality shown in Figure 1.1C at any defined nutrient (nitrogen in this case) input must have particularly efficient acquisition and/or partitioning mechanisms. It has been suggested that postanthesis nitrogen uptake is an important contributory mechanism (Monaghan et al., 2001; Kichey et al., 2007; Bogard et al., 2010), although the molecular and genetic basis for this has not been determined. It may be assumed that deep rooting systems, which have access to untapped nutrient reserves at depth in the soil profile, may be important, along with mechanisms to ensure that such nutrients that are taken up are preferentially allocated to the harvested sink material.

The diversity of inputs to cropping systems

Agricultural systems span a wide range of inputs from none (organic), through extensive (low input), to intensively farmed (high-input) systems. These different situations present quite different challenges in terms of the ideal germplasm required. Targeting improvement of nutrient use efficiency needs to take into account these contrasting agro-ecosystems, the associated agronomic practices, and end-product requirements (e.g., bread vs. feed wheat). In some cases, it is clear that substantial improvements in NUE can be achieved through improved agronomic practice alone. Genetic improvements are likely to be small and incremental by comparison. In low and no input systems, with ever more nutrient capture-efficient varieties, there is a danger of "mining" reserves, leaving land completely unproductive; here the target for genetic improvement needs to be low-nutrient-requiring genotypes combined with improved agronomy to supply minimal nutrition. In many extensive systems, where fertilizers are applied at seed sowing, improved early capture is a critical phenotype. In intensive, high-input systems, conversion to biomass is the principal concern, along with minimization of losses from the system from overfertilization or inappropriate application.

Nutrient use efficiency: critical processes, definitions, mechanisms, and targets for improvement

Within the broad concept of nutrient use efficiency and depending on the nutrient in question, many definitions of efficiency are possible (see Table 1.1). In this work, depending on the nutrient involved, authors use their own definitions, but in all cases these are clearly stated. Table 1.1 lists many of the common definitions of efficient nutrient and fertilizer use. As already indicated, efficient use of any nutrient comprises two fundamental aspects: acquisition efficiency and utilization efficiency (see also Fig. 1.2). For the target of improving crop nutrient use efficiency, it is important to separate the individual processes and identify the respective genes involved, monitoring improvements with the appropriate physiological measures.

Resolving NUE into two component traits, nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE), is a first step to resolving the complexity, and subsequently each of these traits can be subdivided into many specific physiology traits, each of which are complex traits in itself, the result of networks of biochemical pathways, encoded by multiple genes and subject to complex regulatory processes (Figs. 1.2 and 1.3; Nikiforova et al., 2005; Gojon et al., 2009).

Acquisition efficiency and root architecture

Nutrient capture (NUpE) is essentially a root trait, although to be fully expressed, it also requires adequate sinks for temporary storage or final deposition of the nutrients. Efficient acquisition will depend first on root architecture (see Chapter 2), root functions in terms of transporters and exudates(Chapter 10), and often the presence of symbiotic associations such as mycorrhiza (Chapters 3 and 12). As indicated in Figure 1.2, early root establishment is essential for scavenging soil nutrients prior to the application of fertilizer, or alternatively to capture fertilizer applied at the time of sowing. Nutrients will be immediately available in the soil solution, and further availability will be depend on mineralization of organic matter and release from sparingly soluble soil minerals (oxides, clays, etc.). High activity of the high-affinity transporter systems required for uptake into root cells, expressed in the plasma membranes of cells of roots, root tips, root hairs, or in associated organisms (mycorrizha), will be important in this situation as diffusion of nutrients through soil is the rate-limiting factor. In addition, a

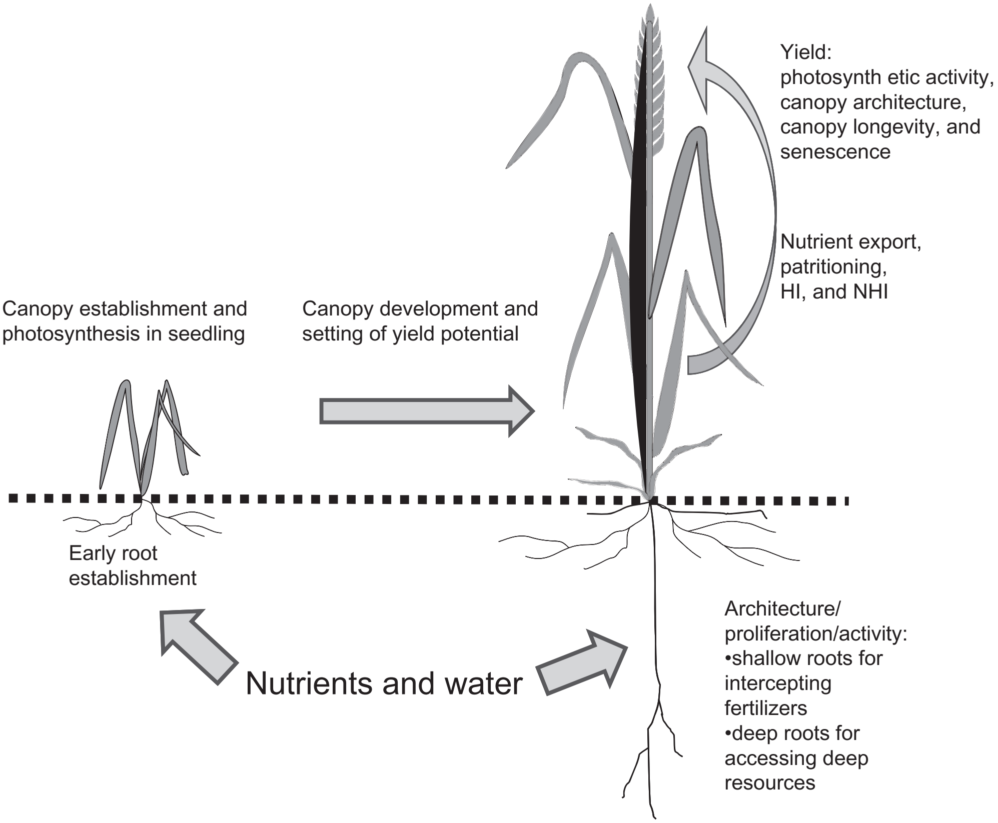

Fig. 1.2. Parameters influencing components of nutrient use efficiency at the crop plant level. Both nutrients and water acquisition depend on root architecture and function. The developing canopy will determine yield; however, for grain crops, nutrient (particularly nitrogen) redistribution from the canopy to the grain (essential for efficient nutrient use and for quality attributes) will negatively affect photosynthesis and limit yield.

well-developed shallow root system will be ideal for intercepting further applications of fertilizer. Deeper roots assume importance with the depletion of surface nutrients, as water near the surface becomes limiting and restricts uptake of nutrients, or in the case of high water supply, for the interception of nutrients that would be potentially leached from the soil profile. In some cases, local proliferation of roots in response to nutrient supply is observed (Drew and Saker, 1978), controlled by specific transcription factors (Zhang and Forde, 1998; Forde 2002).

An alternative approach to enhancing capture mechanisms (root architecture and function) to improve acquisition is to enhance mechanisms for increasing bioavailability of nutrients (e.g., for phosphorus by acid secretions, see Chapters 3 and 12), or to inhibit nitrification losses by the secretion of bio-inhibitors of this process (Subbarao et al., 2007a,b).

Acquisition efficiency and nutrient transporter systems

A key step in mineral nutrient acquisition is the initial transmembrane transport step. In many cases, for any individual nutrient, there are gene families encoding multiple

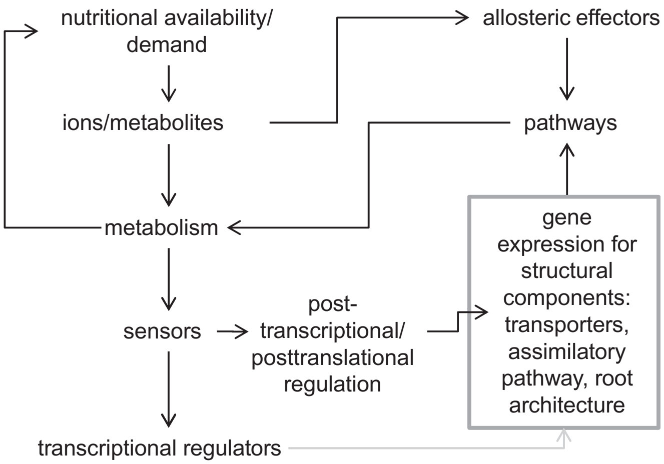

Fig. 1.3. Simple pathway linking supply and demand of nutrients to regulation of gene expression, as envisioned at the cellular level. Complex pathways with multiple components link demands for and availability of nutrients for plant growth, mediated by changes in metabolism, usually as a result of changes in gene expression, but in some cases at the level of enzyme/pathway activity (allosteric regulation).

homologs. In *Arabidopsis*, for example, there are two gene families for nitrate transporters, *NRT1* and *NRT2*, with 53 and 7 members, respectively (see Chapter 10), a gene family of 14 sulfate transporters (Chapter 14 and Hawkesford, 2003) and 9 members of the phosphate transporter family pht1 (Chapter 12 and Smith et al., 2003). While in most cases there are families specific for a single nutrient, there are instances of nonspecificity: Sulfate transporters effectively transport selenate (Shinmachi et al., 2010) and molybdate (Tomatsu et al., 2007; Baxter et al., 2008; Fitzpatrick et al., 2008; Shinmachi et al., 2010). While there is some potential redundancy of function with these large gene families, it has become apparent that there is tissue, developmental, and even membrane specificity with regard to expression patterns. Functionally, there are usually both high and low affinities for the substrate ions, depending on functional requirements: In relation to primary uptake into root cells, the most common functionality is for high-affinity uptake, as required for effective acquisition from soil solutions with low concentrations of ions. Patterns of expression within the root are often complex to effectively transfer the respective ions from the soil solution to the vasculature for transfer to the shoot material. In some instances, vacuolar storage may also play an important part (Kataoka et al., 2004). Many studies have focused on the impacts of nutrient limitation on patterns of transporter expression and the contribution to overall nutrient use efficiency strategies of plants in limiting nutrient availability (Buchner et al., 2004, 2010). For phosphate and sulfate, there is an apparent de-repression system controlling gene expression, facilitating increased expression when nutrient demand exceeds availability (Smith, 2002; Hawkesford and De Kok, 2006). For nitrate, the pattern is more complex, with some transporters induced and others repressed, depending on the presence of nitrate and the nutritional status of the plant (see Chapter 10).

The transporters play essential roles, contributing to nutrient use efficiency, for the most part extremely effectively scavenging nutrients from the soil (potentially present at low concentrations), and particularly in conjunction with effective root proliferation. As targets for improvement of NUE, sophisticated strategies are likely to be important. Modifications to the selectivity (Rogers et al., 2000) may enhance preferential uptake of beneficial ions and exclude toxic ions. Overriding negative feedback mechanisms may facilitate luxury uptake, but appropriate sinks or temporary storage would also be required (see below). One approach that apparently overrides limits on nitrogen uptake is the overexpression of alanine amino transferase in root exodermal tissues, thus channeling nitrogen away from metabolites involved in negative feedback (see Chapter 9). In some instances, enhancing remobilization and optimizing partitioning to harvested organs may require optimization of transporter expression.

Metabolic responses to nutrient availability

Plant responses to nutrient availability are complex and involve changes in pathway fluxes, in activity of pathway enzymes mediated by posttranslation modifications and/or changes in substrate/inhibitor ratios (allosteric effects), as well as changes in expression of genes encoding the pathway enzymes and many additional proteins (Fig. 1.3). The challenge for the plant is to optimize growth and development given the available nutrient inputs. Matching availability to demand may entail many regulatory steps and sensory mechanisms. It is essential to understand these networks before intervention through transgenesis or molecular breeding. For the most part, our knowledge of these regulatory loops is restricted in plants (Gojon et al., 2009).

Nutrient use efficiency, although simply divided into uptake and utilization, encompasses all processes of plant growth and development, and all aspects of metabolism. Potential targets for nutrient use efficiency improvement are therefore diverse. Obvious targets in, for example, nitrogen metabolism (see Chapters 8 and 9) include genes of the assimilatory pathway. Glutamine synthetase has been a specific target for transgenic approaches (Chapter 8), as it is not only involved in primary assimilation but also has a role in efficient recycling of ammonia during senescence processes (Kichey et al., 2006). Generally, results of single-gene manipulation have been disappointing, in part because metabolic pathways form networks that have a great plasticity in responding to perturbations, whether due to gene targeting or environmental fluctuations, for example, in nutrient supply (Wasaki et al., 2003; Palenchar et al., 2004; Nikiforova et al., 2005). Typically, nutrient uptake is balanced by nutritional requirement for growth, and a coordination of pathway expression and activity is seen (Prosser et al., 2001; Hawkesford and De Kok, 2006; Howarth et al., 2008; Gojon et al., 2009) and excess uptake of nutrient is avoided. Excess accumulation of some ions does occur but only to the point at which available storage pools are saturated (for example, nitrate accumulation in vacuoles); this is a strategy to aid with fluctuating supplies of nutrients but is not helpful when one nutrient becomes permanently limiting.

Utilization efficiency

Efficiency of utilization may be defined as biomass production (predominantly fixed carbon) as a function of nutrient taken up (see Table 1.1). This is most often applied for nitrogen, as total canopy nitrogen content reflects the extent of photosynthetically active biomass, as the greatest proportion of the total nitrogen content in this tissue is a major component of proteins involved in photosynthesis. The effectiveness of this capacity in producing harvestable biomass is defined by NUtE. The key attributes that will enhance NUtE are photosynthetic activity, canopy size, longevity, and sink organ capacity (Fig. 1.2).

Photosynthetic activity includes the ability to intercept light, which is clearly linked to canopy architecture and the light harvesting complex density, as well as the biochemistry of the carbon fixation processes, particularly Rubisco, for efficient fixation of carbon dioxide (Parry et al., 2003). An alternative and radical solution is to engineer C4 photosynthesis, which is up to 50% more efficient than C3 photosynthesis, into C3 plants such as rice (Hibberd et al., 2008). Attributes of canopy development and architecture include rapid establishment, followed by proliferation and eventual canopy closure (full coverage of the ground), and then effective architecture to intercept radiant light. Depending on the harvestable product, which may be the canopy itself, or it may be biomass derived from this, for example, woody stem or generative material such as seed, the canopy must be photosynthetically active for as long as possible. Delaying senescence and prolonging the period of photosynthesis results in increased carbon fixation (see Chapters 4 and 5). However, the complexity of processes involved in leaf senescence is highlighted by transcriptome analysis, emphasizing the difficulty in manipulating this process to enhance yield (Gregersen and Holm, 2007). As a target, this process has huge potential for crop improvement, as by definition for a fixed amount of nutrient (nitrogen) taken up, the more carbon that is fixed, the better the NUtE (for a full discussion on the physiology associated with these traits, see Chapter 4).

Sinks

An important attribute for uptake efficiency is having adequate sinks to store acquired nutrients, whether nitrogen or minor but important nutritional components including Fe, Zn, and Se. Adequate sinks will prevent negative feedback regulation on the initial acquisition/assimilatory processes and should provide important remobilizable storage that can be accessed should supply be limiting as well as during production of harvested organs such as seed. Sinks may be subcellular, for example, vacuoles, may be chemical such as nitrogen stores in protein, or may be defined at the organ level, for example, stems (see Chapter 4). Attempts have been made to engineer both metabolism and (Zhu and Galili, 2003) protein sinks to enhance nutritional quality with high methionine, cysteine, or lysine content (Tabe and Higgins, 1998; Nikiforova et al., 2002; see also Chapter 6). As already indicated, one explanation for the remarkable improvement in NUE seen by the overexpression of alanine aminotransferase is that alanine is a local metabolic sink for nitrogen that does not have negative feedback effects on uptake, unlike glutamate (Chapter 9).

HI and partitioning of nutrients

By definition, measures of nutrient use efficiency will be optimum if HI is high and nutrients are partitioned to the harvested material. This ignores the impact at the whole ecosystem level, and there may be merit in not harvesting some nutrients but allowing them to be recycled within the field; this might particularly apply to phosphorus; it does, however, assume that leaching losses will be minimal between crops. In many instances, partitioning to the cropped organ is preferable, for example, nitrogen in the case of grain protein (Chapter 6) and minerals for human nutrition (Chapters 14, 16, and 17). As indicated, a major improvement in yields and NUE was obtained with the introduction of dwarfing genes (into wheat and rice), minimizing the nonharvested fraction of wheat and rice. Although there are efforts to extend the repertoire of dwarfing genes, which may have additional benefits (Ellis et al., 2005), overall, as the HI for many crops has already been optimized, there is likely little benefit from further manipulation of the HI. For those crops for which this is not the case, improving the HI is a high priority. While nutrient harvest index (NHI) for nitrogen is usually high in cereals, this is not the case for all minerals in all crops. Even in wheat, different minerals are partitioned with varied efficiency: selenium and molybdenum were shown to be differentially partitioned to grain (Shinmachi et al., 2010). In *Brassica napus* (oil seed rape, Colza), sulfur is very inefficiently partitioned to the seed (Blake-Kalff et al., 1998).

Manipulating senescence to produce stay-green phenotypes, thus enhancing yield may have a detrimental effect on the HI and NHI. In this case, additional selection for late but rapid nutrient remobilization would be required. Conversely, enhancing the rate of senescence by the introduction of a NAC transcription factor increased remobilization and enhanced grain protein as well as zinc and iron content (Uauy et al., 2006; Waters et al., 2009). However, increasing the onset and rate of senescence may have a negative effect on yield.

Many nutrient deficiencies lead to changes in biomass allocation between roots and shoots, generally increasing the root : shoot ratio (Hermans et al., 2006). This is an adaptive strategy, facilitating the ability to scavenge for nutrients. In nutrient-poor environments, this would be an advantageous trait; however, this does need to be balanced with production of harvestable material. Little is known about the signaling pathways involved; however, the signals may be linked to imbalances in nutrient accumulation in shoot tissues. Signals from the shoots to the roots (Forde, 2002) may be hormonal (Signora et al., 2001) or may be metabolites, for example, carbohydrates (Hermans et al., 2006). Ultimately, partitioning to the cropped organ is of most importance in agricultural production.

Strategies and approaches for the genetic improvement of NUE traits

There is considerable pressure to improve fertilizer use efficiency (see above: economic, environmental, nonrenewable resource use), and this has been traditionally achieved by agronomic practice and breeding for yield in specific environments and agronomic systems. In the case of nitrogen, breeding for yield is equivalent to breeding for nitrogen utilization efficiency at any given nitrogen input, and there has been considerable progress in improving yields and, therefore, the NUtE component of NUE. Generally, wheat varieties responding well at high inputs also respond well at low inputs (Ortiz-Monasterio et al., 1997; Barraclough et al., 2010). However, there is a strong case for selection at varied inputs and for seeking new and untested germplasm to find new alleles for greater NUtE efficiency.

However, acquisition efficiency has been much less specifically selected for, in part due to the difficulties of phenotyping roots. Here, selection at low inputs is vital. Additionally, it will certainly be necessary to introduce wider germplasm pools (landraces, wild relatives) into screening programs, as alleles for high efficiency of acquisition will have almost certainly been lost from the gene pool without the selection pressure for high acquisition efficiency.

Many new technologies for gene discovery (microarrays, deep sequencing tilling transformation SNP detection) are now available and may be combined with established breeding approaches (breeding, quantitative trait loci [QTLs], germplasm screening). In combination with the identification of new traits, technologies for introducing these into modern breeding lines are required (synthetic polyploids, alien introgression, gene transformation; Able et al., 2007). As already mentioned and cautioned by others, single-gene introductions, perhaps through crop transgenesis, are often not successful, particularly when gene selection is from only preexisting biochemical knowledge (Sinclair et al., 2004), almost certainly due to a lack of appreciation of the complexity of the systems being manipulated.

Figure 1.4 outlines an approach for genetic improvement, beginning with a statement of the need to precisely define the trait of interest. Ideally, in order to effectively target the trait, this needs to be resolved to the smallest subcomponent, encoded by just a few genes. After trait prioritization, assessment of variation is required. This may be either natural variation, or variation induced by mutation or by crossing an examination of mapping populations, and will provide material directly for commercial breeding. Variation may also be used to aid in the identification of the target genes. Transcriptome approaches will indicate genes coexpressed with traits of interest; however, these candidates are usually very numerous (Wang et al., 2000, 2001, 2003, 2004; Lu et al., 2005; Gregersen and Holm, 2007). Examination of occurrence across diverse germplasm and expression patterns under multiple conditions will narrow these candidate lists to a few key genes worth further investigation. Definitive implication in crop improvement with respect to NUE may require transgenesis in the crop of interest. Such genetically modified crops may be the end product, or the genes may be used as "perfect" markers for screening other natural populations, avoiding the need for transgenesis. Critically, genes identified by such a route may be more robust than selection based on biochemical pathways alone.

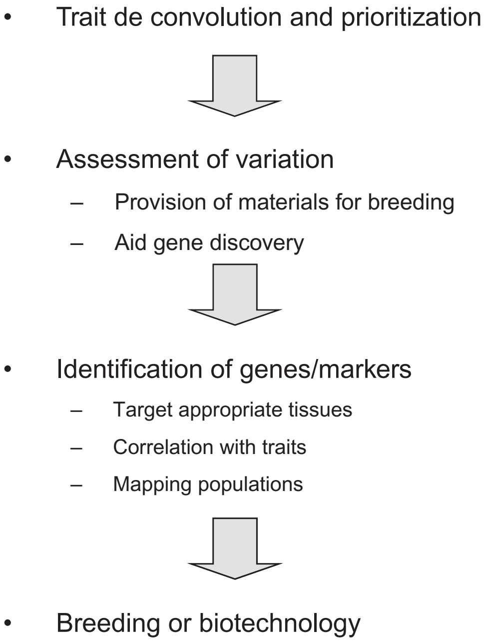

Fig. 1.4. Approaches to finding novel target genes and crop improvement. Germplasm identified with appropriate traits is of direct value for crop improvement. Identified genes will facilitate breeding either as markers or in transgenic approaches.

Prospects

The main targets for improving NUE have been outlined and a case for a thorough understanding of the underlying molecular process has been made. The major targets are improving nutrient capture and interception to avoid losses, modifying requirements (reducing if possible) and enhancing utilization efficiency by generally improving carbon fixation and yield. An ideotype of an idealized set of traits for nutrient use efficiency can be defined and will be crop specific (Foulkes et al., 2009). Furthermore, such ideotypes will be specific to different environments and cropping systems.

Nutrient use efficiency in its broadest sense indicates how effectively a plant is able to capture and utilize nutrients to produce biomass. It is most usually specified for nitrogen as this is a main driver for production. However, healthy and productive crop growth requires a balanced nutrition including several macronutrients and many micronutrients. Irrespective of the quantity needed, all are essential and any limitation will impact on plant growth and crop yields.

In almost all cases, the nutrient in question must be obtained from the pedosphere and therefore uptake processes dependent on architecture and functioning of the roots are critical. Subsequent to this, partitioning within the plant is a vital prerequisite to efficient utilization of the element as part of the plant's growth and developmental cycle. Independent but simultaneous selection for both of these traits must be performed. A radical and alternative solution to providing nitrogen fertilizer would be the transfer of nitrogen fixation capacity, or the ability to form the required symbioses, to nonlegume crops (see Chapter 20).

NUE is an essential component of crop production, and irrespective of the agronomic system, low-input or intense, efficient utilization of valuable resources will be essential for future sustainable food production. NUE is a complex trait that can be broken down into subtraits, all of which are also complex in nature. Few instances can be expected where single genes or a single locus will have a huge benefit; dwarfing genes were an exception. Modern tools and resources available to plant scientists and the agronomy and breeding communities should aid further improvements in NUE and hence crop production. Great variability exists in the extent to which individual crops have been optimized in relation to NUE, and while large improvements may be anticipated for some crops, for the major world grain crops such as wheat maize and rice, smaller incremental improvements are likely. The prospect of step changes in primary production by engineering the photosynthetic process itself will require additional concomitant improvements in nutrient acquisition efficiency.

Acknowledgments

Rothamsted Research is an institute of the Biotechnology and Biological Sciences Research Council of the United Kingdom. The author's research is also supported by the Biotechnology and Biological Sciences Research Council (BB/G022437/1 and BB/C514066/1) and the Department of Environment, Food and Rural Affairs (WGIN project IF0146).

References

Able, J.A., Langridge, P., & Milligan, A.S. (2007) Capturing diversity in the cereals: many options but little promiscuity. *Trends in Plant Science* **12**, 71–79.

Araus, J.L., Slafer, G.A., Royo, C., et al. (2008) Breeding for yield potential and stress adaptation in cereals. *Critical Reviews in Plant Sciences* **27**, 377–412.

Austin, R.B. (1999) Yield of wheat in the United Kingdom: recent advances and prospects. *Crop Science* **39**, 1604–1610.

Barraclough, P.B., Howarth, J.R., Jones, J., et al. (2010) Nitrogen efficiency of wheat: genotypic and environmental variation and prospects for improvement. *European Journal of Agronomy* **33**, 1–11.

Baxter, I., Muthukumar, B., Park, H.C., et al. (2008) Variation in molybdenum content across broadly distributed populations of *Arabidopsis thaliana* is controlled by a mitochondrial molybdenum transporter (MOT1). *PLoS Genetics* **4**, e1000004.

Blake-Kalff, M.M.A., Harrison, K.R., Hawkesford, M.J., et al. (1998) Distribution of sulfur within oilseed rape leaves in response to sulfur deficiency during vegetative growth. *Plant Physiology* **118**, 1337–1344.

Bogard, M., Allard, V., Brancourt-Hulmel, M., et al. (2010) Deviation from the grain protein concentration–grain yield negative relationship is highly correlated to post-anthesis N uptake in winter wheat. *Journal of Experimental Botany* **61**, 4303–4312.

Brancourt-Hulmel, M., Doussinault, G., Lecomte, C., et al. (2003) Genetic improvement of agronomic traits of winter wheat cultivars released in France from 1946 to 1992. *Crop Science* **43**, 37–45.

Buchner, P., Takahashi, H., & Hawkesford, M.J. (2004) Plant sulphate transporters: co-ordination of uptake, intracellular and long-distance transport. *Journal of Experimental Botany* **55**, 1765–1773.

Buchner, P., Parmar, S., Kriegel, A., et al. (2010) The sulfate transporter family in wheat: tissue-specific gene expression in relation to nutrition. *Molecular Plant* **3**, 374–389.

Drew, M.C. & Saker, L.R. (1978) Nutrient supply and growth of seminal root-system in barley. 3. Compensatory increases in growth of lateral roots, and in rates of phosphate uptake, in response to a localized supply of phosphate. *Journal of Experimental Botany* **29**, 435–451.

Ellis, M.H., Rebetzke, G.J., Azanza, F., et al. (2005) Molecular mapping of gibberellin-responsive dwarfing genes in bread wheat. *Theoretical and Applied Genetics* **111**, 423–430.

Evans, L.T. & Fischer, R.A. (1999) Yield potential: its definition, measurement, and significance. *Crop Science* **39**, 1544–1551.

Fischer, R.A.T. & Edmeades, G.O. (2010) Breeding and cereal yield progress. *Crop Science* **50**, S85–S98.

Fitzpatrick, K.L., Tyerman, S.D., & Kaiser, B.N. (2008) Molybdate transport through the plant sulfate transporter SHST1. *FEBS Letters* **582**, 1508–1513.

Forde, B.G. (2002) Local and long-range signaling pathways regulating plant responses to nitrate. *Annual Review of Plant Biology* **53**, 203–224.

Foulkes, M.J., Hawkesford, M.J., Barraclough, P.B., et al. (2009) Identifying traits to improve the nitrogen economy of wheat: recent advances and future prospects. *Field Crops Research* **114**, 329–342.

Gojon, A., Nacry, P., & Davidian, J.C. (2009) Root uptake regulation: a central process for NPS homeostasis in plants. *Current Opinion in Plant Biology* **12**, 328–338.

Gregersen, P.L. & Holm, P.B. (2007) Transcriptome analysis of senescence in the flag leaf of wheat (*Triticum aestivum* L. *Plant Biotechnology Journal* **5**, 192–206.

Hawkesford, M.J. (2003) Transporter gene families in plants: the sulphate transporter gene family—redundancy or specialization? *Physiologia Plantarum* **117**, 155–163.

Hawkesford, M.J. & De Kok, L.J. (2006) Managing sulphur metabolism in plants. *Plant, Cell & Environment* **29**, 382–395.

Hermans, C., Hammond, J.P., White, P.J., et al. (2006) How do plants respond to nutrient shortage by biomass allocation? *Trends in Plant Science* **11**, 610–617.

Hibberd, J.M., Sheehy, J.E., & Langdale, J.A. (2008) Using C-4 photosynthesis to increase the yield of rice—rationale and feasibility. *Current Opinion in Plant Biology* **11**, 228–231.

Howarth, J.R., Parmar, S., Jones, J., et al. (2008) Co-ordinated expression of amino acid metabolism in response to N and S deficiency during wheat grain filling. *Journal of Experimental Botany* **59**, 3675–3689.

Kataoka, T., Watanabe-Takahashi, A., Hayashi, N., et al. (2004) Vacuolar sulfate transporters are essential determinants controlling internal distribution of sulfate in Arabidopsis. *The Plant Cell* **16**, 2693–2704.

Kichey, T., Heumez, E., Pocholle, D., et al. (2006) Combined agronomic and physiological aspects of nitrogen management in wheat highlight a central role for glutamine synthetase. *The New Phytologist* **169**, 265–278.

Kichey, T., Hirel, B., Heumez, E., et al. (2007) In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers. *Field Crops Research* **102**, 22–32.

Lu, C.G., Hawkesford, M.J., Barraclough, P.B., et al. (2005) Markedly different gene expression in wheat grown with organic or inorganic fertilizer. *Proceedings of the Royal Society B-Biological Sciences* **272**, 1901–1908.

Monaghan, J.M., Snape, J.W., Chojecki, A.J.S., et al. (2001) The use of grain protein deviation for identifying wheat cultivars with high grain protein concentration and yield. *Euphytica* **122**, 309–317.

Nikiforova, V., Kempa, S., Zeh, M., et al. (2002) Engineering of cysteine and methionine biosynthesis in potato. *Amino Acids* **22**, 259–278.

Nikiforova, V.J., Kopka, J., Tolstikov, V., et al. (2005) Systems rebalancing of metabolism in response to sulfur deprivation, as revealed by metabolome analysis of Arabidopsis plants. *Plant Physiology* **138**, 304–318.

Ortiz-Monasterio, J.I., Sayre, K.D., Rajaram, S., et al. (1997) Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. *Crop Science* **37**, 898–904.

Palenchar, P.M., Kouranov, A., Lejay, L.V., et al. (2004) Genome-wide patterns of carbon and nitrogen regulation of gene expression validate the combined carbon and nitrogen (CN)-signaling hypothesis in plants. *Genome Biology* **5**, R91.

Parry, M.A.J. & Hawkesford, M.J. (2010) Genetic approaches to reduce greenhouse gas emissions: increasing carbon capture and decreasing environmental impact. In: *Climate Change and Crop Production* (ed. M.P. Reynolds), pp. 139–150. CABI, Wallingford, U.K.

Parry, M.A.J., Andralojc, P.J., Mitchell, R.A.C., et al. (2003) Manipulation of Rubisco: the amount, activity, function and regulation. *Journal of Experimental Botany* **54**, 1321–1333.

Prosser, I.M., Purves, J.V., Saker, L.R., et al. (2001) Rapid disruption of nitrogen metabolism and nitrate transport in spinach plants deprived of sulphate. *Journal of Experimental Botany* **52**, 113–121.

Raun, W.R. & Johnson, G.V. (1999) Improving nitrogen use efficiency for cereal production. *Agronomy Journal* **91**, 357–363.

Robinson, N., Fletcher, A., Whan, A., et al. (2007) Sugarcane genotypes differ in internal nitrogen use efficiency. *Functional Plant Biology* **34**, 1122–1129.

Rogers, E.E., Eide, D.J., & Guerinot, M.L. (2000) Altered selectivity in an Arabidopsis metal transporter. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 12356–12360.

Shinmachi, F., Buchner, P., Stroud, J.L., et al. (2010) Influence of sulfur deficiency on the expression of specific sulfate transporters and the distribution of sulfur, selenium, and molybdenum in wheat. *Plant Physiology* **153**, 327–336.

Signora, L., De Smet, I., Foyer, C.H., et al. (2001) ABA plays a central role in mediating the regulatory effects of nitrate on root branching in Arabidopsis. *The Plant Journal* **28**, 655–662.

Sinclair, T.R., Purcell, L.C., & Sneller, C.H. (2004) Crop transformation and the challenge to increase yield potential. *Trends in Plant Science* **9**, 70–75.

Smith, F.W. (2002) The phosphate uptake mechanism. *Plant and Soil* **245**, 105–114.

Smith, F.W., Mudge, S.R., Rae, A.L., et al. (2003) Phosphate transport in plants. *Plant and Soil* **248**, 71–83.

Subbarao, G.V., Rondon, M., Ito, O., et al. (2007a) Biological nitrification inhibition (BNI)—is it a widespread phenomenon? *Plant and Soil* **294**, 5–18.

Subbarao, G.V., Tomohiro, B., Masahiro, K., et al. (2007b) Can biological nitrification inhibition (BNI) genes from perennial *Leymus racemosus* (Triticeae) combat nitrification in wheat farming? *Plant and Soil* **299**, 55–64.

Tabe, L. & Higgins, T.J.V. (1998) Engineering plant protein composition for improved nutrition. *Trends in Plant Science* **3**, 282–286.

Tilman, D., Fargione, J., Wolff, B., et al. (2001) Forecasting agriculturally driven global environmental change. *Science* **292**, 281–284.

Tilman, D., Cassman, K.G., Matson, P.A., et al. (2002) Agricultural sustainability and intensive production practices. *Nature* **418**, 671–677.

Tomatsu, H., Takano, J., Takahashi, H., et al. (2007) An *Arabidopsis thaliana* high-affinity molybdate transporter required for efficient uptake of molybdate from soil. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 18807–18812.

Uauy, C., Distelfeld, A., Fahima, T., et al. (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* **314**, 1298–1301.

Wang, Y.H., Garvin, D.F., & Kochian, L.V. (2001) Nitrate-induced genes in tomato roots. Array analysis reveals novel genes that may play a role in nitrogen nutrition. *Plant Physiology* **127**, 345–359.

Wang, R.C., Guegler, K., LaBrie, S.T., et al. (2000) Genomic analysis of a nutrient response in Arabidopsis reveals diverse expression patterns and novel metabolic and potential regulatory genes induced by nitrate. *Plant Cell* **12**, 1491–1509.

Wang, R.C., Okamoto, M., Xing, X.J., et al. (2003) Microarray analysis of the nitrate response in Arabidopsis roots and shoots reveals over 1000 rapidly responding genes and new linkages to glucose, trehalose-6-phosphate, iron, and sulfate metabolism. *Plant Physiology* **132**, 556–567.

Wang, R.C., Tischner, R., Gutierrez, R.A., et al. (2004) Genomic analysis of the nitrate response using a nitrate reductase-null mutant of Arabidopsis. *Plant Physiology* **136**, 2512–2522.

Wasaki, J., Yonetani, R., Kuroda, S., et al. (2003) Transcriptomic analysis of metabolic changes by phosphorus stress in rice plant roots. *Plant, Cell & Environment* **26**, 1515–1523.

Waters, B.M., Uauy, C., Dubcovsky, J., et al. (2009) Wheat (*Triticum aestivum*) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. *Journal of Experimental Botany* **60**, 4263–4274.

Zhang, H. & Forde, B.G. (1998) An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. *Science* **279**, 407–409.

Zhu, X.H. & Galili, G. (2003) Increased lysine synthesis coupled with a knockout of its catabolism synergistically boosts lysine content and also transregulates the metabolism of other amino acids in Arabidopsis seeds. *Plant Cell* **15**, 845–853.