



The Genetics of Nitrogen Use Efficiency in Crop Plants

Mei Han,¹ Mamoru Okamoto,² Perrin H. Beatty,¹
Steven J. Rothstein,³ and Allen G. Good^{1,*}

¹Department of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada; email: mhan2@ualberta.ca, pbeatty@ualberta.ca, allen.good@ualberta.ca

²Australian Center for Plant Functional Genomics, The University of Adelaide, PMB1, Glen Osmond, South Australia, 5064, Australia; email: mamoru.okamoto@acpfg.com.au

³Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada; email: rothstei@uoguelph.ca

Annu. Rev. Genet. 2015. 49:9.1–9.21

The *Annual Review of Genetics* is online at
genet.annualreviews.org

This article's doi:
10.1146/annurev-genet-112414-055037

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*Corresponding author

Keywords

N fertilizer, cereals, N uptake, N assimilation, N remobilization, quantitative trait loci

Abstract

In the past 50 years, the application of synthetic nitrogen (N) fertilizer to farmland resulted in a dramatic increase in crop yields but with considerable negative impacts on the environment. New solutions are therefore needed to simultaneously increase yields while maintaining, or preferably decreasing, applied N to maximize the nitrogen use efficiency (NUE) of crops. In this review, we outline the definition of NUE, the selection and development of NUE crops, and the factors that interact with NUE. In particular, we emphasize the challenges of developing crop plants with enhanced NUE, using more classical genetic approaches based on utilizing existing allelic variation for NUE traits. The challenges of phenotyping, mapping quantitative trait loci (QTLs), and selecting candidate genes for NUE improvement are described. In addition, we highlight the importance of different factors that lead to changes in the NUE components of nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE).

Nitrogen use efficiency (NUE): efficiency to uptake and utilize biologically reactive nitrogen from the growth medium, $NUE = N_{UpE} \times N_{UtE} = \text{grain yield (Gw)}/\text{supplied N (Ns)}$

INTRODUCTION

The past five decades have seen some remarkable changes and advances in crop production, from the Green Revolution in the early 1960s to the advent of modern biotechnology in the 1990s. Improved crop management and agronomy combined with improved crop genetics through conventional breeding and genetic engineering have been the major factors behind increased crop production. Crop genetic improvement has been responsible for 50% to 60% of the increases in crop yields and is still a crucial component of any strategy to increase crop yields and nutrient use efficiency (41). Additionally, the large yield increases have also been due to the use of synthetic nitrogen (N), phosphorous (P), and potassium (K) fertilizers. One example of high fertilizer use and increased yields can be seen in the maize breeding programs, where breeders have selected for high seed-density-tolerant, high-yielding genotypes, in synchrony with high levels of applied N fertilizer (40, 42, 98).

Currently, there are two key concerns regarding crop yields and fertilizer application. The first is that traditional breeding strategies to improve crop plants may have reached a plateau. According to the Food and Agriculture Organization of the United Nations, since the mid-1980s global cereal crop yields, including wheat, soy, and maize, have slowed to a growth rate of about 1% annually, and in developed countries, growth of crop yields is closer to zero (47). Thus, it is clear that we face challenges in increasing yield, and many yield predictions are unrealistic (108). The second concern is that further increases in applied N may not result in yield improvements but will lead to serious environmental problems (27, 34, 56, 88, 130, 134, 141). Although the application rates of synthetic N fertilizers used in the United States on most crops increased following World War II, they accelerated markedly after the mid-1960s but have leveled off since the 1980s (32, 59). In contrast, in China between 1980 and 2010, the rate of cereal grain yield increased by 65%; however, the consumption of chemical fertilizers increased by 512% (29, 139). As a consequence, total crop yields in many intensive farming systems have failed to increase in proportion to the increase of chemical fertilizers over the past 20 years, leading to low-nutrient use efficiency and increasing environmental problems (109). To achieve further increases in yield under well-fertilized conditions, we need to select for plants that use fertilizers more efficiently. Simultaneously achieving high crop productivity and high nutrient use efficiency is a major challenge, given the increased global demand for food, feedstock, and biofuels (26, 27, 55, 117). New solutions are needed to increase yields while maintaining, or preferably decreasing, applied N (56, 62).

In this review, we discuss the challenges of developing crop plants with enhanced nitrogen use efficiency (NUE), using more classical genetic approaches based on utilizing existing allelic variation for NUE traits. The alternative of using a transgenic approach is not discussed, as the key aspects of this have been covered in a number of recent reviews (10, 81).

THE BENEFITS OF IMPROVING NITROGEN USE EFFICIENCY

There are two main incentives to improve NUE in crop plants in order to use less N fertilizer: one is the economic gain, the other is N pollution reduction. Although the economic costs associated with inefficient N use are difficult to assess, Raun & Johnson (103) have estimated that every 1% increase in N fertilizer uptake efficiency would result in an approximate US \$2.3 billion savings in annual N fertilizer costs. In Canada, N fertilizers represented approximately 10% (or US \$3.4 billion) of the total farm operating costs in 2010. With annual growth rates of approximately 5%, it is estimated that increased annual fertilizer purchases will add approximately C\$170 million in costs per year to Canadian farmers (3). In addition, there are the unpredictable variations in

costs due to fluctuations in the price of the fossil fuels used to synthesize N fertilizers. Although challenging for all farmers, this volatility has been linked to recent increases in commodity prices and the resulting political instability in many Arab nations over the past decade.

There are many concerns about the negative impact of excess N on the environment. The Europe, the societal damage costs of excess N (e.g., water and air quality) has been estimated to be US \$91–\$466 billion annually (115). These numbers are significantly greater than the costs of excess N estimated by Beatty & Good (10), although it is difficult to calculate these costs with any degree of precision.

DEFINING NITROGEN USE EFFICIENCY

The fundamental approach to developing cultivars with enhanced NUE, in contrast to just improved yield, requires evaluating the plants under both low and high N conditions so as to identify a NUE-efficient genotype and compare its performance with that of other genotypes under various N conditions. Alternatively, one can measure N content directly in different tissue or developmental stages to calculate NUE. A number of different definitions and calculations of NUE include N utilization, N content, and N availability as NUE equation components (38, 49, 57, 85, 86). NUE comprises two key components: N uptake efficiency (NUpE), which is the efficiency of absorption/uptake of supplied N, and N utilization efficiency (NUtE), which is the efficiency of assimilation and remobilization of plant N to ultimately produce grain (57). The simplest definition of plant NUE is the grain yield per unit of supplied N, also represented by the product of NUpE and NUtE. Another method to describe NUE is the utilization index (UI), which factors in the absolute amount of biomass produced per unit of N. NUE can also be described as NUEg, which is grain production per unit of N available. However, a crop plant could produce large amounts of biomass per unit N (high UI) without converting the acquired N to seed production and therefore have a low NUEg. Other NUE calculations take various agronomic and physiological variations into account and are described elsewhere (30, 38, 49, 57, 62). In theory, improving NUE could be achieved by improving either NUpE or NUtE, or one or more of the components of these.

It is only in the recent past that plant breeders and geneticists have begun to select and analyze for improvements in NUE directly. Breeding and selection of improved varieties are generally done under high N supply, where the level of N is not limiting yield. This means that plant breeders select cultivars that perform well under these conditions, and therefore, by definition, if the selection criteria were for increased yield, then improved crop NUE was also selected for by default. The classical example of selecting for a plant's ability to utilize N efficiently was Norman Borlaug's introduction of dwarfing genes and selection for the resulting semidwarf high yielders. These genes (*Rht-B1* and *Rht-D1*), which were originally derived from a cross between a Japanese variety of dwarf wheat (Norin 10) and a high-yielding American variety (Brevor), became the model for the use of dwarfing genes to allow plants to use higher levels of N without the lodging common in the tall varieties (104). The dwarfing genes altered stem strength and plant architecture and indirectly generated plants that could produce much higher yield under high (standardized) levels of fertilizer and hence had enhanced NUE (104). However, since the selection of these high-yielding, semidwarf wheat varieties on high N, what has been observed on a global scale, especially in developed countries, is a plateauing of any more yield increases, even while the crops are grown using the same high N levels. As NUE is a ratio of yield to N supply, this indicates that modern varieties are not improving in their NUE. Conversely, in many parts of the world, especially in developing countries, low-nutrient soils are the norm and often there are neither the finances nor the infrastructure to provide N-based fertilizers to the smallholder farmers. Geneticists now need

N uptake efficiency (NUpE):

the efficiency of absorption/uptake of supplied/available N, $NUpE = \text{plant N} / (N_t) / N_s$

N utilization, or usage, efficiency (NUtE):

the efficiency of assimilation and remobilization of plant N to produce grain, $NUtE = Gw / Nt$

Utilization index (UI):

total plant biomass produced multiplied by the ratio of the total plant biomass to the total plant N

NUEg:

grain production per available N

to select genotypes/hybrids for their ability to yield under low N if they are developing these for smallholder farmers. If there is a strong correlation between yields under high and low N, then a breeder can select for broad adaptability on nutrient-replete soils.

IMPROVING NITROGEN USE EFFICIENCY AND THE AVAILABILITY OF GENETIC VARIABILITY

Given the environmental heterogeneity that exists for nutrients in the soil, it is perhaps not surprising that there is a significant amount of genetic variation and phenotypic plasticity for NUE (20, 35). Therefore, in addressing whether we can use traditional genetics to improve NUE, one needs to determine the level of genetic variation present in the different land races and genotypes of a crop. In order to parse out the contribution of N level from genetic and other environmental effects to plant yield, researchers need to be able to study a defined genetic population under different N conditions. However, as mentioned above, there are other factors to consider besides the genetics, such as the interactions between N uptake and water availability and the interaction between different macronutrients and micronutrients. When selecting for NUE, any variation in the environmental conditions could be as significant as genotype. If genotype rather than phenotype is used for selection, then an understanding of the genes that control the desired traits is also required. A large genotype by environment ($G \times E$) interaction on the expression of target traits is undesirable because it implies that the sought after improvements are not robust and may be observed only under specifically controlled conditions. In addition, the trade-offs associated with other desirable characteristics must also be considered. For example, modifying the root system to increase the uptake of nitrate from the subsoil could have negative effects on the uptake efficiency of less mobile, topsoil-located ions such as phosphate (14, 16, 68). In order to reconcile some of these conflicting demands and to help direct plant breeding, greater emphasis is currently being placed on the design of crop ideotypes for particular cropping systems and end uses.

FACTORS AFFECTING NITROGEN USE EFFICIENCY

The question of whether improvements in NUE result from NU_{pE} , NU_{tE} , or both has been looked at in maize, wheat, and barley. For maize, the relative contributions of NU_{pE} and NU_{tE} to overall NUE varies depending on a number of variables. Bertin & Gallais (13) found that in maize NU_{pE} was more closely correlated to NUE at high N and to NU_{tE} at low N. Moll et al. (85) showed that maize hybrids with similar NUE differed considerably in uptake and utilization efficiencies, such that at low applied N, NU_{tE} contributed more to the variation in NUE, whereas at high applied N, variation in NUE was almost entirely due to differences in NU_{pE} . In contrast, Kamprath et al. (71) reported that genetic improvement of NUE in two maize populations was associated with increased NU_{pE} at low N and increased NU_{tE} at high N. In a comparison of hybrids derived from selection at low N versus high N, Presterl et al. (101) concluded that low-N-developed hybrids had superior N uptake compared with high N hybrids, and both groups had similar N utilization. NUE of tropical hybrids was influenced to similar extents by both NU_{pE} and NU_{tE} (132). Finally, the Illinois long-term maize experiments, which have now run for more than 100 years, have demonstrated that one can perform divergent selection for increased grain protein concentration (GPC), and that this primarily impacts N utilization (125).

For wheat, the question of whether genetic gains in NUE can be explained by NU_{pE} or NU_{tE} depends on the level of N supply and on the study. Studies in the United Kingdom, Mexico, and Finland (50, 89, 97) all found that genetic gains in NUE were explained roughly equally by NU_{pE} and NU_{tE} , but that the importance depended on the level of available N. In an investigation of

wheat cultivars in France, NUpE accounted for more of the genetic variation in NUE at low N than at high N (76). In contrast, for 12 spring wheat varieties in California, NUpE was the most important component of NUE at both low and high N supply (37). In an examination of 39 cultivars at 5N rates in the United Kingdom, cultivar differences in N uptake were found only at the highest 3N rates, and NUtE explained more of the variation in grain yield than NUpE at all 5N rates (8). Similar observations were made by Ortiz-Monasterio et al. (97) and Le Gouis et al. (76), who observed that NUpE was more closely correlated to NUE at low N than at high N. This has also been found to be the case in other species, including barley (5) and oilseed rape (70).

Collectively, these studies suggest that the importance of NUpE and NUtE to NUE varies considerably depending on how these traits are measured or derived, the level of N fertilizer applied, and the germplasm under evaluation. Clearly, significant improvements in NUE require coordinated improvements of both NUpE and NUtE, and it is important to understand the interactions between uptake and utilization, as well as their genetic and environmental regulation (101).

Quantitative trait locus (QTL): a statistical method that links phenotype (trait) to genotype (section of chromosome containing genes)

THE CHALLENGES OF PHENOTYPING NITROGEN USE EFFICIENCY

One of the challenges to improving a phenotype (trait) determined by multiple genes is the collection of high-quality phenotypic data (90). For a complex trait like NUE, it is clear that although we have copious amounts of genetic and sequence data for each crop species, using that data meaningfully to improve phenotype is challenging. As such, experimenters should now focus on the implementation of appropriate experimental design that maximizes phenotyping efforts, rather than on the collection of more genotypic data. The selection of germplasm of appropriate levels of relatedness and the generation of high-quality phenotype data will be the major determinants of the ability to utilize the combined genetic and phenotypic data in the future (90). For example, it is difficult to compare genotypes selected in very different environments, owing to the distinctly different phenology of the genotypes. Many geneticists and breeders regularly evaluate germplasm from geographically diverse breeding programs; however, the North American, Australian, and European germplasm are all so distinctly different that making comparisons of NUE becomes difficult, owing to the many complicating factors affecting yield. A further complication is that for the development of commercial varieties of most crop plants, breeders stay within very limited germplasm (91), and therefore using quantitative trait locus (QTL) data from wide crosses that have generated relatively robust QTL information may not be relevant in developing modern crop varieties of maize, wheat, rice, and other cereal crops. Therefore, despite the significant investments in genomics, improving NUE, or for that matter any quantitative trait, will increasingly depend on the agronomist and accurate phenotyping than on genotyping.

Economically important traits are frequently polygenic; therefore, it is unlikely that a single genotype or recombinant inbred line (RIL) would contain the necessary contrast in the relevant genes underpinning the trait(s). For example, a trait controlled by 10 independent genes would require assessment of more than 3,000 backcrossed F₁ individuals to be 95% confident of identifying at least one F₁ individual heterozygous for all 10 alleles (17). Population sizes required for investigating polygenic traits are very large (>200 RILs) and therefore are resource intensive to phenotype confidently. If this is extended to multiple environmental backgrounds, different fertilizer treatments, and combined traits (e.g., higher yield while maintaining protein content), then the resources required become significant, even given the best genetic material and experimental design.

There is also the difficulty of measuring the trait reliably. Three to five percent increases in yield are fairly standard for differences in maize genotypes (112). However, in order to see this difference in the field, multiple field trials with large numbers of plots must be performed, which becomes very expensive if a large number of different genotypes are tested. Moreover, the researcher must

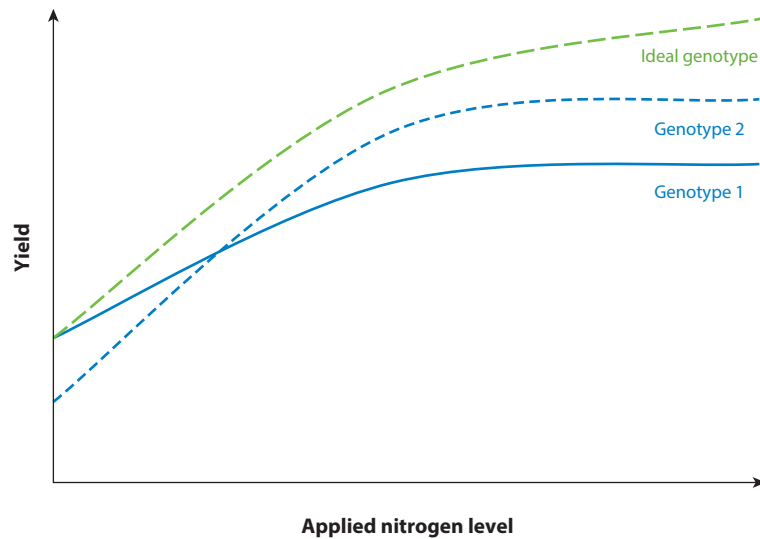


Figure 1

Nitrogen (N) response curve of three different genotypes differing in genetic N efficiency (yield at low N) and N responsiveness (ability to use synthetic N).

be aware of the many sources of inherent experimental variability, such as seed source, that must be accounted for when comparing different genetic lines to detect small quantitative changes in complex traits like NUE.

Several other issues must also be considered when analyzing NUE. First, as demonstrated in **Figure 1**, different genotypes may have very different abilities to use N fertilizers, based on the amount of applied N, which is determined by whether a producer applies significant amounts of synthetic N (developed world farmers) or not (developing world farmers). Genotype 1 is more efficient at low N and is more useful to a subsistence farmer in Africa; therefore, we say it has a high genetic N efficiency, whereas Genotype 2, which is the type that a breeder in the developed world would select, has a lower genetic efficiency but a higher N responsiveness. The ideal genotype has both high genetic N efficiency and high N responsiveness. Provided the traits for N efficiency and N responsiveness are not genetically linked (although they probably are to some degree), one should be able to select for genotypes that perform better under both conditions, requiring the evaluation of genotypes under different N conditions.

Second, one of the key concepts defining the relationship between the N that crops need and the N that harms the environment is nutrient synchrony (22, 31, 107). When N supply (via fertilizer application or organic matter mineralization) is in excess of crop N demand, they are asynchronous and N can accumulate in soils and be susceptible to various loss pathways (**Figure 2**) (58, 99). Current agronomic practices tend to set up the scenario where nutrient supply exceeds plant requirements early in the growing season, because N is applied when plant demand is low or nonexistent (e.g., in spring temperate annual cropping systems). Thus, the challenge becomes having a plant maximize its early N uptake; therefore, traits such as early vigor, an extensive root system, and efficient, developmentally early N-uptake systems are valuable in crops. Later in development, the ability to assimilate and remobilize the available N and associated carbon to the grain is also obviously crucial. **Figure 2** illustrates both this asynchrony and an additional problem associated with measuring NUE, that of developmental time by showing two

Genetic N efficiency:
ability to produce high grain yield with no additional N fertilizer application

N responsiveness:
ability to respond to additional N applications with increased yield

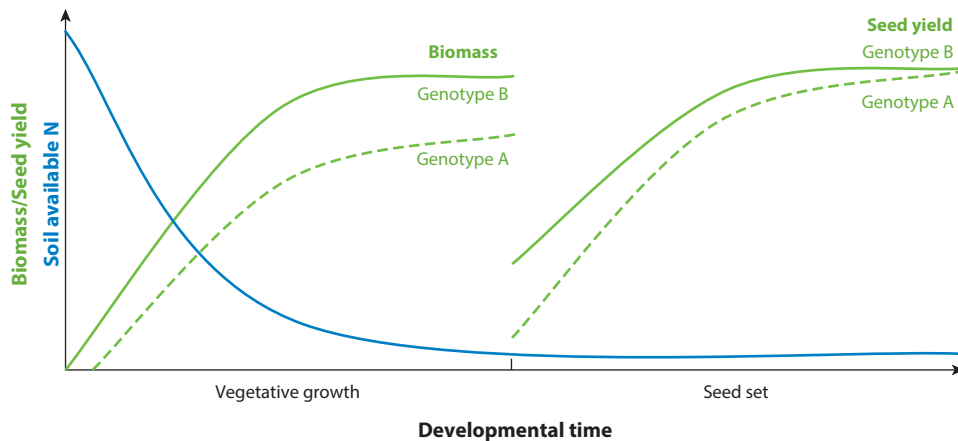


Figure 2

Plant growth and nitrogen availability during plant development illustrating asynchrony and the importance of measuring nitrogen uptake efficiency and nitrogen utilization efficiency.

different genotypes, NU_{pE} and NU_{tE} , which differ in their growth rates, and the amount of available N in the soil. Different genotypes can show the same NUE and yield, even when their NU_{pE} and NU_{tE} are different, depending on the N accumulation in the plant at different times (either total biomass N or seed N). Genotype A is a slower growing plant that achieves lower total N content (lower NU_{pE}) than Genotype B by the beginning of seed set; however, it has a higher NU_{tE} due to a higher ratio between yield and total plant N. These two genotypes in the end produce the same N yield but in very different manners. For example, if Genotype B had 20% greater total N but was 20% less efficient at remobilizing the stored N into the grain, then these two genotypes would have the same N yield. However, if remobilization efficiency could be separate genetically from N storage, yield could be improved by 20%. **Figures 1** and **2** illustrate that geneticists and breeders need to consider whether they are interested in N genetic efficiency, N responsiveness, or both (9). It should be possible to develop genotypes that provide higher yield under lower N while maintaining the ability to convert applied fertilizer into yield.

TRAITS AFFECTING NITROGEN UPTAKE EFFICIENCY

Physiological traits that may affect NU_{pE} include root architecture and any other characteristic that impacts the roots' ability to extract available N from the soil (49). A simple example of improving NU_{pE} would be to increase uptake with expression of more efficient transporters, or increase expression of all the transporters; however, it is unlikely that upregulating transporters only would increase N uptake (61). A second example is to target genes identified to play a part in root architecture (54, 140). However, there have yet to be any examples in which the manipulation of these genes affects N uptake in a crop plant (81)

Studying NU_{pE} and root systems, especially in soil, is still difficult and so it is one area that remains relatively unexplored. The capacity of the root for uptake depends on the degree to which the root extends its absorption area, which is determined by complex root morphology. The lateral roots, which occupy more than 90% of the total length of the root system, play a major role in water uptake. Despite their importance, it has proven difficult to perform field-based root phenotyping (61). To date, studies have been carried out to identify root morphological features

Nutrient synchrony: the extent to which the rate of crop N supply matched the rate of crop N demand

N harvesting index (NHI): percentage of the total N allocated to the grain

such as root mass and depth, root axis length, and lateral branching related to NUE (69, 110, 136). Although the development of better field phenotyping using different scanners looks promising, these techniques currently work only in controlled artificial conditions, and more labor intensive techniques, including field sampling of DNA to quantify the amount to root tissue, are both expensive and challenging

TRAITS AFFECTING NITROGEN UTILIZATION EFFICIENCY

A number of physiological traits can affect the NUtE in crops, including the effect of N on carbohydrate partitioning, the storage of N, and the remobilization of N from senescent tissues, and these have been subdivided into a number of different components by different researchers (67, 77, 93). Gallais & Hirel (52) looked at several components of N remobilization, including stem remobilization, leaf blade remobilization, and total remobilization in a set of RILs in maize and identified many QTLs for these traits. Other researchers have examined N harvesting index (NHI). The minimum N% in the grain at harvest largely determines how much grain can be produced per unit of absorbed N. Van Keulen (128) concluded that the minimum level to which N can be diluted in small grains is approximately 1.6%, and Foulkes et al. (49) reported that current UK winter wheat cultivars had a minimum N% value of approximately 1.25%. If the NHI remains constant, then increasing NUtE results in decreased grain N content (GNC). Raising NUtE associated with lower grain N is feasible in end-use markets for which a high grain starch-to-protein ratio is desirable, e.g., the feed, distilling, and biofuel markets. A lower GNC implies more photosynthesis per unit of absorbed N, and there are a number of metabolic and physiological mechanisms by which this may potentially be achieved. These have been discussed in more detail by Foulkes et al. (49) and include a higher level of photosynthesis per unit N, better postanthesis N remobilization, or the stay-green trait, which is the capacity of a genotype to retain a photosynthetic green leaf for longer than a standard genotype during grain filling (116)

DROUGHT: THE KEY FACTOR THAT INTERACTS WITH NITROGEN UPTAKE EFFICIENCY

Any analysis of different plant genotypes on yield, total N uptake, or NUE will demonstrate a genetic component to NUE, along with a strong environmental component and $G \times E$ (gene \times environment) interaction. However, are there environmental factors that can be monitored and used to provide a better understanding of the analysis required to enhance NUE? Clearly, the most important environmental factor affecting NUE efficiency is water availability. Without sufficient water, plants cannot extract nutrients from the soil and yield is constrained by moisture availability, not N availability. In maize, there are many examples in which drought and NUE have been shown to interact (reviewed in 7). Much of the effort has been to breed for varieties that grow well under the low N soil conditions, common in sub-Saharan Africa. Bänziger et al. (7) found that by selecting for drought tolerance, they were also able to increase the yield, regardless of the amount of additional N applied. Clearly, maize breeders need to consider N application rates when selecting for a drought-tolerant or water-efficient germplasm.

There are many examples from Australia and throughout the world of wheat grown under conditions in which moisture is periodically limiting. In these cases, because grain yield and N removal is driven by water supply, the uncertainties in rainfall will make for uncertainties in N application. In Australia, irrigated wheat yields were approximately 7 Mt/ha and required 125 kg/ha of fertilizer N, whereas dryland wheat yields were approximately 2 Mt/ha and required 27–40 kg/ha of fertilizer N (101a). Carberry et al. (24) reported that Australian rain-fed wheat

producers used only a tenth of the amount of fertilizer compared with Chinese irrigated wheat producers (27 kg/ha versus 260 kg/ha), but also averaged only 2.3 Mt/ha yield versus 6.5 Mt/ha.

Water availability affects NUE of barley as well. Analyzing 10 years of field trial data for barley showed that the year-to-year rankings of different cultivars for yield and NUE were consistent, except in those years where there was a drought and significant water stress on the plants (Y. Anbessa & A.G. Good, unpublished data). In canola, the negative effects of drought are quite similar to those for N limitation, with loss of grain yield, decline of seed oil content, and change in lipid composition (33, 92).

NITROGEN USE EFFICIENCY AND CULTIVAR DEVELOPMENT: HISTORICAL TO CURRENT

It should be recognized that for the majority of plants, their natural selection has primarily been to maximize the conservation of N within the plant tissues rather than the biomass per unit N (35, 113). Therefore, NUE in terms of evolutionary fitness is very different from NUE in an agricultural setting. Historically, the Long Term Broadbalk experimental plots implemented in 1843 at Rothamsted in the United Kingdom have provided a wealth of data that have documented the long-term changes in crop NUE (61). These historical plots have demonstrated the importance of N-based fertilizers, the increase in yield gains from improved crop genetics, and the effect of increased N fertilization levels on N loss into the environment (61). Many organizations, particularly the research stations associated with the CGIAR (Consultative Group for International Agricultural Research) Consortium, such as CIMMYT, have documented genetic variability for NUE in crop plants over multiple environments and years. By comparing the field performances of 10 varieties released from CIMMYT between 1950 and 1985, Ortiz-Monasterio et al. (97) showed that genetic gain for grain yield increase was associated with the improvement of NUE, indicating that breeders selected varieties with improved NUE unconsciously as a result of choosing higher-yielding varieties.

Maize

Many groups have evaluated different germplasm/hybrids for NUE, but only recently have varieties or hybrids been developed with this as a target. Maize is one of the best-studied crops, with many studies looking at yield increases and genetic gains in NUE, based on experiments that examined historic sets of hybrids and genotypes under both low and high N supplies. From the early 1960s to 1990, increased N fertilizer application accompanied early gains in grain yield of single-cross maize hybrids (43). However, with the average N fertilizer rates in the United States remaining relatively static since the early 1980s (126), the selection under a high N input has clearly resulted in maize breeders selecting for N-responsive germplasm (25).

Maize studies can be divided into two groups, namely comparison studies of hybrids from different eras and studies that use inbred lines or mapping populations to genetically dissect traits. Comparing hybrids from different historical times is a common approach to understanding how genetic selection has shaped economically important traits such as yield in maize (23, 28, 42, 131). However, the reality is that the impact of selection on N use has been well documented in only a few cases. Moreover, because most of the current proprietary maize germplasm in the United States used to generate hybrids can be traced to seven progenitor lines (83, 84, 91), the lack of genetic diversity might suggest that maize hybrids would not differ in their N response. However, early studies demonstrated that hybrids varied considerably in the N fertilizer rate required to maximize grain yield (114, 120–122). Several comparisons of older and newer hybrids under contrasting N levels have been reported (28, 45, 96, 118), the results which showed that the newer hybrids

CGIAR
(Consultative Group
for International
Agricultural
Research)

Consortium: global
partnership uniting
organizations like
CIMMYT
(international maize
and wheat
improvement center)
in research for
sustainable agricultural
development

gave greater yields at all levels of fertilizer application (25, 39). Recently CIMMYT breeders reported that, at least in the initial testing, they have developed maize hybrids that yield better on nutrient-depleted African soils and do virtually as well on nutrient-replete conditions (28a). The yield advantage of newer hybrids (compared with older ones) at lower levels of N fertilizer application indicates that NUE has improved over the decades. Although these comparisons seem relatively robust, many studies have shown that significant hybrid \times N interactions exist, even in elite maize germplasm, that are specific to certain environments but are not of sufficient magnitude to merit hybrid specific N recommendations (19, 53, 87, 111). The differences between these studies suggest that the evaluation environment, the hybrids used in a particular study, or other agronomic parameters such as plant density can markedly influence the conclusions drawn from a hybrid \times N rate experiment. For example, Tsai et al. (120) suggested that environmental conditions that are not supportive of high grain yields or elevated residual soil N might obscure genetic differences in response to fertilizer N. Also, large differences in hybrid maturity and lack of adaptation to a particular environment have been proposed as explanations for the apparent genetic differences for N response (53). Thus, whether maize hybrids respond differently than inbred lines to fertilizer N remains difficult to determine. This variability and the limited range of multiple-year genetic studies with large data sets make any comparison unreliable.

Wheat and Barley

In bread wheat, a number of studies have looked at the genetic variation for components of NUE. Barraclough et al. (8) performed the most detailed study, looking at 39 different winter wheat varieties that varied by trait (e.g., height), heritage, and year of registry. Their study showed a large range in variability (between 24% and 42%) for NUE and grain yield, depending on N application rate. As their study represented elite lines, there is no question that there would be significant variation for NUtE in diverse wheat germplasm.

In an analysis of 75 years of breeding for increased grain yield in barley, Bingham et al. (15) found that breeding had resulted in 1.0% and 1.2% per year increases in yield and NUE, respectively. Overall, this increase resulted from the newer varieties having larger grains, a lower GNC, and more grains per unit area than the older varieties. Similarly, modern Argentinian varieties had greater NUE and NIH than older varieties did (2). However, as with other small grain cereals, both these studies found that the increase of yield potential often reduced the GNC (21, 97). Thus, that which can be positive for grain yield may not be good for grain quality. In barley, the importance of GNC is widely known. Although protein is only one of several traits related to malting quality, ranging between 8.5% and 12.5%, this is the most important requirement of the malting industry (51). Because yield improvements in barley are associated with decreases in GNC (see 1), it is still well recognized that adding additional N fertilizer at the appropriate time is often required to produce high-quality malting barley.

MAPPING NITROGEN USE EFFICIENCY QUANTITATIVE TRAIT LOCI

The number of QTLs detected in a given study depends on a number of different factors, including the size and type of mapping population used, the trait being investigated, the number of environments used for phenotyping, environmental conditions, and genome coverage. QTLs reported in the literature generally fall into two groups of genes. The first group includes major genes, with very large effects on highly heritable traits, with each QTL explaining a large portion of the total trait variation in a mapping population. The second and largest group is regulated by many genes, each explaining a small portion of the total trait variation, and numerous QTLs of small additive effects are shared among families. Clearly, the genetic variation for many

quantitative traits involves a number of loci with moderate effects, and a very large number of loci with minor effects (72, 106). Major gene effects can be studied via segregation analysis, as well as evolutionary history; however, when numerous genes with minor effects determine a trait, it is much more challenging, as they usually cannot be investigated individually.

Despite this, researchers have mapped NUE traits in a number of plants. One of the first was carried out by Obara et al. (94), who studied rice. They looked at QTLs associated with NUE and determined whether they cosegregated with glutamine synthetase 1 (GS1) and NADH-GOGAT. The analysis identified seven loci that cosegregated with GS1 activity and six loci that cosegregated with NADH-GOGAT activity. A number of QTLs for agronomic traits related to N use and yield have been mapped to the chromosomal regions containing GS2 in wheat and rice (16, 48, 60, 75, 94, 100, 133, 137, 138, 142), suggesting that the genomic region surrounding GS2 may be valuable for breeding rice and wheat varieties with improved agronomic performance and NUE. However, to date, no one has been able to introduce a GS gene into a NUE-inefficient background and show either enhanced NUE or yield.

For maize, a number of studies have looked at QTLs associated with NUE using segregating maize populations (52). Liu et al. (79) did a meta-analysis for QTLs for yield and yield components identifying a number of meta-QTLs under low and normal N conditions and characterized these by map position, confidence interval, and the proportion of phenotypic variance explained. Meta-analysis revealed 22 QTLs under low N conditions with 4 consensus QTLs found on chromosomes 1 and 4, 2 on chromosomes 3, 5, 6, and 9, and 3 on chromosome 2. Chromosomes 7, 9, and 10 contained only 1 consensus QTL (79). However, these analyses were looking at yield under low N, which is not what some researchers would consider to be NUE. Regardless of the definition of NUE, these studies point out many of the challenges, as there are always multiple QTLs for the trait, and each QTL rarely accounts for a significant amount of variation.

Bordes et al. (18) identified 54 genomic regions associated with grain yield, its components, and other traits through whole-genome association mapping with 196 wheat accessions. The analysis also revealed that 23 genomic regions were N responsive, which may be useful for the wheat breeding programs aiming to improve N responsiveness. More recently, Xu et al. (135) detected QTLs from a wheat RIL population, applying a conditional analysis to detect N and P fertilization effects on QTLs. N treatment influenced QTLs of N concentration in grain, shoots, and NUE traits, whereas P treatment affected a QTL of spikelet number per spike. It may be worthwhile to further investigate the newly detected QTLs, including those on chromosomes 4B and 7A for yield component traits (135). Some QTL studies were incorporated with physiological traits such as N metabolic enzyme activities and N metabolites (48, 60). Although those traits are particularly susceptible to environmental factors and experimental settings (e.g., field versus glasshouse), there are potential outcomes such as metabolic QTLs (mQTLs) that provide us direct evidence to link agronomic traits with potential gene(s) underlying the QTLs (65, 105).

Although there have been a number of QTL maps of barley, the only trait that is usually mapped is yield, and many QTLs, most with minor effects, have been shown to be associated with barley yield (6, 63). Varshney et al. (129) used an association mapping approach to look at genes involved in a number of traits in barley, with the traits grain yield and biomass yield considered to be associated with NUE. Using two separate sites, they were able to map several QTLs involved in these traits. Berger et al. (12) mapped markers associated with a number of key agronomic traits associated with NUE in a genome-wide association study of 329 genotypes of winter barley. They found two QTLs that were consistent between environments and years, 2H at 16.0 cM and 7H at 91.12 cM.

Using genetically defined mapping populations of barley, Mickelson et al. (82) conducted a detailed analysis of QTLs associated with N storage and remobilization and identified a number

of QTLs associated with NUE traits. Unfortunately, owing to lack of consensus markers on this map, and several mapping inconsistencies, only a few loci associated with NUE were identified. For ΔN (the difference between the N acquired by the plant prior to anthesis and the N in the harvest plant product) they found three QTLs on chromosomes 5H and 6H that explained 37.4% of the variation. In barley, Kindu et al. (73) found that although it was possible to detect QTLs for NUE-related traits in two separate years, the QTL analysis of NUE and its components mainly indicated inconsistent results across N levels and years, in line with low correlations among environments for a given trait. However, under the different conditions and between years there were two QTLs for NUtEg (NUtE of grains), NUEg, and NHI that consistently mapped to chromosomes 2H and 3H.

In wheat and barley, there is one group of QTLs that affects both NUE and GPC (64, 123, 124). These loci, known as *Gpc-B1*, affect N reallocation in these plants, resulting in significant differences in NUE and N allocation within the plant. Near-isolines with *Gpc-B1* in Australian varieties showed increased GPC without negative impact on grain yield (44). However, as noted above, GPC has a significant effect on bread quality (wheat) and malt quality (barley) and therefore must be evaluated with this in mind. To improve GPC without any penalty in grain yield is challenging, but some QTLs, such as those detected on chromosomes 1B and 2A for GPC, may be potential candidates for this purpose.

THE CHALLENGE OF IDENTIFYING CANDIDATE GENES FROM MAPPING STUDIES

One of the key reasons for mapping genes is to use the information to identify the candidate genes that affect a trait and either track them (and the trait) with markers or clone the allele. In a meta-analysis, Quraishi et al. (102) identified conserved regions of the genome syntenic between maize, sorghum, rice, and *Brachypodium distachyon* that affect NUE and suggested that there may be evolutionarily conserved regions that exist for NUE within the genome of cereals. One of the conserved regions contained the GS and GOGAT genes, which Quraishi et al. (102) suggest shows a strong link to NUE. However, given the imprecision of these mapping studies, there are many other genes that also cosegregate, and the identification of genes that might affect NUE is still based on our knowledge of the gene's function. For example, the meta-QTL analysis by Quraishi et al. (102) revealed that phenology genes such as *Ppd*, *Vrn*, and a semidwarf gene *Rht* were highly correlated with the NUE-related traits, as described in other studies (60, 75), which indicates that it is essential to address phenology and developmental genes with care when we interpret QTL results. The subsequent attribution of the differences in phenotype to these particular genes is thus still uncertain. Other mapping studies suffer from the same problem, namely that the challenges of mapping the trait prevent any narrowing of the mapping interval to a region of the chromosome that contains a reasonable number of genes. For example, Gallais & Hirel (52) identified a number of chromosomal regions that segregated for traits associated with NUE. However, these regions represented more than 25% of the mapped maize genome. As with previous QTL analyses (66, 78, 94), Gallais & Hirel (52) demonstrated that many of the desired NUE traits cosegregated with GS genes, particularly *GlnA* on chromosome 5. However, as there are multiple GS genes and the mapping intervals are large, it seems that it would be difficult not to find at least one GS gene linked to a chromosomal region that segregated for NUE.

Theoretical studies (74) and computer simulations (4, 11, 127) have demonstrated that, especially for small samples, estimates of the proportion of genotypic variance explained by a QTL can be severely inflated, regardless of the statistical method used. This has a number of consequences for identifying candidate genes or for marker-assisted selection, including attempts to replicate

earlier findings in independent studies, because of the fact that the location and variation explained by that QTL are incorrect. Weightings given to individual marker-trait associations as components of selection indices are often severely biased and have a large sampling error, resulting in overrating the prospects of using marker-assisted selection. In more complex genomes, this makes the prospects of fine mapping and cloning of a particular QTL a utopian adventure. We recently mapped many of the putative candidate genes for NUE in barley and compared these with the regions of the barley linkage groups where QTLs for NUE had been identified. We found that many of the logical candidate genes did not map to a QTL associated with NUE (T. Su & A.G. Good, unpublished results). The difficulty is that by identifying a region that contains putative candidate genes, many researchers have already preselected their genes of interest. Despite the success for genes involved in heavy metal tolerance, we are not aware of a case in which researchers have map-based cloned a NUE (specific) gene.

CONCLUSIONS

Although improving NUE in crop plants represents a significant research challenge, it is nevertheless an area of enormous importance. There have been significant improvements in nutrient management in both developed and developing countries, although further improvements need to be made (109). One novel approach would be to try to develop plants that can fix their own N biologically; the approaches to this are discussed in more detail in the sidebar, Beyond NUE: Toward the Genetics of N-Fixing Plants.

From a farmer's perspective, if given the choice between a high-yielding variety and an N-efficient variety, they invariably select the former, at least in the developed world. Therefore, our goal must be to decrease the optimum fertilizer rate required for a crop while continuing to increase yield. Certainly, nutrient-efficient crops cannot compete if they do not maintain yield, regardless of the source of the genetics (allelic variation or a GMO trait). To some degree, breeders already target this strategy, given that average grain yields have increased steadily in the United States since the early 1980s without increased application rates of N fertilizer (59). Therefore, as has been noted by many researchers, if breeders select for improved yield at a constant N, by definition, NUE improves (61). Ideally, the N response curve of the future varieties will not only show N efficiency but will also be N responsive (62). However, yields will reach a plateau even

BEYOND NUE: TOWARD THE GENETICS OF N-FIXING PLANTS

What if the cereal crops' need for applied N fertilizer was reduced or even eliminated by genetically engineering cereals to fix their own nitrogen using atmospheric N₂? Only diazotrophic bacteria can fix N₂ into biologically reactive ammonia, using ~9 to 20 *nif* genes encoding the enzyme (nitrogenase) and the unique cofactor (FeMoco), both essential for function. Nitrogenase is oxygen labile and needs plenty of ATP and reducing power to catalyze N₂ fixation. The idea of developing N₂-fixing plants is not new; however, the ability to develop them is new. Two genetic methods have been proposed: (a) engineer cereals to allow for diazotrophic symbiosis or (b) stably transform cereals with the *nif* genes needed to make a functional nitrogenase (10, 95). Nitrogenase needs protection from O₂ and access to high levels of ATP and reducing power. Organelles like chloroplasts and mitochondria could provide the right conditions for plant-made nitrogenase. Currently, stable genetic modification of chloroplasts is more technically advanced than genetic modification of mitochondria; however, mitochondria are still a viable choice for enzyme location by nuclear encoding the *nif* genes with a signal peptide sequence that directs the polypeptide into the mitochondria.

if NUE is maximized, as secondary limiting factors such as water, nutrient availability, and/or environmental conditions become relevant.

Many researchers have attempted to map NUE and components of NUE in different crops. However, the challenges have been the degree of phenotypic variation for this complex trait and the difficulty in getting reliable data from field trial studies (80). There are now better statistical tools for molecular mapping, and the need for more careful experimental design and replicate testing has been recognized (90, 119). Moreover, thanks to the decrease in cost of genotyping, genomic selection with whole-genome prediction models has become realistic for plant breeding (36). Although the prediction models and experimental designs need finer tuning (46), this approach, in theory, will enable us to select even for minor QTLs and accelerate the breeding process. However, the reality may well be that although we will be able to map specific QTLs for NUE, these are likely to represent genetic variation specific to the species and environmental conditions. It is extremely unlikely that there will be a single magic bullet in the shape of a single gene or even a single QTL for all crop species that will allow us to develop more nutrient-efficient crops.

SUMMARY POINTS

1. There are two main incentives to improve NUE in crop plants so that they use less N fertilizer; one is economic gain and the other is N pollution reduction. Sutton et al. (115) estimated the societal damage costs of excess N (e.g., water and air quality) in Europe to be US \$91 to \$466 billion annually.
2. There is a significant amount of genetic variation and phenotypic plasticity for NUE. Phenotype can be affected by N uptake and water availability, different macronutrients and micronutrients, and the environment.
3. Two key components comprise NUE: NUpE and NUtE. In theory, improving NUE could be achieved by improving NUpE, NUtE, or both.
4. The importance of NUpE and NUtE to NUE varies considerably depending on how these traits are measured or derived, the level of N fertilizer applied, and the germplasm under evaluation. Significant improvements in NUE require coordinated improvements of both NUpE and NUtE, and it is important to understand the interactions between uptake and utilization.
5. Physiological traits that may affect NUpE include root architecture and any other characteristic that impacts the roots' ability to extract available N from the soil.
6. Economically important traits are frequently polygenic; therefore, it is unlikely that a single genotype or RIL contains the necessary contrast in the relevant genes underpinning the trait(s). The ideal high-NUE genotype has both high genetic N efficiency and high N responsiveness.
7. The number of QTLs detected in a given study depends on a number of different factors, including the size and type of mapping population used, the trait being investigated, the number of environments used for phenotyping, the environmental conditions, and the genome coverage. The numerous genes, each with small effects in the trait, are much more challenging, as they usually cannot be investigated individually. Also, QTL mapping tends to be imprecise, so many other genes cosegregate, and the identification of genes that might affect NUE is still based on our knowledge of the gene's function.

FUTURE ISSUES

1. Although improving NUE in crop plants represents a significant research challenge, it is nevertheless an area of enormous importance. Although breeders and farmers seem to better appreciate a cultivar with better grain yield under the same fertilization condition, our goal should be to decrease the optimum fertilizer rate required for a crop while continuing to increase yield.
2. Researchers need to focus on defining and measuring NUE in a manner that is appropriate to producers. Measuring NUE is difficult, if not impossible, unless researchers use much more care in soil sampling and measuring total plant N.
3. We recommend that all analysis look at N efficiency and N responsiveness under a defined set of soil conditions.
4. Studying NUpE and root systems, especially in soil, is still difficult and so it is one area that remains relatively unexplored.
5. Of the two types of genetic mapping for NUE, association mapping or traditional segregation analysis in defined crosses, both have advantages and disadvantages. However, both approaches are limited in that breeders rarely use the allelic variation identified to develop superior lines, and it will likely prove more useful to identify lines with superior NUE among existing commercial varieties.
6. Analysis of mapping populations in a manner that will yield useful data requires multi-site and multiyear trials. Funding studies that do not provide the resources to conduct proper trials will result in data that are largely useless. Further, researchers should try to take advantage of mapping populations and association populations that have been well studied, as the greater the number of field trials a population has been tested under, the more useful the data set.
7. Researchers need to quantify more clearly the economic and environmental benefits of reducing N applications, and these quantifications need to be used in developing appropriate N applications policies.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We would like to thank Peter Langridge, Trevor Garnett, and Tao Su for helpful discussions on this timely topic. Funding from the Alberta Crop Industry Development Fund (A.G.G.) and the Natural Sciences and Engineering Research Council (A.G.G. and S.J.R.) supported this work.

LITERATURE CITED

1. Abeledo LG, Calderini DF, Slafer GA. 2002. Physiological changes associated with breeding progress in barley. In *Barley Science: Recent Advances from Molecular Biology to Agronomy of Yield and Quality*, ed. GA Slafer, JL Molina-Cano, R Savin, JL Araus, I Romagosa, pp. 361–85. New York: Food Prod. Press

2. Abeledo LG, Calderini DF, Slafer GA. 2008. Nitrogen economy in old and modern malting barleys. *Field Crops Res.* 106:171–78
3. Agriculture and Agri-food Canada. 2012. *Canadian farm fuel and fertilizer: prices and expenses*. Market Outlook Rep. 4. Winnipeg, Manit.: Agric. Agri-food Can.
4. Allison DB, Fernandez JR, Heo M, Zhu S, Etzel C, et al. 2002. Bias in estimates of quantitative-trait-locus effect in genome scans: demonstration of the phenomenon and a method-of-moments procedure for reducing bias. *Am. J. Hum. Genet.* 70:575–85
5. Anbessa Y, Juskiw P, Good A, Nyachiro J, Helm J. 2009. Genetic variability in nitrogen use efficiency of spring barley. *Crop Sci.* 49:1259–69
6. Backes G, Graner A, Foroughi-Wehr B, Fischbeck G, Wenzel G, Jahoor A. 1995. Localization of quantitative trait loci (QTL) for agronomic important characters by the use of an RFLP map in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* 90:294–302
7. Bänziger M, Edmeades GO, Lafitte HR. 1999. Selection for drought tolerance increases maize yields across a range of nitrogen levels. *Crop Sci.* 39:1035–40
8. Barraclough PB, Howarth JR, Jones J, Lopez-Bellido R, Parmar S, et al. 2010. Nitrogen efficiency of wheat: genotypic and environmental variation and prospects for improvement. *Eur. J. Agron.* 33:1–11
9. Beatty PH, Anbessa Y, Juskiw P, Carroll RT, Wang J, Good AG. 2010. Nitrogen use efficiencies of spring barley grown under varying nitrogen conditions in the field and growth chamber. *Ann. Bot.* 105:1171–82
10. Beatty PH, Good AG. 2011. Future prospects for cereals that fix nitrogen. *Science* 333:416–17
11. Beavis W. 1998. QTL analysis: power, precision and accuracy. In *Molecular Dissection of Complex Traits*, ed. A Paterson, pp. 145–62. Boca Raton, FL: CRC Press
12. Berger GL, Liu S, Hall MD, Brooks WS, Chao S, et al. 2013. Marker-trait associations in Virginia Tech winter barley identified using genome-wide mapping. *Theor. Appl. Genet.* 126:693–710
13. Bertin P, Gallais A. 2000. Genetic variation for nitrogen use efficiency in a set of recombinant maize inbred lines. 1. Agrophysiological results. *Maydica* 45:53–66
14. Bingham IJ. 2005. Agronomic approaches for modifying root system of field crops: opportunities and constraints. *Asp. Appl. Biol.* 73:169–78
15. Bingham IJ, Karley AJ, White PJ, Thomas WTB, Russell JR. 2012. Analysis of improvements in nitrogen use efficiency associated with 75 years of spring barley breeding. *Eur. J. Agron.* 42:49–58
16. Bingham IJ, Wu L. 2011. Simulation of wheat growth using the 3D root architecture model SPACSYS: validation and sensitivity analysis. *Eur. J. Agron.* 34:181–89
17. Bonnett DG, Rebetzke GJ, Spielmeier W. 2005. Strategies for efficient implementation of molecular markers in wheat breeding. *Mol. Breed.* 15:75–85
18. Bordes J, Ravel C, Jaubertie JP, Duperrier B, Gardet O, et al. 2013. Genomic regions associated with the nitrogen limitation response revealed in a global wheat core collection. *Theor. Appl. Genet.* 126:805–22
19. Bundy LG, Carter PR. 1988. Corn hybrid response to nitrogen fertilization in the northern corn belt. *J. Prod. Agric.* 1:99–104
20. Byers DL. 2005. Evolution in heterogeneous environments and the potential of maintenance of genetic variation in traits of adaptive significance. *Genetica* 123:107–24
21. Calderini DF, Torres-Leon S, Slafer GA. 1995. Consequences of wheat breeding on nitrogen and phosphorus yield grain nitrogen and associated traits. *Ann. Bot.* 76:315–22
22. Campbell CA, Myers RJK, Curtin D. 1995. Managing nitrogen for sustainable crop production. *Fertil. Res.* 42:277–96
23. Campos H, Cooper M, Edmeades GO, Löffler C, Schussler JR, Ibañez M. 2006. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the U.S. corn belt. *Maydica* 51:369–81
24. Carberry PS, Liang W, Twomlow S, Holzworth DP, Dimes JP, et al. 2013. Scope for improved eco-efficiency varies among diverse cropping systems. *PNAS* 110:8381–86
25. Carlone MR, Russell WA. 1987. Response to plant densities and nitrogen levels for four maize cultivars from different eras of breeding. *Crop Sci.* 27:465–70
26. Cassman KG. 1999. Ecological intensification of cereal production systems: yield potential, soil quality, and precision agriculture. *PNAS* 96:5952–59

27. Cassman KG, Dobermann A, Walters DT, Yang H. 2003. Meeting cereal demand while protecting natural resources and improving environmental quality. *Annu. Rev. Environ. Resour.* 28:315–58
28. Castleberry RM, Crum CW, Krull CF. 1984. Genetic yield improvement of U.S. maize cultivars under varying fertility and climatic environments. *Crop Sci.* 24:33–36
- 28a. Castro MC. 2013. *Nitrogen-use-efficient maize ready for release in Africa*. Mexico City: CIMMYT. <http://blog.cimmyt.org/nitrogen-use-efficient-maize-ready-for-release-in-africa/>
29. Chen X-P, Cui Z-L, Vitousek PM, Cassman KG, Matson PA, et al. 2011. Integrated soil-crop system management for food security. *PNAS* 108:6399–404
30. Crasswell E, Godwin D. 1984. The efficiency of nitrogen fertilizers applied to cereals grown in different climates. In *Advances in Plant Nutrition*, ed. P Tinker, A Lauchli, pp. 1–55. New York: Praeger Publ.
31. Crews TE, Peoples MB. 2005. Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? A review. *Nutr. Cycl. Agroecosyst.* 72:101–20
32. Daberkow S, Taylor H, Huang W. 2000. Nutrient use and management. In *Agricultural Resources and Environmental Indicators*. Econ. Res. Serv. Rep. AH722. Washington, DC: USDA-ERS
33. Danesh-Shahraki A, Nadian H, Bakhshandeh A, Fathi G, Alamisaied K, Gharineh M. 2008. Optimization of irrigation and nitrogen regimes for rapeseed production under drought stress. *J. Agron.* 7:321–26
34. David MB, Drinkwater LE, McIsaac GF. 2010. Sources of nitrate yields in the Mississippi River Basin. *J. Environ. Qual.* 39:1657–67
35. Dawson JC, Huggins DR, Jones SS. 2008. Characterizing nitrogen use efficiency in natural and agricultural ecosystems to improve the performance of cereal crops in low-input and organic agricultural systems. *Field Crops Res.* 107:89–101
36. Desta ZA, Ortiz R. 2014. Genomic selection: genome-wide prediction in plant improvement. *Trends Plant Sci.* 19:592–601
37. Dhugga KS, Waines JG. 1989. Analysis of nitrogen accumulation and use in bread and durum wheat. *Crop Sci.* 29:1232–39
38. Dobermann A. 2005. Nitrogen use efficiency: state of the art. *IFA Int. Workshop* 1:28–30
39. Duvick DN. 1984. Genetic contributions to yield gains of U.S. hybrid maize, 1930 to 1980. In *Genetic Contributions to Yield Gains of Five Major Crop Plants*, ed. WR Fehr, pp. 15–47. Madison, WI: Crop Sci. Soc. Am. Am. Soc. Agron.
40. Duvick DN. 1992. Genetic contributions to advances in yield of U.S. maize. *Maydica* 37:69–79
41. Duvick DN. 2005. The contribution of breeding (*Zea mays* L.). *Adv. Agron.* 86:83–145
42. Duvick DN. 2005. Genetic progress in yield of United States maize (*Zea mays* L.). *Maydica* 50:193–202
43. Duvick DN, Cassman KG. 1999. Post-Green Revolution trends in yield potential of temperate maize in the north-central United States. *Crop Sci.* 39:1622–30
44. Eagles HA, McLean R, Eastwood RF, Appelbee M-J, Cane K, et al. 2014. High-yielding lines of wheat carrying *Gpc-B1* adapted to Mediterranean-type environments of the south and west of Australia. *Crop Pasture Sci.* 65:854–61
45. Echarte L, Rothstein S, Tollenaar M. 2008. The response of leaf photosynthesis and dry matter accumulation to nitrogen supply in an older and a newer maize hybrid. *Crop Sci.* 48:656–65
46. Estaghirou SBO, Ogutu JO, Piepho H. 2014. Influence of outliers on accuracy estimation in genomic prediction in plant breeding. *Genes Genomes Genet.* 4:2317–28
47. Fischer RA, Byerlee D, Edmeades GO. 2009. *Can technology deliver on the yield challenge to 2050?* Presented at FAO Expert Meet. How Feed World 2050, Rome
48. Fontaine J-X, Ravel C, Pageau K, Heumez E, Dubois F, et al. 2009. A quantitative genetic study for elucidating the contribution of glutamine synthetase, glutamate dehydrogenase and other nitrogen-related physiological traits to the agronomic performance of common wheat. *Theor. Appl. Genet.* 119:645–62
49. Foulkes MJ, Hawkesford MJ, Barraclough PB, Holdsworth MJ, Kerr S, et al. 2009. Identifying traits to improve the nitrogen economy of wheat: recent advances and future prospects. *Field Crops Res.* 114:329–42
50. Foulkes MJ, Sylvester-Bradley R, Scott RK. 1998. Evidence for differences between winter wheat cultivars in acquisition of soil mineral nitrogen and uptake and utilization of applied fertilizer nitrogen. *J. Agric. Sci.* 130:29–44

51. Gali VJ, Brown CG. 2000. Assisting decision-making in Queensland barley production through chance constrained programming. *Aust. J. Agric. Resour. Econ.* 44:269–87
52. Gallais A, Hirel B. 2004. An approach to the genetics of nitrogen use efficiency in maize. *J. Exp. Bot.* 55:295–306
53. Gardner CAC, Bax PL, Bailey DJ, Cavalieri AJ, Clausen CR, et al. 1990. Response of corn hybrids to nitrogen fertilizer. *J. Prod. Agric.* 3:39–43
54. Garnett T, Conn V, Kaiser BN. 2009. Root based approaches to improving nitrogen use efficiency in plants. *Plant Cell Environ.* 32:1272–83
55. Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, et al. 2010. Food security: the challenge of feeding 9 billion people. *Science* 327:812–18
56. Good AG, Beatty PH. 2011. Fertilizing nature: a tragedy of excess in the commons. *PLOS Biol.* 9:e1001124
57. Good AG, Shrawat AK, Muench DG. 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends Plant Sci.* 9:597–605
58. Goulding K. 2004. Pathways and losses of fertilizer nitrogen at different scales. In *Agriculture and the Nitrogen Cycle: Assessing the Impacts of Fertilizer Use on Food Production and the Environment*, ed. AR Mosier, KJ Syers, JR Freney, pp. 209–19. Washington, DC: Island Press
59. Grassini P, Eskridge KM, Cassman KG. 2013. Distinguishing between yield advances and yield plateaus in historical crop production trends. *Nat. Commun.* 4:1–11
60. Habash DZ, Bernard S, Schondelmaier J, Weyen J, Quarrie SA. 2007. The genetics of nitrogen use in hexaploid wheat: N utilisation, development and yield. *Theor. Appl. Genet.* 114:403–19
61. Hawkesford MJ. 2012. Improving nutrient use efficiency in crops. *eLS*. doi: 10.1002/9780470015902.a0023734
62. Hawkesford MJ. 2011. An overview of nutrient use efficiency and strategies for crop improvement. In *The Molecular and Physiological Basis of Nutrient Use Efficiency in Crops*, ed. MJ Hawkesford, P Barraclough, pp. 3–19. Chichester, UK: Wiley-Blackwell
63. Hayes PM, Liu BH, Knapp SJ, Chen F, Jones B, et al. 1993. Quantitative trait locus effect and environmental interaction in a sample of North American barley germ plasm. *Theor. Appl. Genet.* 87:392–401
64. Heidlebaugh NM, Trethewey BR, Jukanti AK, Parrott DL, Martin JM, Fischer AM. 2008. Effects of a barley (*Hordeum vulgare*) chromosome 6 grain protein content locus on whole-plant nitrogen reallocation under two different fertilisation regimes. *Funct. Plant Biol.* 35:619–32
65. Hill C, Taylor J, Edwards J, Mather D. 2013. Whole-genome mapping of agronomic and metabolic traits to identify novel quantitative trait loci in bread wheat grown in a water-limited environment. *Plant Physiol.* 162:1266–81
66. Hirel B, Bertin P, Quillere I, Bourdoncle W, Delley C, et al. 2001. Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. *Plant Physiol.* 125:1258–70
67. Hirel B, Le Gouis J, Ney B, Gallais A. 2007. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J. Exp. Bot.* 58:2369–87
68. Ho MD, Rosas JC, Brown KM, Lynch JP. 2005. Root architectural tradeoffs for water and phosphorus acquisition. *Funct. Plant Biol.* 32:737–48
69. Horii H, Nemoto K, Miyamoto N, Harada J. 2006. Quantitative trait loci for adventitious and lateral roots in rice. *Plant Breed.* 125:198–200
70. Horst WJ, Behrens T, Heuberger H, Kamh M, Reidenbach G, Wiesler F. 2003. Genotypic differences in nitrogen use-efficiency in crop plants. In *Innovative Soil-Plant Systems for Sustainable Agricultural Production*, ed. JM Lynch, JS Schepers, I Ünver, pp. 75–92. Paris: OECD
71. Kamprath EJ, Moll RH, Rodriguez N. 1982. Effects of nitrogen fertilization and recurrent selection on performance of hybrid populations of corn. *Agron. J.* 74:955–58
72. Kearsey MJ, Farquhar AGL. 1998. QTL analysis in plants; where are we now? *Heredity* 80:137–42
73. Kindu GA, Tang J, Yin X, Struik PC. 2014. Quantitative trait locus analysis of nitrogen use efficiency in barley (*Hordeum vulgare* L.). *Euphytica* 199:207–21
74. Lande R, Thompson R. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. *Genetics* 124:743–56

75. Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Hanocq E, et al. 2007. Using genotype x nitrogen interaction variables to evaluate the QTL involved in wheat tolerance to nitrogen constraints. *Theor. Appl. Genet.* 115:399–415
76. Le Gouis J, Béghin D, Heumez E, Pluchard P. 2000. Genetic differences for nitrogen uptake and nitrogen utilisation efficiencies in winter wheat. *Eur. J. Agron.* 12:163–73
77. Lea PJ, Azevedo RA. 2007. Nitrogen use efficiency. 2. Amino acid metabolism. *Ann. Appl. Biol.* 151:269–75
78. Limami AM, Rouillon C, Hirel B. 2002. Genetic and physiological analysis of germination efficiency in maize in relation to nitrogen metabolism reveals the importance of cytosolic glutamine synthetase. *Plant Physiol.* 130:1860–70
79. Liu R, Zhang H, Zhao P, Zhang Z, Liang W, et al. 2012. Mining of candidate maize genes for nitrogen use efficiency by integrating gene expression and QTL data. *Plant Mol. Biol. Rep.* 30:297–308
80. Mahjourimajd S, Kuchel H, Langridge L, Okamoto M. 2015. Evaluation of Australian wheat genotypes for response to variable nitrogen application. *Plant Soil*. Submitted
81. McAllister CH, Beatty PH, Good AG. 2012. Engineering nitrogen use efficient crop plants: the current status. *Plant Biotechnol. J.* 10:1011–25
82. Mickelson S, See D, Meyer FD, Garner JP, Foster CR, et al. 2003. Mapping of QTL associated with nitrogen storage and remobilization in barley (*Hordeum vulgare* L.) leaves. *J. Exp. Bot.* 54(383):801–12
83. Mikel MA. 2006. Availability and analysis of proprietary dent corn inbred lines with expired U.S. plant variety protection. *Crop Sci.* 46:2555–60
84. Mikel MA, Dudley JW. 2006. Evolution of North American dent corn from public to proprietary germplasm. *Crop Sci.* 46:1193–205
85. Moll RH, Kamprath EJ, Jackson WA. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agron. J.* 74:562–64
86. Moose S, Below FE. 2009. Biotechnology approaches to improving maize nitrogen use efficiency. In *Molecular Genetic Approaches to Maize Improvement, Biotechnology in Agriculture and Forestry*, ed. AL Kriz, BA Larkins. pp. 65–77. Berlin-Heidelberg, Germ.: Springer-Verlag
87. Mullen R, Thomison PR, Diedrick KA, Henry DC. 2010. Corn response to nitrogen fertilizer as affected by planting date and hybrid. *Crop Manag.* doi:10.1094/CM-2010-0405-01-RS
88. Mulvaney RL, Khan SA, Ellsworth TR. 2009. Synthetic nitrogen fertilizers deplete soil nitrogen: a global dilemma for sustainable cereal production. *J. Environ. Qual.* 38:2295–314
89. Muurinen S, Slafer GA, Peltonen-Sainio P. 2006. Breeding effects on nitrogen use efficiency of spring cereals under northern conditions. *Crop Sci.* 46:561–68
90. Myles S, Peiffer J, Brown PJ, Ersoz ES, Zhang Z, et al. 2009. Association mapping: critical considerations shift from genotyping to experimental design. *Plant Cell* 21:2194–202
91. Nelson PT, Goodman MM. 2008. Evaluation of elite exotic maize inbreds for use in temperate breeding. *Crop Sci.* 48:85–92
92. Norouzi M, Toorchi M, Hosseini Salekdeh G, Mohammadi SA, Neyshabouri MR, Aharizad S. 2008. Effect of water deficit on growth, grain yield and osmotic adjustment in rapeseed. *J. Food Agric. Environ.* 6:312–18
93. Novoa R, Loomis RS. 1981. Nitrogen and plant production. *Plant Soil* 58:177–204
94. Obara M, Kajiura M, Fukuta Y, Yano M, Hayashi M, et al. 2001. Mapping of QTLs associated with cytosolic glutamine synthetase and NADH-glutamyl synthase in rice (*Oryza sativa* L.). *J. Exp. Bot.* 52:1209–17
95. Oldroyd GED, Dixon R. 2014. Biotechnological solutions to the nitrogen problem. *Curr. Opin. Biotechnol.* 26:19–24
96. O'Neill PM, Schepers JS, Caldwell B, Shanahan JF. 2004. Agronomic responses of corn hybrids from different eras to deficit and adequate levels of water and nitrogen. *Agron. J.* 96:1660–67
97. Ortiz-Monasterio JI, Sayre KD, Rajaram S, McMahon M. 1997. Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. *Crop Sci.* 37:898–904
98. Paszkiewicz S, Butzen S. 2010. Corn hybrid response to plant population. *Crop Insights* 17:1–4

99. Peoples MB, Boyer EW, Goulding KWT, Heffer P, Ochwoh VA, et al. 2004. Pathways of nitrogen loss and their impacts on human health and the environment. In *Agriculture and the Nitrogen Cycle: The Scientific Committee on Problems of the Environment*, ed. AR Mosier, KJ Syers, JR Freney, pp. 53–69. Covelo, CA: Island Press
100. Prasad M, Varshney RK, Kumar A, Balyan HS, Sharma PC, et al. 1999. A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat. *Theor. Appl. Genet.* 99:341–45
101. Presterl T, Groh S, Landbeck M, Seitz G, Schmidt W, Geiger HH. 2002. Nitrogen uptake and utilization efficiency of European maize hybrids developed under conditions of low and high nitrogen input. *Plant Breed.* 121:480–86
- 101a. Queensland Gov. 2012. *Wheat-Nutrition*. Brisbane, Aust: Dep. Agric. Fish. <http://www.daff.qld.gov.au/plants/field-crops-and-pastures/broadacre-field-crops/wheat/nutrition>
102. Quraishi UM, Abrouk M, Murat F, Pont C, Foucrier S, et al. 2011. Cross-genome map based dissection of a nitrogen use efficiency ortho-metaQTL in bread wheat unravels concerted cereal genome evolution. *Plant J.* 65:745–56
103. Raun WR, Johnson GV. 1999. Improving nitrogen use efficiency for cereal production. *Agron. J.* 91:357–63
104. Reitz LP. 1970. New wheats and social progress: improved varieties of wheat have helped make possible unprecedentedly high levels of food production. *Science* 169:952–55
105. Riedelsheimer C, Lisec J, Czedik-Eysenberg A, Sulpice R, Flis A, Grieder C. 2012. Genome-wide association mapping of leaf metabolic profiles for dissecting complex traits in maize. *PNAS* 109:8872–77
106. Robertson A. 1967. The nature of quantitative genetic variation. In *Heritage from Mendel*, ed. R Brink, E Styles, pp. 265–80. Madison, WI: Univ. Wisc. Press
107. Robertson GP. 1997. Nitrogen use efficiency in row-crop agriculture: crop nitrogen use and soil nitrogen loss. In *Ecology in Agriculture*, ed. L Jackson, pp. 347–65. San Diego, CA: Academic
108. Semenov MA, Mitchell RAC, Whitmore AP, Hawkesford MJ, Parry MAJ, Shewry PR. 2012. Shortcomings in wheat yield predictions. *Nat. Clim. Change* 2:380–82
109. Shen J, Li Y, Liu X, Luo X, Tang H, et al. 2013. Atmospheric dry and wet nitrogen deposition on three contrasting land use types of an agricultural catchment in subtropical central China. *Atmos. Environ.* 67:415–24
110. Shen L, Courtois B, McNally KL, Robin S, Li Z. 2001. Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection. *Theor. Appl. Genet.* 103:75–83
111. Shepard A, Thomison P, Nafziger E, Mullen R, Clucas C. 2011. Nutridense corn response to nitrogen rates. *Agron. J.* 103:169–74
112. Shi G, Chavas J-P, Lauer J. 2013. Commercialized transgenic traits, maize productivity and yield risk. *Nat. Biotechnol.* 31:111–14
113. Silla F, Escudero A. 2004. Nitrogen-use efficiency: trade-offs between N productivity and mean residence time at organ, plant and population. *Funct. Ecol.* 18:511–21
114. Smiciklas K, Below F. 1990. Influence of heterotic pattern on nitrogen use and yield of maize. *Maydica* 35:209–13
115. Sutton MA, Howard CM, Erisman JW. 2011. The need to integrate nitrogen science and policies. In *The European Nitrogen Assessment*, ed. MA Sutton, CM Howard, JW Erisman, pp. 82–96. Cambridge: Cambridge Univ. Press
116. Thomas H, Smart CM. 1993. Crops that stay green. *Ann. Appl. Biol.* 123:193–219
117. Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. 2002. Agricultural sustainability and intensive production practices. *Nature* 418:671–77
118. Tollenaar M, Nissanka SP, Rajcan I, Bruulsema TW. 1997. Yield response of old and new corn hybrids to nitrogen. *Better Crop* 81:3–5
119. Tong C, Shen L, Lv Y, Wang Z, Wang X, et al. 2012. Structural mapping: how to study the genetic architecture of a phenotypic trait through its formation mechanism. *Brief. Bioinform.* 15:43–53
120. Tsai CY, Dweikat I, Huber DM, Warren HL. 1992. Interrelationship of nitrogen nutrition with maize (*Zea mays*) grain yield, nitrogen use efficiency and grain quality. *J. Sci. Food Agric.* 58:1–8

121. Tsai CY, Huber DM. 1996. Genetic variation of maize hybrids in grain yield response to potassium and inhibiting nitrification. *J. Sci. Food Agric.* 70:263–70
122. Tsai CY, Huber DM, Glover DV, Warren HL. 1984. Relationship of N deposition to grain yield and N response of three maize hybrids. *Crop Sci.* 24:277–81
123. Uauy C, Brevis JC, Dubcovsky J. 2006. The high grain protein content gene *Gpc-B1* accelerates senescence and has pleiotropic effects on protein content in wheat. *J. Exp. Bot.* 57:2785–94
124. Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J. 2006. A *NAC* gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* 314:1298–301
125. Uribelarrea M, Moose SP, Below FE. 2007. Divergent selection for grain protein affects nitrogen use in maize hybrids. *Field Crops Res.* 100:82–90
126. USDA-ERS. 2011. *Nitrogen used on corn, rate per fertilized acre receiving nitrogen, selected states, 1964–2010*. Washington, DC: USDA-ERS. <http://www.ers.usda.gov/Data/FertilizerUse>
127. Utz H, Melchinger A. 1994. Comparison of different approaches to interval mapping of quantitative trait loci. In *Biometrics in Plant Breeding: Applications of Molecular Markers: Proceedings of the 9th Meeting of the EUCARPIA Section Biometrics in Plant Breeding*, ed. J Van Ooijen, J Jansen, pp. 195–201. Wageningen, Neth.: CPRO-DLO
128. Van Keulen H. 1977. Nitrogen requirements of rice, with special reference to Java. *Contrib. Cent. Res. Inst. Agric. Bogor.* 30:1–67
129. Varshney RK, Ribaut J-M, Buckler ES, Tuberosa R, Rafalski JA, Langridge P. 2012. Can genomics boost productivity of orphan crops? *Nat. Biotechnol.* 30:1172–76
130. Vitousek PMM, Naylor R, Crews T, David MB, Drinkwater LEE, et al. 2009. Nutrient imbalances in agricultural development. *Science* 324:1519–20
131. Wang T, Ma X, Li Y, Bai D, Liu C, et al. 2011. Changes in yield and yield components of single-cross maize hybrids released in China between 1964 and 2001. *Crop Sci.* 51:512–25
132. Worku M, Bänziger M, Erley GSA, Friesen D, Diallo AO, Horst WJ. 2007. Nitrogen uptake and utilization in contrasting nitrogen efficient tropical maize hybrids. *Crop Sci.* 47:519–28
133. Wu P, Luo A. 1996. Investigation on genetic background of leaf chlorophyll content variation in rice under nitrogen stressed condition via molecular markers. *Yi Chuan Xue Bao* 23:431–38
134. Wuebbles DJ. 2009. Nitrous oxide: no laughing matter. *Science* 326:56–57
135. Xu Y, Wang R, Tong Y, Zhao H, Xie Q, et al. 2014. Mapping QTLs for yield and nitrogen-related traits in wheat: influence of nitrogen and phosphorus fertilization on QTL expression. *Theor. Appl. Genet.* 127:59–72
136. Yadav R, Courtois B, Huang N, McLaren G. 1997. Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. *Theor. Appl. Genet.* 94:619–32
137. Yamaya T, Obara M, Nakajima H, Sasaki S. 2002. Genetic manipulation and quantitative-trait loci mapping for nitrogen recycling in rice. *J. Exp. Bot.* 53:917–25
138. Yang D-L, Jing R-L, Chang X-P, Li W. 2007. Identification of quantitative trait loci and environmental interactions for accumulation and remobilization of water-soluble carbohydrates in wheat (*Triticum aestivum* L.) stems. *Genetics* 176:571–84
139. Zhang F, Cui Z, Fan M, Zhang W, Chen X, Jiang R. 2011. Integrated soil-crop system management: reducing environmental risk while increasing crop productivity and improving nutrient use efficiency in China. *J. Environ. Qual.* 40:1051–57
140. Zhang H, Forde BG. 1998. An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. *Science* 279:407–9
141. Zhang Q, He K, Huo H. 2012. Cleaning China's air. *Nature* 484:161–62
142. Zhuang J-Y, Lin H-X, Lu J, Qian H-R, Hittalmani S, et al. 1997. Analysis of QTL × environment interaction for yield components and plant height in rice. *Theor. Appl. Genet.* 95:799–808