

The Challenge of Improving Nitrogen Use Efficiency in Maize and Wheat: Towards a More Central Role for Genetic Variability and Quantitative Genetics within Integrated Approaches

Bertrand Hirel¹, Jacques Le Gouis², Bertrand Ney³, André Gallais⁴

¹Unité de Nutrition Azotée des Plantes, UR 511, INRA de Versailles, Route de St Cyr, F-78026 Versailles Cedex, France; Email: hirel@versailles.inra.fr

²Unité de Recherche de Génétique et Amélioration des Plantes. INRA, Domaine de Brunehaut - Estrées-Mons, F-80203, BP 136 Péronne, France

³ Unité Mixte de Recherche AgroParisTech, Environnement et Grandes Cultures, F-78850, Thiverval Grignon, France

⁴Unité Mixte de Recherche de Génétique Végétale, INRA/CNRS/UPS/INAPG, Ferme du Moulon, F-91190 Gif sur Yvette Cedex, France

Abstract

In this presentation, the recent developments and future prospects of obtaining a better understanding of the regulation of nitrogen use efficiency (NUE) in maize and wheat, two of the main crops cultivated in the world, will be presented. In these crops, an increased knowledge of the regulatory mechanisms controlling the plant nitrogen (N) economy is vital for improving NUE and for reducing excessive input of fertilisers, while maintaining an acceptable yield. Using plants grown under agronomic conditions at low and high N fertilisation regimes, it is now possible to develop whole plant physiological studies combined with gene, protein and metabolite profiling to build up a comprehensive picture depicting the different steps of N uptake, assimilation and recycling to the final deposition in the seed. We will provide a critical overview on how our understanding of the physiological and molecular controls of N assimilation under varying environmental conditions in crops, has been improved through the use of combined approaches, mainly based on whole plant physiology, quantitative genetics, forward and reverse genetics approaches. Current knowledge and prospects for future agronomic development and application for breeding crops adapted to lower fertiliser input are explored, taking into account the world economic and environmental constraints in the next century.

Media summary

Understanding of the physiological and molecular controls of N assimilation under varying environmental conditions in maize and wheat, has been improved through the use of combined approaches, based on whole plant physiology, quantitative genetics, forward and reverse genetics approaches.

Key words

Agronomy, genetics, maize, nitrogen, physiology, wheat

Introduction

The doubling of the world agricultural production for the past four decades has been associated with a seven-fold increase in N fertilisation which has caused major detrimental impacts on the diversity and functioning of the non-agricultural bacterial, animal and plant ecosystems. In addition, our studies will also indirectly and partly contribute to fight against the detriments caused to the atmosphere. Firstly, through the complex series of reactions in which N is slowly but continually recycled in the atmosphere under gaseous form which reacts with the stratospheric ozone and the emission of toxic ammonia, with two harmful consequences, depletion of the ozone layer and acid rains. Secondly,

production of mineral fertilisers itself is a high-energy demanding process thus contributing to global warming through release of CO₂.

Between now and 2025, the human population is expected to increase from 6 to 10 billion people. Therefore, the challenge for the next decades will be to accommodate the needs of the expanding world population by developing a highly productive agriculture, while at the same time preserving the quality of the environment. Furthermore, farmers are facing increasing economic pressure with the rising cost of the fossil fuels required for producing N fertilisers.

It is therefore crucial to identify the critical steps controlling NUE, which can be defined as the grain yield or biomass per unit of available N nutrient in the soil (including the residual mineral N present in the soil and those provided by fertilisation). NUE is composed of two components: uptake efficiency (NupE, that is, the ability of plants to take up a given mineral nutrient from the soil such as nitrate, ammonium ions), and utilisation efficiency (NutE, that is, the ability of plants to use the mineral nutrient to produce biomass, grains in particular).

Differences between and within crop species in their ability to grow and yield well on soils with low mineral N availability depend on both NupE and NutE. Considerable inter and intra-specific variability exists in maize and wheat, but the identification of plant characteristics and underlying genes that are involved in NUE is complex because it results from a combination of direct and indirect effects. Combining a genetic with a whole plant molecular physiology approach is therefore the only way to properly investigate genetic variability of processes governing acquisition of soil mineral N.

Nitrogen use efficiency: worldwide economic and environmental stakes

In addition to beneficial impacts on environment, decreasing the use of N fertilisers will bring economic advantages to farmers, considering that mineral fertilisers represent roughly 25% of their operating costs. Higher MUE crop varieties will thus strengthen agriculture competitiveness. It will also help farmers of developing countries to meet the challenge of increasingly expensive fertilisers. By maintaining or even improving the productivity at lower mineral fertiliser use of two crops designed for food and feed, will help to prevent social consequences related to food and fossil fuel shortages that will occur within the next few decades.

Combining whole plant and crop molecular physiology for identifying markers of nitrogen use efficiency

The adaptation of plants to various N levels in soils is a systemic and quantitative process that proceeds from growth to development. Because it is constitutive, it integrates many traits and hence it is polygenic. Therefore, tools to diagnose differential responses either for varied inputs or for genotype differences have to combine many elements that are defined as indicative of function and N economy at a particular stage of development.

A first step for developing these tools was to perform whole plant molecular physiology studies using crops grown in the field. These studies have depicted in a dynamic and integrated manner the changes in various physiological and biochemical markers representative of N uptake, N assimilation and N recycling in both model (Terce-Laforgue et al. 2004) and crop species (Hirel et al. 2005b; Kichey et al. 2006). For example, both in maize and wheat the changes in metabolite concentrations and enzyme activities involved in N metabolism within a single leaf, at different stages of leaf growth and at different periods of plant development during the grain-filling period were investigated (Hirel et al. 2005b; Kichey et al. 2006). Interestingly, in both species it was found that total N, chlorophyll, soluble protein content and GS activity are strongly interrelated. It has therefore been proposed that these four physiological traits are indicators that mainly reflect the metabolic activity of individual leaves with regards to N assimilation and recycling, whatever the level of N fertilization (Hirel et al. 2005a,b; Kichey et al. 2006).

More recently, the use of ¹⁵N-labeling techniques performed in the field to estimate the genetic variability for N uptake, N assimilation and N recycling in different maize and wheat genotypes,

combined with the measurement of physiological traits revealed that GS and NR activities are potential markers to estimate the proportion of N taken up or N remobilized. The N taken up or remobilized is further invested in grain yield elaboration or grain N content respectively (Coque et al. 2006; Kichey et al. 2007). Therefore, ¹⁵N-labelling techniques combined with the use of simple physiological markers may be a way to assist breeders to estimate crop performance under different levels of N nutrition, since both methods allow scoring relatively easily and cheaply when using a large number of genotypes.

In order to increase the potential value of the physio-agronomic indicators identified using whole plant and organ biochemical profiling, it will be necessary to monitor in parallel the changes in the whole spectrum of proteins and genes under different N nutrition conditions in different organs, harvested at various periods of plant development. Although this type of approach will require a huge computational analysis when developed on a large set of genotypes, it will be the only way to identify not only genes and proteins involved in the control of the dynamics of N management throughout the whole plant life cycle but also regulatory genetic and metabolic networks (Hirel et al. 2007). When the value of these physiological and molecular indicators is verified on a large panel of genotypes by performing multiple field trials in different soils and climatic conditions, these indicators will hopefully help breeders when screening the best performing lines under lower N fertilization input.

The next step in the development of diagnostic tools will be to fit them into a precision agriculture framing system combining soil testing, fertilizer application and projected fertilizer requirement to determine fertilizer rate applications under different environmental conditions. This would obviously rely on the possibility of developing easy to use diagnostic tools, either based on kits to measure metabolites, enzyme activity or micro arrays containing a set of marker genes representative of the N physiological status of the plant.

Exploiting the genetic variability for improving nitrogen use efficiency

Crop growth and productivity response and adaptation to both N availability and environmental conditions are a very complex characters controlled by many genes. It is therefore almost impossible to rely only on the results obtained by modulating the expression of a single gene, because all the plant growth parameters interact in a complex way and are constantly changing during plant development. Since all these parameters are subjected to genetic variability, which constitutes the empirical basis of plant breeding, this has led a number of researchers, specific and unique to this consortium to develop quantitative genetic approaches to obtain more information on the genetic basis of complex traits including mineral fertilisers use efficiency. Identifying key genes involved in the control of these traits and associated molecular markers is a prerequisite to conducting a marker assisted selection protocol.

Therefore, we have developed in maize and wheat a genetic approach that relies on gathering and integrating a set of genetic data related to NUE, including genetic maps, QTLs and candidate gene location (Gallais and Hirel, 2003). Meta-analysis of these genetic data has been performed to strongly increase the chance of identifying key genes and physiological and phenotypic traits involved in the control of NUE in relation to plant yield and productivity (Coque et al. 2008). The validity of the candidate gene approach has recently been assessed following the demonstration of the direct role of the enzyme glutamine synthetase (GS) during grain filling in maize (Martin et al. 2006; European Patent 06291570.7 “Improvement of the kernel production of maize through the modulation of glutamine synthetase activity”). This finding constitutes one of the most outstanding progresses in the field of the mineral nutrition in crops opening up possibilities for the development of agronomic applications. It is therefore logical to extend such an approach to other crops cultivated in the world and thus find new candidate genes involved in the control of NUE and find those that are common or specific to a given species.

To increase the value of the candidate gene approach, we have also developed association genetics studies to identify intra-specific candidate gene polymorphism in crops using unique germplasm collections composed of different ancient and modern genotypes originating from various areas in the world. This approach will allow linking of the molecular diversity of these genes to phenotypical traits related to NUE and yield and thus identification of the best performing allele for the trait of interest that can be further used for marker assisted selection.

Towards crop systems biology for a better understanding of nitrogen use efficiency

We are currently collecting data from “omics”-based analyses, field experiments, and organising these data by bioinformatics to develop a system biology methodology that could be applied to crop science in the future – an up until now inexistent approach. Future development of such approach will be able to lean on the knowledge gained from our integrated studies on plant functioning at the spatial (cellular and organ distribution) and temporal (changes in the physiology of the crop during growth and development) scales.

Conclusion

An approach that integrates genetic, physiological and agronomic studies of whole crop N response is essential to elucidate the regulation of NUE and to provide key target selection criteria for breeders and monitoring tools for farmers for conducting a reasoned fertilisation protocol. In particular, this allow the discovery of key genes, enzymes and metabolites by means of a complete and extensive phenotyping, comprising agronomical, physiological and biochemical studies on crops grown under low and high N fertilisation applications. Using maize and wheat as model crops, we are currently developing in parallel:

- A functional genomic approach consisting of a meta-analysis of agronomical, physiological, biochemical (including possibly proteomic) QTLs combined with the data obtained on large-scale transcriptome, proteome and metabolome studies designed to identify N responsive genes for further location on genetic maps. The function of this gene is then validated using forward and reverse genetic and association genetic approaches.
- A whole plant molecular physiology approach to depict in a dynamic and integrated manner the regulation of N uptake, N assimilation and N recycling and their progression during the growth and development under varying N fertilisation treatments. Such integrated studies is now extended by monitoring in parallel the changes in the whole spectrum of metabolites, proteins and genes under different N nutrition conditions in different organs harvested at various periods of plant development. Following integration of the data, these studies will allow increasing the potential value of the molecular and physio-agronomic indicators previously identified. These molecular and physio agronomic indicators can be then used both by breeders to select genotypes with superior NUE and possibly by farmers for optimising fertilization.

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