Genetic variability for pre- and post-flowering nitrogen metabolism in maize in relation to plant architecture and leaf senescence

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Abstract

In this poster, we present recent developments towards a better understanding of the controls of nitrogen use efficiency in the model crop maize. In this species, a better knowledge of the regulatory mechanisms controlling the transition between nitrogen assimilation and nitrogen recycling is vital for improving nitrogen use efficiency and for reducing excessive input of fertilisers without affecting yield. Using plants grown in the field at low and high nitrogen fertilization regimes, classical physiological studies combined with gene expression profiling were developed to build up a model depicting the evolution of source/sink relationships in maize in relation to the evolution of leaf senescence. Using representative physiological markers, the genetic variability of the evolution of the source/sink relationships was also studied. In parallel, ^{15}N labelling experiments were performed using different maize genotypes, thus allowing the demonstration that there are genotype-dependent differences in the capacity to absorb mineral nitrogen before and after silking, as well as in the efficiency of leaf proteins remobilisation. An attempt was also made to characterize the relationships between nitrogen content and light perception during leaf senescence in three-dimensional architectural models. These models may provide a way to identify representative physiological markers that can be further used during a selection process. They could be also be used and for QTLs detection in quantitative genetics.

Media summary

Development of new models depicting source/sink relationships for nitrogen metabolism in relation to leaf senescence during grain filling in different maize genotypes grown at low and high fertilisation input.

Key words

Assimilation, genotypes, maize, nitrogen, remobilisation, senescence.

Introduction

In cereals, at silking, mineral nitrogen uptake from the soil and its subsequent assimilation can both decrease up to a certain threshold, to then be progressively replaced by a recycling of organic nitrogen compounds (mostly proteins) originating from senescing organs such as leaves and stems. These organic nitrogen molecules are then exported to the grain, thus allowing the synthesis of storage proteins. The efficiency and the regulation of nitrogen absorption, nitrogen recycling and nitrogen translocation are all together determinant for the final grain protein content and for grain yield (Masclaux et al., 2001). A better knowledge of the physiological and molecular basis controlling the evolution of these three

biological processes is required for future breeding strategies to select for new genotypes with a better nitrogen use efficiency (NUE) and to optimize the management of nitrogen fertilizers.

Maize was chosen as a model cereal plant because it has the capacity to absorb, assimilate and recycle nitrogen that is required for grain filling. Moreover, in this species, our knowledge of the physiology, the genetics and the genomics has improved, allowing an integrated multidisciplinary approach for nitrogen management at the whole plant level. The final goal of our study was to build an integrative model, describing the source/sink relationships during grain filling, and apply this model to nitrogen management in different genotypes, predicting their subsequent impact on yield and its components.

Several aspects were covered in this project:

Methods

Plant material for field and physiological studies

Two lines (F2 and MBS258) and one hybrid (D?a) were grown in the field on two levels of N fertilization (N^+ = 170kg N/ha and N^- = 30kg N/ha) in 2001 in an experimental field of the Institut National Agronomique, Paris/Grignon, France. The residual N provided by the soil was estimated at about 60 kg/ha. Plants were sawn on the 7th of May. The silking period was the 19th of July for F2, the 25th of July for D?a and the 7th of August for MBS258 and was not significantly affected by the level of N fertilisation. Plant density was 120 000/ha with a space of 80cm between rows (12 rows for D?a, 8 rows for F2 and 7 rows for MBS258 for N^+ and N^- treatments) and of approximately 16 cm between plants. The lines F2 and MBS258 were chosen because they were used to produce the population of RILs for quantitative genetic studies to identify the genetic basis of maize NUE (Hirel et al., 2001). The hybrid D?a is a hybrid already used to modelise plant development under different environmental conditions (Drouet and Bonhomme, 1999).

For measuring the different physiological, three plants were selected for genotype F2, MBS258 and D?a at silking, 15, 35 and 55 days after silking. Leaves were numbered as -3, -1, +1 and +1 considering that leaf 0 corresponds to the leaf located below the ear. At maturity the number of remaining leaves in D?a, MBS and F2 was 16, 18 and 14 respectively. For the detailed physiological study performed on F2, three plants were used for each N^+ and N- feeding condition. Fifteen DAS, the remaining leaves were numbered from 1 to 9 (from bottom to top**)** for N^+ plants and from 1 to 8 for N^- plants. In both N nutrition conditions leaf 4 was the leaf located below the ear. From each leaf, the main midrib was removed and 3-cm2 sections of mesophyll tissue were randomly collected and pooled in two groups of 5 leaf disks. One group was weighed and then lyophilised to determine fresh and dry weights per area. The other was weighed, frozen and used to determine protein quantity and chlorophyll quantity per fresh weight and leaf area. The remaining mesophyll tissue was frozen in liquid nitrogen and immediately reduced to a homogenous powder which was stored at -80?C and used for all further experiments (carbon and nitrogen metabolites contents, enzymes activities measurements and RNA extractions). All the harvesting of fresh material was done concomitantly between 10-12 AM.

Results

1. Study of post-flowering nitrogen remobilisation using ^{15}N labelling experiments.

This study was performed on the two parental lines, Io and F2, originally used to produce the population of recombinant inbred lines used for identifying quantitative trait loci (QTLs) for NUE (Hirel et al., 2001), and on hybrids exhibiting early or delayed leaf senescence. These experiments allowed showing that the capacity of the leaf to store and/or remobilise leaf proteins is variable according to the genotype examined (Fig. 1). It is therefore possible to search for QTLs for these two traits.

Furthermore, by measuring the distribution of total nitrogen in the plant until maturity, we showed that post-flowering nitrogen absorption and leaf nitrogen remobilisation contribute almost equally to grain

nitrogen content. Although already mentioned in the literature (Pan et al., 1995), the occurrence of post-flowering nitrogen absorption in maize has never been clearly demonstrated (For instance see Rajcan and Tollenaar, 1999). This new information is therefore of particular importance for both basic and applied studies on maize nitrogen management during grain filling.

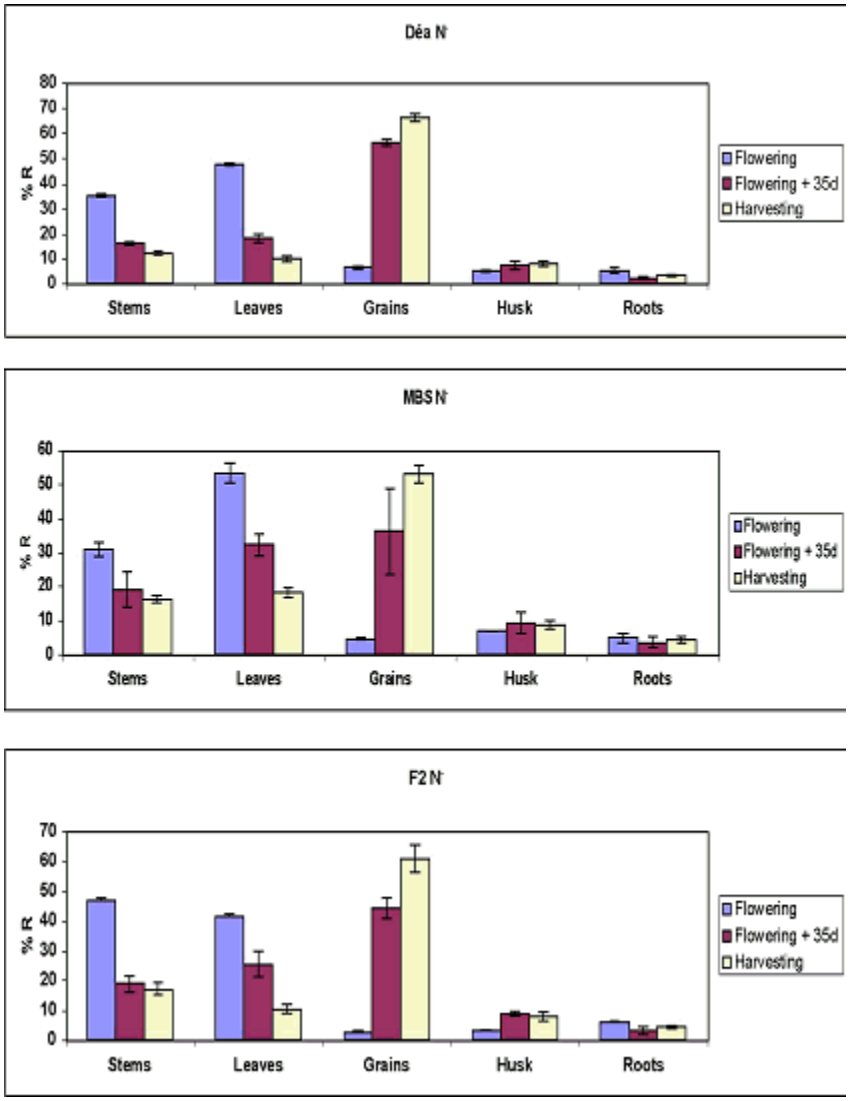

Figure 1: Reallocation of ^{15}N in the three maize genotypes F2, MDS and D?a. %R represents the repartition of ^{15}N incorporated during the labelling period (vegetative stage) in the different organs at flowering, 35 days after flowering and at maturity.

2. Spatial and temporal distribution of physiological markers for nitrogen metabolism.

Nitrogen metabolite distribution and activities of representative marker enzymes for nitrogen metabolism (Terc?-Laforgue et al., 2004) were measured at different leaf stages and at different periods of plant development. In addition, the evolution of the steady state level of the genes encoding these enzymes was monitored.

This study has allowed us to propose a preliminary model depicting the evolution of source/sink relationships in maize in relation to the evolution of leaf senescence. Now an attempt is being made to introduce these physiological markers in plant growth models, in order to provide diagnosis tools on the

physiological nitrogen status of the plant at a given developmental stage. It will also be necessary to verify that this model can be applied to a wide range of genotypes prior the detection of QTLs for representative physiological markers, after their validation in the model.

3. Architectural analysis of leaf senescence in relation to light perception and the evolution of carbon and nitrogen pools within the plant.

The models such as CERES, currently used to describe plant functioning take into account the evolution of leaf senescence only in a very simple way and are not applicable when there is genetic variability. Moreover, a number of authors have shown that the evolution of nitrogen content per unit of leaf surface is strongly correlated with the amount of light absorbed. This observation suggests that an architectural model can provide an explanation of the processes intervening during leaf senescence.

One of our approaches consisted of a three dimensional reconstitution (Drouet et al., 2003) of the maize genotypes Io, F2 and D?a in conjunction with the use of models depicting radiative exchanges. This combination of techniques has allowed characterizing the relationships between nitrogen content and light perception in leaves of the three genotypes at three periods of plant development.

In parallel, senescence profiling along the leaf was monitored in the different leaf stages as a function of low or high nitrogen fertilization regimes. Although we found some similarities, we also observed genotype-dependent differences concerning either the speed to which senescence developed or the order in which leaf stages begin to senesce.

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