Natural variation in Arabidopsis, a tool to identify genetic bases of nitrogen use efficiency

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Abstract

To better understand the genetic bases of nitrogen use efficiency in plants, we studied the response of *Arabidopsis thaliana* to nitrogen limitation. Using a 400 recombinant inbred lines population derived from the cross of Bay-0 and Shahdara accessions, we first isolated several QTL for biomass production, nitrogen, amino acid content or mineral content variations. Then, using heterozygous inbred family lines, we validated the effect of several loci for traits such as leaf area, biomass, amino acid or sulphate content. For the latter, we were able to find a candidate gene (*APR2*). For the amino acid content, the fine mapping of the locus is still in the process. We use recombinant lines at the given locus, which already allowed us to restrict by 90 % the size of the interesting area between markers on the chromosome, opening the way to candidate genes approach.

Media summary

Finding genes involved in plant resistance to N stress will allow selecting new cultivars which should be less exigent for nitrogen fertilizers.

Key Words

Nitrogen, QTL, candidate gene, fine mapping, accession, Arabidopsis

Introduction

Plants do not have the same nutrient requirements, especially for nitrogen. Some species are very exigent, others are less demanding, for example the hardy plants. The genetic bases of the nutrient demand by the plant are still unknown. In order to identify these bases, we have studied the reactions of the model plant *Arabidopsis thaliana* placed in different nitrogen concentrations in the nutrient medium.

Methods

Plants were grown during 35 days in a growth chamber at the INRA Versailles, on sand or non-fertilized compost irrigated with a complete nutrient solution containing as sole nitrogen source either 10 mM nitrate (normal nitrogen supply) or 3 mM nitrate (limited nitrogen supply). The culture was held in short days (8h), at a day/night temperature of 21°C/17°C at a PPFD of 160 $\mu mol.m^{-2}.s^{-1}$. The metabolite contents were determined as described in Loudet et al. 2003.

Results

In a first step, we used a population of Recombinant Inbred Lines (RIL) previously established by our group (Loudet et al. 2002) and derived from the cross of two genetically and geographically distant ecotypes (more precisely "accessions"), Bay-0 (from the plains of Germany) and Shahdara (from the

mountains of Central Asia). Phenotyping the 400 lines of that population for various parameters such as biomass, N content, amino acid content and mineral ion content, we were able to detect (through linked genetic markers) several QTL explaining 20 to 40 % of the total phenotypic variance. Among those QTL, two are particularly interesting (Figure 1). The locus L4, located on chromosome III has been detected on limited N supply and is associated to biomass, total N and free amino acid content variation. Another one, the locus SO3.1, located on chromosome I and detected on both normal and limited N supply, although with different effect, is linked to sulphate content variation.

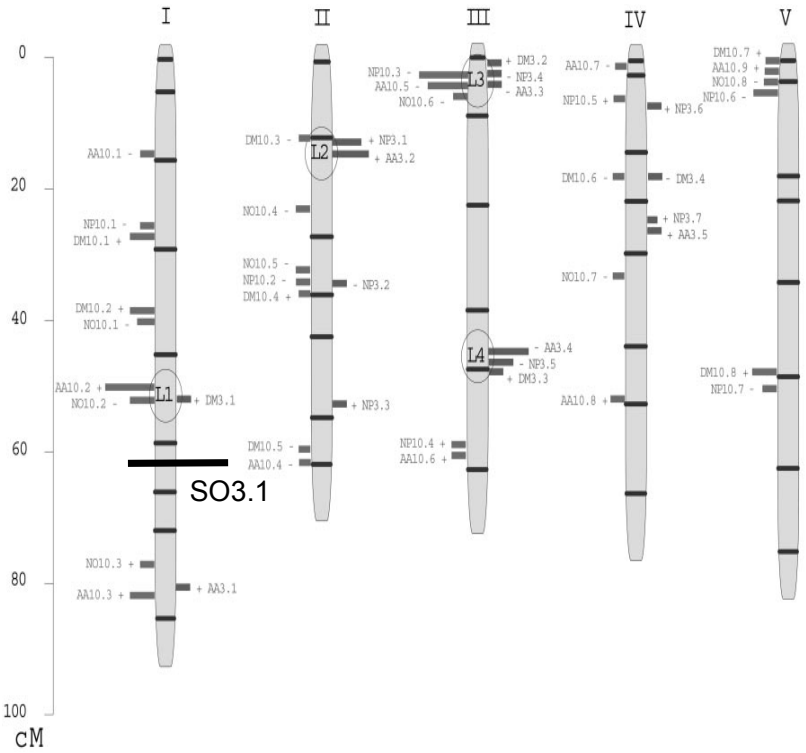

Figure 1. QTL detected in the Bay-0 x Shahdara population. The QTL on the left of the chromosomes are those detected on normal N nutrition, those on the right of the chromosomes correspond to N limited supply. The length of the bar is proportional to the contribution of the QTL.

The second step of that research consists in the validation of the QTL. For that purpose, we used Heterozygous Inbred Family (HIF) lines, descending from the same heterozygous RIL and differing only by the alleles present at the given locus. One set of lines carries the Shahdara allele, while the other set carries the Bay-0 allele. Measuring leaf area, dry matter biomass, amino acid content and sulphate content, we found significant differences between the two sets of lines. That means that the given loci are responsible for the variation of the traits. Thus, we validated the effect of the QTL for the measured traits (sulphate content for SO3.1, and amino acid content for L4).

Then two alternatives are possible: either a gene, which is potentially involved in the quantitative variation of the parameter based on a putative function, is present in the chromosome area where the QTL is located (confidence interval), or no remarkable gene is mapped within the region of interest. In the first case, we can directly use a candidate gene approach, but in the second case we have to go through a fine mapping approach in order to restrict the length of the interval of interest on the chromosome. For the QTL linked to sulphate content (locus SO3.1), we were able to find a candidate gene, but for the locus linked to amino acid content (locus L4), we had to go through fine mapping.

The finding of APR2 gene, successful candidate gene approach (Loudet et al. 2007):
The SO3.1 QTL for shoot sulphate content was detected on chromosome I and explained 48% of the total phenotypic variance in the limited N environment (21% in the normal N supply). Extrapolating the position of the QTL on the physical map, we found that it was very near the gene encoding

adenosine 5'-phosphosulfate reductase (APR2), a key enzyme of assimilatory sulphate reduction pathway. We sequenced the Bay-0 and Shahdara alleles of *APR2* and we found one Single Nucleotide Polymorphism (SNP) resulting in one amino acid change in the protein. Adding new markers on each side of *APR2*, we saw that the position of the reanalysed QTL coincided exactly with the *APR2* gene. To make sure that the polymorphism of this gene was responsible for the observed phenotype, we complemented several Shahdara HIF lines with the Bay-0 *APR2* allele. This restored the Bay-0 sulphate content level in the plants. We thus can conclude that the QTL linked to sulphate content is fully explained by *APR2* allelic variation, illustrating a successful candidate gene approach.

Fine mapping, a school for patience:
In order to do the fine mapping of the locus L4, we used 42 HIF lines which are recombinant at the locus (named rHIF lines), and we developed six new markers. We measured several traits, including amino acid content, in plants grown on a limited N supply. Significant phenotypic differences for those traits have been found between genotypes which are different at the new markers, i.e. carrying either the Shahdara or the Bay-0 allele. This method of fine mapping allowed us to restrict the size of the interesting area from 6800 to 650 kb. The next step will be to clone more precisely that portion in order to find genes involved in these trait variations. In order to do that cloning we have to go through one more run of fine mapping (Figure 2). We have already selected 11 rHIF which are recombinant in the 650 kb region. We are currently developing new markers in this region, and we are going to compare the phenotype (amino acid content) of the rHIF lines. This should allow us to reduce again the size of the QTL to a portion with about twenty genes, in order to be able to find a candidate gene.

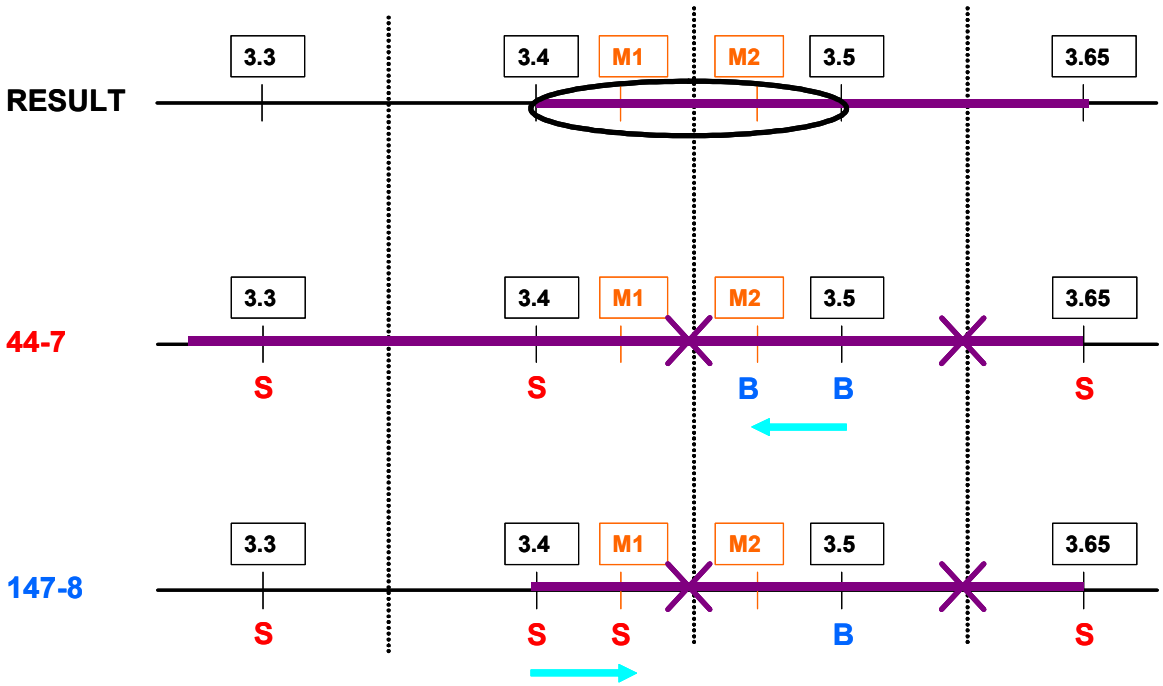

Figure 2. Fine mapping of a QTL: the development of new markers M1 and M2 and the comparison of two rHIF lines (44-7 and 147-8) result in restricting the interval of the QTL from the distance between the markers 3.4 and 3.5 to the distance between M1 and M2 (upper line).

Going further by observing the variability in a core collection of accessions:
We grew the 24 accessions of the core collection of INRA Versailles, which captures almost 96 % of genetic diversity (McKhann et al. 2004), on different N regimes (plethoric or limited) and we measured several traits linked to the growth and development of the plants. We saw contrasted responses among the accessions. For example, when submitted to N-starvation, Can-0 from Spain restricts its root growth, but keeps its phyllochron and leaf number at the same level as the control, while Bay-0 increases its root, but slows down its shoot development (Figure 3).

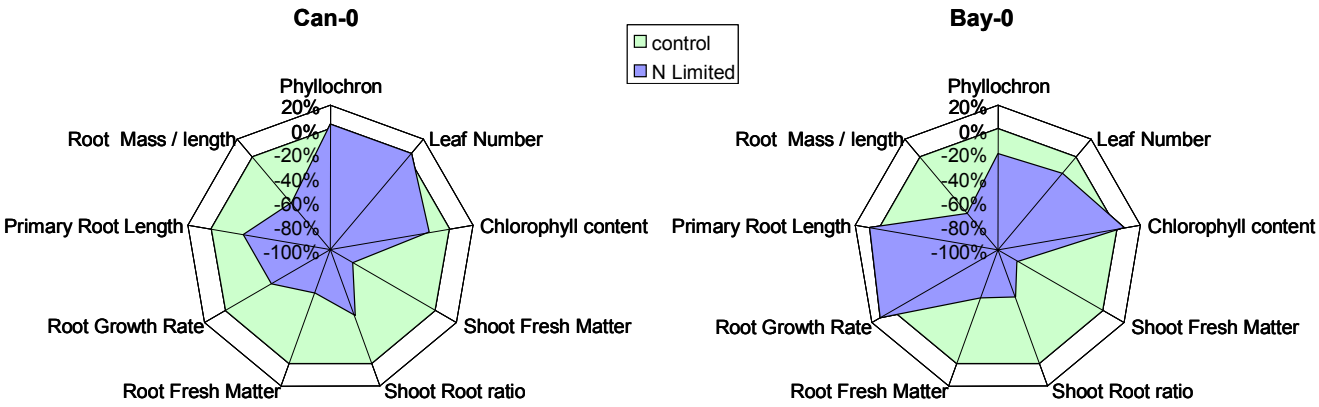

Figure 3. Phenotypic responses profiling of two contrasted ecotypes faced to N limitation. The results are given as percentage of values measured in control plants (plethoric N nutrition).

Conclusion

The quantitative genetics approach used in the present work (observation of natural variation as well as detection of QTL, and even candidate genes method when possible) was applied on the model plant Arabidopsis. This should give interesting results in terms of the discovery of genes involved in the capacity of plants to cope with N shortage. By sequence homology, such genes or markers may be found in crop species, which should be useful tools for crop breeders in order to select new cultivars having better nitrogen use efficiency.

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