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

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Plants have different nutrient requirements: some species are very exigent, others are less demanding.

Our objective is to find the genetic bases of the nutrient demand by the plant. We take the case of Nitrogen Use Efficiency in the model plant *Arabidopsis thaliana.*
Searching for QTL of nitrogen use efficiency in Arabidopsis

- Creation of an Arabidopsis recombinant inbred lines population from two genetically distant ecotypes (Bay 0 and Shahdara)

- Obtaining a genetic map of the population with markers (genotypical data)

- Growing plants on two types of nitrogen nutrition, normal (10 mM) or limiting (3 mM) and measuring several parameters (phenotypical data)

- Correlation between genotypical and phenotypical data for QTL detection
Recombinant Inbred Lines - RILs

Shahdara from Central Asia

Bay-0 from Germany

Single Seed Descent

420 RILs
Bay-0 x Shahdara RIL population

Genetic map of the population of 420 F6 Recombinant Inbred Lines

38 microsatellites markers

Loudet et al, TAG 2002
Phenotyping of the population
View of a set of 24 Arabidopsis lines after 34 days of culture

N+ (10 mM)  N- (3 mM)

(Loudet et al., 2001)
Isolation of several QTL linked to biomass, total N, amino-acid or sulfate content
After isolation of the QTL, we must validate the QTL. The use of Heterozygous Inbred Families (HIF) allows the validation of QTL.
Heterogeneous Inbred Family - HIF

RILXXX (F6)

HIF - Sha
HIF - Het
HIF - Bay
Example of QTL validation using HIFs

Amino acid content

- Mean of ILs 421 B = 173 nmole aa /mg of DM
- Mean of ILs 421 S = 155 nmole aa /mg of DM
Validation of a QTL for sulfate content

068S: 110 nmol SO$_4$/mgDW

068B: 80 nmol SO$_4$/mgDW

HIF068 - Sha

HIF068 - Bay
Then, you may be lucky if a candidate gene is present in the chromosome area where the QTL is located.

That was the case for the QTL of sulfate content. A gene coding for a key enzyme of sulfate assimilatory pathway (APR2) was present in the interval of interest on the chromosome.
Let us have a look at sulfate assimilation...
Confirmation of the candidate gene APR2

APR2 sequenced on Bay-0, Shahdara and 24 ecotypes of Arabidopsis: one Single Nucleotide Polymorphism resulting in one amino acid change in the protein.

Loudet et al 2007
To test the candidate gene APR2, we performed a complementation test

- **Test of dominance**

  - Dominance of Bay-0 allele => suggesting that Shahdara allele is less active => accumulation of sulfate

  - In the complementation test: the Bay-0 allele will be used to complement a HIF-Shahdara

Loudet et al 2007
Transgenic complementation test in Shahdara: restoration of the Bay-0 sulfate content level

Loudet et al 2007
Thus, the QTL linked to sulfate content is fully explained by *APR2* allelic variation, which is a successful candidate gene approach.
If no remarkable gene is mapped in the region of interest, we have to go through a fine mapping approach which allows to restrict the length of the interval on the chromosome.
The use of rHIF lines for fine mapping

We compare the phenotypes of plants which are recombinant in the area of interest.
The QTL interval is restricted from the distance 3.4 – 3.5 to the distance M1 – M2.
This fine mapping approach has to be repeated until the QTL interval is reduced to a portion containing about 20 genes in order to be able to find a candidate gene. So, it is a school for patience!
We can go further by observing the variability among the Arabidopsis genotypes, which are named « accessions ».
Arabidopsis thaliana accessions
Core collection

Restricted set of accessions that encompasses the range of diversity of the full collection. (Exist core collections of 8, 16, 24, 48 accessions)

(Mc khan et al., 2004)
Natural variation in *Arabidopsis thaliana* (core collection of 24 accessions)

28 days of culture on 4 mM nitrate (Sobia Ikram, 2007)
Hydroponic culture of Arabidopsis plants
Phenotypic responses profiling of two contrasted ecotypes faced to N limitation
We were able to distinguish three classes of accessions:
- Class 1, plants which are very affected by N starvation
- Class 2, plants which are less affected
- Class 3, plants which sustain quite well N starvation
After 7 days N starvation, the different genotypes of the core collection can be sorted in three classes.

<table>
<thead>
<tr>
<th>Class</th>
<th>Shoot FW</th>
<th>Root FW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>- 45 %</td>
<td>+ 38 %</td>
</tr>
<tr>
<td>Class 2</td>
<td>- 36 %</td>
<td>+ 78 %</td>
</tr>
<tr>
<td>Class 3</td>
<td>- 23 %</td>
<td>+ 137 %</td>
</tr>
</tbody>
</table>

Values correspond to the decrease or increase in percentage of the control.
The three classes of accessions
Perspectives

- In a model plant, discovery of genes involved in the capacity of plants to cope with N deficiency in the soil
- In crop species, finding of new genes or markers associated with N demand
- Select new cultivars having higher nitrogen use efficiency
And the Palace of Versailles!