

# Detecting the effects of co-adaptation in plant genomes

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*genetic interactions between nucleus and organelle genomes  
Cytoplasmic Male Sterilities*

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*genomic Networks team*  
MIA-Paris, Paris  
*Statistics et genome team*



Math For Genomics  
17/06/2020

# The plant cell metabolism is compartmentalized So is its genome

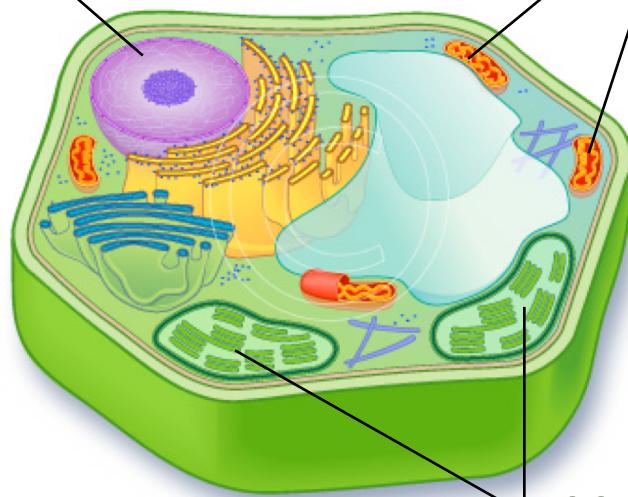
~25,000 genes

nucleus

mitochondria

~70 genes

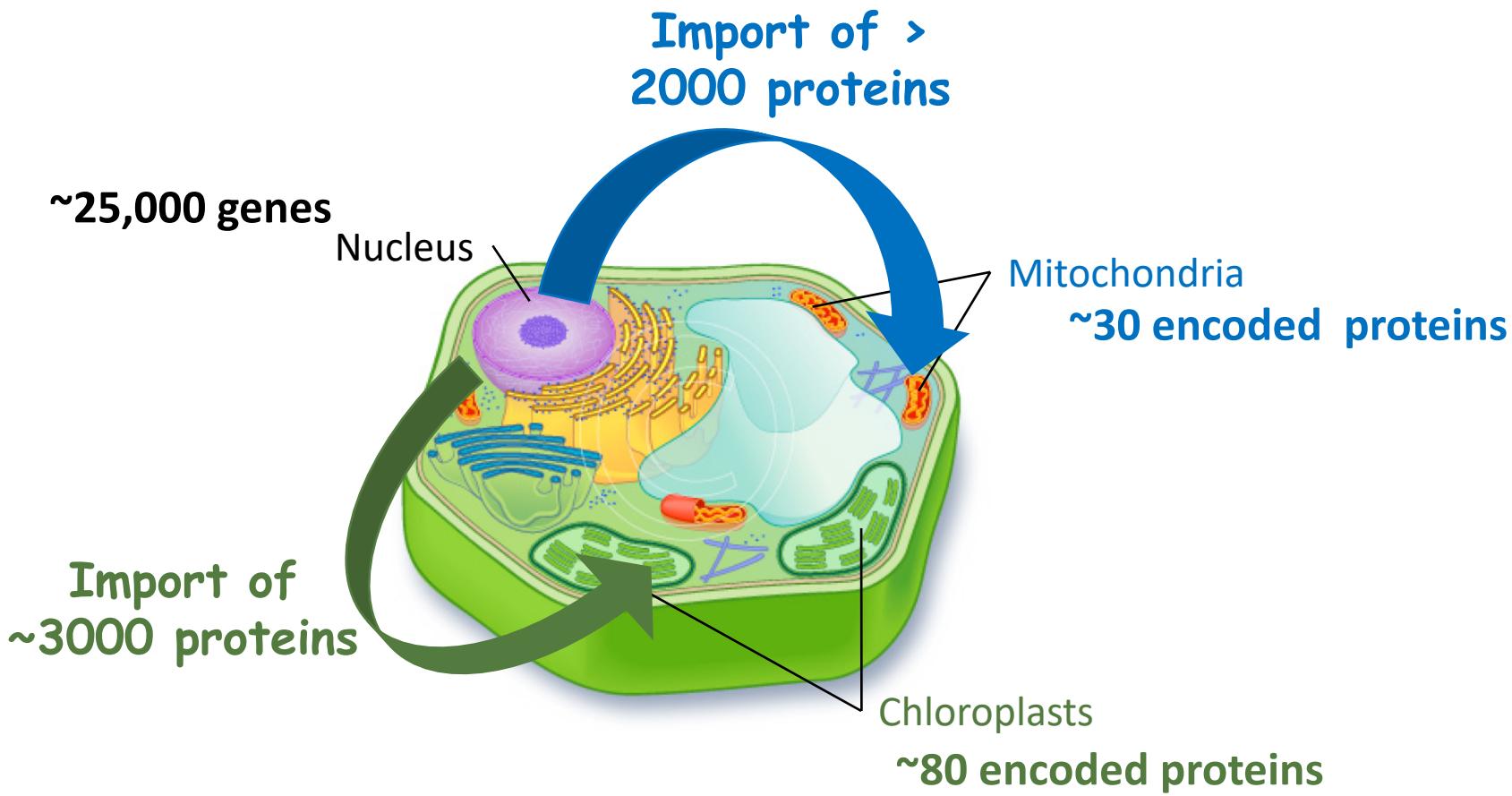
energy metabolism  
redox metabolism  
stress response



chloroplasts  
~130 genes

C assimilation  
N & S assimilation  
Environment sensing

# Mitochondria and chloroplasts are not functionally autonomous

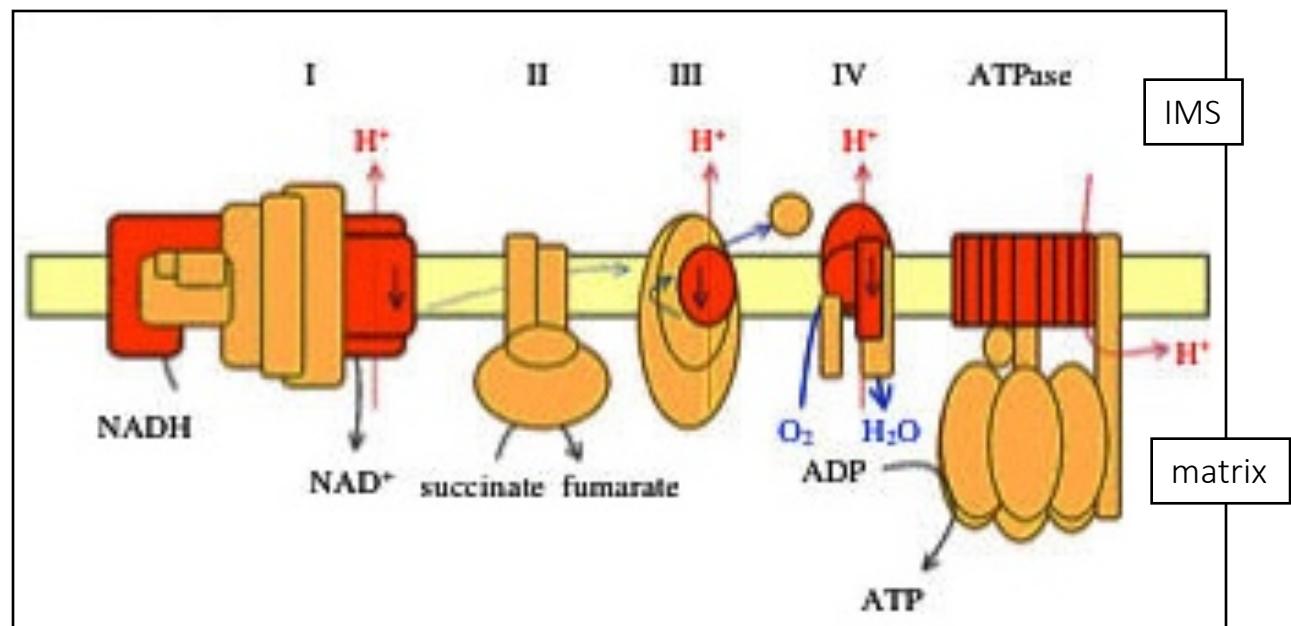


The proper function of plant organelles relies on the interaction between nuclear and organelle genetic units

### Mitochondrial electron transport chain

origin of subunits

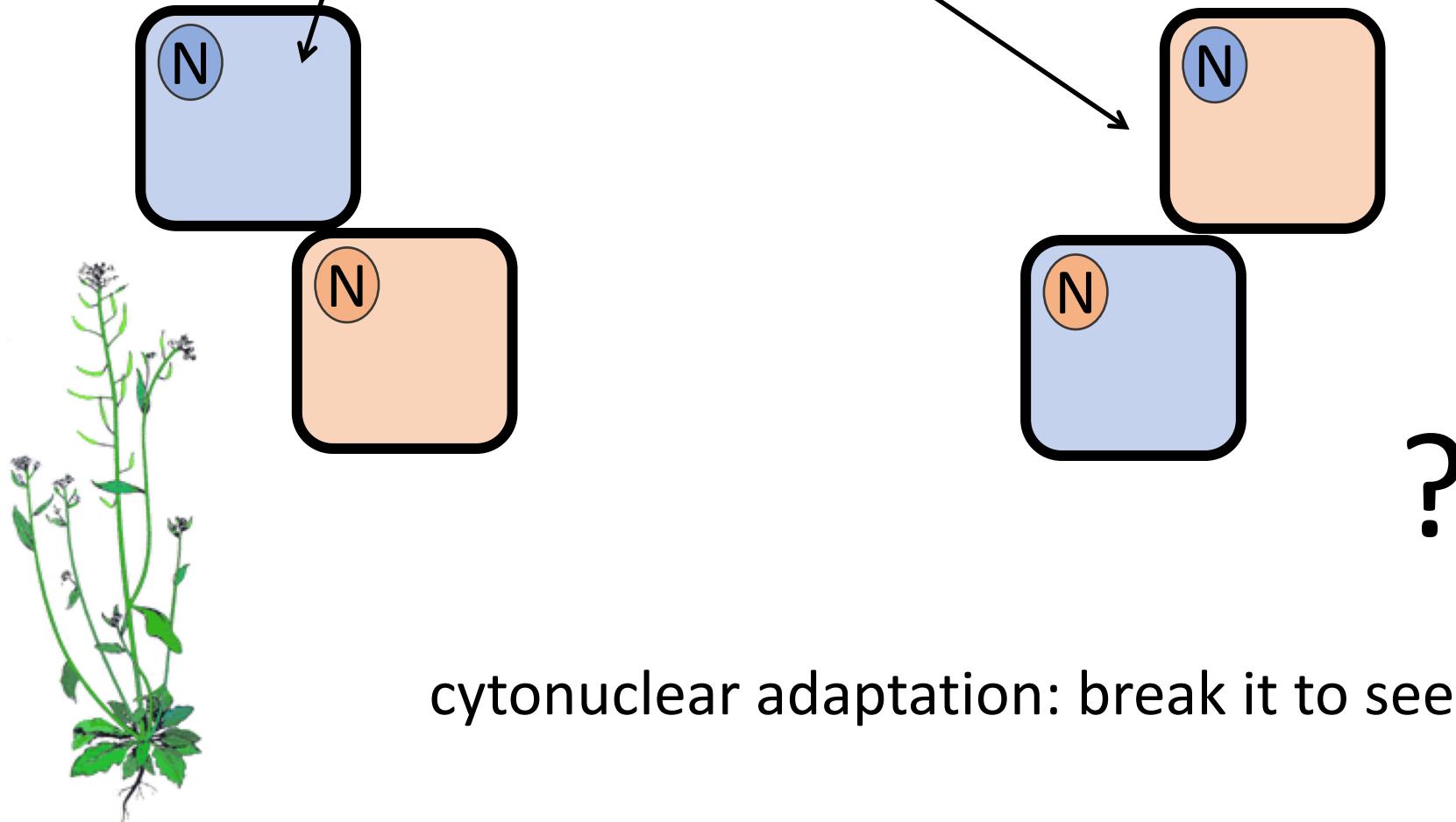
- mitochondrial
- nuclear



“Co-adaptation occurs when a variation in a factor encoded by one compartment will select for a variation in a factor encoded by the other, due to physical interaction between the two factors.” Rand, 2004

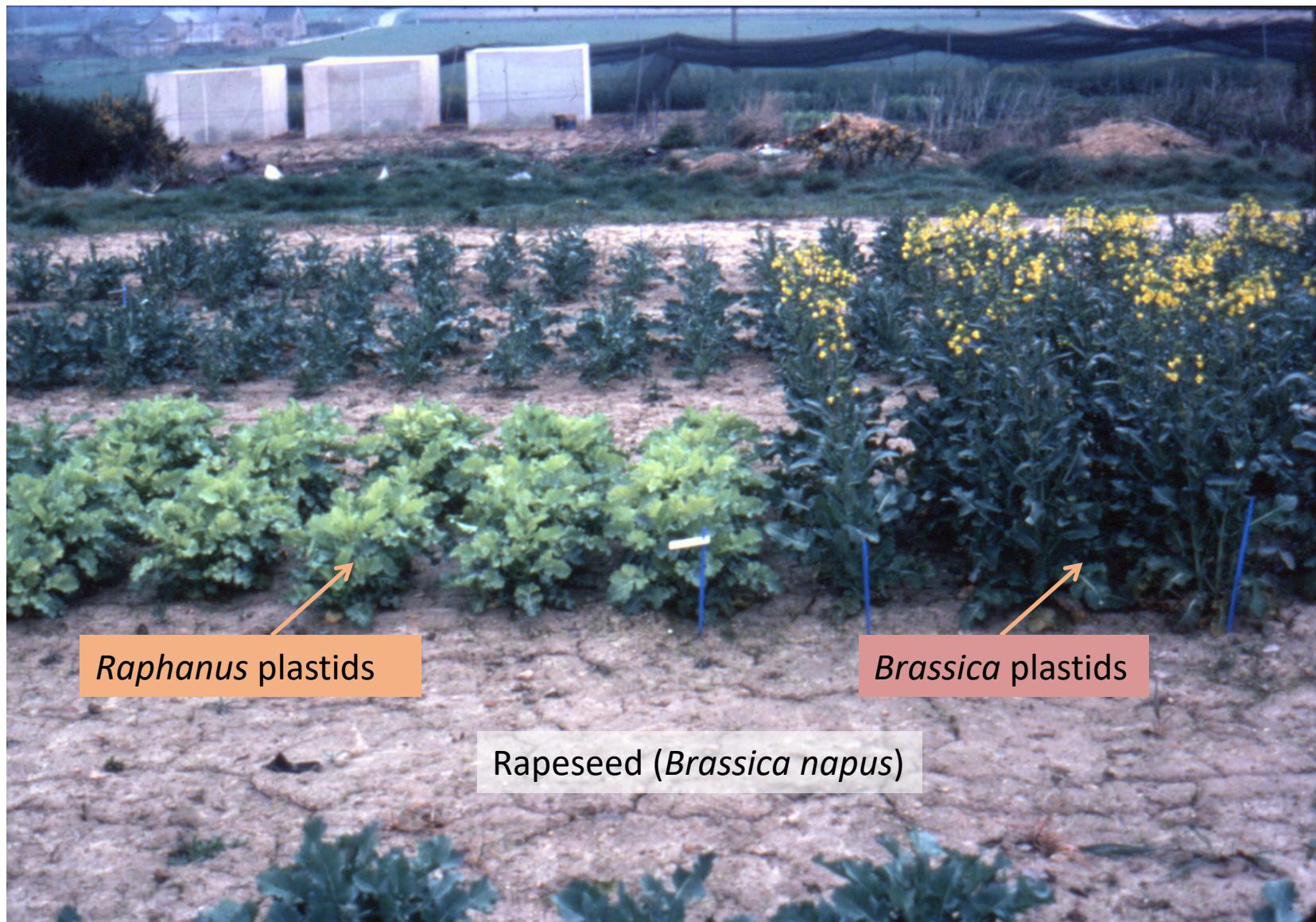
Cytoplasmic and nuclear genomes are expected to be co adapted

"We expect **variation in phenotypic traits** that can contribute to fitness differences between proper and impaired associations of variants." Rand 2004



cytonuclear adaptation: break it to see it!

# Cytonuclear co adaptation : break it to see it



## Biological questions

- Adaptive intra-specific variations in cytoplasm?
- Which phenotypic traits?
- Impact of cytonuclear interactions?

# Biological questions

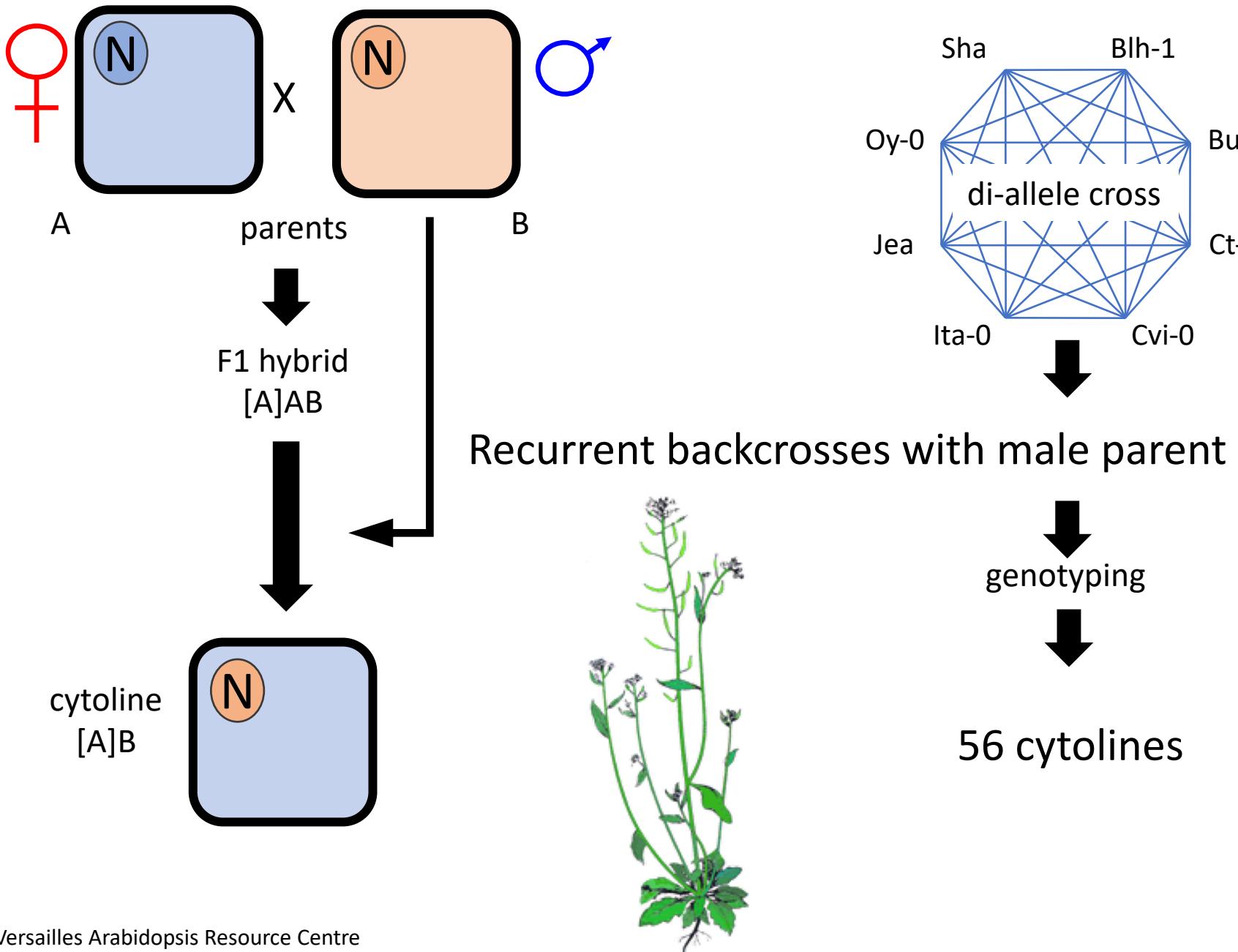
- Adaptive intra-specific variations in cytoplasm?
- Which phenotypic traits?
- Impact of cytonuclear interactions?



The CYTOPHENO Project (2012-2016)

Detection of phenotypes affected  
by variations in organellar genomes  
and by the disruption of cytonuclear co adaptation

# Arabidopsis cytolines: cytoplasm exchange between natural accessions



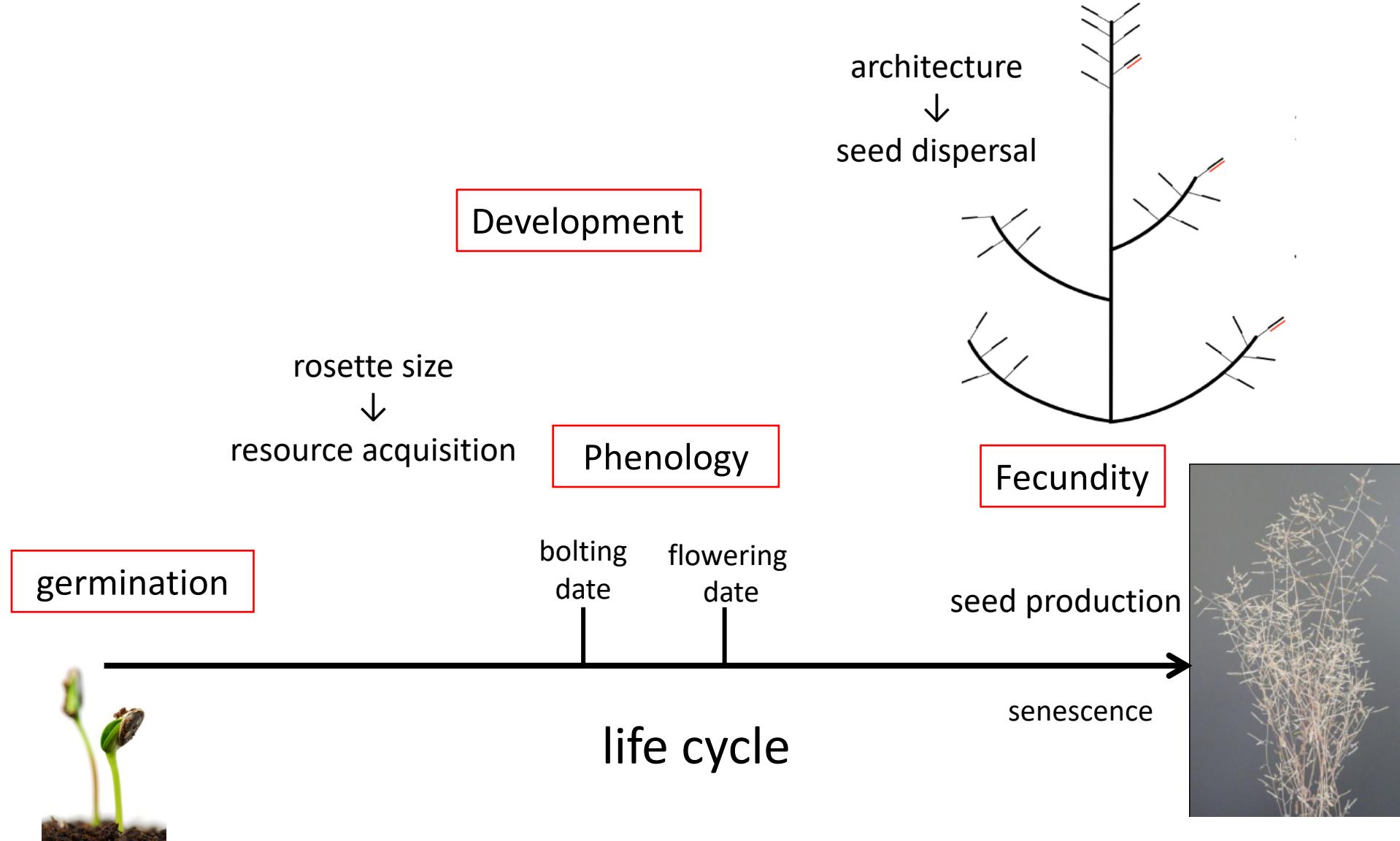
# Experiment 1

## Phenotyping *Arabidopsis* cytolines and their parents for adaptive traits in the field

questions

1. Do intraspecific natural variation in organelle genomes affect adaptive traits?
2. Are adaptive traits under the influence of cytonuclear co adaptation?

# Arabidopsis adaptive traits in nature



# Phenotyping of cytolines in the field

## experimental design

5 Blocks



9 arrays of 66 pods/block



1 plant/pod

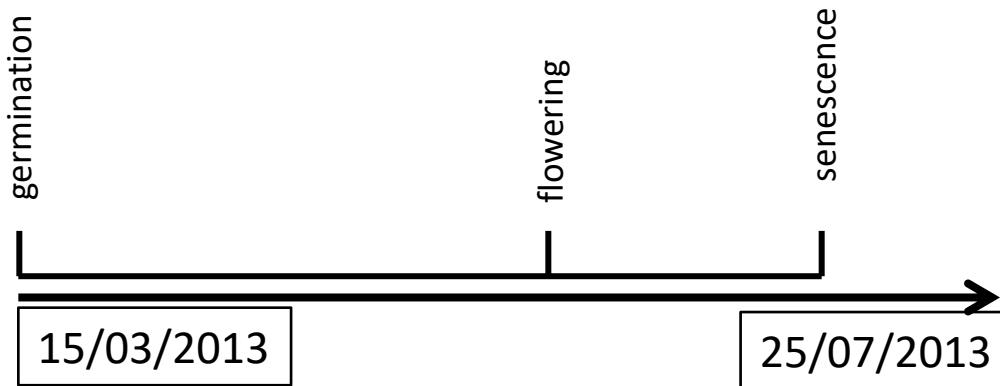
Randomized Complete Block Design

1 array = 11L x 6C

Common garden, University of Lille (North of France)

# Phenotyping of cytolines in the field

## production of data



### 27 quantitative traits

Germination (5)

Resource acquisition (3)

Phenology (4)

Architecture and seed dispersal (5)

Fecundity (10)

+ survival (qualitative trait)

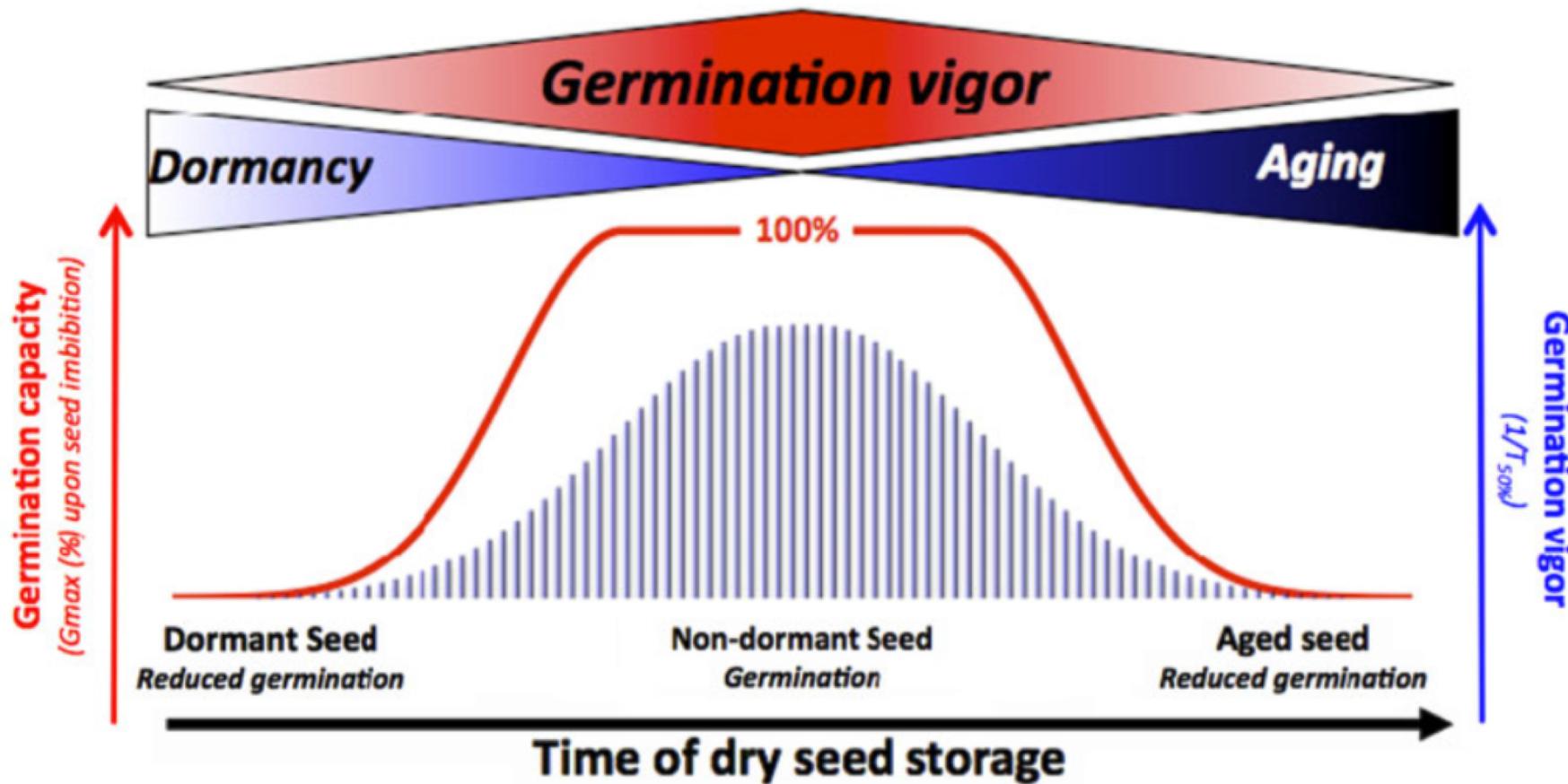
2745 pods sowed  
2228 plants harvested

## Experiment 2

### Phenotyping *Arabidopsis* cytolines and their parents for seed physiological traits

question

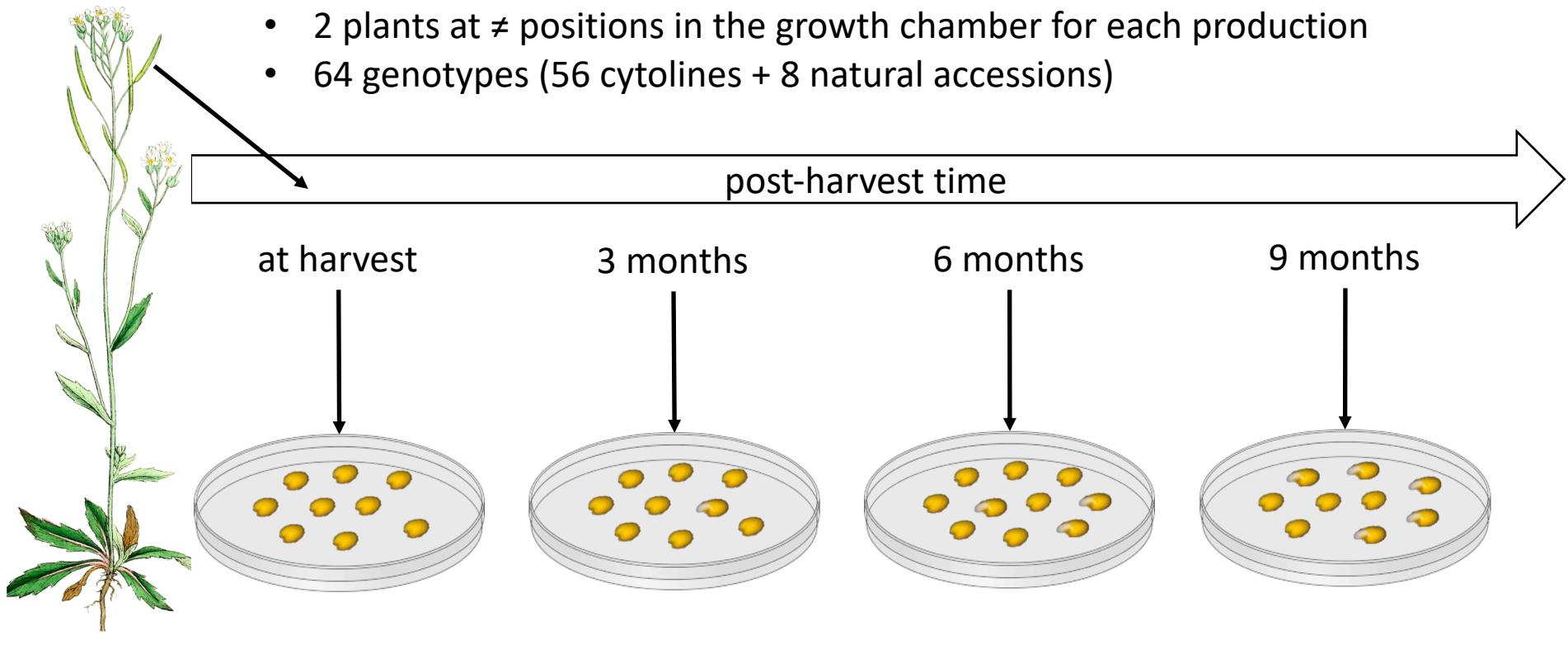
Do cytonuclear interactions affect seed physiology?



# Phenotyping of seed traits : dormancy

## experimental design

- 2 seed productions
- 2 plants at  $\neq$  positions in the growth chamber for each production
- 64 genotypes (56 cytolines + 8 natural accessions)

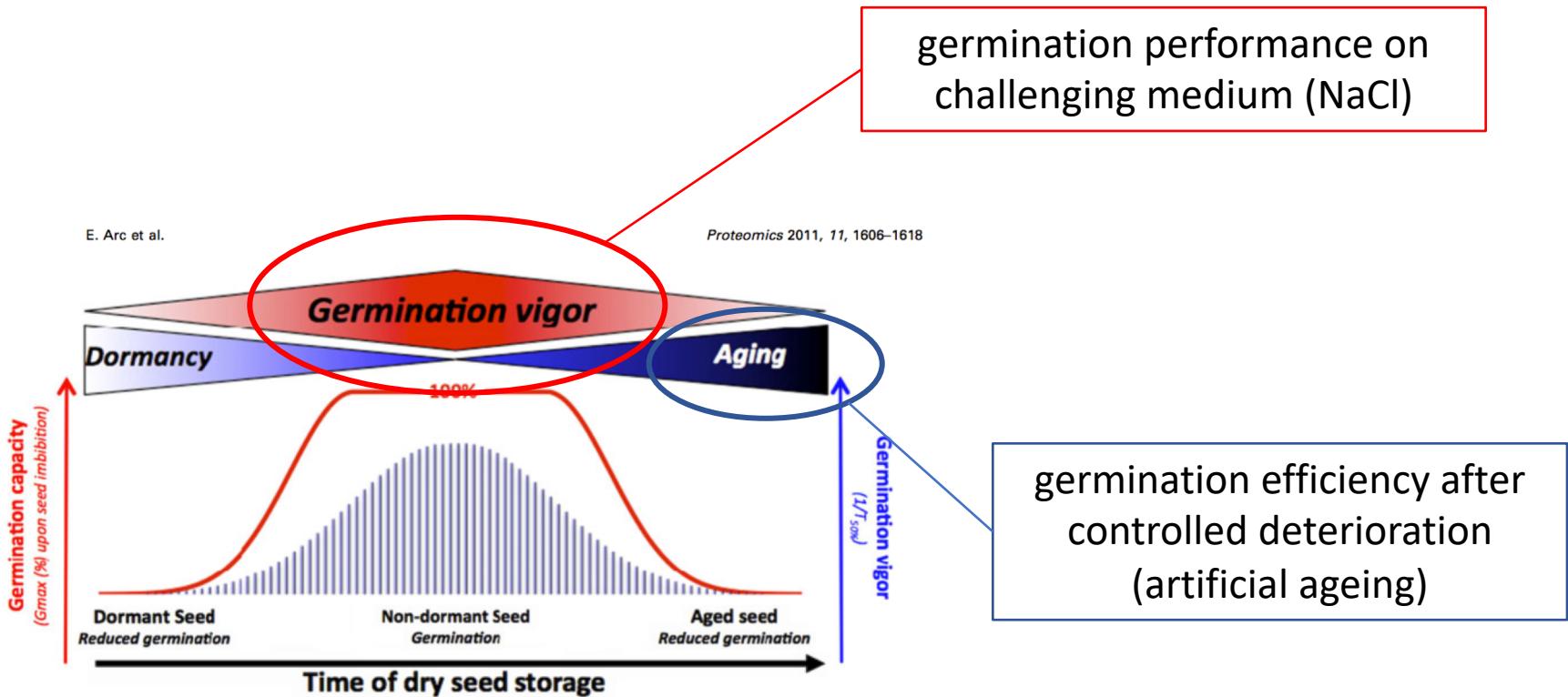


seeds sowed in vitro and incubated at 15°C or 25°C

## production of data

germination (gmax) scored after 96h, 2 technical replicates

# other phenotyped seed traits



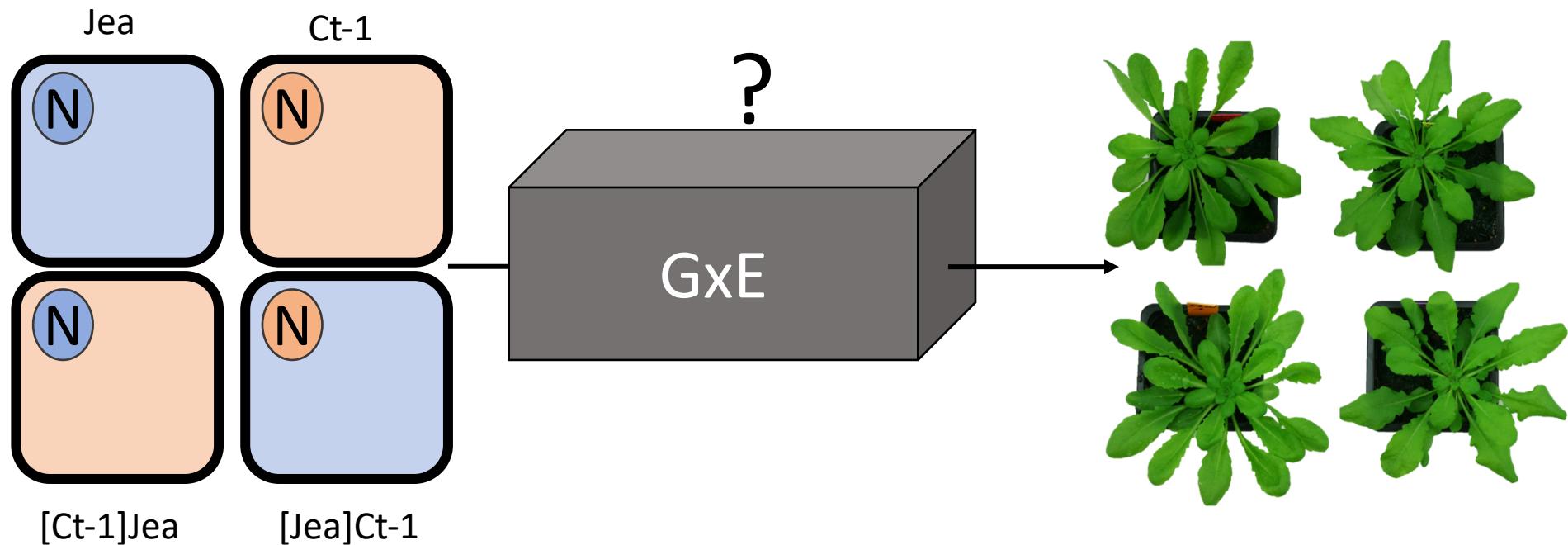
## Experiment 3

Multi-omics phenotyping of *Arabidopsis* cytolines and their parents  
in two nitrogen nutrition conditions

question

Does a disruption of cytonuclear coadaptation modify the molecular response of plants to a nutrition stress?

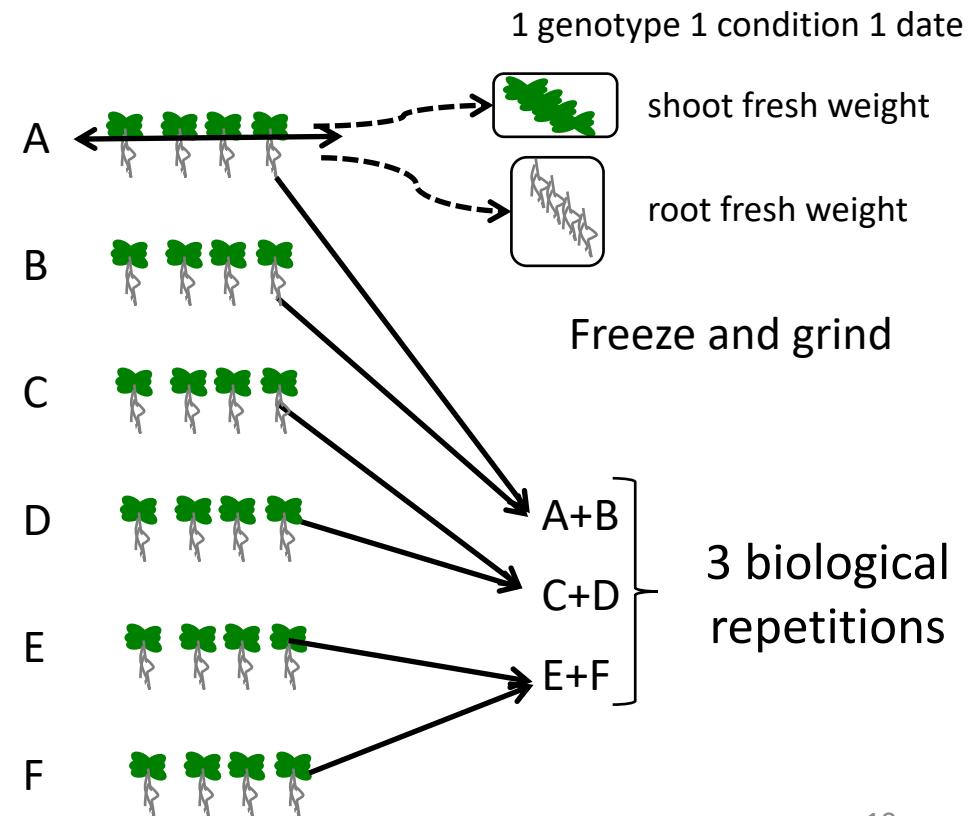
# Molecular phenotyping in two nitrogen nutrition conditions



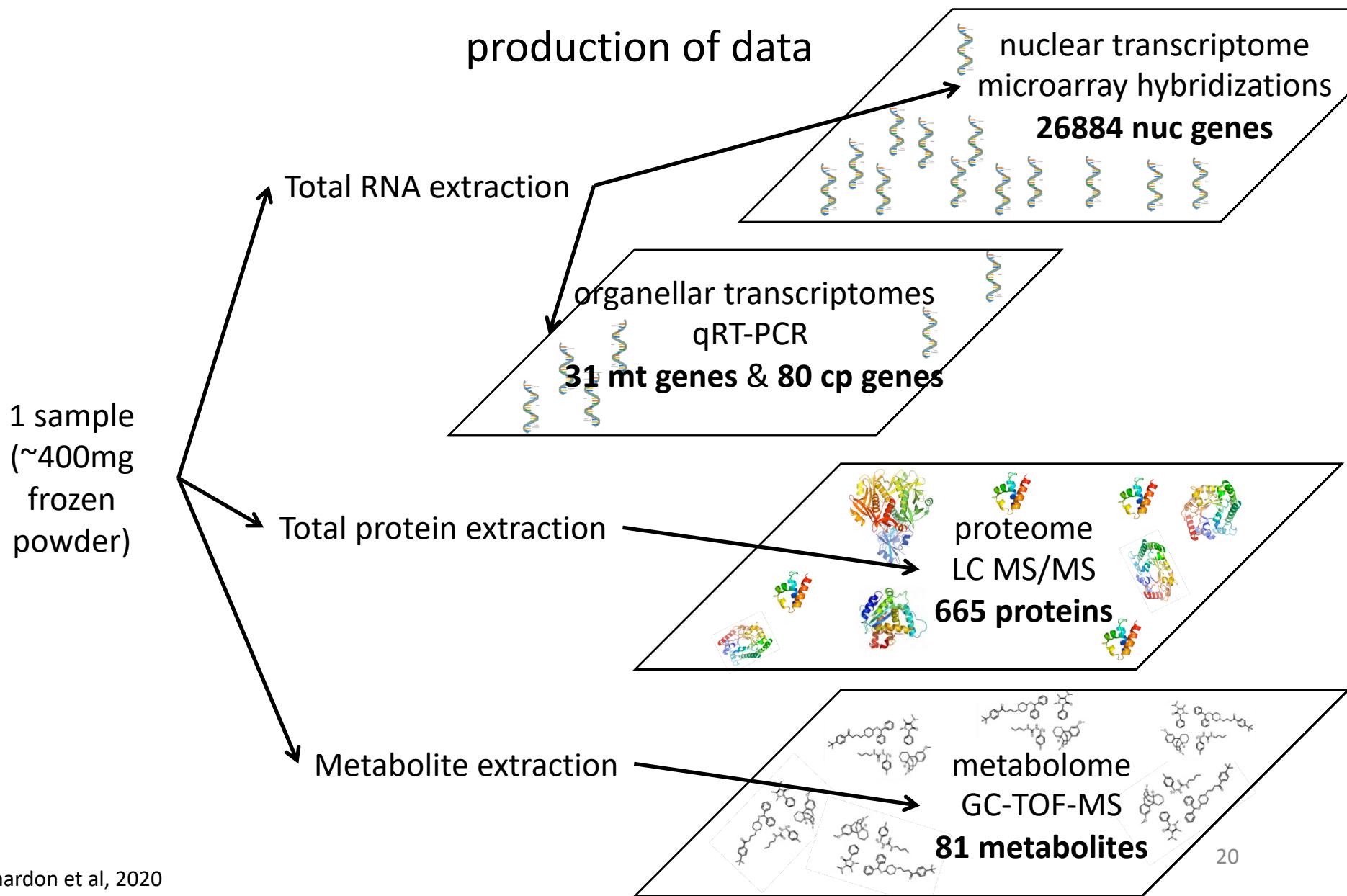
# Phenotyping of seed traits : multi-omics on plants in two nitrogen nutrition conditions

## experimental design

- 4 genotypes : 2 natural accessions and their reciprocal cytolines
- 2 N conditions (standard & starved)
- 6 productions (randomised designs)  
1 production : 4 plants/genotype/N condition



# Molecular phenotyping in two nitrogen nutrition conditions



# Statistical part of the project

Our motivation:

- To be involved in the project at the beginning
- A project with many interactions with biologists
- Several datasets of different nature to study the same question
- No research in statistics but opportunity to do proper statistics from A to Z

People:

- Tristan Mary-Huard
- Benjamin Vittrant (M2 internship)
- Priscilla Monfalet (M2 internship and 6 months of IE)

Our tasks:

- Design of the experiments
- Modelling of the three datasets to answer the questions
- Visualisation of the results
- Validating the biological interpretation of the results
- Writing the M&M of the papers

# Phenotyping Arabidopsis cytolines and their parents for adaptive traits in the field

questions

1. Do intraspecific natural variation in organelle genomes affect adaptive traits?
2. Are adaptive traits under the influence of cytonuclear co adaptation?

for seed physiological traits

question

Do cytonuclear interactions affect seed physiology?

for molecular phenotyping in two nitrogen nutrition conditions

question

Does a disruption of cytonuclear coadaptation modify the molecular response of plants to a nutrition stress?

# Phenotyping Arabidopsis cytolines and their parents for adaptive traits in the field

questions

1. Do intraspecific natural variation in organelle genomes affect adaptive traits?
2. Are adaptive traits under the **influence of cytonuclear co adaptation?**

for seed physiological traits

question

Do **cytonuclear interactions** affect seed physiology?

for molecular phenotyping in two nitrogen nutrition conditions

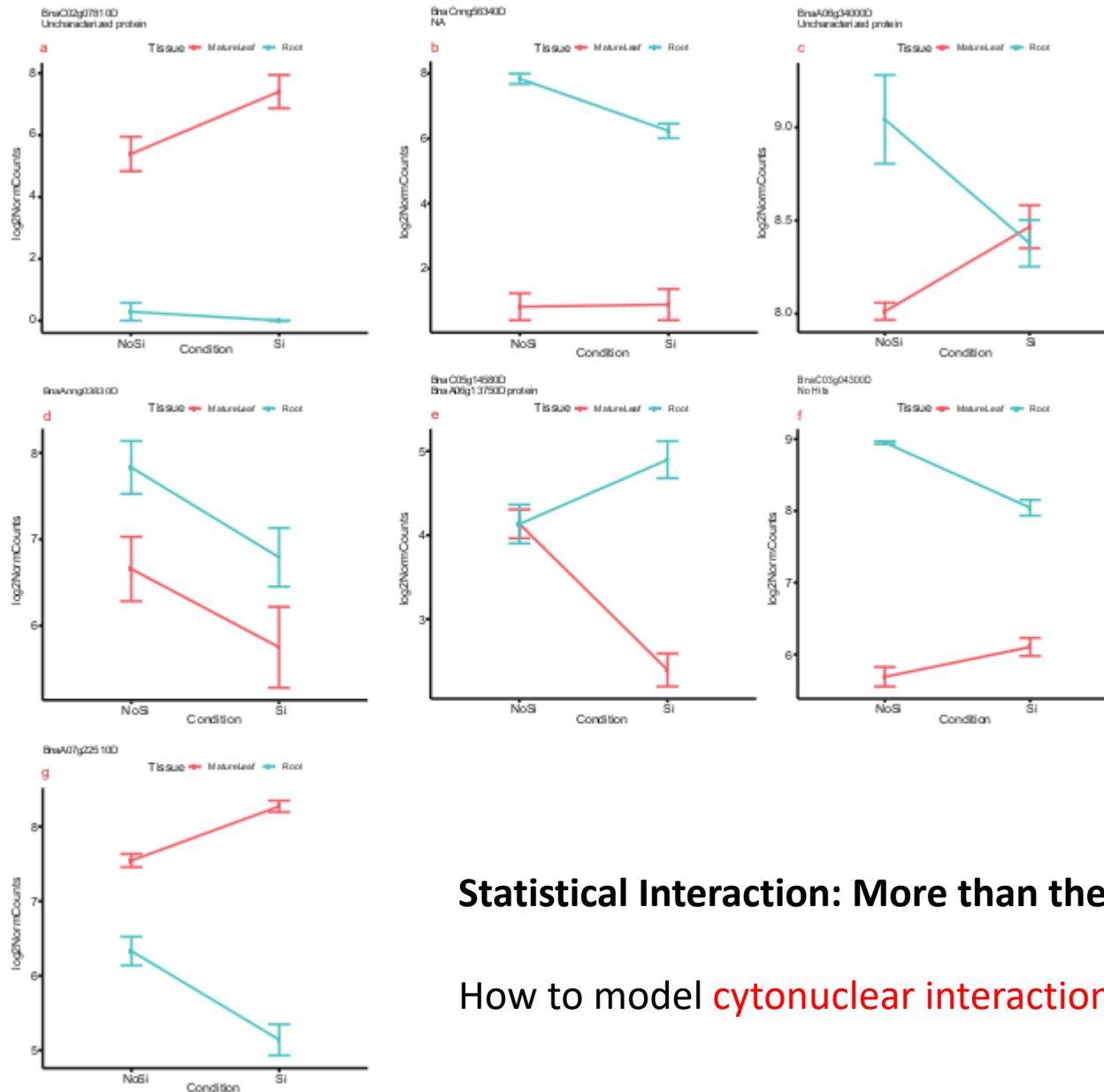
question

Does **a disruption of cytonuclear coadaptation** modify the molecular response of plants to a nutrition stress?

Several formulations of the SAME question

Do the nucleus and the cytoplasm interact together ?

# What is an interaction ?



**Statistical Interaction: More than the Sum of its Parts**

How to model **cytonuclear interactions** in the three datasets ?

# Phenotyping of cytolines in the field

Roux et al, 2016

27 quantitative traits

+

1 qualitative trait

Germination (5)

Survival trait (1)

Resource acquisition (3)

Phenology (4)

Architecture and seed dispersal (5)

Fecundity (10)



5 Blocks

9 arrays /block

1 array = 11L x 6C

$$Y_{bncij} = \mu + \alpha_b + \beta_n + \gamma_c + (\alpha\beta)_{bn} + (\beta\gamma)_{nc} + L_{i(b)} + C_{j(b)} + E_{bncij}$$



Random effects within the block  
25

Gaussian error  


# Phenotyping of cytolines in the field

Roux et al, 2016

$$Y_{bncij} = \mu + \alpha_b + \beta_n + \gamma_c + (\alpha\beta)_{bn} + (\beta\gamma)_{nc} + L_{i(b)} + C_{j(b)} + E_{bncij}$$

What traits are under a **genetic effect** ?

What traits are under a **cytoplasmic effect** ?

Are adaptive traits under the **influence of cytonuclear co adaptation?**

For (k,l) a couple of parents, we tested

$$[(\beta\gamma)_{kl} - (\beta\gamma)_{kk}] - [(\beta\gamma)_{lk} - (\beta\gamma)_{ll}] = 0 \quad \text{versus} \quad [(\beta\gamma)_{kl} - (\beta\gamma)_{kk}] - [(\beta\gamma)_{lk} - (\beta\gamma)_{ll}] \neq 0$$

Global FDR control on all the pairs (k, l) and all the traits at 5%

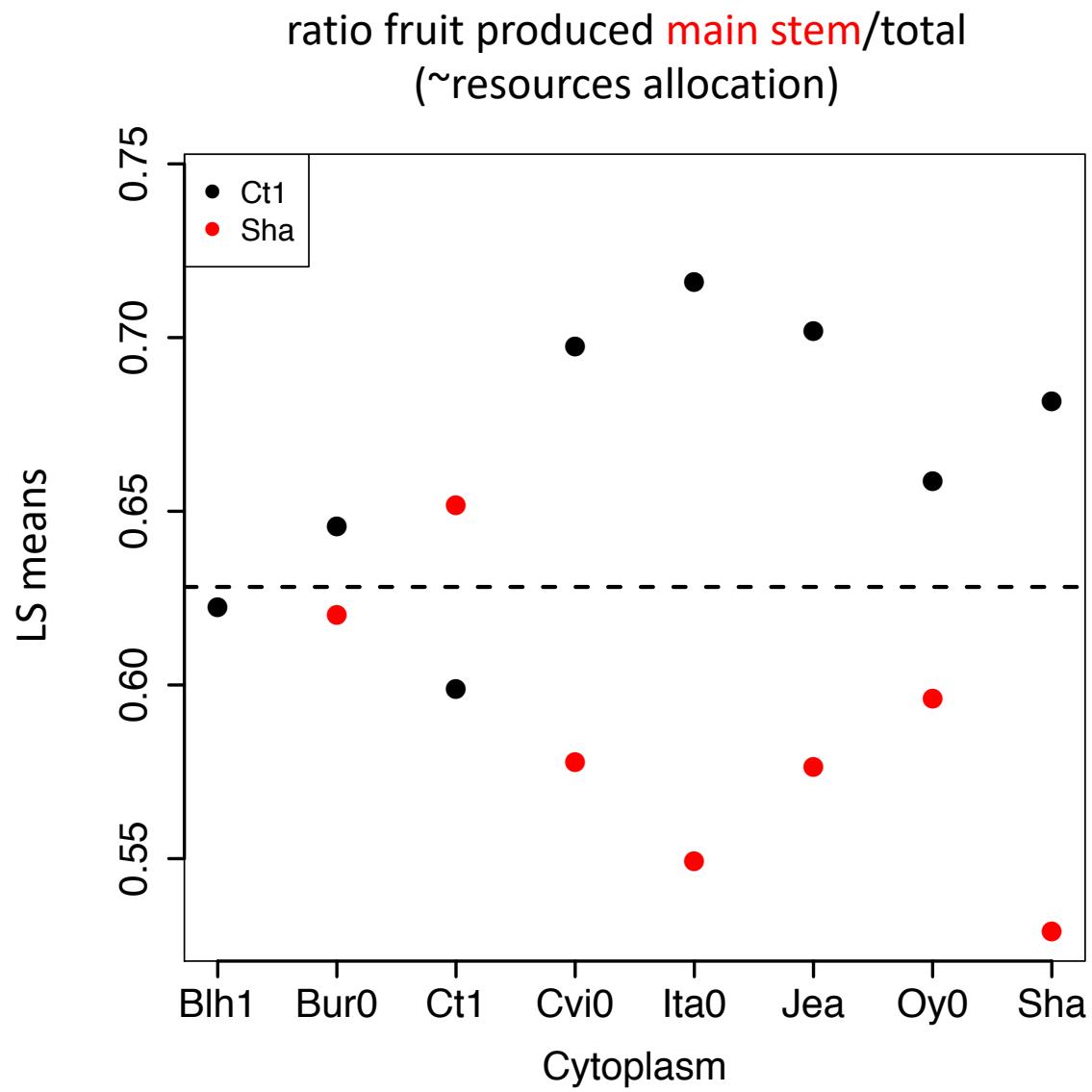
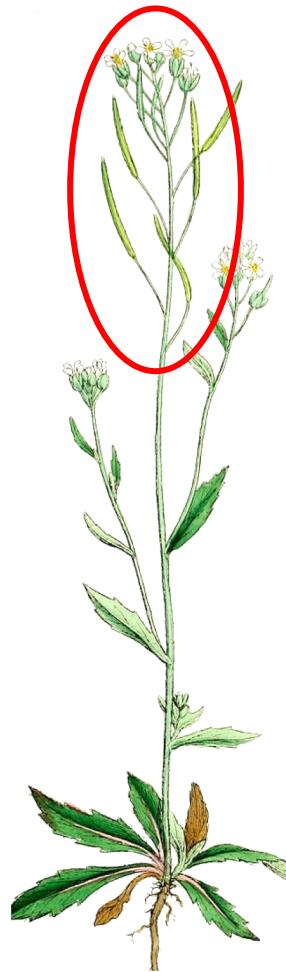
# Global effects of nucleus, cytoplasm, and cytonuclear interactions on phenotype

| Phenotypic class  | Model terms |     |         |     |           |     |                     |     |                 |     |                                 |  |
|---|-------------|-----|---------|-----|-----------|-----|---------------------|-----|-----------------|-----|---------------------------------|--|
|   | Block       |     | Nucleus |     | Cytoplasm |     | Cytoplasm × nucleus |     | Block × nucleus |     | Variance structure <sup>†</sup> |  |
|   | F           | P   | F       | P   | F         | P   | F                   | P   | F               | P   |                                 |  |
| Germination   |             |     |         |     |           |     |                     |     |                 |     |                                 |  |
| Germination time  | 18.05       | *** | 96.46   | *** | 0.55      | NS  | 2.94                | *** | 5.36            | *** | hmg                             |  |
| Germination percentage 4 das                                      | 37.24       | *** | 221.52  | *** | 0.86      | NS  | 1.72                | **  | 4.02            | *** | hmg                             |  |
| Germination percentage 5 das                                      | 5.67        | *** | 127.64  | *** | 2.11      | NS  | 2.63                | *** | 2.25            | *** | htg                             |  |
| Germination percentage 6 das                                      | 3.04        | *   | 125.78  | *** | 1.3       | NS  | 2.69                | *** | 3.69            | *** | hmg                             |  |
| Germination percentage 13 das                                     | 15.46       | *** | 52.55   | *** | 4.65      | *** | 1.7                 | **  | 2.53            | *** | htg                             |  |
| Resource acquisition  |             |     |         |     |           |     |                     |     |                 |     |                                 |  |
| Rosette surface area 28 das                                       | 11.45       | *** | 259.35  | *** | 5.29      | *** | 2.8                 | *** | 1.82            | *   | hmg                             |  |
| Rosette perimeter 28 das  | 8.75        | *** | 111.98  | *** | 3.54      | **  | 1.62                | **  | 2.34            | *** | htg                             |  |
| Rosette diameter at flowering                                     | 3.57        | *   | 33.93   | *** | 2.59      | *   | 1.62                | **  | 1.83            | *   | hmg                             |  |
| Phenology   |             |     |         |     |           |     |                     |     |                 |     |                                 |  |
| Bolting time  | 1.75        | NS  | 2648.29 | *** | 3.1       | *   | 2.53                | *** | 2.8             | *** | htg                             |  |
| Flowering interval  | 2.38        | NS  | 57.59   | *** | 0.96      | NS  | 1.55                | *   | 2.98            | *** | htg                             |  |
| Reproductive period   | 5.65        | **  | 32.1    | *** | 1.09      | NS  | 2.75                | *** | 1.88            | *   | htg                             |  |
| Length of life cycle  | 12.7        | *** | 273.23  | *** | 5.21      | *** | 5.43                | *** | 5.02            | *** | htg                             |  |
| Architecture and seed dispersal                                   |             |     |         |     |           |     |                     |     |                 |     |                                 |  |
| Height from soil to the first fruit on the main stem <sup>‡</sup> | 14.29       | *** | 227.55  | *** | 8.22      | *** | 3.94                | *** | 3.07            | *   | htg                             |  |
| Maximum height <sup>‡</sup>                                       | 11.95       | *** | 101.59  | *** | 7.08      | *** | 4.11                | *** | 2.38            | *   | htg                             |  |
| Number of basal branches <sup>‡</sup>                             | 0.28        | NS  | 9.01    | *** | 1.11      | NS  | 0.67                | NS  | 0.56            | NS  | hmg                             |  |
| Number of primary branches <sup>‡</sup>                           | 0.78        | NS  | 681.29  | *** | 1.68      | NS  | 1.87                | **  | 1.88            | NS  | htg                             |  |
| Total number of branches <sup>‡</sup>                             | 0.98        | NS  | 491.58  | *** | 1.71      | NS  | 1.74                | **  | 1.77            | NS  | htg                             |  |
| Fecundity   |             |     |         |     |           |     |                     |     |                 |     |                                 |  |
| Total fruit length = proxy of total seed production <sup>‡</sup>  | 4.36        | **  | 54.04   | *** | 0.54      | NS  | 0.77                | NS  | 1.04            | NS  | htg                             |  |
| Total fruit length on the main stem <sup>‡</sup>                  | 0.7         | NS  | 124.16  | *** | 1.22      | NS  | 0.92                | NS  | 0.95            | NS  | htg                             |  |
| Fruit number on the main stem <sup>‡</sup>                        | 1.6         | NS  | 124.64  | *** | 1.32      | NS  | 0.96                | NS  | 1.02            | NS  | htg                             |  |
| Mean fruit length on the main stem <sup>‡</sup>                   | 0.41        | NS  | 210.03  | *** | 1.28      | NS  | 4.34                | *** | 2.07            | NS  | htg                             |  |
| Total fruit length on primary branches <sup>‡</sup>               | 3.27        | *   | 29.29   | *** | 0.51      | NS  | 0.99                | NS  | 1.25            | NS  | htg                             |  |
| Fruit number on primary branches <sup>‡</sup>                     | 13.14       | *** | 70.08   | *** | 1.79      | NS  | 1.87                | **  | 2.42            | *** | htg                             |  |
| Mean fruit length on primary branches <sup>‡</sup>                | 3.8         | **  | 95.28   | *** | 0.85      | NS  | 3.76                | *** | 1.56            | NS  | htg                             |  |
| Ratio of seeds produced on the main stem <sup>‡</sup>             | 3.84        | **  | 31.76   | *** | 2.33      | NS  | 1.6                 | *   | 1.8             | *   | hmg                             |  |
| Ratio of seeds produced on primary branches <sup>‡</sup>          | 3.62        | *   | 29.91   | *** | 2.26      | NS  | 1.85                | **  | 1.61            | *   | hmg                             |  |
| Percentage of aborted fruit <sup>‡</sup>                          | 4.63        | NS  | 85.51   | *** | 1.64      | NS  | 10.36               | *** | 1.61            | NS  | htg                             |  |
| Survival  | 91.55       | *** | 69.12   | *** | 20.95     | *** | 29.51               | *** | 10.03           | *** | hmg                             |  |

# Majority of adaptive traits are influenced by cytonuclear interactions

| Traits                              | Nucleus effect | Cytoplasm effect | Cyto x nuc effect |
|-------------------------------------|----------------|------------------|-------------------|
| Germination (5)                     | 5              | 1                | 5                 |
| Resource acquisition (3)            | 3              | 3                | 3                 |
| Phenology (4)                       | 4              | 2                | 4                 |
| Architecture and seed dispersal (5) | 5              | 2                | 4                 |
| Fecundity (10)                      | 10             | 0                | 6                 |
| Survival (1)                        | 1              | 1                | 1                 |

# Sha and Ct-1 nuclear alleles have contrasted phenotype outputs in alien cytoplasmic backgrounds

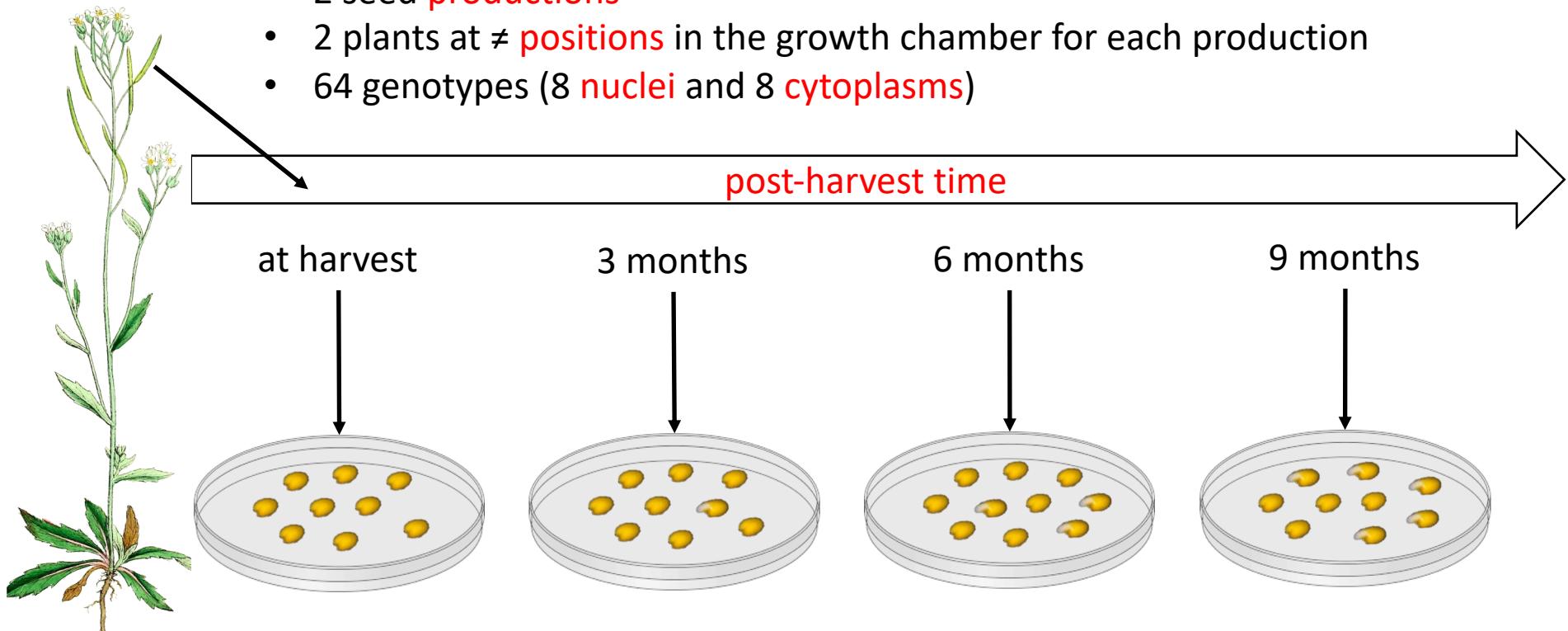


# Phenotyping of seed traits: dormancy

Boussardon et al, 2019

Response variable: percentage of germinated seeds after 96h

- 2 seed **productions**
- 2 plants at  $\neq$  **positions** in the growth chamber for each production
- 64 genotypes (8 **nuclei** and 8 **cytoplasms**)



seeds sowed in vitro and incubated at 15°C or 25°C

# Statistical analysis of seed dormancy

Large discrepancy observed between post-harvest times  
-> analysis per post-harvest time

Large number of missing data for some genotypes  
-> incomplete and unbalanced design

Five explicative factors (shelf, nucleus, cytoplasm, temperature)  
-> starting from the most complete model, nested models were sequentially fitted and the best one according to BIC was selected.

Then the contrasts relevant to the addressed question were tested  
p-values were adjusted using the Bonferroni procedure to control the family-wise error rate (FWER) at 5%.

# Statistical analysis for a post-harvest time of 3 months

The selected model was the one with all the first and second order terms plus 4 third order interactions

- shelf x cytoplasm x nucleus (SCN)
- harvest x cytoplasm x nucleus (HCN)
- shelf x harvest x nucleus (SHN)
- harvest x temperature x nucleus (HTN)

Effect of each foreign cytoplasm ( $c$ ) in each natural accession nuclear background ( $c'$ ,  $n'$ ) was tested with the null hypothesis using the contrast

$$\begin{aligned} & (Cc - Cc') + (Cn' - Cn'c') \\ & + 1/2 \sum t \text{ in } \{1,2\} (Tc tc - Tc tc') \\ & + 1/2 \sum h \text{ in } \{1,2\} (Hc hc - Hc hc') \\ & + 1/2 \sum h \text{ in } \{1,2\} (HCN hcn' - HC hc'n') \\ & + 1/2 \sum s \text{ in } \{1,2\} (Sc sc - Sc sc') \\ & + 1/2 \sum S \text{ in } \{1,2\} (SCN hcn' - SC hc'n') \end{aligned}$$

Hence the cytoplasm effects in each nuclear background should be interpreted as averaged on the germination temperatures, the harvests and the shelves.

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Effect of each foreign cytoplasm (c) in each natural accession nuclear background (c', n') was tested with the null hypothesis using the contrast

$$\begin{aligned} & (Cc - Cc') + (Cn' - Cn)c' \\ & + 1/2 \sum t \text{ in } \{1,2\} (Tc tc - Tc tc') \\ & + 1/2 \sum h \text{ in } \{1,2\} (Hc hc - Hc hc') \\ & + 1/2 \sum h \text{ in } \{1,2\} (HCN hcn' - HC hc'n') \\ & + 1/2 \sum s \text{ in } \{1,2\} (Sc sc - Sc sc') \\ & + 1/2 \sum S \text{ in } \{1,2\} (SCN hcn' - SC hc'n') \end{aligned}$$

Hence the cytoplasm effects in each nuclear background should be interpreted as **averaged on the germination temperatures, the harvests and the shelves.**

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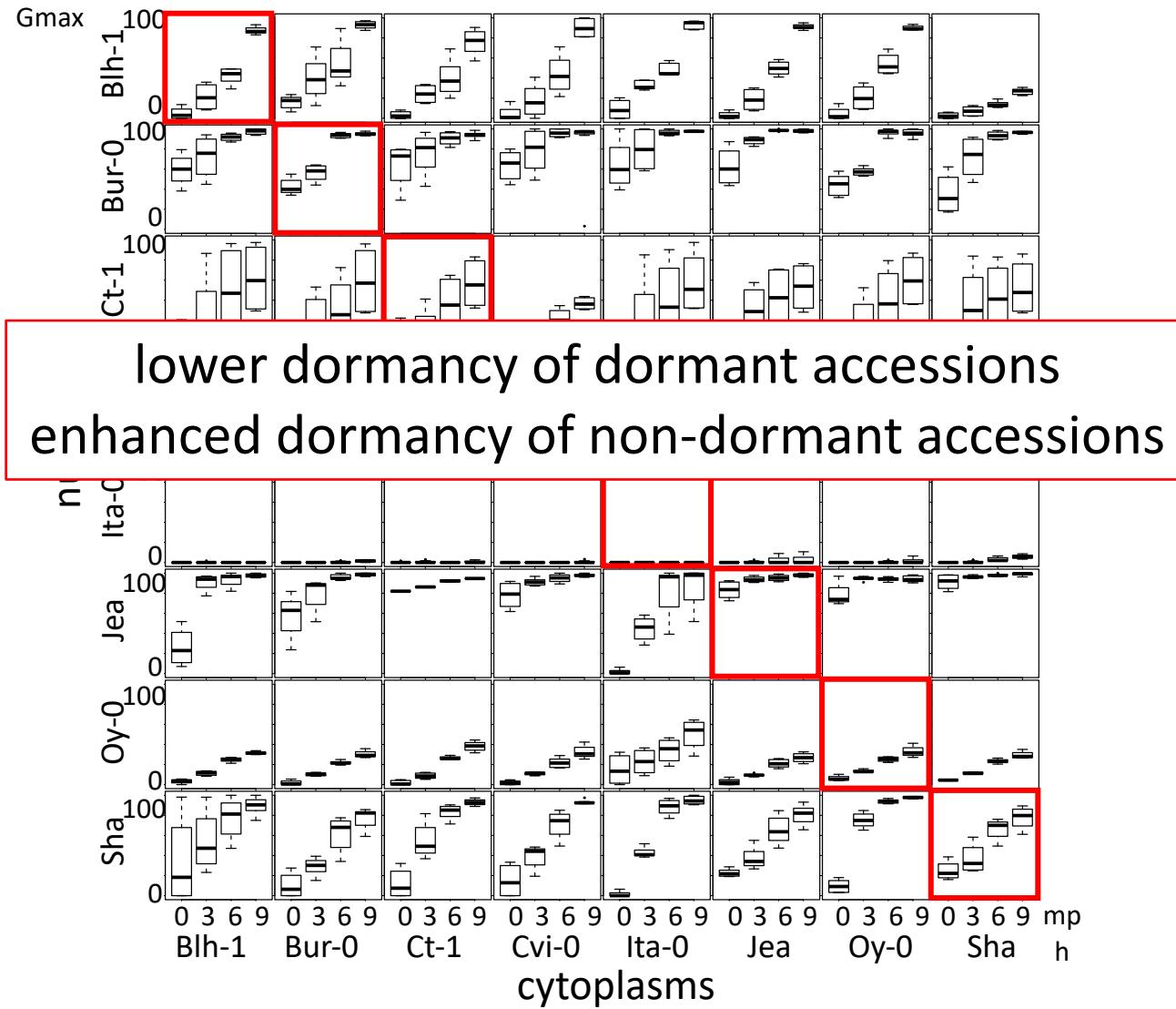
- shelf x cytoplasm x nucleus (SCN)
- harvest x cytoplasm x nucleus (HCN)
- shelf x harvest x nucleus (SHN)
- harvest x temperature x nucleus (HTN)

An effect of the cytonuclear interacting combination was tested for all pairs of cytoplasms ( $c, c'$ ) and for all pairs of nuclei ( $n, n'$ ).

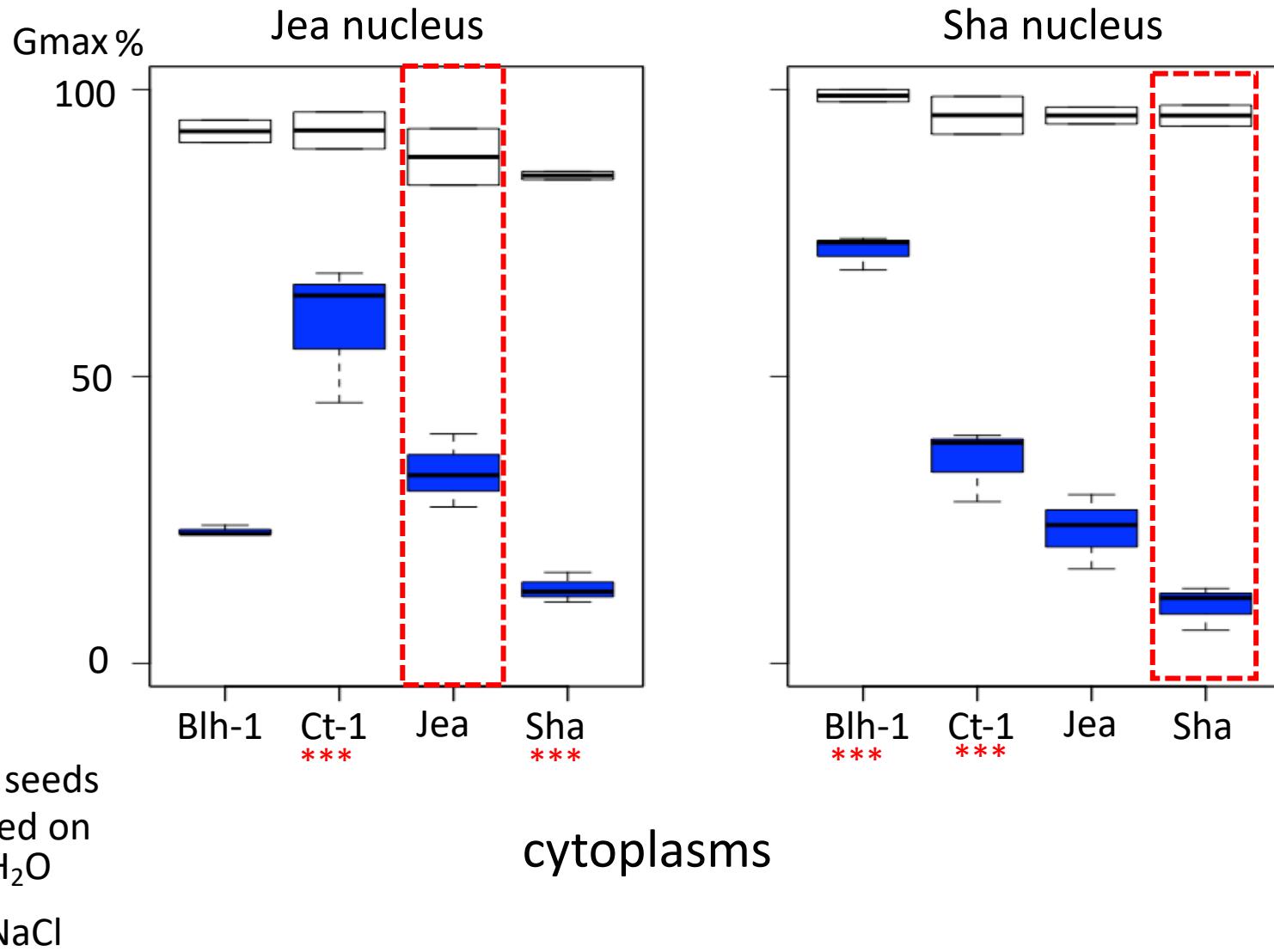
As no third order interaction involving both nucleus and cytoplasm was included in the selected model, cytonuclear interacting combinations could be tested **independently from other model terms**.

$$H_0 \{ (CN cn - CN c'n) - (CN cn' - CN c'n') = 0 \}$$

# novel cytonuclear combinations can modify dormancy depth and release

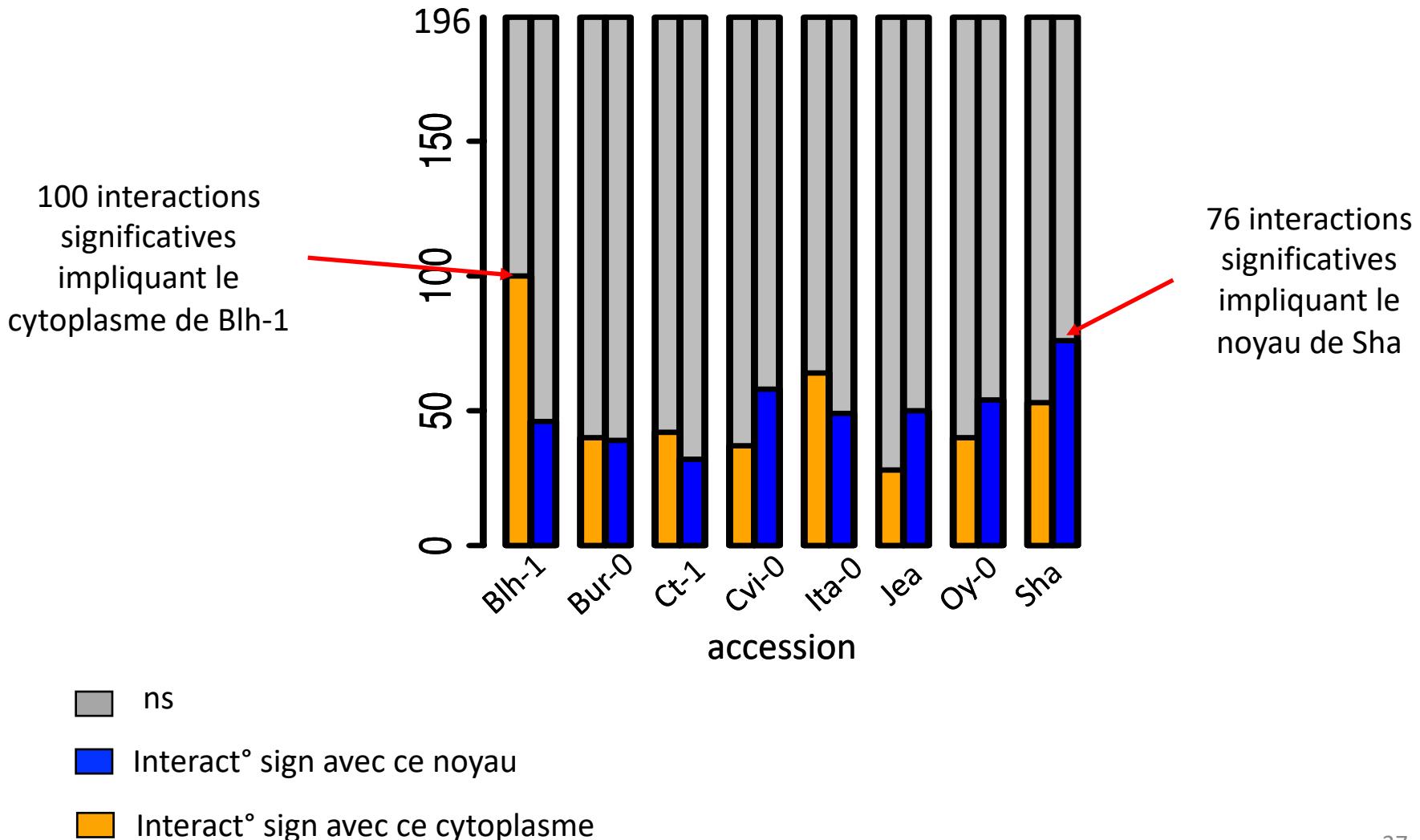


# novel cytonuclear combinations can modify germination performance



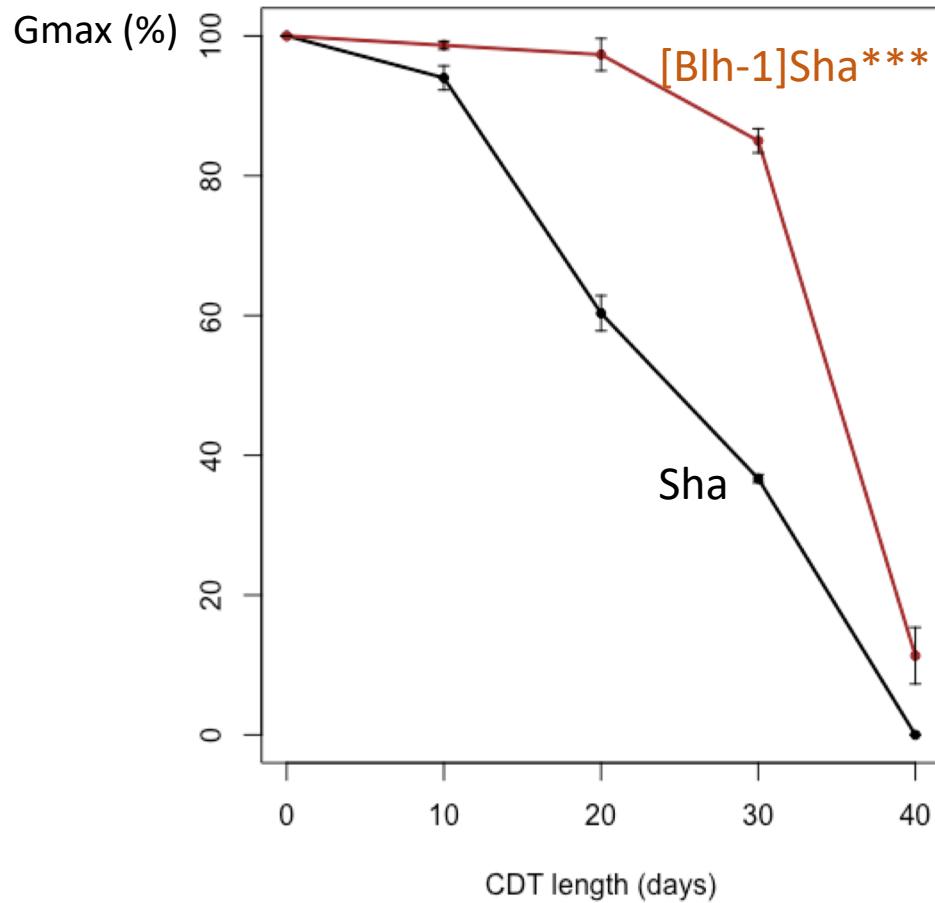
# Diversité du nombre d'interactions significatives impliquant chaque fonds nucléaire et chaque fonds cytoplasmique

Chaque noyau et chaque cytoplasme est testé dans 196 combinaisons

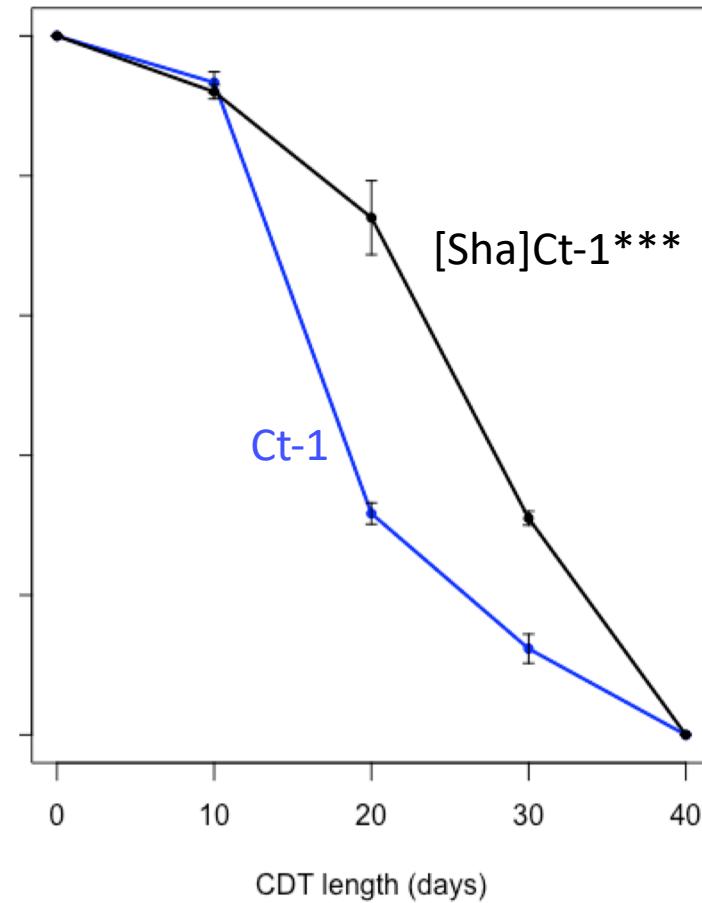


# new cytonuclear combination can enhance seed longevity

Nucleus Sha



Nucleus Ct-1



# Molecular phenotyping in two nitrogen nutrition conditions

Chardon et al, 2020

nuclear transcriptome  
microarray hybridizations  
26884 nuc genes

organellar transcriptomes  
qRT-PCR  
31 mt & 80 cp genes

proteome  
LC MS/MS  
665 proteins

metabolome  
GC-TOF-MS  
81 metabolites

- Specific pre-processing
- Normalisation step to remove technical biaises
- Signal analysis to answer the biological questions with an ANOVA
- p-values were adjusted to control FDR at 5%
- The scripts and datasets used are available as a git project [https://forgemia.inra.fr/GNet/cytopheno\\_omics](https://forgemia.inra.fr/GNet/cytopheno_omics)

**The main task was to translate the biological questions into contrasts**

# Molecular phenotyping in two nitrogen nutrition conditions

Chardon et al, 2020

Each type of accumulation was modeled with a three-way ANOVA

$$Y_{ijkr} = \mu + C_i + N_j + A_k + CN_{ij} + CA_{ik} + NA_{jk} + CNA_{ijk} + E_{ijkr}$$

**the nitrogen supply across both cytoplasms and both nuclei,**

mean(all genotypes in nitrogen starvation) – mean(all genotypes in control nitrogen supply).

**the nucleus origin across both nitrogen supplies and both cytoplasms**

mean(genotypes with Jea nucleus in both nitrogen conditions) – mean(genotypes with Ct-1 nucleus in both nitrogen conditions)

**a cytoplasm × nucleus interaction effect across both nitrogen supplies**

mean(both cytolines in both nitrogen conditions) –

mean(both parental lines in both nitrogen conditions).

**a cytoplasm × nuclear × nitrogen interaction effect.**

[mean(**both parental lines in nitrogen starvation**) – mean(**both parental lines in control nitrogen supply**)] – [mean(**both cytolines in nitrogen starvation**) – mean(**both cytolines in control nitrogen supply**)].

$$E(Y_{\text{cnt}}) = \mu + C_C + N_{nT} + T_T + (CN)_{cn} + (CT)_{ct} + (NT)_{nt} + (CNT)_{cnt}$$

avec  $\begin{cases} C_{CT} = 0 & N_{CT} = 0 & T_{NO} = 0 & (CN)_{ct,n} = 0 & (CT)_{ct,t} = 0 \\ c, ct = 0 & & & c, cn = 0 & (CT)_{c,no} = 0 \end{cases}$

contraintes d'identifiabilité.

$(NT)_{ct,t} = 0$        $(CNT) = 0$   
 $c, No = 0$        $c, cn, t$   
 $c, ct, t$   
 $c, n, No = 0$ .

Cela veut dire que le modèle à

$\mu$  = l'intercept = expression de Ct:Ct en condition NO

$C_{Jea} = (Jea:Ct)_{No} - (Ct:Ct)_{No}$  = la différence d'expression entre Jea:Ct et Ct:Ct en NO

$N_{Jea} = (Ct:Jea)_{No} - (Ct:Ct)_{No}$  = la différence d'expression entre Ct:Jea et Ct:Ct en NO

$T_{N4} = (Ct:Ct)_{N4} - (Ct:Ct)_{No}$  = la différence d'expression entre N4 et NO pour Ct:Ct

$(CN)_{Jea,Jea}$  = la différence d'expression entre (les parents) et (les cytolignées) à NO.

$(CT)_{Jea,N4}$  = la différence d'expression entre N4 et NO pour Jea:Ct et la  $\bar{m}$ ème différence pour Ct

$(NT)_{Jea,N4}$  = la différence d'expression entre N4 et NO pour Ct:Jea et la  $\bar{m}$ ème différence pour Ct

$(CNT)_{Jea,Jea,N4} = [(la \ somme \ des \ parents) - (la \ somme \ des \ cytolignées)] \ en \ N4 - [ ] \ en \ NO$

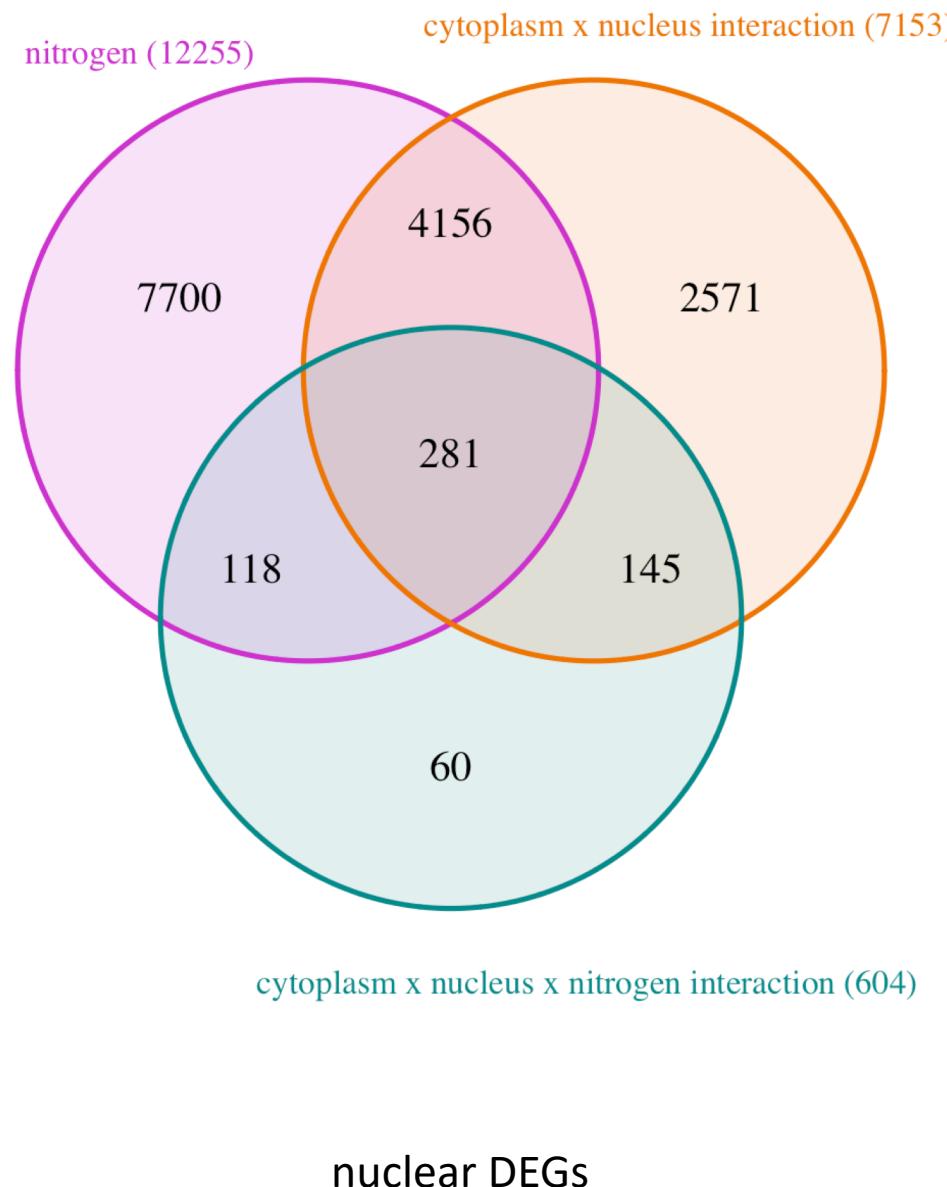
effet interact noyan x azote

$$\begin{aligned}
 & \left[ \frac{1}{2} \sum_{C \in \text{Sea, Ch}} Y_{C \text{ Sea No}} - \frac{1}{2} \sum_{C \in \text{Sea, Ch}} Y_{C \text{ Ch No}} \right] - \left[ \frac{1}{2} \sum_{C \in \text{Sea, Ch}} Y_{C \text{ Sea N4}} - \frac{1}{2} \sum_{C \in \text{Sea, Ch}} Y_{C \text{ Ch N4}} \right] \\
 & \left[ (CN)_{\text{Sea Sea}} + (CN)_{\text{Ch Sea}} - (CN)_{\text{Sea Ch}} - (CN)_{\text{Ch Ch}} \right] - \left[ (CN)_{\text{Sea Sea}} + (CN)_{\text{Ch Sea}} + (CN)_{\text{Sea Ch}} - (CN)_{\text{Ch Ch}} \right] \\
 & \left[ (CT)_{\text{Sea No}} + (CT)_{\text{Ch No}} - (CT)_{\text{Sea No}} - (CT)_{\text{Ch No}} \right] - \left[ \right. \\
 & \frac{1}{2} \left[ 2(NT)_{\text{Sea No}} - 2(NT)_{\text{Ch No}} \right] - \frac{1}{2} \left[ 2(NT)_{\text{Sea N4}} - 2(NT)_{\text{Ch N4}} \right] - \frac{1}{2} (CNT)_{\text{Sea Sea N4}} \\
 & = \left[ (NT)_{\text{Sea No}} - (NT)_{\text{Ch No}} \right] - \left[ (NT)_{\text{Sea N4}} - (NT)_{\text{Ch N4}} \right] - \frac{1}{2} (CNT)_{\text{Sea Sea N4}} \\
 & = - (NT)_{\text{Sea N4}} - \frac{1}{2} (CNT)_{\text{Sea Sea N4}} \\
 & = \boxed{(NT)_{\text{Sea No}} + \frac{1}{2} (CNT)_{\text{Sea Sea No}}}
 \end{aligned}$$

Interaction cyto x azote

$$\begin{aligned}
 & - (CT)_{\text{Sea N4}} - \frac{1}{2} (CNT)_{\text{Sea Sea N4}} \\
 & \boxed{(CT)_{\text{Sea No}} + \frac{1}{2} (CNT)_{\text{Sea Sea No}}}
 \end{aligned}$$

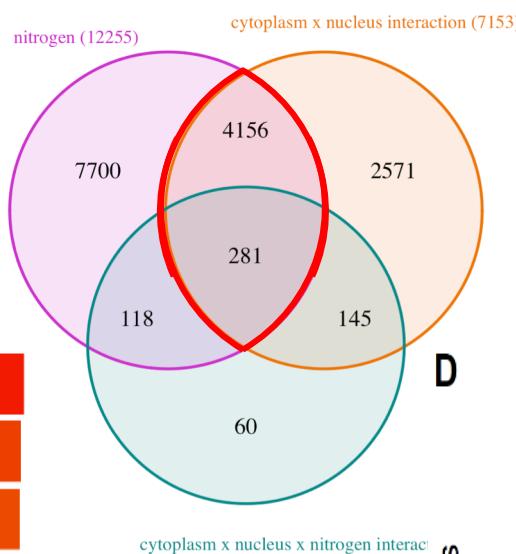
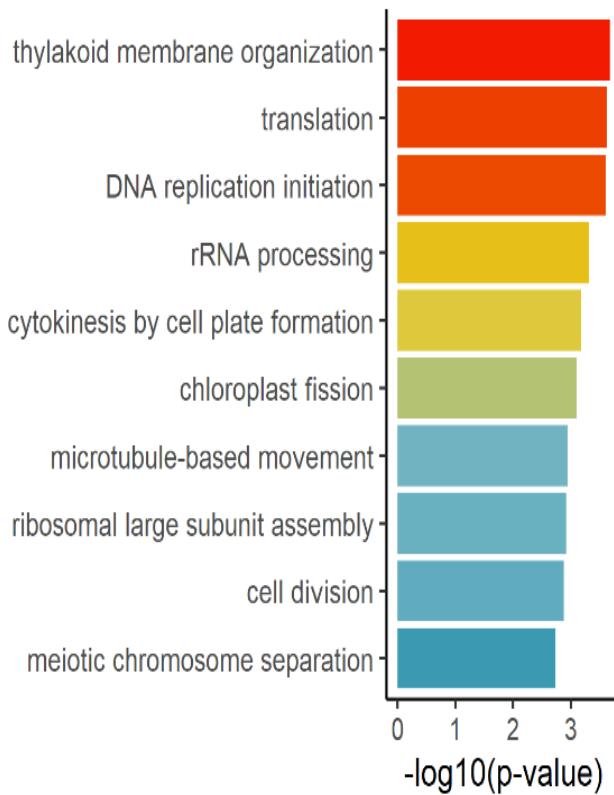
# cytolines have a sensible different molecular response to N starvation than their natural parents



# cytolines have a sensible different molecular response to N starvation than their natural parents

same effect of N starvation and disruption of coadaptation

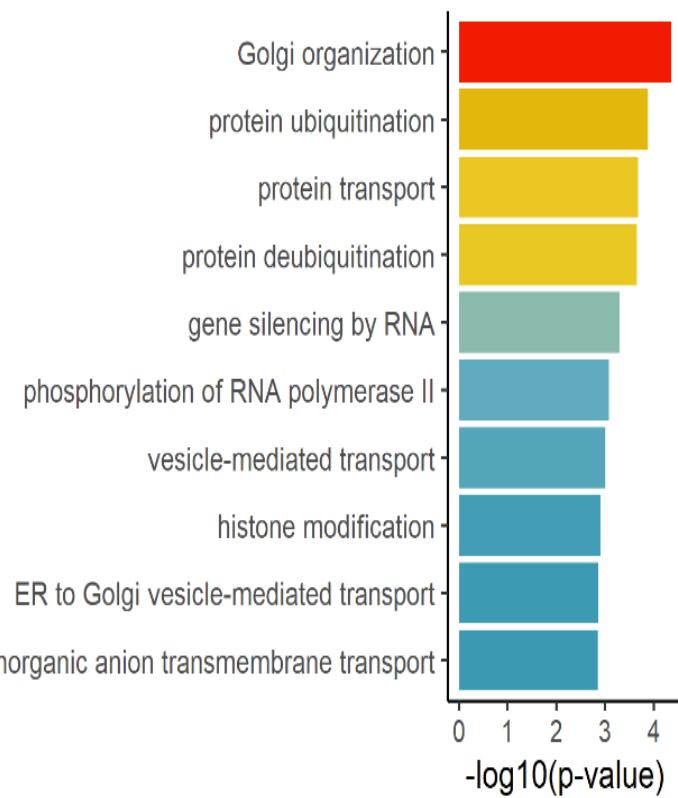
C



D

opposite effects of N starvation and disruption of coadaptation

Biological process



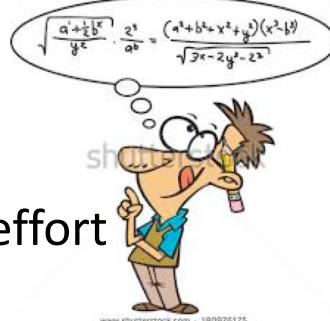
# general conclusions and take home messages

Interactions between nuclear and organellar genomes are worth considering

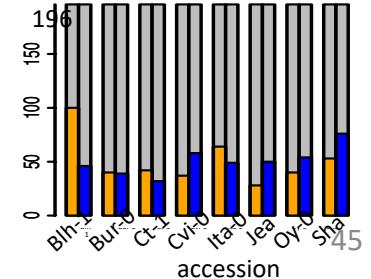
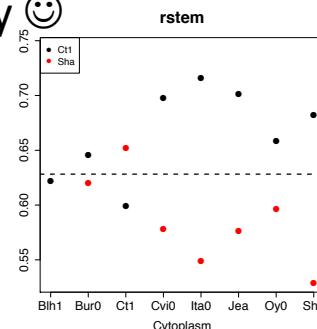
- ✓ Intraspecific variation in cytoplasm genomes is relevant for adaptive phenotypes
- ✓ Traits impacted by cytonuclear interactions are relevant to plant breeding
- ✓ New cytonuclear genetic combination may have improved performances



Interactions ... between biologists and statisticians are worth the effort



- importance of open and frequent exchanges -> improvement of mutual understanding
- improvement of the biologist's skills in statistical modeling ☺
- improvement of the statistician's skills in biology ☺
- unexpected outputs from statistical analyses



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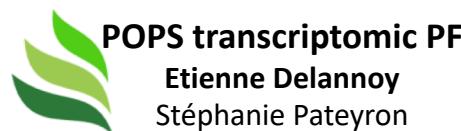
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