

Towards a molecular understanding of Aluminium genotoxicity for crop improvement

Lieven De Veylder - Lisbon 08-05-2014

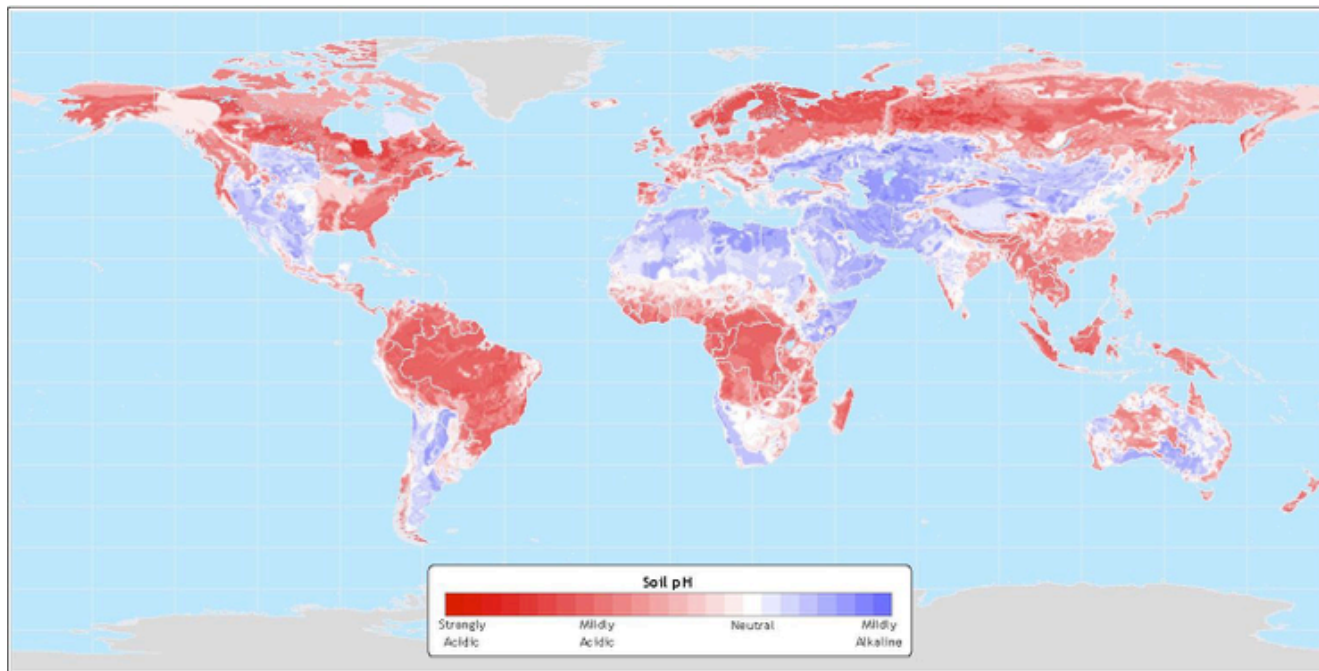


ERA-NET for Coordinating
Action in Plant Sciences



Al toxicity is a global problem

- Occurs in acid soil environments (pH < 5.5)
 - Upwards of 50% of arable land is acidic (shown in red)



- Al is the 3rd most abundant element and most abundant metal
- Al speciates to Al^{3+} in acidic conditions

Symptoms of Al toxicity

- 📌 Severe inhibition of root growth
- 📌 Biological targets include cell wall, PM, and nucleus
- 📌 Biochemical basis of Al-dependent root phenotype unknown



How do plants cope with Al?



Al resistance mechanisms:

- Effectively exclude Al from the root (release of organic acids, rhizosphere alkalization, ...)

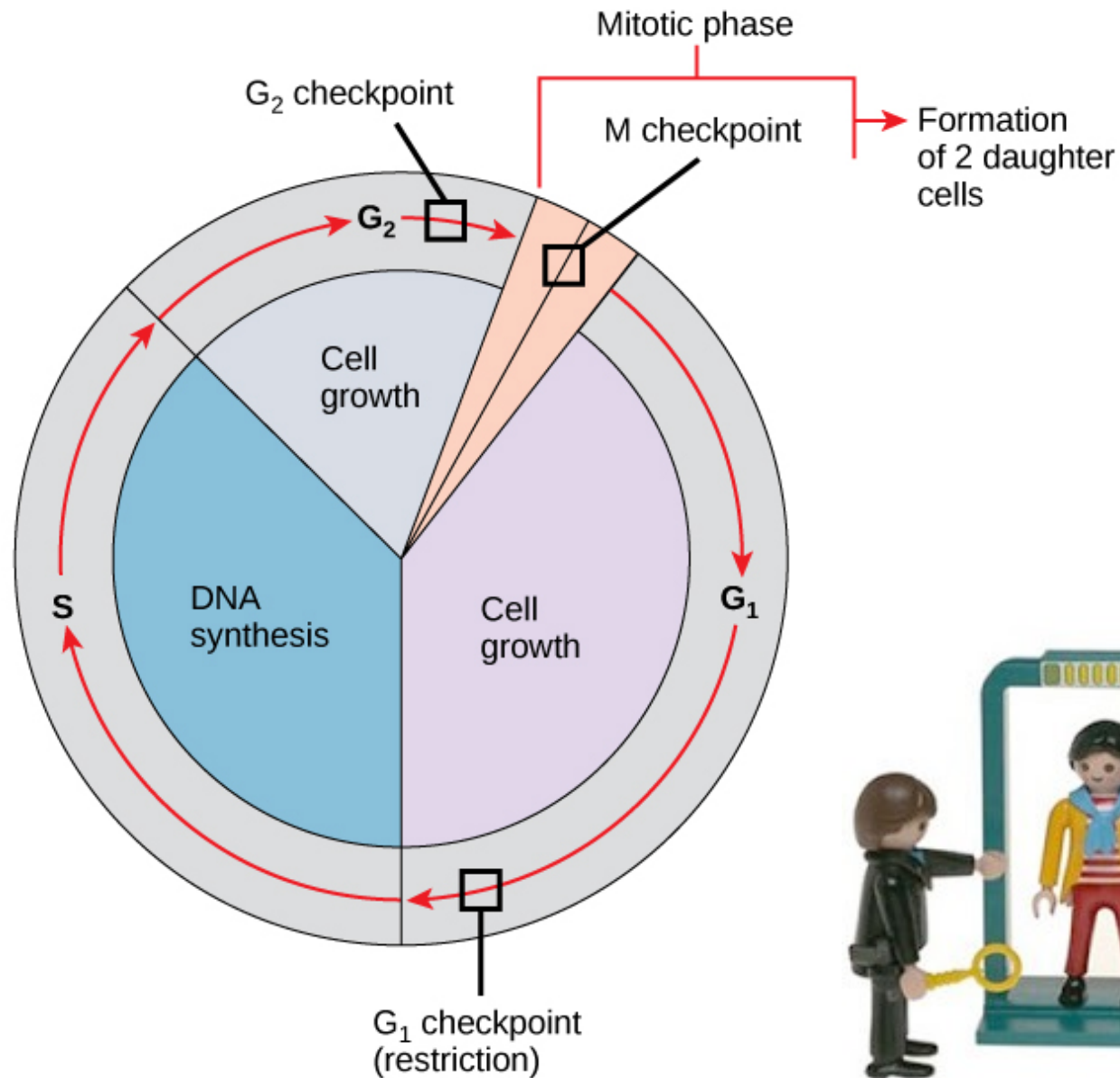


Al tolerance mechanisms:

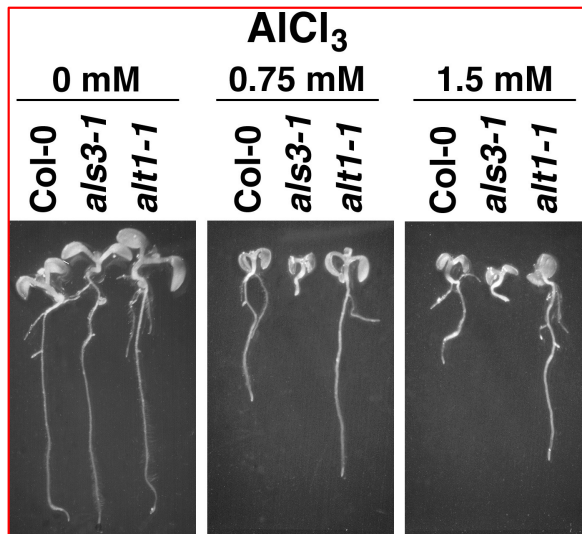
- Al tolerant species sequester internalized Al
- Other unidentified strategies

Current work shows that by short circuiting DNA damage checkpoint signaling cascades, Al tolerance can be conferred in Arabidopsis

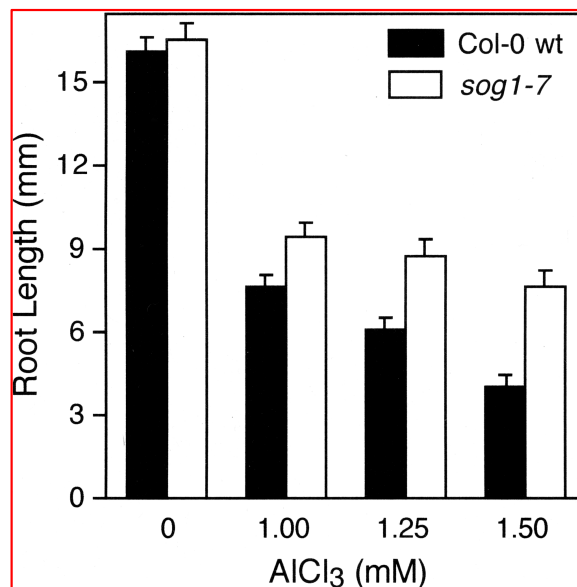
DNA checkpoint control safeguard cell integrity



Checkpoint mutants rescue Al hypersensitivity phenotype

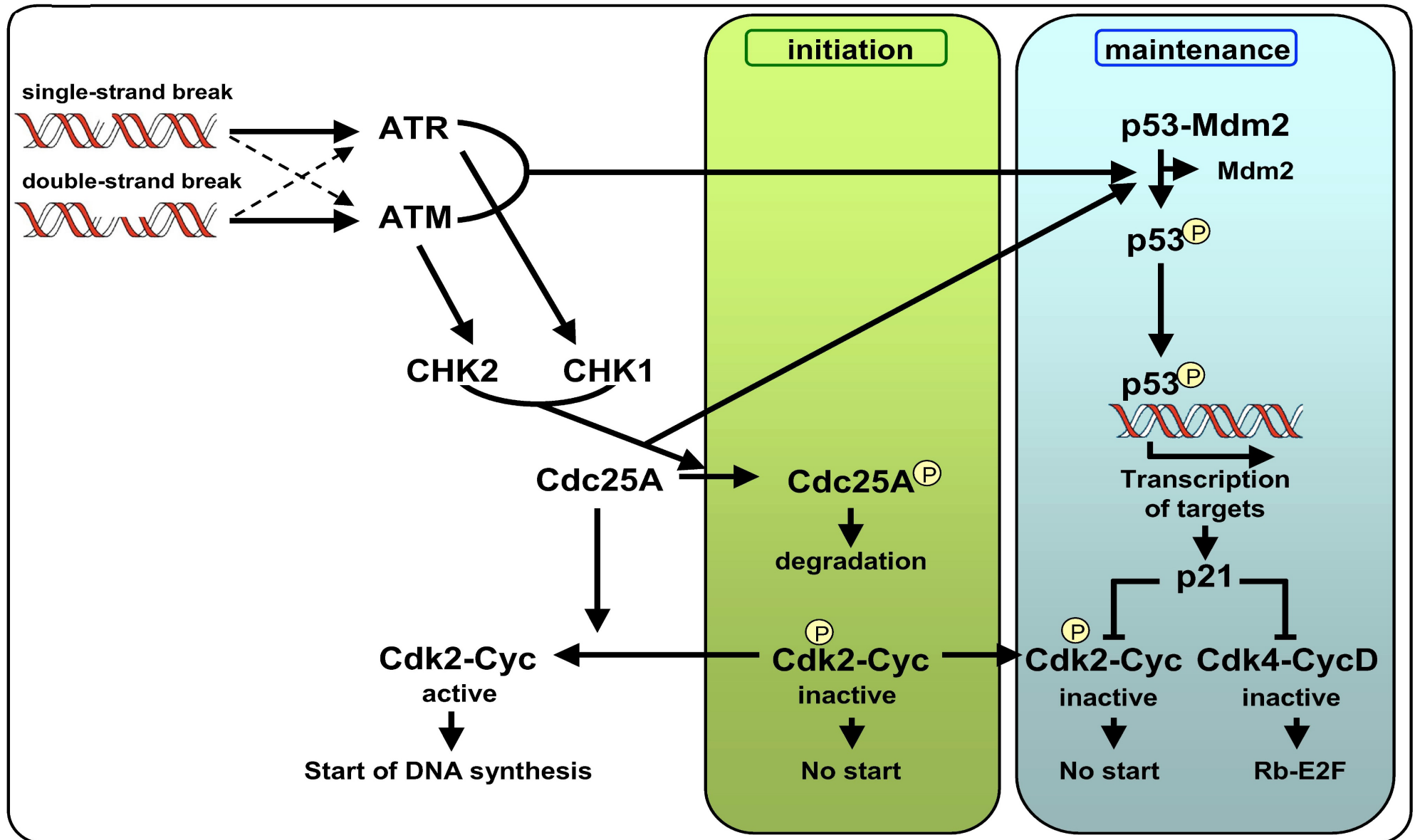


A suppressor mutagenesis screen looking for mutations that restore growth to an Al hypersensitive Arabidopsis mutant recovered 4 cell cycle checkpoint genes

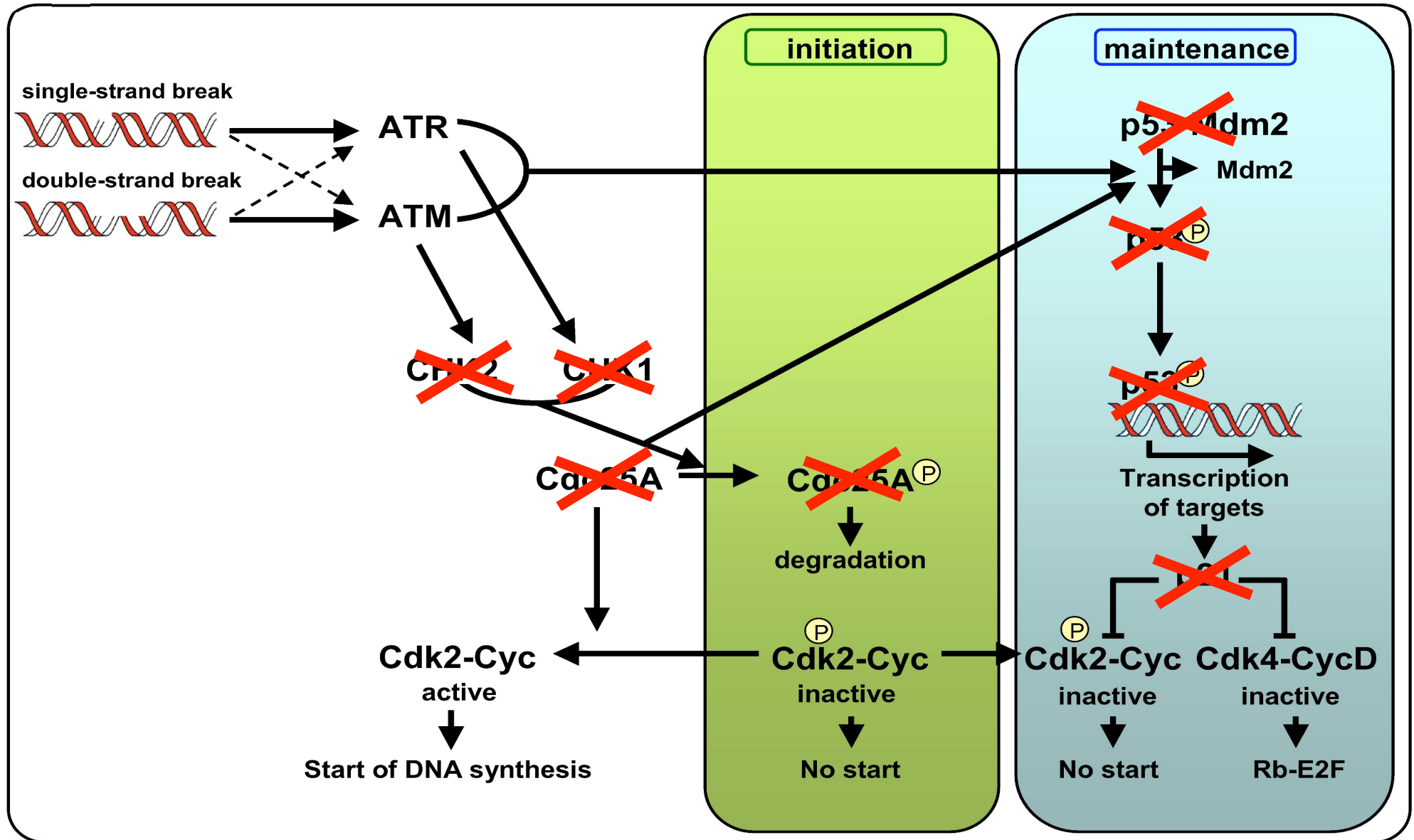


Al induces a cell cycle arrest that push cells into differentiation and consequently stoppage of root growth

Checkpoint control in mammals



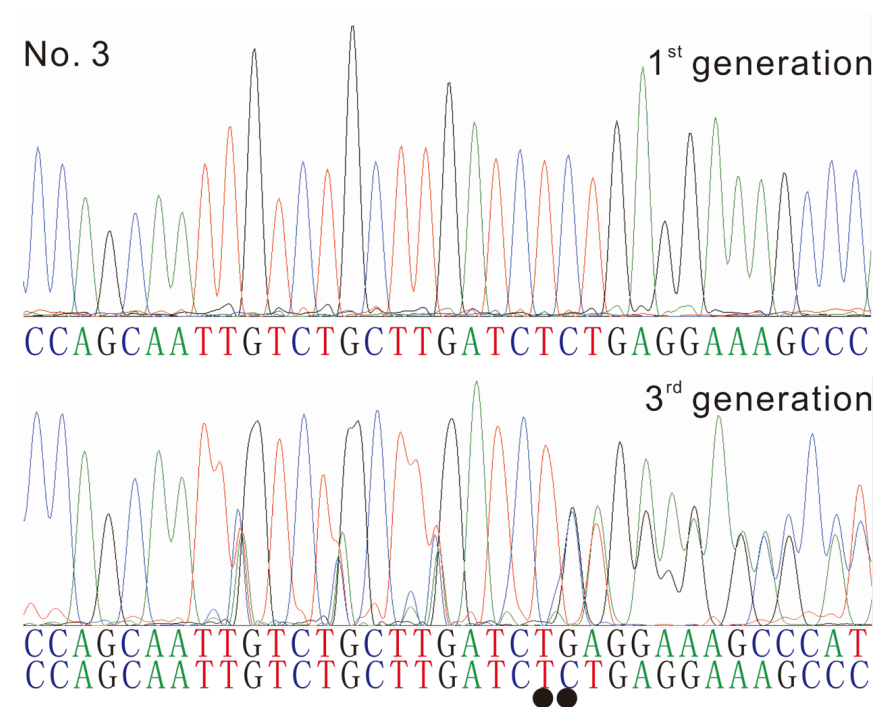
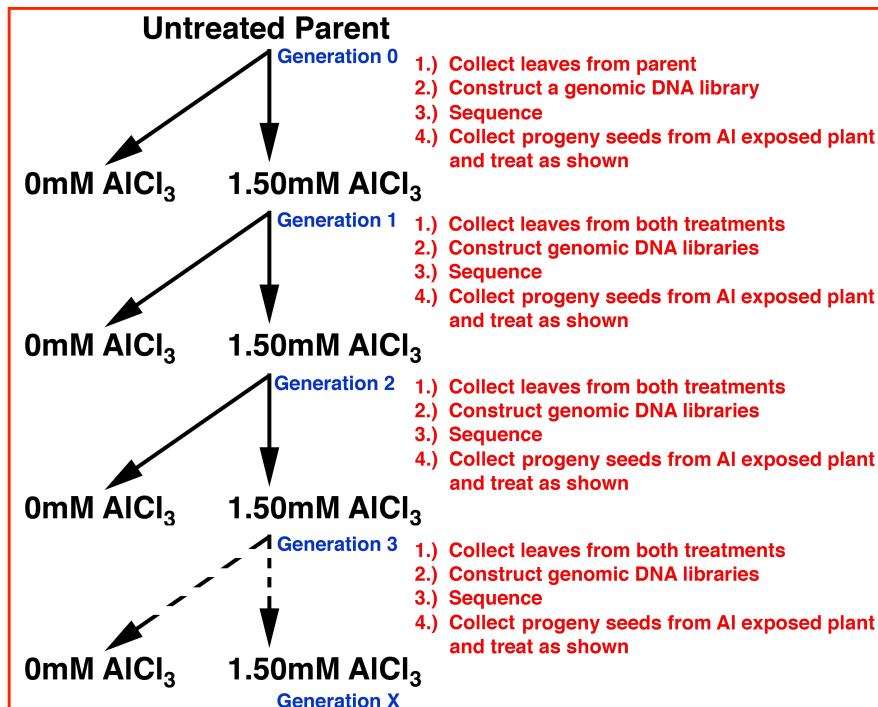
Checkpoint control in plants



AI-UCIDATE Objectives

- 📌 What exactly is AI doing at the level of DNA?
- 📌 Which molecular mechanisms underly the AI-dependent cell cycle checkpoint?
- 📌 Can AI checkpoint control knowledge be transferred to crop species?

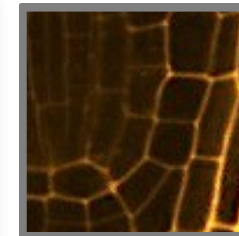
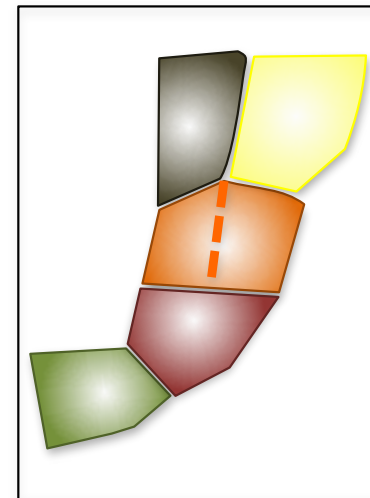
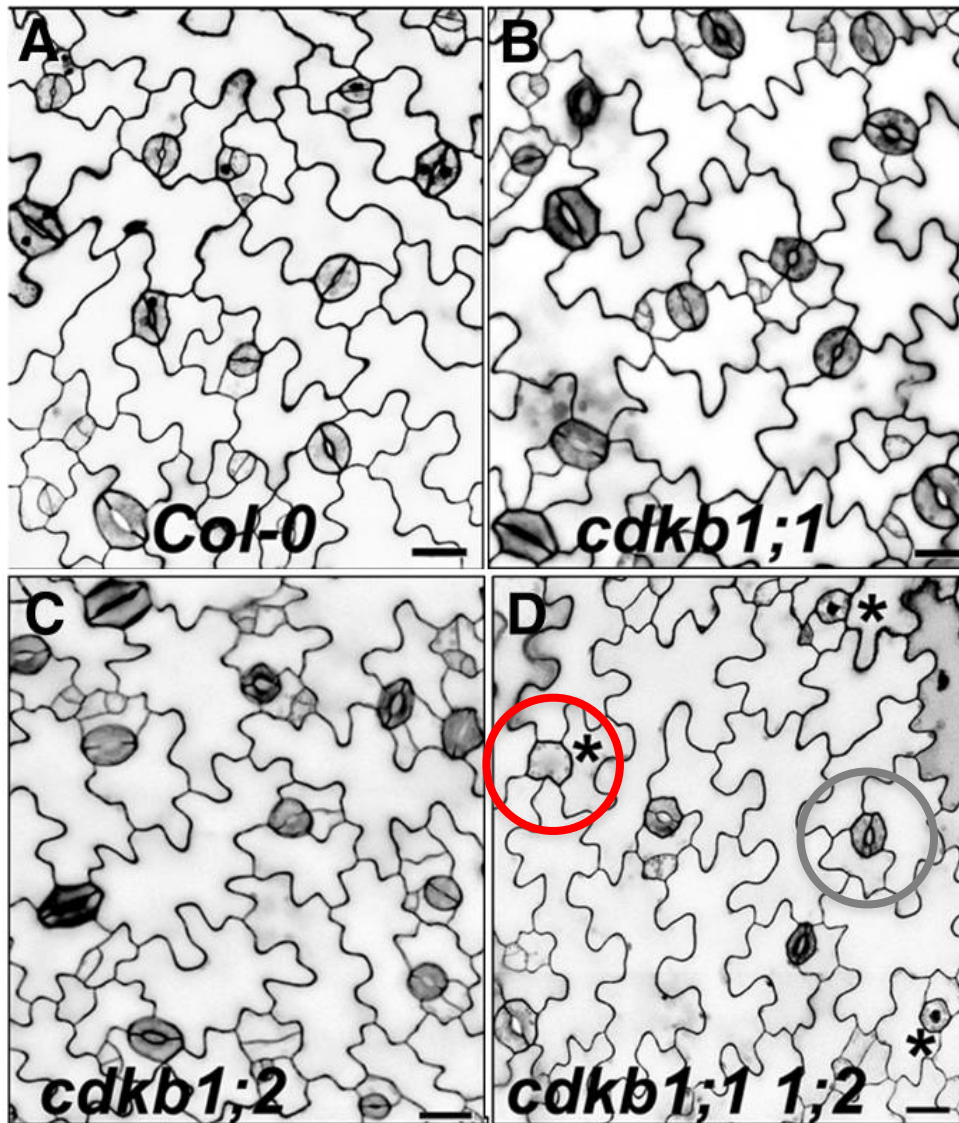
How does Al affects DNA?



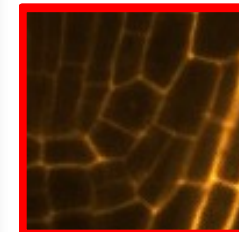
Which molecular mechanisms underly Al-dependent checkpoint activation?

- 📌 Comparative transcriptome analysis of Arabidopsis and Barley Al-stressed root meristems
- 📌 Phenotypic analysis of knockouts through kinematic growth measurements
- 📌 Identification of novel checkpoint regulators through use of yeast one-hybrid, tandem affinity purification, and CDKB mutant suppressor screen and phosphoproteomics

CDKB suppressor screen

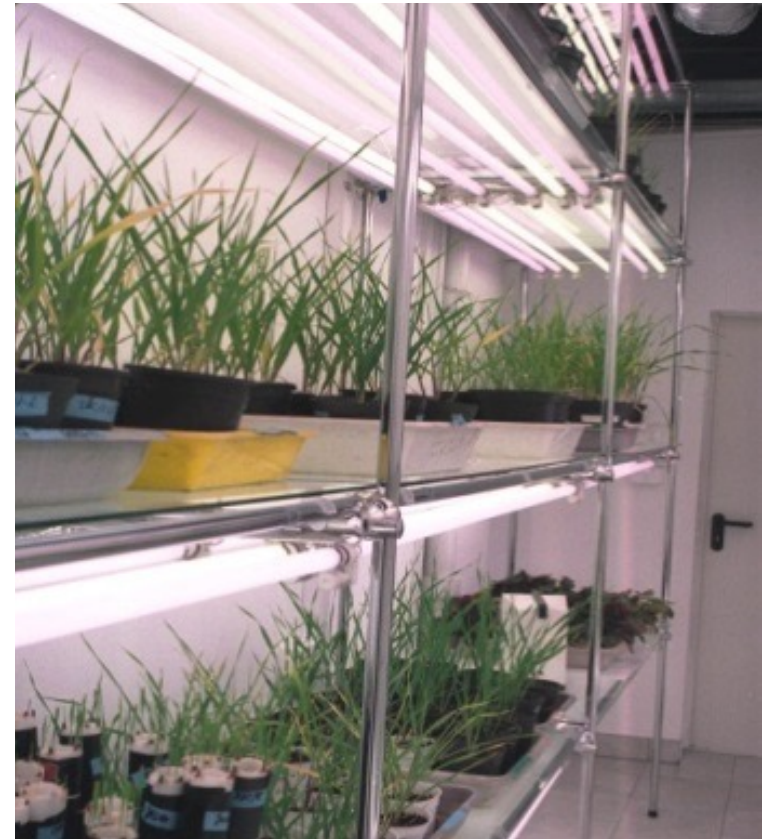


normal (fast)
cell division
of CEID

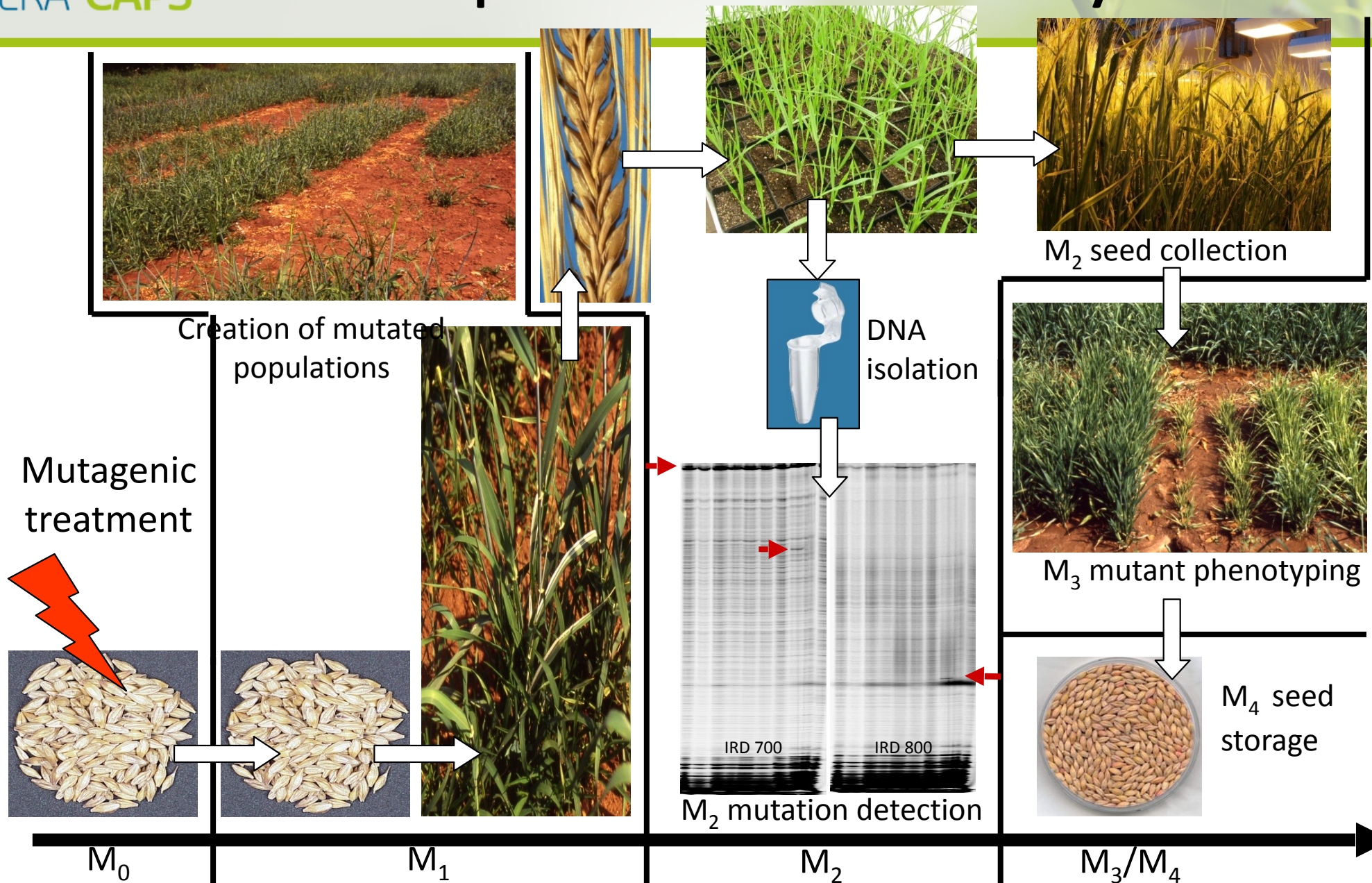


delayed
cell division
of CEID

Can AI checkpoint control knowledge be transferred to crop species?



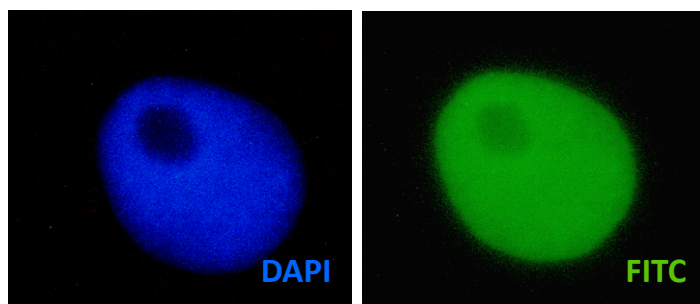
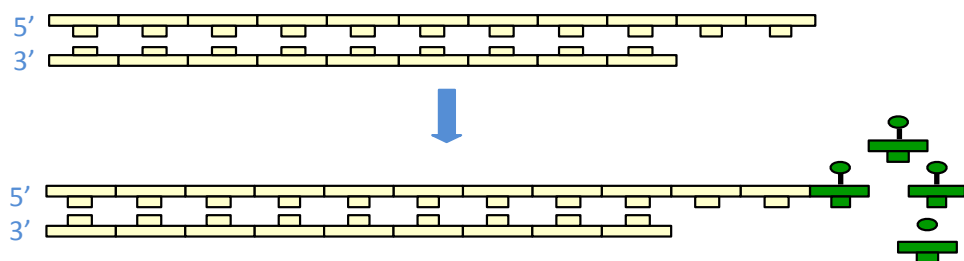
Development of TILLING platform in barley



Develop DNA damage tests in barley

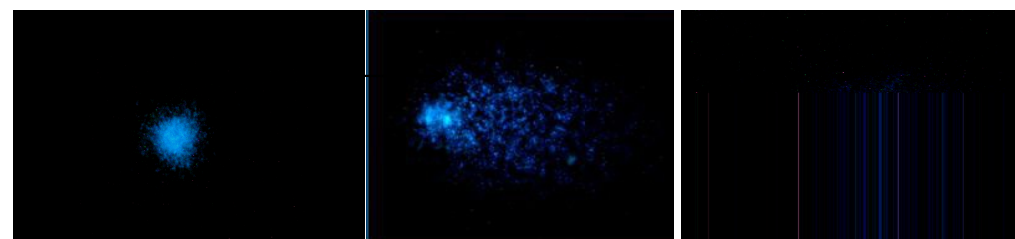
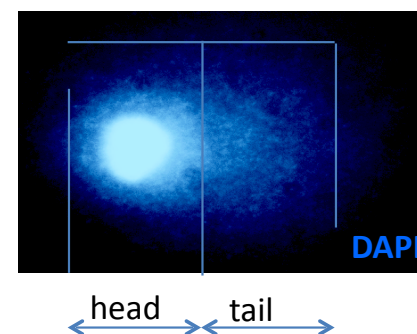
TUNEL test

TdT - mediated dUTP Nick End Labelling



Analysis of the frequency of FITC labelled nuclei (with DNA damage)

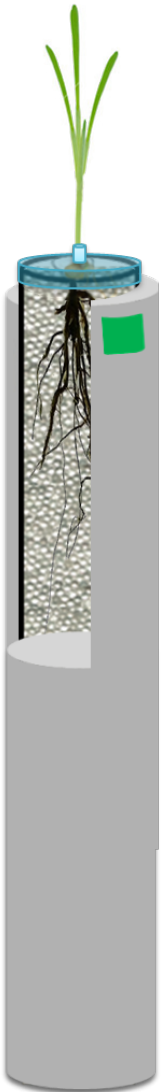
Comet assay



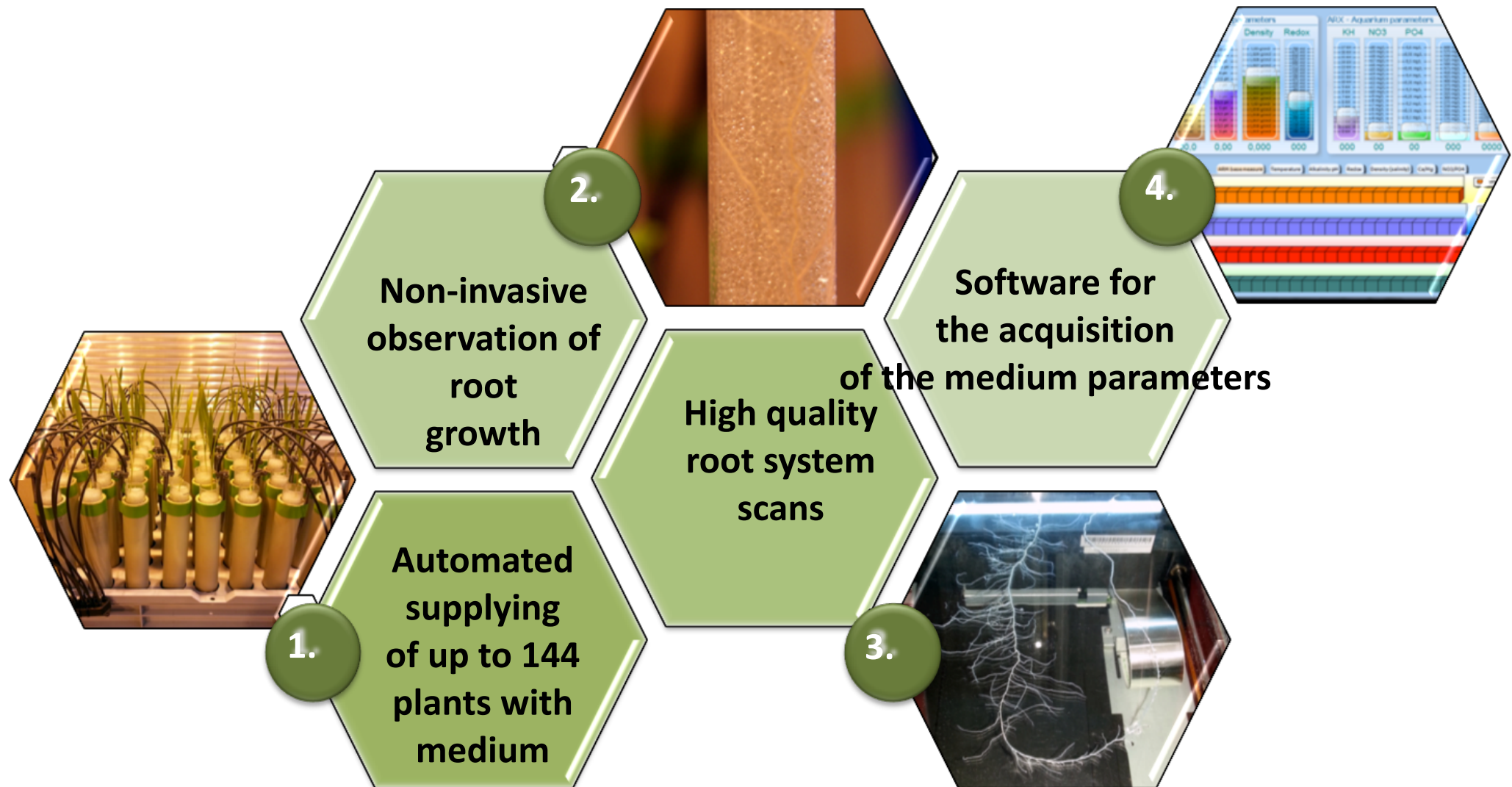
No damage

Nuclei with different level of DNA damage

A semi-hydroponics system for root phenotyping of cereals



A semi-hydroponics system for root phenotyping of cereals



AI-UCIDATE Partners



Lieven De Veylder



Arp Schnittger



Paul Larsen



Iwona Szarejko



Thanks



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