MATE: A PROMISSING GENE FOR ALUMINUM TOLERANCE IN SUGARCANE

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ALUMINUM TOLERANCE

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- In the world 50% of the potentially arable soils are acids.



- Latosoil, distrofic and acid.
- pH ≤ 5
  - ↓ N, P, K, Ca, Mg
- ↑ Al^{3+}.

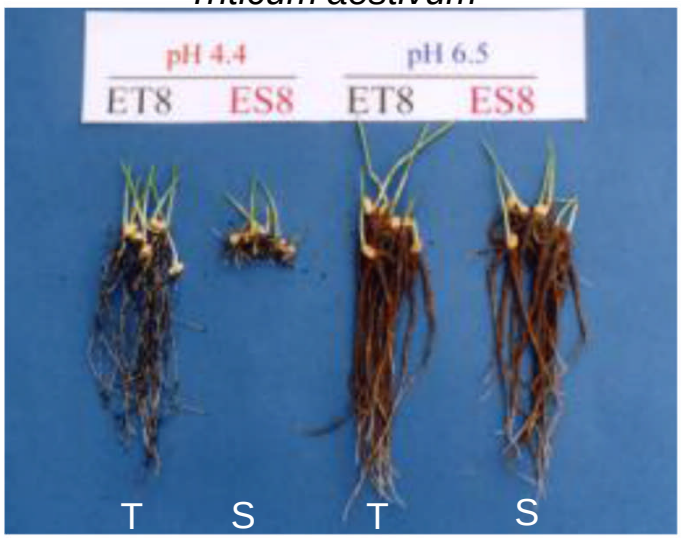
Aluminum is present in clay soil, therefore after sucessive hydrolises it stays in a toxic form, being harmfull to plant development.

Sugarcane ideal pH 6 – 7

Damage caused by aluminum in plants

INHIBITION OF GROWTH

Triticum aestivum

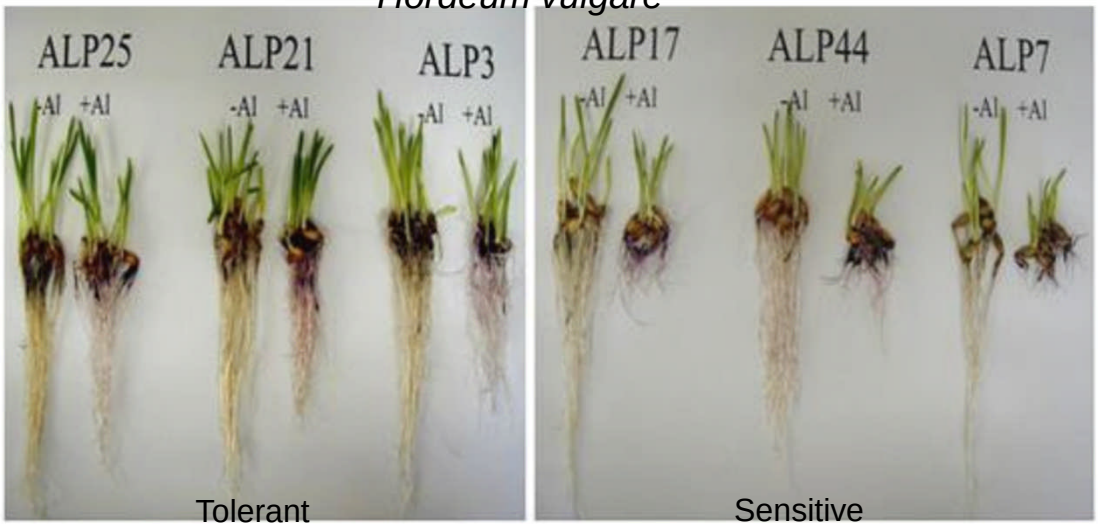


Zea mays





Hordeum vulgare



Wheat, Barley and Maize cultures from studies describing tolerance and sensibility to aluminum in grass.

Source: Ma et al, 1993; Sasaki et al, 2004; Magalhães et al, 2007; Cançado et al, 2008; Kochian, 2013.

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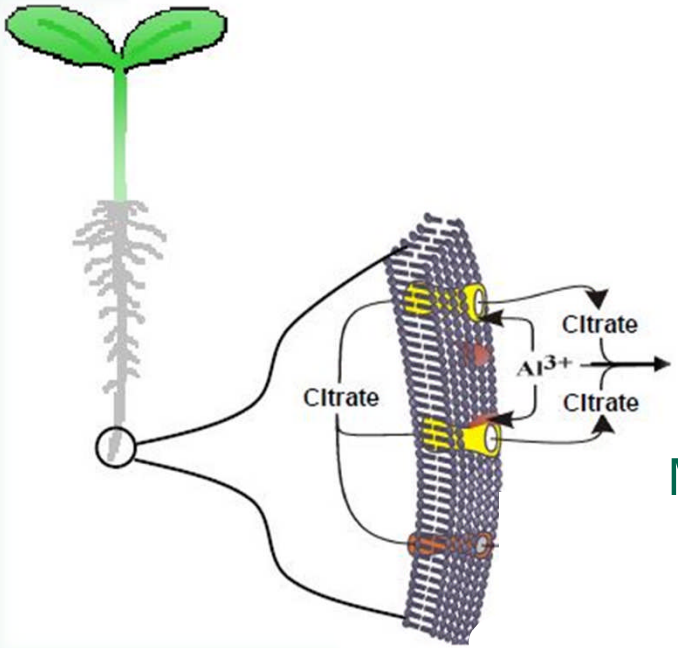
Why the *MATE* gene may be a promise?

nature genetics

A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum

Jurandir V Magalhaes[1,6], Jiping Liu[2], Claudia T Guimarães[1], Ubiraci G P Lana[1], Vera M C Alves[1], Yi-Hong Wang[2,5], Robert E Schaffert[1], Owen A Hoekenga[2], Miguel A Piñeros[2], Jon E Shaff[2], Patricia E Klein[3], Newton P Carneiro[1], Cintia M Coelho[1,5], Harold N Trick[4] & Leon V Kochian[2,6]

VOLUME 39 | NUMBER 9 | SEPTEMBER 2007 NATURE GENETICS

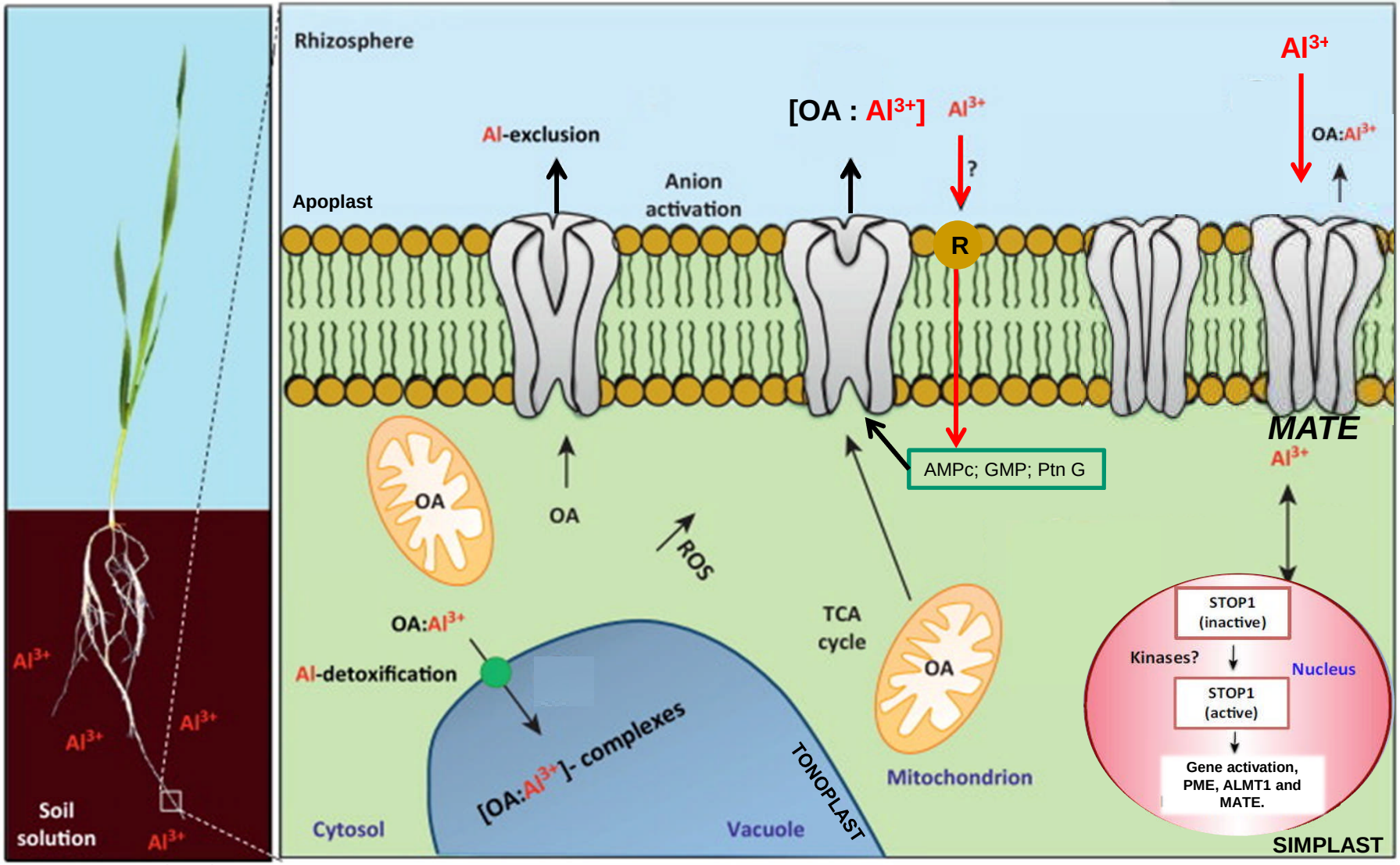


- *Arabidopsis* by Liu et al. (2008)
- *Hordeum vulgare* by Furukawa et al. (2007)
- *Secale cereale* by Collins et al. (2008)
- *Triticum* sp by Ryan et al. (2009)
- *Zea mays* by Maron et al. (2010)

Citrate + Al^{3+} = Non toxic complex

MATE acts in the exudation of citrate, for anionic channel by eletrochemical gradient.

Mechanisms of Aluminum Tolerance



Source: Nunes-Nesi et al, 2014.

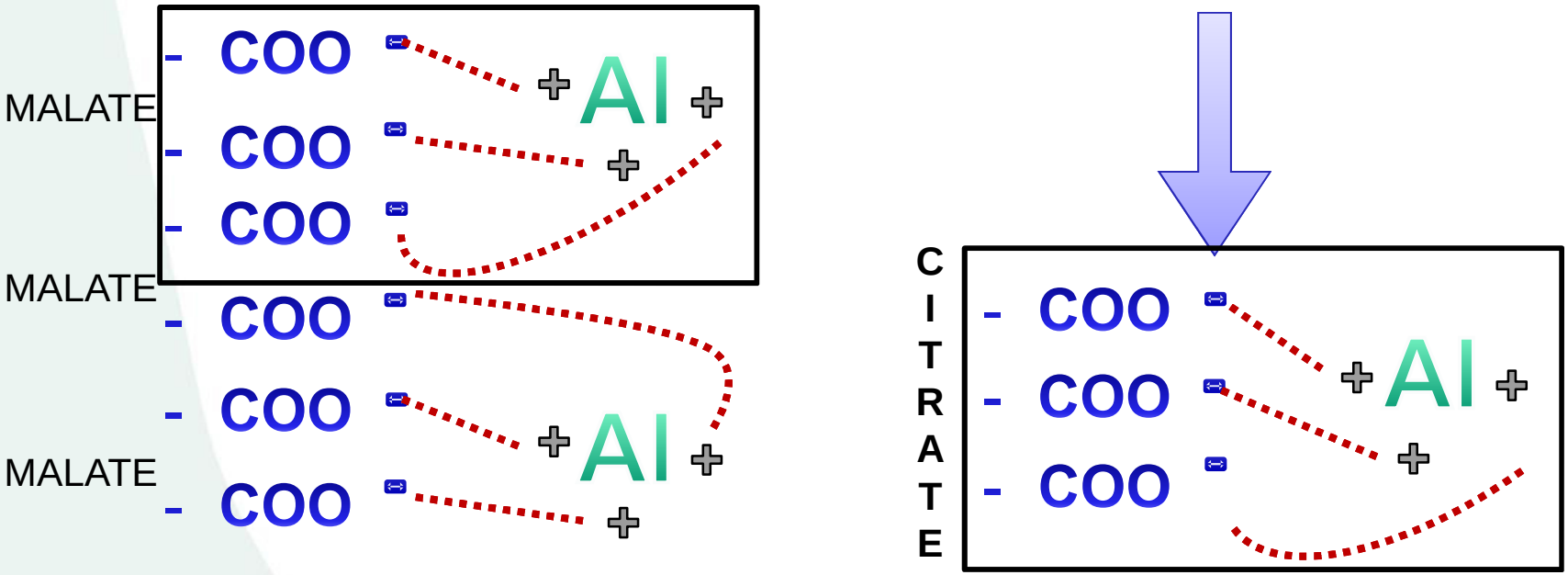
- Aluminum interacts with a membrane receptor, resulting in a signal that will be transmitted to the nucleus, causing an unknown kinase to activate the STOP transcription factor, which is responsible for transcription of genes, that encode transmembrane proteins *ALMT1* and *MATE*.

ALMT1

Aluminum-activated Malate Transporter

MATE

Multidrug and Toxic Compound Extrusion



The aim of this work in to transform Sugarcane and *Setaria viridis* using *MATE* gene.

Sugarcane Transformation

Biotechnology Reports 6 (2015) 41–44

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Short communication

A simple and highly efficient *Agrobacterium*-mediated transformation protocol for *Setaria viridis*☆

Polyana Kelly Martins, Ana Paula Ribeiro, Bárbara Andrade Dias Brito da Cunha, Adilson Kenji Kobayashi, Hugo Bruno Correa Molinari *

Genetics and Biotechnology Laboratory, Embrapa Agroenergy, Brasília, DF, Brazil



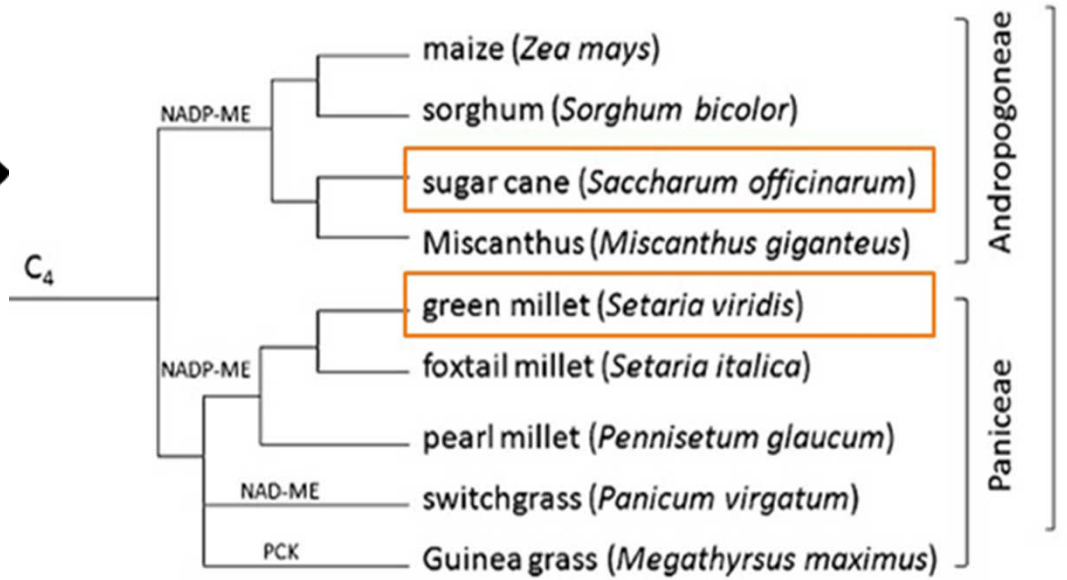
Source: Molinari et al., 2007.

Why *Setaria viridis?*

Grass; Short size; Short life cycle; Prolific seed production; Phylogenetically close to sugarcane; C4 metabolism.

Proof of Concept

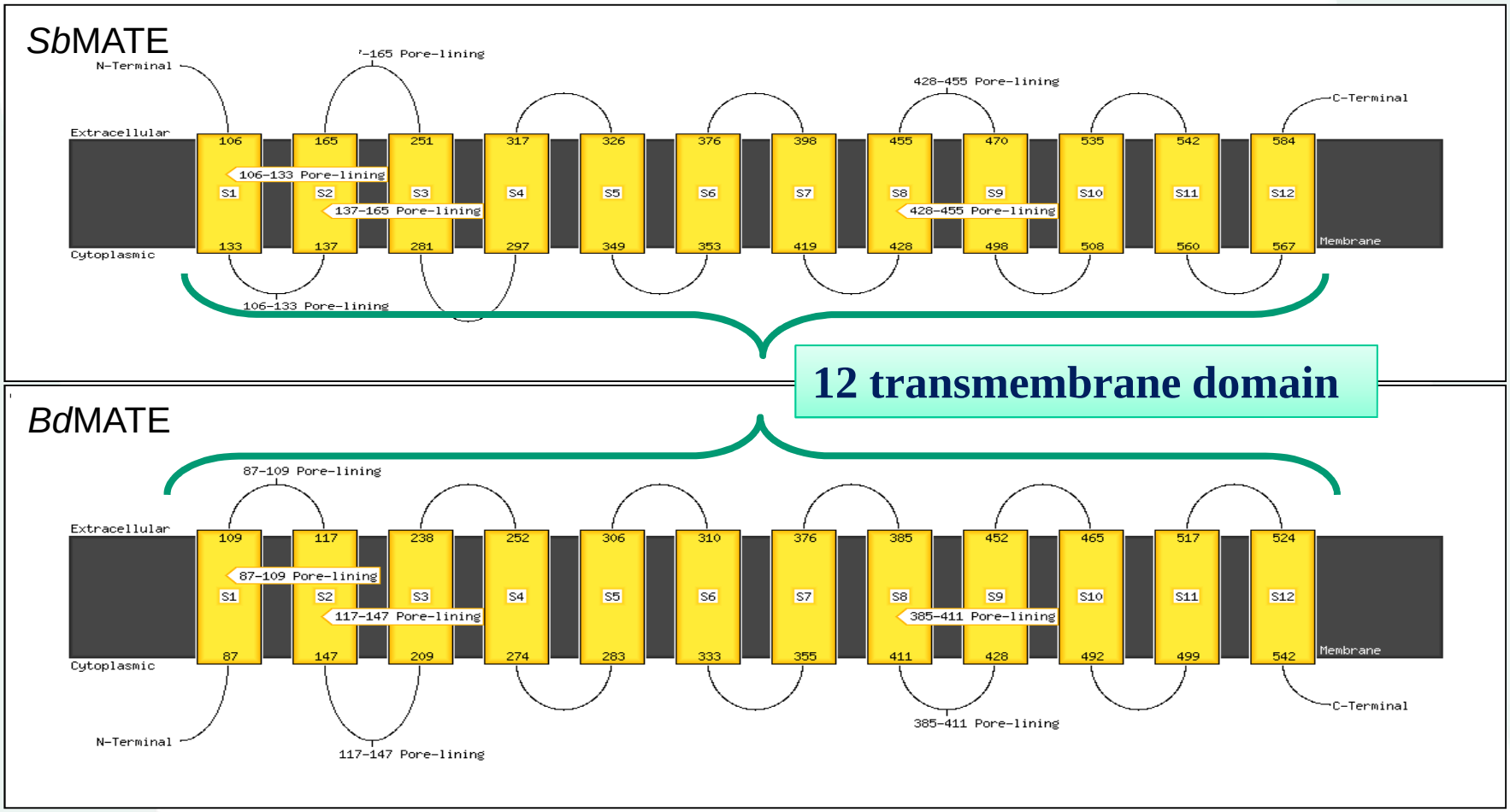




Setaria is considered suitable as model plant for aluminum tolerance studies in sugarcane.



*Sb*MATE – Multidrug and toxic compound extrusion - MATE family

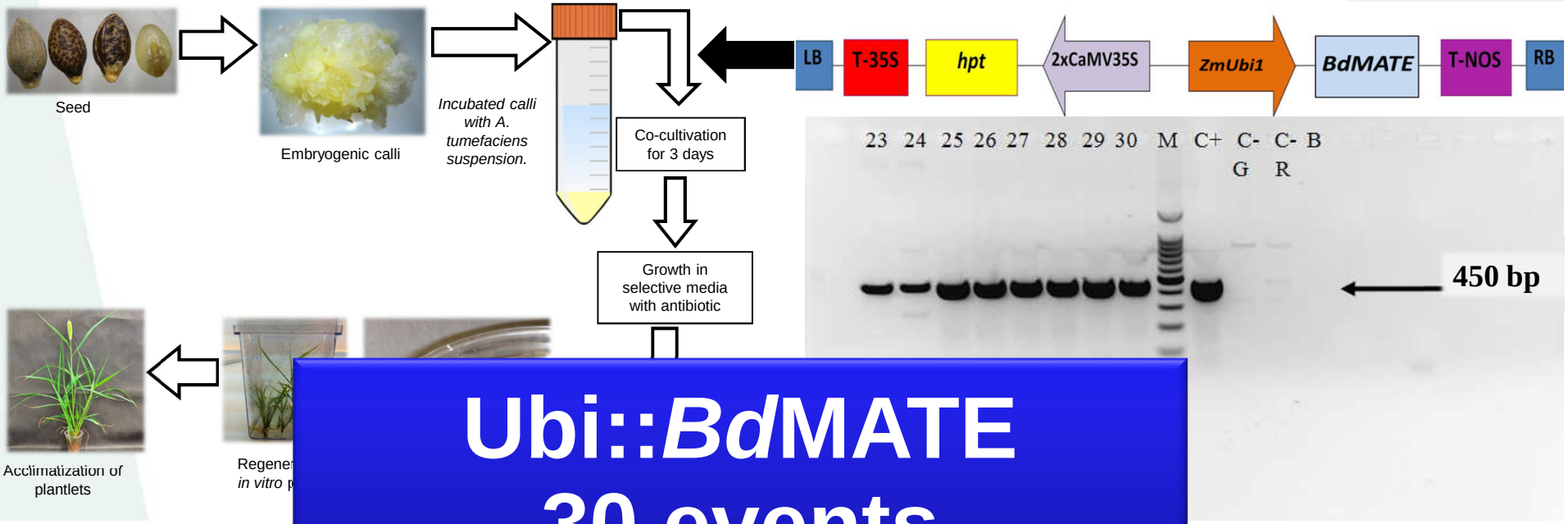


Ortologue in *B. distachyon*

- Large multifunctional transporter family involved in the transport of organic solutes out of the cytoplasm.

Source: Magalhães et al., 2007.

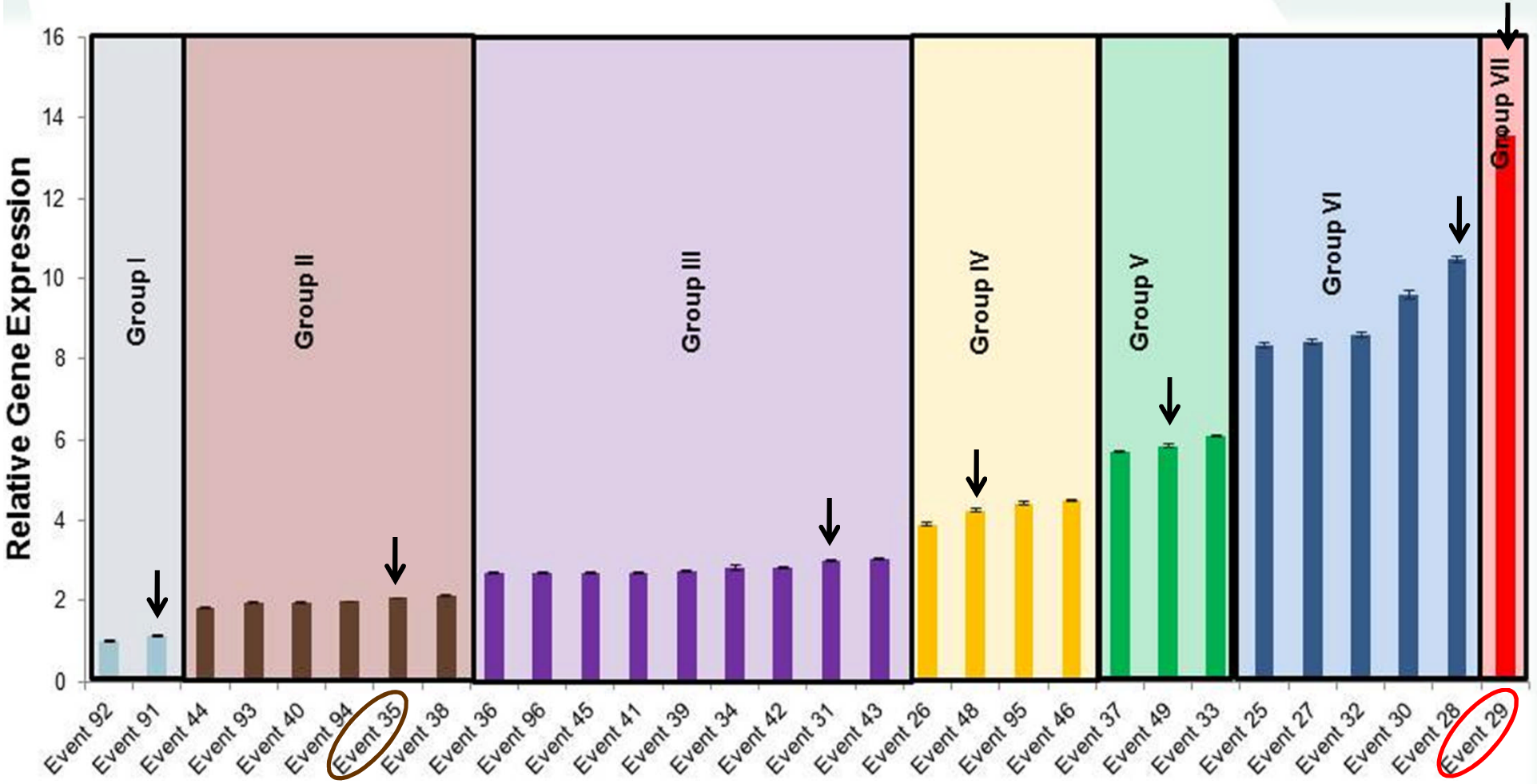
Genetic transformation of *Setaria viridis* by *Agrobacterium tumefaciens*



Ubi::*Bd*MATE
30 events

Construct	Experiment	Strain	Number of calli	Number of PCR positive plants	Transformation efficiency (%)
Ubi1::*Bd*MATE	1	EHA105	94	9	10
	2		55	16	**29**
	3		69	6	9

Events *Bd*MATE *screened* by RT-qPCR



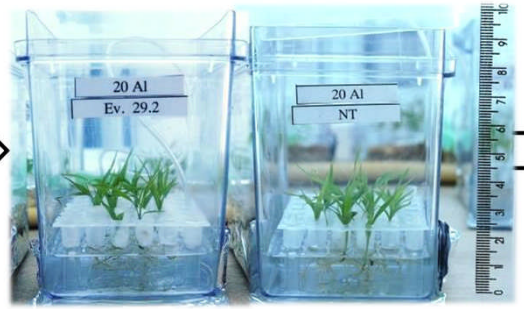
- The events were divided into 7 groups, according to their statistic differences.
- The arrows indicate the selected events for mendelian segregation. And all the events are single insertion, since they had a 3:1 mendelian ratio on the χ test.
- The circled events were submitted to experimental aluminum assay.

Evaluation Protocol for Aluminum Tolerance

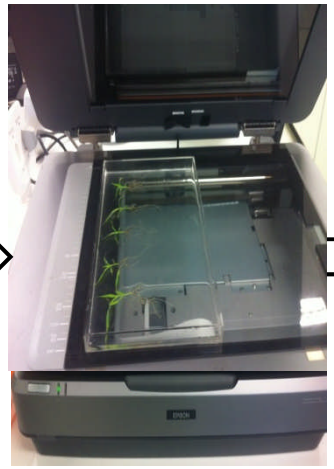
- ***Setaria viridis* non-transgenic**



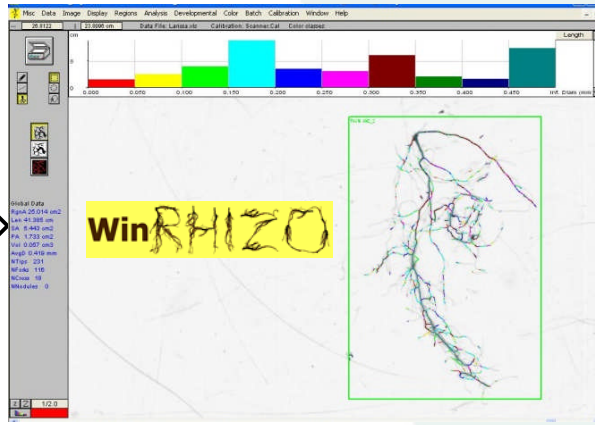
Plantlets after 7 days of *in vitro* germination



Hydroponic system for *S. viridis*



Digital scanner for image aquisition



Software WinRhizo for analyse of total root length.

- Experiments:
  - Nutritive solution and $CaCl_2$.
  - Concentrations of $CaCl_2$.
  - Concentrations of aluminum and respectives free activities of Al^{3+}

Solution/ Reference	Specie
Arnon and Hoagland (1950)	Setaria
Magnavaca et al. (1987)	Sorghum, Corn
Camargo et al. (1981)	Wheat
$CaCl_2$ 500 µM Sasaki, et al. (2004)	Wheat

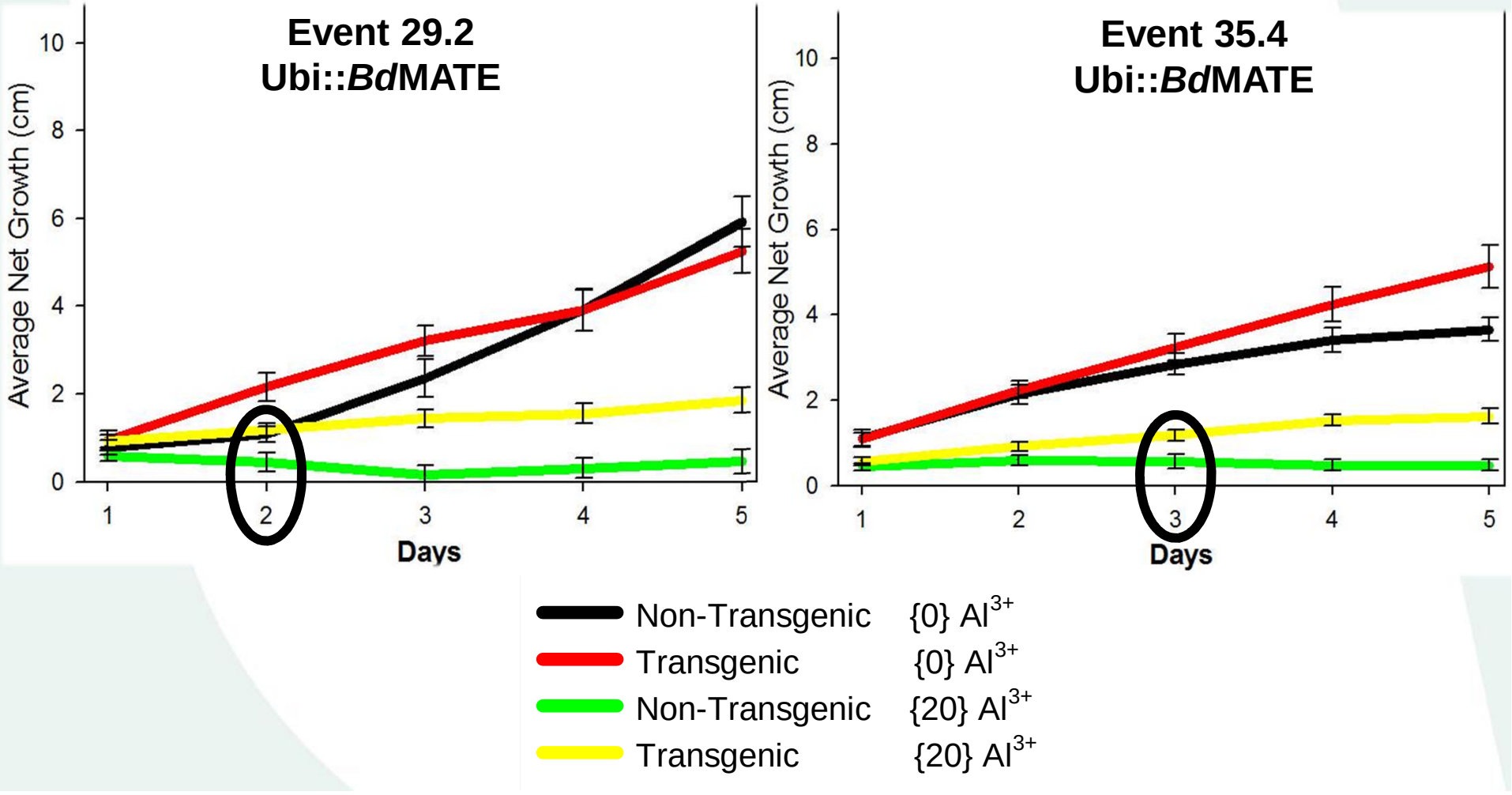
A final aluminum tolerance assay was established:

- **Solution of $CaCl_2$ 500 µM; Free activity {20} µM de Al^{3+}, pH 4.2.**

- **Aluminum tolerance assay with homozygous transgenic lines.**

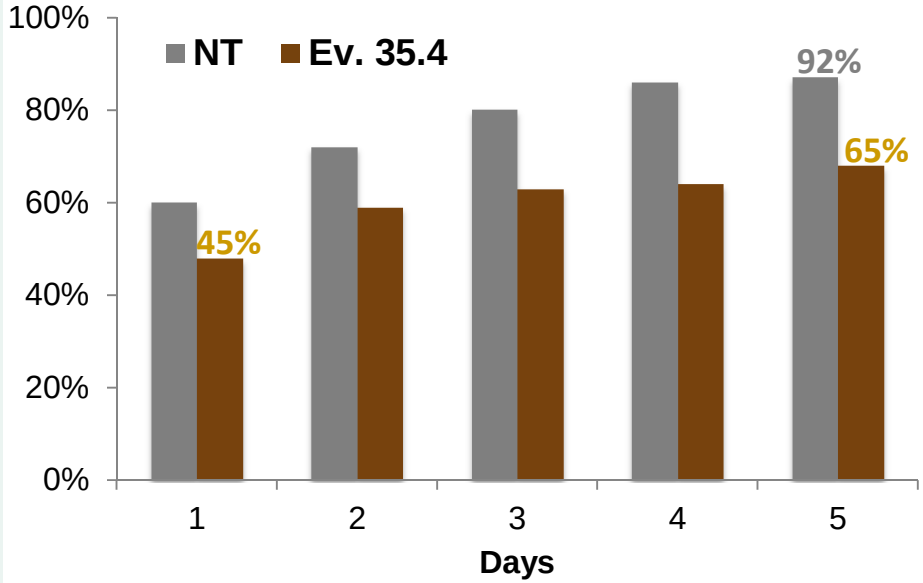
Root Net Growth

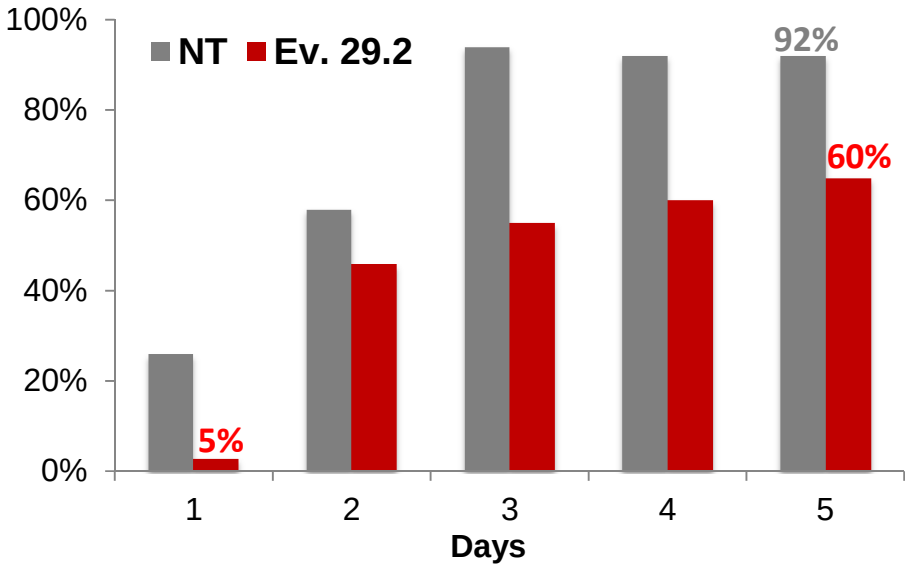
Solution of $CaCl_2$ 500 µM; {20} µM Al^{3+}; pH 4.2; and 20 plantlets by treatment.



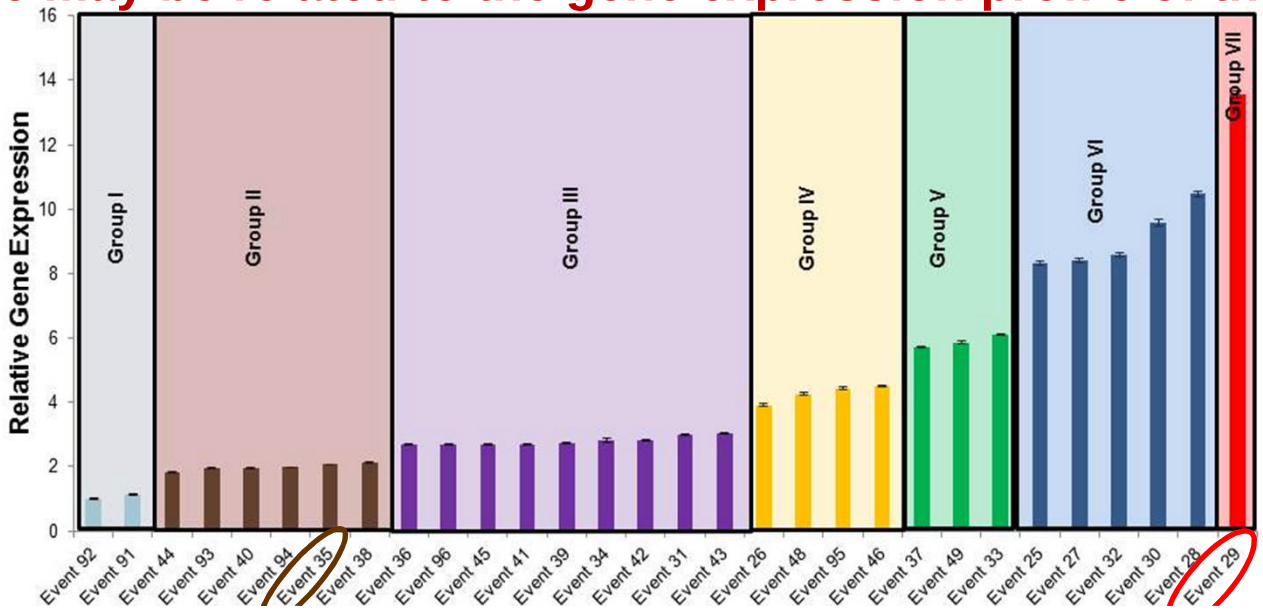
Root Growth Inhibition (%)

$$\frac{\overline{X}\ \text{RRG} + \text{Aluminum}}{\overline{X}\ \text{RRG} - \text{Aluminum}}$$





The response may be related to the gene expression profile of these events.



Conclusions

- ✓ 30 transgenic events were obtained of *S. viridis* overexpression the *MATE* gene of *Brachypodium distachyon.*
- ✓ A protocol of avaliation and selection to aluminum was established.
- ✓ Two homozygous lines *Zm*Ubi1::*Bd*MATE showed tolerance to {20} µM of Al^{3+}.



Underway

➢ Sugarcane

- Biolistic genetic transformation using the construct *Zm*Ubi1::*Sb*MATE.
- Development of a hydroponic protocol to evaluate response to Al^{3+}.









Sugarcane/Setaria Team

Embrapa Agroenergia



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Adilson Kenji Kobayashi, PhD



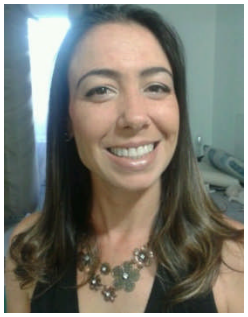
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CAPES

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Thank you!!!

Valoriser la ressource canne
eRcane

11th Germplasm and Breeding & 8th Molecular Biology Workshops