

Modelo Cartesiano do mecanismo de ação dos herbicidas mimetizadores das auxinas

Baseado no princípio do desbalanço energético nas membranas plasmáticas da célula

By:
Marcelo Rodrigues Alves de Figueiredo &
Pedro J. Christoffoleti

Mecanismo de ação do 2,4-D

- ✓ Auxina é um hormônio multifuncional
- ✓ É bem conhecido que as auxinas induz o processo de sinalização de expressão gênica
- ✓ Mas a sinalização citoplasmática é pouco conhecida



Xu et. al., 2010 – *Cell*, n.143, p.99-110.

Objetivo é explorar a polarização da célula e sua relação com o processo de Inter digitação das células de pavimentação celular (PC – inter digitação)

ABP1

Rho GTPases, ROP2 and ROP6

PINFORMED 1 – PIN1

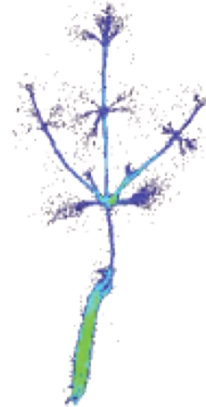
Modulação da expansão celular

Fase I - Estimulação

(0 a 5 horas depois do tratamento)

Reação rápida da membrana plasmática

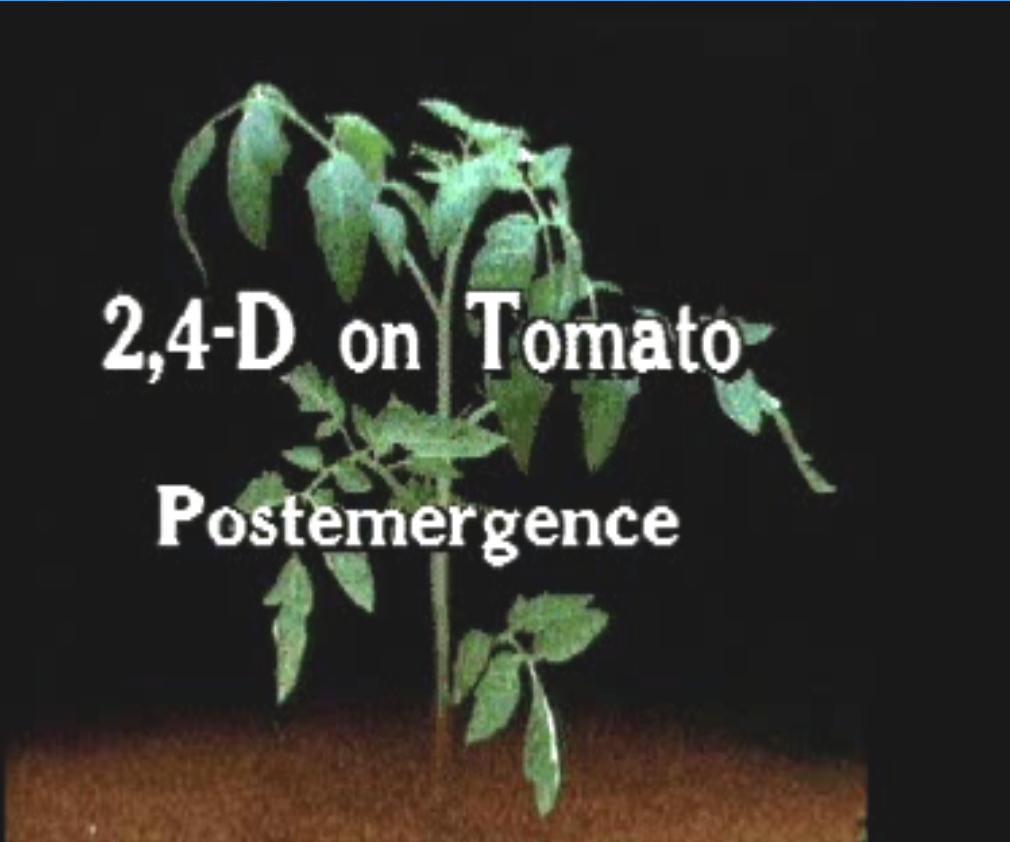
Autoradiograph



30 min



5 hours

A photograph of a young tomato plant seedling with several green leaves, growing out of a black plastic nursery pot. The soil in the pot is a reddish-brown color. The background of the photo is black.

2,4-D on Tomato

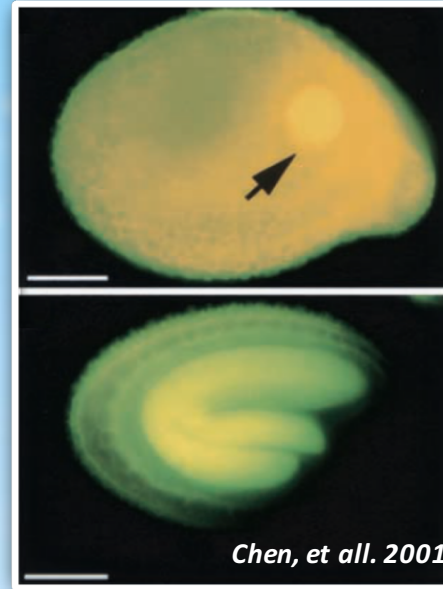
Postemergence



“AUXIN-BINDING PROTEIN” (ABP1)

- ✓ Ancoradas na membrana plasmática,
- ✓ Retidas no reticulo endoplasmático
- ✓ Aumenta a atividade do complexo de Golgi;
- ✓ pH ideal para ligação com auxina é 5,5
- ✓ Não possui nenhuma ligação à pH 7,0;
- ✓ Relacionadas à rápidas respostas celulares.

Divisão celular é aberrante em mutantes *ABP1*



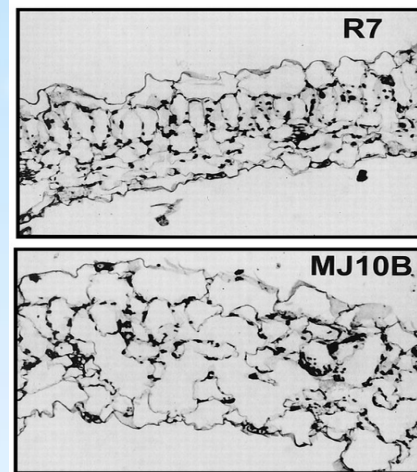
Mutante *ABP1*:

Embrião permanece no Estádio Globular

Selvagem:

Embrião com formação normal – Estádio Torpedo

Folhas de tabaco tratadas - NAA 10 μ M

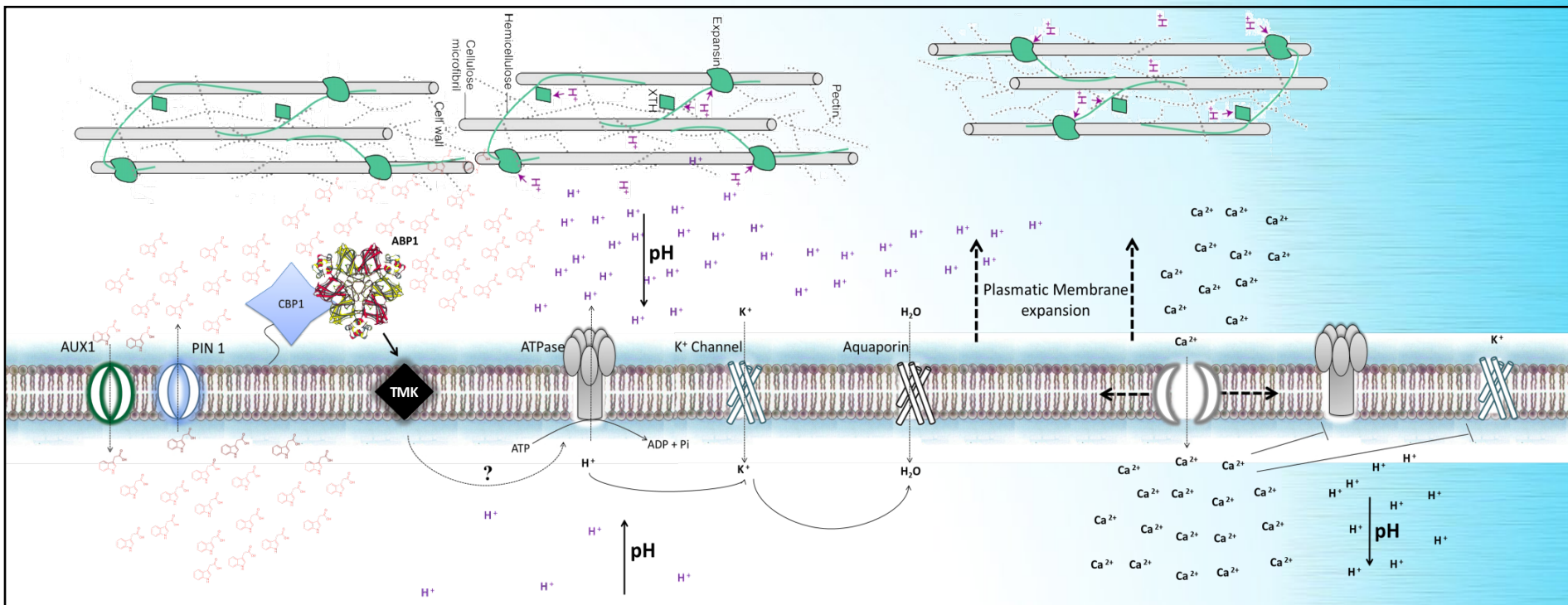


Plantas controle

Plantas com super-expressão de ABP1

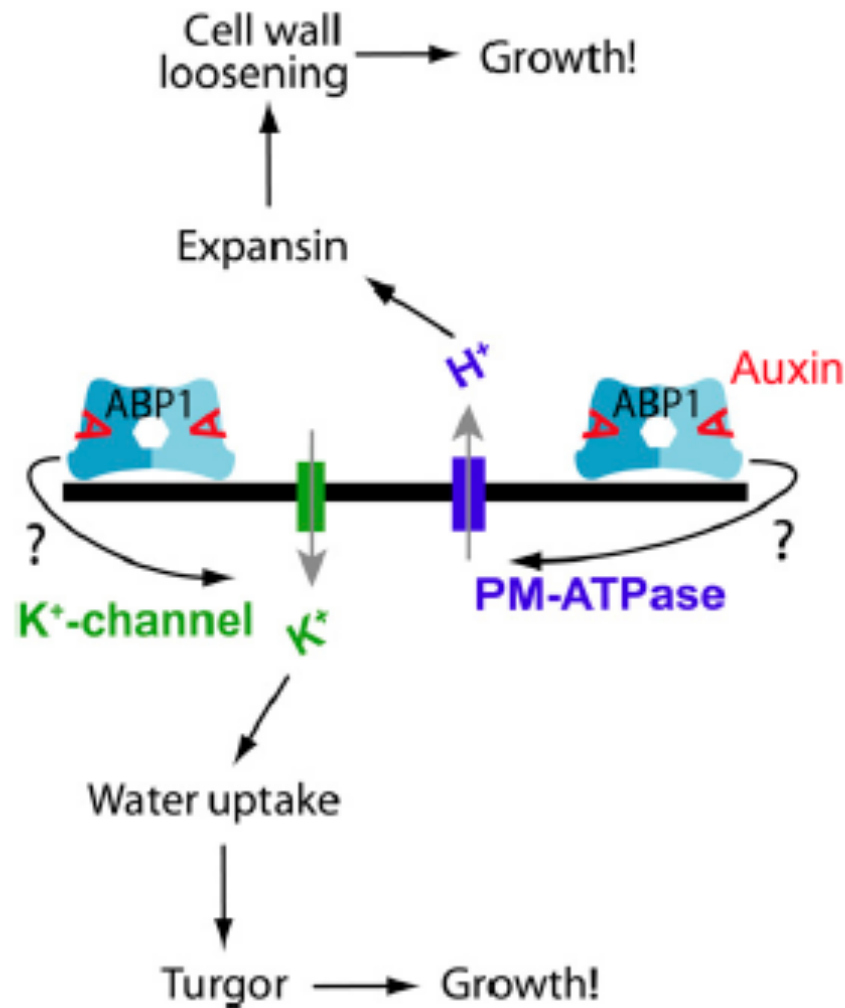
Células 2,5x mais expandidas

Princípio do desbalanço energético na plasmalema celular e afrouxamento da parede celular



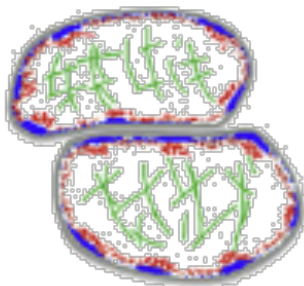
- ✓ Ligação da ABP1 com a auxina
- ✓ Hiper polarização da membrana plasmática
- ✓ Fluxo de K⁺
- ✓ Expansão celular

Expansão celular regulada pela ABP1

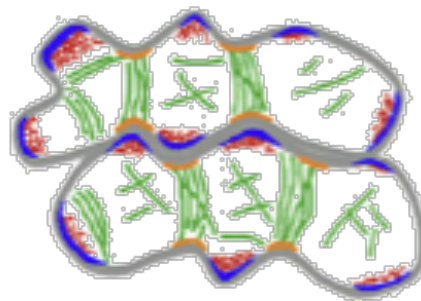


ABP1 na Pavimentação Celular - Morfogênese

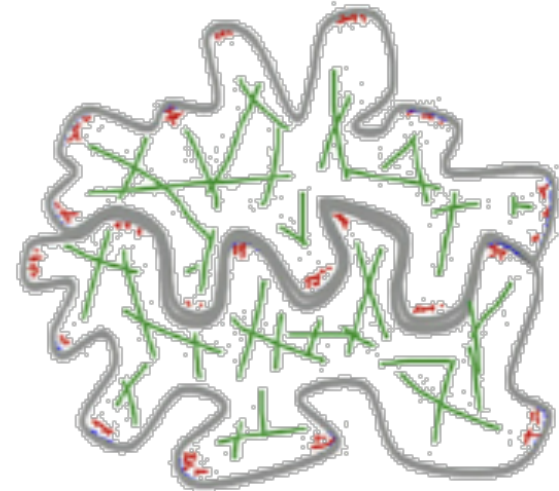
■ Active ROP2
■ Active ROP6
■ F-actin
■ Microtubules



Stage 1



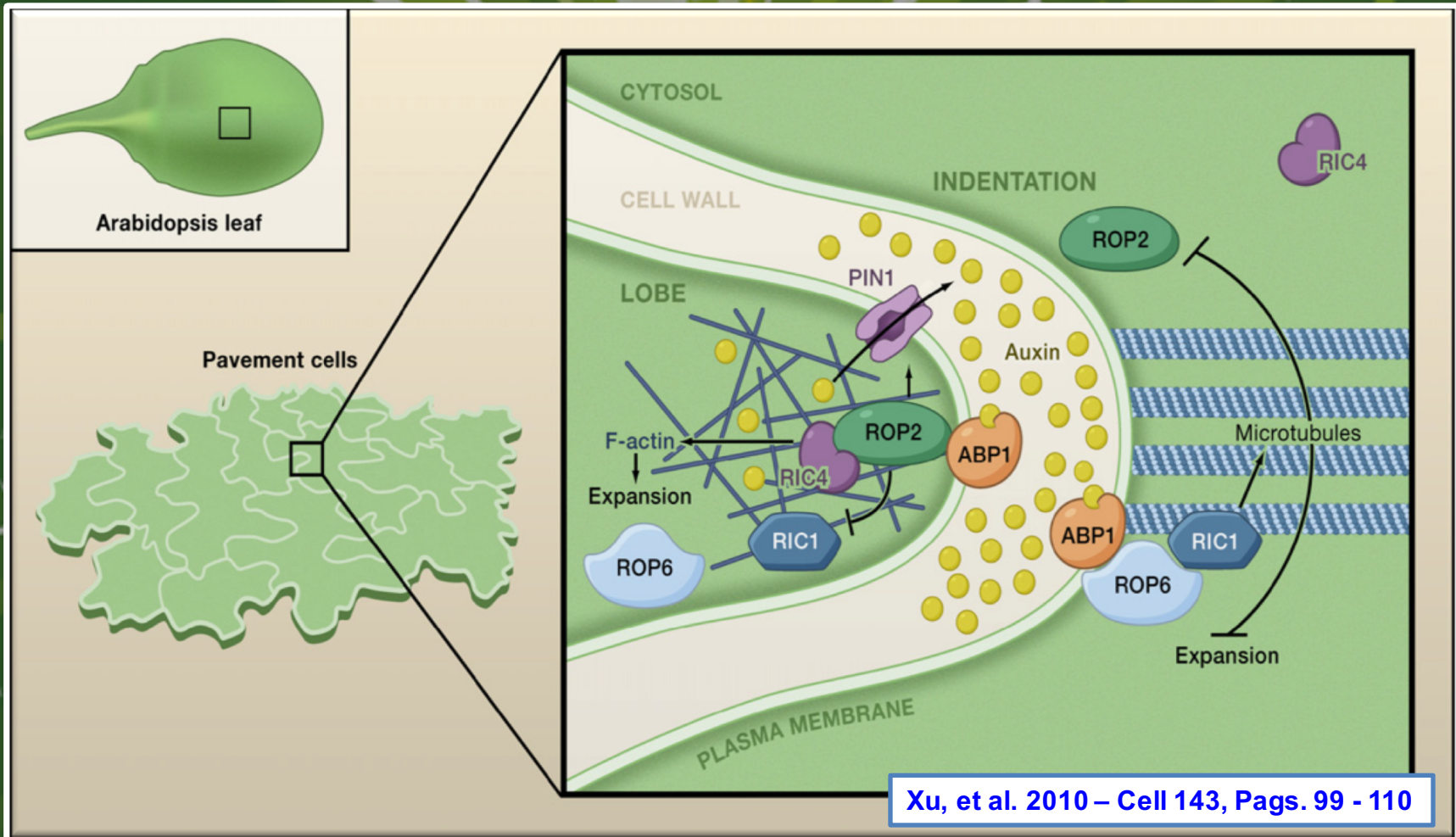
Stage 2

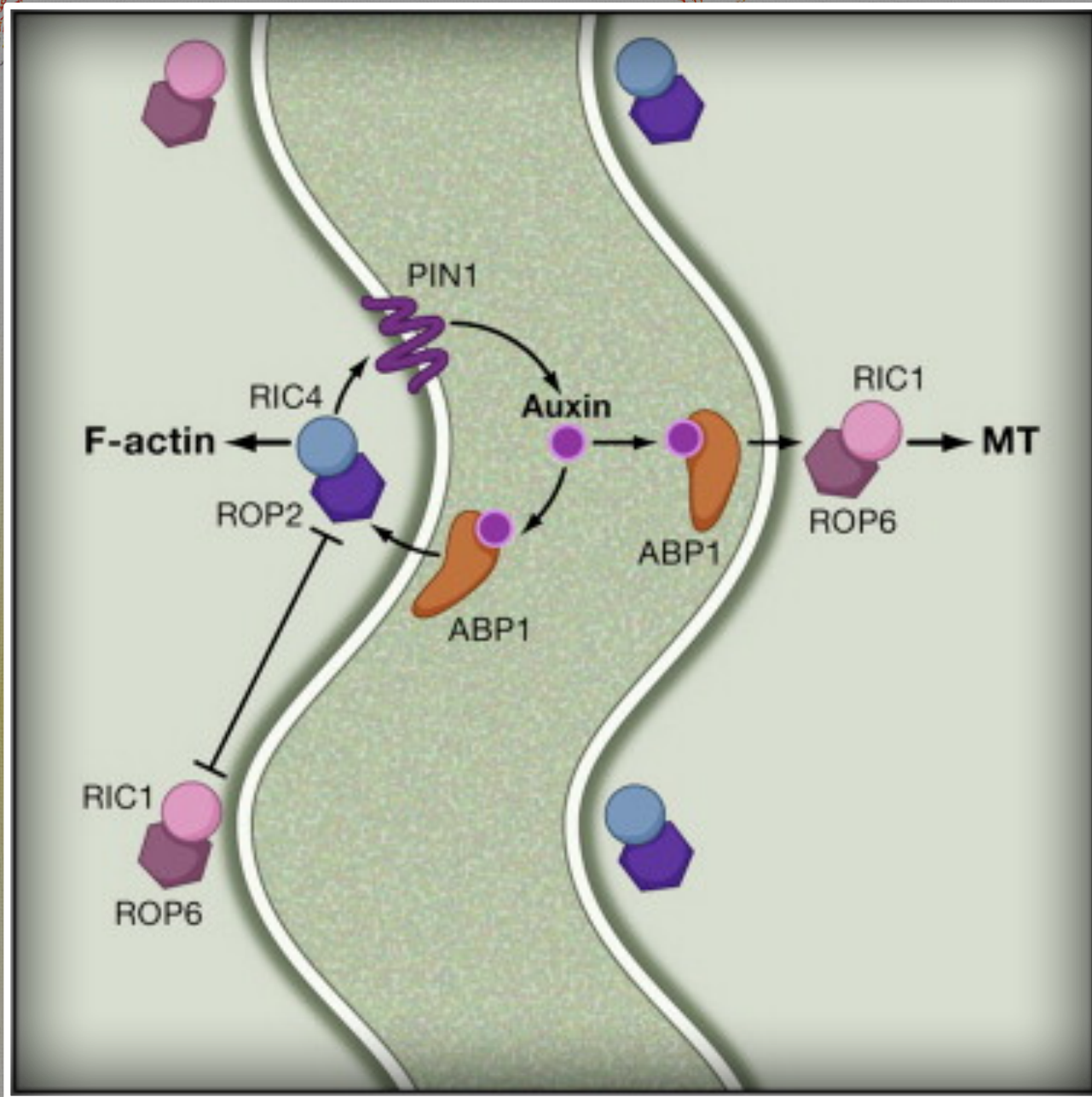


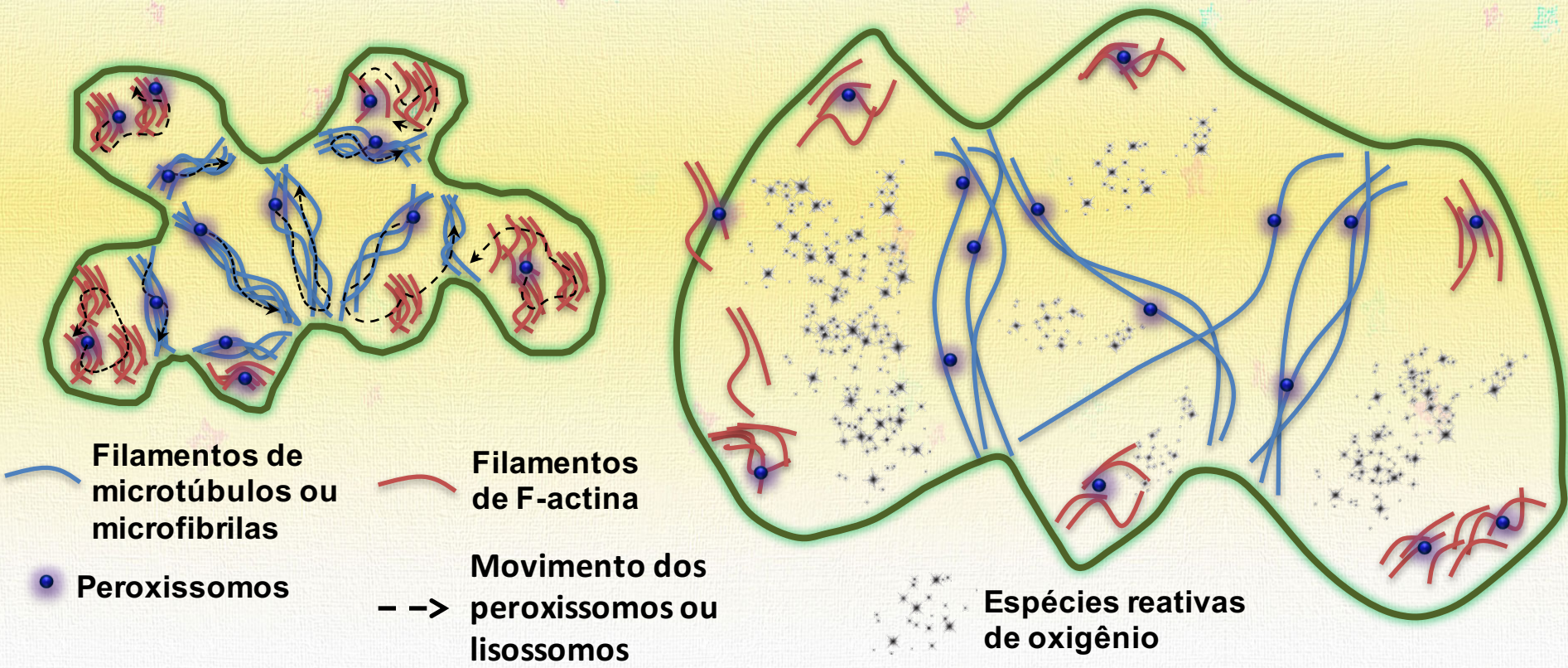
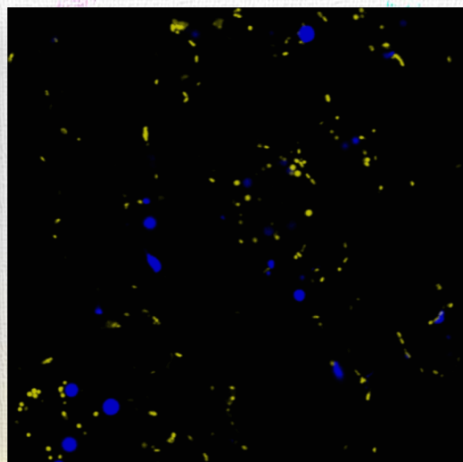
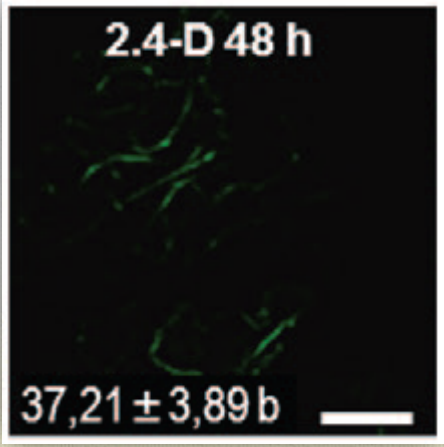
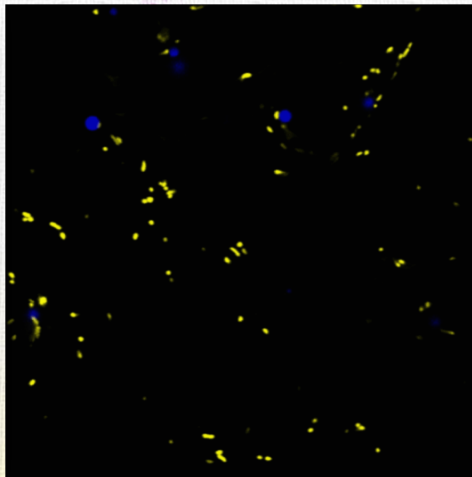
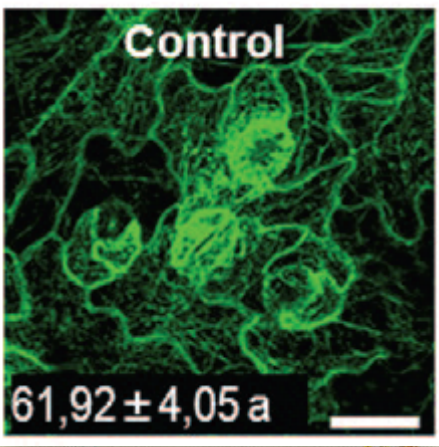
Stage 3

Auxina controla o desenvolvimento de inter-digitação entre Células

- ABP1 – regula a atividade das GTPase ROP2 e ROP6 na inter-digitação







Fase II - Inibição

(5 a 24 horas depois do tratamento)

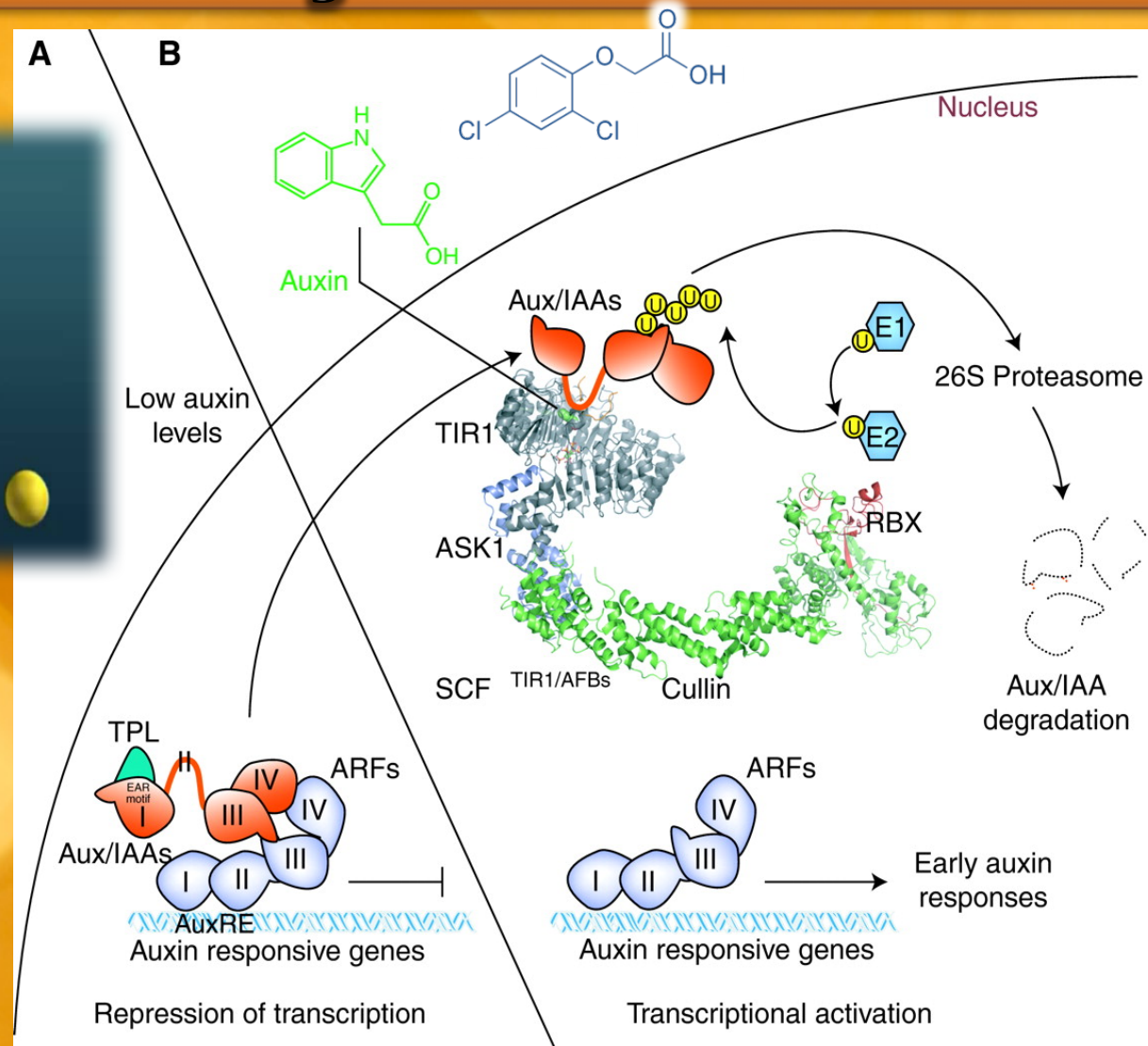
Ativação das respostas dos genes



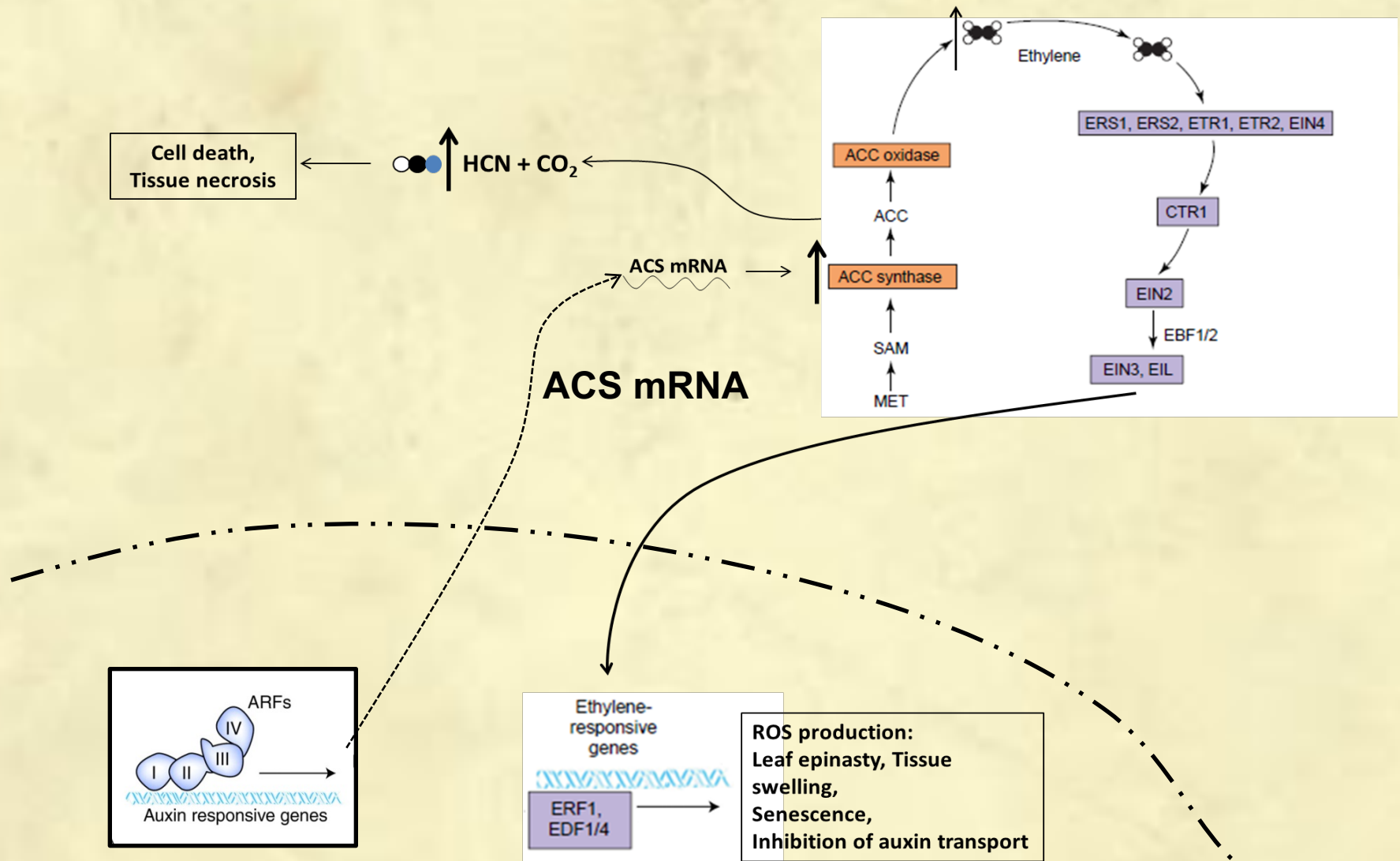
A auxina liga-se á vários compostos

- ✓ TIR1AFB
 - ✓ SKP2A
 - ✓ E3 ligasse SCF (complexo)
- 24 hours

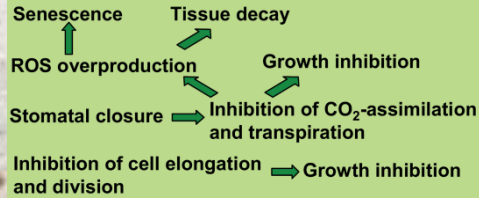
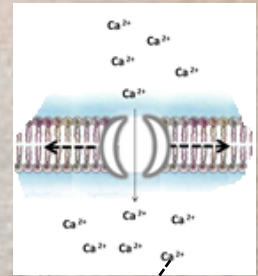
TIRs/AFBs: atuam na complexação da rede de sinalização dentro da célula



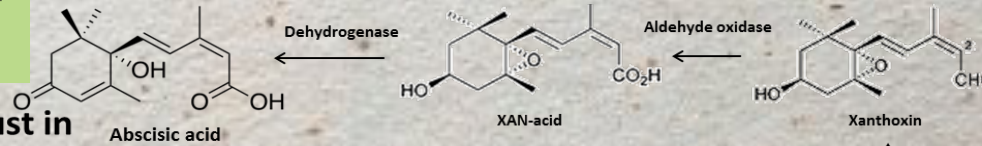
Mecanismo da síntese de etileno e respostas



Produção de ABA e descompartmentalização



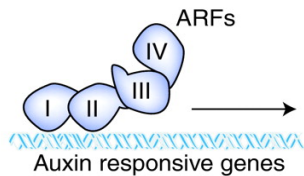
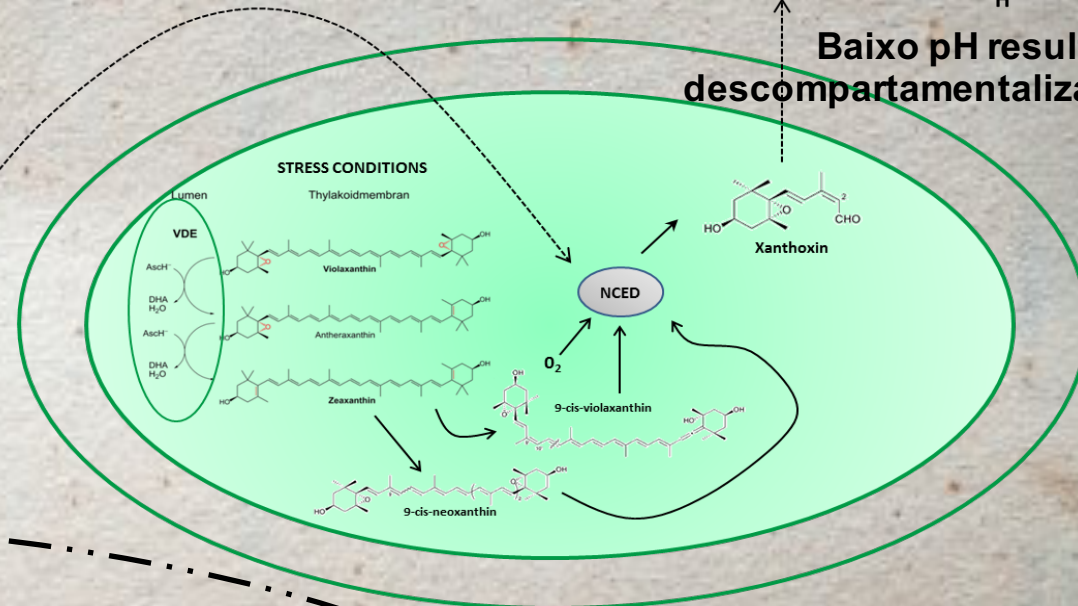
Active just in low pH (6,5) in Cytoplasm



Baixo pH resulta na descompartmentalização do ABA

NCED mRNA

NCED



Estresse permanente

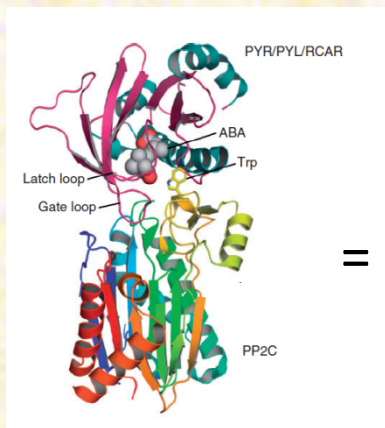
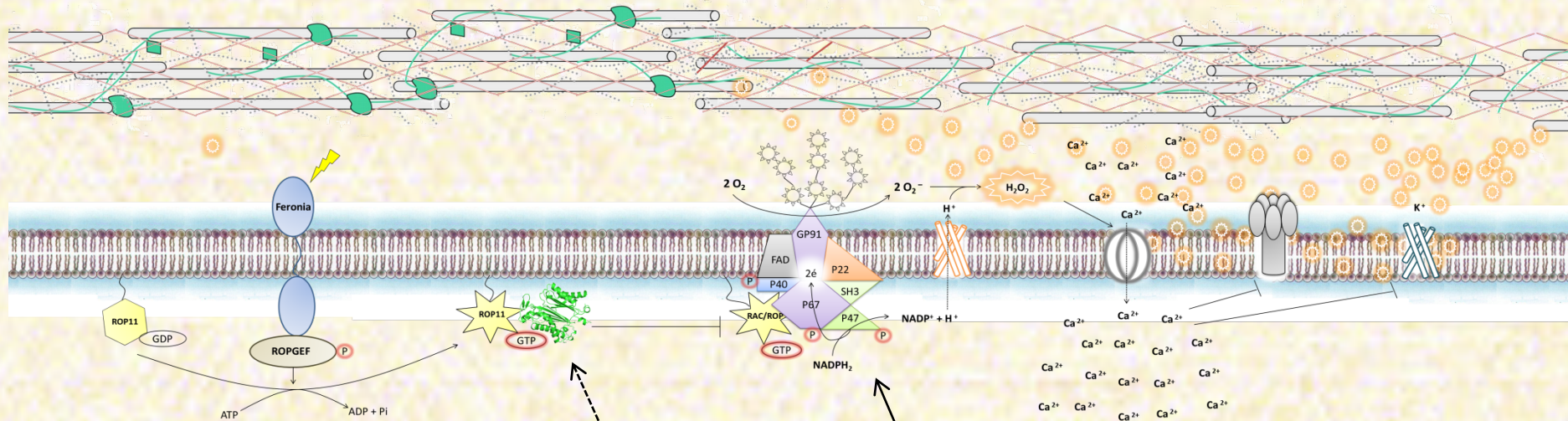
(24 a 72 horas depois do tratamento)

Declínio da atividade metabólica

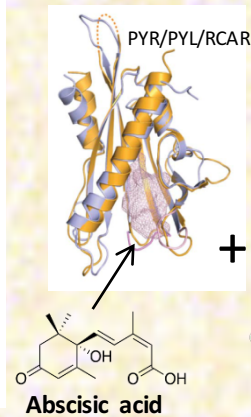


Twisting, strapping of leaves, and auxin-like injury symptoms.

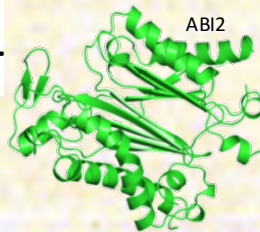
ABA: Regulação



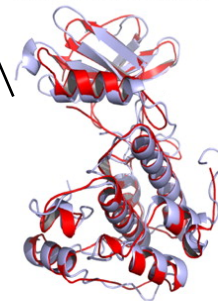
=



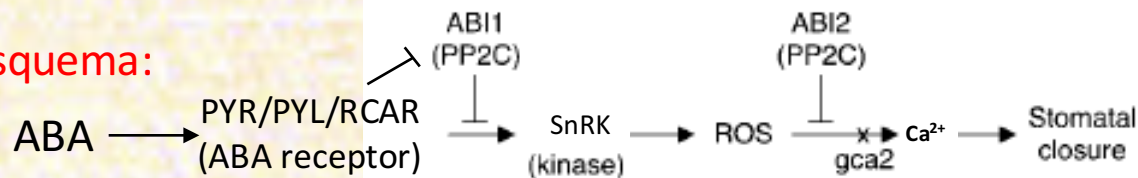
or



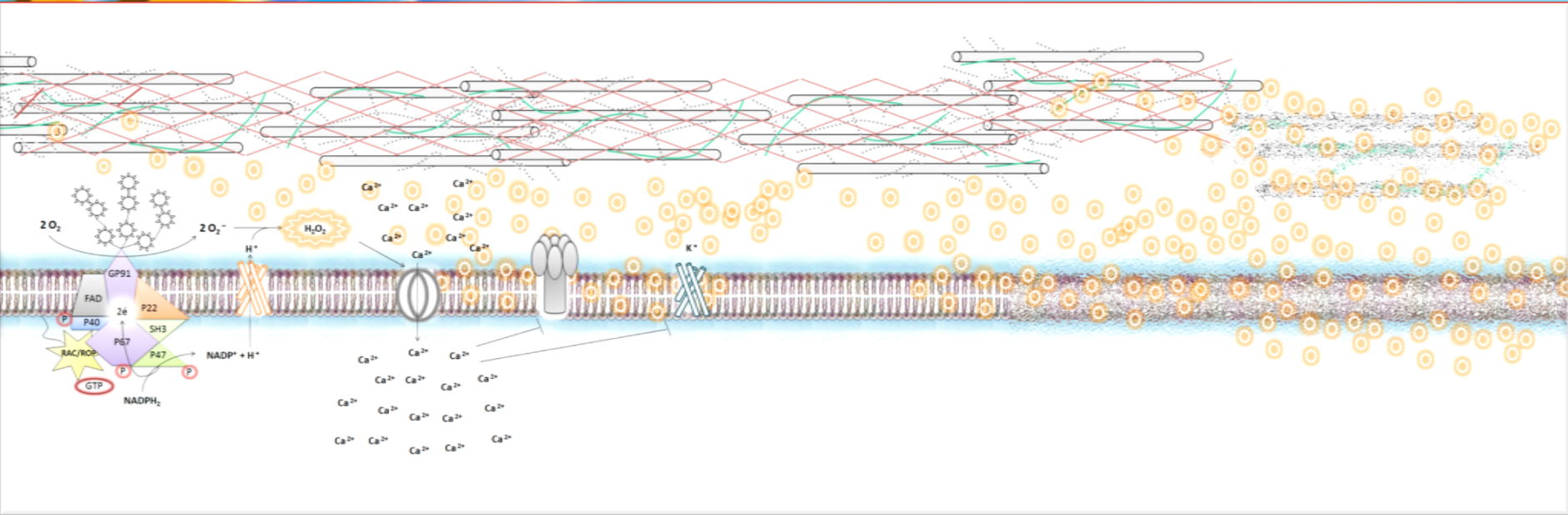
closed conformation



Esquema:



Membrane Redox Status: Fluidity and Disorder



The over production of ROS induces changes in intracellular redox status of becoming the oxidant (electron acceptor), affecting the synthesis or activities associated with redox systems. Initially, the main system will be changed by the high amount of ROS produced is again the cell wall

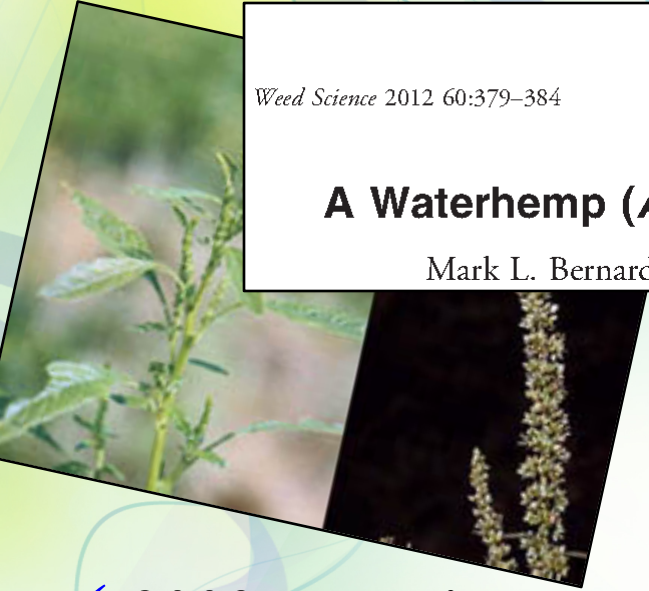
Finally, the radicals are able to penetrate deeply into the lipid head phospholipids existing in the membrane region, promoting unsaturation of the chains that make up lipids, leading to greater fluidity and consequent disorder, leading to leakage of the cytosol, culminating in cell death

Resistência ao 2,4-D

- Primeiros casos em 1957
- 30 relatos de resistência aos inibidores de auxinas
- Resistência ao 2,4-D (14 casos)
 - *Sphenoclea zeylanica* (3 países)
 - *Sisymbrium orientale* (resistência múltipla – ALS)
 - *Sinapis arvensis*
 - *Raphanus raphanistrum* (4 países)
 - *Papaver rhoeas*
 - *Limnophila erecta* (resistência múltipla – ALS)
 - *Limnocharis flava*
 - *Lactuca serriola*
 - *Fimbristylis miliacea*
 - *Daucus carota*
 - *Commelina diffusa*
 - *Cirsium arvense*
 - *Carduus pycnocephalus* e *Carduus nutans*
 - *Amaranthus tuberculatus* (=A. rudis)

A Waterhemp (*Amaranthus tuberculatus*) Population Resistant to 2,4-D

Mark L. Bernards, Roberto J. Crespo, Greg R. Kruger, Roch Gaussoin, and Patrick J. Tranel*

- 
- ✓ **2009 – ensaios de casa de vegetação – R/S = 10**
 - ✓ **História de 12 anos de uso de 2,4-D, algumas vezes com aplicações múltiplas no ano**
 - ✓ **Plantas mostraram sintomas característicos de epinastia mas depois recuperaram o suficiente para produzir sementes**
 - ✓ **Provavelmente a mutação não está associada a ABP1 mas nas etapas seguintes**

21 DAT

Susceptible

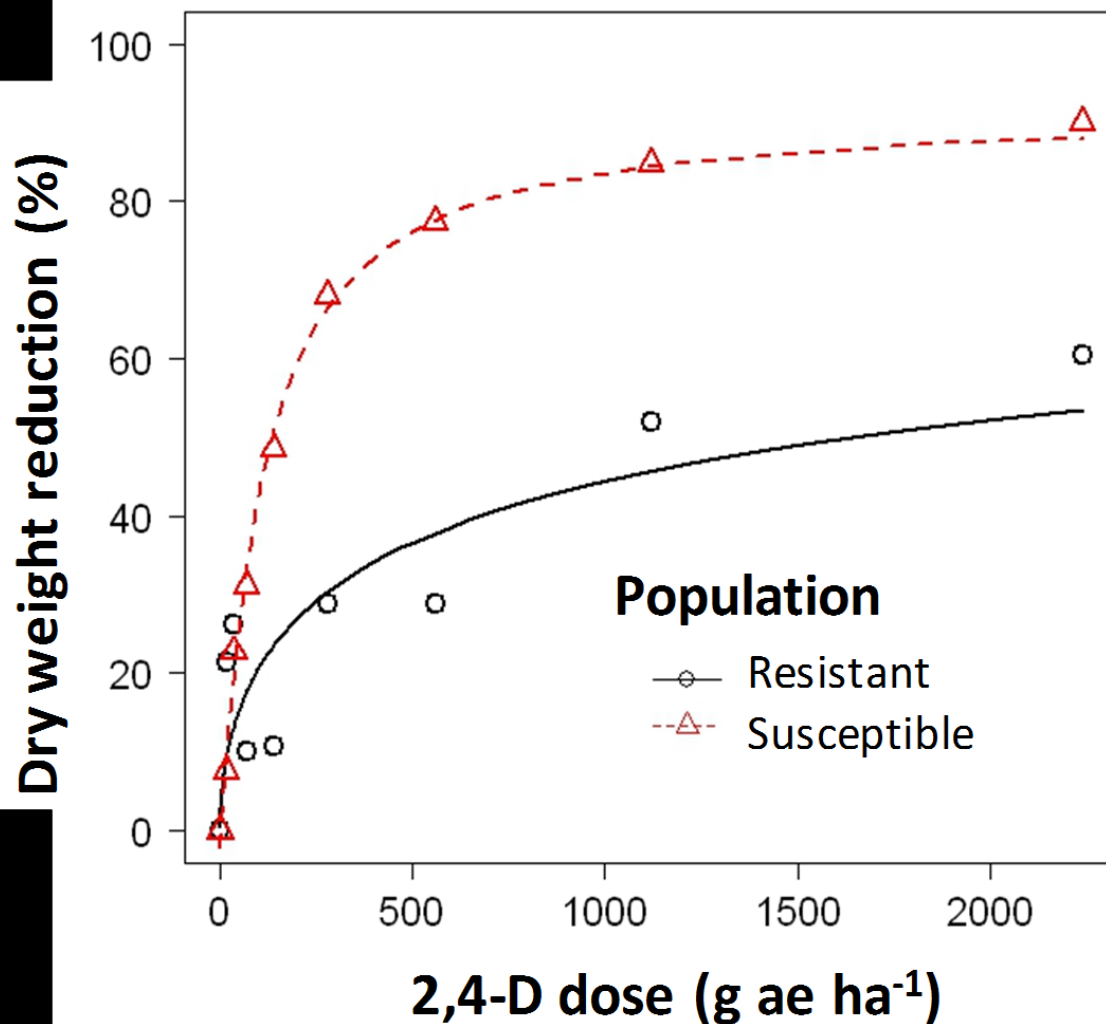


Resistant



0 18 35 70 140 280 560 1120 2240
2,4-D dose, g ae ha⁻¹

Greenhouse Dose Response 21 DAT



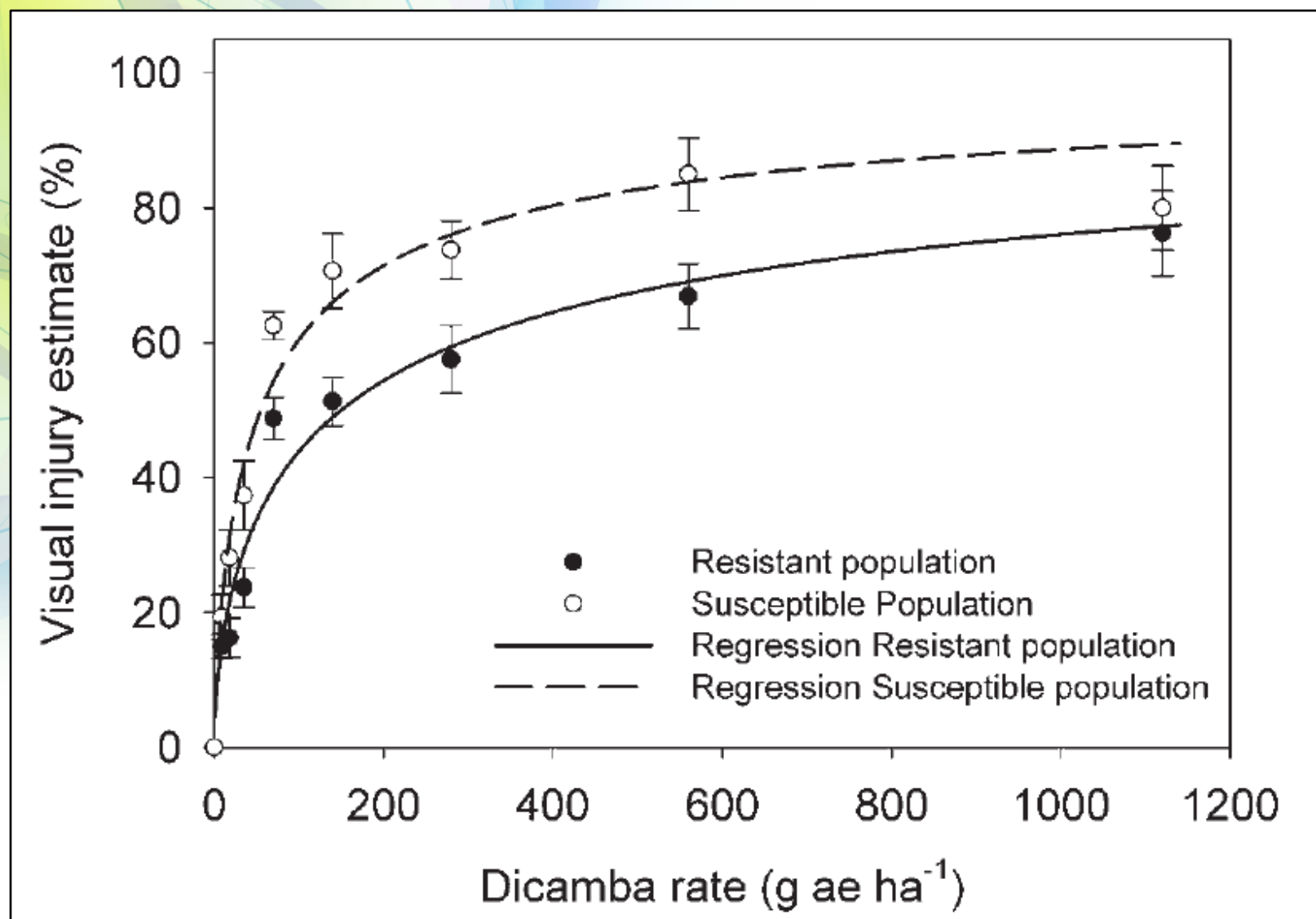
ED50

R:S – 9.1

ED90

R:S – 111

G. Kruger, UNL



Resistance of a Prickly Lettuce (*Lactuca serriola*) Biotype to 2,4-D

Ian C. Burke, Joseph P. Yenish, Dennis Pittmann, and Robert S. Gallagher*

Table 1. Percent fresh weight reduction of four-leaf prickly lettuce at 3 wk after treatment (WAT) with glyphosate and 2,4-D mixtures, averaged across greenhouse experiments.

2,4-D rate	Glyphosate rate	Fresh weight reduction ^a	
		Susceptible	Resistant
g ae/ha		%	
430	0	63 a	9 b
860	0	55 a	2 b
1,720	0	93 a	57 b
0	420	25 a	23 a
0	840	24 a	8 a
0	1,680	85 a	92 a
430	420	83 [*] a	17 b
860	840	92 [*] a	25 b
1,720	1,680	96 a	93 a
LSD (0.05)		7	10

Mecanismos de resistência ao 2,4-D

Nível 1 – resistência na transcrição gênica dos ARFs

- ✓ A planta apresenta epinastia, mas a oxidação do tecido não é acentuado
- ✓ Significa que o complexo SCF TIR/AFB não está realizando a destruição do AUX/IA
- ✓ Dessa forma podem haver alterações nesse complexo enzimático que estão impedindo que o 2,4-D faça a ligação entre o TIR e o Aux/IAA

Nível 2 – resistência na ABP1

- ✓ A nível de membrana plasmática
- ✓ A planta não sofre epinastia mas somente apresenta oxidação dos tecidos

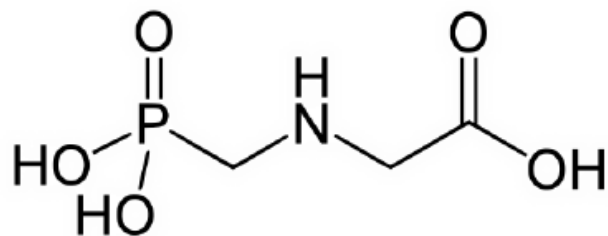
Formulations Interaction Study:

❖ Glyphosate Isopropylamine Salt 356 g ae/L

❖ Glyphosate Ammonium Salt 480 g ae/L

❖ Glyphosate Potassium Salt 506 g ae/L

❖ Glyphosate DMA 480g ae/L



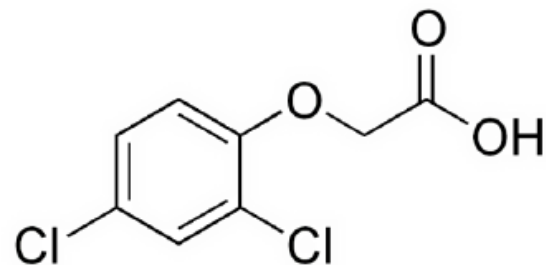
❖ 2,4-D DMA 669 g ae/L

❖ 2,4-D Colex-D 456 g ae/L

For all treatments:

Glyphosate: 280 g a.i./ha

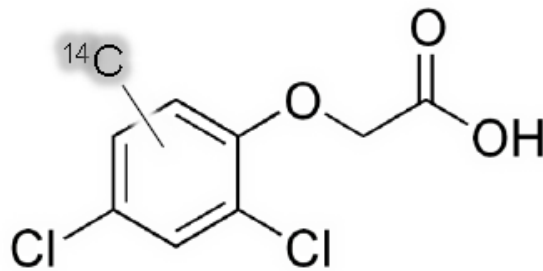
2,4-D: 280 g a.i./ha



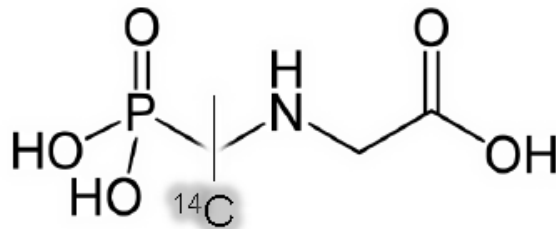
Laboratory Studies:

Formulations Absorption

Plants with six true leaves;



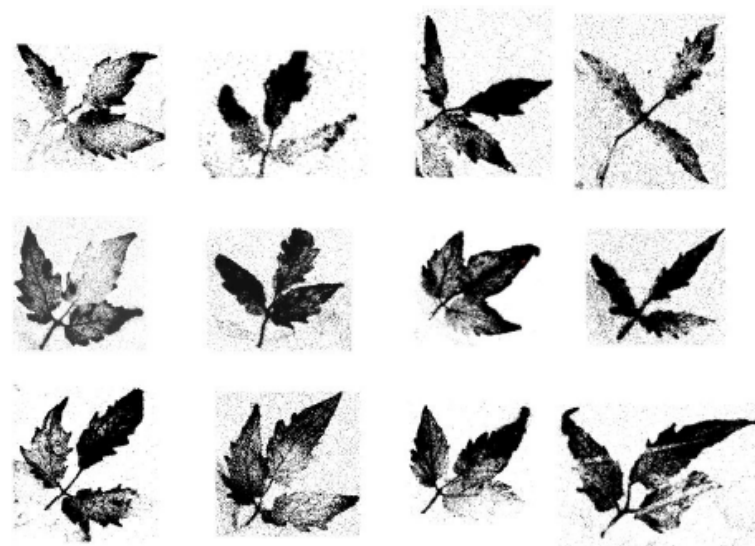
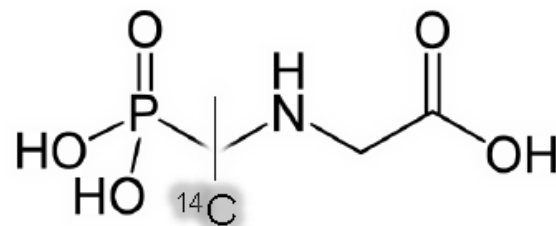
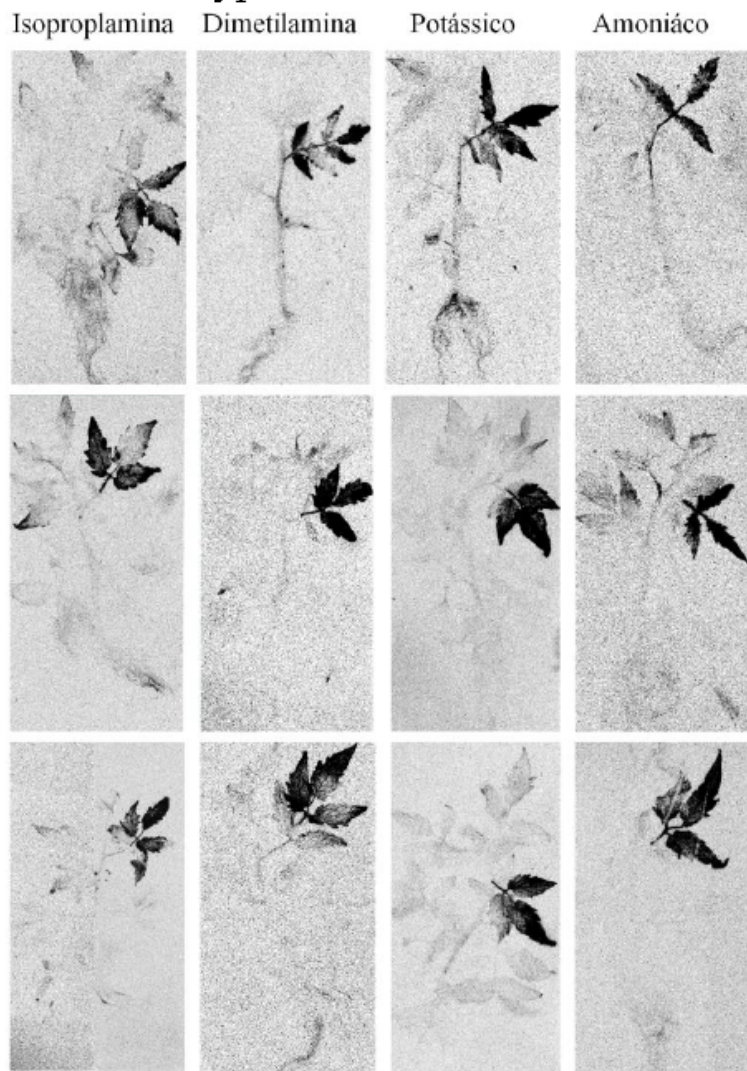
Applied with microsyringe, in 10 different points of the third leaf in the last three leaflets.



Treatments:

- a) ^{14}C -glyphosate
- b) ^{14}C -glyphosate plus 2,4-D
- c) ^{14}C -2,4-D
- d) ^{14}C -2,4-D plus glyphosate

Glyphosate Formulations



Radio-Labeled Glyphosate (280 g a.i. ha⁻¹)

		ISO	DMA	K	NH ₄
		% of absorbed radiation			
2,4-D (280 g a.i. ha ⁻¹)	-----	82.66 A/a	87.08 a	91.14 a	69.29 b
	DMA	86.01 AB	87.12	86.44	73.94
	Choline	90.89 B	88.87	89.18	82.5

Glyphosate Formulations

Isopropilamina

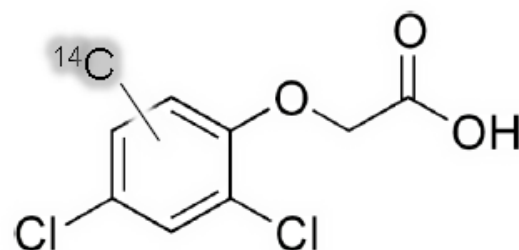
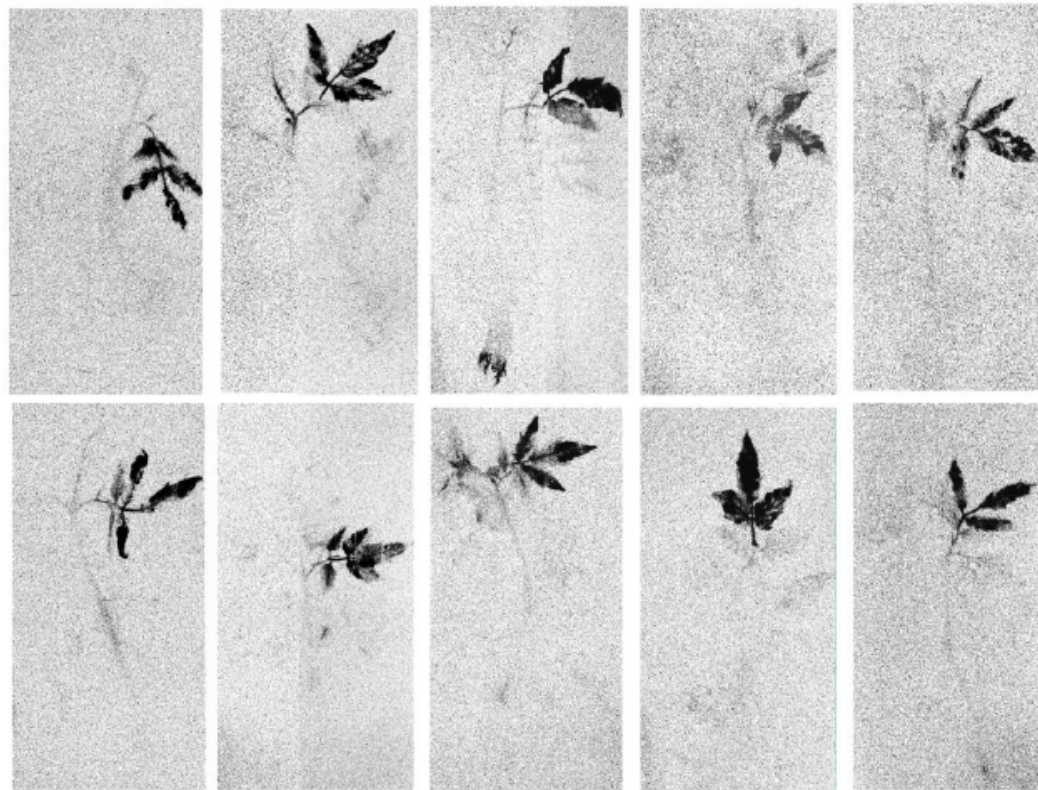
Dimetilamina

Potássico

Amoníaco

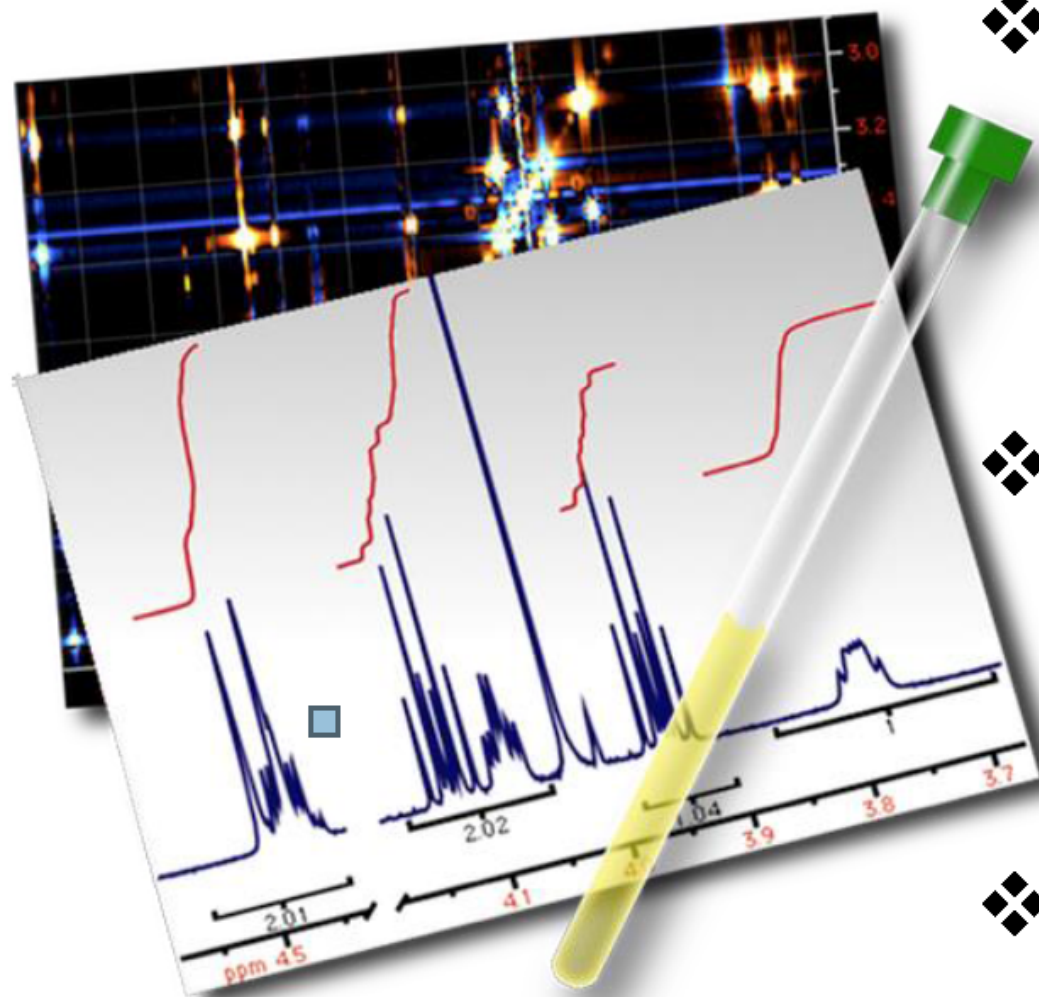
Dimetilamina

Choline



% absorbed radiation		Glyphosate (280 g a.i. ha ⁻¹)				
		-----	ISO	DMA	K	NH ₄
Radio-Labeled	DMA	68.71 A/b	83.86 a	88.42 a	80.51 a	62.52 b
2,4-D	Cholina	79.41 B	92.48	91.56	90.82	76.32
(280 g a.i. ha ⁻¹)						

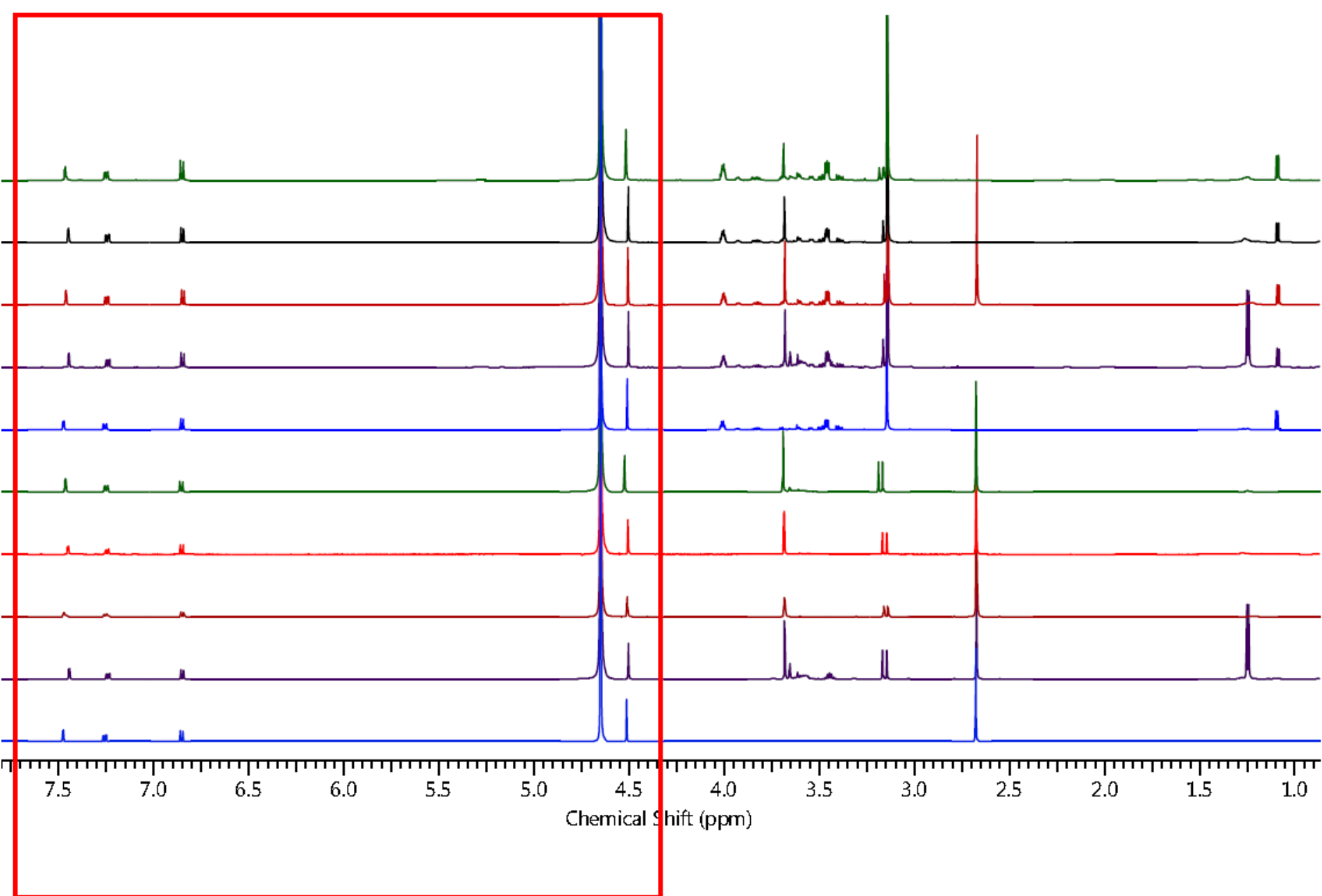
Laboratory Studies: Nuclear Magnetic Resonance



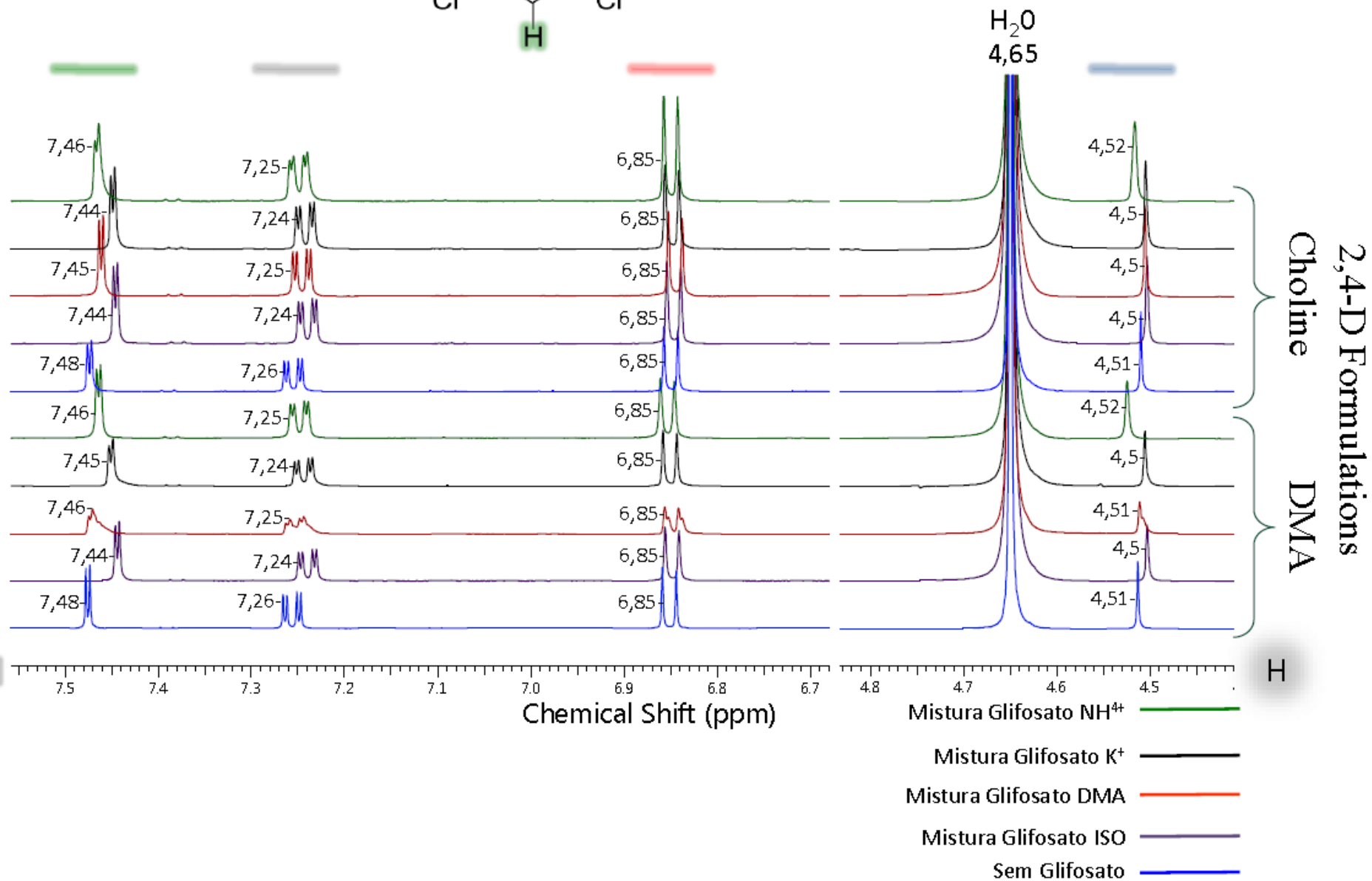
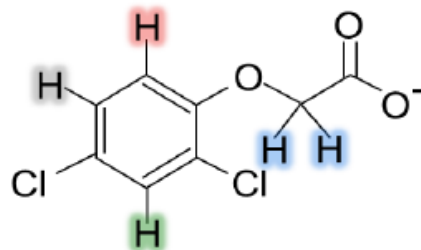
- ❖ Study the atomic environment
- ❖ Glyphosate and 2,4-D formulations;
- ❖ Carrier solvent: deuterium oxide (D_2O).



^1_1H



2,4-D



Observações finais

“The fact that resistance to 2,4-D has evolved in at least one waterhemp population should be emphasized to corn, soybean, and cotton producers to show that proper Stewardship of these new Technologies is critical for maintaining their efficacy”

Bernards et al., 2012

Weed Science 2012 60:379–384



A Waterhemp (*Amaranthus tuberculatus*) Population Resistant to 2,4-D

Mark L. Bernards, Roberto J. Crespo, Greg R. Kruger, Roch Gaussoin, and Patrick J. Tranel*



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19 3429 4190 ramal 209

19 99727 8314

Perguntas?



Thank you

A young tomato plant with several green leaves is growing out of a pot of brown soil. The plant is centered in the frame against a solid black background. The leaves are bright green and have a serrated edge. The stem is thin and upright.

2,4-D on Tomato

Postemergence