



# ECFG15

ROME • ITALY 2020



SAPIENZA  
UNIVERSITÀ DI ROMA

The **Crz1** transcription factor  
regulates lipid metabolism and  
fumonisin production in  
***Fusarium verticillioides***

Marzia Beccaccioli, PhD

# *Fusarium verticillioides* and maize infection

*F. verticillioides* infect the maize and produce mycotoxins

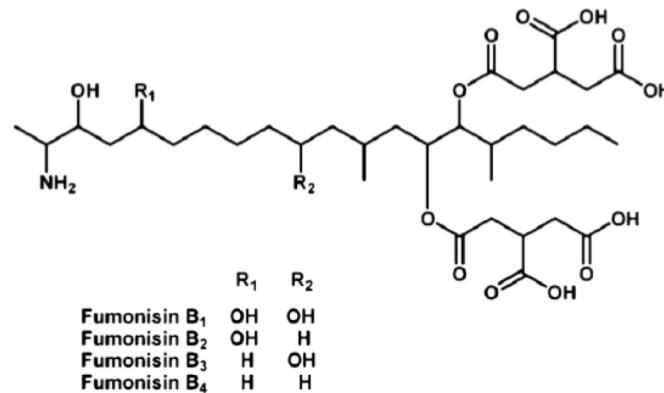
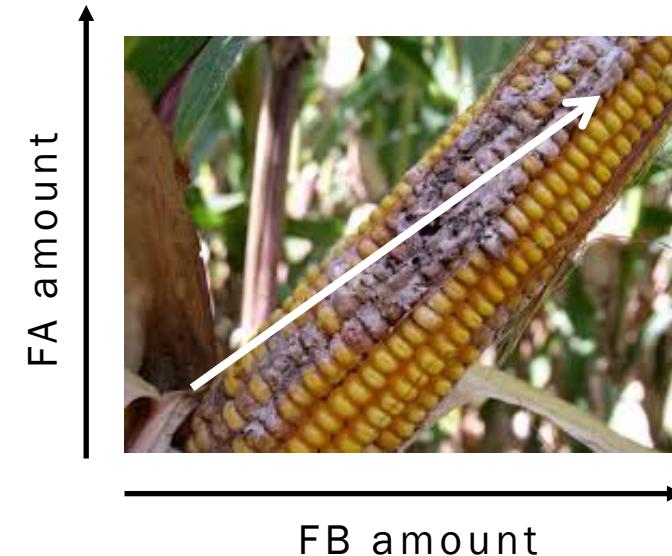


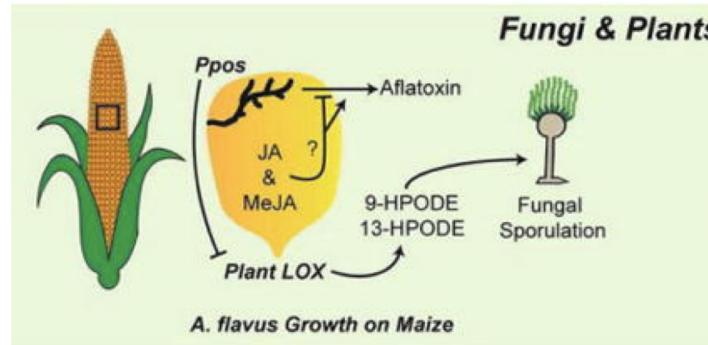
Figure 1. Chemical structures of fumonisins B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, and B<sub>4</sub>.

Fumonisins are positively correlated to the total **fatty acid**: oleic and linoleic acid amounts

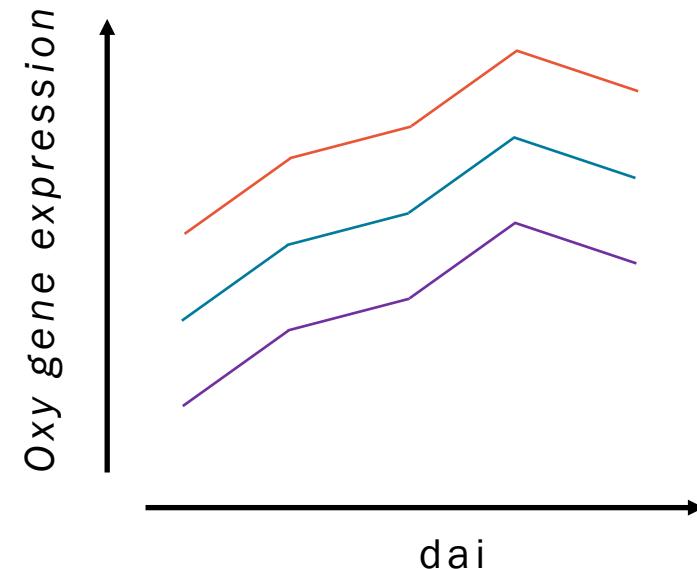


# *Fusarium verticillioides* and maize infection

Oxylipins can regulate the fungal sporulation and the mycotoxin production

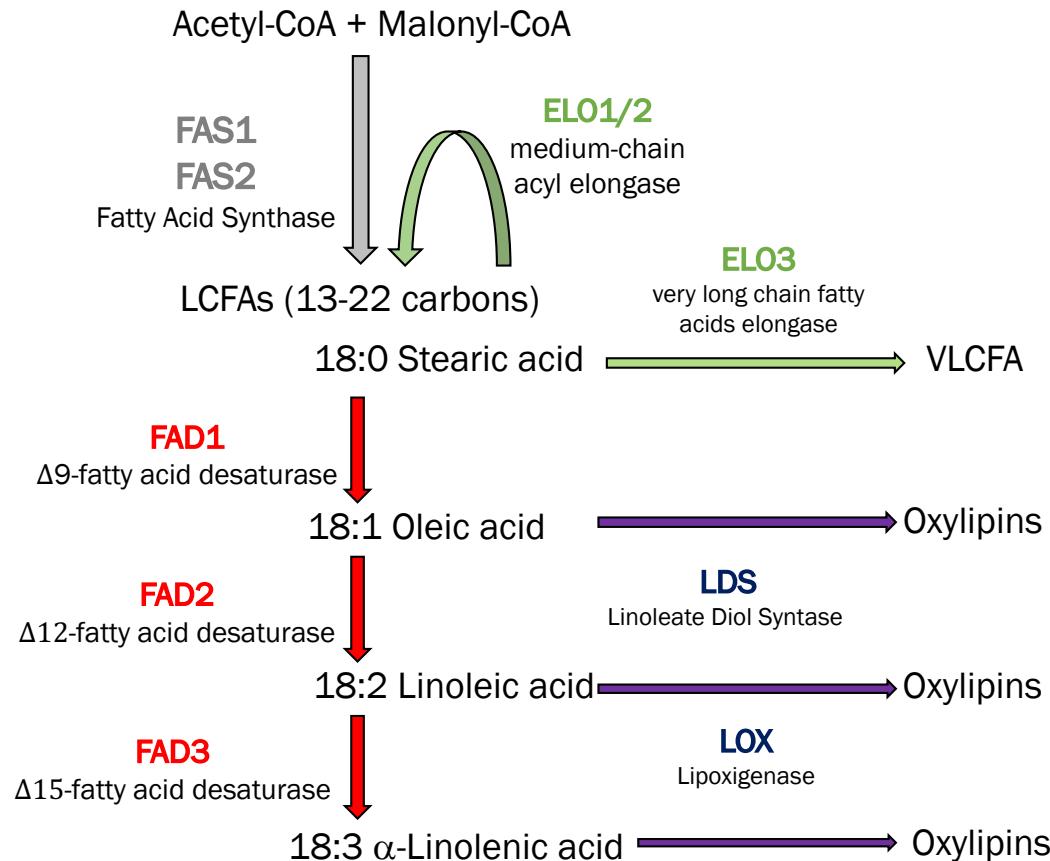


Synchronized expression among the oxylipin-related genes in *F. verticillioides* during maize infection



# Fatty acids and Fatty Acid Related Genes (FARGs) in *F. verticillioides*

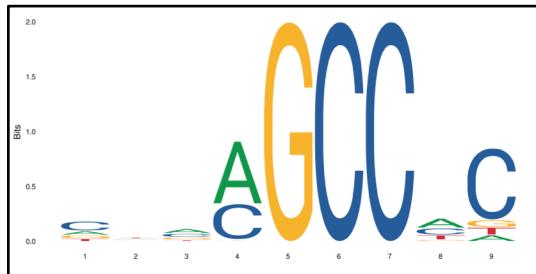
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Is there a transcription factor influencing the  
lipid metabolism?

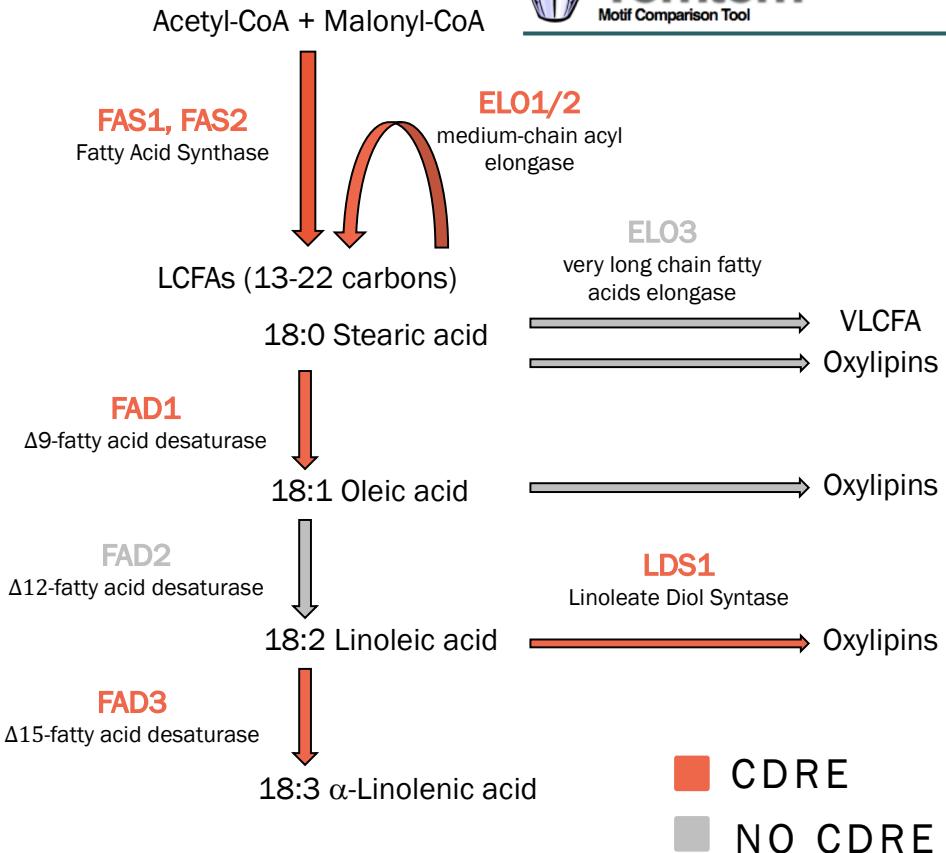
# Research of a common transcription factor

Crazy1 (Crz1) is our best candidate

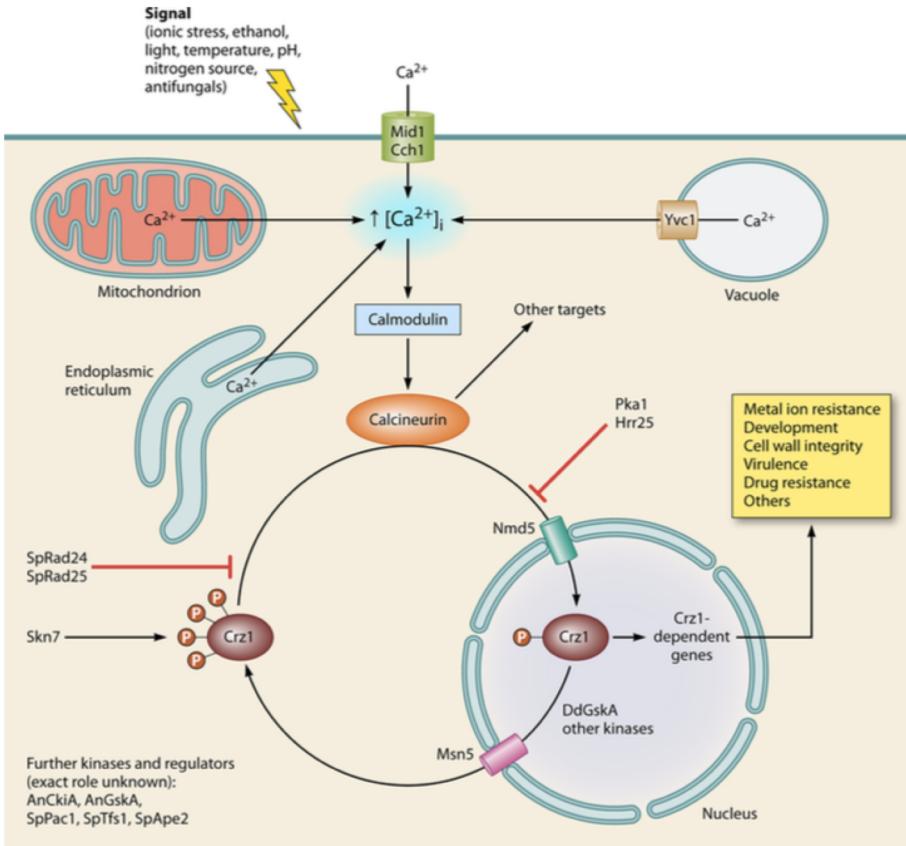


consensus sequence of *S. cerevisiae*

Crz1 binds the **CDREs**  
(calcineurin-dependent response elements)



# The transcription factor Crz1

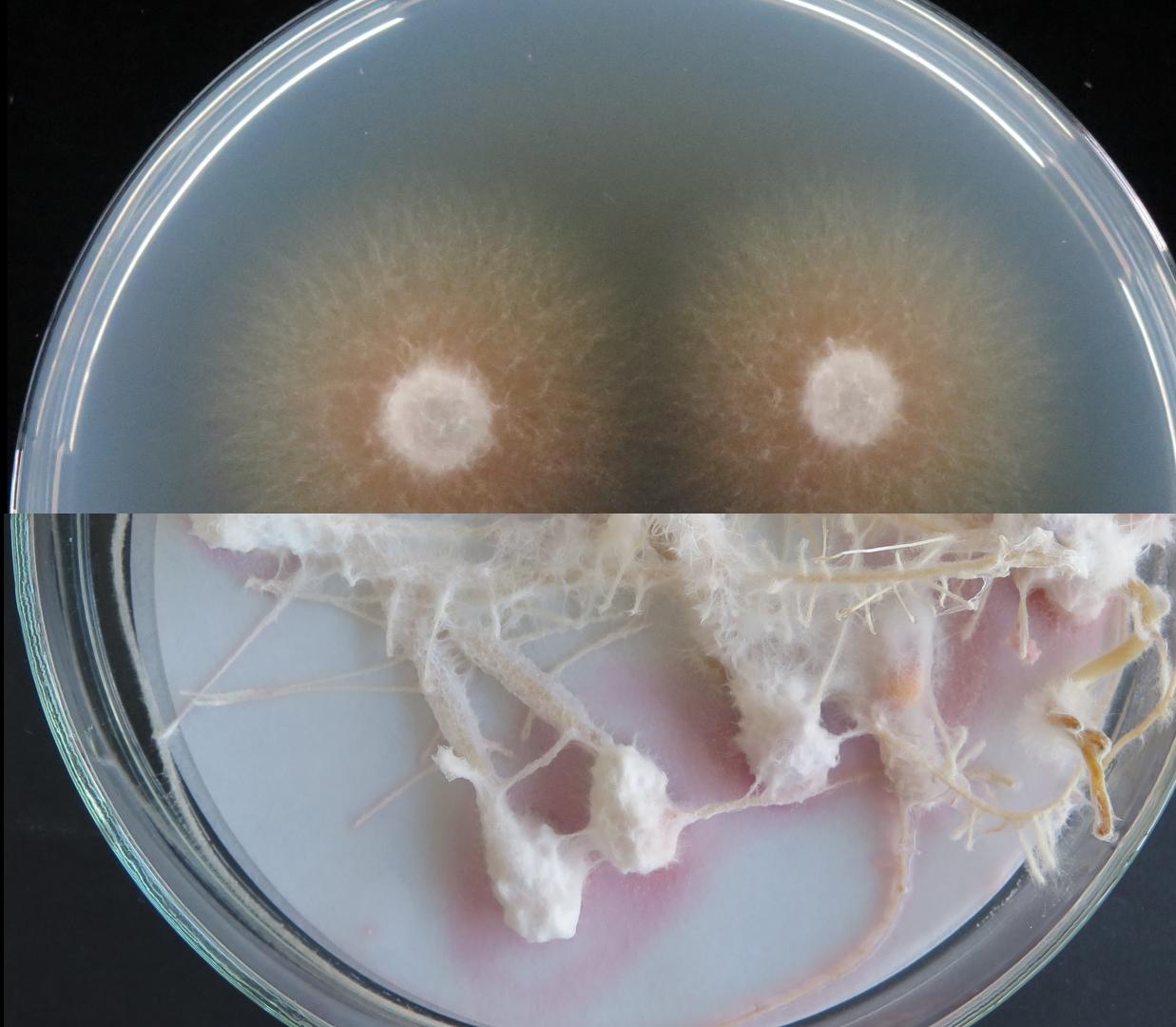


## Crz1 Signalling in lower eukaryotes

- ✓ Cell wall biogenesis
- ✓ Regulation of «filamentous structures»
- ✓ Lipid and sterol metabolism
- ✓ Virulence

*crz1Δ deletion mutant*

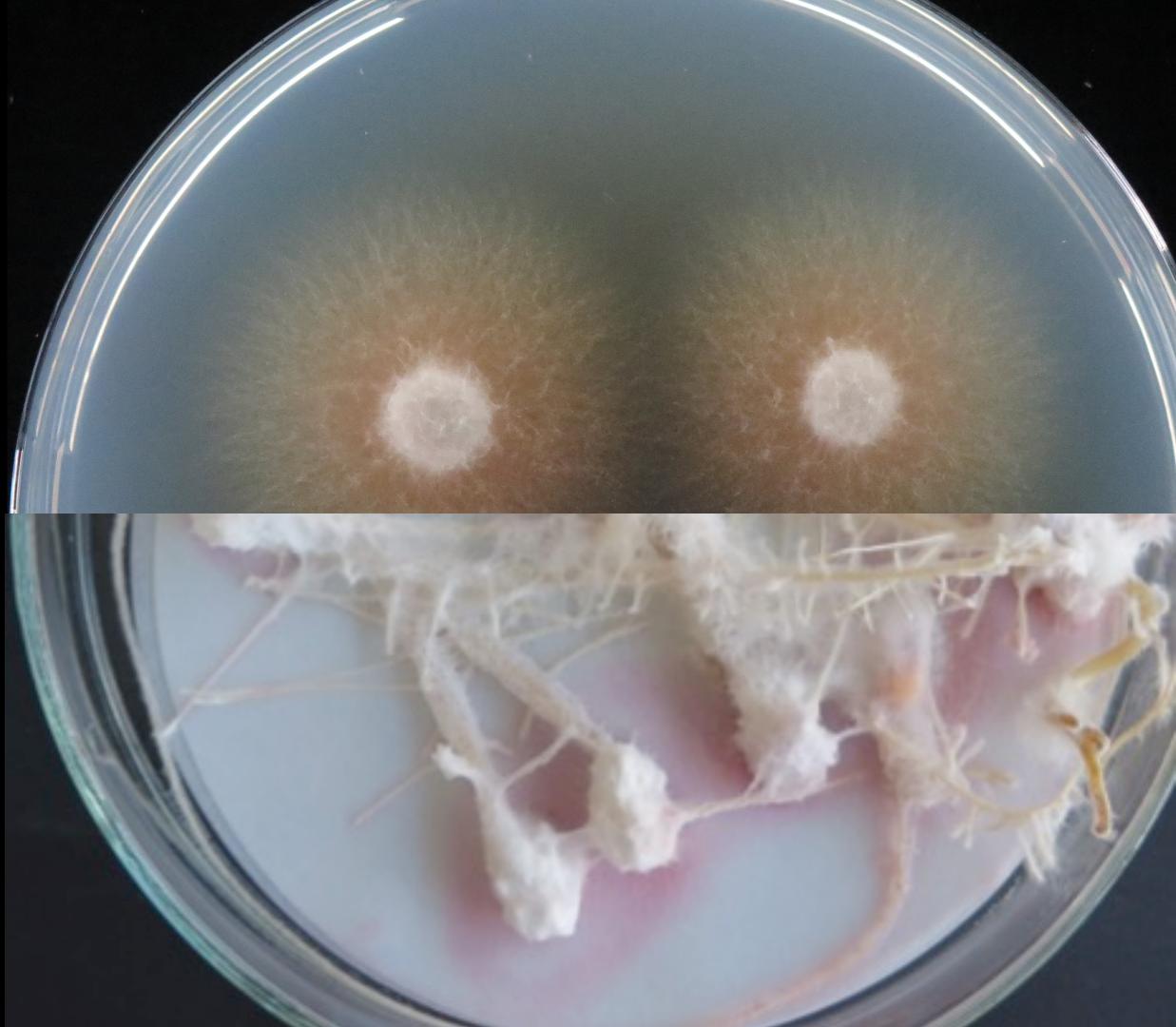
✓ *in vitro* assay



✓ *in vivo* assay

*crz1Δ deletion mutant*

✓ *in vitro assay*



✓ *in vivo assay*

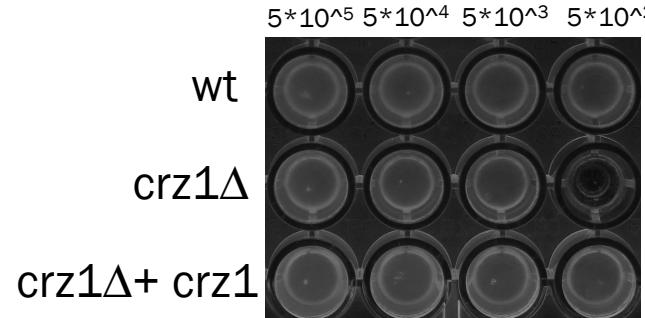
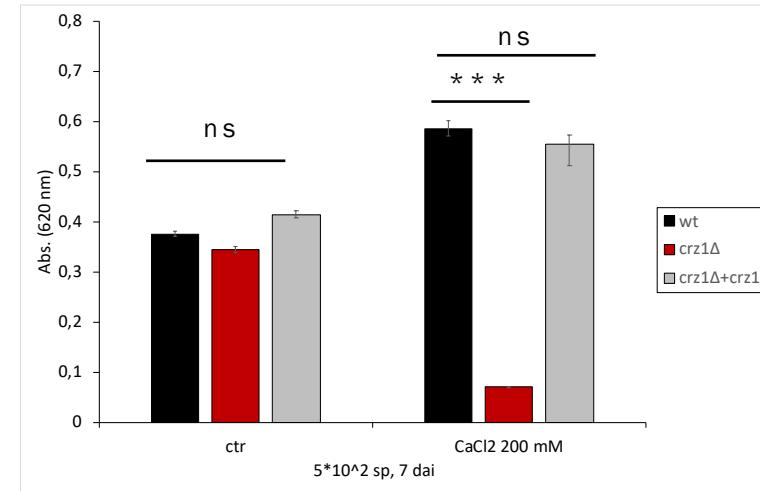
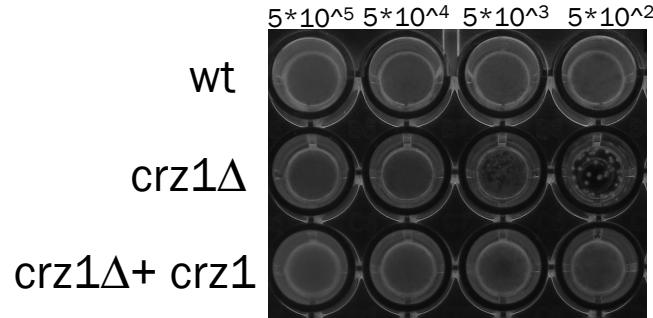
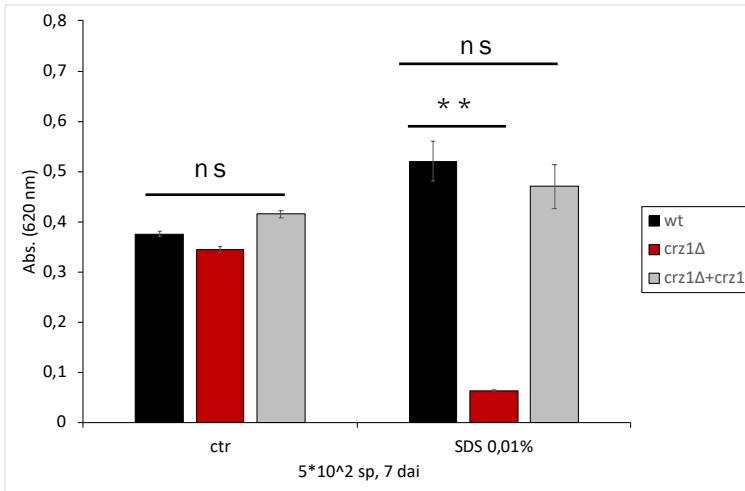
## *in vitro* analysis

1. Phenotypic characterization of mutant strain
2. Expression of FARGs
3. Evaluation of fatty acid content
4. Evaluation of oxylipin content

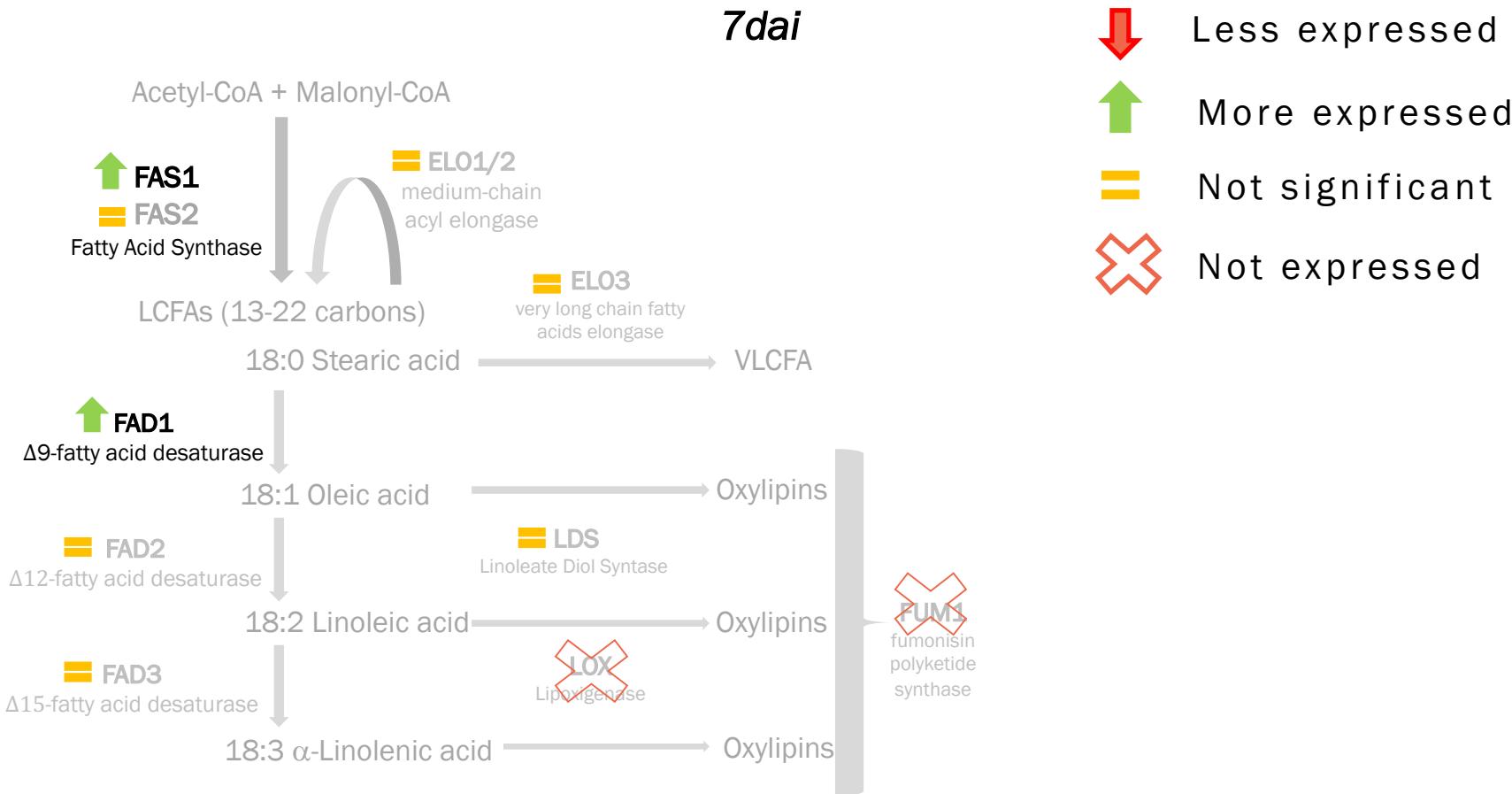


# 1. Phenotypic characterization of the mutant strain

## Multi well test and absorbance measurement



## 2. Expression of FARGs in *crz1Δ*



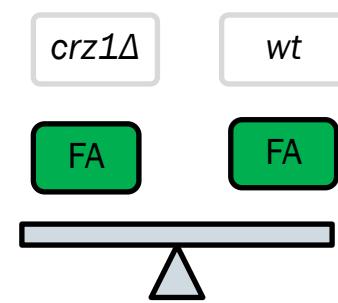
### 3. Evaluation of fatty acid content by LC-MS/MS

*in vitro assay*

7dai	C16:0	C16:1			
wt	18505,27	20611,2			
crz1Δ	15955,6	15240,36	ns		
crz1Δ+crz1	21412,81	25941,54			
	C18:0	C18:1	C18:2	C18:3	
wt	38110,3	35428,91	1114063	26641,09	
crz1Δ	35883,14	35743,96	935470,1	19527,17	ns
crz1Δ+crz1	43397,66	54541,54	1261564	22672,88	
	C22:0	C24:0	C24:1		
wt	274212,1	10242,37	5996,129		
crz1Δ	248362,7	8024,708	6654,588		ns
crz1Δ+crz1	335361,2	11217,76	8746,386		

Fatty acid content is not affected by the deletion of crz1 after 7 dai

ANOVA+Tukey test p<0,05



### 3. Evaluation of oxylipin content by LC-MS/MS

*in vitro* assay

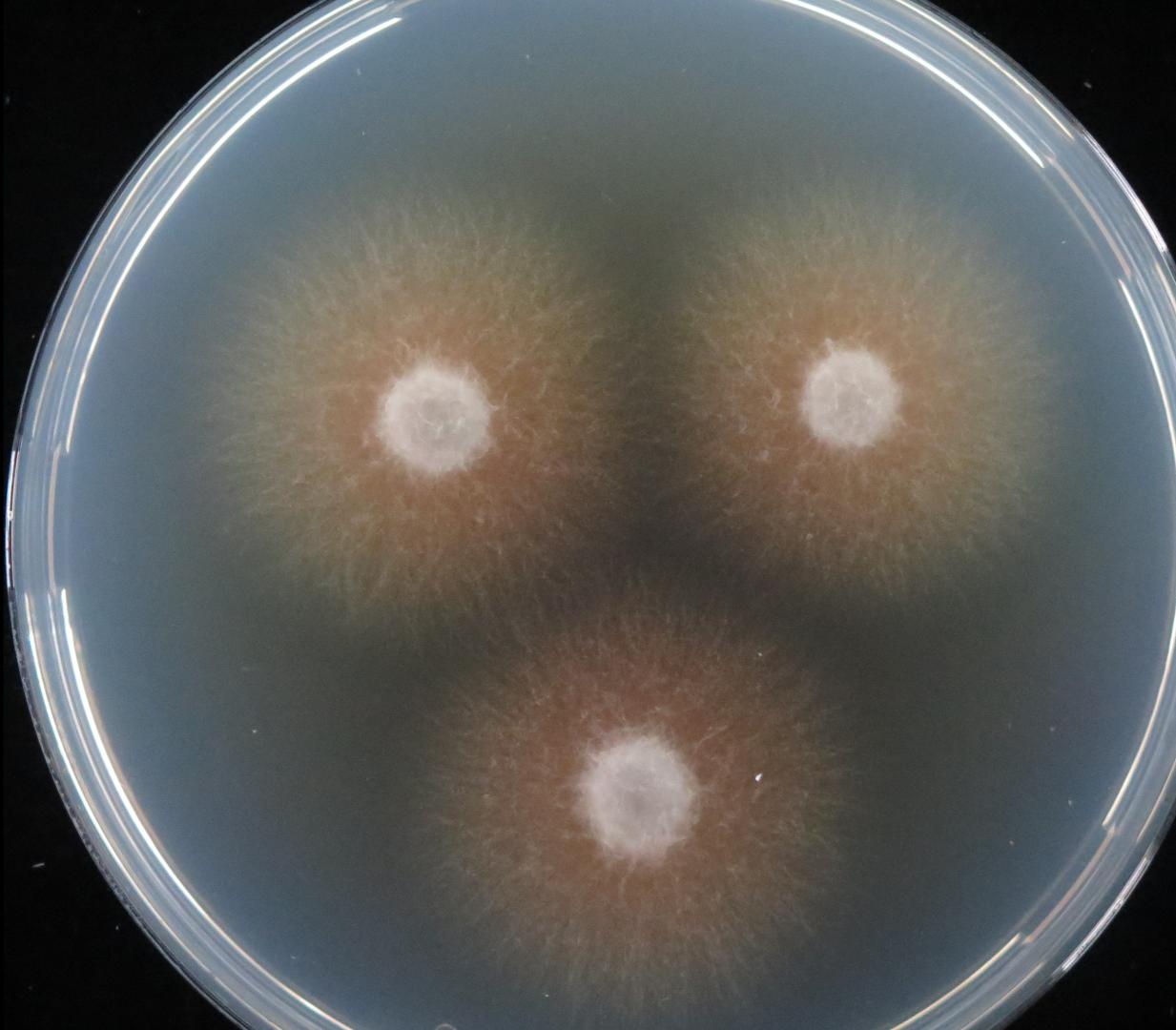
		C18:2 oxylipins						
		12,13-DiHOME	13-HODE	9,10-DiHOME	9-HODE			
7dai		7068,07	27895,69	b	11742,21	a	10963,76	b
wt		3891,41	4788,87	c	4212,63	b	20089,99	a
crz1Δ		5509,82	40133,55	a	10990,45	a	13286,65	ab
crz1Δ+crz1								

- Few oxylipins are formed *in vitro*, and deriving by C18:2
- 9-HODE is more present in the mutant strain

*crz1Δ deletion mutant*

✓ *in vitro* assay

✓ *in vivo* assay



*in vivo assay*



wt



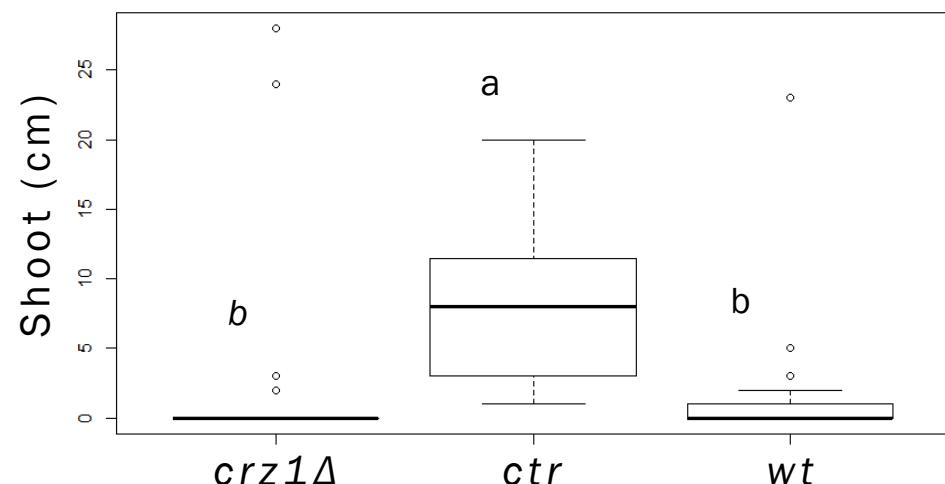
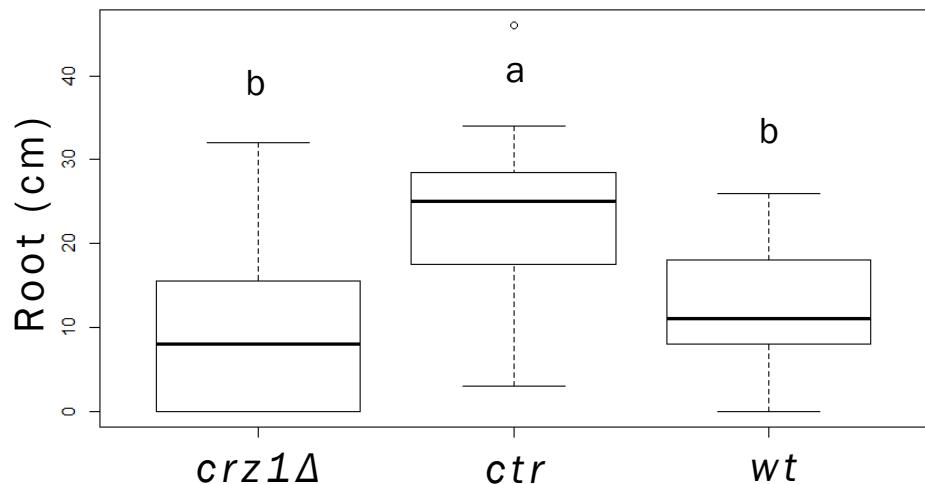
ctr



crz1 $\Delta$

1. Evaluation of root and shoot length
2. Expression of FARGs
3. Evaluation of fatty acid content
4. Evaluation of oxylipin content
5. Mycotoxin quantification

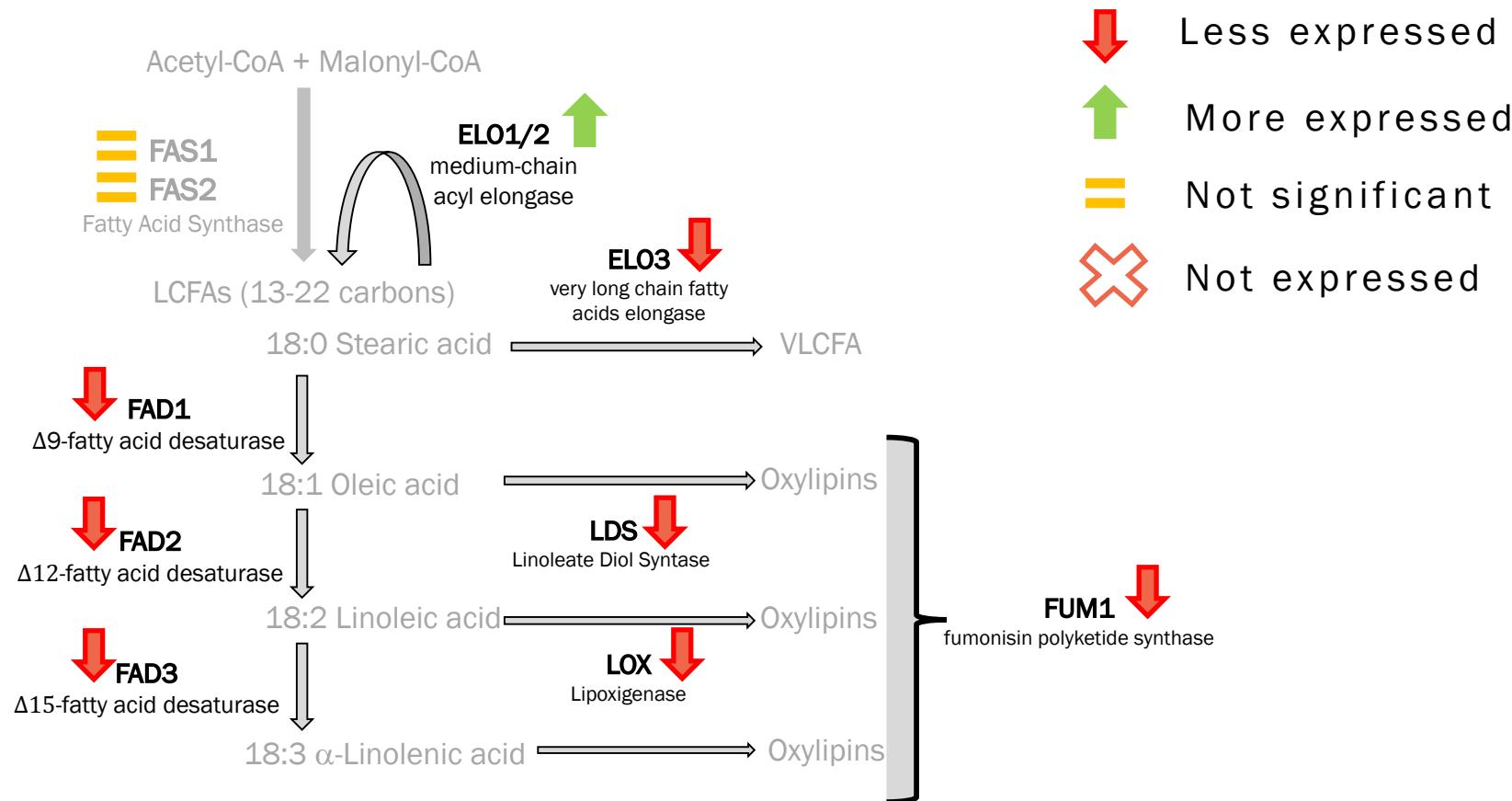
# 1. Evaluation of root and shoot length



The deletion seems not influence the elongation of the roots and shoots

ANOVA+Tukey test  $p<0,05$  \*

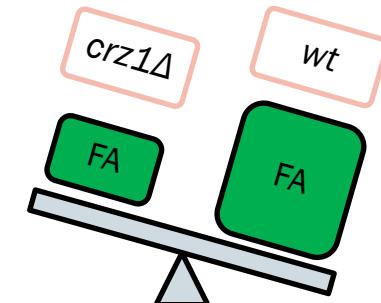
## 2. Expression of FARGs in *Fv crz1Δ*



### 3. Evaluation of fatty acid content by LC-MS/MS

#### *in vivo assay*

	C16:0	C16:1		
ctr	42945,15	38304,71 c		
wt	62415,11 ns	150146,09 a		
crz1Δ	49366,84	62239,79 b		
	C18:0	C18:1	C18:2	C18:3
ctr	231757,51 b	2099025,99 b	10358731,70 c	397810,76 b
wt	1277638,87 a	3579922,01 a	20858932,99 a	1249527,15 a
crz1Δ	437069,87 b	3821183,67 a	15962769,90 b	524905,68 b
	C22:0	C24:0	C24:1	
ctr	11934031,14 b	1054537,77 b	42615,96 b	
wt	17484177,78 a	1989223,42 a	121122,55 a	
crz1Δ	11550367,55 b	1939211,21 a	56406,70 b	



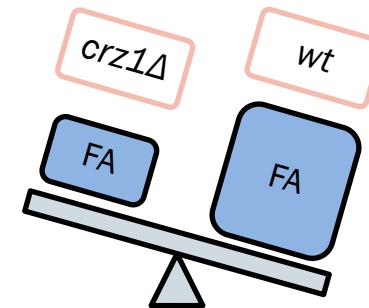
- Fatty acids appear to be affected by the deletion (pink and orange)
- C18:1 and C24:0 are present both in maize kernel infected with *wt* and *crz1Δ* strain (green)

## 4. Evaluation of oxylipin content by LC-MS/MS

*in vivo*

C18:1	ctr	10-HpOME 5408,46	b							
	wt	14900,71	a							
	crz1Δ	5475,03	b							
C18:2	ctr	12,13-DiHOME 52055,94	b	9,10-DiHOME 58420,52038	c	9-HpODE 419263,4	c	9-HODE 230911,5	b	13-HpODE 10302,38
	wt	202590,59	a	343733,2828	a	1389484	a	526858,8	a	15187,82
	crz1Δ	81396,05	b	116756,24	b	692811,9	b	325912,1	b	12354,66
C18:3	ctr	9-HOTrE 11423,41	b	13-HOTrE 122779,0503	b	9-oxoOTrE 26103,44	b			
	wt	20707,01	a	192465,1767	a	47217,42	a			
	crz1Δ	12078,90	b	141929,1348	b	28323,17	b			

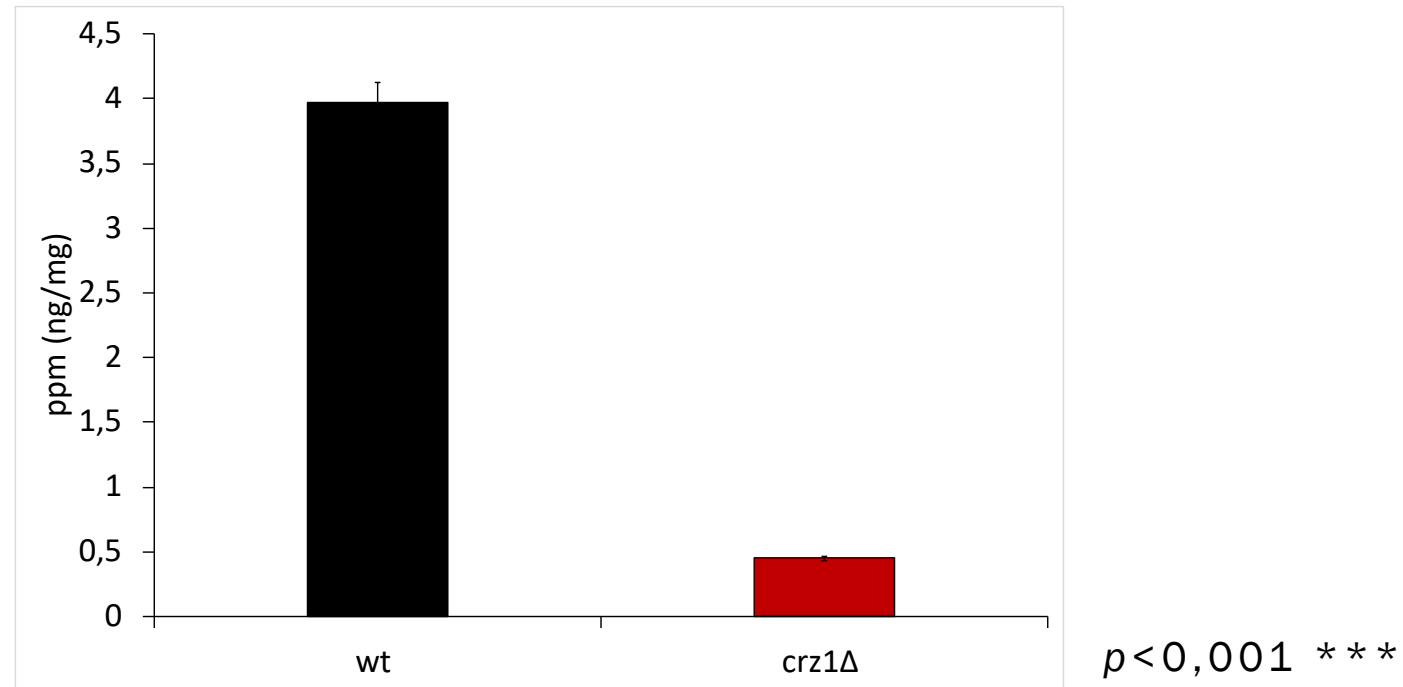
All the oxylipins analysed decrease in the mutant strain (pink and orange )



ANOVA+Tukey test p<0,05

## 5. Mycotoxin quantification

### kernel assay



Mycotoxin content (FB1+FB2) drastically decrease after 14 dai in *crz1Δ* mutant strain

## *In vitro*

- ✓ Mutant strain is more susceptible to the stress but FARGs and lipid are not significantly affected

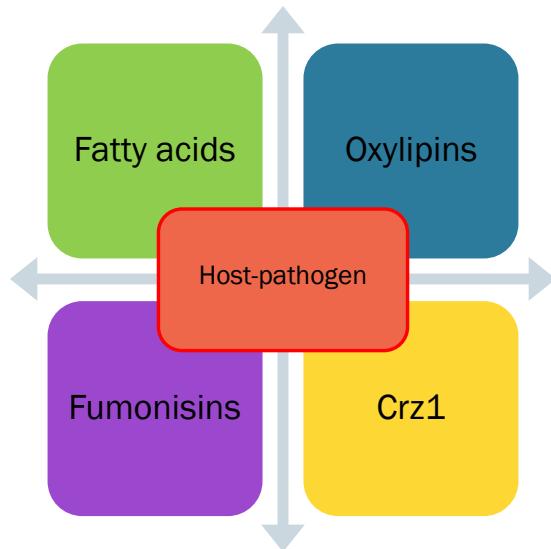
## *Host-pathogen*

- ✓ FARGs expression is reduced in the mutant
- ✓ Fatty acid and oxylipin content is affected by the deletion of *crz1*
- ✓ Lack of fatty acids and oxylipins may influence the production of fumonisins

The transcription factor Crz1, found in *Fusarium verticillioides*, is a novel regulator of lipid metabolism affecting fumonisins during the interaction with the plant host.

# Future perspectives

- *RNAseq analysis for the kernel assay, to understand more about the gene expression*
- *ChIPSeq analysis to identify the binding sites Crz1.*





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Consiglio per la ricerca in agricoltura  
e l'analisi dell'economia agraria



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**Thank you  
for your attention!**



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