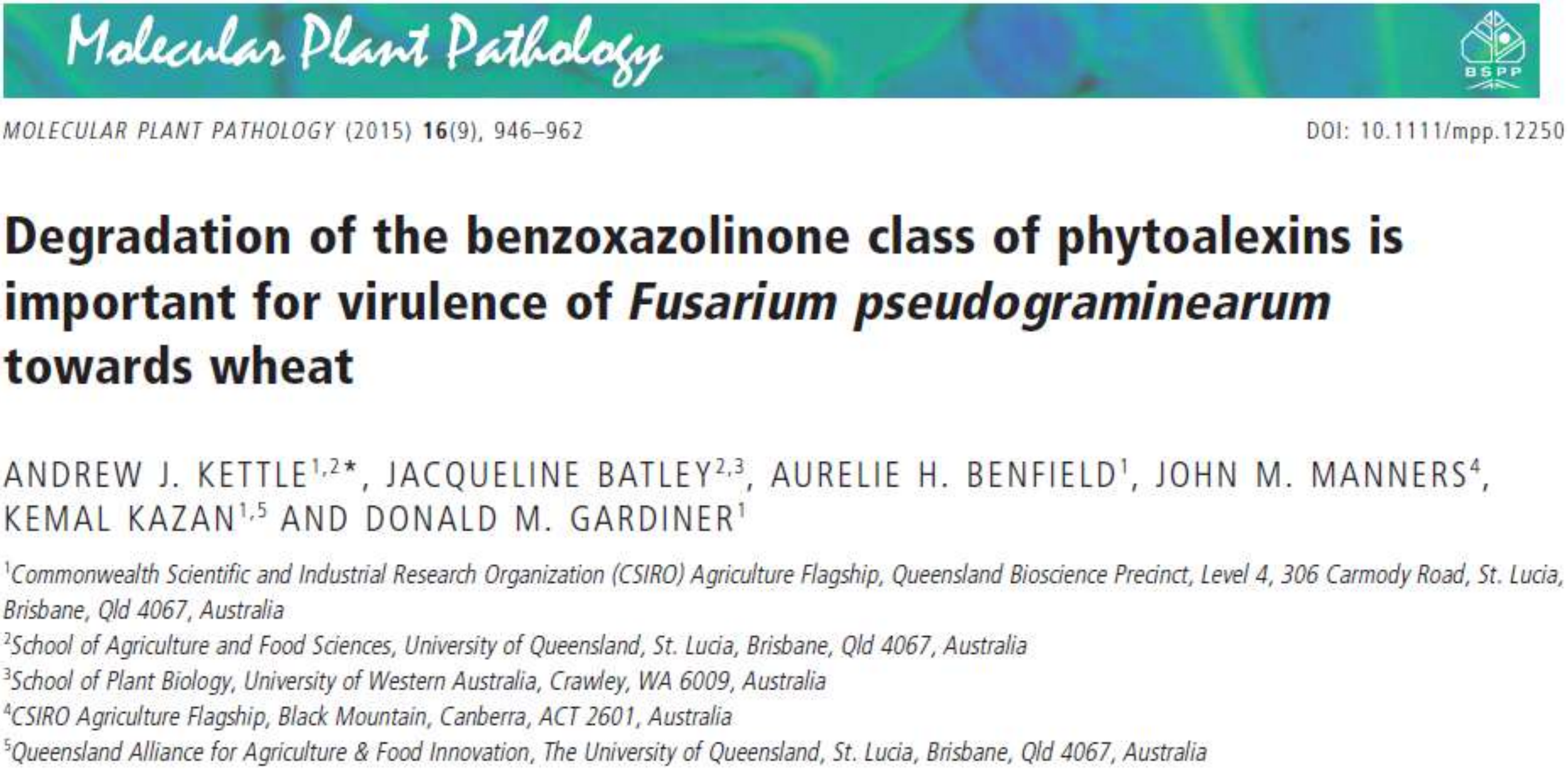

Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2015) **16**(9), 946–962 DOI: 10.1111/mpp.12250

Degradation of the benzoxazolinone class of phytoalexins is important for virulence of *Fusarium pseudograminearum* towards wheat

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Bárbara Marçon

Contextualização

Cevada, trigo e milho (família Poacea) produzem benzoxazolinone, classe de fitoalexinas, contra o ataque de pragas e patógenos. Alguns fungos possuem mecanismos de detoxificação desses compostos, mas isso não significa que este seja um fator de virulência.

Fusarium verticillioides X Milho

Grupo de genes FDB2

Codifica N-malonyltransferase

Envolvida na detoxificação de benzoxazolinones (fitoalexina da família Poaceae)

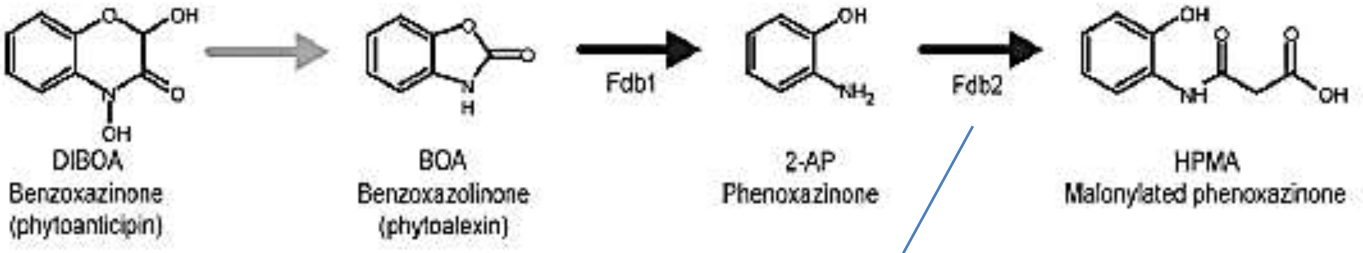
🚫 **Fator de virulência**

São insensíveis às fitoalexinas do trigo ou superam sua ação tóxica pela detoxificação?

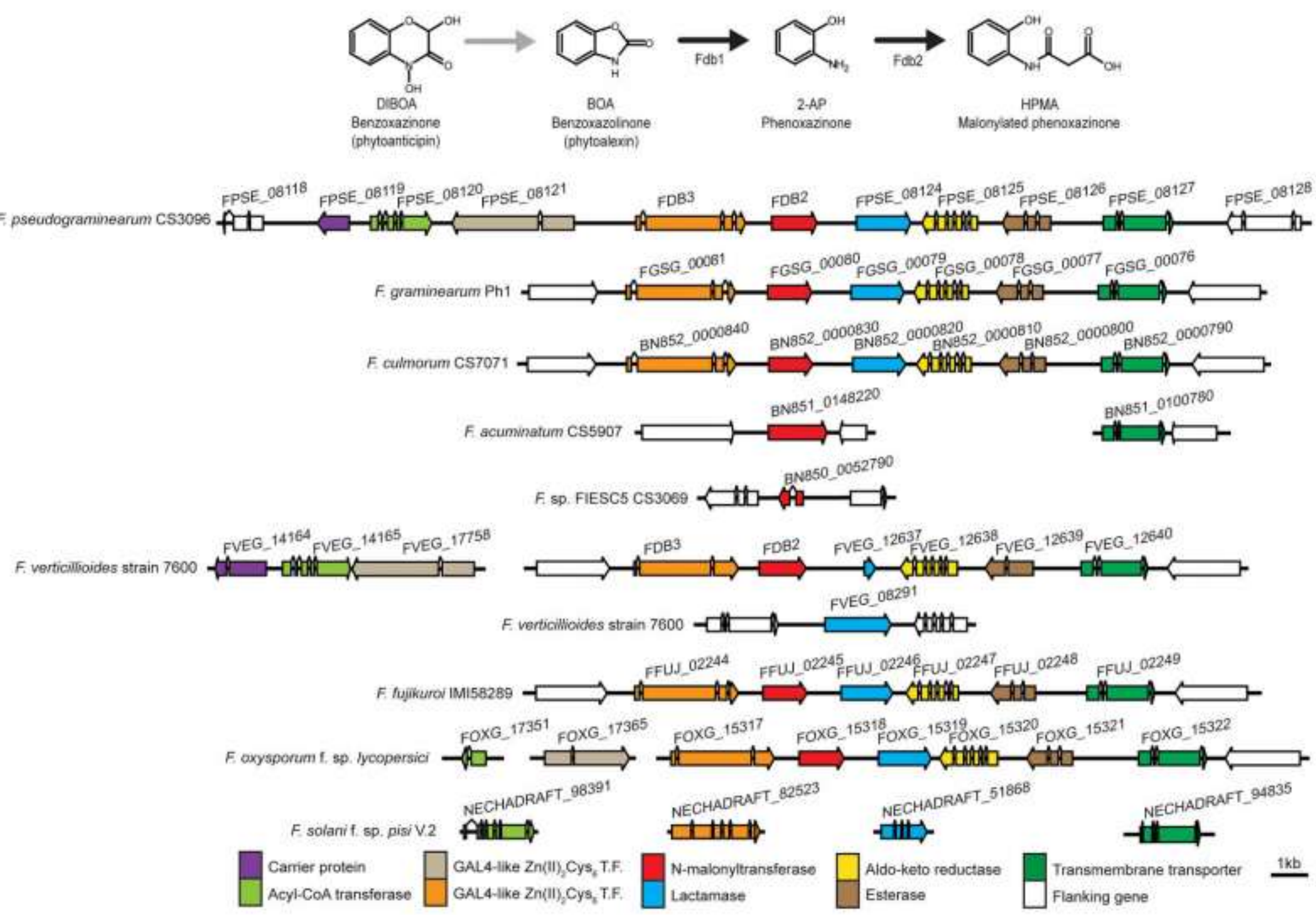
Benzoxazolinones mais abundantes:
Bezoxazolin-2-one – **BOA**
6-methoxy-benzoxazolin-2-one - **MBOA**

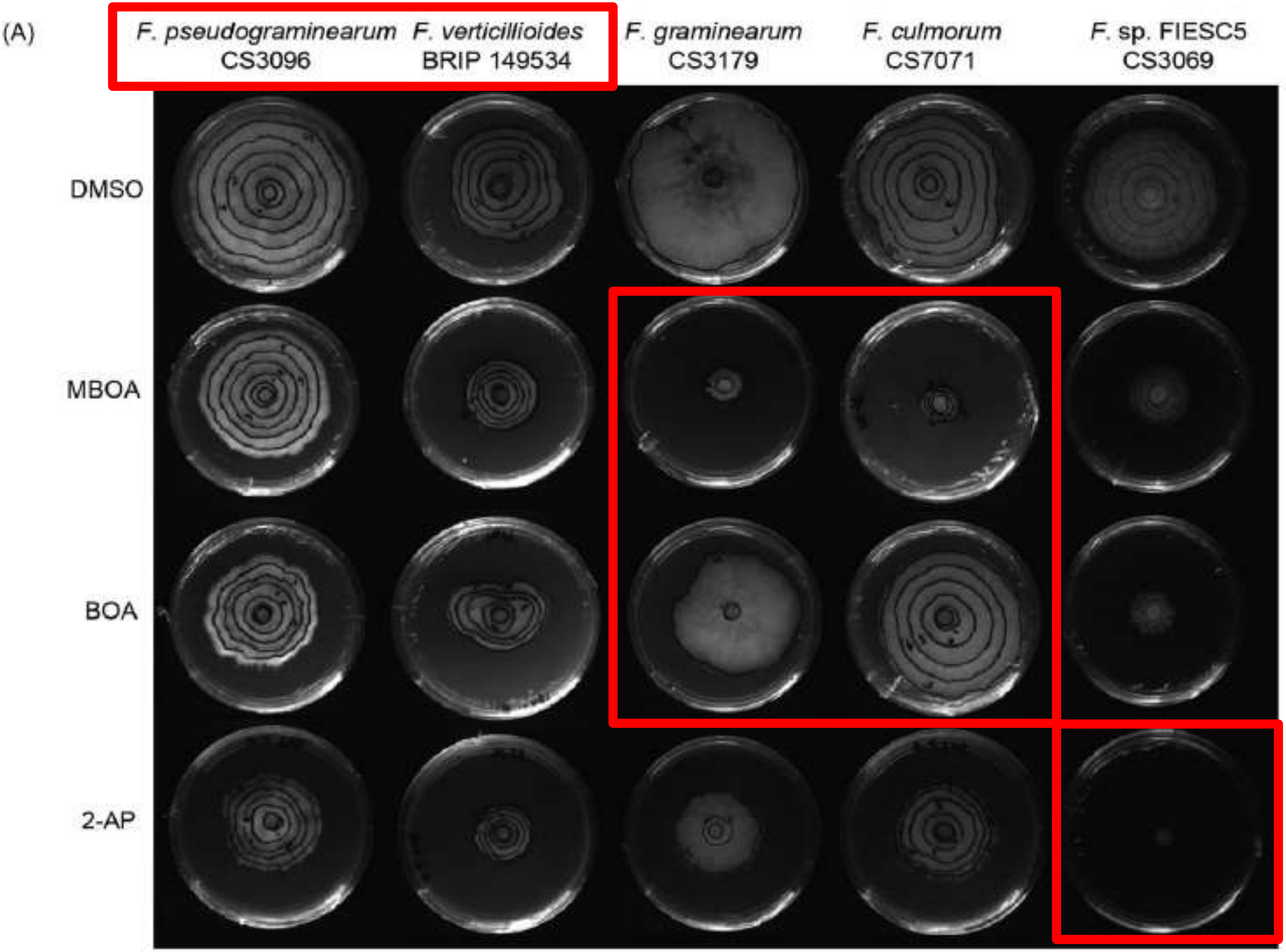
Reação de detoxificação de BOA e MBOA no patossistema *F. verticillioides* X milho

Tóxico → não tóxico


OH
O
N
OH
O
DIBOA
Benzoxazinone
(phytoanticipin)
O
N
H
O
BOA
Benzoxazolinone
(phytoalexin)
Fdb1
OH
NH2
2-AP
Phenoxazinone
Fdb2
OH
NH
O
O
OH
HPMA
Malonylated phenoxazinone

Codifica N-malonyltransferase


DIBOA
Benzoxazinone
(phytoanticipin)
BOA
Benzoxazolinone
(phytoalexin)
Fdb1
2-AP
Phenoxazinone
Fdb2
HPMA
Malonylated phenoxazinone
F. pseudograminearum CS3096
FPSE_08118
FPSE_08119
FPSE_08120
FPSE_08121
FDB3
FDB2
FPSE_08124
FPSE_08125
FPSE_08126
FPSE_08127
FPSE_08128
F. graminearum Ph1
FGSG_00081
FGSG_00080
FGSG_00079
FGSG_00078
FGSG_00077
FGSG_00076
F. culmorum CS7071
BN852_0000840
BN852_0000830
BN852_0000820
BN852_0000810
BN852_0000800
BN852_0000790
F. acuminatum CS5907
BN851_0148220
BN851_0100780
F. sp. FIESC5 CS3069
BN850_0052790
F. verticillioides strain 7600
FVEG_14164
FVEG_14165
FVEG_17758
FDB3
FDB2
FVEG_12637
FVEG_12638
FVEG_12639
FVEG_12640
F. verticillioides strain 7600
FVEG_08291
F. fujikuroi IMI58289
FFUJ_02244
FFUJ_02245
FFUJ_02246
FFUJ_02247
FFUJ_02248
FFUJ_02249
F. oxysporum f. sp. lycopersici
FOXG_17351
FOXG_17365
FOXG_15317
FOXG_15318
FOXG_15319
FOXG_15320
FOXG_15321
FOXG_15322
F. solani f. sp. pisi V.2
NECHADRAFT_98391
NECHADRAFT_82523
NECHADRAFT_51868
NECHADRAFT_94835
Carrier protein
Acyl-CoA transferase
GAL4-like Zn(II)2Cys6 T.F.
GAL4-like Zn(II)2Cys6 T.F.
N-malonyltransferase
Lactamase
Aldo-keto reductase
Esterase
Transmembrane transporter
Flanking gene
1kb


(A)
F. pseudograminearum CS3096
F. verticillioides BRIP 149534
F. graminearum CS3179
F. culmorum CS7071
F. sp. FIESC5 CS3069
DMSO
MBOA
BOA
2-AP

Sensibilidade das espécies: 5 dias na presença de 0,5 mg mL^{-1} de BOA e MBOA

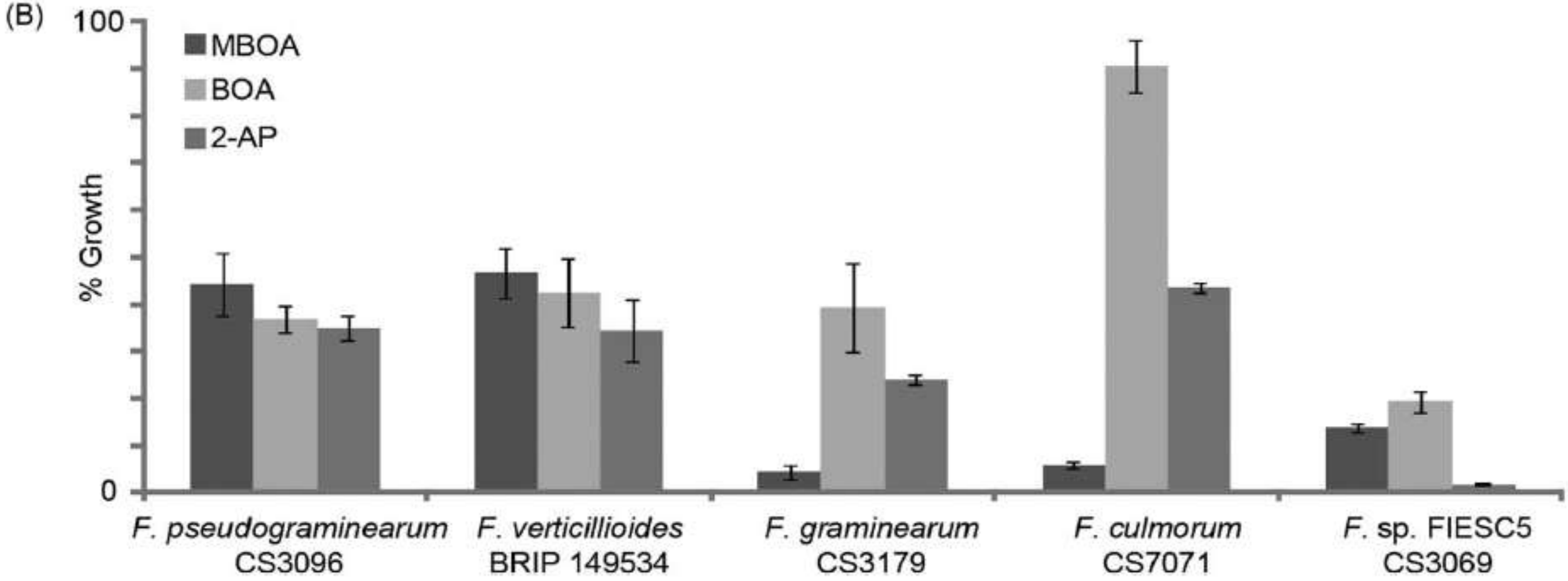
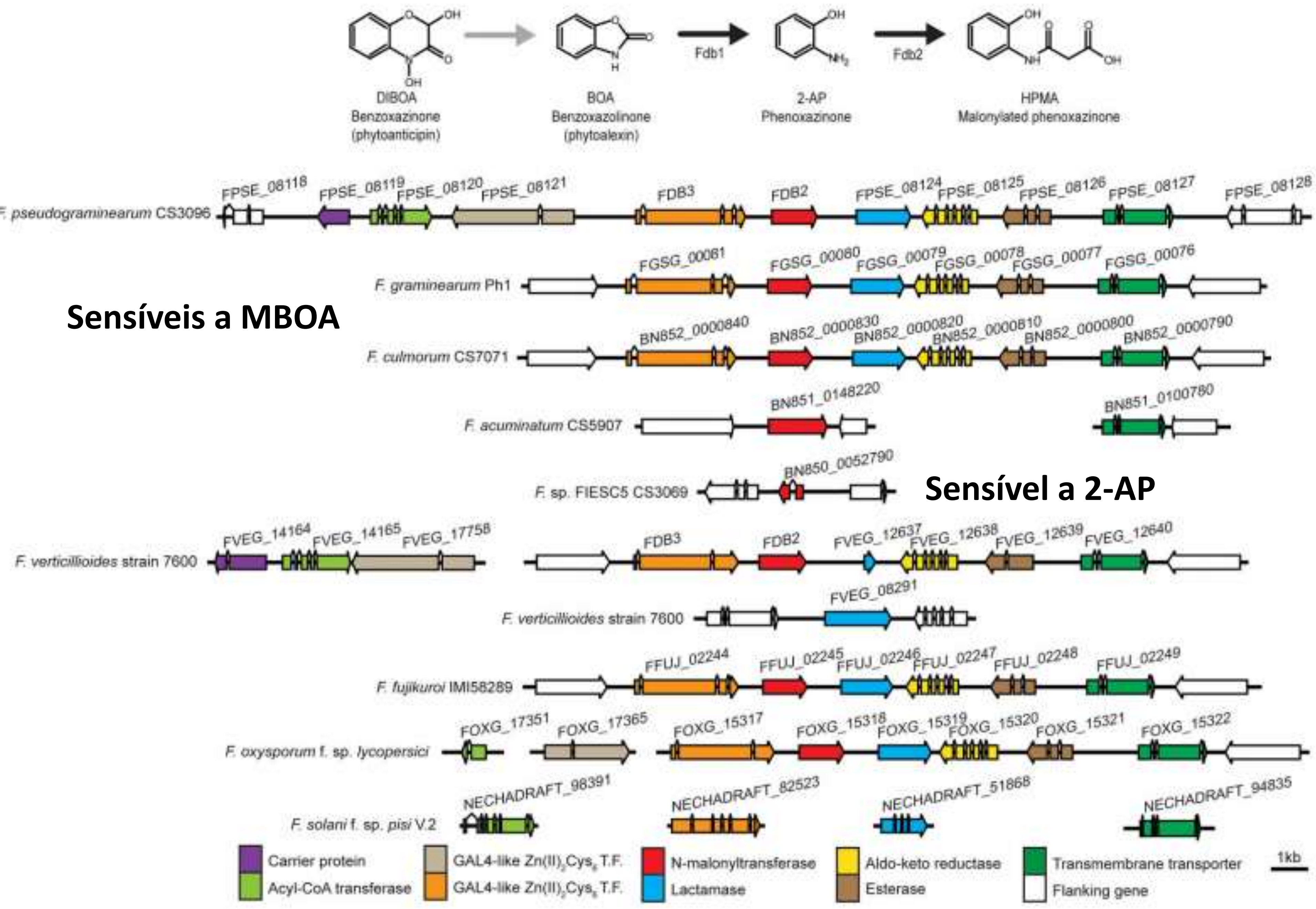


Fig. 2 Relative growth of wheat-associated fusaria in the presence of benzoxazolin-2-one (BOA), 6-methoxy-benzoxazolin-2-one (MBOA) and 2-aminophenol (2-AP). (A) Example photographs of *Fusarium pseudograminearum* (CS3096), *F. culmorum* (CS7071), *F. verticillioides* (BRIP 149534), *F. graminearum* (CS3179) and *F.* sp. FIESC5 (CS3069) grown on benzoxazolinones- and BOA detoxification intermediate (2-AP)-amended half-strength potato dextrose agar (PDA). Rings marked on the plates indicate the daily hyphal growth edge positions. *Fusarium graminearum* (CS3179) marked at 2 and 5 days post-inoculation (dpi) only. All compounds were tested at 0.5 mg/mL, with an equal percentage (v/v) of dimethylsulfoxide (DMSO) used as a control. (B) Quantification of relative growth rates of five *Fusarium* spp. when grown for 5 days on half-strength PDA amended with BOA, MBOA, 2-AP or DMSO only. Error bars represent the standard error of the mean for five biological replicates.


DIBOA
Benzoxazinone
(phytoanticipin)
BOA
Benzoxazolinone
(phytoalexin)
Fdb1
2-AP
Phenoxazinone
Fdb2
HPMA
Malonylated phenoxazinone
F. pseudograminearum CS3096
FPSE_08118
FPSE_08119
FPSE_08120
FPSE_08121
FDB3
FDB2
FPSE_08124
FPSE_08125
FPSE_08126
FPSE_08127
FPSE_08128
F. graminearum Ph1
FGSG_00081
FGSG_00080
FGSG_00079
FGSG_00078
FGSG_00077
FGSG_00076
F. culmorum CS7071
BN852_0000840
BN852_0000830
BN852_0000820
BN852_0000810
BN852_0000800
BN852_0000790
F. acuminatum CS5907
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F. sp. FIESC5 CS3069
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FVEG_17758
FDB3
FDB2
FVEG_12637
FVEG_12638
FVEG_12639
FVEG_12640
F. verticillioides strain 7600
FVEG_08291
F. fujikuroi IMI58289
FFUJ_02244
FFUJ_02245
FFUJ_02246
FFUJ_02247
FFUJ_02248
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F. oxysporum f. sp. lycopersici
FOXG_17351
FOXG_17365
FOXG_15317
FOXG_15318
FOXG_15319
FOXG_15320
FOXG_15321
FOXG_15322
F. solani f. sp. pisi V.2
NECHADRAFT_98391
NECHADRAFT_82523
NECHADRAFT_51868
NECHADRAFT_94835
Carrier protein
Acyl-CoA transferase
GAL4-like Zn(II)2Cys6 T.F.
GAL4-like Zn(II)2Cys6 T.F.
N-malonyltransferase
Lactamase
Aldo-keto reductase
Esterase
Transmembrane transporter
Flanking gene
1kb

Sensíveis a MBOA

Sensível a 2-AP

A gene cluster involved in benzoxazolinone detoxification is conserved in multiple (7) *Fusarium* species

- *FDB3*, encoding a GAL4-like Zn(II)2Cys6 **transcription factor** proposed to **regulate the *FDB* cluster or other responses to a benzoxazolinone signal**

- *FDB2*, encoding an *N*-malonyltransferase

- *FPSE_08124*, encoding a lactamase probably performing the function of Fdb1

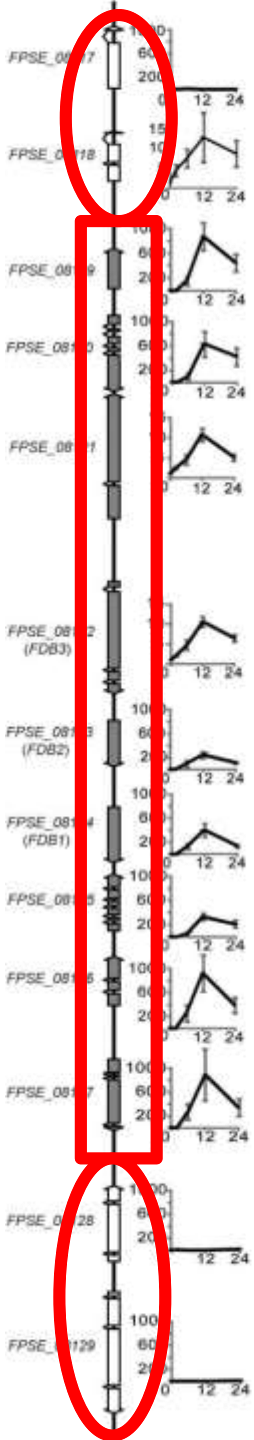
- *FPSE_08125*, encoding an aldo–keto reductase possibly associated with a ketone component of **BOA and MBOA degradation**

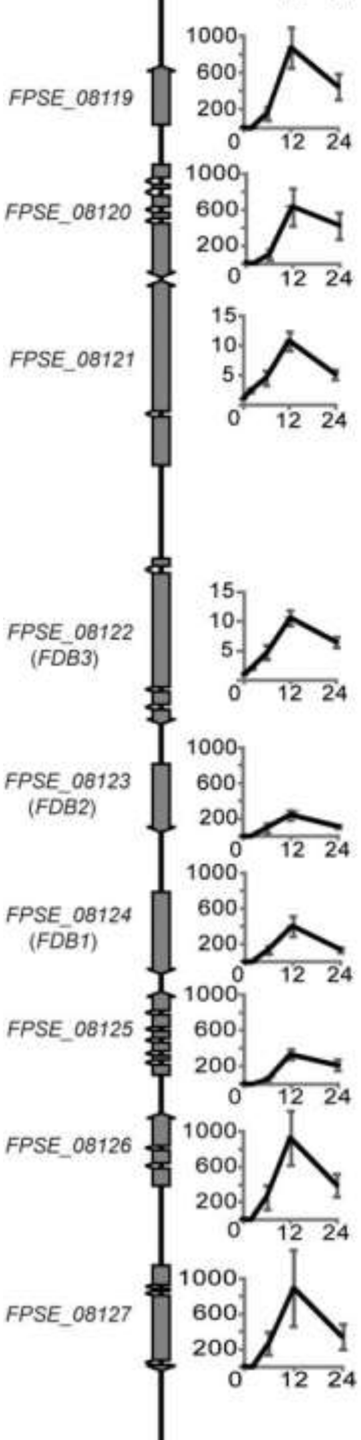
- *FPSE_08126*, encoding an esterase

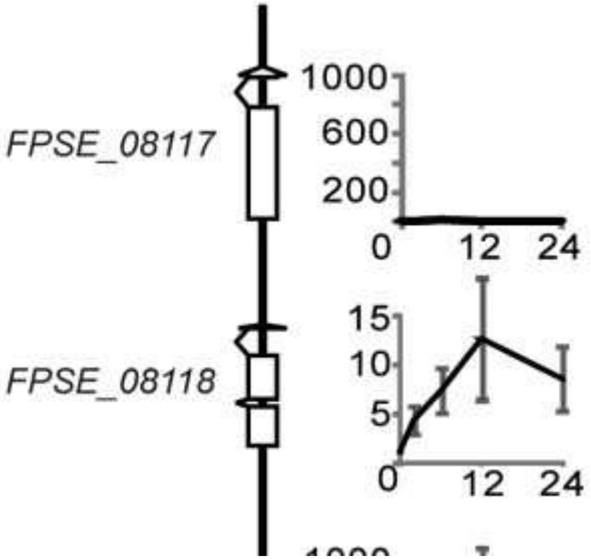
- *FPSE_08127*, encoding a **transmembrane transporter** proposed to shuttle **metabolites of benzoxazolinone degradation**

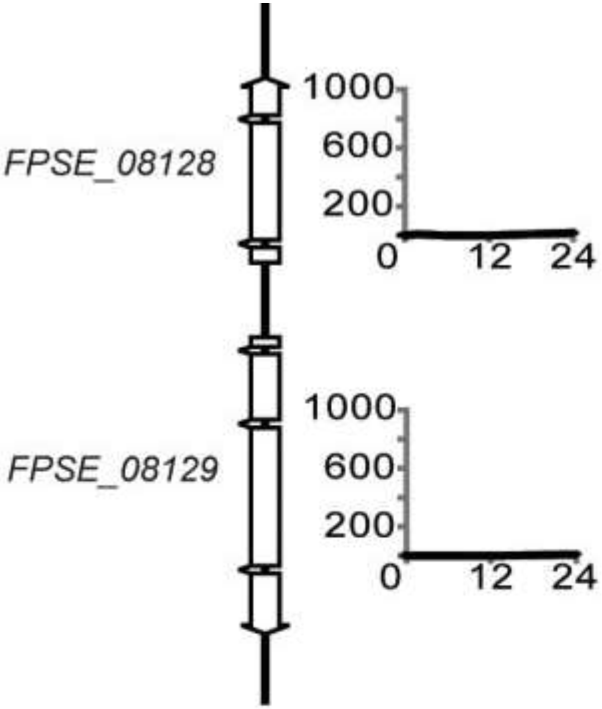
The *FDB* cluster genes show significant co-expression patterns after BOA treatment

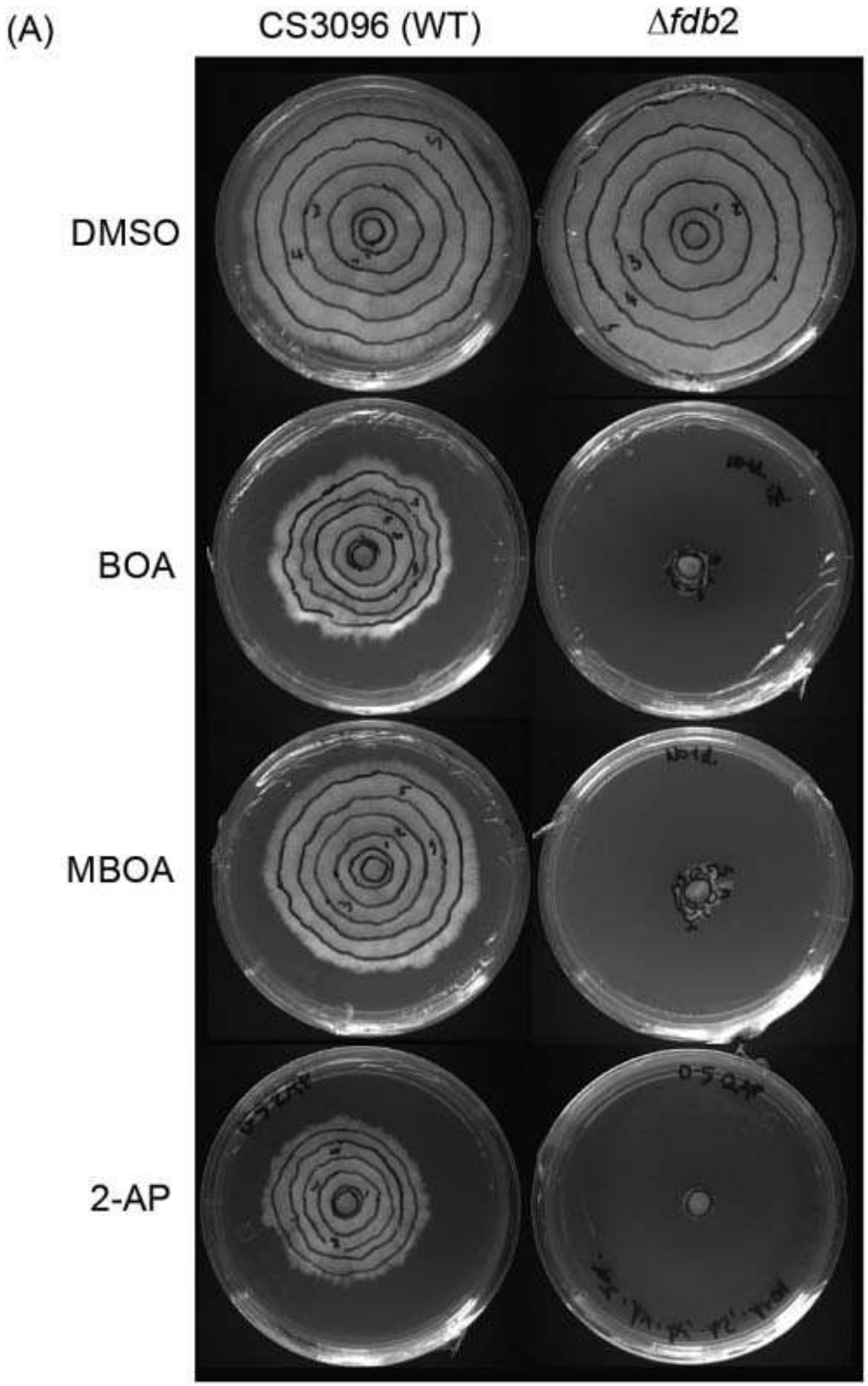
To determine whether the genes in the putative *FDB* cluster are co-expressed, their expression was assessed by reverse transcriptase-quantitative polymerase chain reactions (RT-qPCRs) at multiple time points after application of BOA to *F. pseudograminearum*




FPSE_08119
FPSE_08120
FPSE_08121
FPSE_08122
(FDB3)
FPSE_08123
(FDB2)
FPSE_08124
(FDB1)
FPSE_08125
FPSE_08126
FPSE_08127
1000
600
200
15
10
5
0
12
24


FPSE_08117
FPSE_08118
1000
600
200
15
10
5
0
12
24

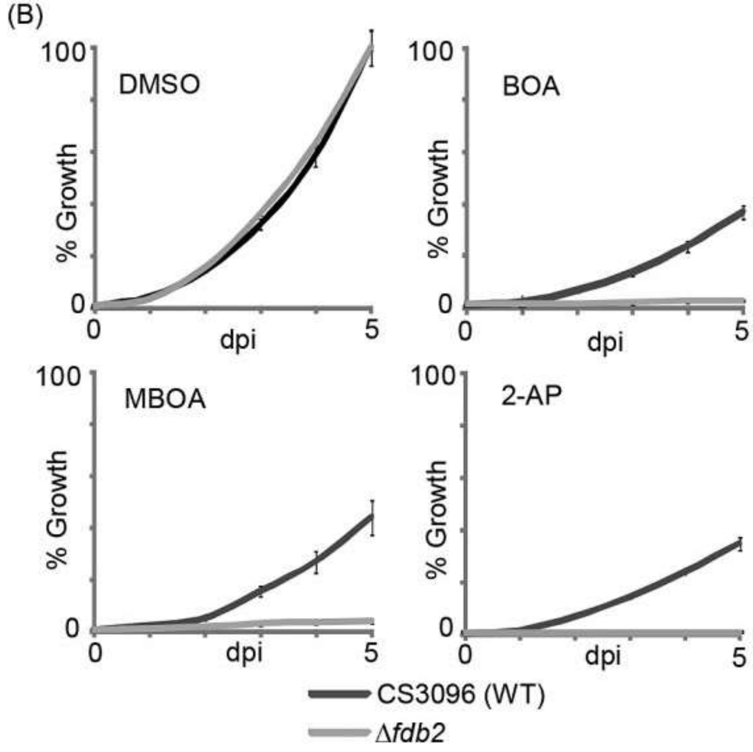

FPSE_08128
FPSE_08129
1000
600
200
0
12
24


(A)
CS3096 (WT)
Δfdb2
DMSO
BOA
MBOA
2-AP

CS3096 (WT) – *F. pseudogramineaum*

Δfdb2 strain - *F. pseudogramineaum* FDB cluster knock-out

FDB2 cluster é necessário como fator de virulência



Conclusões

- FDB cluster contribui para virulência de *F. pseudograminearum* em trigo
- *F. verticillioides* possui genes de FDB cluster em diferentes loci
- *F.* sp. FIESC5: rota alternativa de detoxificação sem formação de intermediário 2-AP
- Incapacidade de *F. graminearum* para processar MBOA independe de FDB
- 3 genes exibem muita semelhança entre as espécies F. *pseudograminearum* e *F. verticillioides*