SeedBioProtect project: Evaluation of seed treatment to control damping off in wheat and tomato

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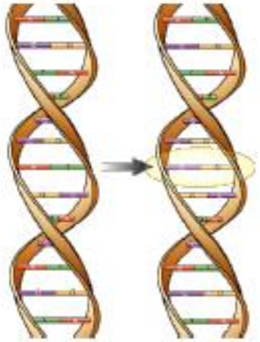
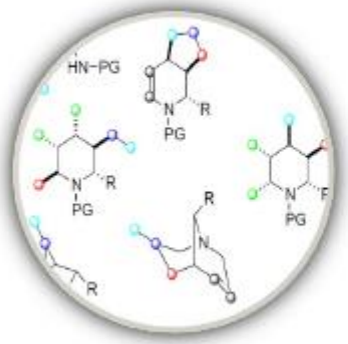
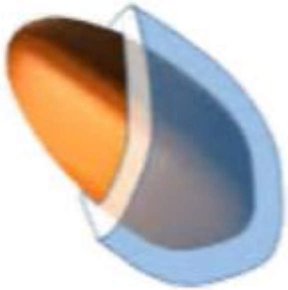




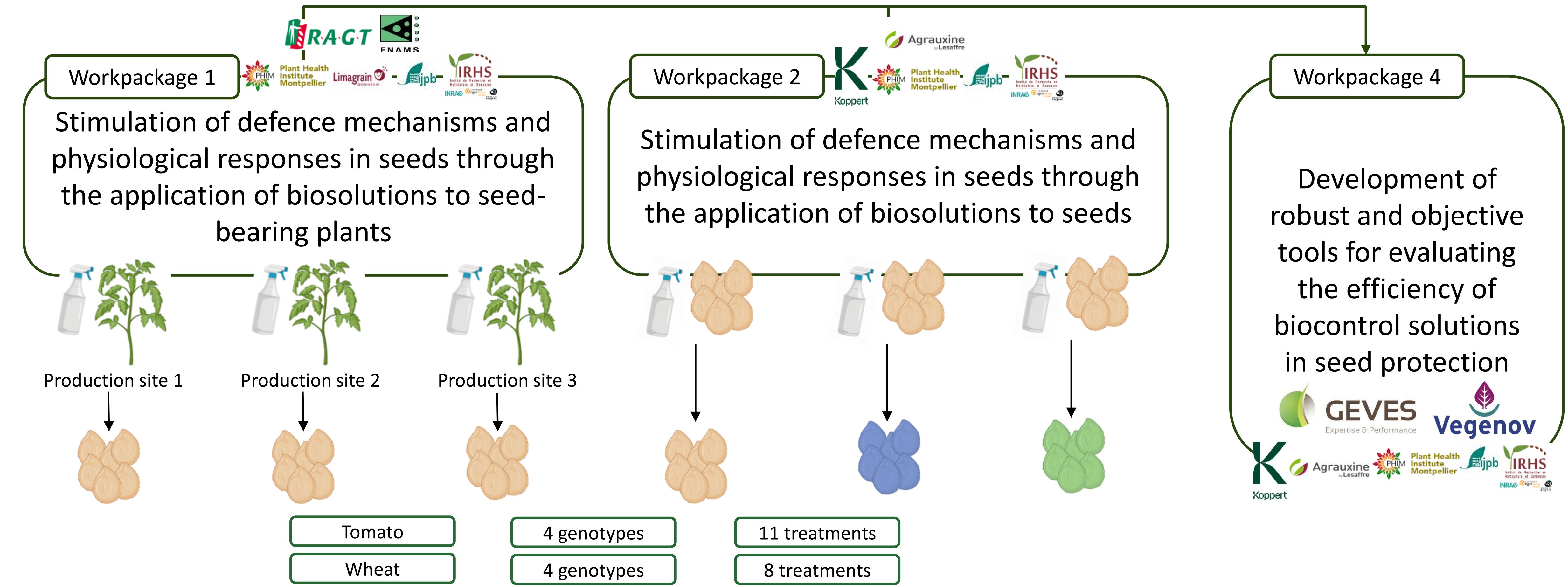
Aim : Characterise the metabolic and molecular defence mechanisms of seeds during their development, maturation and germination in order to devise new protection strategies.

Hypothesis:

- **Biocontrol efficiency depends on genotype**
- **Biocontrol agents triggers seed-specific defences**
- Response to biocontrol treatment varies during seed development & germination
- Microorganisms persist & can be transmitted across generation

Aim : Characterise the metabolic and molecular defence mechanisms of seeds during their development, maturation and germination in order to devise new protection strategies.

Figure : Presentation of the SeedBioProtect project workpackages



PART 1
Evaluation of the efficiency of biocontrol products on three pathosystems using phenotypic observation

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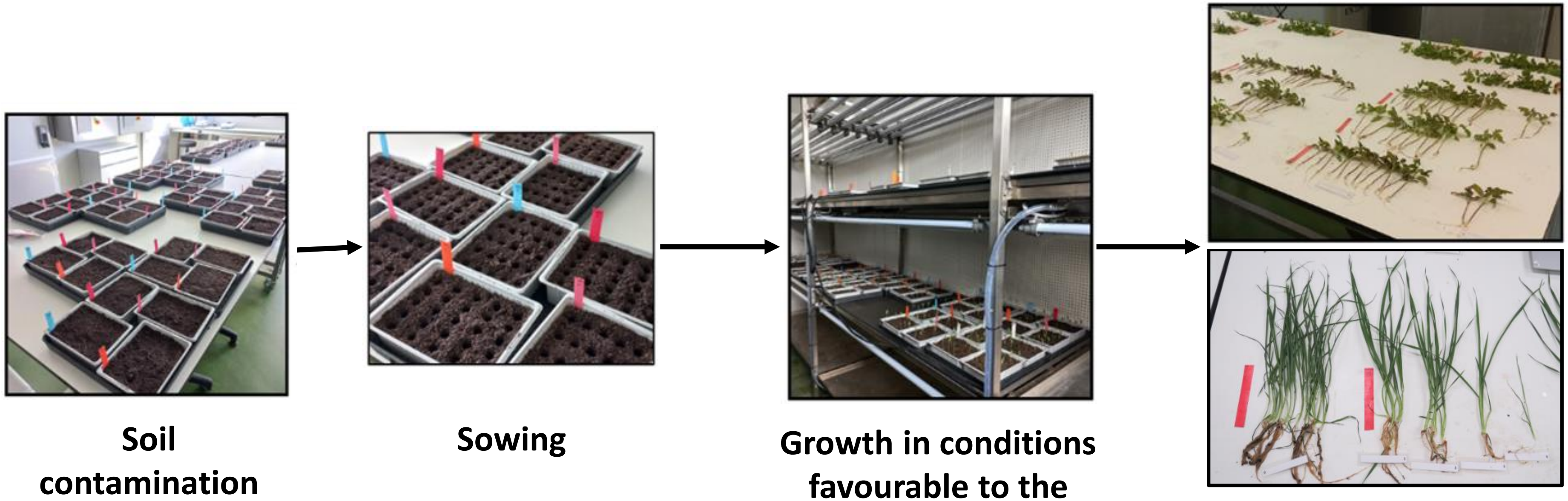
Evaluation of the efficiency of biocontrol products

<u>Figure :</u> Description of the experimental protocol (Image credit: Emma Joubert).



Soil contamination → **Sowing** → **Growth in conditions favourable to the development of symptoms** → **Final measurement** → **Disease index calculation**

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Evaluation of the efficiency of biocontrol products on *Rhizoctonia solani* /Tomato

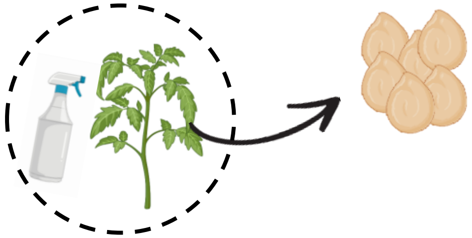
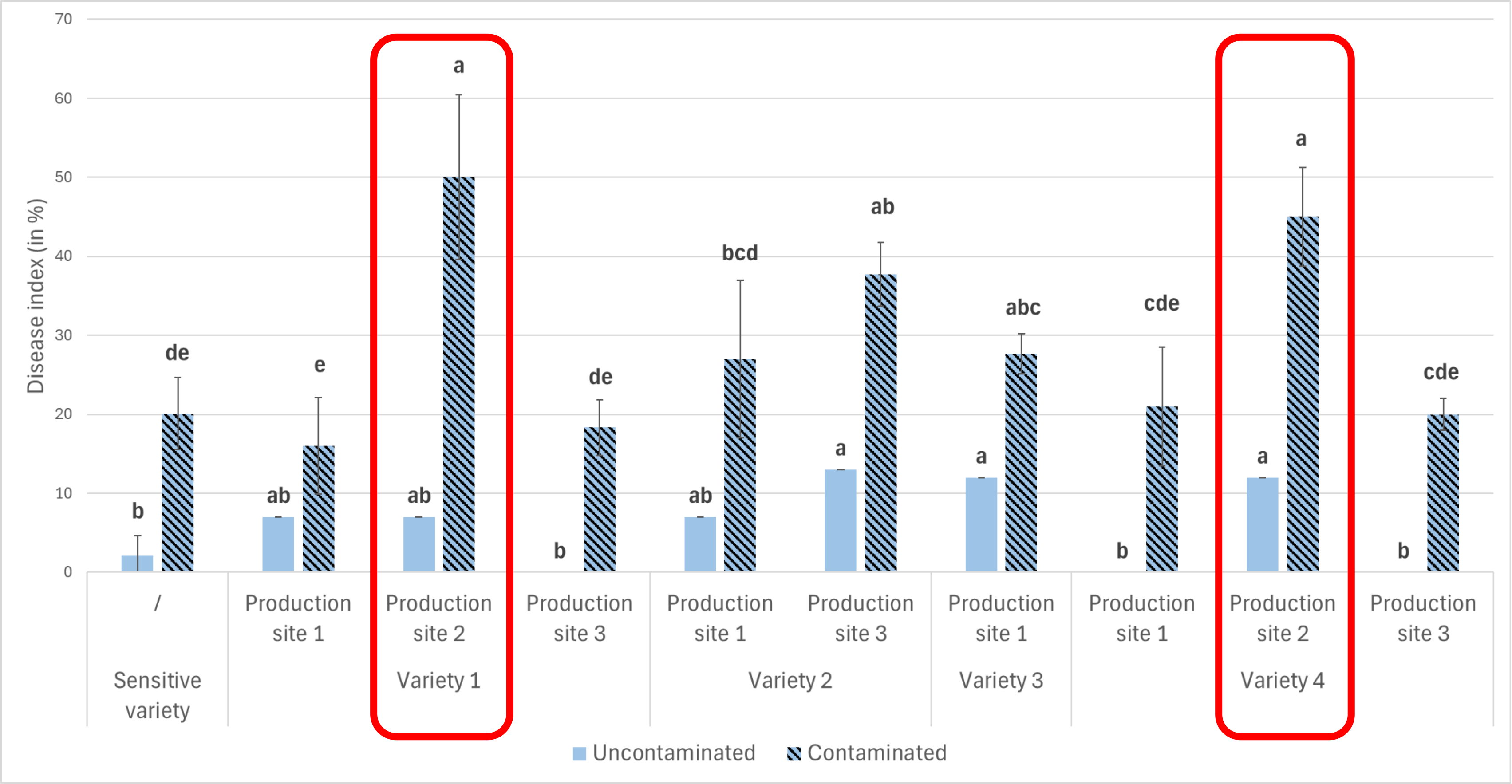


Figure : Disease severity in water-treated control by variety and production site.
Different letters indicate significant differences between modalities (Kruskal-Wallis test).



- Varietal sensitivity appears to vary with the production site.

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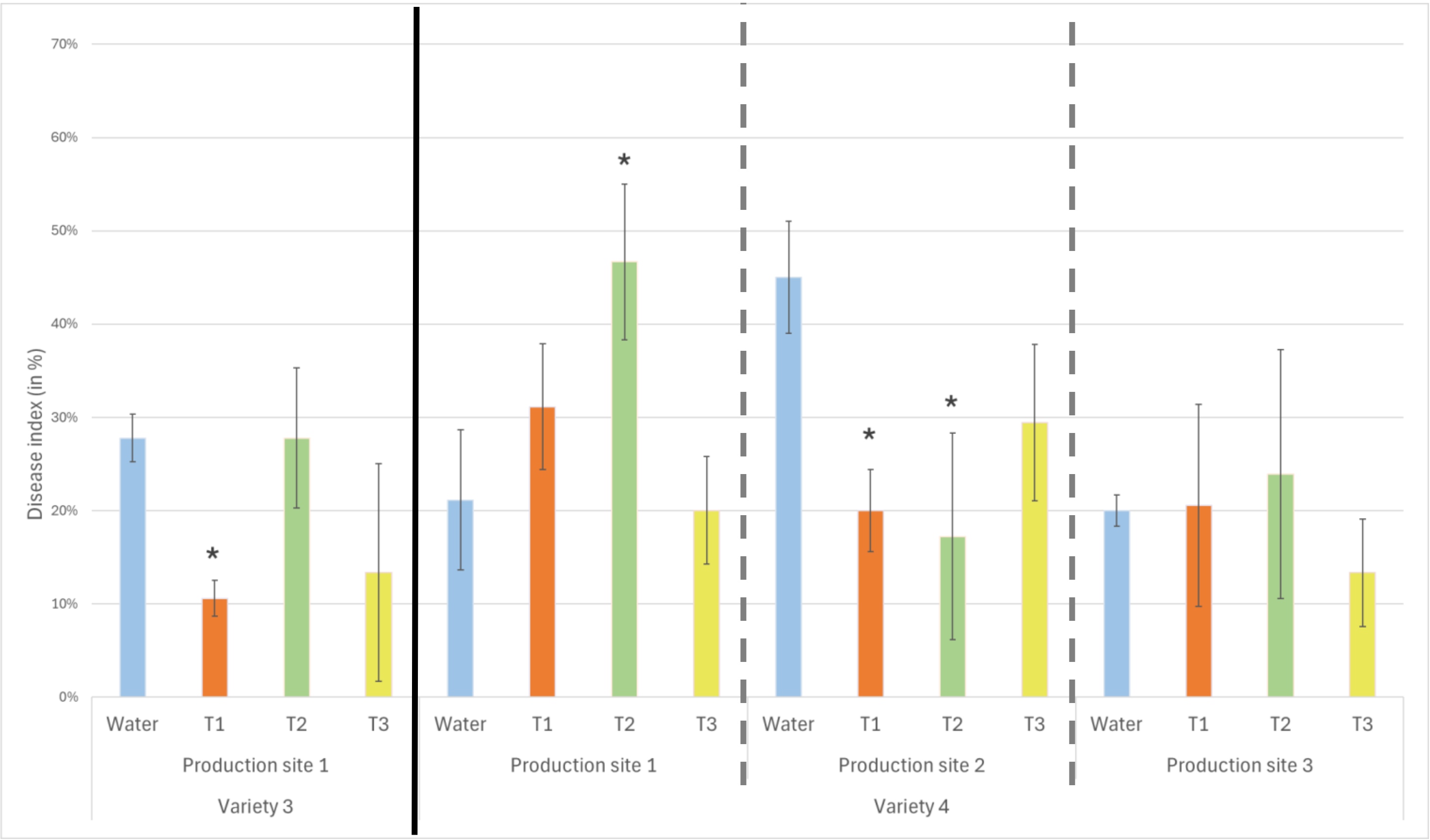
Expertise & Performance

Evaluation of the efficiency of biocontrol products on *Rhizoctonia solani* /Tomato

Figure : Disease index according to Index of diseases according to treatment/variety/production site combinations (location).
Asterisks (*) indicate treatments that are significantly different from the water-treated control for the same variety at the given site (ANOVA $p \leq 0.05$ followed by Tukey's test, $p \leq 0.05$). * : $p \leq 0.05$; ** : $p \leq 0.01$; *** : $p \leq 0.001$.

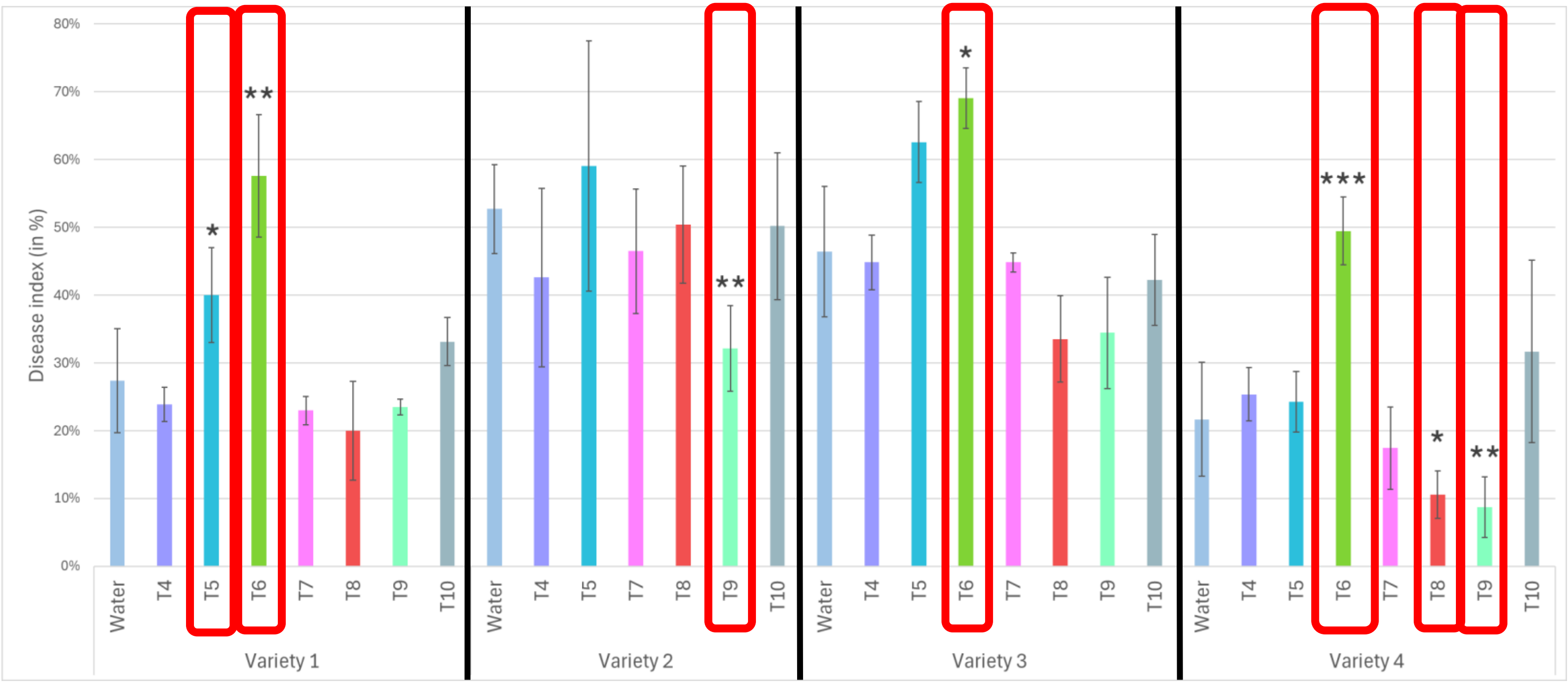


- No treatment efficiency was observed on varieties 1 and 2.
- Significant differences were found between some treatments and the control depending the production sites for varieties 3 and 4.
- Neither variety nor production site significantly affected treatment efficiency.

Evaluation of the efficiency of biocontrol products on *Rhizoctonia solani* /Tomato

<u>Figure :</u> Disease index according to treatment/variety combinations.

Asterisks (*) indicate treatments that are significantly different from the water-treated control for the same variety at the given site (ANOVA $p \leq 0.05$ followed by Tukey's test, $p \leq 0.05$). * : $p \leq 0.05$; ** : $p \leq 0.01$; *** : $p \leq 0.001$.

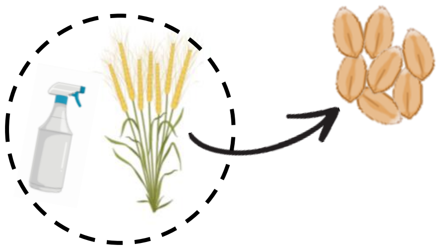


- Significant increase in the disease index for the treatment 5 on the variety 1
- Significant increase in the disease index for the treatment 6 on varieties 1, 3 and 4.

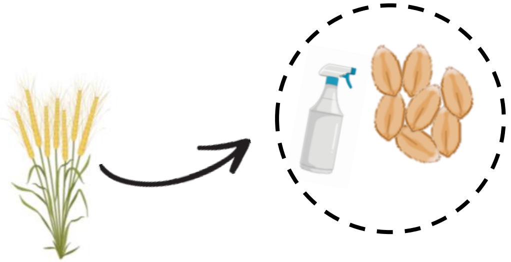
- Significant decrease in the disease index for the treatment 8 on the variety 4
- Significant decrease in the disease index for the treatment 9 on varieties 2 and 4.

- Significant effect of variety on the efficiency of treatments 5, 6, 8 and 9.

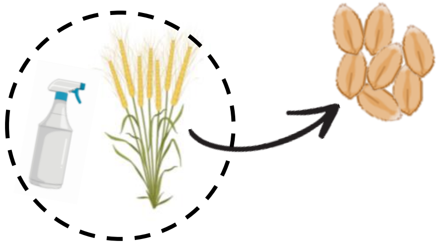
Evaluation of the efficiency of biocontrol products on *Pythium irregulare* / Wheat



- No significant treatment efficiency was observed across all conditions studied.

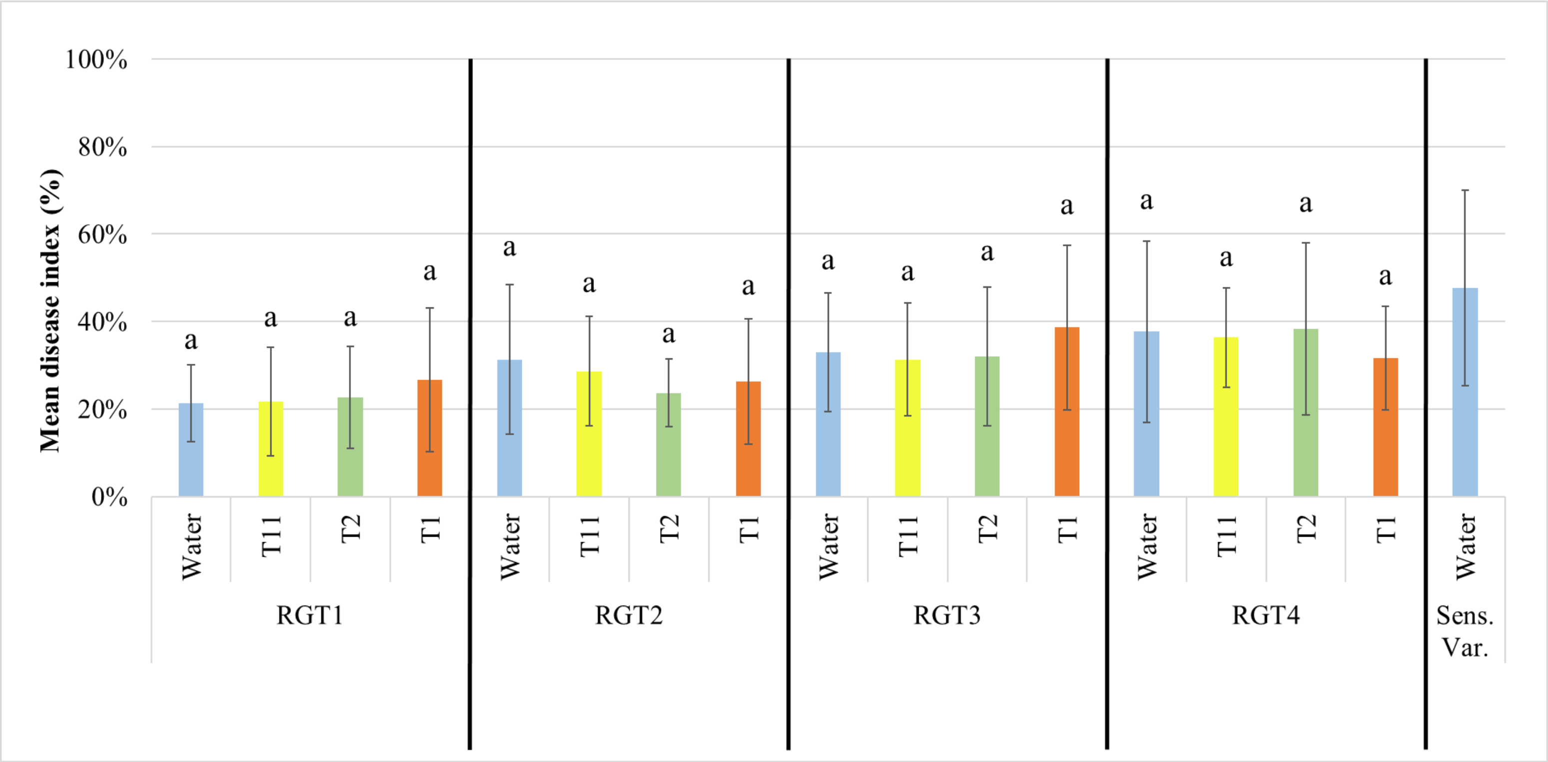


- There is no significant effect of the varieties on treatment effectiveness.



Evaluation of the efficiency of biocontrol products on *Fusarium graminearum*/ Wheat

Figure : Histogram from mixed model ANOVA analysis. Letters indicate significant differences.



- No significant effect of treatments or varieties on the protection against *F. graminearum*
- Significant effect of seed production site (data not shown)

Evaluation of the efficiency of biocontrol products on *Fusarium graminearum*/ Wheat

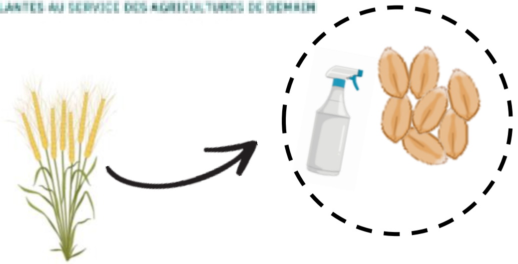
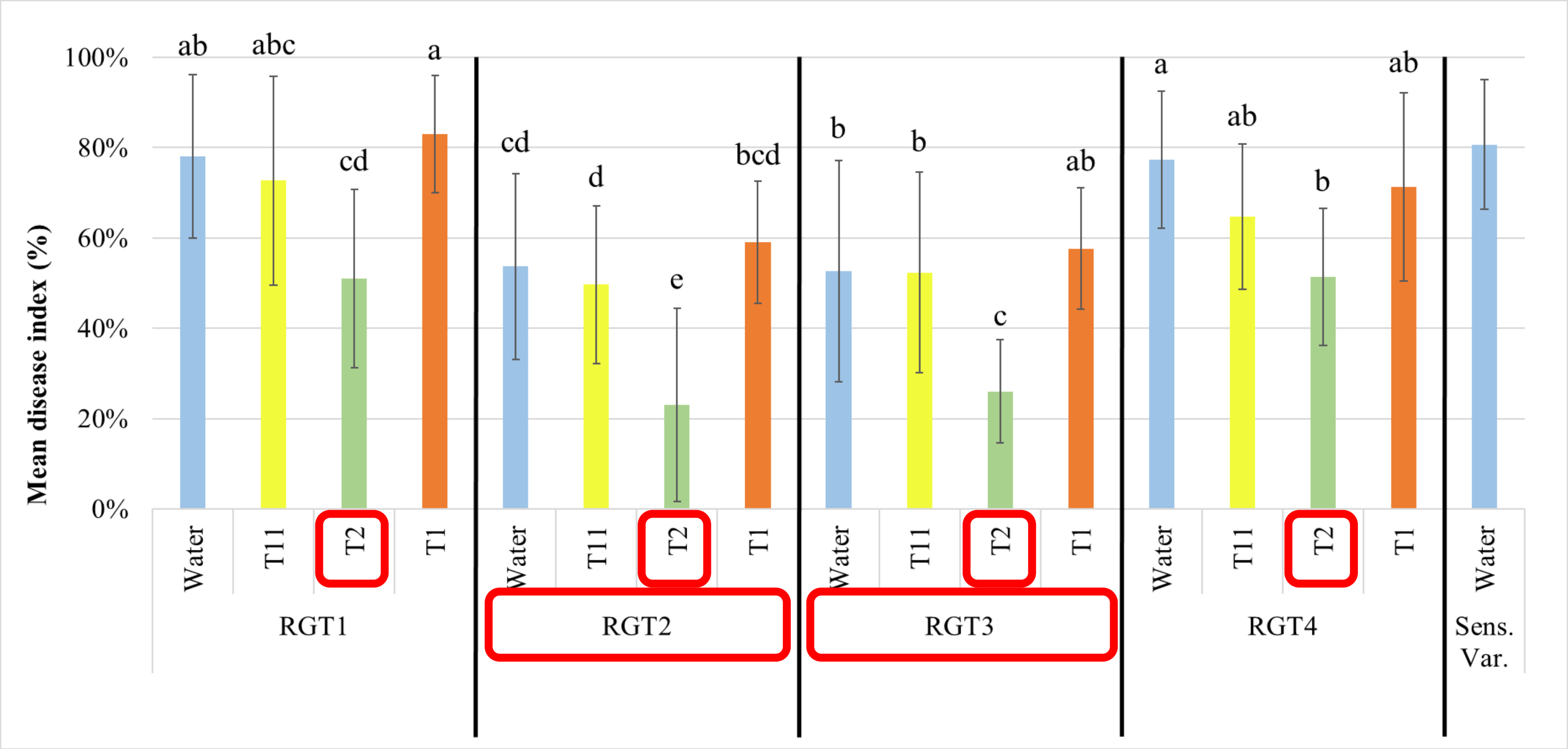
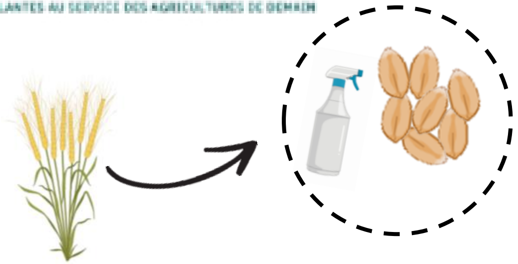


Figure : Histogram from mixed model ANOVA analysis. Letters indicate significant differences.

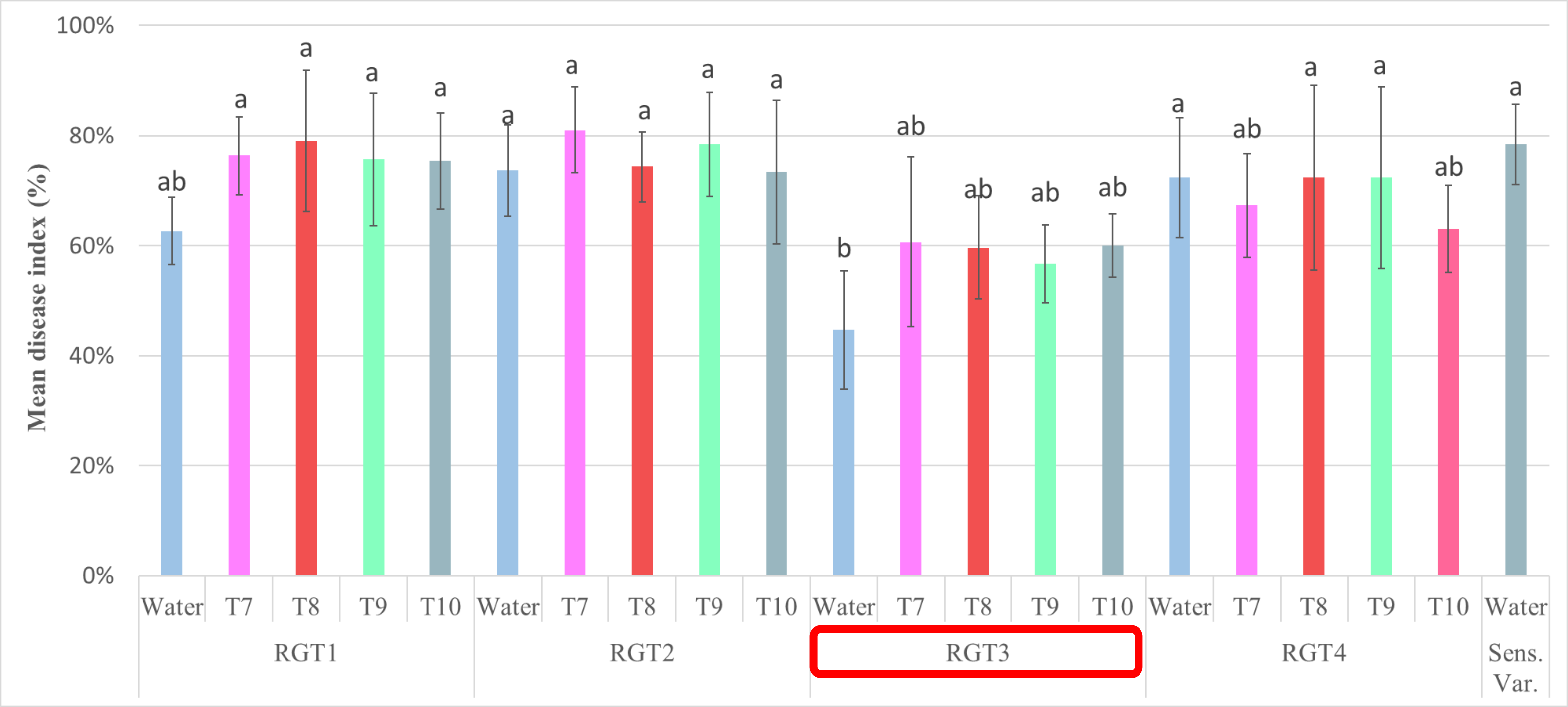


- Significant effect of **treatment** and **varieties** on the protection against *F. graminearum*
- Significant effect of **biological replicate**



Evaluation of the efficiency of biocontrol products on *Fusarium graminearum*/ Wheat

<u>Figure :</u> Histogram from mixed model ANOVA analysis. Letters indicate significant differences.



- Significant effect of **<u>biological replicate</u>** and **<u>varieties</u>** on the protection against *F. graminearum*
- No significant effect of treatments on the protection against *F. graminearum*

PART 2

Development of a set of RT-qPCR markers for the evaluation of the expression of gene involved in plant defense on **seeds**

Aim of the RT-qPCR tool :

- Evaluation of the elicitor mode of action of **biocontrol treatment** on seeds?
- Evaluation of the basic defense level of seeds for different **varieties** (after H2O treatment for example)?
- Evaluation of the defense level of **varieties** after **biocontrol treatment** of seeds?

How to proceed?

	Cinetic time 1			Cinetic time 2			Cinetic time 3		
	Moda 1	Moda 2	Moda 3	Moda 1	Moda 2	Moda 3	Moda 1	Moda 2	Moda 3
PR1	4,37	4,96	0,31	7,23	6,19	0,05	5,56	4,2	-0,25
PR2	3,8	2,94	1,12	4,57	4,01	2,60	4,12	3,57	-1,25
PR4	4,73	4,94	-0,56	7,98	6,96	0,57	6,06	4,37	-0,99
PR5	2,7	2,39	2,11	3,08	2,88	2,73	2,94	2,61	-2,68
PR8	2,27	3,33	-0,15	4,89	4,35	0,38	4,23	2,37	-0,80
PR14	1,44	2,8	-0,14	-1,15	1,99	0,69	0,34	-0,48	-1,87
PR15	-1,36	-2,14	0,39	0,04	-0,19	0,17	-0,49	-1,92	0,45
PAL	0,25	4,1	0,57	1,03	4,48	0,68	2,08	0,69	1,20
CHS	2,7	2,39	2,11	3,08	2,88	2,73	2,94	2,61	-2,68
DFR	2,27	3,33	-0,15	4,89	4,35	0,38	4,23	2,37	-0,80
ANS	-1,2	-1,15	-1,69	-0,52	-0,08	0,31	-1,1	-1,21	-3,01
HMGR	-0,44	-0,12	-0,05	-1,82	-1,59	0,15	-1,21	-0,98	0,70
FPPS	-1,36	-2,14	0,39	0,04	-0,19	0,17	-0,49	-1,92	0,45
Far	-0,01	-0,15	0,75	-0,08	-0,41	-0,32	0,05	-0,19	1,21
CSL	-0,12	-0,06	-0,71	-1,96	-0,38	-0,39	-1,28	-0,34	-0,25
APOX	-1,01	-0,63	-0,98	0,39	-0,26	0,16	-0,38	-0,67	-3,07
GST	1,88	1,92	-0,50	3,37	2,74	0,28	2,33	1,94	-1,05
POX	2,63	2,19	-0,42	4,57	4,28	0,31	3,93	3,08	-0,50
CalS	-0,02	0,15	0,37	0,18	0,39	-0,31	0,24	0,2	1,17
Pect	1,19	1,54	0,21	0,45	0,95	-1,08	2,24	1,12	0,64
CAD	-0,37	-0,23	-0,26	-1,37	-0,92	0,30	-0,47	-0,51	0,71
EDS1	1,57	1,45	0,10	3,02	2,36	0,51	2,06	0,97	0,26
WRKY	-0,07	0,08	0,67	0,22	-0,01	0,15	-0,12	-0,4	0,28
LOX2	-0,47	-0,16	-0,04	0,87	-0,07	-0,13	0,13	-0,06	-1,09
JAR	3,05	2,62	0,59	0,47	1,31	0,57	1	1,57	-1,77
ACCO	6,96	6,37	-0,23	7,86	7,35	-0,51	6,21	5,48	-0,72
EIN3	5,59	5,36	0,23	1,47	2,66	0,22	1,21	1,58	-1,10

Repression ⟷ Induction

- Germination in incubator for 24 hours (25°C, continuous light)
- Grinding (liquid nitrogen)
- RNA extraction
- Reverse transcriptase
- Mix and qPCR on a selected set of markers
- Data analysis

For tomato, selection of RT-qPCR markers among :

... The 28 markers of the **qPFD® tool** (licence INRAE available at Vegenov)

Classes et sous-classes de défense		Code	Noms des gènes
Barrières chimiques et/ou physiques	Protéines PR	PR-1	Pathogenesis-related protein 1
		PR-2	Pathogenesis-related protein 2 (glucanases)
		PR-4	Pathogenesis-related protein 4 (hevein-like)
		PR-5	Pathogenesis-related protein 5 (thaumatin-like, osmotin)
		PR-8	Pathogenesis-related protein 8 (class III chitinase)
		PR-14	Pathogenesis-related protein 14 (lipid transfer protein)
		PR-15	Pathogenesis-related protein 15 (oxalate oxidase)
	Voie des phénylpropanoïdes	PAL	Phenilalanine ammonia-lyase
		CHS	Chalcone synthase
		DFR	Dihydroflavonol reductase
		ANS	Anthocyanidin synthase
		PPO	Polyphenol oxidase
	Voie des isoprénoïdes	HMGR	Hydroxymethyl glutarate-CoA reductase
		FPPS	Farnesyl pyrophosphate synthase
		Far	(E,E)-alpha-farnesene synthase
	Voie de la cystéine	CSL	Cystéine lyase
	Stress oxydant	APOX	Ascorbate peroxidase
		GST	Glutathion S-transférase
		POX	Peroxidase
	Modifications pariétales	CalS	Callose synthase
		Pect	Pectin methyl esterase
		CAD	Cinnamyl alcool dehydrogenase
Signalisation hormonale	Voie de l'acide salicylique	EDS1	Disease resistance protein EDS1
		WRKY	WRKY transcription factor 30
	Voie de l'acide jasmonique	LOX2	Lipoxygenase AtLOX2
		JAR	Jasmonate resistant 1
	Voie de l'éthylène	ACCO	1-aminocyclopropene-1-carboxylate oxidase
		EIN3	EIN3-BINDING F BOX PROTEIN 1

... 16 markers from **Scientific publications**

Code amorce	Classe de défense	Référence bibliographique
b1	Voies des phénylpropanoïdes	(Ali Safaie-Farahani & S Mohsen Taghavi, 2017)
b2	Protéine PR	(Molinari S. & Leonetti P., 2019)
b3	Protéine PR	(Molinari S. & Leonetti P., 2019)
b4	Protéine PR	(Molinari S. & Leonetti P., 2019)
b5	Protéine PR	(Ali Safaie-Farahani & S Mohsen Taghavi, 2017)
b6	Protéine PR	(Ali Safaie-Farahani & S Mohsen Taghavi, 2017)
b7	Protéine PR	(Ali Safaie-Farahani & S Mohsen Taghavi, 2017)
b8	Protéine PR	(Hany H A El-Sharkawy et al., 2023)
b9	Voie du stress oxydatif	(Ali Safaie-Farahani & S Mohsen Taghavi, 2017)
b10	Voie du stress oxydatif	(Molinari S. & Leonetti P., 2019)
b11	Voie de l'acide jasmonique	(Ali Safaie-Farahani & S Mohsen Taghavi, 2017)
b12	Voie de l'acide jasmonique	(Molinari S. & Leonetti P., 2019)
b13	Modifications structurales	(Molinari S. & Leonetti P., 2019)
b14	Modifications structurales	(Ali Safaie-Farahani & S Mohsen Taghavi, 2017)
b15	Modifications structurales	(Ali Safaie-Farahani & S Mohsen Taghavi, 2017)
b16	Voie de l'éthylène	(Molinari S. & Leonetti P., 2019)

... 28 markers (targetting 21 genes) from **Transcriptomic analysis** performed in the SeedBioProtect project

Code amorce	Classe de défense	Code amorce	Classe de défense
d1	Famille ABC Transport membranaire	d15	Voie de l'acide jasmonique
d2	Famille CYP Composés de défense	d16	Voie de l'acide abscissique
d3	Protéines de photosynthèse	d17	Voie des gibbérellines
d4	Protéines de photosynthèse	d18	Voie des phosphates
d5	Protéines de photosynthèse	d19	Voie des phosphates
d6	Protéines de photosynthèse	d20	Voie des phosphates
d7	Protéine PR	d21	Voie des phosphates
d8	Modifications pariétales	d22	Voie des phosphates
d9	Modifications pariétales	d23	Modifications pariétales
d10	Défense structurale	d24	Voie du stress oxydatif
d11	Récepteur spécifique	d25	Voie du stress oxydatif
d12	Voies des phénylpropanoïdes	d26	Voie du stress oxydatif
d13	Voie de l'acide jasmonique	d27	Voie du stress oxydatif
d14	Voie de l'acide jasmonique	d28	Voie du stress oxydatif

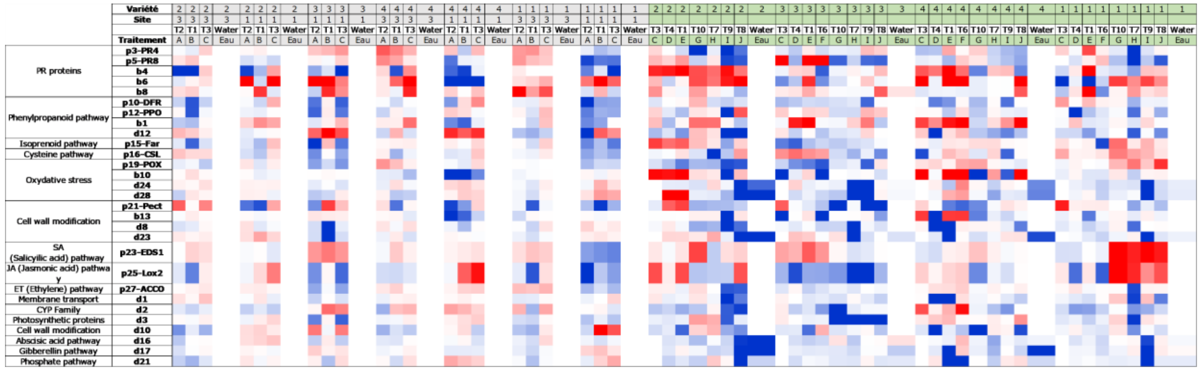
29 markers selected corresponding to a large diversity of defense pathways involved in response to different types of stress (biotic/abiotic)

RT-qPCR set designed for tomato seeds

Defense classes and sub-classes		Code	Gene names
Chemical and/or Physical barriers	PR proteins	p3-PR4	Pathogenesis-related protein 4 (hevein-like)
		p5-PR8	Pathogenesis-related protein 8 (class III chitinase)
		b4	NC
		b6	NC
		b8	NC
		d7	NC
	Phenylpropanoid pathway	p10-DFR	Dihydroflavonol reductase
		p12-PPO	Polyphenol oxidase
		b1	NC
		d12	NC
	Isoprenoid pathway	p15-Far	(E,E)-alpha-farnesene synthase
	Cysteine pathway	p16-CSL	Cystéine lyase
	Oxydative stress	p19-POX	Peroxidase
		b10	NC
		d25	NC
		d28	NC
	Cell wall modification	p21-Pect	Pectin methyl esterase
		b13	NC
		d9	NC
		d10	NC
		d23	NC
	Famille CYP	d2	NC
Hormonal signaling	SA (Salicyilic acid) pathway	p23-EDS1	Disease Resistance protein EDS1
	JA (Jasmonic acid) pathway	p25-Lox2	Lipoxygenase
	ET (Ethylene) pathway	p27-ACCO	1-aminocyclopropane-1-carboxylate oxidase
	Abscisic acid pathway	d16	NC
	Gibberellin pathway	d17	NC
Other metabolic function	Photosynthetic proteins	d3	NC
	Phosphate pathway	d22	NC

Origin of markers: qPFD® tool - Scientific publications - Transcriptomic analyses

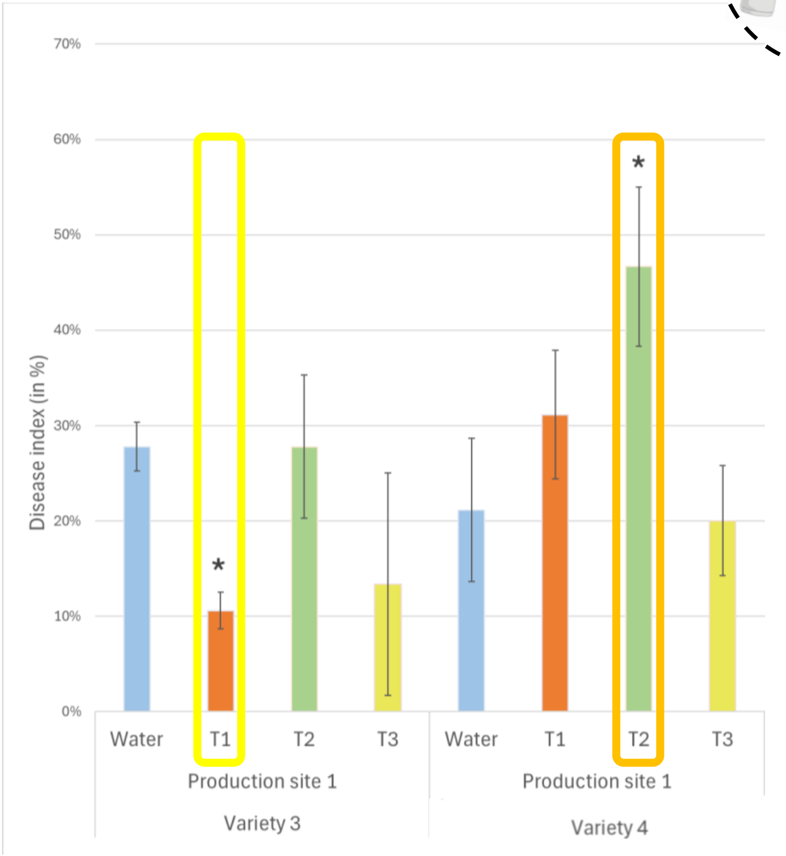
Tested on SeedBioProtect modalities



Many datas!

Focus on some RT-qPCR datas

Rhizoctonia solani /Tomato



			Variety 3	Variety 4
			Production site 1	
		Water	T1	T2
PR proteins	p3-PR4			
	p5-PR8			
	b4			
	b6			
	b8			
Phenylpropanoid pathway	p10-DFR			
	p12-PPO			
	b1			
	d12			
Isoprenoid pathway	p15-Far			
Cysteine pathway	p16-CSL			
Oxydative stress	p19-POX			
	b10			
	d24			
	d28			
Cell wall modification	p21-Pect			
	b13			
	d8			
	d10			
	d23			
SA (Salicyilic acid) pathway	p23-EDS1			
JA (Jasmonic acid) pathway	p25-Lox2			
ET (Ethylene) pathway	p27-ACCO			
Membrane transport	d1			
CYP Family	d2			
Photosynthetic proteins	d3			
Abscisic acid pathway	d16			
Gibberellin pathway	d17			
Phosphate pathway	d21			

CONSORTIUM biocontrôle

SeedBioProtect

PlantAlliance
DES PLANTES AU SERVICE DES AGRICULTURES DE DEMAIN

For wheat, selection of RT-qPCR markers among :

... The 28 markers of the **qPFD® tool** (licence INRAE available at Vegenov)

Classes et sous-classes de défense		Code	Noms des gènes
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		PR-8	Pathogenesis-related protein 8 (class III chitinase)
		PR-14	Pathogenesis-related protein 14 (lipid transfer protein)
		PR-15	Pathogenesis-related protein 15 (oxalate oxidase)
	Voie des phénylpropanoïdes	PAL	Phenilalanine ammonia-lyase
		CHS	Chalcone synthase
		DFR	Dihydroflavonol reductase
		ANS	Anthocyanidin synthase
		PPO	Polyphenol oxidase
	Voie des isoprénoïdes	HMGR	Hydroxymethyl glutarate-CoA reductase
		FPPS	Farnesyl pyrophosphate synthase
		Far	(E,E)-alpha-farnesene synthase
	Voie de la cystéine	CSL	Cystéine lyase
	Stress oxydant	APOX	Ascorbate peroxidase
		GST	Glutathion S-transférase
		POX	Peroxidase
	Modifications pariétales	CalS	Callose synthase
		Pect	Pectin methyl esterase
		CAD	Cinnamyl alcool dehydrogenase
Signalisation hormonale	Voie de l'acide salicylique	EDS1	Disease resistance protein EDS1
		WRKY	WRKY transcription factor 30
	Voie de l'acide jasmonique	LOX2	Lipoxygenase AtLOX2
		JAR	Jasmonate resistant 1
	Voie de l'éthylène	ACCO	1-aminocyclopropene-1-carboxylate oxidase
		EIN3	EIN3-BINDING F BOX PROTEIN 1

... 22 markers from **Scientific publications**

Primer code	Defense class	Reference
b17	PR proteins	(Ghalia Mustafa, 2017)
b18	PR proteins	(Amber N. Hafeez, 2025)
b19	PR proteins	(Amber N. Hafeez, 2025)
b20	PR proteins	(Hany H.A. El-Sharkawy, 2023)
b21	PR proteins	(Ghalia Mustafa, 2017)
b22	Phenylpropanoid pathway	(Ghalia Mustafa, 2017)
b23	Oxydative stress	(Claire Guérin, 2019)
b24	Oxydative stress	(Abderrakib Zahid, 2023)
b25	Oxydative stress	(Abderrakib Zahid, 2023)
b26	Oxydative stress	(Abderrakib Zahid, 2023)
b27	Oxydative stress	(Abderrakib Zahid, 2023)
b28	Oxydative stress	(Abderrakib Zahid, 2023)
b29	Oxydative stress	(Abderrakib Zahid, 2023)
b30	Oxydative stress	(Ghalia Mustafa, 2017)
b31	Oxydative stress	(Marcelo P. Giovanini et al, 2005)
b32	Oxydative stress	(Marcelo P. Giovanini et al, 2005)
b33	Oxydative stress	(Marcelo P. Giovanini et al, 2005)
b34	Oxydative stress	(Marcelo P. Giovanini et al, 2005)
b35	JA (Jasmonic acid) pathway	(Ghalia Mustafa, 2017)
b36	Reference gene	(Ghalia Mustafa, 2017)
b37	Reference gene	(Hany H.A. El-Sharkawy, 2023)
b16	ET (Ethylene) pathway	(Sergio Molinari, Paola Leonetti, 2019)

... 40 markers (targetting 34 genes) from **Transcriptomic analysis** performed in the SeedBioProtect

Code amorce	Classe de défense	Code amorce	Classe de défense
t1	Régulation transcription	t21	Xyloglucan endotransglucosylase/hydrolase
t2	Processus catabolique	t22	Patatin
t3	Régulation transcription	t23	LOB domain-containing protein
t4	Réponse de défense	t24	Peroxidase
t5	Stress oxydatif	t25	Dirigent protein
t6	Reproduction	t26	BHLH domain-containing protein
t7	Réponse aux bactéries/chamignons	t27	AAA+ ATPase domain-containing protein
t8	Stress oxydatif	t28	Stress responsive protein
t9	Processus catabolique	t29	Amine oxidase
t10	Reproduction	t30	O-methyltransferase domain-containing protein
t11	Organisation cellulaire	t31	Peroxidase
t12	Réponse au stress osmotique	t32	Flavonoid 3'-monooxygenase
t13	Voie de l'éthylène	t33	CASP-like protein
t14	Processus catabolique des polyamines	t34	Protein TIFY (Jasmonate ZIM domain-containing protein)
t15	Voie des phénylpropanoides	t35	Protein TIFY (Jasmonate ZIM domain-containing protein)
t16	Régulation transcription	t36	AAA+ ATPase domain-containing protein
t17	Transport vésiculaire	t37	Pathogenesis-related protein 1-8
t18	Voie des phénylpropanoides	t38	Pectin acetylesterase
t19	Voie des phénylpropanoides	t39	Peroxidase
t20	Voie des phénylpropanoides	t40	4-coumarate--CoA ligase

➡ Set of markers to test on wheat seeds RNA for the selection of the more relevant markers

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Conclusion and Outlook :

- The susceptibility of a variety to a phytopathogen appears to vary depending on the seed production site.
  - Know the quality of the seed lots on which the evaluations are carried out.

- The efficiency of tested biocontrol products could vary depending on the variety studied.
  - Working with different varieties when evaluating biocontrol products

- Variety tolerance to a phytopathogen seems to be more efficient than seed treatments, even if some few cases treatment improve seed protection

- RT-qPCR markers to study expression of genes involves in seed defenses, are proposed and more data will be acquired in the next months to valid their relevance -> A new tool soon available

- qPCR markers for the detection of key damping off micro-organisms have been validated and could be used for their detection in various matrix (seed, soils, etc.)

→ Project ending in December 2025 - To be continued with SUPRASEED

Revolutionising seed protection and enhancement with cutting-edge innovation for sustainable agriculture.

Thank you for your attention

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