



Phenotypic characterization of the wheat-septoria interaction

Réunion du Groupe Céréales



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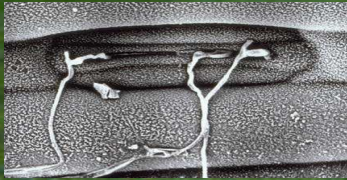
09 / 04 / 2015

Wheat-septoria interaction

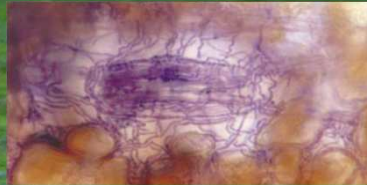
Infectious cycle of *Zymoseptoria tritici*

Biotrophic stage (asymptomatic)

Penetration through stomata



Colonisation of the sub-stomatal cavity



Necrotrophic stage

Pycnides formation



Maturation of pycnidiospores



Infection of SEEDLINGS



Infection of ADULT PLANTS



Wheat-septoria interaction

Specificities of the pathosystem

- ❖ *Zymoseptoria tritici* is a hemibiotrophic fungal pathogen with two phases of infection: a biotrophic (asymptomatic) phase followed by a necrotrophic phase
- ❖ *Z. tritici* populations have a high level of diversity and a weak structuration (importance of both sexual and asexual reproductions)
 - possibility to perform controlled crosses between *Z. tritici* isolates
- ❖ *Z. tritici* isolates show an incomplete specificity towards different cultivated wheat species: bread wheat and durum wheat, etc
- ❖ A large number of resistance sources is available: 18 *STB* resistance genes, ~89 QTLs mapped, and many uncharacterized resistances (the resistance gene *STB6* is present in more than 15% European bread wheat varieties)
 - some qualitative resistance phenotypes but mostly a quantitative variation in resistance/susceptibility



GENETIC RESISTANCES IN BREAD WHEAT

Projet Investissements d'Avenir BREEDWHEAT - Breeding for economically and environmentally sustainable wheat varieties: an integrated approach from genomics to selection. 2011-2019.

Project European FP7 KBBE WHEALBI - Wheat and barley legacy for breeding improvement. 2014-2018.



Resistance genetics in bread wheat

Objectives

Development of reliable tools and protocols to characterize the wheat-septoria interactions under controlled conditions and under field conditions

Actions

- ❖ Characterize a set of 110 French *Zymoseptoria tritici* isolates for their virulences and their level of aggressiveness
- ❖ Identification of resistance sources from bread wheat cultivars (controlled conditions and field disease tests) and evaluation of their effectiveness

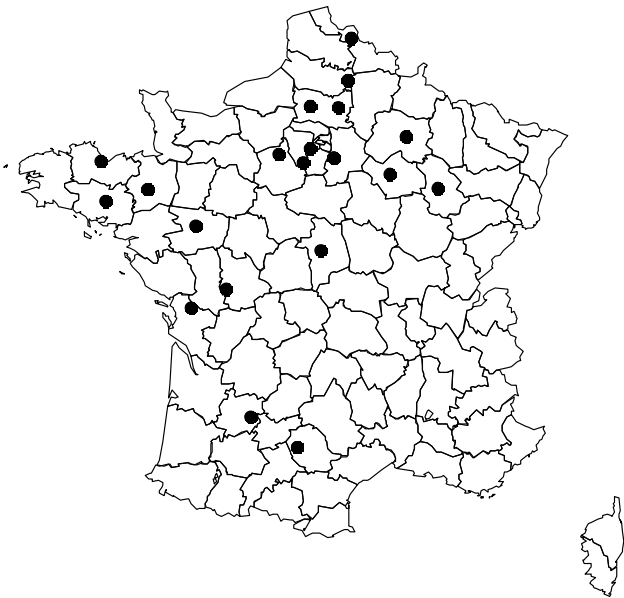
Breedwheat Panel: 220 French bread wheat cultivars

- ❖ Set-up a set of differential lines for their resistance to *Z. tritici*

Seedling evaluation of Breedwheat Panel

Characterizing a collection of French isolates

- ❖ A collection of 2,236 *Zymoseptoria tritici* isolates from France 2009-2010
 - 13 geographic populations from 3 regions (FSOV 2008-11)
 - 781 isolates genotyped with microsatellites (FSOV 2008-11)
 - 110 isolates genotyped and pathotyped (ANR GANDALF)



- 518 isolates (66.9%) had unique genotypes
- A weak geographic structure in the French populations of *Z. tritici*

A Ducasse, L Gout, TC Marcel (INRA BIOGER)

Seedling evaluation of Breedwheat Panel

Characterizing a collection of French isolates

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 - 13 geographic populations from 3 regions (FSOV 2008-11)
 - 781 isolates genotyped with microsatellites (FSOV 2008-11)
 - 110 isolates genotyped and pathotyped (ANR GANDALF)

- ❖ Evaluation of the Breedwheat Panel:
 - Seedlings: IPO-323 and IPO-90006 used to discriminate cultivars carrying *STB6* (2 REPs)
 - Seedlings: IPO-09415 and IPO-09455 used as virulent and aggressive isolates (4 REPs)
 - Field: IPO-09415 has been selected to evaluate the Biotech Panel at the adult plant stage (also used as reference isolate by the GEVES)

Seedling evaluation of Breedwheat Panel

Inoculation protocol on seedlings



16 days old wheat seedlings



Inoculation of the 2nd leaf by brushing 1×10^6 spores / ml



3 days in high humidity

Seedling evaluation of Breedwheat Panel

Inoculation protocol on seedlings



Climate cells:

>70%RH

16 hrs light

18°C/22°C

Notations:

Quantitative visual
assessments

CHL/NEC/PYC

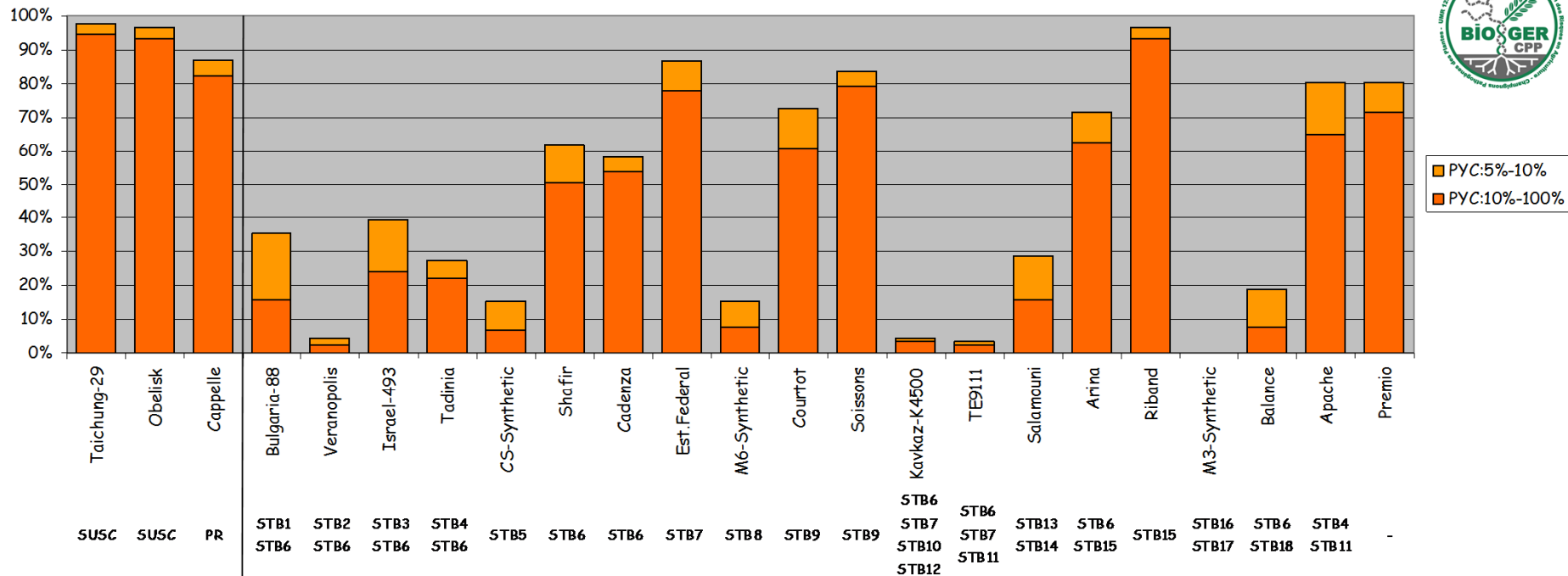
Scans for image
analysis*

Seedling evaluation of Breedwheat Panel

Frequency of virulences among 92 French septoria isolates

Fréquence des virulences de 92 isolats de *Zymoseptoria tritici* collectés en France 2009-2010

BIOGER-CPP
TC Marcel



rmx La virulence est postulée lorsque l'isolat sporule sur plus de 5% (orange foncé et clair) ou plus de 10% (orange foncé) de la surface inoculée. Les inoculations sont réalisées sur la première vraie feuille de plantules de blé en appliquant l'inoculum avec un pinceau (10⁶ spores par mL). La lecture des symptômes est réalisée 21 jours après l'inoculation. Les données présentées ne consistent qu'en une seule réplication (moyenne de 3 feuilles).

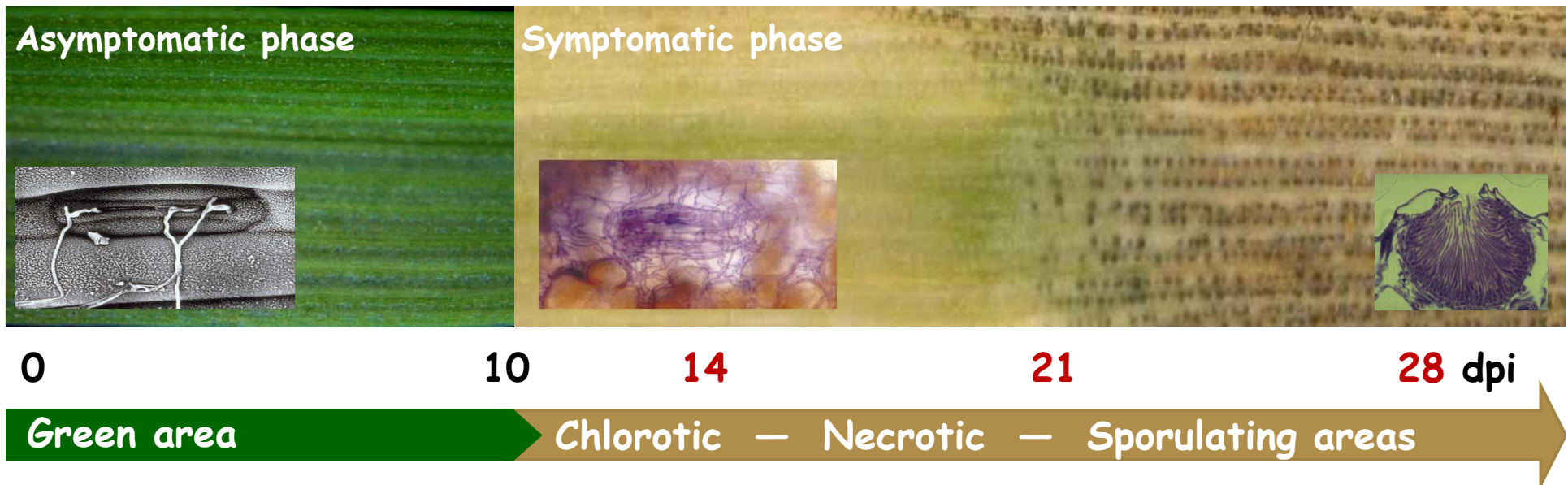
Selection of isolates cumulating a maximum of virulences to known Stb genes and with the highest level of aggressivity

Seedling evaluation of Breedwheat Panel

Seedling Disease Index

100%

%Green %Chlorosis **%Necrosis** **%Sporulation**



Seedling evaluation of Breedwheat Panel

Seedling Disease Index

* SDI: Seedling Disease Index at 21 dpi [scale 0-10]

$$(100 - \% \text{Green} + \% \text{Necrosis} + \% \text{Sporulation}) / 30$$

$$(200 - (2 * \% \text{Green}) + (1 * \% \text{Necrosis}) + (3 * \% \text{Sporulation})) / 60$$

$$[(201 - (2 * \% \text{Green}) + (1 * \% \text{Necrosis}) + (3 * \% \text{Sporulation})) / 60] - [1/60]$$

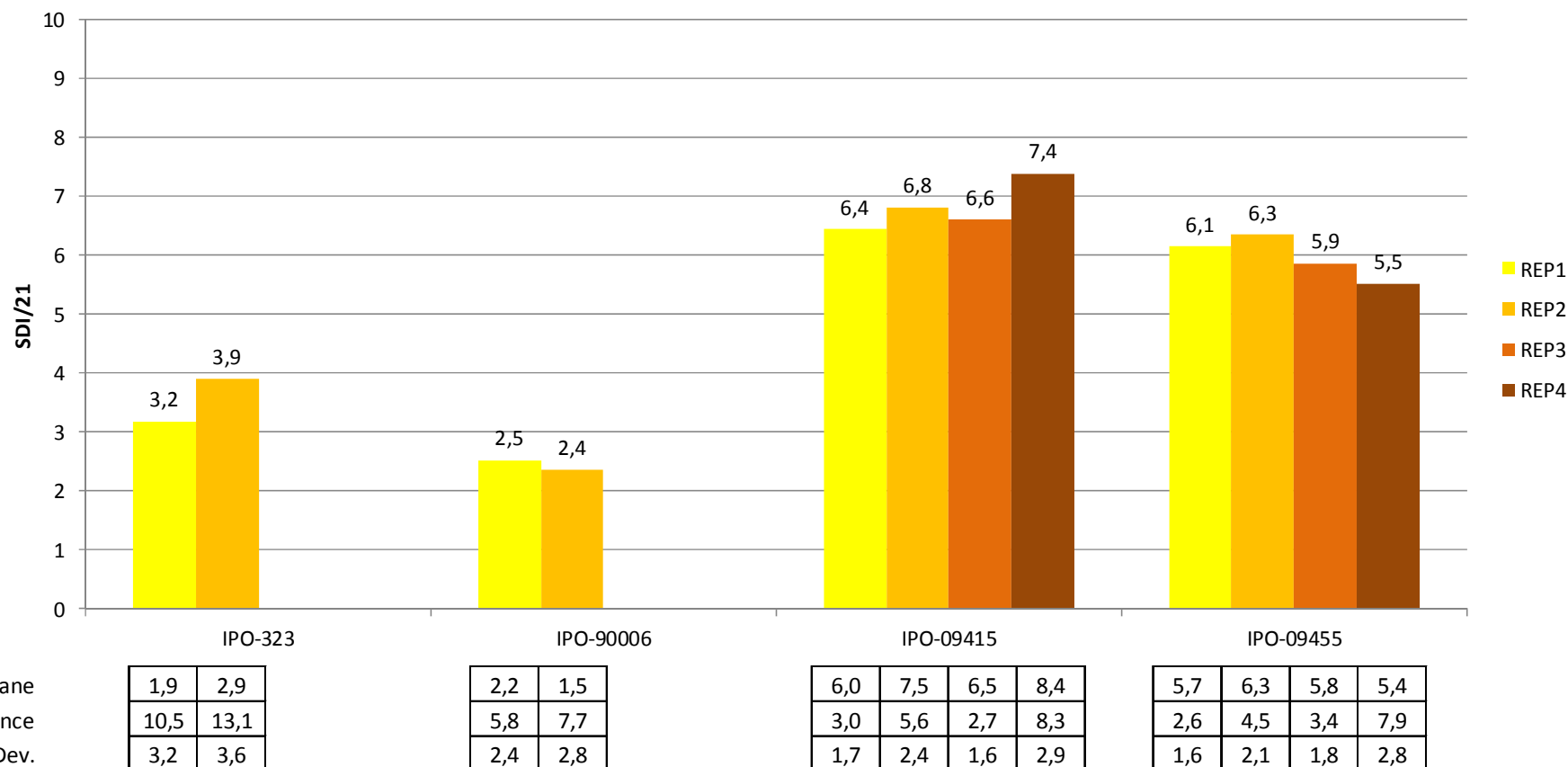
%Green	%Necrosis	%Sporulation	DI
100	0	0	0,0
50	0	0	1,7
0	0	0	3,3
0	100	0	5,0
0	50	25	5,4
0	100	50	7,5
0	100	100	10,0



Seedling evaluation of Breedwheat Panel

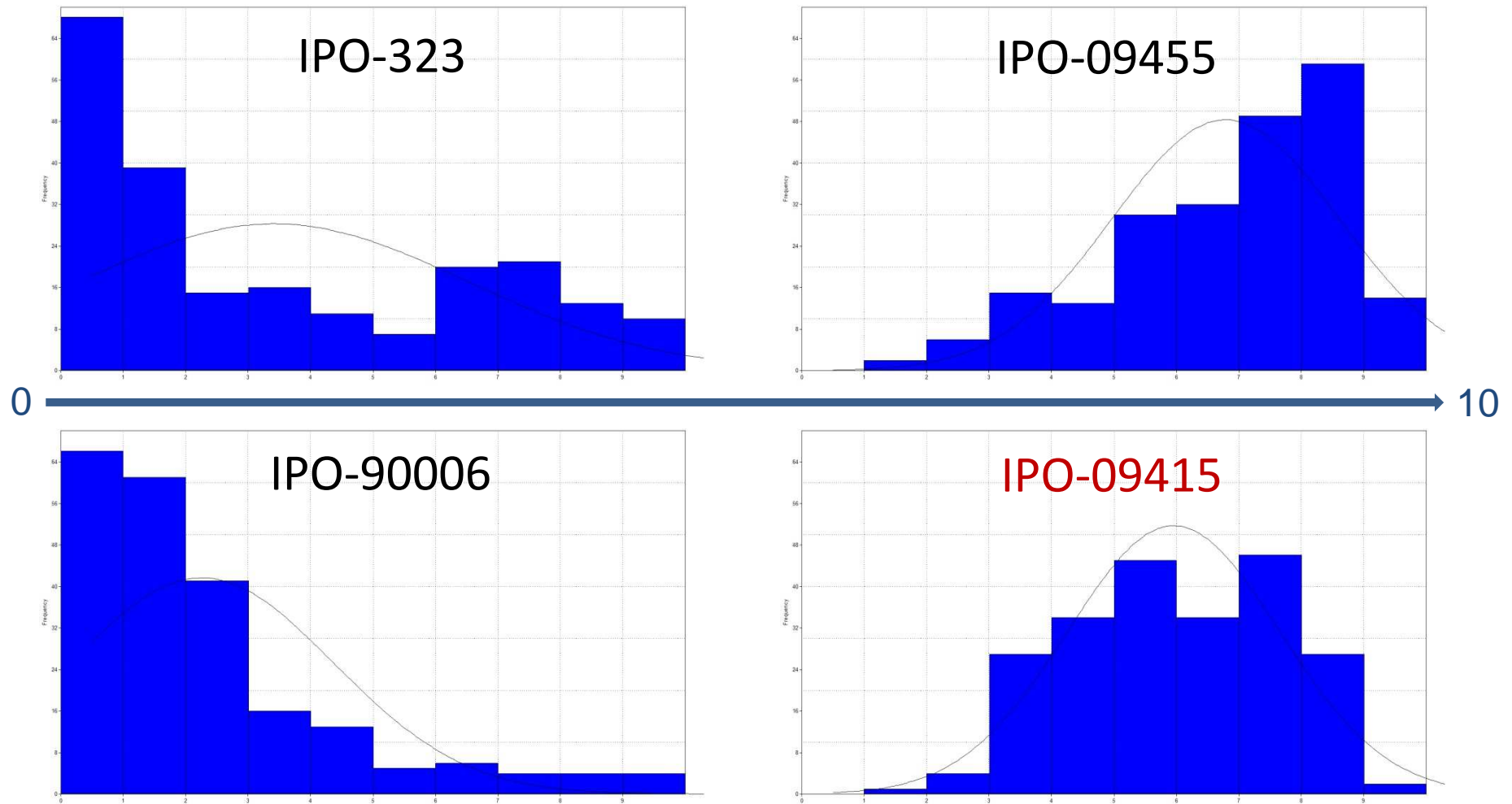
Mean SDI/21 over the panel in each repetition

Mean Seedling Disease Index (SDI) at 21 dpi over the Biotech Panel



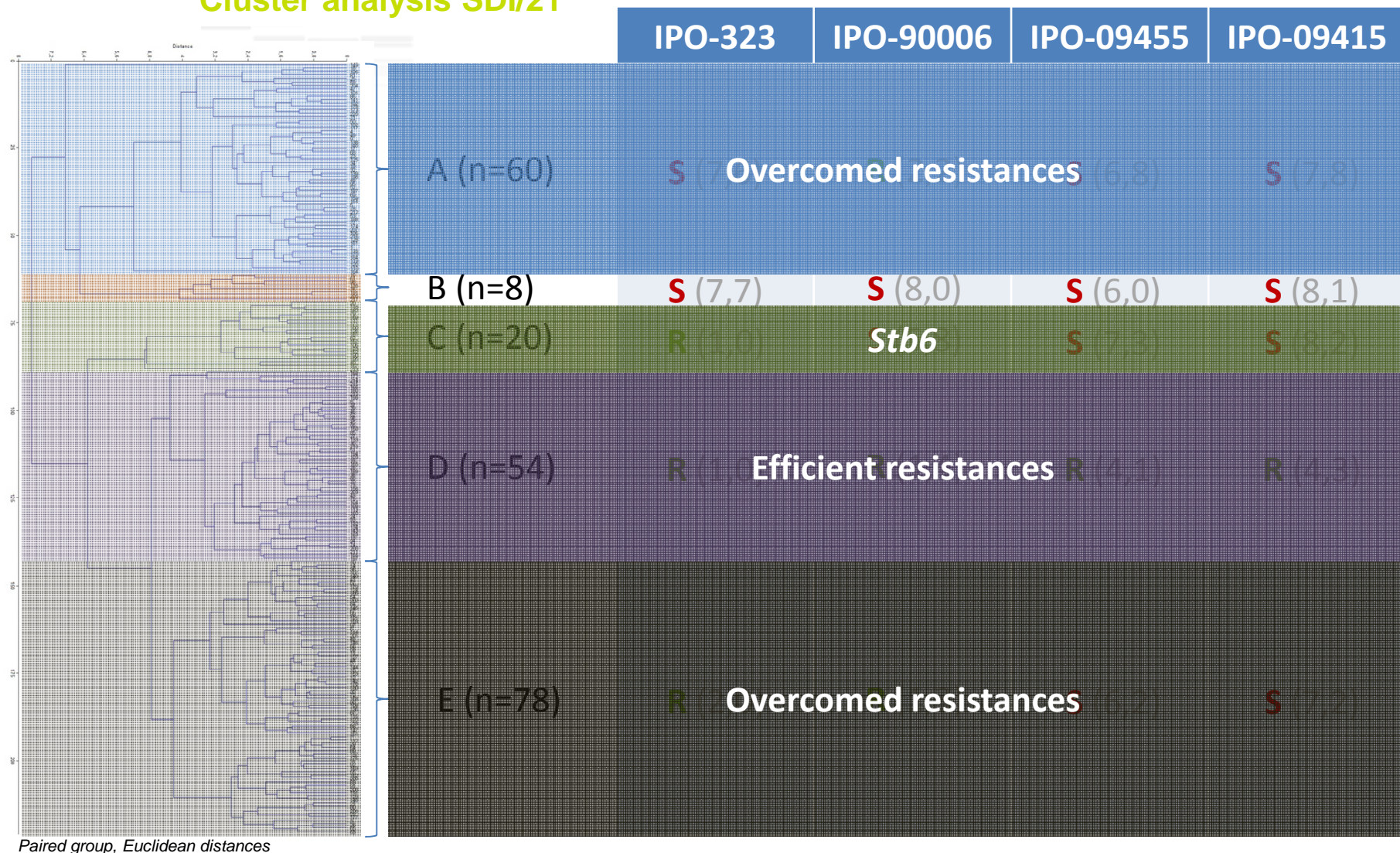
Seedling evaluation of Breedwheat Panel

Frequency distributions SDI/21



Seedling evaluation of Breedwheat Panel

Cluster analysis SDI/21



Seedling evaluation of Breedwheat Panel

Efficient resistances

Group D: efficient resistances

- ❖ 7 cultivars have less than 10% sporulating area at 28 dpi (SPO/max) on all isolates

Code BW	Variety	IPO-323	IPO-90006	IPO-09415	IPO-09455	
155	CELLULE	0,0	0,0	0,0	0,0	} SPO/max
166	MH09-17	0,0	0,0	0,0	0,0	
211	NOGAL	0,0	0,0	0,0	0,0	
70	ARISTOTE	0,0	0,3	0,0	0,0	
180	ACCOR	0,5	0,0	5,7	0,0	
76	BOREGAR	0,0	0,7	3,3	6,7	
73	AZZERTI	0,0	0,0	10,0	2,7	

- ❖ 12 cultivars have less than 10% sporulating area at 28 dpi (SPO/max) over the mean of the four isolates

93	PLAYER	0,0	2,3	17,3	0,0	} SPO/max
66	SY_EPSON	0,0	0,0	6,0	16,3	
74	BAROK	0,0	16,8	0,0	5,7	
94	RENAN	0,0	19,8	0,0	4,0	
37	TIMING	0,0	0,8	23,7	1,7	
132	ACOUSTIC	0,0	3,8	26,7	0,0	
197	MIROIR	0,0	21,8	5,0	4,7	
62	SANKARA	0,0	0,0	16,7	17,3	
200	PRIMO	0,0	0,0	0,0	36,3	
8	AMBITION	0,0	0,3	38,0	0,0	
156	FLAMENKO	0,0	5,5	17,3	17,0	
150	EPIDOC	0,0	1,7	30,0	9,3	

Conclusions

Seedling evaluation of the Breedwheat Panel

- ❖ A set of 110 *Zymoseptoria tritici* isolates has been characterized for their virulences and for their level of aggressiveness
- ❖ IPO-09415 is one of the most aggressive isolates on French elite material
- ❖ Development of a disease index to characterize wheat-*Z. tritici* interactions in controlled experiments on seedlings (SDI)
- ❖ 7 highly resistant and 12 moderately resistant cultivars have been identified at seedling stage with four isolates of *Z. tritici* differing for their virulences

Field evaluation of the Breedwheat Panel

WP2 – Breedwheat Field Trials

❖ Breedwheat Field Trials, Septoria stress:

→ 2011-2012 network

→ 2012-2013 network

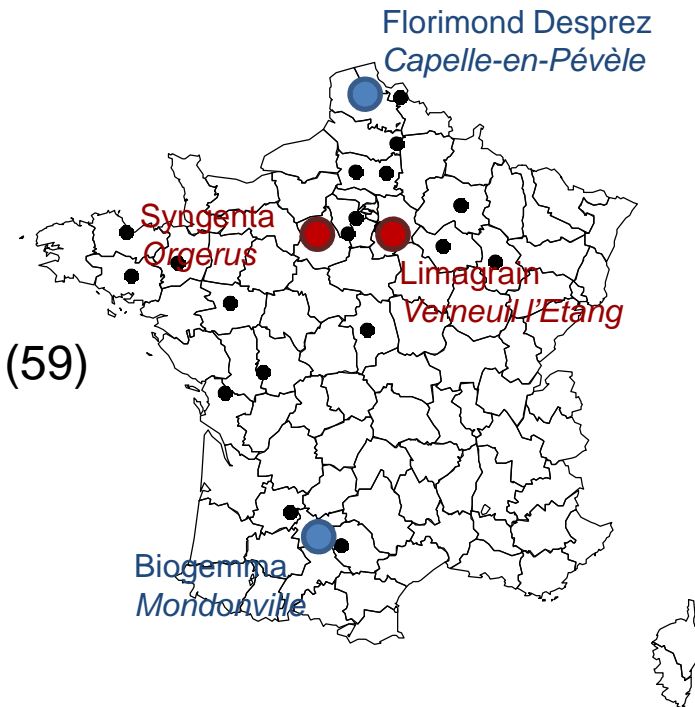
● Florimond Desprez – Capelle-en-Pévèle (59)

● Biogemma – Mondonville (31)

→ 2013-2014 network

● Syngenta – Orgerus (78)

● Limagrain – Verneuil l'Étang (91)



Septoria trial at Orgerus (78) - SYNGENTA



Septoria trial at Verneuil l'Etang (77) - LIMAGRAIN



Septoria trial at Verneuil l'Etang (77) - LIMAGRAIN





Field evaluation of the Breedwheat Panel

Notation scale



Bioger's note

2 notes
Scale 0-9

9 ... 5 ... 0
←————→
9 ... 5 ... 0
←————→

Breeder's note

1 note
Scale 0-9

6 7 8 9
————→

5

4

3

2

1

0



FLAG (F): 5 leaves scored / accession

F-1: 5 leaves scored / accession

* FDI: Field Disease Index [scale 0-100]

$$[(F_{\text{mean}}+1)/10] * [(F-1_{\text{mean}}+1)/10] * 100$$



Field evaluation of the Breedwheat Panel

Notation scale

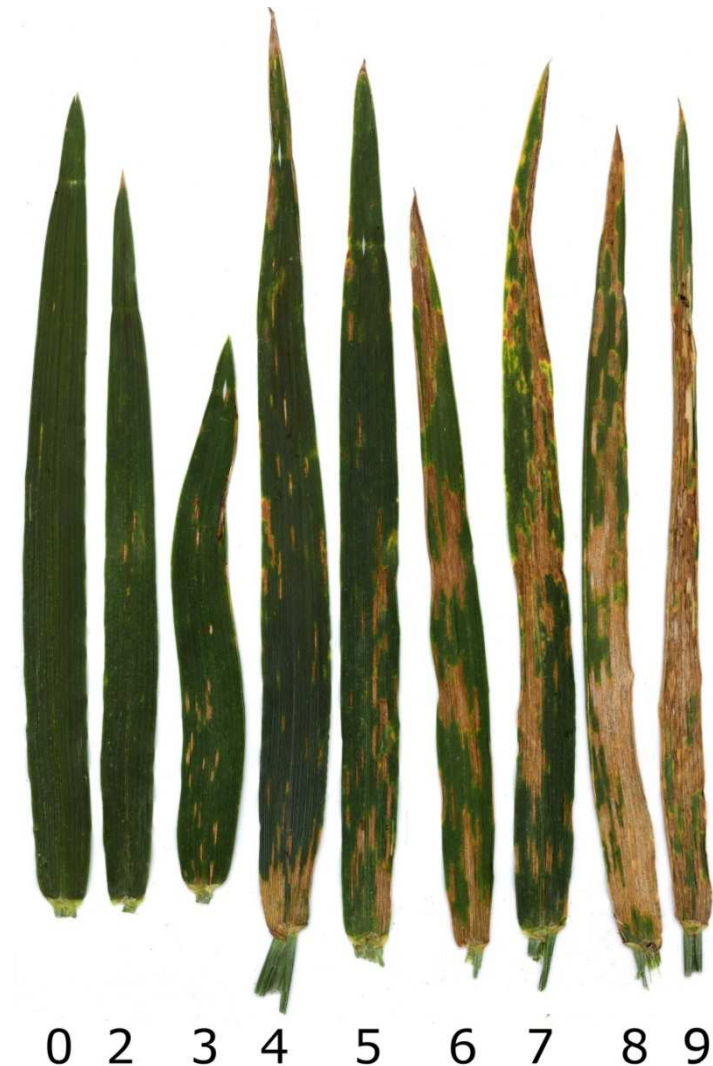
single lesions



merging lesions



- 0: immune
- 1: one single sporulating lesion
- 2: two to five single sporulating lesions
- 3: more than five sporulating lesions
- 4: mix of single and merging lesions
- 5: only merging sporulating lesions
- 6: 10% to 25% sporulating leaf area
- 7: 25% to 50% sporulating leaf area
- 8: 50% to 75% sporulating leaf area
- 9: over 75% sporulating leaf area



Field evaluation of the Breedwheat Panel

Breedwheat field evaluation 2013-2014

BREEDHWHEAT FIELD EVALUATION 2013-2014:

- 2 sites (Orgerus & Verneuil l'Etang)
- 2 repetitions
- 2 dates of notation



Notations:

- 4/5 June
- 18/19 June

Notations:

- 11/12 June
- 23 June



➤ Strong incidence of yellow rust infestation on both sites (10 to 15 cultivars)

Field evaluation of the Breedwheat Panel

Coefficients of determination between repetitions

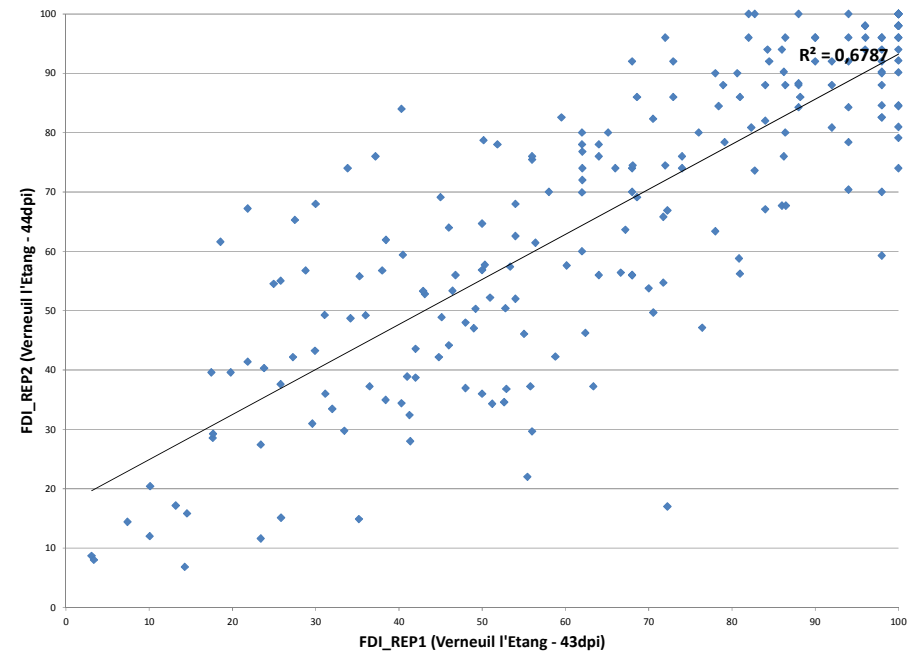
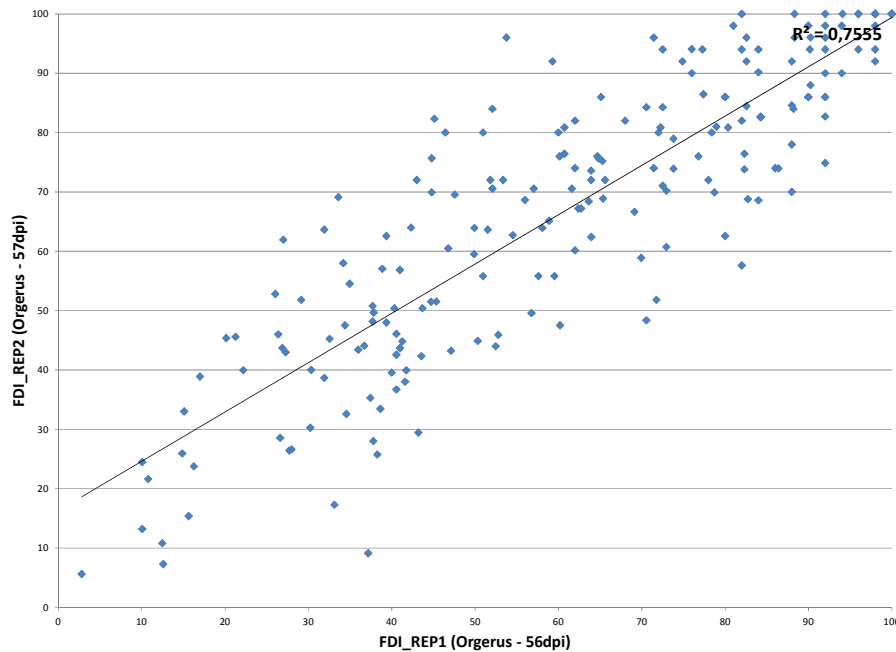


Notations:

- 4/5 June
- 18/19 June

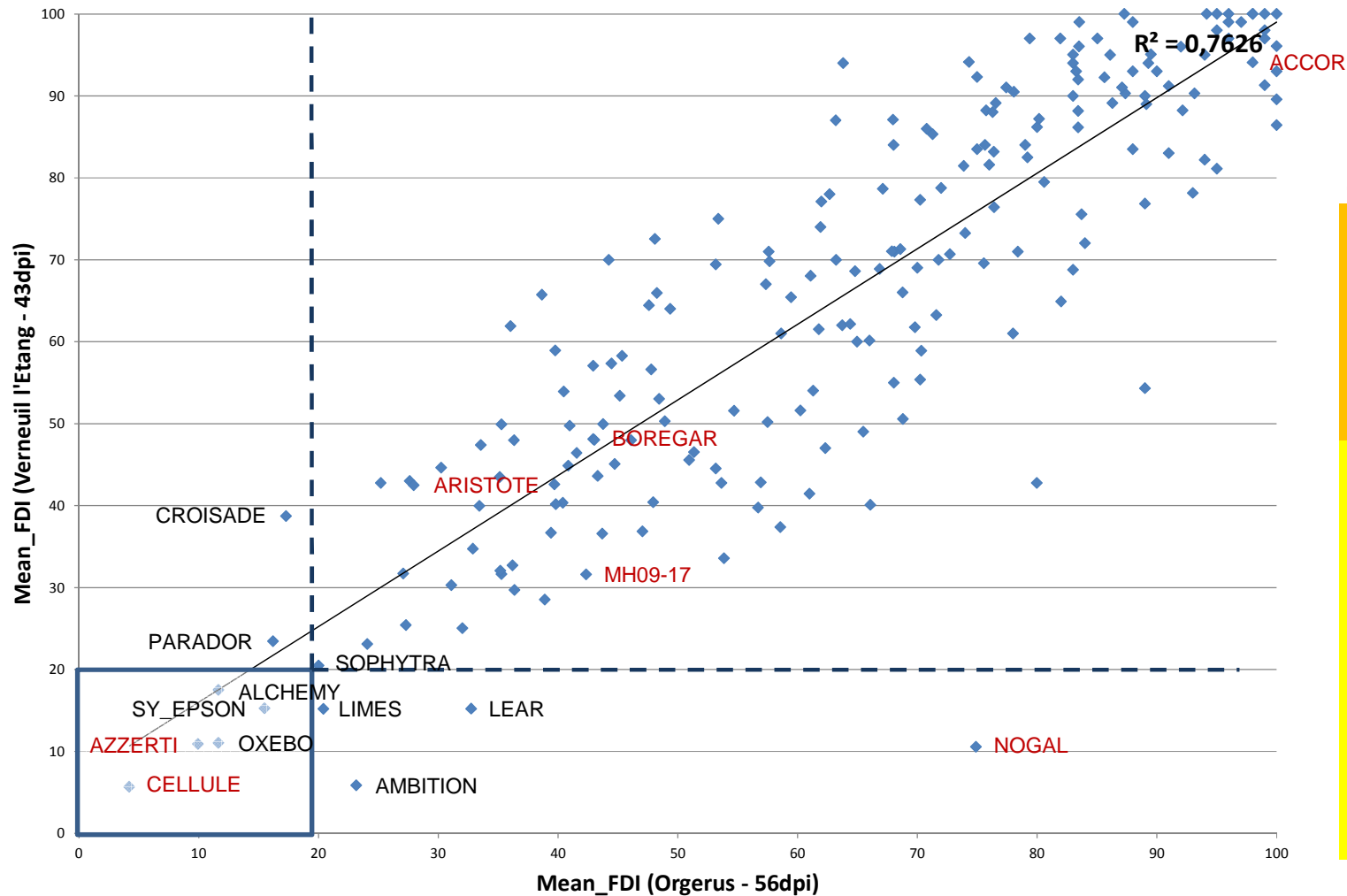
Notations:

- 11/12 June
- 23 June



Field evaluation of the Breedwheat Panel

Coefficient of determination between sites



Seedling Evaluation

Code BW Variety

155	CELLULE
166	MH09-17
211	NOGAL
70	ARISTOTE
180	ACCOR
76	BOREGAR
73	AZZERTI
93	PLAYER
66	SY_EPSON
74	BAROK
94	RENAN
37	TIMING
132	ACOUSTIC
197	MIROIR
62	SANKARA
200	PRIMO
8	AMBITION
156	FLAMENKO
150	EPIDOC

Field evaluation of the Breedwheat Panel

Coefficients of correlation between seedling and field notations

IPO-09415

Pearson
correlation
coefficients

Field / Orgerus

Field / Verneuil

Seedling

ORG14-ZT-FDI/56-R1
ORG14-ZT-FDI/57-R2
VER14-ZT-FDI/43-R1
VER14-ZT-FDI/44-R2
09415-SDI/21
09415-SPO/max

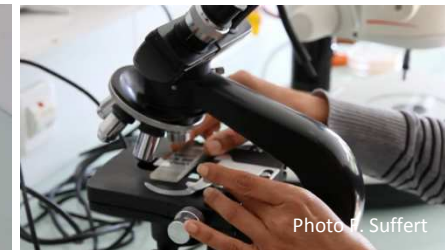
ORG14-ZT- FDI/56-R1	ORG14-ZT- FDI/57-R2	VER14-ZT- FDI/43-R1	VER14-ZT- FDI/44-R2	09415-SDI/21	09415-SPO/max
*					
0,868	*				
0,844	0,785	*			
0,808	0,783	0,823	*		
0,278	0,300	0,306	0,394	*	
0,338	0,356	0,386	0,452	0,852	*

Factors explaining the lost of correlation between field and seedling data:

- Confounding effects (plant architecture, days to heading, ...)
- Plant developmental stage effect on the expression of the resistance
- Environmental effect (temperature, light, humidity, ...)
- Presence of natural inoculum introducing genetic biais →

Field evaluation of the Breedwheat Panel

Re-sampling of septoria isolates



- 139 isolates were isolated from Breedwheat trials at Orgerus and Verneuil l'Etang, mostly from cv. APACHE
- 93 isolates genotyped with 12 microsatellite markers to determine their hamplotype

BMC
Research Notes

TECHNICAL NOTE

Open Access

Development of a rapid multiplex SSR genotyping method to study populations of the fungal plant pathogen *Zymoseptoria tritici*

Angélique Gautier^{1†}, Thierry C Marcel^{1†}, Johann Confais¹, Charles Crane², Gert Kema³, Frédéric Suffert¹ and Anne-Sophie Walker^{1*}

Field evaluation of the Breedwheat Panel

Re-sampling of septoria isolates



Occurrence of IPO-09415 haplotype in field trials resampled isolates:

		Orgerus	Verneuil l'Etang
APACHE (S)	Flag	14/15 (93%)	9/14 (64%)
	Low	14/17 (82%)	8/12 (67%)
AZZERTI (R)	Flag	-	2/5
	Low	2/3	0/5
RENAN (R)	Flag	-	0/3
Nursery edges		0/11*	0/8
TOTAL		46 isolates	47 isolates

* 3/11 isolates correspond to the haplotype of Syngenta's reference isolate

Conclusions

Seedling evaluation of the Breedwheat Panel

- ❖ A set of 110 *Zymoseptoria tritici* isolates has been characterized for their virulences and for their level of aggressiveness
- ❖ IPO-09415 is one of the most aggressive isolates on French elite material
- ❖ Development of a disease index to characterize wheat-*Z. tritici* interactions in controlled experiments on seedlings (SDI)
- ❖ 7 highly resistant and 12 moderately resistant cultivars have been identified at seedling stage with four isolates of *Z. tritici* differing for their virulences

Field evaluation of the Breedwheat Panel

- ❖ Development of a disease index to characterize wheat-*Z. tritici* interactions in field trials (FDI)
- ❖ The reproducibility of *Z. tritici* phenotyping obtained in controlled experiments on seedlings or in field trials is good
- ❖ The correlation between SDI and FDI is low (0.28 – 0.39)

Perspectives

- ❖ Evaluate the resistant genotypes (seedling group D) with a set of isolates selected for their virulences against known *STB* genes
- ❖ Propose a new set of bread wheat differential lines for their resistance to *Zymoseptoria tritici*
- ❖ Study the resistance mechanism of different *STB* resistance genes
- ❖ Perform association mapping analyses to identify resistance factors with « controlled conditions seedling phenotypes against 4 isolates and with « field phenotypes » against IPO-09415
- ❖ Perform association mapping analyses to identify virulence and aggressivity genetic factors in the French population of *Z. tritici*



GENETIC RESISTANCES IN DURUM WHEAT

Project CTPS SEPTODUR - Contribution à la durabilité de la lutte génétique et chimique contre la septoriose du blé dur par la caractérisation des populations du complexe d'espèces responsable de cette maladie. 2013-2015.

Project CTPS DUROMAL - Résistance durable aux maladies foliaires chez le blé dur : développement d'outils d'aide à la sélection. 2014-2017.



Genetic resistances in durum wheat

Objectives

Development of the tools and protocols to help for the selection of durum wheat cultivars durably resistant to septoria leaf blotch (*Z. tritici*)

Actions

- ❖ Identification of resistance sources from European cultivars (controlled conditions and field disease tests)
- ❖ Set-up a set of differential lines for their resistance to *Zymoseptoria tritici*
- ❖ Characterize a set of *Z. tritici* isolates (World and French)
- ❖ Genetically map resistance genes from most resistant cultivars

Genetic resistances in durum wheat

Evaluation of French durum wheat cultivars

		IPO-95052 PVC21	L6-595 PVC21	L6-702 PVC21	L6-1035 PVC21
1	Ainzen I	61	41	59	74
	AUS-1	0	0	0	0
	Prospero	19	0	1	3
	Orobel	4	1	6	16
	Cando	11	2	6	16
	CD11_SEPT_04	0	0	0	17
	Plusur	11	0	0	22
	CD11_SEPT_07	39	0	0	29
	Obelix	6	4	3	29
	Nafar	17	5	8	31
	Cossur	15	0	8	31
	Postifur	8	0	1	34
	Prodigal	42	12	7	54
	Athoris	1	1	8	54
	08-D-2450	23	0	0	65
	CD11_SEPT_02	49	0	12	67
	Portedur	56	0	1	69
	Joyau	46	0	4	84
	Lemur	44	0	6	88
	CD11_SEPT_10	17	20	6	93
	Marakas	27	0	1	98
	SV Gibraltar	35	0	20	82
	SV Blanco	21	14	23	47
	Lloyd	40	4	25	48
	Ramirez	21	0	25	61
	Atoudur	19	4	26	70
	SV Enzo	25	9	29	70
	Auris	32	0	29	17
	SV Carmo	14	5	31	43
	Zenath-Bouteille	69	3	31	64
	SV Cysco	51	3	35	62
	Surmesur	46	0	37	91
	Bakardi	45	10	38	61
	CD11_SEPT_11	44	6	41	76
	Augur	1	36	1	67
	CD11_SEPT_06	10	2	43	70
	Sculptur	32	2	46	73
	Miradoux	37	6	47	38
	Cessur	47	7	47	91
	Acalou	43	1	48	88
	CD11_SEPT_01	75	1	48	57
	Actisur	33	17	48	74
	Loukoum	37	19	49	78
	Volcani-447	90	1	49	85
	Byblos	49	14	50	91
	Sachem	73	2	55	79
	Orlu	44	9	56	81
	Alexis	58	0	67	72
	CD11_SEPT_08	57	15	77	97
	Karur	33	20	25	72
	Musclur	50	21	35	83
	Fabulis	52	23	39	80
	Reglisse	34	23	27	60
	Ralief	39	24	39	74
	Babylone	24	24	29	70
	CD11_SEPT_05	24	24	65	94
	Murano	35	25	47	85
	Qualidou	43	25	39	51
	Lyliou	37	25	56	42
	Kanakis	60	26	37	86
	Liberdur	46	27	35	80
	Pictur	66	31	64	94
	Isildur	56	32	31	75
	Ginseng	65	32	55	93
	Nautur	26	32	58	85
	Asterix	45	33	53	56
	CD11_SEPT_03	48	35	37	83
	Brennur	39	35	48	72
	Floridou	28	38	58	71
	Pescadou	53	38	39	67
	CD11_SEPT_09	64	41	61	94
	Anvergur	72	43	34	92
	Tablur	51	49	54	86
	Pharaon	57	62	74	96
	Luminur	77	65	84	97

■ 9 variétés ou lignées ont un bon niveau de résistance aux 4 isolats testés

■ 52% des variétés testées semblent porter des résistances spécifiques contournées par au moins un des 3 isolats Français, dont SCULPTUR et MIRADOUX

■ 35% des variétés testées sont sensibles aux trois isolats Français

■ Certaines variétés sont particulièrement sensibles et pourraient être utilisées comme témoins: LUMINUR, PHARAON, GINSENG, PICTUR, TABLUR

Résistant <20%; Modéré <50%; Sensible <100%



Genetic resistances in durum wheat

Constitution of a European durum wheat panel



A European panel of 200 durum wheat cultivars

- 6 reference cultivars: Joyau, Cando, Prodigal, Lloyd, AUS-1
- 66 cultivars proposed by GIE Blé Dur (France, Italia, Spain)
 - 5 controls: Sculptur, Pharaon & Fabulis (S), Babylone & Nobilis (R)
- 128 cultivars representative of durum wheat breeding in Italia (1915- 2007)
(Association panel from Laidò et al. 2013, 2014)

Evaluation of the European durum wheat panel

- With 4 isolates in controlled conditions on seedlings
- With 2 isolates in field trial (Arvalis – Villiers-le-Bâcle, 2015)

Isolates and Populations

INRA BIOGER, Grignon

Aurélie Ducasse

Lilian Gout

Henriette Goyeau

Marc-Henri Lebrun

PRI (the Netherlands)

Gert G.H.J. Kema

ARVALIS

David Gouache

Phenotypic characterization

INRA BIOGER

Béatrice Beauzoone

Gwilherm Gazeau

Martin Willigsecker

Breadwheat Field Trials

ETS FLORIMOND DESPREZ

Denis Beghin

Olivier Robert

SYNGENTA

Lucie Pericard

Baptiste Robin

Patrice Senellart

LIMAGRAIN Europe

Jérémy Derory

Céline Duque

Durum wheat

GIE Blé Dur

ARVALIS

CRA (Italia)