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Article

Monitoring and surveillance of aerial mycobiota of rice paddy through DNA metabarcoding and qPCR

Supplementary Materials:

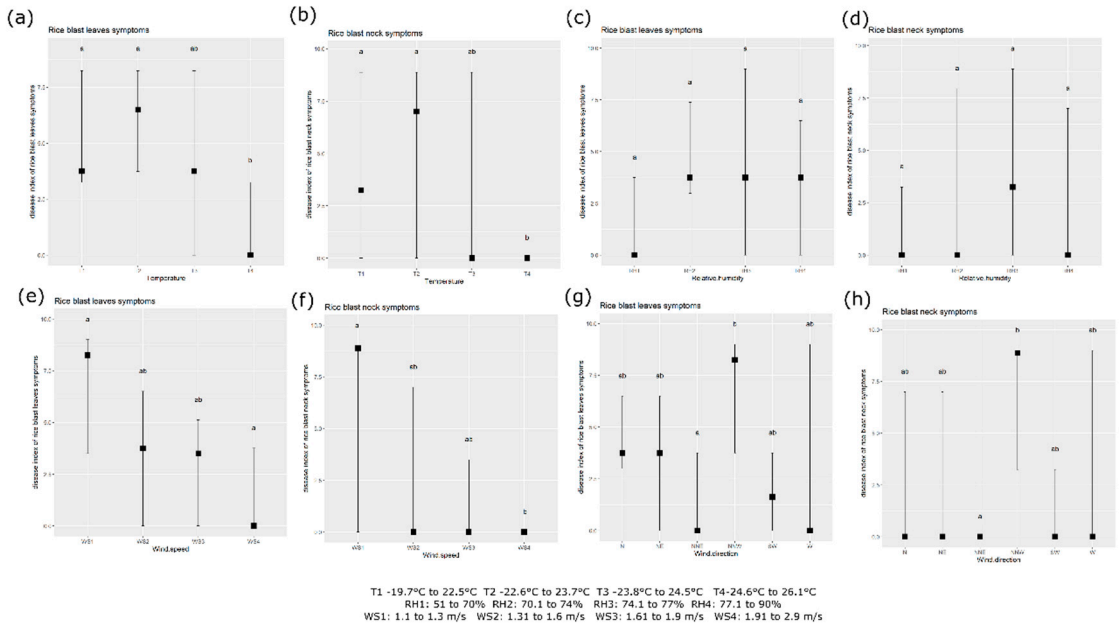

Figure S1. Representation of the leaves (a, c, e, g) and neck (b, d, f, h) blast symptoms index and the weather data. A and B. Kruskal-Wallis rank sum with Dunn test of the groups determined by the temperature quartiles. C and D: Kruskal-Wallis rank sum with Dunn of the groups determined by the relative abundance quartiles. E and F: Kruskal-Wallis rank sum with Dunn of the groups determined by the wind speed quartiles. G and H Kruskal-Wallis rank sum with Dunn of the groups determined by the wind direction.

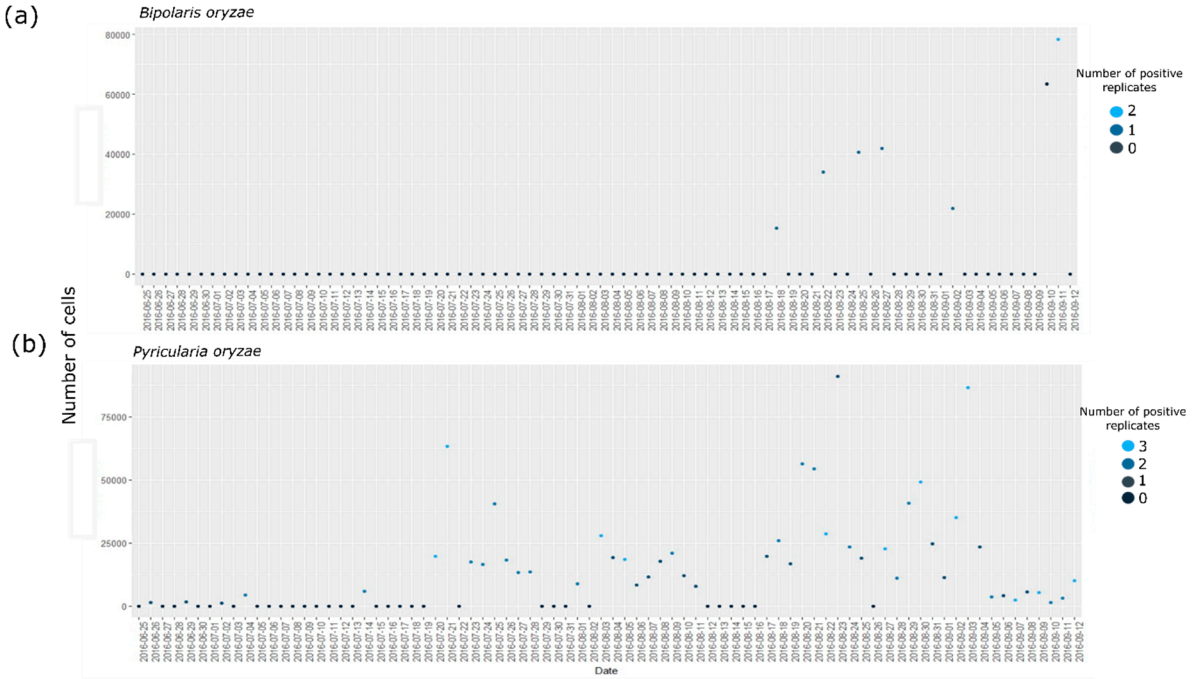

Figure S2. Number of cells of *Bipolaris oryzae* (a) and *Pyricularia oryzae* (b) present among the trial calculated according the Ct of the qPCR assays.

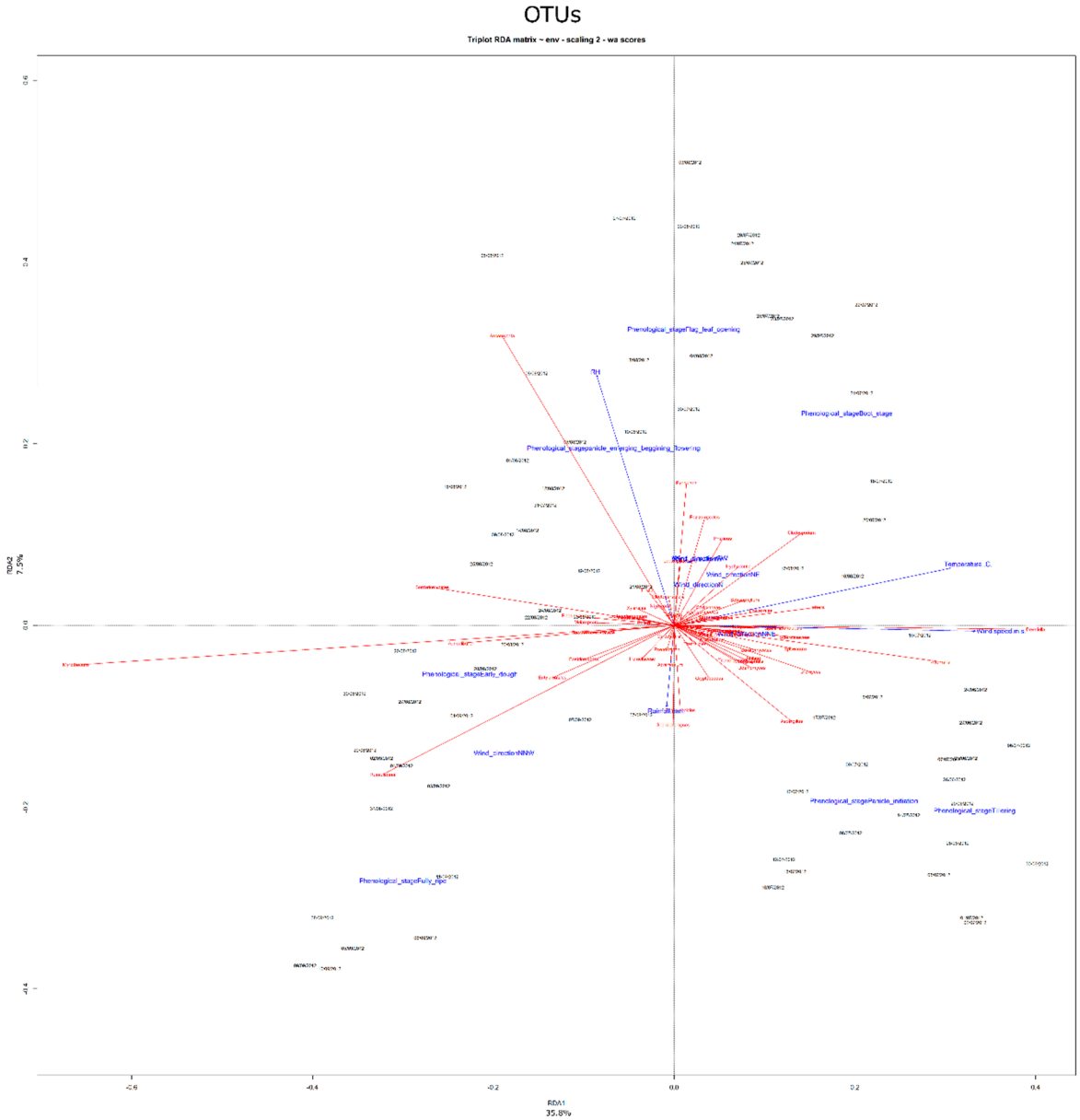

Figure S3. Redundancy Analysis (RDA) representing the Axis 1 and 2 among the OTUs and the weather data and phenological stages. Proportion explained by two first constrained ordinations are reported as percentages in the axes.

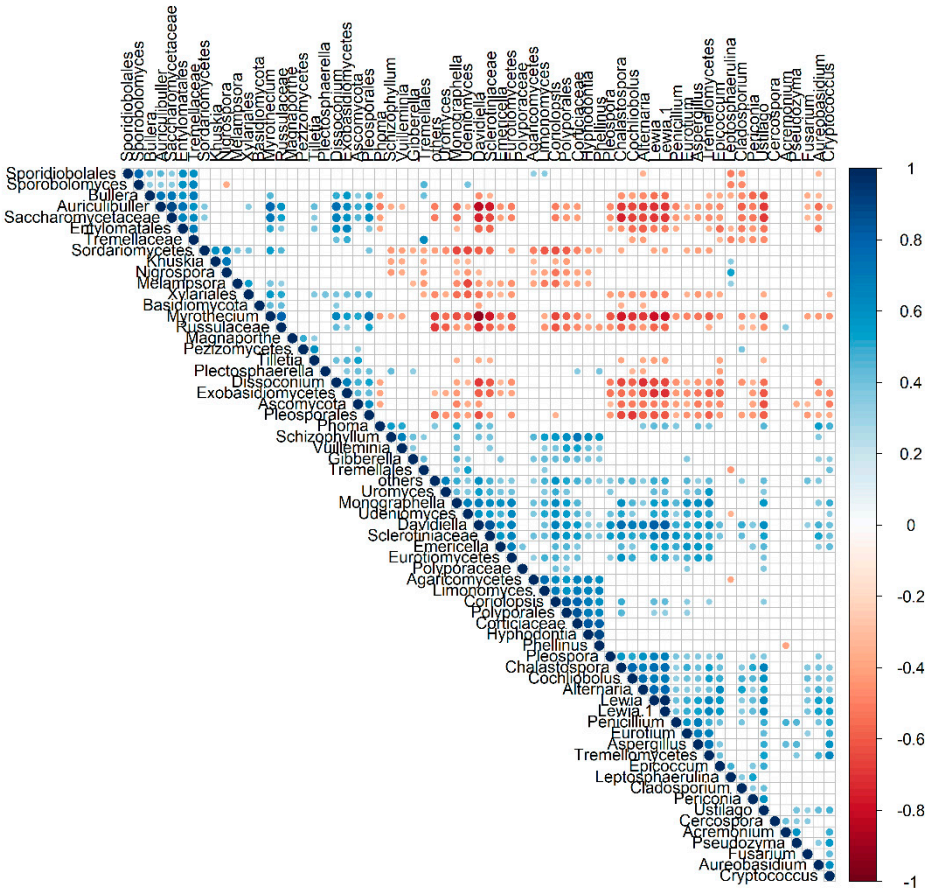

Figure S4. Significant co-occurrence and co-exclusion relationships between species. Spearman's rank correlation matrix of the genera identified with > 0.2% abundance in at least 20 samples. The plots only represent significant differences FDR < 0.01. Strong correlations are indicated by large circles, whereas weak correlations are indicated by small squares. The colour of the scale bar denotes the nature of the correlation, with 1 indicating perfectly positive correlation (dark blue) and -1 indicating a perfectly negative correlation (dark red).

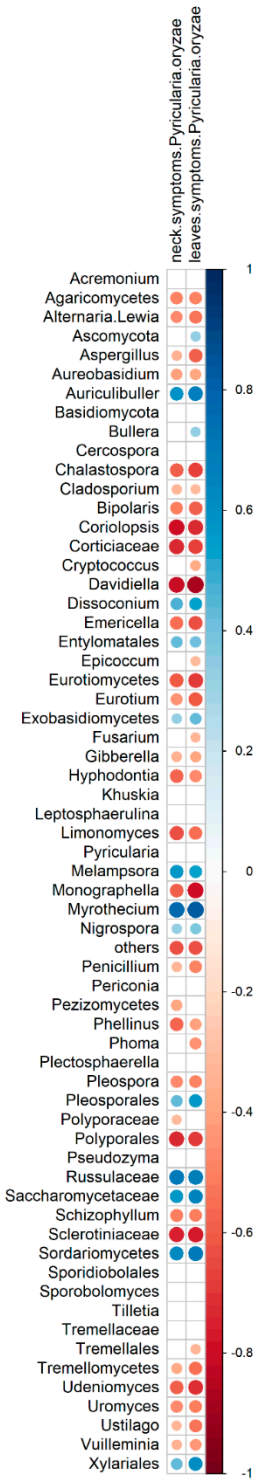

Figure S5. Significant correlation between OTUs and the *Pyricularia* leaves and neck symptoms using Spearman's rank correlation matrix of the genera identified with > 0.2% abundance in at least 20 samples. The plots only represent significant differences FDR < 0.01. Strong correlations are indicated by large circles, whereas weak correlations are indicated by small circles. The colour of the scale bar denotes the nature of the correlation, with 1 indicating perfectly positive correlation (dark blue) and -1 indicating a perfectly negative correlation (dark red).

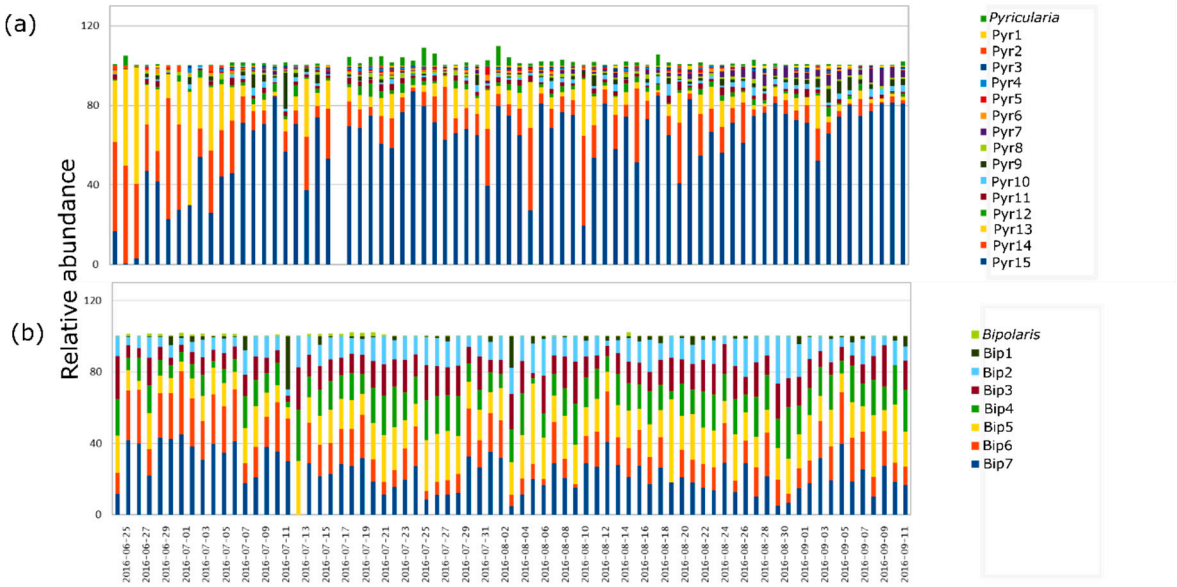

Figure S6. Distribution of the oligotypes of *Pyricularia* (a) and *Bipolaris* (b) over time.

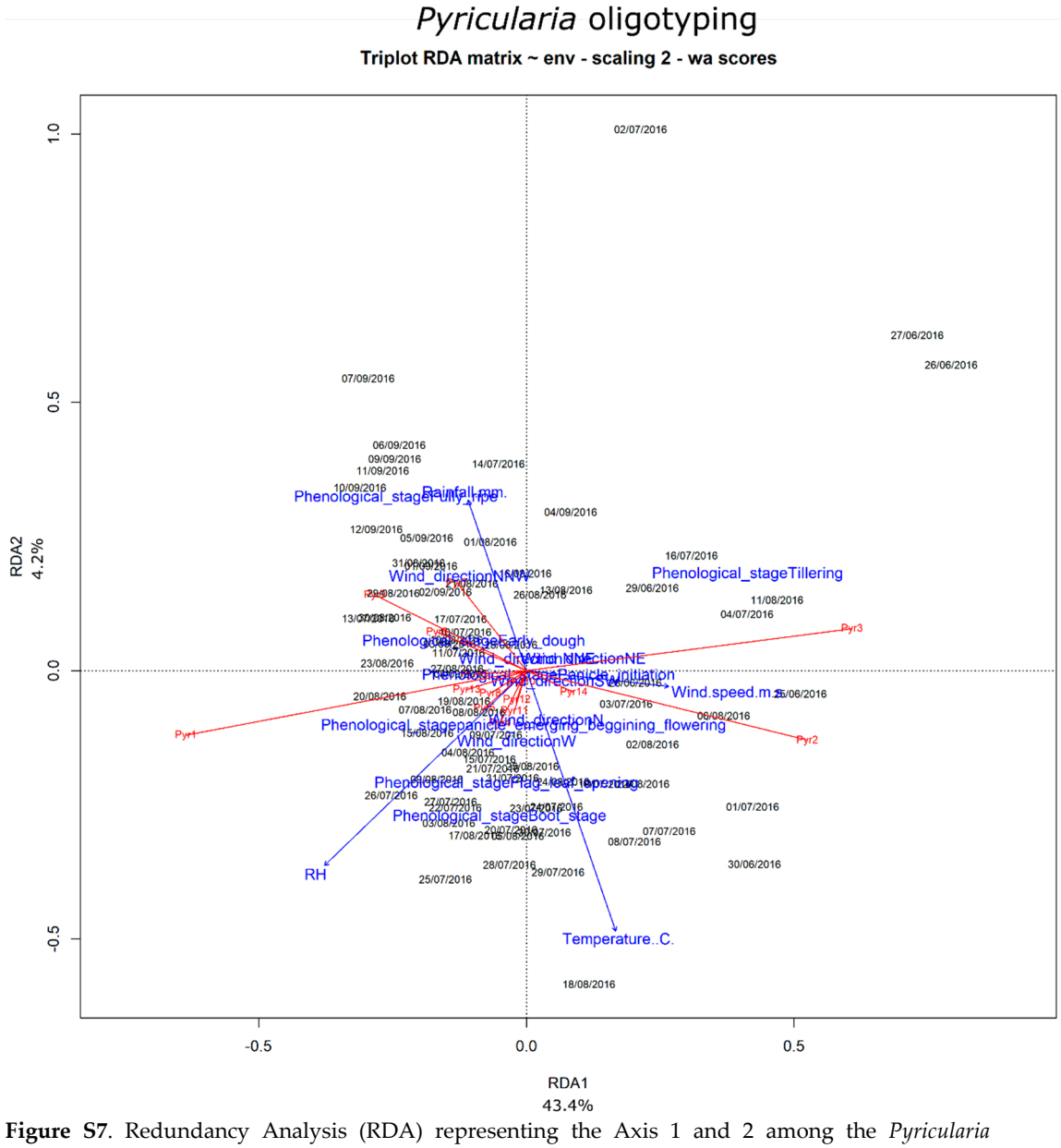

Figure S7. Redundancy Analysis (RDA) representing the Axis 1 and 2 among the *Pyricularia*

oligotypes and the weather data and phenological stages. Proportion explained by two first constrained ordinations are reported as percentages in the axes.

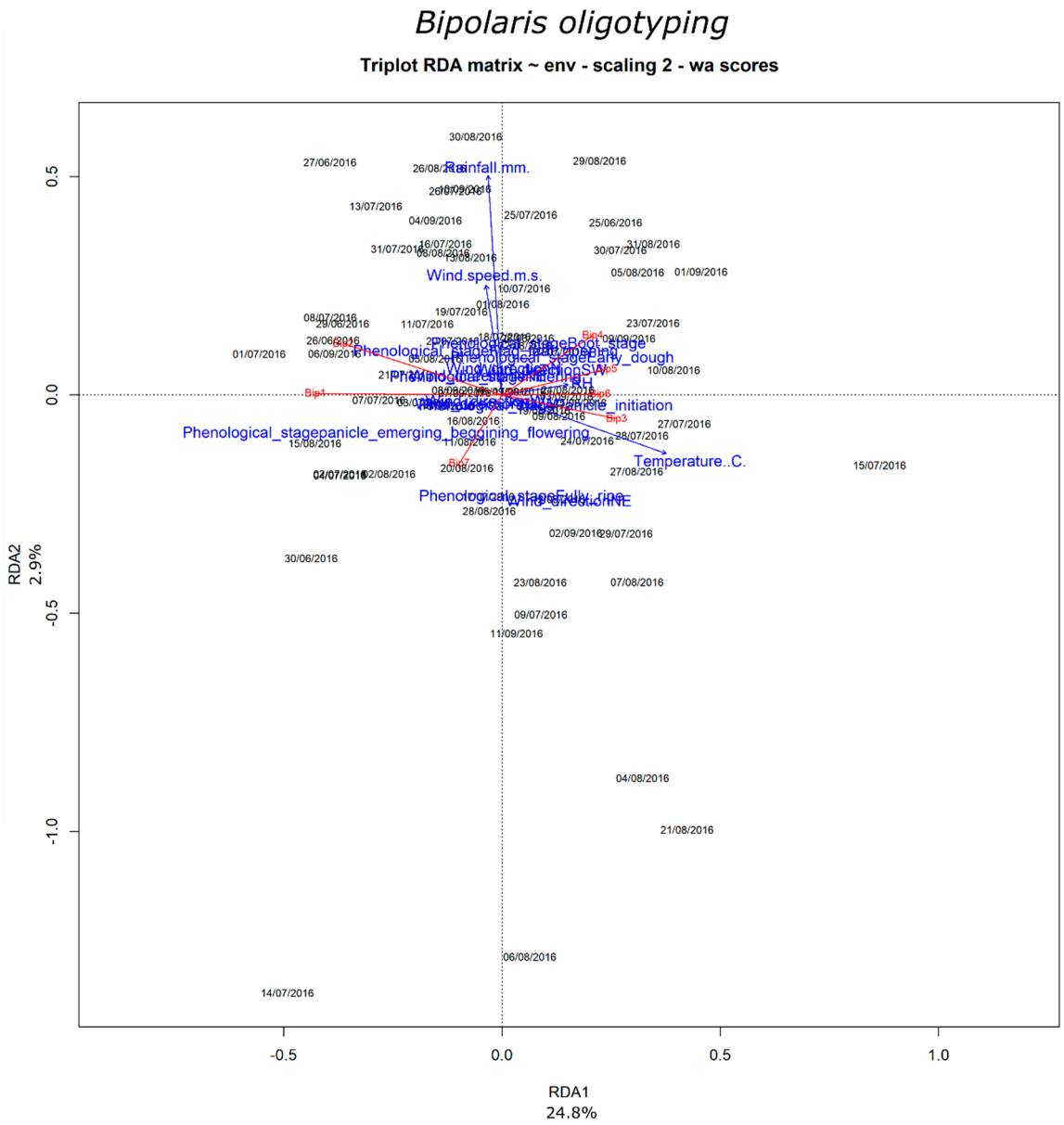

Figure S8. Redundancy Analysis (RDA) representing the Axis 1 and 2 among the *Bipolaris* oligotypes and the weather data and phenological stages. Proportion explained by two first constrained ordinations are reported as percentages in the axes.

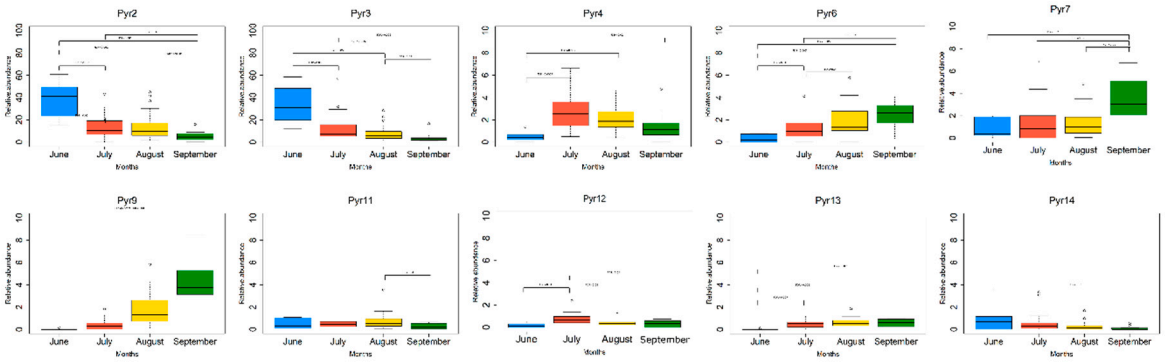

Figure S9. Abundance (%) of *Pyricularia* oligotypes significantly different (FDR < 0.05) among the sampling month (June, July, August and September).

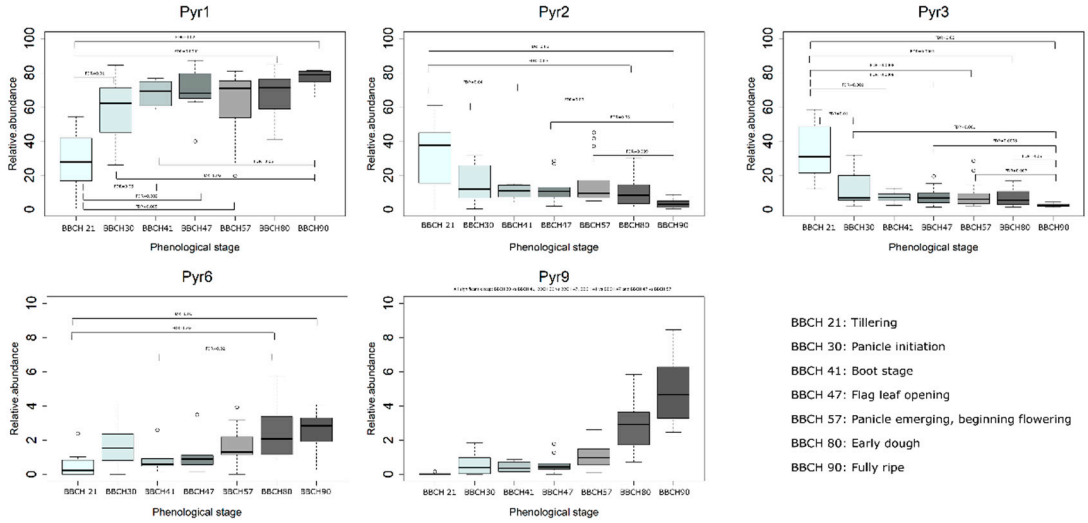

Figure S10. Abundance (%) of *Pyricularia* oligotypes significantly different (FDR < 0.05) among the growth stages (BBCH 21: tillering; BBCH 30: panicle initiation; BBCH 41: boot stage; BBCH 47: leaf opening; BBCH 57: panicle emerging; BBCH 80: early dough; and BBCH 90: fully ripening).

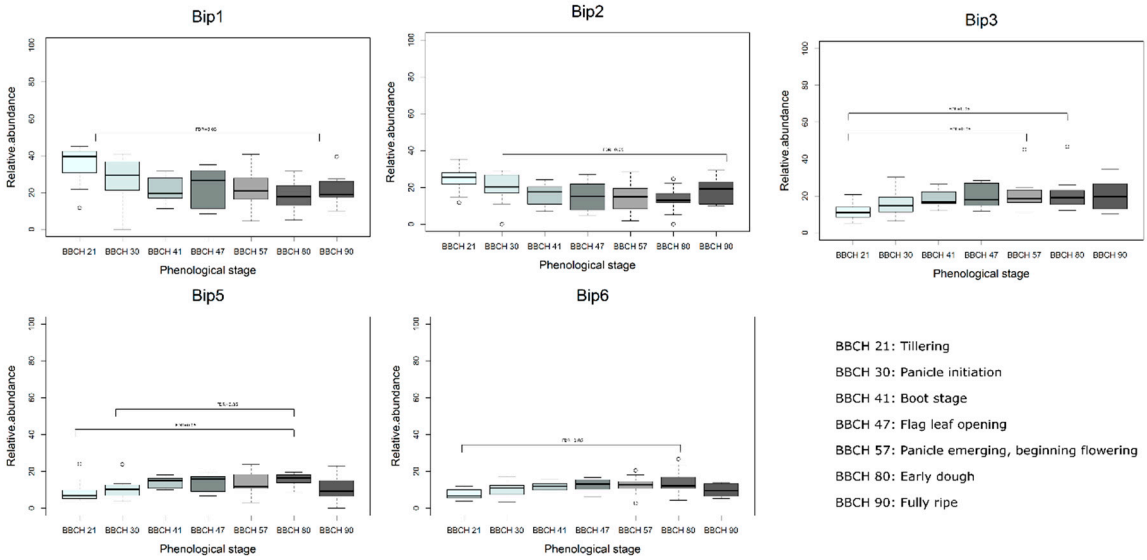

Figure S11. Abundance (%) of *Bipolaris* oligotypes significantly different (FDR < 0.05) among the growth stages (BBCH 21: tillering; BBCH 30: panicle initiation; BBCH 41: boot stage; BBCH 47: leaf opening; BBCH 57: panicle emerging; BBCH 80: early dough; and BBCH 90: fully ripening).

Table S1. Growth stages of the rice paddy during the trial.

DATA	PHENOLOGICAL STAGE	IDENTIFICATION KEYS OF RICE
07/05/2016	Sowing in water	
14/05/2016	BBCH:10-11	Imperfect leaf unrolled, tip of first true leaf visible- First leaf unfolded
30/05/2016	BBCH:13-14	3 leaves unfolded
14/06/2016	BBCH:21	Beginning of tillering: first tiller detectable
21/06/2016	BBCH:22-23	2-3 tillers detectable
05/07/2016/	BBCH:30-34	Panicle initiation or green ring stage: chlorophyll accumulates in the stem tissue, forming a green ring-Internode elongation or jointing stage: internodes begin to elongate, panicle more than 2 mm long (variety-dependent)
19/07/2016	BBCH:41	Early boot stage: upper part of stem slightly thickened, sheath of flag leaf about 5 cm out of penultimate leaf sheath
26/07/2016	BBCH:47-49	Flag leaf sheath opening-open
04/08/2016	BBCH:57-61	70% of panicle emerged- Beginning of flowering: anthers visible at top of panicle
20/08/2016	BBCH:80	Early dough
05/09/2016	BBCH:90	Fully ripe: grain hard, difficult to divide with thumbnail

Table S2. Meteorological conditions during the trial in the rice paddy.

Date	**Rainfall (m*m*)**	*Temperature (°C)*	*Relative Humidity (%)*	*Wind Speed* **(m/s)**	*Wind Direction*
25/06/2016	0	24.3	73	2.8	NNE
26/06/2016	0	24.4	71	1.9	NE
27/06/2016	0	24	66	1.6	N
28/06/2016	0	23.6	69	2.0	NNE
29/06/2016	0	24.8	67	2.0	NNE
30/06/2016	0.2	24.5	76	1.6	W
01/07/2016	0.4	25	71	1.3	N
02/07/2016	0	23.8	81	1.8	NNE
03/07/2016	11.4	25.1	75	1.2	N
04/07/2016	0	23.3	74	2.2	NE
07/07/2016	0	25.1	77	1.7	N
08/07/2016	0	26.1	74	2.1	NE
09/07/2016	0	27.7	69	1.2	SW
10/07/2016	0	27.5	72	1.7	NNE
11/07/2016	0	26.1	78	2.2	NNE
13/07/2016	13.6	21.8	78	2.4	N
14/07/2016	15.2	19.7	63	2.5	NNW
15/07/2016	0.4	20.3	51	2.9	NNE
16/07/2016	0	21.9	59	1.6	SW
17/07/2016	0	22.8	68	1.8	NNE
18/07/2016	0	24.6	68	1.5	NNW
19/07/2016	0	25.0	72	1.2	N
20/07/2016	0	26.7	70	1.6	N
21/07/2016	0	26.0	69	1.6	NNE
22/07/2016	0.6	22.5	88	2.0	N
23/07/2016	2.2	21.5	88	1.9	W
24/07/2016	0.2	24.1	76	1.2	W

25/07/2016	0	25.1	78	1.2	W
26/07/2016	0	26.4	72	2.1	NNE
27/07/2016	0	24.1	79	2.2	N
28/07/2016	0.6	24.5	74	1.3	SW
29/07/2016	0	25.1	76	1.7	N
30/07/2016	0	26.3	74	1.4	N
31/07/2016	0	23.0	87	2.1	NNE
01/08/2016	19.6	24.0	78	1.7	NE
01/08/2016	19.6	24.0	78	1.7	NE
02/08/2016	0	23.8	74	1.8	NNE
03/08/2016	0	24.4	81	1.4	N
04/08/2016	0	25.1	77	2.0	NE
05/08/2016	2	21.1	81	2.2	NNE
06/08/2016	1	22.3	68	1.5	N
07/08/2016	0	22.1	71	1.5	N
08/08/2016	0	22.9	71	1.3	NNW
09/08/2016	0	23.0	77	1.8	NNE
09/08/2016	0	23.0	77	1.8	NNE
10/08/2016	3.8	22.2	65	1.8	NNE
11/08/2016	4.4	19.9	66	1.8	N
13/08/2016	0	21.2	75	1.3	NNW
15/08/2016	0	24.5	72	1.4	N
16/08/2016	0	25.0	69	1.6	SW
17/08/2016	0	23.7	74	1.3	N
18/08/2016	0	23.6	76	1.6	N
19/08/2016	0	23.3	79	1.7	NE
20/08/2016	0	22.4	91	1.8	NE
21/08/2016	11	23.4	76	1.6	NNE
22/08/2016	0	21.1	66	1.9	NNW

23/08/2016	0	21.3	73	1.3	NNW
24/08/2016	0	22.2	73	1.3	N
25/08/2016	0	23.7	72	1.4	NE
26/08/2016	0	23.6	74	1.3	NNW
27/08/2016	0	23.6	74	1.3	NNW
27/08/2016	0	23.4	73	1.5	NNW
28/08/2016	0	24.5	72	1.4	N
29/08/2016	0	24.6	75	1.4	NNW
30/08/2016	3.4	21.3	87	2.2	N
31/08/2016	2	23.1	78	1.3	NNW
01/09/2016	0	23.9	75	1.1	W
02/09/2016	0	23.8	77	1.3	N
03/09/2016	0	23.9	78	1.2	N
04/09/2016	0	23.8	79	1.2	N
05/09/2016	0	23.4	70	1.6	NNE
06/09/2016	0	22.5	62	2.2	NNW
07/09/2016	0	21.2	68	1.8	NNW
08/09/2016	0	22.3	70	1.3	N
09/09/2016	0	22.5	74	1.2	NNW
10/09/2016	0	23.0	70	1.3	NNW
11/09/2016	0	22.7	75	1.1	NNW
12/09/2016	0	22.8	75	1.1	W

Table S3. Rice blast severity, indicated as disease index from 0 to 9, measured in the field during the cropping season (2016).

	19th July	*26th July*	*2nd August*	*10th August*	*17th August*	*23rd August*	*30th August*
NECK	0.0	0.0	0.0	3.2	7.0	8.9	9.0
LEAVES	3.0	3.7	4.0	3.2	6.5	8.2	9.0

Table S4. Alpha diversity values over time.

DATE	MONTH	SHANNON	CHAO1	OBSERVED OTUS	GOODS COVERAGE	ESC %
25/06/2016	June	5.728	1458.127	894.000	0.974	97.423
26/06/2016	June	5.657	1140.546	688.000	0.981	98.082
27/06/2016	June	6.256	1372.420	867.333	0.977	97.724
28/06/2016	June	5.390	1362.449	779.667	0.977	97.691
29/06/2016	June	5.211	1228.353	704.000	0.979	97.916
30/06/2016	June	5.455	1011.506	648.667	0.983	98.255
01/07/2016	July	4.765	1149.141	641.333	0.980	98.020
02/07/2016	July	5.362	1417.719	777.000	0.976	97.564
03/07/2016	July	5.014	1016.376	614.333	0.983	98.300
04/07/2016	July	4.900	1353.234	721.333	0.976	97.624
07/07/2016	July	4.494	1032.670	603.667	0.982	98.234
08/07/2016	July	5.091	1287.180	753.000	0.977	97.737
09/07/2016	July	5.631	949.915	560.333	0.986	98.588
10/07/2016	July	4.989	966.985	586.667	0.984	98.411
11/07/2016	July	5.132	1338.811	708.667	0.977	97.714
13/07/2016	July	5.402	1074.169	647.000	0.982	98.199
14/07/2016	July	5.255	1140.763	659.667	0.981	98.084
15/07/2016	July	4.734	861.833	544.000	0.986	98.648
16/07/2016	July	5.493	1156.065	725.333	0.982	98.178
17/07/2016	July	5.556	963.686	597.000	0.986	98.563
18/07/2016	July	5.304	1140.787	651.667	0.980	97.970
19/07/2016	July	4.390	1003.993	568.667	0.983	98.290
20/07/2016	July	4.871	889.239	533.500	0.986	98.571
21/07/2016	July	4.445	939.747	524.333	0.984	98.429
22/07/2016	July	4.458	918.721	547.333	0.985	98.500
23/07/2016	July	4.894	907.367	545.333	0.985	98.500
24/07/2016	July	5.704	1515.070	861.667	0.974	97.364
25/07/2016	July	5.078	765.592	441.667	0.990	98.956
26/07/2016	July	4.558	967.513	510.333	0.983	98.348
27/07/2016	July	5.140	731.388	431.667	0.989	98.875
28/07/2016	July	5.063	698.921	389.333	0.990	99.008
29/07/2016	July	5.061	1393.957	708.000	0.977	97.707
30/07/2016	July	4.595	1070.864	586.667	0.981	98.130
31/07/2016	July	5.540	873.635	534.000	0.987	98.727
08/08/2016	August	4.853	1148.918	537.333	0.981	98.126
09/08/2016	August	5.107	1032.232	566.000	0.983	98.259
10/08/2016	August	5.047	837.209	524.333	0.987	98.665
11/08/2016	August	5.544	1667.186	918.667	0.970	97.027
13/08/2016	August	5.173	968.854	540.667	0.984	98.377

15/08/2016	August	5.029	828.607	484.000	0.987	98.729
16/08/2016	August	5.127	1272.575	698.667	0.978	97.797
17/08/2016	August	4.534	827.134	454.667	0.986	98.627
18/08/2016	August	4.719	1231.400	614.500	0.979	97.906
19/08/2016	August	4.604	1197.655	549.000	0.980	98.038
20/08/2016	August	4.776	769.431	444.000	0.988	98.764
21/08/2016	August	4.597	1220.272	543.333	0.980	97.991
22/08/2016	August	4.619	1586.372	729.667	0.974	97.393
23/08/2016	August	4.602	1099.142	532.000	0.981	98.126
24/08/2016	August	5.028	1372.590	696.667	0.976	97.620
25/08/2016	August	4.582	1319.198	565.000	0.979	97.916
26/08/2016	August	4.401	1009.029	496.000	0.983	98.298
27/08/2016	August	4.715	1169.571	570.833	0.980	98.009
28/08/2016	August	4.592	1069.230	536.500	0.981	98.125
29/08/2016	August	4.651	1158.798	508.500	0.982	98.218
30/08/2016	August	4.183	1034.337	453.667	0.984	98.365
31/08/2016	August	4.917	1185.976	559.000	0.981	98.072
01/08/2016	August	5.211	1330.252	700.667	0.978	97.766
02/08/2016	August	4.899	1110.422	583.333	0.981	98.090
03/08/2016	August	5.016	831.902	412.667	0.989	98.870
04/08/2016	August	4.718	551.565	336.000	0.992	99.193
05/08/2016	August	5.079	1675.116	836.667	0.971	97.098
06/08/2016	August	5.584	1700.250	907.333	0.970	97.009
07/08/2016	August	5.394	1098.419	624.667	0.982	98.186
01/09/2016	September	4.516	1081.610	498.667	0.982	98.246
02/09/2016	September	4.582	1351.799	571.667	0.978	97.837
03/09/2016	September	4.175	1142.402	488.000	0.982	98.167
04/09/2016	September	4.955	1566.092	741.667	0.973	97.337
05/09/2016	September	4.422	1400.603	599.000	0.978	97.751
06/09/2016	September	4.554	1239.060	587.667	0.979	97.905
07/09/2016	September	5.148	1460.947	679.000	0.975	97.514
08/09/2016	September	4.908	1372.419	658.667	0.977	97.683
09/09/2016	September	4.336	1215.225	503.333	0.981	98.076
10/09/2016	September	4.539	1095.600	504.000	0.982	98.188
11/09/2016	September	3.857	985.095	437.667	0.984	98.388
12/09/2016	September	4.731	1587.578	657.333	0.975	97.525

Table S5. Average relative abundance (%) of the OTUs throughout the season.

OTU	Relative abundance (%)	OTU	Relative abundance (%)
Cladosporium	20.177	*Gibberella*	0.334
Alternaria	9.88	*Ustilago*	0.269

Myrothecium	7.312	*Pleospora*	0.231
Ascomycota	7.026	*Acremonium*	0.225
Epicoccum	5.787	Saccharomycetaceae	0.223
Basidiomycota	5.292	*Aureobasidium*	0.204
Sporidiobolales	5.154	*Tilletia*	0.195
Sordariomycetes	4.448	Exobasidiomycetes	0.174
Davidiella	4.299	Agaricomycetes	0.164
Russulaceae	2.727	*Dissoconium*	0.15
Leptosphaerulina	2.333	*Emericella*	0.147
Entylomatales	1.852	*Periconia*	0.144
Pyricularia	1.574	*Eurotium*	0.142
Auriculibuller	1.516	*Vuilleminia*	0.134
Pleosporales	1.357	Polyporaceae	0.134
others	1.332	*Pseudozyma*	0.13
Sporobolomyces	0.975	*Plectosphaerella*	0.13
Tremellaceae	0.88	*Penicillium*	0.13
Lewia	0.673	Tremellales	0.124
Bipolaris	0.568	*Limonomyces*	0.12
Uromyces	0.544	Eurotiomycetes	0.118
Hyphodontia	0.527	Tremellomycetes	0.115
Fusarium	0.516	Xylariales	0.115
Polyporales	0.502	*Chalastospora*	0.115
Schizophyllum	0.497	*Udeniomyces*	0.11
Coriolopsis	0.488	*Bullera*	0.109
Aspergillus	0.46	*Nigrospora*	0.101
Sclerotiniaceae	0.451	*Monographella*	0.096
Pezizomycetes	0.412	*Cercospora*	0.094
Melampsora	0.388	*Phoma*	0.094
Phellinus	0.346	*Khuskia*	0.086
Cryptococcus	0.339	Corticiaceae	0.078

Table S6. Identification of the oligotypes of *Pyricularia* and *Bipolaris* showing the BLASTn results the percentage of similarity and the GenBank accession numbers of the most similar sequences.

OLIGOTYPING	*BLAST RESULTS*	*SIMILARITY*	*GENBANK ACCESSION NUMBER*
Bip1	*Bipolaris austrostipae/Bipolaris cynodontis/Bipolaris coffeana*	99%	NR_147491/ HF934930/ HE792944
BIP2	*B. austrostipae/B. cynodontis/B. coffeana*	100%	NR_147491/ HF934930/ HE792944
BIP3	*Curvularia buchloes/Curvularia hawaiiensis/Curvularia spicifera/Bipolaris buchloes/Bipolaris maydis/Curvularia australiensis/Cochliobolus* sp./*Curvularia* sp.	99%	NR_147487/ HG778990/ HG778989/ HG778989/ JN207242/ HF934914/ KJ909765/ KJ913699/ KC986957/ JN207283/ JN207258/ FJ235087/ JN207242
BIP4	*C. buchloes/C. hawaiiensis/C. spicifera/B. buchloes/B. maydis/C. australiensis/Cochliobolus* sp./*Cochliobolus* sp.	100%	NR_147487/ HG778990/ HG778989/ HG778989/ JN207242/ HF934914/ KJ909765/ KJ913699/ KC986957/ JN207283/ JN207258/ FJ235087/ JN207242
BIP5	*C. buchloes/C. hawaiiensis/C. spicifera/B. buchloes/B. maydis/C. australiensis/Cochliobolus* sp./*Cochliobolus* sp.	100%	NR_147487/ HG778990/ HG778989/ HG778989/ JN207242/ HF934914/ KJ909765/ KJ913699/ KC986957/ JN207283/ JN207258/ FJ235087/ JN207242
BIP6	*C. buchloes/C. hawaiiensis/C. spicifera/B. buchloes/B. maydis/C. australiensis/Cochliobolus* sp./*Cochlibolus* sp.	100%	NR_147487/ HG778990/ HG778989/ HG778989/ JN207242/ HF934914/ KJ909765/ KJ913699/ KC986957/ JN207283/ JN207258/ FJ235087/ JN207242
BIP7	*Curvularia inaequalis*	99%	KX674660
PYR1	*Magnaporthe grisea*	99%	DQ493955
PYR2	*Pyricularia oryzae*	99%	KM816794
PYR3	*P .oryzae*	99%	KM816794
PYR4	*M. grisea*	99%	DQ493955
PYR5	*M. grisea*	99%	DQ493955
PYR6	*M. grisea*	99%	DQ493955
PYR7	*P. oryzae/P. grisea*	99%	JQ747492/ JF414841
PYR8	*M. grisea*	99%	DQ493955

PYR9	*M. grisea*	99%	DQ493955
PYR10	*P. oryzae*	99%	KM816794
PYR11	*P. oryzae*	99%	KM816794
PYR12	*M .grisea*	99%	DQ493955
PYR13	*M. grisea*	99%	DQ493955
PYR14	*M. grisea*	99%	FN555109
PYR15	*M. grisea*	99%	DQ493955

Table S7. Correlation of Spearman between the *Pyricularia* oligotypes and the number of cells calculated with a qPCR with TaqMan probe designed by Su'udi et al. 2013. The table reports the rho value and the p-value of each correspondence.

OLIGOTYPE	IDENTIFICATION WITH BLASTN	RHO	P-VALUE
Pyr1	*Magnaporthe grisea*	**0.47**	1.76e-05
Pyr2	*Pyricularia oryzae*	0.16	0.18
Pyr3	*P. oryzae*	0.08	0.49
PYR4	*M. grisea*	0.42	0.0002
PYR5	*M. grisea*	0.46	3.13e-05
PYR6	*M. grisea*	0.39	0.0006
PYR7	*P. oryzae/M. grisea*	0.30	0.009
PYR8	*M. grisea*	0.49	7.592e-06
PYR9	*M. grisea*	0.56	1.342e-07
PYR10	*P. oryzae*	0.33	0.003
PYR11	*P. oryzae*	0.38	0.0007
PYR12	*M. grisea*	0.36	0.001
PYR13	*M. grisea*	0.47	2.117e-05
PYR14	*M. grisea*	0.17	0.15
PYR15	*M. grisea*	0.48	1.174e-05