Supplementary Materials: Changes in the *Fusarium* Head Blight complex of malting barley in a three-year field experiment in Italy

Giovanni Beccari, Antonio Prodi, Francesco Tini, Umberto Bonciarelli, Andrea Onofri, Souheib Oueslati, Marwa Limayma and Lorenzo Covarelli

Table S1. Incidence (%) of the different fungal genera isolated from the malting varieties in the three experimental years.

Variety	*Fusarium*						*Aspergillus*						*Penicillum*						*Epicoccum*						*Alternaria*					
	2011		2012		2013		2011		2012		2013		2011		2012		2013		2011	2012		2013			2011 [a]	2012		2013		
	%	±SE [b]	%	±SE	%	±SE	%	±SE	%	±SE	%	±SE	%	±SE	%	±SE	%	±SE	%	%	±SE	%	±SE		%	%	±SE	%	±SE	
Belgravia	32	3.5	25	1.5	20	2.4	11	1	26	0.8	3	0.7	4	1.2	11	1.3	2	0.5	0	3	0	2	0.5		0	29	4.5	57	2.7	
Concerto	33	1.9	12	1.2	19	1.9	9	1.5	29	2.5	0	0	14	2.4	16	1.2	1	0.4	0	2	0.6	2	0.5		0	23	1	75	1.8	
Esterel	32	2.2	18	2.1	22	1.7	2	1.6	13	1.3	1	0.4	9	1	24	4.3	0	0	0	0	0	6	1.4		0	41	5.9	60	2.2	
Grace	33	1.6	13	1.5	15	2.1	5	0.6	24	2.2	1	0.7	11	1	7	1.3	0	0	0	3	1.5	12	1.8		0	37	1.3	60	3.2	
Prague	31	1.7	15	1.3	10	2.1	15	0.5	25	2.6	0	0	1	0.8	16	1.2	1	0.4	0	4	1.2	5	1.7		0	20	1.4	56	2.4	
Propino	37	2.2	22	1.7	18	2.4	2	1	50	9.8	2	1.1	16	0.1	18	1.7	0	0	0	0	0	9	1.1		0	28	3.4	66	4.1	
Quench	31	2.2	21	1.9	14	1.6	7	1.3	31	3.7	0	0	6	1.9	20	1.4	2	0.5	0	1	0.5	6	1.2		0	29	2.5	60	2.2	
Scarlett	33	2.5	29	1.7	19	2.1	5	1.3	28	2.8	1	0.7	12	1.8	17	3.9	1	0.5	0	1	0.5	3	0.7		0	34	1	63	2.1	
Sunshine	41	2.2	28	2.6	27	2.5	5	1	20	0.8	2	0.7	12	2.2	27	0.5	1	0.4	0	0	0	6	1.6		0	26	1.3	66	3.3	
Violetta	47	2.9	18	1	20	2.6	19	1.3	16	2.2	2	1.1	6	1.5	11	1.5	6	1.8	0	0	0	7	0.9		0	34	1.7	51	3.7	
Wintmalt	40	2.1	17	3.3	13	1.3	18	0.6	43	5.7	2	0.7	11	0.5	22	2.9	0	0	0	3	1.5	5	1.2		0	17	3.4	55	3.5	
Average	35.5		19.8		17.9		8.9		27.7		1.3		9.3		17.2		1.3		0	1.5		5.7			0	28.9		60.8		
Significance of effects [c]																														
Year	2E-16						2E-16						2E-16						0.000044						2E-16					
Variety	0.0048						0.105						0.447						0.0032						0.078					
Year × Variety	0.047						0.000024						0.000024						0.23						0.013					

[a] In 2011 *Alternaria* incidence was not observed; [b] Standard Error; [c] *P*-levels from F tests in ANOVA

Table S2. *F. graminearum* chemotypes in 2011, 2012 and 2013.

Year	Chemotypes (number of *F. graminearum* strains)		
	NIV	**15ADON**	**3ADON**
2011	0	6	0
2012	4	0	0
2013	0	6	2

Table S3. Total counts of isolates observed for the different *Fusarium* species in the 11 malting barley varieties.

Variety	*F. poae*	*F. avenaceum*	*F. tricinctum*	*F. graminearum*	*F. culmorum*	FIESC [a]	*F. proliferatum*
Belgravia	5	0	0	1	0	0	0
Concerto	6	2	0	2	0	1	0
Esterel	0	5	0	3	0	0	0
Grace	3	1	0	0	0	1	0
Prague	5	3	0	0	0	2	0
Propino	6	3	4	1	1	0	0
Quench	15	4	0	6	0	1	10
Scarlett	4	3	0	0	0	0	0
Sunshine	0	4	0	1	0	2	0
Violetta	6	3	8	4	0	0	0
Wintmalt	7	5	0	0	0	0	0

[a] *Fusarium incarnatum-equiseti* species complex

Table S4. Characteristics of the malting barley varieties analyzed in this study.

Variety	Row	Type of grain	Habitus	Height of vegetation	Resistance to lodging	Resistance to diseases		Country of origin	Main destination
						Puccinia hordei	*Rhynchosporium secalis*		
Belgravia	Two	Covered	Spring	High	Medium	Medium	High	UK	Malting
Concerto	Two	Covered	Spring	Medium	High	Medium	Medium	UK-Spain	Malting
Esterel	Six	Covered	Winter	High	Low	Low	High	France	Malting/feed
Grace	Two	Covered	Spring	Low	Medium	Medium	Medium	Germany	Malting
Prague	Two	Covered	Spring	Low	High	Medium	Medium	UK	Malting
Propino	Two	Covered	Spring	Medium	Medium	Low	Low	UK	Malting
Quench	Two	Covered	Spring	Medium	High	Medium	Medium	UK	Malting
Scarlett	Two	Covered	Spring	Medium	High	Low	Low	Germany	Malting
Sunshine	Two	Covered	Spring	High	Medium	Medium	Medium	Germany	Malting
Violetta	Two	Covered	Winter	High	Medium	Low	High	Germany	Malting
Wintmalt	Two	Covered	Winter	Low	Low	Medium	High	Germany	Malting

Table S5. Sowing, anthesis and harvesting dates of malting barley in the three experimental years.

Year	Sowing	Anthesis	Harvest
2011	10/12/2010	From 1/5 to 31/5/2011	22/6/2011
2012	22/12/2011	From 1/5 to 31/5/2012	27/6/2012
2013	13/12/2012	From 10/5 to 9/6/2013	17/7/2013

Table S6. Primer sequences, product sizes and annealing temperatures used for PCR identification of *Fusarium* species and chemotype characterization.

Species/ Gene target	Primers	Sequences (5′-3′)	Product size (bp)	Annealing temp. (°C)	Reference
F. graminearum	Fg16F Fg16R	CTCCGGATATGTTGCGTCAA GGTAGGTATCCGACATGGCAA	420	54	[1]
F. avenaceum	FaF FaR	CAAGCATTGTCGCCACTCTC GTTTGGCTCTACCGGGACTG	920	63	[2]
F. culmorum	Fc51F Fc51R	ATGGTGAACTCGTCGTGGC CCCTTCTTACGCCAATCTCG	570	54	[1]
F. poae	FpsF FpoR	CGCACGTATAGATGGACAAG CAGCGCACCCCTCAGAGC	400	61	[3]
F. equiseti	FeqF FeqR	GGCCTGCCCGATGCGTC CGATACTGAAACCGACCTC	990	66	[3]
F. sporotrichioides	FsporF LansporR	CGCACAACGCAAACTCATC TACAAGAAGAGCGTGGCGATAT	332	66	[4]
Translation Elongation Factor 1-alpha	EF1 EF2	ATGGGTAAGGA(A/G)GACAAGAC GGA(G/A)GTACCAGT(G/C)ATCATGTT	700	53	[5,6]
TRI12 (NIV)	12NF(F)	TCTCCTCGTTGTATCTGG	840	53	[7]
TRI12 (15ADON)	12-15F(F)	TACAGCGGTCGCAACTTC	670		
TRI12 (3ADON)	12-3F(F)	CTTTGGCAAGCCCGTGCA	410		
TRI12	12CON(R)	CATGAGCATGGTGATGTC	-		

References

1. Nicholson, P.; Simpson, D.R.; Weston, G.; Rezanoor, H.N.; Lees, A.K.; Parry, D.W.; Joyce, D. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. *Physiol. Mol. Plant Pathol.* **1998**, *53*, 17–37.
2. Doohan, F.M.; Parry, D.W.; Jenkinson, P.; Nicholson, P. The use of species specific PCR based assays to analyse *Fusarium* ear blight of wheat. *Plant Pathol*. **1998**, *47*, 197–205.
3. Jurado, M.; Vazquez, C.; Patino, B.; Gonzalez-Jaen, M.T. PCR detection assays for the trichothecene-producing species *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium poae*, *Fusarium equiseti* and *Fusarium sporotrichioides*. *Syst. Appl. Microbiol.* **2005**, *28*, 562–568.
4. Wilson, A.; Simpson, D.; Chandler, E.; Jennings, P.; Nicholson, P. Development of PCR assays for the detection and differentiation of *Fusarium sporotrichioides* and *Fusarium langsethiae*. *FEMS Microbiol. Lett.* **2004**, *233*, 69–76.
5. O'Donnell, K.; Kistler, H.C.; Cigelnik, E.; Ploetz, R.C. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2044–2049.
6. Geiser, D.M.; Jimenez-Gasco, M.D.; Kang, S.C.; Makalowska, I.; Veeraraghavan, N.; Ward, T.J.; Zhang, N.; Kuldau, G.A.; O'Donnell, K. FUSARIUM-ID v. 1.0: A DNA sequence database for identifying *Fusarium*. *Eur. J. Plant Pathol.* **2004**, *110*, 473–479.
7. Ward, T.J.; Bielawski, J.P.; Kistler, H.C.; Sullivan, E.; O'Donnell, K. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. *Proc. Nat. Acad. Sci. USA* **2002**, *99*, 9278–9283.