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Components of partial disease resistance detected using a detached leaf assay in CIMMYT Fusarium head blight resistant wheat germplasm

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Abstract

One hundred and nineteen entries from the CIMMYT International Wheat and Maize Improvement Centre 2004/05 Fusarium head blight (FHB) resistance screening nursery were evaluated as possible sources of novel components of partial disease resistance (PDR), against FHB and *Microdochium nivale* snow mould, detected using a detached leaf assay. In addition the FHB resistant cvs Arina, Alsen and Frontana and 21 European wheat genotypes were included for comparison. There was wide variation among CIMMYT entries for the PDR components incubation period, latent period and lesion length ($P < 0.001$) and European lines for incubation and latent periods ($P < 0.001$). The CIMMYT entries with the longest latent periods were not superior to cv. Arina, the best European source of this PDR component identified to date. Notably the CIMMYT lines exhibiting the longest latent periods had *Aegilops squarrosa* (878) in their pedigree, indicating that *Ae. squarrosa* (878) may be a source of enhanced resistance detected by latent period. Macroscopic observation suggested that the underlying mechanisms contributing to latent period may differ among the CIMMYT germplasm and European sources of long latent period such as cv. Arina. Among the CIMMYT germplasm, incubation period was only weakly correlated with latent period ($r = 0.25$; $P < 0.01$); this also was the case among European genotypes ($r = 0.36$; $P < 0.05$) supporting previous findings that these PDR components are largely under separate genetic control. However, the correlation was higher on a subset of the most resistant and susceptible lines for latent period ($r = 0.73$ and $r = 0.44$; incubated at 10 °C and 15 °C, respectively). While a number of the European lines had latent periods that were comparable to cv. Arina many were significantly shorter indicating potential for improvement in this PDR component. Adaptations to the experimental design utilized in the present experiments for the efficient evaluation of large numbers of genotypes utilizing the detached leaf assay are discussed.

Introduction

Fusarium head blight (FHB) resistance breeding is a key priority of breeders world-wide. There is no complete resistance to FHB in wheat although many sources of high resistance have been identified. However, due to the complex nature of FHB resistances, which are not fully understood, it has proved difficult to combine these resistance sources routinely into commercial cultivars. Potential problems include poor agronomic characteristics of resistance sources (Mesterhazy, 1995; Chen et al., 1997), possible interactions when combining diverse resistance sources (Browne et al., 2005), resistances based on head morphology in exotic germplasm (Oliver et al., 2005) which may not be useful in developing commercial cultivars, and possible yield penalty associated with some resistance mechanisms. In addition, germplasm selection for FHB resistance in one environment may not provide germplasm with effective resistance in other environments (Dardis and Walsh, 2003).

In an effort to improve our understanding of the underlying mechanisms, FHB resistance has been classified by 'Types' of resistance (Schroeder and Christensen, 1963) chromosomal location in molecular mapping studies (Van Ginkel et al., 1996, Otto et al., 2002; Gervais et al., 2003; Liu and Anderson, 2003, Shen et al., 2003 Paillard et al., 2004) and components of partial disease resistance (PDR) (Diamond and Cooke, 1999; Browne and Cooke, 2004; Browne and Cooke, 2005a). All methods show resistance is partial and complex. Types of resistance are defined principally as resistance to initial infection (Type I) and subsequent spread of infection (Type II). In molecular mapping, many genes governing resistance to FHB are involved in a diversity of germplasm, which frequently differ in chromosomal location (Otto et al., 2002; Gervais et al., 2003; Liu and Anderson, 2003; Shen et al., 2003; Paillard et al., 2004). Components of PDR identified using *in vitro* assays have been related to FHB resistance in the whole plant (Diamond and Cooke, 1999; Browne and Cooke, 2004; Browne and Cooke, 2005a; Browne et al., 2005). Similarly morphological and developmental characteristics related to FHB resistance include narrow flower opening (Gilsinger et al., 2005) anther extrusion (Taylor, 2004) heading date and plant height (Parry et al., 1995), which reduce the rate of disease epidemic development and can also be considered as components of PDR. In the detached leaf assay, the PDR components incubation period (period from inoculation to the first appearance of symptoms), latent period (period from inoculation to sporulation) and lesion length are measured (Diamond and Cooke, 1999; Browne and Cooke, 2004). In the seed germination assay it is hypothesized that resistance to *Fusarium* spp., measured as percentage germination following infection, is related to resistance specific to the developing grain on the wheat head (Browne and Cooke, 2005a). A similar approach to pre-screening PDR components of FHB resistance in barley using *in vitro* ground grain and detached leaf assays has been proposed by Kumar et al. (2005).

Pre-screening for specific PDR components against FHB using rapid *in vitro* techniques and morphological characteristics may be of use in achieving favourable combinations of resistance components at a greater frequency in FHB resistance breeding. Gilsinger et al. (2005) also suggest breeding wheat lines for both morphological avoidance, such as narrow flower opening, and physiological resistance to FHB which may be valuable in future breeding. Indirect selection for FHB resistance based on anther extrusion has been of value in selection of early generation material in years of low FHB incidence (Taylor, 2004) although it is too early to say how useful selection based on anther extrusion and flower opening will be on a wider scale (Taylor, 2004).

The objectives of this research were to evaluate a subset of 119 wheat lines from the FHB resistance breeding programme at CIMMYT as potential enhanced sources of the PDR component latent period, and to investigate any potential implications for plant breeding.

Materials and methods

Wheat genotypes used in this study comprised a selection of 119 wheat lines from the CIMMYT 2004/05 FHB resistance screening nursery, many of which included *Aegilops squarrosa* lines (wide crosses/synthetic wheats) in their pedigree (Table 1). In addition 23 European wheat lines were evaluated including the FHB resistant line Arina obtained from two separate sources (Table 2), the US cv. Alsen (Tables 1 and 2) (derived from Sumai 3) (Table 1) and the Brazilian cv. Frontana (Figures 1 and 2). The European cultivars were selected from spring and winter European germplasm to represent a range of responses to FHB (Table 2). The detached leaf assays were conducted based on the methodology described by Browne and Cooke (2004). Segments 5 cm in length from the mid-section of the first leaf of 2 week-old plants were harvested, and placed adaxial surface uppermost on the surface of 0.5% water agar. Leaf segments were inoculated at the centre of the adaxial surface with a 10 μl droplet of *Microdochium nivale* spore suspension of isolate OP2A adjusted to 1×10^6 conidia ml^{-1} (Browne and Cooke, 2004). The detached leaves were then incubated at 10 °C, except Experiment 2 where the

Table 1. Experiment 1: Disease reactions for the CIMMYT wheat genotypes from the 2004–2005 FHB screening nursery inoculated with *Microdochium nivale* in the detached leaf assay

Entry no.	Cross	Incubation period (days)				Latent period (days)			
		Repeat 1	Repeat 2	Repeat 3	Mean	Repeat 1	Repeat 2	Repeat 3	Mean
254	RUSS/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROS*A (878)	2.67	3.00	2.25	2.64	12.75	11.58	8.42	10.92
256	RUSS/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	3.00	3.08	2.42	2.83	11.75	11.25	9.00	10.67
255	RUSS/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	3.00	3.42	2.08	2.83	9.92	13.00	8.50	10.47
261	RUSS/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	3.50	3.67	2.08	3.08	10.25	11.92	9.08	10.42
257	RUSS/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	3.42	3.17	2.42	3.00	10.25	11.25	9.50	10.33
233	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	3.17	3.42	2.33	2.97	9.33	10.67	10.17	10.06
260	RUSS/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	3.17	3.42	2.75	3.11	9.75	13.00	7.42	10.06
259	RUSS/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	2.83	2.75	2.00	2.53	11.50	11.42	7.17	10.03
230	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	3.67	3.83	2.67	3.39	10.08	10.67	8.92	9.89
239	VERDE/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/*AE.SQUARROSA* (205)/3/3*BUC	3.25	3.50	2.42	3.06	10.00	9.92	9.33	9.75
231	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.75	3.33	2.50	2.86	9.17	9.17	10.42	9.58
100	MILAN/AMSEL//CBRD	2.72	2.75	2.25	2.57	10.25	10.92	7.17	9.44
223	RUSS/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/*AE.SQUARROSA* (205)/3/3*BUC	3.00	2.33	2.17	2.50	10.33	9.17	8.83	9.44
237	RUSS/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	3.08	3.33	2.83	3.08	8.00	10.42	9.92	9.44
294	WHT/4/BCN/3/68112/WARD//*AE.SQUARROSA* (369)	3.08	3.00	2.25	2.78	9.08	8.42	10.75	9.42
232	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	3.33	3.42	2.33	3.03	10.50	10.17	7.50	9.39
236	RUSS/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	2.92	3.00	2.33	2.75	11.33	9.83	7.00	9.39
332	GAN/*AE.SQUARROSA* (437)	3.17	2.67	2.58	2.81	10.25	9.58	8.33	9.39
251	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.83	3.42	2.33	2.86	10.00	9.83	7.50	9.11
306	CEP24	2.75	2.67	2.50	2.64	8.00	8.42	10.42	8.94
265	RUSS/6/MAYOOR/5/CS/TH.CU//GLEN/3/ALD/PVN/4/CS/LE.RA//2*CS/3/CNO79	2.83	3.33	2.50	2.89	10.17	9.75	6.75	8.89
258	RUSS/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	2.83	3.00	2.17	2.67	10.08	9.42	7.08	8.86
329	MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	3.08	3.08	2.75	2.97	10.58	7.17	8.83	8.86
278	VERDE/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.67	3.08	2.25	2.67	9.42	9.42	7.53	8.79
266	RUSS/6/OPATA/5/CPI/GEDIZ/3/GOO//JO69/CRA/4/*AE.SQUARROSA* (223)	3.00	4.08	2.58	3.22	9.00	9.42	7.83	8.75
322	MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.58	2.92	2.15	2.55	8.42	10.08	7.73	8.74
221	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.97	3.58	2.42	2.99	9.56	8.83	7.75	8.71
273	VERDE/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.75	2.50	2.08	2.44	8.67	9.25	8.17	8.69
248	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.83	3.97	2.42	3.07	8.75	9.33	7.83	8.64
275	VERDE/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	3.08	3.00	2.33	2.81	8.08	9.83	7.92	8.61
43	BR 23	2.42	3.08	2.67	2.72	8.83	8.83	8.08	8.58
15	MILAN/SHA7	2.58	3.33	2.42	2.78	8.67	9.25	7.75	8.56
250	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.92	3.25	2.42	2.86	9.58	9.75	6.25	8.53
289	VERDE/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	2.83	2.83	2.08	2.58	10.58	8.67	6.33	8.53
276	VERDE/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.67	3.75	2.33	2.92	9.08	10.08	6.33	8.50
274	VERDE/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	3.25	2.50	2.17	2.64	9.42	9.58	6.42	8.47
46	EHAL//CHUANMAI18/BAU	2.75	3.33	2.61	2.90	8.17	9.00	8.22	8.46
247	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.92	3.42	2.19	2.84	8.25	10.33	6.72	8.44

Table 1. Continued

Entry no.	Cross	Incubation period (days)				Latent period (days)			
		Repeat 1	Repeat 2	Repeat 3	Mean	Repeat 1	Repeat 2	Repeat 3	Mean
49	LI169/LE2177	2.42	2.42	2.25	2.36	8.83	9.00	7.42	8.42
234	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	3.17	3.33	2.50	3.00	8.92	8.83	7.50	8.42
235	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	3.00	4.08	2.42	3.17	9.58	8.67	7.00	8.42
240	VERDE/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/*AE.SQUARROSA* (205)/3/3*BUC	3.42	3.92	2.67	3.33	8.17	9.83	7.25	8.42
213	ALTAR 84/AE.SQ//OPATA	2.92	3.00	2.67	2.86	6.83	10.17	8.17	8.39
227	VERDE/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/*AE.SQUARROSA* (205)/3/3*BUC	2.83	2.58	2.50	2.64	7.25	10.92	7.00	8.39
249	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	3.17	3.33	2.50	3.00	9.50	8.33	7.33	8.39
44	CEP 24-INDUSTRIAL	2.75	2.67	2.17	2.53	8.58	9.42	6.75	8.25
82	ICAB/ITIJ	2.44	2.11	2.00	2.19	9.22	8.28	7.25	8.25
290	VERDE/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	3.08	3.33	2.08	2.83	8.25	10.58	5.92	8.25
305	VSOL/N.BOZU//PEL 73101/LRI	3.08	3.33	2.42	2.94	8.75	8.17	7.83	8.25
14	CATBIRD	3.17	3.58	2.75	3.17	8.42	8.33	7.92	8.22
226	VERDE/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	3.25	3.25	2.25	2.92	9.17	9.00	6.50	8.22
244	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	3.17	2.92	2.02	2.70	9.33	8.08	7.18	8.20
262	RUSS/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	3.08	3.42	2.42	2.97	8.92	9.50	6.17	8.19
84	K.ESCORPION	2.92	2.89	2.17	2.66	9.33	8.67	6.50	8.17
212	RUSSELL-DR	2.33	2.75	2.42	2.50	8.67	7.75	8.08	8.17
330	MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	3.00	3.42	2.42	2.94	8.33	9.25	6.92	8.17
245	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.83	3.17	2.25	2.75	9.25	8.83	6.33	8.14
252	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.92	3.50	2.08	2.83	7.92	9.17	7.33	8.14
293	WHT/4/BCN/3/68112/WARD//*AE.SQUARROSA* (369)	3.00	3.17	2.42	2.86	8.67	9.08	6.67	8.14
267	RUSS/6/OPATA/5/CPI/GEDIZ/3/GOO//JO69/CRA/4/*AE.SQUARROSA* (223)	3.00	3.58	2.75	3.11	9.25	8.42	6.67	8.11
287	VERDE/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	3.00	2.67	2.25	2.64	7.42	8.92	7.92	8.08
238	VERDE/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	2.92	2.92	2.25	2.69	8.33	9.42	6.42	8.06
253	REEDER/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	2.92	3.25	2.58	2.92	8.42	8.33	7.33	8.03
272	VERDE/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.83	3.25	2.33	2.81	7.75	10.33	5.92	8.00
288	VERDE/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	3.50	3.33	2.42	3.08	9.33	6.58	8.08	8.00
144	HEILO	2.58	2.67	2.33	2.53	9.58	6.25	8.08	7.97
268	RUSS/6/OPATA/5/CPI/GEDIZ/3/GOO//JO69/CRA/4/*AE.SQUARROSA* (223)	3.25	3.42	2.33	3.00	7.83	9.50	6.58	7.97
225	VERDE/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	2.78	3.25	2.67	2.90	7.64	8.67	7.58	7.96
325	MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	3.08	3.25	2.58	2.97	7.50	9.50	6.83	7.94
282	VERDE/3/BCN//DOY1/*AE.SQUARROSA* (447)	2.58	3.67	2.42	2.89	7.67	7.67	8.42	7.92
243	IVAN/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.67	2.75	2.25	2.56	8.33	8.50	6.83	7.89
281	VERDE/3/BCN//DOY1/*AE.SQUARROSA* (447)	2.33	3.67	2.58	2.86	8.83	7.25	7.58	7.89
228	WHT/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.50	2.83	2.25	2.53	7.75	8.17	7.67	7.86
224	VERDE/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.58	3.26	2.25	2.70	8.33	8.46	6.75	7.85
67	ALTAR 84/*AE.SQUARROSA*(224)//2*OPATA	2.17	2.92	2.33	2.47	8.25	8.42	6.83	7.83
246	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.83	3.75	2.58	3.06	8.67	8.50	6.33	7.83

284	VERDE/3/BCN//DOY1/*AE.SQUARROSA* (447)	2.67	3.08	2.08	2.61	8.33	7.83	7.33	7.83
292	WHT/6/MAYOOR/5/CS/TH.CU//GLEN/3/ALD/PVN/4/CS/LE.RA//2*CS/3/CNO79	3.08	2.92	2.25	2.75	8.25	8.75	6.50	7.83
219	VERDE*2/4/BCN/3/68112/WARD//*AE.SQUARROSA* (369)	3.33	3.42	2.08	2.94	8.58	7.92	6.83	7.78
270	VERDE/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	3.17	3.42	2.83	3.14	8.17	8.83	6.33	7.78
327	OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	2.42	2.92	2.17	2.50	8.58	7.50	7.17	7.75
333	GAN/*AE.SQUARROSA* (437)	3.75	3.00	2.75	3.17	8.50	8.50	6.25	7.75
18	CATBIRD	3.17	3.50	2.78	3.15	7.67	9.58	5.83	7.69
321	MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.83	3.33	2.33	2.83	9.33	7.33	6.42	7.69
280	VERDE/3/BCN//DOY1/*AE.SQUARROSA* (447)	3.33	4.33	2.69	3.45	7.58	9.42	6.03	7.68
47	BCHA/MILAN	2.92	2.67	2.33	2.64	8.14	8.08	6.58	7.60
271	VERDE/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.58	3.00	2.42	2.67	8.50	7.83	6.42	7.58
218	VERDE*2/3/BCN//DOY1/*AE.SQUARROSA* (447)	3.00	3.42	2.42	2.94	8.58	7.75	6.33	7.56
222	RUSS/6/MAYOOR/5/CS/TH.CU//GLEN/3/ALD/PVN/4/CS/LE.RA//2*CS/3/CNO79	2.58	2.67	2.17	2.47	8.00	8.67	5.83	7.50
104	SABUF/7/ALTAR 84/*AE.SQUARROSA* (224)//YACO/6/CROC_1/*AE.SQUARROSA*	2.50	3.33	2.25	2.69	7.50	9.17	5.75	7.47
	(205)/5/BR12*3/4/IAS55*4/CI14123/3/IAS55*4/EG,AUS//IAS55*4/ALD								
48	ECOL	2.92	2.50	2.11	2.51	7.75	7.50	6.97	7.47
215	SCOOP_1/*AE.SQUARROSA* (434)//CETA/*AE.SQUARROSA* (895)/3/MAIZ	2.75	2.58	2.00	2.44	8.00	8.42	5.92	7.44
328	MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.75	2.83	2.42	2.67	8.50	7.33	6.33	7.39
216	MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.58	3.50	2.08	2.72	7.50	8.42	6.17	7.36
19	SITELLA	2.72	2.83	2.00	2.52	8.31	8.33	5.42	7.35
326	MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.75	3.17	2.58	2.83	8.50	7.50	6.00	7.33
37	TAMOI-INIA	2.83	3.00	2.00	2.61	7.25	8.92	5.75	7.31
177	YANG87-158	3.08	3.08	2.42	2.86	9.17	6.67	6.00	7.28
269	VERDE/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.75	2.83	2.25	2.61	7.83	7.00	7.00	7.28
324	MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	2.75	2.75	2.25	2.58	7.67	8.33	5.83	7.28
334	BCN//DOY1/*AE.SQUARROSA* (447)	2.58	3.08	2.25	2.64	7.67	7.33	6.75	7.25
217	VERDE//NORM*2/*AE.SQUARROSA* (178)/3/RUSS	2.83	2.83	2.33	2.67	7.17	7.67	6.67	7.17
229	VERDE*2/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.50	3.17	2.08	2.58	7.83	7.17	6.50	7.17
323	MAYOOR/5/CS/TH.CU//GLEN/3/ALD/PVN/4/CS/LE.RA//2*CS/3/CNO79	2.67	3.08	2.08	2.61	7.25	8.25	5.92	7.14
242	WHT/4/BCN/3/68112/WARD//*AE.SQUARROSA* (369)	3.25	3.67	2.58	3.17	6.33	7.42	7.58	7.11
279	VERDE/3/BCN//DOY1/*AE.SQUARROSA* (447)	3.00	3.25	2.42	2.89	6.83	8.42	6.08	7.11
331	CETA/*AE.SQUARROSA* (1029)	2.92	2.83	2.25	2.67	8.25	7.00	5.83	7.03
277	VERDE/3/MAYOOR//TK SN1081/*AE.SQUARROSA* (222)	3.33	2.50	2.42	2.75	7.67	7.00	6.25	6.97
Alsen		2.25	3.00	2.42	2.56	7.33	7.67	5.83	6.94
286	VERDE/3/BCN//DOY1/*AE.SQUARROSA* (447)	3.08	2.83	2.33	2.75	6.67	7.33	6.75	6.92
291	WHT/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.92	3.17	2.58	2.89	7.67	6.83	6.25	6.92
335	ALTAR 84/*AE.SQUARROSA* (224)//2*YACO	3.25	3.25	2.33	2.94	6.33	7.25	7.17	6.92
25	PROINTAMILENIUM	3.00	2.92	2.19	2.70	7.08	6.97	6.47	6.84
214	OPATA/5/CPI/GEDIZ/3/GOO//JO69/CRA/4/*AE.SQUARROSA* (223)	3.00	3.17	2.50	2.89	7.17	6.92	6.42	6.83

Table 1. Continued

Entry no.	Cross	Incubation period (days)				Latent period (days)			
		Repeat 1	Repeat 2	Repeat 3	Mean	Repeat 1	Repeat 2	Repeat 3	Mean
283	VERDE/3/BCN//DOY1/*AE.SQUARROSA* (447)	2.78	2.75	2.25	2.59	6.42	8.08	5.75	6.75
241	WHT/6/OPATA/5/CPI/GEDIZ/3/GOO//JO69/CRA/4/*AE.SQUARROSA* (223)	3.08	2.83	2.33	2.75	7.33	6.50	6.25	6.69
264	RUSS/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.42	2.58	2.00	2.33	7.42	7.08	5.42	6.64
263	RUSS/6/SABUF/5/BCN/4/RABI//GS/CRA/3/*AE.SQUARROSA* (190)	2.25	3.08	2.08	2.47	5.92	8.42	5.33	6.56
220	WHT*2/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/*AE.SQUARROSA* (878)	3.08	2.58	2.67	2.78	6.67	6.25	6.67	6.53
285	VERDE/3/BCN//DOY1/*AE.SQUARROSA* (447)	2.67	2.50	2.17	2.44	6.25	6.67	5.92	6.28
	Mean	2.90	3.13	2.35	2.79	8.57	8.78	7.14	6.94
	LSD				0.39				1.39
	cv %				14.9				18.4

Ranked in order of decreasing length of latent period

experiment was repeated at an incubation temperature of 15 °C in addition to 10 °C, under a 24 h diurnal cycle of white light (12 h light/12 h dark).

In Experiment 1, the 119 CIMMYT entries and the US cv. Alsen were screened in experiments repeated three times (Table 1). Within each experiment an experimental unit consisted of one Petri dish containing four detached leaves and was replicated three times. Overall, across the three repeats of the experiment, and three replications within each experiment, the means for each cultivar were based on 36 detached leaves (nine Petri dishes × four leaves).

In Experiment 2, genotypes were selected based on the results of Experiment 1 (Table 1). Those lines exhibiting the longest latent periods (lines 233, 254, 255, 256, 257, 259, 260 and 261), intermediate latent periods (lines 253 and 258) and the shortest latent periods (lines 220, 241, 263, 264 and 285) were evaluated with Frontana and the Swiss cvs Arina and Orsino. The experimental design was the same as for Experiment 1.

In Experiment 3, a range of European wheat germplasm was evaluated using the detached leaf assay along with the Swiss wheat cv. Arina and US cv. Alsen. The experimental unit consisted of one Petri dish with four detached leaves and five replications. The experiment was repeated twice.

Assessments of symptom appearance and sporulation were carried out daily under a compound microscope measuring incubation period and latent period across all experiments. In addition, lesion length was measured in Experiment 2 at an incubation temperature of 10 °C (chlorotic area visible by placing the Petri dishes over a light box) at 7 and 10 days post-inoculation.

Statistical analysis

ANOVA were conducted using Genstat V software. Experiment 1, containing 119 CIMMYT lines and the US cv. Alsen, was analysed as a randomized block design with wheat line and repeat of the experiments as factors. Similarly, Experiment 2, conducted with incubation temperatures of 10 °C and 15 °C, was analysed with wheat line and the repeat of the experiments as factors for each incubation temperature individually. Experiment 3 containing the European cultivars was analysed as two separate experiments with wheat entry as the single factor. All correlations were conducted using Microsoft Excel.

Results

Experiment 1

Among the CIMMYT lines developed from diverse sources of FHB resistance, there was a large range in the expression of the PDR components incubation ($P < 0.001$) and latent periods ($P < 0.001$) (Table 1) detected by the detached leaf assay. There were no significant wheat entry × experiment repeats interactions (Table 1). The correlation between the individual experiments ranged from $r = 0.33$ to 0.44 for incubation period and from $r = 0.33$ to 0.49 for latent period. Notably the lines 254, 256, 255, 261, 257, 260 and 259 derived from *Ae. squarrosa* (878) showed the longest latent periods of all FHB nursery lines tested. The lines with the shortest latent periods were 241, 264, 263, 220 and 285 shorter than that of Alsen and included one genotype, entry 220, also derived from *Ae. squarrosa* (878). The PDR component incubation period (mean of three experiments) was poorly correlated with latent period ($r = 0.25$).

Experiment 2

The most and least resistant lines (Figure 1) selected from Experiment 1 were further tested with the detached leaf assay and incubated at 10 and 15 °C with the Swiss commercial cvs Orsino, Arina and Brazilian FHB-resistant cv. Frontana. There were highly significant differences among cultivars ($P < 0.001$) for incubation period (Figure 1a, c), latent period (Figure 1b, d), and lesion length (Figure 2a, b). The relative incubation and

Table 2. Experiment 3: Disease reactions for a range of European wheat genotypes inoculated with *Microdochium nivale* in the detached leaf assay

Line	Incubation period			Latent period		
	Repeat 1	Repeat 2	Mean	Repeat 1	Repeat 2	Mean
Kamerat (NK97017)	3.65	2.83	3.24	12.05	9.45	10.75
Arina[1]	4.15	2.90	3.53	11.85	9.50	10.68
NK02016	3.55	2.90	3.23	11.40	9.85	10.63
Magnifik	3.20	2.55	2.88	10.75	10.10	10.43
Arina[2]	3.70	3.65	3.68	11.15	9.40	10.28
Solitar	3.00	2.05	2.53	10.50	8.90	9.70
Altos	4.00	3.10	3.55	9.80	9.15	9.48
NN03023	3.95	2.97	3.46	10.40	8.22	9.31
NK01050	4.10	2.65	3.38	9.65	8.50	9.08
Bjørke	3.85	3.35	3.60	8.75	9.30	9.03
SW Maxi	2.95	2.55	2.75	9.65	8.30	8.98
Bussard	3.80	3.00	3.40	9.35	8.50	8.93
SW Topper	3.72	3.50	3.61	9.05	8.75	8.90
Romanus	3.80	2.70	3.25	10.10	7.65	8.88
Turkis	3.60	3.10	3.35	8.80	8.85	8.83
Zentos	3.95	2.87	3.41	9.05	7.97	8.51
NK01063	3.45	2.95	3.20	8.70	8.10	8.40
NK03029	3.25	2.75	3.00	9.25	7.35	8.30
Opus	3.90	2.60	3.25	8.85	7.65	8.25
SvP72017	3.50	2.45	2.98	9.05	7.40	8.23
Mjølner	2.90	2.55	2.73	8.80	7.05	7.93
Tarso	3.60	2.75	3.18	8.00	7.10	7.55
Mikon	3.30	2.85	3.08	7.50	7.40	7.45
Alsen	2.45	2.45	2.45	7.20	6.80	7.00
Mean	3.56	2.83		9.57	8.39	
LSD	0.65	0.47		1.58	1.24	
cv %	14.6	13.2		13.1	11.8	

[1]Arina seed supplied from Norway
[2]Arina seed supplied from Agroscope, Switzerland

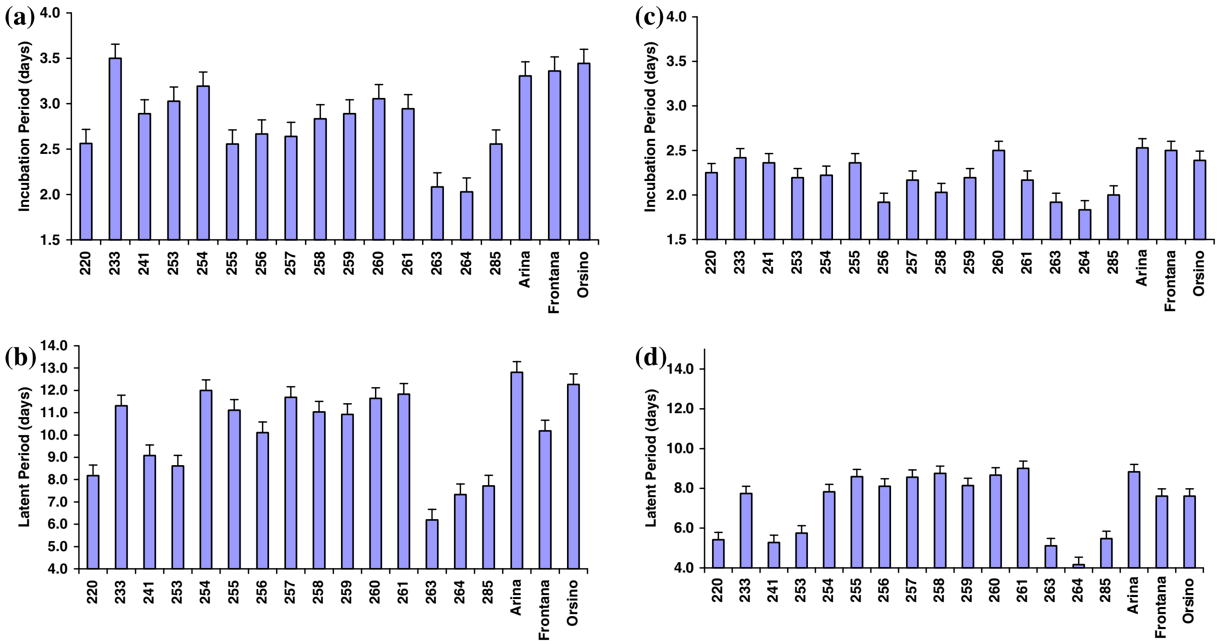

Figure 1. Experiment 2: Disease reactions for the CIMMYT wheat genotypes, selected from Experiment 1 and cvs Arina, Frontana and Orsino inoculated with *Microdochium nivale* in the detached leaf assay. Incubated at 10 °C: (a) incubation period and (b) latent period; incubated at 15 °C: (c) incubation period and (d) latent period. Bars represent standard error of the mean.

latent periods for the entries selected from the CIMMYT nursery reflected that observed in the mass screening (Table 1). However, among this subset of genotypes the correlation between incubation and latent periods was higher (10 °C: $r = 0.73$; 15 °C: $r = 0.44$). Notably, no genotype from the CIMMYT nursery showed latent periods longer than that of the Swiss FHB resistant cv. Arina at either 10 °C or 15 °C (Figure 1b, d). The results for lesion length measured on day 7 (Figure 2a) and day 10 (Figure 2b) at 10 °C reflected the variation in latent period ($r = -0.89$: $P < 0.001$; lesion length day 7 and $r = -0.91$; $P < 0.001$; lesion length day 10). None of the CIMMYT entries had lesion lengths shorter than that of the Swiss cv. Arina.

Experiment 3

There were highly significant differences among entries of European germplasm for both incubation and latent periods (Table 2). The correlation between the two experiments for incubation period was $r = 0.51$; $P < 0.01$ and for latent period $r = 0.76$; $P < 0.001$. Correlation between incubation and latent periods means of both experiments was low at $r = 0.36$; $P < 0.05$. Unlike the CIMMYT lines, none of the European lines had latent periods shorter than Alsen. However, a number of lines exhibited latent periods comparable to that observed in Arina. Reflecting the commercial cultivars, the Norwegian breeding lines (identified by the prefix NK in Table 2) similarly showed a large range for latent period, NK02016 having the longest. Many of the cultivars showed incubation periods as long as Arina but with shorter latent periods notably in the wheat cvs Bjorke and Topper.

Discussion

There was wide variation in the PDR components incubation period, latent period and lesion length among genotypes in the CIMMYT FHB nursery. The purpose of this research was to quantify, among CIMMYT wheat, which has provided an important source for FHB resistance breeding particularly in the USA (Browne et al., 2005), the variation in the PDR components detected in the

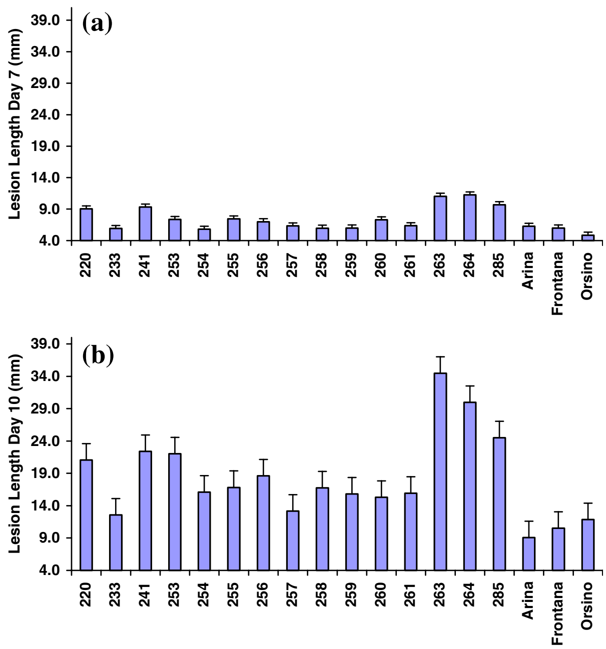

Figure 2. Experiment 2: Disease reactions for the CIMMYT wheat genotypes, selected from Experiment 1 and cvs Arina, Frontana and Orsino inoculated with *Microdochium nivale* in the detached leaf assay. Incubated at 10 °C: (a) lesion length at day 7 post-inoculation, (b) lesion length at day 10 post-inoculation. Bars represent standard error of the mean.

in vitro detached leaf assay. This is an important step for plant breeding both towards understanding the potential utility of exotic resistance sources and the specific PDR components detected *in vitro*, and provides a basis for elucidating the mechanisms of resistance against FHB. None of the lines evaluated had a latent period superior to the Swiss cv. Arina. As yet, it is not clear if the polygenic mechanisms, detected using the detached leaf assay to identify PDR components, are of similar or different combinations among the CIMMYT lines from those detected in Arina. Macroscopic observations of lines with the longest latent periods indicated that the CIMMYT lines were not as effective at inhibiting mycelial growth around the stomata as would be expected based on the relative latent periods, an observation supported by the comparison of lesion lengths. These observations could relate to the dramatically lower sucrose contents in leaf tissue of CIMMYT lines, compared to those observed in commercially acceptable European cultivars (Browne and Brindle, unpublished), and possibly lower levels of readily available substrates for pathogen development and sporulation.

The PDR component latent period is of particular significance in European wheat where longer latent periods have been related to greater FHB resistance (Diamond and Cooke, 1999; Browne and Cooke, 2004) and the PDR component lesion length to snow mould resistance (Ergon and Tronsmo, 2006). Among the European wheat cultivars evaluated, a number had latent periods comparable to that of Arina. However, many genotypes had medium or poor resistance in this characteristic similar to that recorded for wheat cultivars on the UK recommended list (Browne and Cooke, 2004). Among the European genotypes evaluated there was a wide range of anthesis dates and consequently a large effect of heading date on FHB resistance was observed (data not presented). The influence of latent period was only detected using multivariate analysis. This illustrates that FHB resistance consists of multiple PDR components some of which are detected in the detached leaf assay (Diamond and Cooke, 1999; Browne and Cooke, 2004; Browne et al., 2005), seed germination assay (Browne and Cooke, 2005a), morphological and developmental characteristics associated with disease avoidance and escape (Parry et al., 1995), narrow flower opening (Gilsinger et al., 2005) and anther extrusion (Taylor, 2004). It is notable that in the UK recommended list cultivars evaluated for resistance in the detached leaf and seed germination assays (Browne and Cooke, 2004, 2005a), the range of anthesis dates and anther extrusion was more limited. This allowed the effect of the PDR components detected using the detached leaf and seed germination assays to be observed more clearly using single linear correlations, although for the assessment of multiple PDR components simultaneously multiple linear regression techniques are more appropriate.

Wide crosses have been identified as potential sources of novel FHB resistance (Cai et al., 2005; Oliver et al., 2005) and the related Graminaceae barley and oat which exhibit greater inhibition of sporulation with markedly longer latent periods than observed in European wheat (Browne and Cooke, 2005b). The present work evaluated potential sources of enhanced expression of the PDR component latent period among lines from the CIMMYT FHB nursery, combining diverse sources of FHB resistance. Although no superior sources of extended latent period were identified, among the CIMMYT lines, a breeding nursery utilising diverse exotic sources of FHB resistance may not be optimal for selection of this PDR component. However, it was notable that the CIMMYT FHB nursery genotypes with the longest latent period shared the common parent *Ae. squarrosa* (878). This suggests that although not found in the present study, further screening of *Ae. squarrosa* (878), synthetic wheats, and those derived from wide crosses using the detached leaf assay could identify breeding sources which exhibit greatly enhanced latent periods.

The importance of latent period among European wheat contrasts with that of incubation period among various lines of other provenances. These include the US FHB resistant cv. Alsen in the present study, CIMMYT FHB nursery germplasm (Browne and Cooke, 2004), the 2002 Southern US FHB screening nursery (Browne et al., 2005) and subsequently in the 2005 Southern US FHB screening nursery (Browne et al., unpublished), where shorter incubation periods relate to greater FHB resistance. It is hypothesized that this dichotomy reflects the different genetic control of highly effective FHB resistance in exotic germplasm (Browne and Cooke, 2004). A greater understanding of the relationships among resistance mechanisms across growth stages using metabolomics and molecular techniques is desirable to further understand the implications of these observations. This should help elucidate potential interactions between diverse sources of FHB resistance thereby increasing the efficiency of FHB resistance breeding. Overall, incubation period and latent period were poorly correlated with each other supporting the view that while some mechanisms governing both may be similar, others are independent (Browne and Cooke, 2004, 2005a). However, many sources of short incubation period have been identified, most notably entry 28 from the southern US FHB screening nursery 2002 (Browne et al., 2005) which combines a very short incubation period with relatively long latent period. This suggests that the CIMMYT genotypes evaluated are not necessarily a useful source of the PDR component incubation period for breeding, although the importance of the genetic background for the expression of FHB resistance relating to short incubation periods is unclear.

The current database of PDR components provides information on CIMMYT germplasm, which is of importance in many breeding programmes worldwide and which may be of use in plant breeding. For example lines with short latent periods and long lesion lengths previously related to snow mould susceptibility (Ergon and Tronsmo, 2006) may be particularly unsuitable sources of FHB resistance where snow mould resistance is of importance. In addition, a method for efficiently pre-screening groups of 120 genotypes for the PDR components detected in the detached leaf assay is shown. This is of relevance for pre-screening using the detached leaf assay in plant breeding and for identifying sources of enhanced expression of the PDR component latent period. Selection for FHB resistance based on anther extrusion (Taylor, 2004) and similarly pre-screening for specific PDR components against FHB using rapid *in vitro* techniques may be of use for achieving favourable combinations of resistance components at greater frequencies, although this remains to be tested on a wider scale within a breeding programme. Differentiation among cultivars could be improved with additional replicates. However, for initial mass screening, in European germplasm where latent period is of greatest importance, a single experiment of three replicates and measuring only lesion length, which has been consistently closely correlated with latent period (Diamond and Cooke, 1999; Browne and Cooke, 2004; Browne et al., 2005) may be more efficient to exclude the most susceptible genotypes. The most promising germplasm can be evaluated with more detailed measurements of incubation period and latent period. This may differ among CIMMYT germplasm and resistance derived from provenances in the US southern FHB screening nursery where short incubation period is significantly related to FHB resistance (Browne et al., 2005).

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