Eur J Plant Pathol (2009) 123:229–234
DOI 10.1007/s10658-008-9353-7

Investigation into components of partial disease resistance, determined *in vitro*, and the concept of types of resistance to *Fusarium* head blight (FHB) in wheat

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Received: 21 April 2008 / Accepted: 7 July 2008 / Published online: 11 September 2008

Abstract This work presents an analysis of the relationship between components of partial disease resistance (PDR) detected using *in vitro* detached leaf and seed germination assays, inoculated with *Microdochium majus*, and *Fusarium* head blight (FHB) resistance to *Fusarium graminearum* assessed using point inoculation, termed Type II resistance. Relationships between *in vitro*-determined PDR components and FHB resistance using techniques which inoculate the wheat spike uniformly, termed Type I resistance (incidence and severity), have been reported previously. In this study shorter incubation periods, longer latent periods and shorter lesion lengths in the detached leaf assay and higher germination rates in the seed germination assay were related to greater FHB resistance measured by single point inoculation (Type II), collectively explaining 54% of the variation. Overall the relationships observed for Type II FHB resistance were similar to previous findings for Type I resistances. However, the relative magnitude of effects of the individual PDR components determined *in vitro* varied between FHB disease resistance parameters. Resistance in seed germination and latent period in the detached leaf assay were more strongly related to resistance assessed by point inoculation (Type II) and severity-Type I as opposed to incubation period which was most strongly related to disease incidence-Type I. The results provide evidence that individual components of partial disease resistance differentially affect aspects of FHB disease progression in the wheat spike. This work supports the view that the current model of types of resistance is an oversimplification of the interacting mechanisms underlying expression of FHB resistance.

Keywords Detached leaf assay · *Microdochium majus* · Seed germination assay

Fusarium head blight (FHB) Type 1 resistance testing in plant breeding is predominantly conducted with inoculum evenly spread over the wheat head, assumed to be resistance to initial infection (Schroeder and Christensen 1963). A number of methods are utilised to achieve the even application of inoculum over the wheat head, typically spray inoculation, natural infection, or spreading infected corn or wheat during crop development. Resistance is subsequently measured as disease incidence and severity or a combination of both. Type II resistance was hypothesised as resistance to spread, determined by point inoculation of a single spikelet (Schroeder and Christensen 1963) conducted in the glasshouse. More recently, disease incidence in field-inoculated nurseries has been regarded as being more strongly related with resis-

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tance to initial infection, and disease severity more closely with resistance to spread (Bai and Shanner 1994). However it is not well understood how partial disease resistance (PDR) components determined *in vitro* (Diamond and Cooke 1999; Browne and Cooke 2004; Browne 2007) relate to the relationship between Type I and Type II resistances to FHB. Investigation of PDR components are utilised across a range of plant pathogen systems (Herrera-Foessel et al. 2007; Onfroy et al. 2007; Sandoval-Islas et al. 2007). For partial resistance to *Puccina hordei* in barley, the relative ratio of mature pustules is suggested for potential use in breeding programmes, germplasm screening and use in fundamental studies (Jiang et al. 2007).

In FHB it been suggested that the high correlations observed between Type I and II resistances cannot be explained by independent effects (Thomas et al. 2007), the authors suggesting that combined Type I and II effects are a common feature in the expression of genes affecting FHB resistance in wheat. In addition to incidence and severity, additional aspects of FHB infection have been loosely described as additional types of resistance resulting from Type I inoculation, including *Fusarium*-damaged kernels (FDK) and deoxynivalenol (DON) concentration (Mesterhazy et al. 1999). However both FDK and DON have been related, in part, to the cumulative effect of PDR components detected in detached leaf and seed germination assays (Browne 2007). In investigations of components of partial disease resistance to *Phytophthora sojae* in soybean (Mideros et al. 2007) and potato blackleg caused by *Erwinia carotovora* (Allefs et al. 1996) the authors similarly suggest that partial resistance is the result of combinations of various resistance mechanisms with the cumulative effect of the different components influencing the total level of resistance. In FHB it has been suggested that the individual PDR components not only influence the total level of resistance but also have a variable individual influence on disease development parameters (Browne et al. 2005; Browne 2007). For example, the PDR component incubation period was most closely related to FHB disease incidence (Browne et al. 2005) while resistance detected in the seed germination assay related to a greater decline in *Fusarium*-damaged kernels and a smaller reduction in the mycotoxin DON than would be expected from the visual symptoms of FHB on the wheat spike (Browne 2007).

FHB disease is caused by a complex of fungi, principally *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium avenaceum* although in cooler regions *Microdochium majus* assumes a greater importance (Parry et al. 1995). Nevertheless resistance is considered to be common across *Fusarium* species (Parry et al. 1995). Van Eeuwijk et al. (2004) reported that resistance to FHB in wheat was horizontal and non-species specific across a range of *Fusarium* spp. and *M. majus*. *Microdochium majus* has been used in detached leaf assays because it allows more distinct symptoms to be observed (Diamond and Cooke 1999; Browne and Cooke 2004). Partial disease resistance components detected in the *M. majus* detached leaf assay have been correlated to FHB in wheat inoculated with *F. culmorum* (Diamond and Cooke 1999; Browne and Cooke 2004) and *F. graminearum* (Browne et al. 2005; Browne 2007). Resistance in the seed germination assay was highly correlated across *F. graminearum*, *F. avenaceum*, *F. culmorum*, *M. majus* and *Microdochium nivale* indicating common resistance between *M. majus* and other *Fusarium* spp. in the *in vitro* assay (Browne and Cooke 2005). The objective of this work was to investigate the relationship of individual PDR components determined using the *in vitro* detached leaf and seed germination assays to the concept of types of resistance to FHB particularly Type I and Type II.

This paper presents new analyses exploring the relationships between Type II FHB resistance and previously published data on PDR components detected in the detached leaf and seed germination assays (Browne et al. 2005; Browne 2007). Methods, data and discussion of results for the detached leaf assay (incubation period, latent period and lesion length) and *F. graminearum*-inoculated field nursery evaluations (incidence, severity, FDK and DON content) of the 30 soft red winter wheat lines entered in the 2002 Uniform Southern Fusarium Head Blight Nursery (USFHBN) are published in Browne et al. (2005), and for the seed germination assay in Browne (2007). In these previously published data, *M. majus* isolates were utilised for both the detached leaf and seed germination assays. Assessments of Type I resistance conducted by the USFHBN contributors utilised isolates of *F. graminearum* (Browne et al. 2005). For the new analyses presented in this paper, point FHB resistance data inoculation (Type II resistance) was provided courtesy of the participants of the 2002 USFHBN. Resistance was determined by point inoculation with *F. graminearum* in the glasshouse, in replicated experiments across five locations, with overall means presented in Fig. 1. Analysis of variance was conducted as a randomised block design with locations treated as blocks. There was significant variation among USFHBN entries ($P<0.001$) for Type II resistance (point inoculation and subsequent spread of infection through the wheat spike) which was strongly correlated with both Type I disease severity and to a lesser extent with Type I incidence ($r=0.79$; $P<0.001$ and $r=0.67$; $P<0.001$ respectively). Although single linear correlations between latent period in the detached leaf assay and Type II FHB resistance were not significant, there was a significant, albeit low, negative single linear correlation between resistance in the seed germination assay and the percentage of diseased spikelets ($r=-0.37$; $P<0.05$; Fig. 2). In addition, the PDR component, incubation period, was significantly correlated with Type II resistance; shorter incubation periods related to greater disease resistance ($r=0.63$; $P<0.001$).

Multivariate analysis was conducted using the general linear model procedure of Statistical Analysis System (SAS) software (SAS Institute, Inc.). The SAS STEPWISE procedure was utilised to explore the relationships between the independent variables incubation period, latent period and lesion length from the detached leaf assay, germination rate in the seed germination assay, and the dependent variable Type II resistance. This multiple linear regression procedure used the forward selection method with respect to the independent variables entered into the model. Collectively, the PDR components detected *in vitro* in the detached leaf assay and in the germination assay explained 54% of the variation for resistance to disease spread, termed Type II (Table 1). Greater resistance in the seed germination assay, shorter incubation periods and longer latent periods in the detached leaf assay were related to lower FHB disease resulting from point inoculation (Table 1). The overall relationships between PDR components determined in the *in vitro* detached leaf assay and seed germination assay were similar to those reported for Type I resistances (Browne 2007). However, the relative proportion of variation explained by the individual PDR components differed between Type I and Type II inoculation techniques (Table 1). Resistance determined in the PDR component, latent period, and germination rate in the seed germination assay, were more closely related to Type II resistance (partial $R^2=0.12$; $P<0.05$ and $R^2=0.11$; $P<0.05$ respectively) than Type I incidence (partial $R^2=0.01$; NS and $R^2=0.07$; $P<0.05$ respectively). While the PDR component, incubation period, had a clear effect on Type II resistance (partial $R^2=0.29$; $P<0.01$), this was considerably less than that for Type I disease incidence (partial $R^2=0.45$; $P<0.001$). These findings reflect those reported for FHB Type I parameter severity and FDK, more closely associated with the later stages of

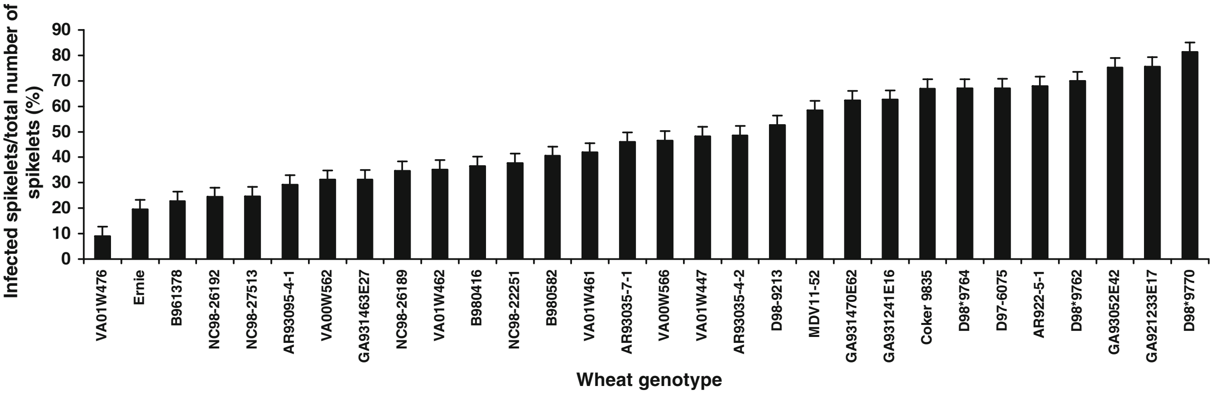

Fig. 1 *Fusarium* head blight (FHB) Type II resistance (means of five locations) of entries in the US 2002 uniform soft red wheat FHB screening nursery. Severity was calculated as the percentage of infected spikelets/total number of spikelets. The assessment date which varied between the five locations was between 21–28 days post-inoculation. Y-*error bars* represent standard error of the mean ($P<0.001$)

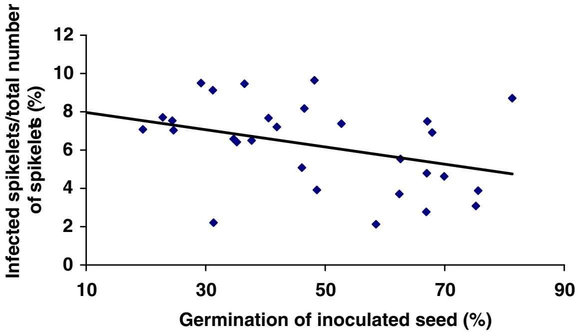

Fig. 2 Single linear correlation between seed germination rate in the *M. majus*-inoculated seed germination assay and point inoculation (Type II) with *F. graminearum* (infected spikelets/ total number of spikelets; $r=-0.37$; $P<0.05$)

FHB disease development, and for which resistance in the seed germination assay and latent period were of relatively greater importance (Browne 2007).

The present research illustrates the complex nature of FHB resistance and the utility of multiple linear regression techniques. This is particularly the case where the individual effect of PDR components are weak and are not readily detected using single linear correlations. While the physiological basis for the PDR components determined in the detached leaf and seed germination assays are not fully understood, biochemical correlates of the PDR component latent period in the detached leaf assay have been identified in an NMR-based metabolomics study (Browne and Brindle 2007). These biochemical correlates were hypothesised by the authors to relate to the relative abundance of preferred carbon and nitrogen sources influencing the relative growth rate of the fungi. The metabolites include those known to influence the growth of *Fusarium* spp. such as betaine, choline, glutamine/ glutamate and alanine, in addition to trans-aconitate (Browne and Brindle 2007).

The variation reported here in the relative effects of the individual PDR components and FHB disease resistance (Type I and Type II) supports in part the hypothesis of Schroeder and Christensen (1963), that Type I resistance relates to resistance to initial infection, and Type II resistance relates to resistance to disease spread. Consistent with the diverse sources of FHB resistance contained within the 2002 USFHBN, it is clear that important resistance components present were not detected by either the detached leaf or seed germination assays. This is to be expected as partial resistance to FHB is found in a diversity of germplasm sources that frequently differ with respect to the chromosomal regions governing resistance (Van Ginkel et al. 1996; Otto et al. 2002; Gervais et al. 2003; Liu and Anderson, 2003; Shen et al. 2003; Somers et al. 2003; Paillard et al. 2004; Zwart et al. 2007). Zhang et al. (2008) screening the US Department of Agriculture small grains collection reported that the novelty and types of FHB resistance in these selections should be further characterised using molecular markers and different inoculation techniques. The current findings support the utility of multiple screening techniques and the emerging view that the current model of types of resistance is an oversimplification of the interacting mechanisms underlying expression of FHB resistance. Further investigations, across a range of techniques, are desirable to further elucidate the combinations of physiological and morphological resistance components expressed.

Table 1 Multivariate analysis of independent variables from the detached leaf assay (incubation period, latent period, lesion length) and seed germination assay using the forward selection procedure in proc-stepwise regression to explain dependent FHB resistance variables from field nurseries

FHB variable/step	Variable entered	Model R^2	*P* value
Incidence			
1	Incubation period	0.45	< 0.001
2	Lesion length	0.54	0.031
3	RSGA	0.61	0.044
4	Latent period	0.62	0.303
Severity			
1	Incubation period	0.27	0.003
2	RSGA	0.41	0.018
3	Latent period	0.48	0.088
4	Lesion length	0.49	0.362
Point Inoculation			
1	Incubation period	0.29	0.002
2	Latent period	0.41	0.024
3	RSGA	0.52	0.021
4	Lesion length	0.54	0.410

Type I-Incidence, Type I-Severity (from Browne 2007) and glasshouse inoculation (point inoculation, termed Type II)

Acknowledgements Dr. Paul Murphy, North Carolina State University, and the contributors to the 2002 US southern soft red winter wheat FHB screening nursery are gratefully acknowledged for the provision of the multi-site point inoculated (Type II) FHB resistance data.

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