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RESEARCH PAPERS

An *in vitro* assay for pre-screening resistance to Fusarium head blight in durum wheat

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Summary. Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is a wheat disease that causes yield losses and mycotoxin accumulation in the grain. In germplasm of durum wheat (*Triticum turgidum* L. ssp. *durum*), genetic variability for resistance is scarce. To develop an improved assay for FHB resistance in wheat, we evaluated the effect of *F. graminearum* inoculation on seed germination and seedling growth and relationships of these parameters with disease severity. A genotype set was examined, comprising ten durum wheats as well as two common wheats, including susceptible and introgressed resistant lines. Genotypes were additionally screened using molecular markers linked to known FHB resistance QTLs. Significant differences were detected among inoculated genotypes for germination, coleoptile length, coleoptile weight, root weight and disease severity. Seedling variables were shown to be correlated across experiments validating repeatability of the *in vitro* assay. Using multiple regression analysis, coleoptile length was correlated with FHB severity ($P<0.05$) and explained 63% of head disease. Introgressed genotypes carrying resistance alleles/QTLs when inoculated showed nil or slight reductions in seedling growth, in comparison to controls, and lower disease severities than the corresponding original genotypes, suggesting that the seedling test can detect genetic differences in FHB resistance. Three genotypes performed well in the seedling assay and showed moderate severity even though they lacked the targeted QTLs, suggesting they harbor resistance genes not screened herein. Based on relative coleoptile length and Fisher statistical significance, a ranking of genotypes was established, consisting of three groups with best, intermediate and poorest performance in relation to fungal effect. The advantageous features of this method reside in its statistical basis and predictive ability of head disease. The *in vitro* assay allows rapid and early determination of resistance/susceptibility to *F. graminearum,* suitable for evaluating germplasm collections or segregating progenies.

Key words: *Fusarium graminearum,* FHB, scab, *Triticum turgidum* ssp. *durum.*

Introduction

Durum wheat (*Triticum turgidum* L. ssp. *durum*, $2n = 4x = 28$ AABB) is an important cereal crop for human consumption worldwide. This type of wheat is preferred for preparing high quality pasta products due to the high protein content and gluten strength of the grain. Fusarium head blight (FHB) or scab, mainly caused by *Fusarium graminearum* Schwabe, continues to be one of the most serious diseases affecting cereal crops (McMullen *et al.*, 2012). This pathogen infects the heads of wheat plants during flowering and the early stages of kernel development, especially under humid and warm weather conditions, causing significant yield losses and quality reduction. FHB-infected grains also cause food safety concerns due to mycotoxin accumulation, mainly by deoxynivalenol (DON) (Fernández Pinto *et al.*, 2013). Severe and frequent FHB epidemics cause direct and secondary economic losses (Nganje *et al.*, 2004; Galich *et al.*, 2006). Current strategies to mitigate FHB attack include varietal selection, disease forecasting, fungicides and crop rotation (Gil-

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bert and Haber, 2013) and bacterial antagonists as biocontrol agents (Shi *et al.*, 2014).

FHB resistance sources within durum wheat germplasm are scarce. The moderately resistant cultivars released by North Dakota State University are derived from adapted North American germplasm (Elias and Manthey, 2007), but most improved durum lines carry resistance derived from exotic sources. Langdon-Dic3A-10 is a resistant recombinant substitution line derived from crosses of *T. turgidum* spp. *durum* var. Langdon with wild emmer wheat *T. turgidum* spp. *dicoccoides* ($2n = 4x =$ AABB; Otto *et al.*, 2002; Stack *et al.*, 2002). Through extensive intergeneric hybridization, Jauhar and Petersen (2008) developed a disomic alien addition line named Langdon-DGE1 ($2n = 28 + 2$), with the 1E chromosomes from *Lophopyrum elongatum* that confer FHB resistance. Two new promising genetic stocks, DGE-2 and DGE-3, were recently released; in those, the 1E chromosome pair from *L. elongatum* replaces the 1A pair in DGE-2 and the 1B pair in DGE-3 (Jauhar and Peterson, 2012; 2013). Genes enhancing FHB resistance have been discovered in Tunisian durum lines (Huhn *et al.*, 2012) and cultivated emmer wheat *T. turgidum* spp. *dicoccum* (Buerstmayr *et al.*, 2012). An attempt to transfer FHB resistance from hexaploid wheat using Langdon D-genome disomic substitution lines is ongoing (Cai, 2013).

Molecular maps have enabled the dissection of FHB resistance to some defined genomic regions or quantitative trait loci (QTLs), and linked markers are available for screening. Otto *et al.*, (2002) mapped chromosome 3A in a Langdon-Dic3A recombinant inbred chromosome line population and identified a single QTL (*Qfhs.ndsu-3AS*) that explained approximately 55% of the genetic variation in FHB resistance. In addition, a genomic region derived from common wheat cultivar Sumai-3 is significantly associated with FHB resistance, and was mapped to chromosome 3B (*Qfhs.ndsu-3BS*; Anderson *et al.*, 2001). Diagnostic microsatellite alleles have also been identified as being linked to *Qfhs.ndsu-3AS* (Hartel *et al.*, 2004), *Qfhs.ndsu-3BS* (Matus-Cádiz *et al.*, 2006) and 1E *L. elongatum* chromosomes (Jauhar *et al.*, 2009).

Genetic resistance to FHB in wheat comprises several mechanisms. Schroeder and Christensen (1963) originally proposed a two component model for resistance: Type I, or disease incidence, operates against the initial infection and is usually experimentally measured based on the number of infected spikelets after spray inoculation; Type II, or severity, operates by limiting the spread of the fungus in infected tissues and can be evaluated using the number of infected spikelets per spike after inoculation of the central zone of the spike. Three additional components are related to resistance to kernel infection (Mesterházy, 1995), to mycotoxin accumulation (Miller *et al.*, 1985) and to the ability to maintain yield under infection (Mesterházy, 1995).

Plant phenotyping is critical for the selection of superior genotypes, and methods for disease assessment include visual scoring of head symptoms, calculating the percentage of diseased grains at harvest and determining the yield relative to non-inoculated samples (Buerstmayr *et al.*, 2009). However, environmental factors, such as temperature and humidity, influence field evaluation of FHB resistance and complicate phenotyping and breeding efforts. Seedling tests have been proposed as alternative methods for resistance screening that allow for early evaluation, short testing times and controlled experimental conditions. Mesterházy (1978) pioneered the use of artificial inoculations in Petri dishes and the greenhouse in comparison to field data; however that research failed to establish that adult plant stage resistance could be predicted on the basis of seedling tests. In a detached leaf assay, the latent period, i.e., the elapsed time from inoculation to sporulation, was the variable most closely correlated with Type I FHB resistance, but this correlation was not stable and depended on germplasm origin (Browne and Cooke, 2004). A subsequent study found that the germination percentage of inoculated seeds was moderately correlated with Type I FHB resistance. However, this was not correlated with any of the resistance components detected in the previous detached leaf assay, suggesting that the resistance factors expressed in these two *in vitro* assays are distinct (Browne and Cooke, 2005).

Coleoptile growth rate (Brennan *et al.*, 2003) and lesion length (Wu *et al.*, 2005) have also proven to be useful variables for evaluating the pathogenicity of different *Fusarium* species and *F. graminearum* strains in common wheat. More recent studies have successfully quantified the aggressiveness of *F. graminearum* strains using seed and floret inoculations in a small sample of durum cultivars (Purahong *et al.*, 2012; 2014). Information concerning *in vitro* responses of durum germplasm to *F. graminearum* is very scarce, and there are no available reports evaluating the ef-

fectiveness of seedling assays in detecting wheat lines carrying FHB resistance QTLs, compared to the susceptible genotypes from which they were derived.

The aim of the current study was to investigate the utility of a seedling assay for identification of FHB resistant genotypes in durum wheat. To achieve this objective, we evaluated the effect of *F. graminearum* inoculation on seed germination and seedling growth in a set of durum and common wheat genotypes, and examined the relationship between seedling and head inoculation assays. The inclusion of previously characterized genotypes and the use of DNA markers for detection of known resistance QTLs enabled evaluation of the effectiveness of the seedling test for recognizing wheat sources with FHB resistance.

Materials and methods

Plant material

Assays were performed using three sets of wheat materials: i) four durum commercial cultivars from Argentina, ACA 1801F, 'Buck Platino' (BPLA), 'Buck Esmeralda' (BESM), 'Bonaerense Inta Facón' (BIFAC), two genotypes from USA, 'Kofa' and UC1113, and 'Ciccio' from Italy. 'Kofa' has shown mechanisms for resistance related to DON content (Cirlini *et al*., 2014); the levels of resistance of the remaining lines are unknown; ii) the durum cultivar 'Langdon' and the derived resistant lines Langdon-Dic3A (Otto *et al*., 2002) and Langdon-DGE1 (Jauhar and Peterson, 2008); and iii) the common wheat line ACA 601 and the derived line ACA 601S3, obtained at the Asociación Cooperativas Argentinas (ACA, Argentina) by crossing the susceptible line ACA 601 with the resistant line Sumai 3, followed by six marker-assisted backcrosses using markers associated with FHB resistance (Anderson *et al.,* 2001). The seed samples were obtained from Buck Seed experimental plots planted at Pieres, Argentina (38°24'00"S - 58°40'00"W).

Molecular analyses

The presence of alleles conferring resistance was tested in 12 durum and common wheat lines using four linked SSR loci. DNA was extracted from leaf tissue following a modified CTAB method (Saghai-Maroof *et al.,* 1984). The *Xgwm2* locus localized near to the *Qfhs.ndsu-3AS* resistance region (Otto *et al.,* 2002) was checked using the primer combination L: CTGCAAGCCTGTGATCAACT and R: CATTCTCAAATGATCGAACA (Röder *et al.,* 1998). The flanking markers *Xgwm493* and *Xgwm533* localized 7 cM apart (Anderson *et al.,* 2001) were used to detect the presence of the *Qfhs.ndsu-3BS* region using the primer combinations L: TTCCCATAACTAAAACCGCG and R: GGAACATCATTTCTGGACTTTG for *Xgwm493* and L: AAGGCGAATCAAACGGAATA and R: GTTGCTTTAGGGGAAAAGCC for *Xgwm533* (Röder *et al.,* 1998). The *Xedm74* locus was used to screen for the presence of 1E *L. elongatum* chromosomes (Jauhar *et al.,* 2009) using the primer combination L: GTAAGTCGACCGAGGAGACG and R: CCTTCTTGCTTGGCATTCTC (Mullan *et al.,* 2005).

PCR reactions were each performed in a total volume of 25 μL containing 0.25 μM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, 1 × Taq buffer, 1 U *Taq* polymerase (Invitrogen), and 75 ng DNA template. The PCR profile consisted of an initial denaturation step of 3 min at 94ºC, 45 cycles of 1 min at 94ºC, 1 min at the annealing temperature, 2 min at 72ºC, and a final extension step of 10 min at 72ºC. Annealing temperatures were 52°C for markers *Xgwm493*, *Xgwm533* and *Xgwm2*, and 60°C for *Xedm74*. Amplification reactions were performed by a MyCycler Thermal Cycler (Bio-Rad). Products were resolved in 2% (w/v) agarose gel electrophoresis and visualized after staining with ethidium bromide.

Preparation of fungal cultures

The *F. graminearum* strain KBC7 was isolated from common wheat seeds and classified as a DON producing strain by Cecilia Fernochi, Universidad Nacional de Río Cuarto, Argentina. A macroconidial suspension of this strain was cultured in liquid mungbean medium for 7 d at 25ºC as described by Bai *et al.* (2000). The medium was then removed by multiple centrifugation steps (12,000 rpm, 10 min) in distilled water. The conidium concentration was directly quantified under an optical microscope with a Neubauer chamber, and diluted to the desired concentration.

Seedling blight assay

The *in vitro* assay was performed three times (Experiments I, II and III). In each experiment, two plates

with 20 seeds per accession were imbibed in 4 mL of *F. graminearum* conidium suspension (2×10^5 conidia mL^{-1}), or in sterile distilled water (control plate). Fresh inoculum was used for each experiment. After 15 min, the excess suspension was decanted and the inoculated seeds were planted on filter paper placed on 0.5% agar in Petri dishes, and were then incubated at 15ºC with 16 h photoperiod (Browne and Cooke, 2005). Six days after inoculation, four variables were recorded: germination, coleoptile length, coleoptile weight and root weight. Germinated seeds were counted in each inoculated 20-seed plate and expressed as a percentage of that of the non-inoculated control plate. Roots and coleoptiles were collected from each seedling and their lengths were measured using calipers and weights were determined using an analytical balance. Coleoptile and root measurements were recorded in each germinated individual and expressed as a percentage of the non-inoculated plate mean.

Head blight assay

The FHB severity assay consisted of two experiments (Experiments I and II). In each experiment, 15 to 20 plants per genotype were grown in plastic pots under greenhouse conditions (25±3ºC day, 11±1ºC night; 14 h light). Diammonium phosphate fertilization was applied during growth. Five to 26 spikes per genotype were each point inoculated in each experiment during early anthesis by injecting 10 μL of *F. graminearum* conidium suspension (1×10^5 conidia mL^{-1}) into the basal florets of the two central spikelets. The ears were wrapped in clear polythene bags and plants placed in a growth chamber with high relative humidity maintained by spraying with water, a temperature of 24±1ºC and 16 h light. After 3 d, the bags were removed and plants were taken back to the greenhouse. Disease severity was estimated as the percentage of symptomatic spikelets/spike at 21 d post-inoculation (dpi), with a total of 119 spikes evaluated in Experiment I and 132 in Experiment II.

Statistical analyses

For the *in vitro* assay, the experimental unit corresponded to the 20-seed plate. For each genotype, the means of inoculated plates across three experiments and the corresponding control plates were compared using Student's *t* test. For the subsequent analyses, the variables were expressed as percentages of the control values. The relationships between seedling variables and experiments were evaluated by the parametric Pearson correlation. The differences in genotype responses to *F. graminearum* for seedling variables and severity were evaluated using analysis of variance (ANOVA) under a randomized complete block design, with blocks consisting of the three experiments. In the case of seedling variables, the ANOVA model also included the component of variance attributable to seedling differences within plates. Coleoptile and root weights were transformed using, respectively, the square root and natural log (ln) transformations, to meet ANOVA assumptions. The mean values of the genotypes were compared using the Fisher's Least Significant Difference test.

Multiple linear regression analysis was used to explore the relationships between the independent seedling variables of the *in vitro* assay and severity as the dependent variable, using the backward elimination method. The significance level of a variable to be added to the model was set at 0.15.

All statistical analyses were performed using Infostat software version 2010 (Di Rienzo *et al.*, 2010).

Results

Molecular screening for resistance alleles

The SSR markers associated with FHB resistance are shown in Table 1. After amplification of the *Xgwm2* locus, Langdon-Dic3A produced the ≈200 bp fragment, linked to resistance allele at *Qfhs.ndsu-3AS* region (Hartel *et al.*, 2004). In ACA 601S3, amplification of markers *Xgwm493* and *Xgwm533* flanking the *Qfhs.ndsu-3BS* region gave rise, respectively, to fragments of ≈210 bp and ≈160 bp. These two alleles have been associated with resistance (Matus-Cádiz *et al.*, 2006). The *Xedm74* marker linked to *L. elongatum* 1E chromosomes was amplified in durum Langdon-DGE1 and common wheat ACA 601 and ACA 601S3 producing an ≈400 bp fragment (Jauhar *et al.*, 2009). The amplification of the *Xedm74* marker in common wheat had been previously demonstrated to represent co-amplification of *L. elongatum* 1E and *T. aestivum* 1D chromosomes (Jauhar and Peterson, 2011).

Therefore, the presence of *Qfhs.ndsu-3AS* and *Qfhs.ndsu-3BS* resistance QTLs was confirmed, respectively, in the introgressed lines Langdon-Dic3A and ACA 601S3, using linked markers. Molecular analysis also demonstrated that the other wheat gen-

Table 1. Molecular screening of 12 wheat genotypes using SSR markers associated with Fusarium head blight resistance. For each tested genotype, (+) denotes presence of the allele linked to resistance, (*) presence of a different allele and (–) no amplification, at the indicated locus.

QTL/chromosome	*Qfhs.ndsu-3AS*	*Qfhs.ndsu-3BS*		1E *L. elongatum*
SSR locus	*Xgwm2*[1]	*Xgwm493*[2]	*Xgwm533*[2]	*Xedm74*[3]
Genotype				
ACA 601	*	*	*	+
ACA 601S3	*	+	+	+
Langdon	*	*	*	-
Langdon-Dic3A	+	*	*	-
Langdon-DGE1	*	*	*	+
ACA 1801F	*	*	*	-
BIFAC	*	*	*	-
BESM	*	*	*	-
BPLA	*	*	*	-
Kofa	*	*	*	-
UC1113	*	*	*	-
Ciccio	*	*	*	-

[1] Hartel *et al.,* 2004; [2]Matus-Cádiz *et al.,* 2006; [3]Jauhar *et al.,* 2009; [3]Jauhar and Peterson, 2011

otypes tested lack the SSR alleles associated to these two QTLs.

In vitro assay

Compared to the water control, seedlings growing in the presence of *F. graminearum* showed mycelia on the seed surfaces and reddish discolouration of coleoptiles and roots (Figure 1 A and B). When the mean values of inoculated and control plates for germination, coleoptile length, coleoptile weight and root weight were compared, 19 out of 48 comparisons (four seedling variables, 12 genotypes) revealed statistically significant differences according to the Student's *t* test (Table 2). For Langdon-Dic3A, Langdon-DGE1 and ACA 601S3, there were no significant differences between the inoculated and control mean values, indicative of seedling resistance to *F. graminearum* in those lines. Inoculated and control seedlings of UC1113 and 'Ciccio' were significantly different for all comparisons, suggesting a strong effect of the fungus on the growth of these genotypes. The remaining genotypes showed significant differences in one, two or three comparisons relative to controls (Table 2).

Correlation values for the four variables and the three experiments are shown in Table 3. Nine of 12 possible comparisons for each variable between experiments were significantly correlated. In addition, 15 of 18 comparisons between variables within experiments showed significant correlations.

ANOVA detected significant differences between genotypes for all the seedling variables and non-significant differences between experiments (Table 4). The results of Fisher´s LSD comparisons are shown in Table 5. There were substantial differences in seed germination between genotypes, with reductions ranging from 0 to 59.5% relative to non-inoculated controls. The reductions in coleoptile and root growth also varied widely between genotypes, ranging from 0 (no detectable inoculation effect) to 64.8%.

In vitro inoculation experiments thus revealed pronounced differences in germination and seedling growth between genotypes and acceptable consistency among experiments.

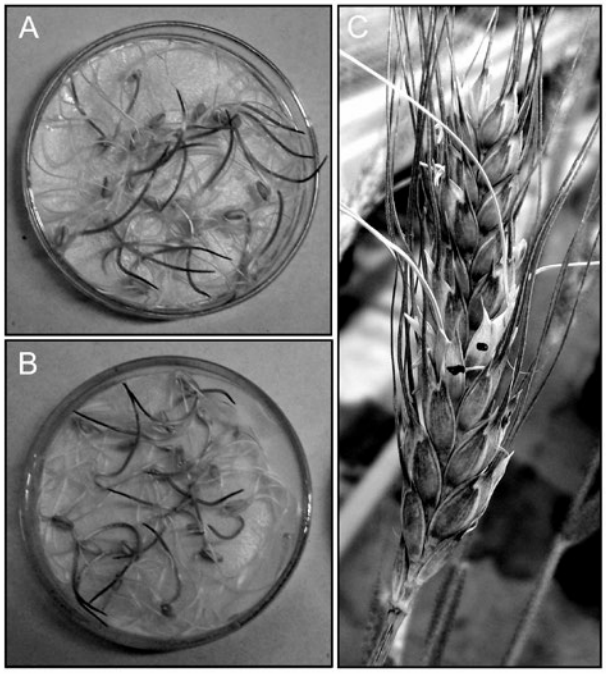

Figure 1. Seedlings and head of Langdon-Dic3A durum wheat. Six-d-old seedlings in control (water) (*A*) and *Fusarium graminearum*-inoculated (*B*) treatments. Damaged spikelets at 21 dpi (*C*).

Table 2. Mean comparisons between *Fusarium graminearum* inoculated and control (water) plates of four seedling variables across three experiments for 12 wheat genotypes.

Wheat genotype	Germination	Coleoptile length	Coleoptile weight	Root weight
'Kofa'			*	
BIFAC	*			
UC1113	*	*	*	*
ACA 1801F				*
BESM	*	**	*	
BPLA			*	
'Ciccio'	**	*	*	*
Langdon-Dic3A				
'Langdon'		*		*
Langdon-DGE1				
ACA 601		*	*	
ACA 601S3				

Student's *t* test: * $P \leq 0.05$; ** $P \leq 0.01$. Empty cells were not statistically significant.

Severity

Point inoculation of central spikelets conducted to assess FHB resistance revealed statistically significant differences in disease severity both for genotypes and experiments (Table 4). The genotype by experiment interaction was not significant suggesting that genotypes differed consistently in the levels of head infection across experiments. Severity of disease, calculated as the average percentage of affected spikelets per head at 21 dpi, ranged from 32.3 to 99% (Figure 1C; Table 5). Langdon-Dic3A, ACA 601S3, BIFAC and 'Kofa' showed the lowest infection levels, with severity values below 57%, whereas ACA 601, UC1113, 'Ciccio' and 'Langdon' were the most affected genotypes, with severity values of 77–99%.

Overall, the introgressed lines showed lower severities that did the original cultivars, and two cultivars not previously evaluated also presented low levels of spike damage.

Combination of seedling and head blight assays

A multiple linear regression analysis based on means of seedling variables across experiments was performed with average severity as the dependent variable. The coleoptile length was related to severity ($P<0.05$) and explained 63% of head disease. The addition of the other variables did not contribute significantly to the model (Table 6). Coleoptile length showed a highly significant negative correlation with disease severity (r = -0.8; $P<0.001$), and a ranking of genotypes in terms of these two disease estimations is shown in Figure 2. The two statistically defined (Fisher's LSD test) groups of genotypes with contrasting responses are termed "best performance" and "poorest performance", with the remainder listed as "intermediate".

Discussion

More rapid and accurate methods to identify resistance to *Fusarium* infection are in demand for cere-

Table 3. Correlation coefficients (Pearson test) between seedling variables (% of control) measured in Experiments I, II and III of a Petri-dish assay after inoculation with *Fusarium graminearum*.

Variable		Germination			Coleoptile length			Coleoptile weight			Root weight	
		I	II	III	I	II	III	I	II	III	I	II
Germination	I											
	II	**0.73****										
	III	**0.65***	**0.74****									
Coleoptile length	I	**0.83*****	**0.73****	**0.72****								
	II	**0.64***	**0.59***	**0.51**	**0.86*****							
	III	**0.58***	**0.44**	**0.58***	**0.63***	0.55						
Coleoptile weight	I	**0.68****	**0.61***	**0.71****	**0.93*****	**0.8*****	0.46					
	II	**0.77*****	**0.6***	0.55	**0.87*****	**0.94*****	**0.62***	**0.76*****				
	III	0.53	0.41	0.55	**0.65***	**0.69****	**0.95*****	0.53	**0.68***			
Root weight	I	0.41	0.33	0.46	**0.75*****	**0.76*****	0.3	**0.9*****	**0.62***	0.47		
	II	**0.79*****	0.52	0.37	**0.82*****	**0.81*****	**0.69****	**0.64***	**0.9*****	**0.68***	0.5	
	III	0.48	0.34	**0.57***	**0.69****	**0.72****	**0.89*****	**0.64***	**0.68***	**0.97*****	**0.63***	**0.65***

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

al breeding. One of the main tasks in testing for FHB resistance is to achieve experimental consistency. In the present study, the finding of significant correlations of seedling variables between experiments shows evidence of acceptable repeatability of the *in vitro* assay to assess disease resistance.

The seedling variables evaluated were not equally informative for FHB prediction. Coleoptile length was consistently associated with Type II resistance (severity) whereas the other variables did not significantly increase the level of head disease explanation. This is not surprising considering the high correlation values observed between root and coleoptile measurements. In contrast to previous *in vitro* assays of *T. aestivum* germplasm (Browne and Cooke, 2005; Browne, 2007), a significant association between germination and head infection was not observed in our study. However, those experiments used *Microdochium majus*, a non-toxin-producing species which also causes FHB, and heads were spray inoculated, which is more indicative of Type I resistance. In addition, the weight of roots and coleoptiles were included in our seedling blight assay, unlike previous assays.

Coleoptile growth rate has been utilized effectively to examine the aggressiveness of different *Fusarium* species (Brennan *et al.*, 2003) and *F. graminearum* strains (Wu *et al.*, 2005; Purahong *et al.*, 2012) in wheat. This allowed for determination of an "*aggressiveness index*", based on a sequential percentage of healthy coleoptiles, germination reduction and coleoptile length reduction to be applied to quantify the aggressiveness of 25 *F. graminearum* strains, and significant correlation with ear disease level was observed (Purahong *et al.*, 2012). Here we describe a seedling assay that explains 63% of head blight disease evaluated in the greenhouse and that distinguishes previously characterized susceptible and resistant genotypes. When considered together, these independent studies provide strong evidence for the usefulness of seedling assays for FHB evaluation concerning both the pathogen and the host.

There were substantial differences between genotypes in germination and seedling growth after inoculation with *F. graminearum*, suggesting differences in ability to resist pathogen effects at early growth stages. A ranking of genotypes according to the relative decrease of coleoptile length in infected seedlings

Table 4. Analysis of variance (ANOVA) of seedling variables (% of control) and disease severity (% symptomatic spikelets/spike) of 12 wheat genotypes after inoculation with *Fusarium graminearum*.

Variable	Source of variance	DF	MS	*F* value
Germination (%)	Experiment	2	27.6	0.2 ns
	Genotype	11	837.1	6.4 ***
	Error	22	130.1	
	Total	35		
Coleoptile length (%)	Experiment	2	215.0	0.1 ns
	Genotype	11	9262.1	4.9 **
	Error	22	1873.1	3.9 ***
	Within plates	503	483.5	
	Total	538		
Coleoptile weight (%)	Experiment	2	4.6	0.5 ns
	Genotype	11	47.2	4.8 **
	Error	22	9.8	4.7 ***
	Within plates	503	2.1	
	Total	538		
Root weight (%)	Experiment	2	0.8	0.5 ns
	Genotype	11	4.9	3.2 **
	Error	22	1.5	12.2 ***
	Within plates	503	0.1	
	Total	538		
Severity	Experiment	1	5589.8	7.7 **
	Genotype	11	5515	7.6 ***
	Experiment *Genotype	11	1187.2	1.6 ns
	Error	227	722.8	
	Total	250		

DF = degree of freedom; MS = mean squares; ns: not significant; ** $P \leq 0.05$; *** $P \leq 0.0001$.

and Fisher significance allowed the identification of genotypes with better, poorer and intermediate levels of performance (Figure 2). The reliability of this genotype order was validated by its highly significant correlation with disease severity (r = -0.8) and its agreement with previous Type II resistance evaluations performed on some of the genotypes included in this study (Stack *et al.*, 2002; Jauhar and Peterson, 2008). The four genotypes with the lowest values for coleoptile growth were those having the highest levels of head disease (Table 5, Figure 2). Moreover, two of these, 'Ciccio' and UC1113, presented the greatest number of significant differences between inoculated and non-inoculated seedling means. Similarly, the four genotypes ranked best according to coleoptile growth showed the lowest values of spike damage. There were two differences in "intermediate" group members that do not invalidate the outcome of the present test because groups of genotypes with the extreme responses were conserved. Although based on a small sample of

Table 5. Means of seedling variables (% of control) and disease severity (% symptomatic spikelets/spike) for 12 wheat genotypes after inoculation with *Fusarium graminearum*.

Genotype	Germination	Coleoptile length	Coleoptile weight	Root weight	Severity
Langdon-Dic3A	100 a	99.5 ab	95.4 a	90.9 ab	32.3 a
Langdon-DGE1	96.9 a	97.6 ab	96 a	96.5 a	63.2 bcd
ACA 601S3	93.3 a	104.1 a	103.4 a	94.6 a	49.9 b
'Langdon'	90 ab	75.3 cd	67.9 cde	54.1 cde	99 g
ACA 1801F	89.1 ab	95.9 ab	91 abc	86.5 abcd	72.5 cde
BPLA	88.3 ab	81.9 bc	69.7 bcd	54.6 bcde	69.5 bcde
BIFAC	87.5 ab	96.5 ab	93.1 ab	90 abc	55 bc
ACA 601	84.6 ab	67.2 cd	53.9 de	38.9 e	77.3 def
'Kofa'	81.5abc	101 ab	91.2 abc	89.1 abc	56.4 bc
BESM	72.3 bc	81.4 bcd	70.7 bcd	75.9 abcd	69.2 bcde
'Ciccio'	62.3 c	65.1 cd	58.1 de	48.4 de	92.9 fg
UC1113	40.5 d	57.6 d	43.6 e	35.2 e	84.3 efg

Values within a column followed by the same letters are not significantly different (Fisher's LSD test, $\alpha = 0.05$).

Table 6. Multivariate analysis of independent variables in a *Fusarium graminearum*-mediated Petri dish test (average of three experiments) using backward selection to explain FHB severity (average of two experiments) under greenhouse condition. Variables with *P* values greater than 0.15 were not added to the final model and are reported as not significant (ns).

FHB variable	Variable entered	*P* value	Model R^2
Severity	Coleoptile length (%)	0.0021	0.63
	Coleoptile weight (%)	ns	
	Root weight (%)	ns	
	Germination (%)	ns	

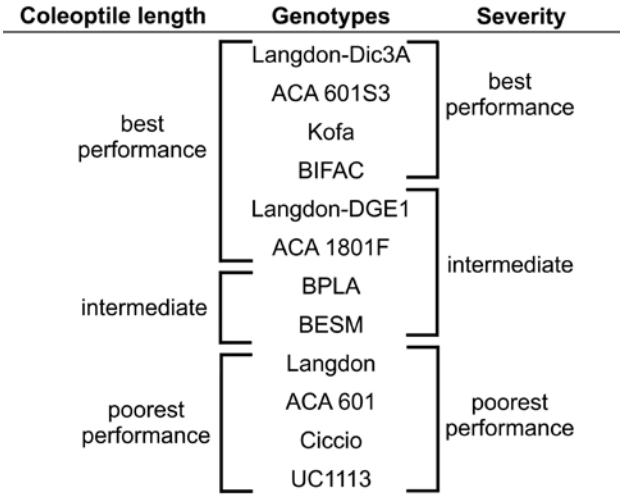

Figure 2. Ranking of 12 wheat genotypes based on coleoptile length in a *Fusarium graminearum*-mediated Petri dish test and on FHB severity following point inoculation of spikes in the greenhouse.

selected genotypes, our results indicate that the level of resistance or susceptibility to *F. gramineaurm* can be recognized at early stages of plant growth. This could lead to improved throughput of segregating progenies in breeding programmes, and of germplasm collections when evaluating for resistance to FHB.

The introgressed lines Langdon-DGE1 and Langdon-Dic3A of durum wheat and ACA 601S3 of common wheat performed better than the original cultivar 'Langdon' and ACA 601. These results are in agreement with the molecular screening utilizing markers linked to QTLs/chromosomes previously

associated with resistance, and when combined, support the concept that the variability in response to infection is caused by genetic differences that are expressed both in the seedling and the reproductive (seed production) stages. They also reveal that evaluation methods based on the differential growth of infected seedlings allow for the identification of important FHB resistance sources both in durum and common wheat. This in contrast to previous *in vitro* detached leaf assays that were unable to identify resistance determinants of Sumai3 (Browne and Cooke, 2004). Further, the better performance of ACA 601S3 in comparison to the original ACA 601 constitutes new evidence for the stability of the Sumai3 *Qfhs.ndsu-3BS* region in a different genetic background.

The present study also allowed the characterization of some cultivars for which information about resistance had not been previously reported. UC1113 showed high FHB susceptibility whereas genotypes 'Kofa' and BIFAC were much less affected. None of the resistance QTLs evaluated here were detected in the latter cultivars, and further investigation is necessary to identify the genetic determinants of their resistance. A recent study demonstrated that 'Kofa' is able to efficiently convert deoxynivalenol (DON) mycotoxin into its conjugated form DON-3G (Cirlini *et al.*, 2014).

The biological explanation for an association between the seedling and adult plant responses to infection remains unknown, but it can be hypothesized that similar genetic pathways become activated at both developmental stages. The genetic basis of FHB resistance is highly complex but is beginning to be understood. QTLs for resistance have been associated with passive factors, such as plant height, spike architecture and flowering date (Buerstmayr *et al.*, 2012), and also with variability in specific genes. Some specific compounds of wheat anthers (choline, betaine) were thought to be responsible for fungal growth stimulation (Strange *et al.*, 1974), but in a later study, a substantial role of floral structures in resistance to *F. graminearum* is denied (Engle *et al.*, 2004). Multiple signaling pathways that are involved in spike response to *F. graminearum* infection (Ding *et al.*, 2011) have been found to be similarly regulated in inoculated seedlings (Li *et al.*, 2010; Cho *et al.*, 2012). Moreover, some genes up-regulated during infection affect plant growth and stem cell division (Cho *et al.*, 2012). It is possible that lipid transfer and deoxynivalenol resistance genes associated with 5A (*Qfhs.ifa-5A*) and 3B (*Qfhs.ndsu-3BS*) QTLs in common wheat (Schweiger *et al.*, 2013) are also components of resistance at the seedling stage. In addition, the observed correlations between parameters of coleoptiles and roots within each experiment could reflect concurrent gene expression in different tissues/organs. Improved understanding of these putative parallel mechanisms is expected as comparative gene expression studies across developmental stages increase in number.

Since only ten durum wheat and two common wheat genotypes were tested here, further research using a larger sample of lines is needed to validate our results. However, our study included three of the most important resistance sources at both the tetraploid and hexaploid levels, i.e. *T. turgidum* spp. *dicoccoides*, *L. elongatum* and *T. aestivum* Sumai3. The *in vitro* analysis can be expanded as additional resistance sources become available (Buerstmayr *et al.*, 2012; Huhn *et al.*, 2012; Jauhar and Peterson, 2012, 2013; Cai, 2013), and it would be worthwhile to include different *F. graminearum* strains. The seedling assay described here, based on a minimum of three experiment replications, can be realized in a relatively short period of time, making it a convenient protocol for evaluation of a large number of progeny at early breeding stages. Hence, evaluation of FHB resistance under greenhouse or field conditions could be restricted to a narrower sample of selected genotypes with best performance in the seedling assay. The Petri dish assay has a low cost approach, and can be carried out off-season. This assay may also constitute a model system for biochemical and genetic studies of plant defense responses.

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