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Spike architecture traits associated with type II resistance to fusarium head blight in bread wheat

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Abstract Fusarium head blight (FHB) remains a devastating disease in bread wheat (*Triticum aestivum* L.). Genetic resistance to FHB is complex; aside from active physiological resistance, plant developmental and morphological traits indirectly affect disease progression and provide a passive resistance mechanism. Here, the relationship between FHB Type II resistance and spike architecture traits was evaluated in a recombinant inbred line (RIL) population of bread wheat in field experiments during two crop seasons under a completely randomized block design with two replications. Point inoculation was carried out at anthesis of each RIL. Disease severity at 21 days post inoculation (dpi), area under the disease progress curve (AUDPC) comprising severity measurements at 12, 17 and 21 dpi, rachis length, spike density, number of spikelets per spike, number of florets per spike and number of florets per spikelet were determined. The population showed significant variation for all traits. Heritability was moderate-high for FHB severity (0.69) and AUDPC (0.63) and high for spike architecture traits (0.74–0.92). FHB severity at 21 dpi and AUDPC were significantly associated with number of

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florets per spike [$r = 0.38$ ($P < 0.001$) and $r = 0.31$ ($P < 0.01$) respectively] and with the number of florets per spikelet [$r = 0.28$ ($P < 0.01$) and $r = 0.27$ ($P < 0.05$) respectively], reflecting a greater spread of the fungus in spikes with higher floret number. These results suggest that the number of florets per spike and per spikelet should be considered in FHB resistance breeding efforts, because selection of lines with higher number of florets could lead to a correlated selection response towards increased FHB levels under field conditions.

Keywords *Fusarium graminearum* · *Triticum aestivum* · Inflorescence traits · Passive resistance

Introduction

Fusarium head blight (FHB), also known as head scab, is one of the most devastating diseases of wheat, frequently causing epidemics in many wheat-growing areas of the world (Lori et al. 2003; Mazzilli et al. 2007). This disease is prevalent in regions with prolonged warm and humid climatic conditions in the period from flowering to the soft dough stage of kernel development (Bai and Shaner 1994; Sutton 1982).

Although many *Fusarium* species can cause FHB, *Fusarium graminearum* Schwabe is one of the main pathogens associated with the disease in many countries of the world, including Argentina (Malbrán and Lori 2014; Schroeder and Christensen 1963; Sutton 1982). The fungus invades the spikes predominantly by direct penetration and colonizes the rachis and the spikelets. FHB infection leads to severe losses not only in grain yield but also in quality, decreasing seed germination and flour baking properties (McMullen et al. 1997). Damaging effects are further aggravated by the accumulation of mycotoxins produced by the fungus in the grains, which render them unfit for human or animal consumption (Kendrick 1992). Different control strategies such as crop rotation, tillage practices and fungicide application have been proposed to reduce the impact of FHB. However, these agronomic practices have a limited success. Therefore, the use of FHB-resistant cultivars is still the most reliable and consistent strategy for minimizing losses caused by the disease (CIMMYT 2019).

Resistance to FHB is a complex trait: it is quantitatively inherited, significantly affected by environmental conditions, and subjected to strong genotype-by-environment interactions (Bai and Shaner 1994). Resistance to FHB is the result of passive and active mechanisms (Mesterhazy 1995; Rudd et al. 2001). Passive resistance includes morphological and developmental traits (for example: plant height, spike architecture and flowering date) which alter conditions for initial infection and subsequent fungal growth in the spike (Buerstmayr and Buerstmayr 2015). Also, active resistance mechanisms comprise biochemical pathways that produce compounds that affect the pathogen during and/or after infection (Wiese 1987). The two main types of resistance to FHB are resistance to initial infection (Type I) and resistance to spread of the pathogen within the spike after infection (Type II) (Schroeder and Christensen 1963). In this way, considering that plant architecture can play a significant role in disease resistance, establishing the relationship between FHB resistance and architectural traits affecting disease development could be an advantageous strategy for accelerating the development of resistant varieties (Zhu et al. 1999).

So far, several plant morphological and developmental traits have been investigated for their association with FHB resistance. However, most of these studies have been carried out for FHB Type I resistance. Thus, resistance to initial infection has been correlated with flower opening and duration of flower opening (Pugh et al. 1933; Zhang et al. 2018), extent of anther extrusion/retention (Skinnes et al. 2005; Kubo et al. 2013; Buerstmayr and Buerstmayr 2015), plant height (Gervais et al. 2003; Steiner et al. 2004) and flowering date (Buerstmayr et al. 2012; Steiner et al. 2004), among others. On the other hand, there are only a few studies about the effect of morphological traits on Type II resistance (Steiner et al. 2019; Buerstmayr et al. 2020).

Since Fusarium head blight is a floral infection disease (Arthur 1891), once penetration occurs on the inner surfaces of the lemma and palea or on the upper portion of the ovary, fungal hyphae spread downwards to the rachilla and rachis node by inter- and intracellular growth. When the hyphae reach the rachis, they spread upwards and downwards the entrance point through vascular bundles in the rachis (Kang and Buchenauer 2000). Then, it may be hypothesized, for example, that wheat plants which exhibit a longer

rachis or lower inflorescence compactness have a lower disease progress and severity by reducing the speed with which *Fusarium* hyphae can extend into the spike. The aim of this research was to study the relationships between FHB Type II resistance and spike architecture traits, in order to enhance the current knowledge on this complex pathosystem from a breeding standpoint.

Materials and methods

Plant material

A biparental population of 126 recombinant inbred lines (RILs) was developed from a cross between 'Baguette 10' and 'Klein Chajá', two spring bread wheat cultivars of very different genetic background, agronomically adapted for cultivation in Argentina. This population was generated at the Instituto Nacional de Tecnología Agropecuaria (INTA) (Alonso et al. 2018; Martino et al. 2015; Mirabella et al. 2016). Both parental cultivars have medium FHB resistance level and differ in spike architecture.

Fusarium head blight resistance evaluation

Field experiments

The RIL population, the parental cultivars and four commercial checks were tested in field experiments at the INTA Balcarce Experimental Station (37°46′15″ S; 58°18′ 24″ W; 112 m.a.s.l.), Buenos Aires province, Argentina in two consecutive years (2016 and 2017). In each crop season, two experiments were carried out, which differed in their sowing date by about one month; this resulted in a total of four experiments (environments). Experiments were arranged as randomized complete block designs with two blocks (or replications). Sowing dates, sowing density and crop management were as described in Franco et al. (2021).

Inoculation technique and disease assessment

A macroconidial suspension of *F. graminearum* -isolate 'SP1'- was used for inoculation. This isolate was previously characterized by its aggressiveness and it was used in this study because it caused the highest disease severity in two consecutive field tests (Malbrán et al. 2014, 2012). The macroconidial suspension was prepared as described by Malbrán et al. (2012) and the concentration was adjusted to ~100,000 spores ml^{-1} using a haemocytometer.

Anthesis date, defined as the date in which 50% of the spikes of each individual plot was flowering-Zadoks growth stage 65 (Zadoks et al. 1974)-, was recorded for each genotype in all plots. Ten flowering spikes per plot were randomly picked and tagged with a numbered label for identification. The spikes were inoculated using the point inoculation (PI) technique as described in Franco et al. (2021).

Development of FHB symptoms was followed individually on each inoculated spike. The number of infected spikelets per spike was determined visually at 12, 17, and 21 days post inoculation (dpi). FHB severity was estimated as the proportion of infected spikelets in a spike at 21 dpi (number of infected spikelets divided by the total number of spikelets per spike). The Area Under the Disease Progress Curve (AUDPC) was calculated for each spike, according to Shaner and Finney (1977) as:

$$AUDPC = \sum_{i=1}^{n} \frac{(S_i + S_{i+1})}{2} * (t_{i+1} - t_i)$$

where Si = disease severity at the *ith* observation, t_i = days at the *ith* observation, and n = total number of observations.

Evaluation of spike architecture traits

Spike architecture traits were evaluated in all RILs and the parental cultivars. Rachis length, number of spikelets per spike and spike density were determined in all inoculated spikes. Rachis length was measured as the distance in cm between the top and the bottom node of the rachis. Number of spikelets per spike was the total number of fertile spikelets per spike. The average number of spikelets per cm of rachis length was calculated and used as an estimation of spike density. Number of florets per spike was counted on 15 randomly chosen spikes per plot from the two field experiments carried out in 2016 and one field experiment in 2017. The average number of florets per spikelet was estimated as the number of florets in the spike divided by the total number of spikelets in the spike.

Statistical analysis

All statistical analyses were performed using R software (R Core Team 2013). All the data was analyzed fitting linear mixed models with the *lme* function from package *nlme* (Pinheiro et al. 2013). Residuals were tested for normality and homoscedasticity and a log-transformation was performed for FHB severity and AUDPC to normalize residuals. Models for logarithm of FHB severity and AUDPC were fitted considering the anthesis date as a fixed factor and genotypes and genotypes x environment interaction as random factors according to Franco et al. (2021).

Models for spike traits were fitted considering environment and block within environment as fixed effects and genotypes and genotype x environment interaction as random effects:

$$y_{ijk} = \mu + \alpha_j + \beta_{k(j)} + \tau_i + \gamma_{j(i)} + \varepsilon_{ijk}$$

where y_{ijk} is the logarithm of the response variable on block "k" of line "i" in the environment "j", μ is the mean value of the of response variable,α_j is the fixed effect of the environment "j", $\beta_{k(j)}$ is the fixed effect of the block "k" in the environment "j", τ_i is the random effect of line "i", $\gamma_{j(i)}$ is the random interaction effect between line "i" and environment "j", and $\varepsilon_{ijk(s)}$ is the random error of the observation on repetition "k" of line "i" in the environment "j".

Assumptions on this model are: $\tau_i \sim N\left(0; \sigma_g^2\right)$, $\gamma_{j(i)} \sim N\left(0; \sigma_{ge}^2\right)$ and $\varepsilon_{ijk} \sim N\left(0; \sigma_{res}^2\right)$ all are independent of each other.

Sequential restricted maximum likelihood ratio tests were performed to determine the significances of the random effects of lines and lines by environment interactions. For this, three linear models were fitted with the package *nlme*: Model 1 or complete model included the design fixed effects and genotypes and genotype x environment interaction as random effects, Model 2 omitted the random effect of genotype by environment interaction and Model 3 omitted the random effect of genotype by environment interaction and random effect of the genotype. Sequential restricted maximum likelihood ratio tests were performed for Model 1 versus Model 2 to test the significance of the random effects of genotype by environment interaction, and Model 2 versus Model 3 to test the significance the random effects of the genotype.

For all the variables, best linear unbiased predictors (BLUPs) were obtained for all RILs and parental cultivars. Variance components were estimated by the restricted maximum likelihood (REML) method (Milliken and Johnson 2001) and broad-sense heritabilities (H^2) were estimated from variance components according to Hallauer et al. (2010), as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \left(\frac{\sigma_{ge}^2}{e}\right) + \left(\frac{\sigma_{res}^2}{re}\right)}$$

where σ_g^2 is the genotypic variance, σ_{ge}^2 is the genotype x environment interaction variance, σ_{res}^2 is the error variance; e is the number of environments, and r is the number of replications per experiment.

To determine the significance of the genetic correlation between all the evaluated traits, Pearson correlation tests were performed with the obtained BLUPs. Also, phenotypic correlation analysis was carried out with individual data for each experiment.

Results

FHB severity and AUDPC

Despite the great environmental variation recorded among environments as well as among inoculation dates (Figs. S1 and S2), FHB symptoms were present in all field experiments and evaluated genotypes, with an overall 70% incidence in conidia-inoculated spikes. Mean values of the parental cultivars and means, minimum and maximum scores and standard deviations of the RIL population for each experiment as well as for the overall mean across all experiments for FHB severity and AUDPC are presented in Table 1. Sequential restricted maximum likelihood ratio tests revealed highly significant variation due to genotypes for both variables ($P < 0.01$) (Table 2). The RIL population showed continuous variation for these variables across the conducted experiments (Fig. 1). Averaged across experiments, FHB severity varied between 0.14 and 0.69, and the AUDPC, between 91.5 and 513.2. The parental cultivars exhibited an intermediate performance, although Baguette 10 showed lower FHB severity mean but higher AUDPC mean than Klein Chajá. For both variables, transgressive

Table 1 Means, minimum (Min) and maximum (Max) values and standard deviations (SD) for FHB severity and AUDPC in the Baguette 10 × Klein Chajá RIL population (N = 126) and parental cultivars, as evaluated in four field experiments carried out in Balcarce, Argentina

Trait	Year	Experiment	Means of parental cultivars		Values for RIL population			
			Baguette 10	Klein Chajá	Mean	Min	Max	SD
FHB Severity	2016	1	0.69	0.46	0.38	0.05	1.0	0.24
	2016	2	0.40	0.46	0.51	0.06	1.0	0.20
	2017	1	0.18	0.44	0.24	0.05	0.9	0.15
	2017	2	0.25	0.36	0.27	0.05	1.0	0.18
	Overall mean		**0.38**	**0.43**	**0.34**	**0.05**	**1**	**0.23**
AUDPC	2016	1	544.1	218.9	274.7	10.3	1088.0	202.5
	2016	2	350.9	262.7	368.4	11.0	924.4	193.8
	2017	1	135.0	280.8	167.3	11.9	892.7	112.6
	2017	2	192.6	259.9	214.4	8.5	855.1	128.9
	Overall mean		**305.7**	**255.6**	**252.2**	**8.5**	**1088.0**	**177.0**

Table 2 Sequential restricted maximum likelihood ratio tests to determine the significances of the random effects of lines and line by environment interactions for severity and AUDPC

Trait	Model[a]	df[b]	AIC[c]	BIC[d]	logLik[e]	Test	L. Ratio[f]	*P*-value
FHB Severity	1	29	1.704.002	1.843.400	− 8.230.008			
	2	28	1.709.290	1.843.881	− 8.266.449	1 vs. 2	728.811	0.0069
	3	27	1.816.911	1.946.696	− 8.814.558	2 vs. 3	10.962.179	< 0.0001
AUDPC	1	29	1.864.524	2.003.922	− 9.032.619			
	2	28	1.864.798	1.999.389	− 9.043.989	1 vs. 2	227.400	0.1316
	3	27	1.933.160	2.062.945	− 9.395.801	2 vs. 3	7.036.224	< 0.0001

[a]Model 1 is the complete model. Models 2 and 3, sequentially omit the random effect of line by environment interaction and random effect of the lines

[b]Degrees of freedom

[c]Akaike information criterion

[d]Bayesian information criterion

[e]Log-Likelihood

[f]Likelihood-ratio test

segregation (i.e., the occurrence of RILs with more extreme values than those of the parents) was observed in all experiments.

Spike architecture traits

The mean spike architecture traits' values of the parental cultivars and means, minimum and maximum scores and standard deviations of the RIL population for each experiment as well as for the overall mean across all experiments are presented in Table 3. The RIL population showed significant variation for rachis length, spike density, spikelets per spike, florets per spike and florets per spikelet across all experiments (Table 4). A bell-shaped frequency distribution was observed for each of the spike architecture traits evaluated in the population (Fig. 2). The parental cultivars showed intermediate values for all the variables. 'Baguette 10' exhibited a higher spike density than 'Klein Chajá', due to a higher number of

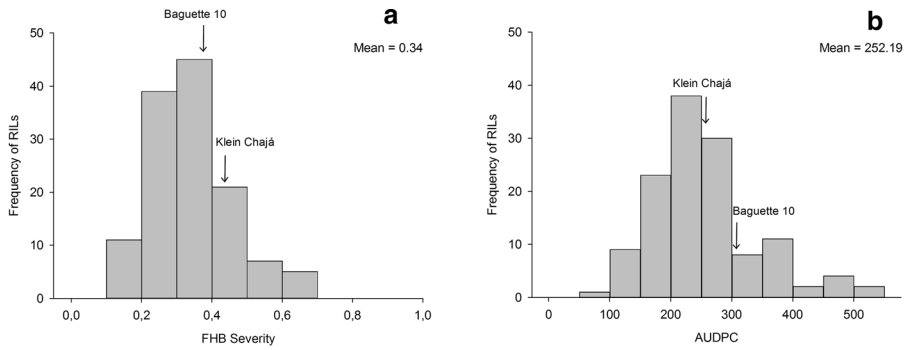

Fig. 1 Frequency distribution of **a** FHB severity and **b** Area under the disease progress curve (AUDPC)-average of four field experiments carried out in Balcarce, Argentina- in the Baguette 10 × Klein Chajá RIL population (N = 126). The values of the parental cultivars are indicated with arrows

Table 3 Means, minimum (Min) and maximum (Max) values and standard deviations (SD) for spike architecture traits in the Baguette 10 × Klein Chajá RIL population (N = 126) and parental cultivars, as evaluated in field experiments carried out in 2016 and 2017 at Balcarce, Argentina

Trait	Year	Experiment	Means of parental cultivars		Values for RIL population			
			Baguette 10	Klein Chajá	Mean	Min	Max	SD
Rachis length	2016	1	8.2	9.7	8.8	6.3	12.1	0.9
	2016	2	8.5	10.4	9.3	6.5	12.4	1.0
	2017	1	7.7	10.0	8.8	6.3	11.7	0.9
	2017	2	9.0	9.4	8.5	6.4	11.3	0.9
	Overall mean		**8.3**	**9.9**	**8.9**	**6.3**	**12.4**	**1.0**
Spike density	2016	1	2.2	1.6	2.0	1.5	2.7	0.2
	2016	2	2.3	1.7	1.9	1.5	2.6	0.2
	2017	1	2.2	1.6	1.9	1.4	2.8	0.2
	2017	2	2.1	1.5	1.9	1.4	2.5	0.2
	Overall mean		**2.2**	**1.6**	**1.9**	**1.4**	**2.8**	**0.2**
Spikelets per spike	2016	1	18.0	15.3	17.3	13.2	20.8	1.4
	2016	2	19.0	17.8	17.7	14.4	21.0	1.3
	2017	1	17.3	15.7	16.9	13.0	23.4	1.4
	2017	2	18.3	14.2	16.4	12.4	20.3	1.4
	Overall mean		**18.2**	**15.8**	**17.1**	**12.4**	**23.4**	**1.5**
Florets per spike	2016	1	42.2	38.9	42.4	13.3	64.3	7.8
	2016	2	31.5	47.6	44.2	12.2	68.1	9
	2017	1	33.3	40.8	39.3	26.7	56.7	5.2
	Overall mean		**36.5**	**42.4**	**42.0**	**12.2**	**68.1**	**7.8**
Florets per spikelet	2016	1	2.34	2.5	2.4	0.8	3.6	0.4
	2016	2	1.65	2.7	2.5	0.7	3.9	0.5
	2017	1	1.91	2.6	2.3	1.5	3.4	0.4
	Overall mean		**1.97**	**2.6**	**2.4**	**0.7**	**3.9**	**0.4**

spikelets in the spike and a shorter rachis. Also, 'Baguette 10' showed a lower number of florets per spikelet and per spike than did 'Klein Chajá'. Transgressive segregation was observed in all experiments.

Table 4 Sequential restricted maximum likelihood ratio tests to determine the significances of the random effects of lines and lines by environment interactions for the spike architecture traits

Trait	Model[a]	df[b]	AIC[c]	BIC[d]	logLik[e]	Test	L. Ratio[f]	*P*-value
Rachis length	1	11	2.330.097	2.383.961	− 1.154.049			
	2	10	2.333.287	2.382.254	− 1.156.643	1 vs 2	518.951	0.0227
	3	9	2.634.656	2.678.726	− 1.308.328	2 vs 3	30.336.916	< 0.0001
Spike density	1	11	− 12.522.349	− 11.983.713	6.371.175			
	2	10	− 12.499.499	− 12.009.830	6.349.750	1 vs 2	42.850	0.0385
	3	9	− 6.146.743	− 5.706.041	3.163.372	2 vs 3	6.372.756	< 0.0001
Spikelets per spike	1	11	3.147.756	3.201.620	− 1.562.878			
	2	10	3.155.215	3.204.182	− 1.567.608	1 vs 2	94.590	0.0021
	3	9	3.486.162	3.530.232	− 1.734.081	2 vs 3	3.329.469	< 0.0001
Florets per spike	1	9	4.697.509	4.738.456	− 2.339.755			
	2	8	4.701.134	4.737.531	− 2.342.567	1 vs 2	562.414	0.0177
	3	7	4.844.716	4.876.564	− 2.415.358	2 vs 3	14.558.264	< 0.0001
Florets per spikelet	1	9	6.671.720	7.079.892	− 3.245.860			
	2	8	6.724.175	7.086.994	− 3.282.087	1 vs 2	724.547	0.0071
	3	7	8.317.668	8.635.135	− 4.088.834	2 vs 3	16.134.930	< 0.0001

[a]Model 1 is the complete model. Models 2 and 3, sequentially omit the random effect of line by environment interaction and random effect of the lines

[b]Degrees of freedom

[c]Akaike information criterion

[d]Bayesian information criterion

[e]Log-Likelihood

[f]Likelihood-ratio test

Variance components and heritabilities

Variance component analysis by REML revealed that σ_g^2 was greater than σ_{ge}^2 for all variables (Table 5). Medium to high heritability values were observed for FHB severity (0.69) and AUDPC (0.63), indicating that a high portion of the observed phenotypic variation was caused by the genotypic component. As expected, heritability values were high for spike architecture traits (between 0.74 and 0.92).

Correlation analysis

Genetic correlation coefficients between BLUPs of FHB severity, AUDPC, rachis length, spike density, spikelets per spike, florets per spike and florets per spikelet are shown in Table 6. The strongest genetic correlation coefficient ($r = 0.94$, $P < 0.001$) was detected between FHB severity and AUDPC.

Both variables showed a moderate and significant association with the number of florets per spike ($r = 0.38$, $P < 0.001$ and $r = 0.31$, $P < 0.01$, respectively) and with the number of florets per spikelet ($r = 0.28$, $P < 0.01$ and $r = 0.27$, $P < 0.05$ respectively). Also, a significant, positive correlation ($r = 0.59$, $P < 0.001$) was found between the number of florets per spike and number of florets per spikelet. Rachis length, number of spikelets per spike and spike density did not show association with either FHB severity or AUDPC. As it was expected, the number of spikelets per spike was positively correlated with both rachis length ($r = 0.58$, $P < 0.001$) and spike density ($r = 0.27$, $P < 0.01$). Additionally, phenotypic correlation coefficients for each individual experiment are shown in Tables S1, S2 and S3. The significant positive associations between FHB severity and florets per spike were observed for all three experiments and between FHB severity and florets per spikelet in the two experiments of 2016. Also, AUDPC was

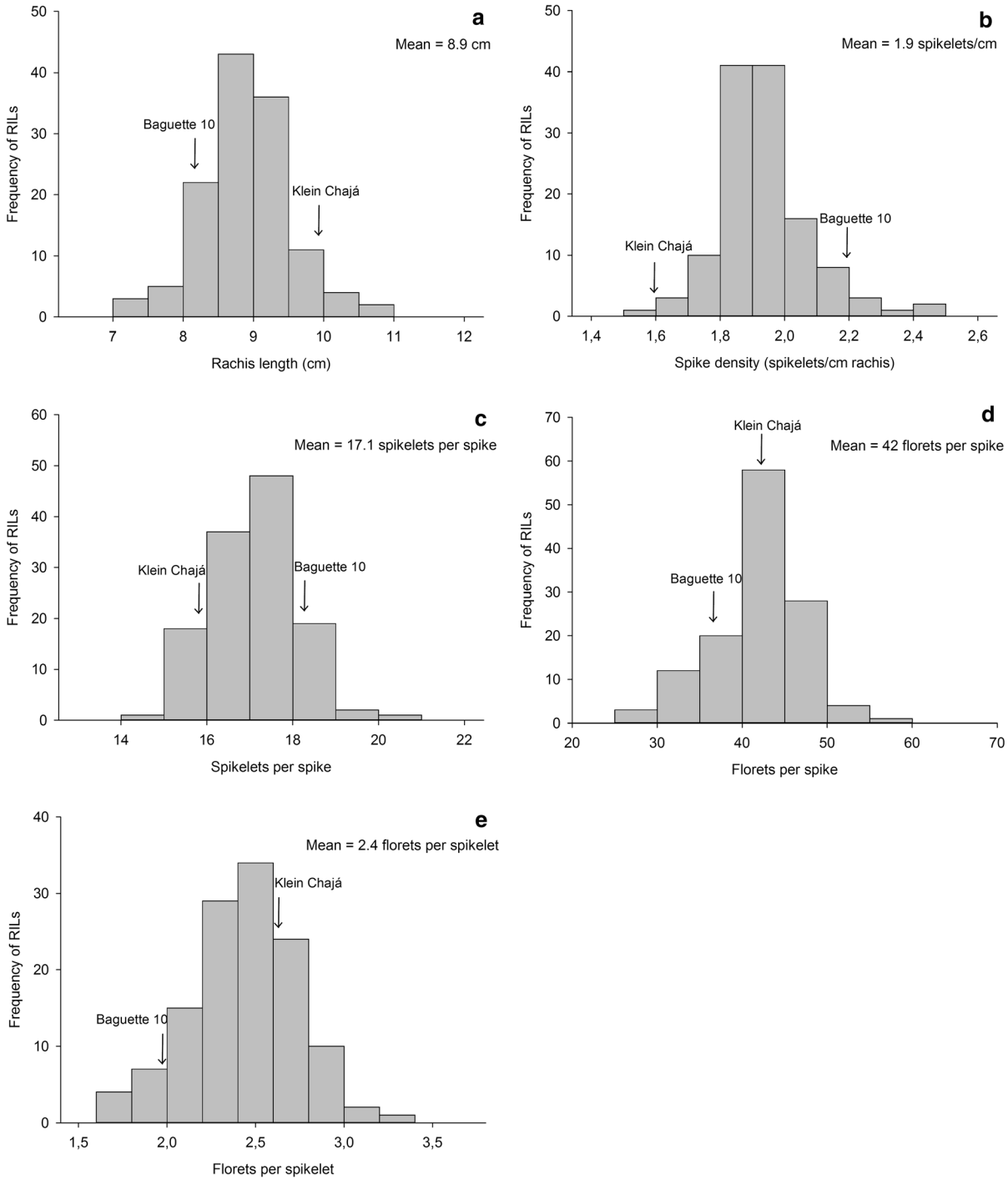

Fig. 2 Frequency distribution of **a** Rachis length, **b** Spike density, **c** Spikelets per spike, **d** Florets per spike and **e** Florets per spikelet-average of four field experiments carried out in Balcarce, Argentina- in the Baguette 10 × Klein Chajá RIL population (N = 126). The values of the parental cultivars are indicated with arrows

significantly correlated with florets per spike in the experiment 1 in 2016 and experiment 1 in 2017 and with florets per spikelet in the experiment 1 and 2 in 2016.

Table 5 Variance component estimates (genotypic, genotype x environment interaction and residual variances) and broad-sense heritability (H^2) for the analyzed traits

Trait	Genotypic variance (σ_g^2)	Genotype x environment interaction variance (σ_{ge}^2)	Residual variance (σ_{res}^2)	Broad-sense heritability (H^2)
FHB severity [a]	0.09	0.04	0.24	0.69
AUDPC[a]	0.08	0.03	0.32	0.63
Rachis length[a]	0.33	0.05	0.41	0.84
Spike density[a]	0.02	0.001	0.01	0.92
Spikelets per spike[a]	0.81	0.16	0.91	0.84
Florets per spike[b]	19.9	5.28	31.40	0.74
Florets per spikelet[a]	0.07	0.02	0.09	0.81

[a]Data of 126 RILs, 4 environments (2 years × 2 experiments), two blocks within experiment

[b]Data of 126 RILs, 3 environments (2 experiments in 2016 and 1 experiment in 2017)

Table 6 Genetic correlation coefficients between FHB severity, AUDPC, rachis length, spike density, spikelets per spike, florets per spike and florets per spikelet in the Baguette 10 × Klein Chajá RIL population (N = 126), evaluated in field experiments in 2016 and 2017 at Balcarce, Argentina

	FHB severity	AUDPC	Rachis length	Spike density	Spikelets per spike	Florets per spike
AUDPC	0.94***					
Rachis length	0.12	0.10				
Spike density	− 0.13	− 0.13	− 0.61***			
Spikelets per spike	0.01	0.03	0.58***	0.27**		
Florets per spike	0.38***	0.31**	0.29***	− 0.08	0.27*	
Florets per spikelet	0.28**	0.27*	− 0.01	− 0.19*	− 0.19*	0.59***

*Correlation significant at the 0.05 level;

**Correlation significant at the 0.01 level;

***Correlation significant at the 0.001 level

Discussion

Passive resistance mechanisms act through expression of morphological and developmental features which alter conditions for initial infection and allow the plant to avoid contact with the pathogen or prevent the disease development once the contact has taken place (Mesterhazy 1995). To date, most studies dealing with the association between plant morphological/developmental traits and FHB resistance have focused on Type I resistance (Pugh et al. 1933; Gervais et al. 2003; Zhang et al. 2018; Steiner et al. 2004). However, little has been investigated about the effect of passive mechanisms on Type II resistance.

In a recent work, we found that the anthesis date is correlated with Type II resistance and that the prevailing environmental conditions during this stage affect the F. *graminearum* spread within the spike (Franco et al. 2021). Thus, considering this trait allows a more precise and objective characterization of the level of FHB Type II resistance. In the same way, gaining insight into the associations between Type II resistance to FHB and the architecture of the spike may lead to a better understanding of the implicated mechanisms in the resistance, and an increase of the resistance to FHB through the introgression of such desirable traits.

In this study, FHB Type II resistance was evaluated in a RIL population of bread wheat, developed from the cross between two cultivars with moderate level of resistance to FHB and contrasting spike architecture, after implementing a precise point inoculation technique at anthesis under field conditions. Also, several spike architectural traits which might alter fungal colonization of the spike were evaluated. The RIL population used in this study showed large genetic variation for both FHB severity and AUDPC across all experiments. A continuous distribution with transgressive segregation towards lower and higher values for the two variables was observed. The population also segregated for the spike architecture traits and showed a continuous normal frequency distribution with transgressive variation for all the evaluated attributes. This supports the quantitative inheritance nature of all the studied attributes. The high broad-sense heritability values obtained for FHB severity ($H^2 = 0.69$), AUDPC ($H^2 = 0.63$) and all the spike traits (H^2 between 0.74 and 0.92) indicate that a large proportion of the variation among the evaluated lines was due to genetic effects, particularly considering that the experiments performed in this study spanned a wide array of environmental conditions.

In relation to the associations evaluated here, the strong and significant level of genetic correlation between the two variables associated with the disease, FHB severity and AUDPC ($r = 0.94$, $P < 0.001$), coincides with that documented by other authors (Malbrán et al. 2012; Mourelos et al. 2014) and strengthen the idea that both traits are under the same genetic control (Groth et al. 1999).

In this study, FHB severity and AUDPC were moderately, positively, and significantly associated with both the total number of florets per spike and the number of florets per spikelet. Likewise, the correlation observed between the number of florets per spike and per spikelet was high and significant. To the best of our knowledge, this is the first report in which these correlations are evidenced in bread wheat. The associations found here could be explained by the fact that these spike traits can provide a microclimate of high humidity in the spike, favoring the fungal spread and sporulation within the spike, increasing the level of disease.

Spike density is a function of two traits -rachis length and number of spikelets per spike- (Faris et al. 2014). In the present study, spike density was not correlated with severity or with AUDPC. Some studies have found that genotypes with compact spikes usually exhibit higher disease levels. For instance, Buerstmayr et al. (2011) and Steiner et al. (2004) found that laxer spikes were significantly associated with an increase in FHB Type II resistance, arguing that genotypes with more compact spikes have a faster disease dissemination than do genotypes with lax spikes due to the fact that compactness may facilitate pathogen spread to adjacent nodes more easily or because of the microclimate conditions that are generated in the more compact spikes. However, there are some reports indicating variable associations between these attributes depending on the population studied (Buerstmayr et al. 2012).

No association between rachis length and FHB Type II resistance was detected in the present study. This is consistent with results reported by Somers et al. (2003) who, studying different associations between FHB and morphological and phenological variables under controlled conditions, found no correlation between spike length and FHB Type II resistance. However, Buerstmayr et al. (2011) reported a negative and significant association ($r = -0.27$) between spike length and AUDPC.

The number of spikelets on the spike was not correlated either with FHB severity or AUDPC. These results are in agreement with that reported by Buerstmayr et al. (2011), who, studying the association between different morphological characters and type II resistance to FHB in a population of *Triticum macha* Dek.et Men. x *T. aestivum* L., also found no significant association between the number of spikelets and the progression of the disease. Similarly, Liu et al. (2007) reported lack of correlation between the number of spikelets per spike and type II resistance to FHB.

It is important to highlight that while correlation coefficients between variables reported in the bibliography are generally estimated from the means of the variables studied (phenotypic correlations), in this study we calculated both phenotypic correlations for each experiment and genetic correlations (using the BLUPs for each variable). An important property of BLUPs is the shrinkage towards the mean, which is often a desirable statistical property as it increases accuracy, while maximizing the correlation of true genotype values and predicted genotype values (Piepho et al. 2008).

In summary, the results shown here suggest that the number of florets per spike and the number of florets per spikelet should be considered in FHB resistance breeding efforts, because selection of lines with higher number of florets could lead to a correlated selection response towards increased FHB levels under field conditions.

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Author contributions MFF, ACP, GAL and IM designed the study. Field experiments were designed by MGC, ACP and MFF. Inoculum was prepared by IM and field experiments were performed by MFF, JSP, MPA, ACP and NEM. Statistical analyses were performed by MFF with the contribution of MGC; production of figures and tables was performed by MFF with the contribution of ACP and MGC. The manuscript was written by MFF with the contribution of ACP, MGC and GL. All authors read and approved the final manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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