Increasing Grain Yield in Bread Wheat (*Triticum aestivum* L.) by Selection for High Spike Fertility Index

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ABSTRACT

Spike fertility index (SF) has been proposed as a promising selection criterion for increasing grain yield (GY) in bread wheat. Here, changes in GY and related traits after simulated selection (10% intensity) for high SF or high GY *per se* were assessed in two RIL populations: (I) Avalon/Glupro and (II) Baguette 10/Klein Chajá, and in (III) advanced lines from a breeding program. Grain yield, SF, grain number per unit area (GN), grain weight (GW), test weight (TW) and grain protein content (GPC) were determined. Regardless of the environmental conditions, simulated selection for high SF always resulted in GN increases (between 1.6 and 27.4%). Average GY increase observed after selection for high SF (11.5%; N=10; SEM=5.8) did not differ (p=0.92) from the average GY increase observed after

selection for GY *per se* (11.8%; N=10; SEM=4.9). Grain weight, GPC and TW tended to decrease with selection for high SF; however, these trade-offs might be avoided by concurrent selection. Our findings validate the use of SF as a selection criterion for increasing grain yield in bread wheat.

KEYWORDS: grain yield, grain number; fruiting efficiency; breeding; selection; genetic progress

INTRODUCTION

Grain yield is one of the key targets of bread wheat (*Triticum aestivum* L.) breeding programs worldwide. Under a scenario of increased global demand for grains and limited possibilities of expanding cropping areas, breeding efforts must focus on raising annual yield increase rates (CIMMYT, 2019). One way to achieve this is by using yield-related, high-throughput phenotypic variables as selection criteria.

Grain yield in wheat is determined mainly by the number of grains per unit area (GN) and, to a lesser extent, by grain weight (GW). Therefore, variations in GN best explain yield variation (Abbate et al., 1998; Borrás et al., 2004; Elía et al., 2016; Ferrante et al., 2017; Lynch et al., 2017; Shearman et al., 2005; Slafer et al., 1990). However, this is a difficult trait to select for in early generations of a breeding program, in which not enough seed is available. Fischer (1984) proposed that, under non-limiting growing conditions (i.e., without water or nutrient limitations and in absence of pests and diseases), GN is determined by the product of (i) the duration of rapid spike growth period, (ii) crop growth rate during the spike growth period, (iii) dry weight partitioning to spikes during the spike growth period, and (iv) the number of grains per unit of spike dry weight, i.e., a “spike fertility” index (SF) (Abbate et al., 1998) or “fruiting efficiency” (Ferrante et al., 2012). The spike dry weight at anthesis is a complex trait to measure; thus, chaff dry weight at maturity has been proposed as its surrogate (Abbate et al., 2013). Then, the spike fertility index at maturity [also termed fruiting efficiency at

57 maturity; Fischer and Rebetzke (2018)] is calculated as the number of grains produced per
58 unit of chaff dry weight.

59 There is extensive evidence in the literature that GN is tightly linked to SF (Abbate et al., 1998;
60 Acreche et al., 2008; Alonso et al., 2018b; Foulkes et al., 2015; Shearman et al., 2005; Terrile et
61 al., 2017). In the reference method (9), SF determination is carried out at anthesis, but this is
62 destructive and very time consuming. A high throughput, non-destructive method for assessing
63 the trait in small samples at maturity has then been proposed (Abbate et al., 2013). In addition,
64 SF at maturity has been shown to have high heritability, substantial genetic variability and low
65 genetic by environment interaction (Alonso et al., 2018b; Martino et al., 2015; Mirabella et al.,
66 2016). Because of all these features, SF has been regarded as a promising selection criterion for
67 increasing GN (and yield) in breeding programs (Abbate et al., 2013; Elía et al., 2016; Fischer,
68 2007, 2011; Fischer & Rebetzke, 2018; Foulkes et al., 2010; García et al., 2014; González et al.,
69 2011; Lázaro & Abbate, 2012; Martino et al., 2015; Mirabella et al., 2016; Slafer et al., 2015;
70 Terrile et al., 2017). As a matter of fact, Alonso et al. (2018b) have shown that selection for
71 high SF, either solely or in combination with selection for high grain yield, effectively
72 increases yield, resulting in higher and more stable yields than if selecting for high yield *per*
73 *se*. These encouraging results, obtained with a single RIL population under non-limiting
74 environmental conditions, need further validation before such selection strategies can be
75 implemented in a breeding program. Also, the assessment of possible trade-offs that could
76 arise as a byproduct of these schemes should be carried out. Therefore, the aim of this work
77 was to determine the effectiveness of selection for high SF in raising grain yield and its effect
78 on other variables, by carrying out simulated selection and analyzing the response to this
79 selection in a combination of diverse genetic materials (RILs and advanced lines) and an array
80 of environmental conditions.

81 MATERIALS AND METHODS

82 *Field experiments and plant material*

83 Field experiments were carried out at the Balcarce (37°45' S; 58°18' W; 130 m a.s.l.) and
84 Marcos Juárez (32°43'S; 62°06'W; 112 m a.s.l.) Agricultural Experimental Stations of the
85 Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina. Characteristics of
86 experiments are summarized in Table 1.

87 Experiments were conducted under conventional tillage with no nutrient limitations; weeds,
88 pests and fungal diseases were chemically controlled. As described in Table 1, experiments
89 were grouped into Sets I, II and III. In Set I, a biparental population of 90 recombinant inbred
90 lines (RIL) derived from the cross between 'Avalon' and 'Glupro' was evaluated in
91 experiments BAG15 and BAG16. 'Avalon' is a cultivar from the UK with high protein
92 content, whereas 'Glupro' is a cultivar from the USA with the introgression of the *GPC-BI*
93 gene (Uauy et al., 2006) for high protein content. In Set II, a biparental population of 146 RIL
94 derived from the cross between 'Baguette 10' and 'Klein Chajá' (Alonso et al., 2018b;
95 Martino et al., 2015; Mirabella et al., 2016) was evaluated in experiments BFE13, BFE14,
96 BFE15 and MJFE17. Both parents are Argentinean spring bread wheat cultivars of good
97 agronomic performance, with contrasting SF and yield components. In Set III, breeding lines
98 derived from crosses between cultivars of diverse origin (CIMMYT, Europe, Argentina),
99 advanced through a modified bulk selection scheme and selected for grain yield and
100 agronomic performance in a wheat breeding program (INTA Balcarce Experimental Station),
101 were evaluated in experiments BLE16 and BLE17 (28 lines), and BLP17 and BLE18 (36
102 lines).

103 *Data collection*

104 Grain yield was determined by mechanical harvest of the whole plot (when three-row plots
105 were used) or its five central rows (when seven-row plots were used; Table 1). In the former
106 case, the plot (and harvested) area was 3.5 m², with an inter-plot distance of 0.3 m; in the
107 latter case, the harvested area was 5 m². Grain moisture was obtained from a grain subsample
108 of each plot to calculate dry grain yield (GY; g/m²). Grain weight (GW; mg) was determined

109 by counting a 1000-grain sample with an electronic counter, weighing it and dividing total
110 weight by 1000. Grain number per square meter was then calculated as the quotient between
111 GY and GW $\times$ 1000.

112 Spike fertility index was determined at maturity on a sample of 15-20 spikes drawn at random
113 from the harvestable area of each plot prior to mechanical harvest. Spikes were cut at the
114 lowest spikelet level, counted, weighed, and threshed. Spike chaff dry weight (g) was
115 calculated as the difference between total spike dry weight (i.e. before threshing) and total
116 grain weight. Grains were counted using an electronic counter. Then, SF (grains/g) was
117 calculated as the quotient between the number of grains and spike chaff dry weight per sample
118 (Abbate et al., 2013).

119 Test weight (TW) was measured using a Schopper chondrometer on a 250 g grain sample and
120 expressed as kg/hl. Grain protein content (GPC; %) was determined indirectly by NIRS using
121 a TEC NIR 256 Inlab NIR in 100 g grain samples, previously cleaned and dried at 60°C to
122 constant weight.

123 *Trait responses after simulated selection*

124 Data from three sets of experiments (Table 1) were used to evaluate responses in GY and
125 other traits after simulated selection for high SF. At each set, the 10% top lines with the
126 highest SF values were selected, based on SF data generated in experiments BAG16 (Set I),
127 BFE13, BFE14, BFE15 and MJFE17 (Set II) and BLE16, BLE17, BLP17 and BLE18 (Set
128 III). This means that, at each set, SF was assessed in only *some* experiments (Table 1).
129 However, the performance of those selected lines and the simulated response after selection
130 (i.e., their differential performance compared with that of the population mean) was evaluated
131 based on data generated in *all* experiments within each set. This also included the experiments
132 in which SF itself was determined, as an independent spike sample was drawn before harvest
133 for SF determination, whereas the response variables were evaluated at harvest. In order to
134 compare the effects of selection for high SF with those for high GY *per se*, a similar approach
135 was followed by carrying out simulated selection for high GY *per se* (10% intensity) and
136 assessing trait responses in all experiments within each set. However, as there is an obvious

137 bias in assessing GY response after selection for GY *per se* in the same experiment, these
138 cases were excluded from the analysis.

139 Responses after selection for SF or GY *per se* were calculated for GY and other traits as the
140 difference between the mean values of the ‘selected group’ and the general mean of the
141 population at each response experiment. They were then expressed as percentage of the
142 population mean. For each set of experiments, an overall response mean was also calculated.

143 *Statistical analysis*

144 Grain yield was analyzed with a linear fixed effects model including experiments and
145 genotypes and genotype by experiment interaction effects nested in sets of experiments.
146 Welch’s t-test was used to compare trait responses (in %) after the two simulated selection
147 strategies (i.e., for high SF vs. for high GY *per se*). For this comparison, only those cases that
148 had data for side by side comparisons were used.

149 **RESULTS**

150 Experiments included in Sets I to III were carried out under different environmental
151 conditions (i.e. different locations, years, growing conditions, plot size, etc.; Table 1). In
152 addition, conspicuous environmental differences between crop seasons due to temperature,
153 radiation and rainfall were observed (Fig. 1). This resulted in significant differences in
154 average grain yield between experiments ($p < 0.0001$); GY differences between genotypes
155 ($p < 0.0001$) were also evident across experiments (Tables 2 and 3). As a consequence of the
156 observed environmental differences and the genetic constitution of the populations under
157 study, conspicuous phenotypic variation was also observed in the remaining traits (Table 3).
158 When analyzed by set, very different population means for all the variables were observed
159 across experiments in each of the three sets, reflecting environmental variation between years
160 and/or locations (Table 3). A summary of results of simulated selection for high SF carried
161 out in each of the three sets is presented in Table 4 (for responses in GY and GN) and Table
162 S1 (for responses in GW, TW and GPC). Selection of the top 10% lines with the highest SF in
163 experiments BAG16 (Set I), BFE13, BFE14, BFE15 and MJFE17 (Set II) and BLE16 and
164 BLE17, BLP17 and BLE18 separately (Set III) always increased GN, and also increased GY

except for a few cases of slight decrease in Set III. For each set of experiments, the overall mean response (%) in GY and GN after simulated selection for SF is shown in Table 4. Only for set III the overall mean was negative for GY (-2.0%), while it was positive for GN (8.6%). Across the three set of experiments, GY and GN respectively increased by 5.6 and 13.4% as a result of simulated selection for high SF (Table 4), as compared with the population means. On the other hand, selection responses after simulated selection by high GY *per se* ranged between -9.3% and 46.0% in GY and between -6.8% and 51.2% in GN, considering all experiments. As occurred with simulated selection for high SF, responses after selection for high GY *per se* were positive for all cases except for a few in set III (Table 4).

Both simulated selection strategies (i.e., by high SF and by high GY *per se*) were compared in terms of the average response attained in GN and GY (Table 5). As a result, both yielded substantially similar average responses ($p>0.05$) in GN and GY, although the variance was several fold higher when using GY *per se* as the selection criterion for responses in GN.

As for other yield-related traits, simulated selection for high SF consistently lowered GW (overall decrease of 5.3%; Table S1). As for TW, response after selection for high SF was slightly positive in Set I and slightly negative in Set II, but not significant. Grain protein content, only determined in Set I, was decreased by 6.9% as a result of selection for high SF. On the other hand, average responses after simulated selection for high GY *per se* were positive (Table S1).

DISCUSSION

Grain yield increase is of paramount importance in every bread wheat breeding program. However, annual increase rates have been steadily declining and would hardly meet the present and future demand. In a recent review, Fischer and Rebetzke (2018) proposed focusing on a number of breeding targets for accelerating grain yield genetic gains, among which they included fruiting efficiency/spike fertility index (SF). When evaluating several selection strategies under different environmental conditions, Alonso et al. (2018b) found that simulated selection for high SF, either solely or along with selection for high grain yield, always resulted in higher and more stable yields than if selecting for high yield alone. In the

193 present study, we show that, regardless of the genetic material or the environmental
194 conditions, selection for high SF alone virtually always increases grain yield in bread wheat.

195 Independently of the set of experiments, selection for high SF always resulted in GN increases
196 (between 1.6 and 27.4%) and in GY increases in most cases, except for a few in Set III (Table
197 4). These latter cases, however, occurred in a population of advanced breeding lines, which
198 underwent selection in previous generations as opposed to the RIL populations used in sets I
199 and II. Also, this was the population with the least number of individuals (N=28/36; Table 1).
200 The top 10% lines with the highest SF values amounted to only 3-4, which could have
201 increased sampling error. Nevertheless, overall results suggest that one can select lines with
202 high SF on the basis of data collected from a very small spike sample and sometimes in a
203 different environment than the one in which yield is determined (even including lower yield
204 potential locations as it is Marcos Juárez as compared with Balcarce, in this study) (Menéndez
205 & Satorre, 2007) and expect, at the minimum, a meager increase in yield and/or GN.

206 Regarding the substantial variation in the magnitude of the responses to selection observed
207 across sets and experiments within sets (Table 3), several possible underlying causes could be
208 considered. For instance, variations might have occurred due to unmeasured sampling errors,
209 sample size, and different genetic and genetic by environment interaction effects of the traits
210 under evaluation. In this sense, additional data sets comprising a wider spectrum of
211 germplasm and environments/years of evaluation should be incorporated in the analysis in
212 order to reduce these errors. On the other hand, the heritability of each trait as well as the
213 degree of association between the trait used for selection and the response trait might have
214 been other sources of variation in the observed responses to selection. For instance, in the case
215 of the RIL population of Set II, the heritability values of the traits ($h^2_{SF}=0.84$; $h^2_{GY}=0.28$;
216 $h^2_{GN}=0.35$; Alonso et al. 2018b) and the correlations between the traits, both genetic
217 ($r_{SF-GY}=0.36$, $r_{SF-GN}=0.55$; Alonso et al. 2018b) and phenotypic ($r_{SF-GY}=0.29$, $r_{SF-GN}=0.44$;
218 Table S2), provide an idea of how these relationships could affect the responses. Spike
219 fertility index, used here as a selection criterion, has high heritability with low environmental
220 influence, which makes it fairly stable across successive generations. The trait response after
221 such selection is expected to be positively correlated with the degree of association between
222 the selection and evaluation traits; indeed, in this study, the response was better in GN than in
223 GY. In turn, the year-to-year variations in the response of these traits are in line with their

224 heritabilities (low to moderate) and with their great sensitivity to environmental effects.
225 Therefore, selecting for high SF would give stability to the response in GN and GY, whereas
226 these latter traits' low/moderate values of both heritability and genetic/phenotypic correlation
227 with SF would increase variability in such response.

228 Some trade-offs were observed: in general, GW, GPC and TW tended to decrease with
229 selection for high SF. Nevertheless, genotypes with all high SF, GY, GW, GPC and TW were
230 detected (data not shown); therefore, these trade-offs could be avoided by concurrent selection
231 for all traits. Furthermore, all three populations used in the present study had a relatively low
232 number of individuals if considering the scale of an actual breeding program. Provided that
233 the prerequisite of existence of genetic variability for the traits is met (as it was the case in the
234 present study; Table 3), increasing population numbers is likely to yield even better results
235 when selecting for high SF. This way, while increasing GY and GN, one could concurrently
236 maintain desirable GW, TW and GPC values. Phenotypic correlations between variables were
237 generally positive (Table S2) except for the ones between GN and GW ($r=-0.18$) and SF and
238 GW ($r=-0.2$). These low values, however, confirm the idea that the negative relationship
239 between these variables is not complete.

240 Independently of the genetic constitution of the breeding material and/or the environmental
241 conditions, selections for high SF, as determined in a small spike sample, appear to result in
242 consistently increased grain number per unit area (and yield) in bread wheat. As virtually
243 similar average responses to selection in GN and GY were attained when using SF or GY *per*
244 *se* as selection criteria (Table 5), selection for SF alone has the potential of increasing the
245 throughput and efficiency of the breeding process, while lowering costs. Spike fertility index
246 determination entails drawing samples in the field, weighing and threshing (which, although
247 cheaper, might be more labor-intensive and time-consuming than GY determination), but it is
248 a process amenable to automation. This could translate into a massive screening of lines for
249 SF in small plots and subsequent multi-environment yield trials that only include a subset of
250 the lines initially screened. This turns SF into a very promising selection target in breeding
251 programs aimed at increasing grain yield. Nevertheless, the effectiveness of carrying out
252 selection for high SF in spaced plants, as it would be the case of early generations of a
253 breeding program, remains yet to be established.

254 DATA AVAILABILITY

255 The dataset of the study is available from the authors upon reasonable request.

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Tables

Table 1. Description of field experiments carried out in this study, as organized in Sets of experiments (exps.) I to III. Genetic material included in each set, N (number of individuals), exp. name, location, year, exp. design (RCBD=randomized complete block design; ARCBD=augmented randomized complete block design), No. reps. (number of replications), growing conditions, plot dimensions and data collected (SF=spike fertility index; GY=grain yield; GN=grain number per unit area; GW=grain weight; TW=test weight; GPC=grain protein content). Data from exps. BFE13, BFE14 and BFE15 have been partially published by Alonso et al. (2018a;b).

Set of exps.	Genetic material	N	Exp. name	Location	Year	Sowing date	Exp. design	No. reps.	Growing conditions	Plot dimensions				Data collected					
										Area (m ²)	No. rows	Row length (m)	Inter-row spacing (m)	SF	GY	GN	GW	TW	GPC
I	RIL population	90	BAG15	Balcarce	2015	June 19 th	RCBD	2	Rainfed	3.5	3	5	0.2		X	X	X	X	X
	(Avalon/Glupro)	90	BAG16	Balcarce	2016	July 14 th	RCBD	2	Rainfed	3.5	3	5	0.2	X	X	X	X	X	X
II	RIL population	146	BFE13	Balcarce	2013	June 23 rd	RCBD	2	Irrigated	3.5	3	5	0.2	X	X	X	X	X	
	(Baguette 10/ Klein	146	BFE14	Balcarce	2014	July 25 th	RCBD	2	Irrigated	7	7	5	0.2	X	X	X	X	X	
	Chajá)	146	BFE15	Balcarce	2015	July 15 th	RCBD	2	Irrigated	7	7	5	0.2	X	X	X	X	X	
		146	MJFE17	Marcos Juárez	2017	June 15 th	ARCBD	1	Rainfed	0.9	3	1	0.3	X					
III	Advanced	28	BLE16	Balcarce	2016	August 8 th	ARCBD	1	Rainfed	7	7	5	0.2	X	X	X			
	breeding lines	28	BLE17	Balcarce	2017	June 14 th	ARCBD	1	Irrigated	7	7	5	0.2	X	X	X			
		36	BLP17	Balcarce	2017	August 6 th	ARCBD	1	Rainfed	7	7	5	0.2	X	X	X	X		
		36	BLE18	Balcarce	2018	June 11 th	RCBD	2	Irrigated	7	7	5	0.2		X				

356 *Table 2. Analysis of variance for grain yield (g/m^2) across nine experiments. All experiments*
 357 *from Sets I to III (Table 1) were included in the analysis except for MJFE17, which plot size*
 358 *(0.9 m^2) was deemed too small for accurate grain yield determination.*

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Source of variation	DF	Mean Square	F	P-value
Experiment	8	5155433	530.03	<0.0001
Genotype (set)	236	33324	3.43	<0.0001
Genotype*experiment (set)	499	19126	1.97	<0.0001
Error	693	9727		

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Table 3. Mean ($\bar{x}$) and standard deviation (S) of spike fertility index (SF, grains/g), grain yield (GY, g/m²), grain number per unit area (GN, grains/m²), grain weight (GW, mg), test weight (TW, kg/hl) and grain protein content (GPC, %) of I: Avalon/Glupro RIL population (N=90, experiments BAG15 and BAG16); II: Baguette 10/Klein Chajá RIL population (N=146, experiments BFE13, BFE14, BFE15 and MJFE17) and III: advanced breeding lines (N=28, experiments BLE16 and BLE17; N=36, experiments BLP17 and BLE18).

Set of Experiments	Experiment	SF		GY		GN		GW		TW		GPC	
		$\bar{x}$	S	$\bar{x}$	S	$\bar{x}$	S	$\bar{x}$	S	$\bar{x}$	S	$\bar{x}$	S
I	BAG15			515.9	125.0	12990	3254	39.9	3.2	79.9	2.4	16.0	1.2
	BAG16	70.5	11.2	314.1	81.9	9788	2554	32.3	3.4	77.1	2.1	14.5	0.7
II	BFE13	98.3	9.2	717.0	185.9	21960	6283	33.0	4.2	77.8	11.5		
	BFE14	91.9	11.7	367.1	89.4	9964	2687	37.4	4.6	73.8	2.2		
	BFE15	89.5	9.5	749.1	123.5	17824	3457	42.5	3.8	80.3	1.8		
	MJFE17	101.7	12.1	432.5	134.9			32.4	4.7				
III	BLE16	81.5	10.2	498.9	108.4	14755	3004	34.2	5.3				
	BLE17	83.9	9.4	448.2	84.9	11302	2139	39.7	4.9				
	BLP17	86.6	11.8	383.9	46.0	11015	1770	35.3	4.1				
	BLE18			618.1	193.6								

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Table 4. Response after simulated selection for high spike fertility index (SF) and grain yield (GY) per se (i.e., selection of the top 10% lines with the highest SF or GY values at each of three sets of experiments (exps.) carried out between 2013 and 2018 in Balcarce and Marcos Juárez, Argentina) in grain yield (GY; g/m²) and grain number per unit area (GN; grains/m²). Grain yield responses after selection for GY per se in the same experiment are not shown, as they were not included in the analysis (see Materials and Methods).

Set of Exps.	Selection Exp.	Response Exp.		Selection for high SF		Selection for high GY		
				Response	Response	Response	Response	
				in GY	in GN	in GY	in GN	
I	BAG16	BAG16	Population mean (N=90)	313.7	9760		9788	
			Mean of 10% lines (N=9) selected for SF or GY in exp. BAG16	396.2	12431		14496	
			Response after simulated selection (%)	26.3	27.4		48.1	
		BAG15	Population mean (N=90)	515.5	12971	515.5	12971	
			Mean of 10% lines (N=9) selected for SF or GY in exp. BAG16	599.5	15176	743.7	17752	
			Response after simulated selection (%)	16.3	17.0	44.3	36.9	
		BAG15	Population mean (N=90)				12966	
			Mean of 10% lines (N=9) selected for GY in exp. BAG15				19598	
			Response after simulated selection (%)				51.2	
		BAG16	Population mean (N=90)			313.7	9788	
			Mean of 10% lines (N=9) selected for GY in exp. BAG15			458.2	14257	
			Response after simulated selection (%)			46.0	45.7	
	Mean (±SD) response after simulated selection in set I (%)				21.3±7.1	22.2±7.4	45.2±1.2	48.5±7.2
	II	MJFE17	BFE13	Population mean (N=146)	719.5	22092		
				Mean of 10% lines (N=15) selected for SF in exp. MJFE17	721.0	24313		
Response after simulated selection (%)				0.2	10.1			
		BFE14	Population mean (N=146)	367.4	9978			
			Mean of 10% lines (N=15) selected for SF in exp. MJFE17	419.0	12592			
			Response after simulated selection (%)	14.0	26.2			
		BFE15	Population mean (N=146)	749.1	17763			
			Mean of 10% lines (N=15) selected for SF in exp. MJFE17	752.0	19387			
			Response after simulated selection (%)	0.4	9.1			
		BFE13	Population mean (N=146)	719.5	22092		22092	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE13	772.4	25615.5		30890	
			Response after simulated selection (%)	7.3	16.0		39.4	
		BFE14	Population mean (N=146)	367.4	9978	367.4	9978	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE13	397.2	10811	444	11714	
			Response after simulated selection (%)	8.1	8.3	20.9	17.4	
		BFE15	Population mean (N=146)	749.1	17763	749.1	17763	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE13	790.8	19626	772.5	18522	
			Response after simulated selection (%)	5.6	10.5	3.1	4.3	

Set of exps.	Selection exp.	Response exp.		Selection for high SF		Selection for high GY		
				Response	Response	Response	Response	
				in GY	in GN	in GY	in GN	
II (cont)	BFE14	BFE13	Population mean (N=146)	719.5	22092	719.5	22092	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE14	763.0	24166	903	29246	
			Response after simulated selection (%)	6.0	9.1	25.5	6.9	
		BFE14	Population mean (N=146)	367.4	9978		9978	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE14	430.5	12259		13918	
			Response after simulated selection (%)	17.2	22.9		39.5	
		BFE15	Population mean (N=146)	749.1	17763	749.1	17763	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE14	778.7	19310	759.5	18746	
			Response after simulated selection (%)	4.0	8.7	1.4	5.5	
	BFE15	BFE13	Population mean (N=146)	719.5	22092	719.5	22092	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE15	745.8	24528	771.8	23610	
			Response after simulated selection (%)	3.6	11.0	7.2	6.9	
		BFE14	Population mean (N=146)	367.4	9978	367.4	9978	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE15	408.5	12294	393	10767	
			Response after simulated selection (%)	11.2	23.2	7.0	7.9	
		BFE15	Population mean (N=146)	749.1	17763		17763	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE15	783.4	20006		20816	
			Response after simulated selection (%)	4.6	12.6		17.2	
	Mean (±SD) response after simulated selection in set II (%)			6.9±5.1	14.0±6.5	10.9±9.9	16.1±14.1	
	III	BLE16	BLE16	Population mean (N=28)	498.9	14755		14755
				Mean of 10% lines (N=3) selected for SF or GY in exp. BLE16	457.1	15962		17905
Response after simulated selection (%)				-8.4	8.2		21.3	
BLE17			Population mean (N=28)	449.6	11413	449.6	11413	
			Mean of 10% lines (N=3) selected for SF or GY in exp. BLE16	445.1	12122	407.8	10638	
			Response after simulated selection (%)	-0.1	6.2	-9.3	-6.8	
BLE17			Population mean (N=28)	449.6	11413		11413	
			Mean of 10% lines (N=3) selected for SF or GY in exp. BLE17	424.6	11600		13563	
			Response after simulated selection (%)	-5.5	1.6		18.8	
BLE16		Population mean (N=28)	498.9	14755	498.9	14755		
		Mean of 10% lines (N=3) selected for SF or GY in exp. BLE17	565.8	17157	514	15455		
		Response after simulated selection (%)	13.4	16.3	3.0	4.7		
BLP17		BLP17	Population mean (N=36)	383.9	11015		11015	
			Mean of 10% lines (N=4) selected for SF or GY in exp. BLP17	367.4	12195		12793	
			Response after simulated selection (%)	-4.3	10.7		16.1	
		BLE18	Population mean (N=36)	609.6		609.6		
			Mean of 10% lines (N=4) selected for SF or GY in exp. BLP17	566.6		639.1		
			Response after simulated selection (%)	-7		4.8		
BLP18		BLP17	Population mean (N=36)			383.9	11015	
			Mean of 10% lines (N=4) selected for GY in exp. BLP18			389.4	11165	
			Response after simulated selection (%)			1.4	1.4	
Mean (±SD) response after simulated selection in set III (%)			-2.0±8.1	8.6±5.4	0.0±6.3	9.3±11.2		
Overall mean response after simulated selection (%)			5.6	13.4	12.9	20.1		

380 *Table 5. T test for comparison of the average response in grain number per unit area (GN;*
 381 *grains/m²) and in grain yield (GY; g/m²) attained after selection for high spike fertility index*
 382 *(SF) vs. for high GY per se (data extracted from Table 4; only those cases with available data*
 383 *for side by side comparisons were included).*

Variable	Selection criterion	N	Trait response after selection		DF	T	P-value
			(%)				
			Mean	SEM			
GN	SF	16	13.1	1.73	21	-1.09	0.29
	GY <i>per se</i>	16	17.8	3.9			
GY	SF	10	11.5	5.8	17	0.09	0.92
	GY <i>per se</i>	10	11.8	4.9			

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Figures

Figure 1

Figure legends

Fig. 1. Mean temperature (left) and accumulated rainfall plus irrigation (right) during the June-December period (wheat growing season) at Balcarce (Bce) in 2013 to 2018 and in Marcos Juárez (MJ) in 2017. Stripped bars show accumulated water by irrigation.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

ACP, MPA and NEM designed the study; ACP, MPA, NEM, JSP, MFF, and ML performed experiments at Balcarce and LSV performed the experiment at Marcos Juárez; MPA, NEM and JSP processed SF samples; NEM, MPA and ACP analyzed the data; MFF prepared all figures of the manuscript; ACP wrote the paper with input from all authors.

Table S1. Response to simulated selection for high spike fertility (SF) or grain yield (GY) per se (i.e., selection of the top 10% lines with the highest SF or GY values at each of three sets of experiments (exps.) carried out between 2013 and 2018 in Balcarce and Marcos Juárez, Argentina) in spike fertility index (SF; grains/g), grain weight (GW; mg), test weight (TW; kg/hl) and grain protein content (GPC; %).

Set of exps.	Selection exp	Response exp.	Response to selection for high SF				Response to selection for high GY				
			SF	GW	TW	GPC	SF	GW	TW	GPC	
I	BAG15	BAG15	Population mean (N=90)					39.9	79.9	81.4	
			Mean of 10% lines (N=9) selected for GY in exp.					40.4	83.5	111.1	
		BAG15									
		Response after simulated selection (%)						4.6	4.6	36.5	
	BAG16	BAG16	Population mean (N=90)				70.6	32.3	77.15	45.1	
			Mean of 10% lines (N=9) selected for GY in exp.				81	32.4	79.4	61	
		BAG15									
		Response after simulated selection (%)					14.2	0.3	2.9	35.2	
	BAG16	BAG16	Population mean (N=90)	70.6	32.3	77.1	14.5	70.6	32.3	77.1	45.1
			Mean of 10% lines (N=9) selected for SF or GY in exp. BAG16	89.7	31.6	79.0	13.8	82.2	33.9	80	64.6
			Response after simulated selection (%)		-2.0	2.5	-4.9	16.5	5.0	3.7	43.3
		BAG15	BAG15	Population mean (N=90)		39.9	79.9	16.0		39.9	79.9
			Mean of 10% lines (N=9) selected for SF or GY in exp. BAG16		39.8	82.4	14.6		42.3	83.7	105.6
			Response after simulated selection (%)		-0.4	3.2	-9.0		5.9	4.8	29.7
II	MJFE17		BFE13	Population mean (N=146)		33.0	79.4				
			Mean of 10% lines (N=15) selected for SF in exp. MJFE17		30.6	77.6					
			Response after simulated selection (%)		-7.2	-2.2					
		BFE14	BFE14	Population mean (N=146)		37.4	73.8				
			Mean of 10% lines (N=15) selected for SF in exp. MJFE17		34.1	72.3					
			Response after simulated selection (%)		-8.8	-2.0					
	BFE15		BFE15	Population mean (N=146)		42.6	80.3				
			Mean of 10% lines (N=15) selected for SF in exp. MJFE17		39.3	79.4					
			Response after simulated selection (%)		-7.8	-1.2					

Set of exps.	Selection exp	Response exp.		Response to selection for high SF				Response to selection for high GY			
				SF	GW	TW	GPC	SF	GW	TW	GPC
II (cont)	BFE13	BFE13	Population mean (N=146)	98.4	33.0	79.4		98.4	33	79.4	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE13	112.8	31.9	79.3		100.2	32.8	76.6	
			Response after simulated selection (%)		-3.5	-0.13		1.8	-0.7	-3.5	
		BFE14	Population mean (N=146)		37.4	73.8		92	37.4	73.8	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE13		37.5	74.0		94.1	38.3	74.1	
			Response after simulated selection (%)		0.3	0.3		2.3	2.4	0.5	
		BFE15	Population mean (N=146)		42.6	80.3		89.4	42.6	80.3	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE13		40.4	79.7		90.8	42.1	80.1	
			Response after simulated selection (%)		-5.0	-0.9		1.6	-1.1	-0.4	
	BFE14	BFE13	Population mean (N=146)		33.0	79.4		98.4	33	79.4	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE14		31.8	78.3		102.7	32.8	76.6	
			Response after simulated selection (%)		-3.6	-1.4		4.3	-0.7	-3.5	
		BFE14	Population mean (N=146)	92.0	37.4	73.8		92	37.4	73.8	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE14	109.4	35.4	73.5		101	37	73.9	
			Response after simulated selection (%)		-5.5	-0.4		9.9	-1.5	0.2	
		BFE15	Population mean (N=146)		42.6	80.3		89.4	42.6	80.3	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE14		40.5	79.4		95.2	40.6	79.2	
			Response after simulated selection (%)		-4.9	-1.1		6.5	-4.7	-1.5	
	BFE15	BFE13	Population mean (N=146)		33.0	79.4		98.4	33	79.4	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE15		31.4	78.8		103.5	33.4	80	
			Response after simulated selection (%)		-4.8	-0.8		5.2	1.1	0.8	
		BFE14	Population mean (N=146)		37.4	73.8		92	37.4	73.8	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE15		33.8	72.9		96.1	37.1	74.5	
			Response after simulated selection (%)		-9.6	-1.2		4.5	-0.9	1.0	
		BFE15	Population mean (N=146)	89.4	42.6	80.3		89.4	42.6	80.3	
			Mean of 10% lines (N=15) selected for SF or GY in exp. BFE15	103.9	39.5	79.5		95	41.9	80.5	
			Response after simulated selection (%)		-7.3	-1.1		6.3	-1.5	0.2	

Set of exps.	Selection exp	Response exp.		Response to selection for high SF				Response to selection for high GY			
				SF	GW	TW	GPC	SF	GW	TW	GPC
III	BLE16	BLE16	Population mean (N=28)	81.5				81.5			
			Mean of 10% lines (N=3) selected for SF or GY in exp. BLE16	92.9				84.3			
			Response after simulated selection (%)					3.4			
			BLE17	Population mean (N=28)					84.5		
		Mean of 10% lines (N=3) selected for GY in exp. BLE16					89.3				
		Response after simulated selection (%)					5.7				
	BLE17	BLE17	Population mean (N=28)	84.5				84.5			
			Mean of 10% lines (N=3) selected for SF or GY in exp. BLE17	98.3				80.5			
			Response after simulated selection (%)					-4.8			
			BLE16	Population mean (N=28)					81.5		
		Mean of 10% lines (N=3) selected for GY in exp. BLE17					82.8				
		Response after simulated selection (%)					1.6				
	BLP17	BLP17	Population mean (N=36)	86.6	35.3			86.6	35.3		
			Mean of 10% lines (N=4) selected for SF or GY in exp. BLP17	104.5	30.3			77.4	36.7		
			Response after simulated selection (%)		-14			-10.6	4.0		
			BLP18	Population mean (N=36)					86.6	35.3	
		Mean of 10% lines (N=4) selected for GY in exp. BLP18					84.9	35.3			
		Response after simulated selection (%)					-1.9	0.0			
	Overall mean response after simulated selection (sets I, II and III; %)				-5.6	-0.5	-7.0	3.9	0.8	0.8	36.2

417 *Table S2. Pearson correlation coefficients between spike fertility index (SF, grains/g), grain*
 418 *yield (GY, g/m²), grain number per unit area (GN, grains/m²), grain weight (GW, mg) and test*
 419 *weight (TW, kg/hl), calculated with data from sets of experiments I, II and III. All coefficients*
 420 *are significant ($p < 0.05$) except where noted (ns).*

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	GY	GN	GW	TW
SF	0.29	0.44	-0.20	0.00 _{ns}
GY		0.91	0.24	0.28
GN			-0.18	0.19
GW				0.21

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