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MOLECULAR MARKER ANALYSIS OF SPIKE FERTILITY INDEX AND RELATED TRAITS IN A BREAD WHEAT RECOMBINANT INBRED LINE POPULATION

ANÁLISIS DE MARCADORES MOLECULARES PARA EL ÍNDICE DE FERTILIDAD DE ESPIGA Y CARACTERES ASOCIADOS EN UNA POBLACIÓN DE LÍNEAS ENDOCRIADAS RECOMBINANTES DE TRIGO PAN.

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ABSTRACT

Spike fertility index (SF) has been well established as an ecophysiological trait related to grain number per unit area and a promising selection target in wheat breeding programs. Scarce information on the molecular basis of SF is available thus far. In this study, a preliminary molecular marker analysis was carried out in a RIL population derived from the cross between two Argentinean cultivars with contrasting SF to identify candidate genomic regions associated with SF. Twenty-four microsatellites and two functional markers that had been found to co-segregate with SF in a bulked-segregant analysis of the F_3 generation of the population were analyzed. Phenotypic data were collected from three field experiments carried out during 2013, 2014 and 2015 growing seasons at Balcarce, Argentina. Two genomic regions associated with SF in chromosomes 5BS and 7AS were detected, which merit further investigation.

Key words: selection, genomic regions, grain number, yield, QTL, spike fertility index, fruiting efficiency

RESUMEN

El índice de fertilidad de espiga (FE) ha sido propuesto como un carácter ecofisiológico asociado con el número de granos por unidad de área y como criterio de selección prometedor para los programas de mejoramiento de trigo. Sin embargo, la información sobre las bases moleculares de la FE aún es escasa. En este estudio, se realizó un análisis preliminar de marcadores moleculares en una población RIL derivada del cruce entre dos cultivares argentinos con FE contrastante con el objetivo de identificar regiones genómicas candidatas asociadas con el carácter. Se analizaron 24 microsatélites y dos marcadores funcionales que se había encontrado que se co-segregaban con la FE en un análisis de segregantes en *"bulk"* en la generación F_3 de la población. Se recopilaron datos fenotípicos de tres experimentos de campo llevados a cabo durante las temporadas de cultivo 2013, 2014 y 2015 en Balcarce, Argentina. Se detectaron dos regiones genómicas asociadas con la FE en los cromosomas 5BS y 7AS, que mostraron ser estables a través de los años de evaluación. Este trabajo aporta información novedosa acerca de las bases moleculares de la FE, las cuales deberán ser estudiadas con mayor profundidad.

Palabras clave: selección, regiones genómicas, número de granos, rendimiento, QTL, indice de fertilidad de espiga, eficiencia de fructificación

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The authors contributed equally to this work (*ex-aequo*).

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most important field crops in the world. It provides ~20% of human food calories and protein (FAO 2018). Prospects indicate a steady growth in the global population, which will be encompassed by an increase in food demand. However, this demand will hardly be attained through the expansion of farming areas (Albajes *et al.* 2013). Breeding efforts should rather concentrate on achieving higher grain yield-increase rates (Reynolds *et al.* 2012).

Grain yield in wheat is more strongly associated with grain number per unit area (hereinafter referred to as GN m^{-2}) than it is with grain weight (Sadras 2007; Fischer 2011). Hence, breeding efforts have focused on increasing grain yield through increasing GN m^{-2} (Slafer *et al.* 2014, 2015; Lo Valvo *et al.* 2018). However, this is a difficult trait to select for in early breeding stages. Thus, the use of GN m^{-2}-related traits as selection targets may be helpful to increase grain yield at the pace it is required (Slafer 2003; Fischer and Rebetzke 2018). A conceptual model proposed by Fischer (1984) suggests that, under non-limiting growing conditions, GN m^{-2} in wheat can be considered as the product of (i) the duration of the spike growth period, (ii) the crop growth rate during the spike growth period, (iii) the dry weight partitioning to spikes during the spike growth period and (iv) the number of grains per unit of spike chaff dry weight, i.e. a spike fertility index (SF), also termed "fruiting efficiency" (Ferrante *et al.* 2012).

Many authors have described SF as an ecophysiological component which explains a substantial proportion of the differences in GN m^{-2} between cultivars (Acreche *et al.* 2008; Gonzalez *et al.* 2014; Aisawi *et al.* 2015; Gonzalez-Navarro *et al.* 2016), with high stability across environments (Abbate *et al.* 2013; Elía *et al.* 2016; Guo *et al.* 2016) and moderate to high heritability (Martino *et al.* 2015; Mirabella *et al.* 2016; Alonso *et al.* 2018b). In turn, a fast and high-throughput method was developed for SF determination at maturity, using as few as 15 spikes per plot (Abbate *et al.* 2013). Adding up all these features, SF emerges as a promising trait to select for in the early generations of breeding programs, in which little seed is available for GN m^{-2} determinations (Fischer and Rebetzke 2018). Furthermore, a recent study showed that the use of SF as a selection criterion, either solely or in combination with selection for high yield, effectively increased yield, resulting in superior and more stable grain yields than selecting just for high yield (Alonso *et al.* 2018b).

Molecular markers associated with agronomically valuable traits have been successfully used to select for in wheat breeding programs' early generations (Collard and Mackill 2008). For example, some authors reported microsatellites linked to traits as plant height (Wang *et al.* 2010; Zhang *et al.* 2011), grain number per spike (Quarrie *et al.* 2005, 2006; Hai *et al.* 2008) and yield *per se* (Kobiljski *et al.* 2007). The availability of molecular markers associated with SF would be very helpful for increasing the genetic gain per selection cycle. Marker-assisted selection could allow seed or plantlet selection of transgressive genotypes at early generations of segregating populations, and molecular characterization of the crossing block. Despite the prospective relevance of SF for wheat breeding, about the genetic and molecular control of this trait little is known, except for a couple of recent studies which respectively reported a QTL for SF in chromosome 2AL (candidate gene *CO4*; Guo *et al.* 2017) and a significant effect of photoperiod sensitivity genes *Ppd-B1* and *Ppd-D1* on SF (Ramirez *et al.* 2018). In the present study, a preliminary molecular marker analysis was carried out in a recombinant inbred line (RIL) population segregating for SF in order to identify candidate genomic regions associated with this trait. These results provide valuable information as a first step in mapping QTL/genes controlling SF and possibly other related traits.

MATERIALS AND METHODS

Phenotypic data generation

In the present study, molecular marker analysis of SF and related traits was carried out with previously published phenotypic data (Alonso *et al.* 2018a, b). A brief description of the mapping population, experiments, environmental conditions, and measurements and calculations, is included below. For further details see Alonso *et al.* (2018a).

Plant material. A mapping population of 146 recombinant inbred lines (RILs) derived from the cross between the Argentinean spring bread wheat cultivars 'Baguette 10' and 'Klein Chajá' was used in all field experiments. Both parental cultivars were also included. 'Baguette 10' and 'Klein Chajá' were commercially released in 2000 and 2002 respectively and are contrasting for SF and other yield-related traits (Martino *et al.* 2015; Alonso *et al.* 2018a, b).

Field experiments. During the 2013, 2014 and 2015 crop seasons, field experiments were carried out at the experimental station of the Instituto Nacional de Tecnología Agropecuaria (INTA) Balcarce (37° 45' S; 55° 18' W; 130 m a.s.l.), Balcarce, Buenos Aires, Argentina. Experiments are fully described in Alonso *et al.* (2018a, b).

Measurements and calculations. Plant height was measured from the ground to the ear tip at maturity; the average of two measurements per plot was registered. At the same time, a sample of 20 spikes was drawn at random from the three or five central rows of each plot and air-dried for further SF determination according to Abbate *et al.* (2013). Briefly, the sample was weighed (total weight) and threshed, and grains were weighed

(grain weight) and counted (grain number). Spike fertility index was calculated as the quotient between grain number and chaff weight (*i.e.*, the difference between total weight and grain weight).

Grain yield was determined by mechanical harvest. For grain weight determination, a clean and dry subsample of ~30 g was taken from the yield sample, weighted and counted in an automatic counter. Grain number m^{-2} was calculated as the quotient between grain yield and grain weight. Grain test weight was measured using a Schopper cylinder.

Molecular marker analysis

Genomic DNA extraction from fresh tissue of ten-days-old seedling leaves was carried out according to Haymes *et al.* (1996). Approximately 200 molecular markers were analyzed for polymorphism between the parents of the RIL population. Bulked segregant analysis (Michelmore *et al.* 1991) was carried out in the F_3 generation of the population (Deperi, 2012). Those markers which showed co-segregation with SF were used in the present study (24 microsatellites and two functional markers, Table 1, Fig. S1). In all cases, PCR reactions were performed using a final volume of 15 µl in a Veriti™ (Applied Biosystems) thermal cycler. The reaction buffer contained 1X *Taq* DNA Polymerase buffer (Promega), 0.8 U *Taq* DNA Polymerase (Promega), 0.2 mM of each dNTP, 0.2 µM of each primer, 1.5 mM of $MgCl_2$ and 100 ng of genomic DNA (template). Cycling conditions were as follows: 3' initial denaturation at 95°C, 18 cycles of 30" denaturation at 95°C, 30" annealing at 65°C to 56°C ("touchdown") and 30" extension at 72°C, followed by 22 additional, similar cycles but with annealing at 56°C, and 5' final extension at 72°C. Primer names and sequences, linkage group, allele sizes and cycling conditions for each molecular marker are detailed in Table 1. Amplified fragments were separated and analyzed through horizontal electrophoresis in 2% agarose gels in 1X TBE buffer, stained with GelRed® (Biotium) during 15 min at 100V and exposed to UV light. Also, fragments were analyzed through electrophoresis in denaturing 6% polyacrylamide-urea gels (Sambrook *et al.* 2001) stained with silver nitrate following the protocol described by Benbouza *et al.* (2006). In this case, fragment visualization was performed by exposing gels to white light. Allelic variants were assigned to each RIL according to their parent of origin, as 'B' for 'Baguette 10' and 'K' for 'Klein Chajá'. A few heterozygous individuals were detected and discarded from further analyses.

Statistical analysis

Statistical analysis was performed using the package *nlme* (Pinheiro *et al.* 2017) of the Rsoftware (R-Core Team 2017). A linear fixed effects model including year, genotype, block nested in year, and genotype-by-year interaction effects on phenotypic variables, was used. Variances from the model were used to calculate broad-sense heritability (H^2) for each trait according to Hallauer *et al.* (2010) as:

$$H^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_e^2/re + \hat{\sigma}_{ge}^2/e + \hat{\sigma}_g^2}$$

with $\hat{\sigma}_g^2$ as genotypic variance, $\hat{\sigma}_e^2$ as environmental variance, $\hat{\sigma}_{ge}^2$ as the genotype-by-environment (year) variance, r as the number of replications or blocks nested in environment and e as the number of environments (years).

In order to detect genomic regions associated with the evaluated traits, a linear fixed effects model was run for each marker, including marker, year, block nested in year, and the marker-by-year interaction effects. Bonferroni correction was applied in multiple comparisons using a family-wise error rate of 0.05. When a significant marker-by-year interaction effect was detected, the marker effect was analyzed for each year individually. When a significant marker-trait association was detected, the percentage of phenotypic variation explained by the marker was calculated as the quotient between the sum of squares of the marker and the total sum of squares x 100. The marker effect was calculated as the difference between the mean in the group 'B' and the mean in the group 'K'.

Haplotypes were constructed with one marker per region associated with SF. In chromosome 7AS, the chosen marker was the one with the lowest p-value. Differences between these groups were tested with the Tukey test (α=0.05).

Table 1. Molecular markers used in this study.

Linkage group	Marker	Type	Reference	Primer sequences
2AL	*Xgwm372*	SSR	Röder *et al.* (1998)	F AATAGAGCCCTGGGACTGGG R GAAGGACGACATTCCACCTG
2AS	*Xwmc63*	SSR	Somers and Isaac (2004)	F GTGCTCTGGAAACCTTCTACGA R CAGTAGTTTAGCCTTGGTGTGA
2BL	*Xwmc317*	SSR	Somers and Isaac (2004)	F TGCTAGCAATGCTCCGGGTAAC R TCACGAAACCTTTTCCTCCTCC
3BS	*Xgwm493*	SSR	Röder *et al.* (1998)	F TTCCCATAACTAAAACCGCG R GGAACATCATTTCTGGACTTTG
3DS	*Xgwm314*	SSR	Röder *et al.* (1998)	F AGGAGCTCCTCTGTGCCAC R TTCGGGACTCTCTTCCCTG
4BL	*Xgwm495*	SSR	Röder *et al.* (1998)	F GAGAGCCTCGCGAAATATAGG R TGCTTCTGGTGTTCCTTCG
4DL	*Xgwm194*	SSR	Röder *et al.* (1998)	F GATCTGCTCTACTCTCCTCC R CGACGCAGAACTTAAACAAG
4DS	*RhtD1*	Functional marker	Ellis *et al.* (2002)	DF CGCGCAATTATTGGCCAGAGATAG DF2 GGCAAGCAAAAGCTTCGCG MR2 CCCATGGCCATCTCGAGCTGCTA WR2 GGCCATCTCGAGCTGCAC
5AL	*VrnA1*	Functional marker	Xue *et al.* (2008)	F GCGCAACAAGATCAGACTCA R ACGCTTATATGGGCTGGAAG
5AL	*Xbarc151*	SSR	http://www.scabusa.org	F TGAGGAAAATGTCTCTATAGCATCC R TGAGGAAAATGTCTCTATAGCATCC
5AL	*Xgwm291*	SSR	Röder *et al.* (1998)	F CATCCCTACGCCACTCTGC R AATGGTATCTATTCCGACCCG
5AL	*Xgwm293*	SSR	Röder *et al.* (1998)	F TACTGGTTCACATTGGTGCG R TCGCCATCACTCGTTCAAG
5AL	*Xgwm304*	SSR	Röder *et al.* (1998)	F AGGAAACAGAAATATCGCGG R AGGACTGTGGGGAATGAATG
5BL	*Xgwm335*	SSR	Röder *et al.* (1998)	F CGTACTCCACTCCACACGG R CGGTCCAAGTGCTACCTTTC
5BL	*Xgwm213*	SSR	Röder *et al.* (1998)	F TGCCTGGCTCGTTCTATCTC R CTAGCTTAGCACTGTCGCCC
5BS	*Xgwm540*	SSR	Röder *et al.* (1998)	F TCTCGCTGTGAAATCCTATTTC R AGGCATGGATAGAGGGGC
5DS	*Xgwm190*	SSR	Röder *et al.* (1998)	F GTGCTTGCTGAGCTATGAGTC R GTGCCACGTGGTACCTTTG
6AS	*Xgwm427*	SSR	Röder *et al.* (1998)	F AAACTTAGAACTGTAATTTCAGA R AGTGTGTTCATTTGACAGTT
6BL	*Xgwm626*	SSR	Röder *et al.* (1998)	F GATCTAAAATGTTATTTTCTCTC R TGACTATCAGCTAAACGTGT
7AS	*Xgwm282*	SSR	Röder *et al.* (1998)	F TTGGCCGTGTAAGGCAG R TCTCATTCACACACAACACTAGC
7AS	*Xgwm332*	SSR	Röder *et al.* (1998)	F AGCCAGCAAGTCACCAAAAC R AGTGCTGGAAAGAGTAGTGAAGC
7AS	*Xpsp3050*	SSR	Bryan *et al.* (1997)	F CCGATAAAAGTTTAGCGACCC R TAACTCACCTGCGAACTGTG
7AS	*Xpsp3094.1*	SSR	Bryan *et al.* (1997)	F ACCAGGAGAGATAGTCGTTAGGC R TTTGTACACCATGATAGGCTTCC
7AS	*Xwmc790*	SSR	Somers and Isaac (2004)	F CGACAACGTACGCGCC R CGACAACGTACGCGCC
7BL	*Xgwm344*	SSR	Röder *et al.* (1998)	F CAAGGAAATAGGCGGTAACT R ATTTGAGTCTGAAGTTTGCA
7BS	*Xgwm46*	SSR	Röder *et al.* (1998)	F GCACGTGAATGGATTGGAC R TGACCCAATAGTGGTGGTCA

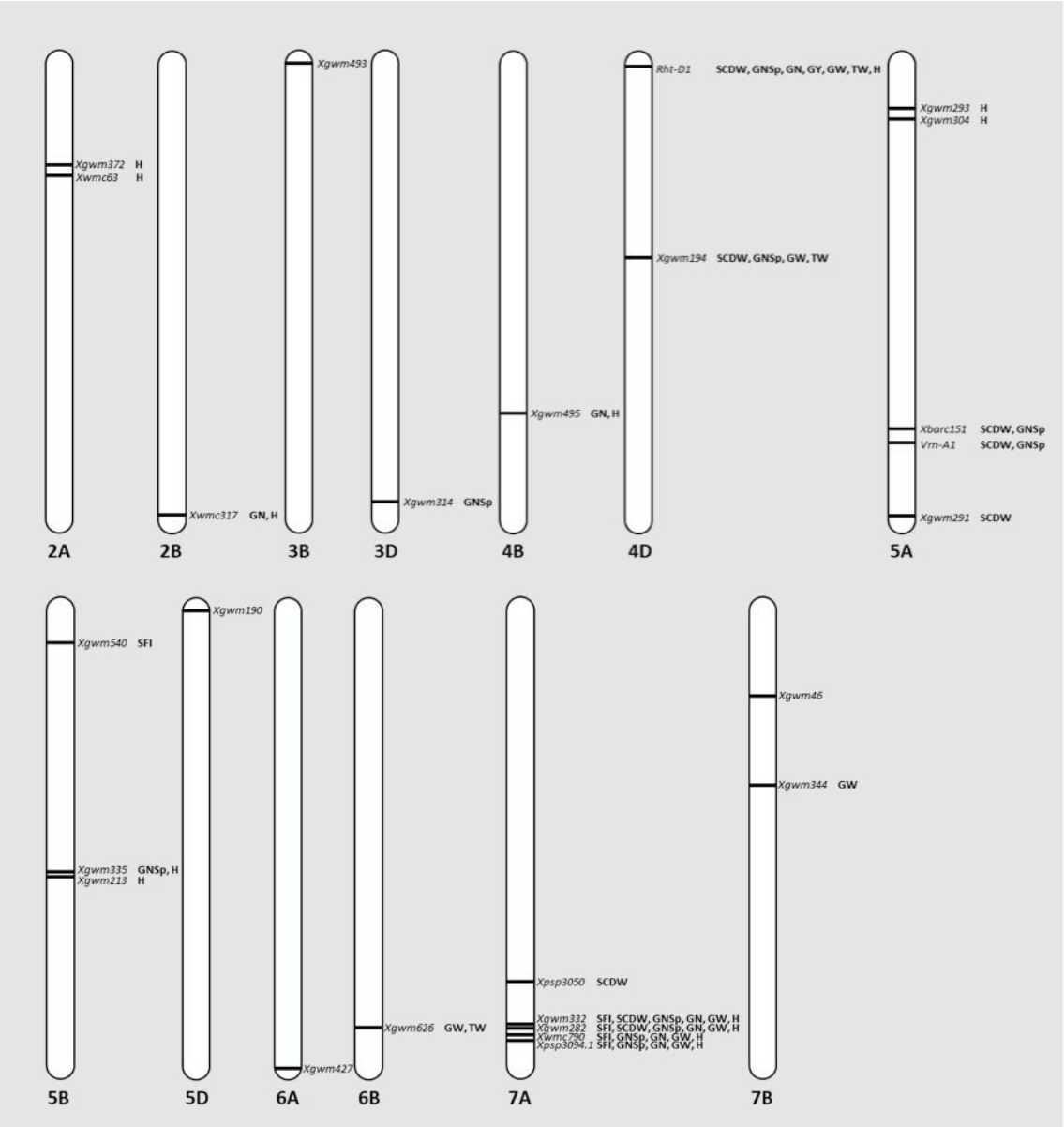

Figure S1. Approximate chromosome location of polymorphic markers used in this study and traits with which markers were associated.

RESULTS AND DISCUSSION

Environmental conditions

The environmental conditions under which the experiments were performed are fully described in Alonso *et al.* (2018a). Conspicuous inter-annual environmental variation was observed, even though all experiments were carried out with no water or nutrient limitations and with chemical control of pests and fungal diseases.

Phenotypic variation of RIL population

Phenotypic data description is fully detailed in Alonso *et al.* (2018a, b). Evaluated traits showed a bell-shaped and symmetrical distribution across all years (Fig. S2). Mean standard deviation and coefficient of variation for the analyzed traits in the RIL population are presented in Table 2. 'Baguette 10' had higher values of SF, grain yield and GN m^{-2} than those of 'Klein Chajá'. Significant effects of genotype, year and genotype-by-year interaction were detected for all the traits in the RIL population (Table 3). However, the genetic variance was always greater than genotype-by-year interaction variance. All traits showed moderate to high broad-sense heritability (Table 3), which is essential for meaningful QTL detection. Although field evaluations comprising a larger number of environments are needed, these results suggest stability in SF, in line with previous findings (Abbate *et al.* 2013; Elía *et al.* 2016; Gonzalez-Navarro *et al.* 2016; Mirabella *et al.* 2016). This further supports the possibility of using molecular markers linked to the trait.

Molecular marker analysis

A total of 24 out of 200 SSR markers, plus two functional markers, were analyzed in the RIL population for their polymorphism between the parents and co-segregation with SF in the F_3 generation. Even though a very low number of polymorphic markers was found, 55 significant single marker-trait associations were detected (Tables 4, 6; Fig. S1). A significant year effect was found in all cases, whereas significant marker-by-year interaction effects ($p<0.05$) were detected in only eleven out of 55 cases (Tables 4, 6). No crossover interactions were found. Genome coverage reached by these polymorphic markers was low and markers were not evenly distributed. Also, many linkage groups were not covered. This may lead to biased results, with phenotypic variation only being explained by covered regions. Nevertheless, significant regions explaining an interesting amount of variation (R^2=0.6 to 24%; Tables 4, 6 and 7) were detected for the reported traits.

Markers associated with spike fertility index

Five markers were associated with SF (Table 4), one on chromosome 5BS (*Xgwm540*) and four on chromosome 7AS. The highest proportion of explained variance for a marker in SF was that of *Xwmc790* (R^2=3.7%) with a positive effect of allele 'B'. On chromosome 5BS, genotypes carrying the 'K' allele in marker *Xgwm540* showed the highest SF values (Table 4). Presence of high SF alleles in the low SF parent is expected, as transgressive segregation was observed in this population (Fig. S2) (Martino *et al.* 2015; Alonso *et al.* 2018b). Such alleles are interesting because further variability for the trait can be exploited even in "bad" genotypes, for stacking favorable minor alleles. Thus, the selection of extreme superior phenotypes could further increase SF and, in extension, raise grain yield (Slafer *et al.* 2015; Fischer and Rebetzke 2018).

Haplotypes constructed with these two markers yielded four genotypic groups. In all cases (Table 5), haplotype *Xwmc790-B/ Xgwm540-K* showed the highest SF, whereas the haplotype *Xwmc790-K/Xgwm540-B* showed the lowest SF ($p<0.05$). Haplotypes with the remaining allele combinations had intermediate SF values. Also, haplotype ranking was the same across years. On average, SF difference between extreme haplotypes was ~7%; according to results reported by Alonso *et al.* (2018b), this could represent a difference in GN m^{-2} potentially associated with a significant grain yield increase.

Markers associated with other traits

Spike chaff dry weight. Eight marker-trait associations were detected in four chromosomic regions, with effects ranging between 3.6 and 4.4% (Table 6). Two of these markers, on chromosome 7AS, co-localized with SF as well. All markers on 7AS showed a positive effect of allele 'B', except for *Xpsp3050*. When analyzed by year, a significant negative effect of *Vrn-A1-B* on 5AL was observed at all three years, but the magnitude of such effect varied across years (Table 7).

Grain number per spike. Ten marker associations with GN/spike were detected in four chromosomic regions (Table 6). These results are partly coincident with the ones reported by Quarrie *et al.* (2005, 2006) and Hai *et al.* (2008). These authors detected QTL associated with grain number per spike in chromosome 7AS of a doubled haploid population. Marker effect ranged between 3.4 and 7.6% (Table 6). *RhtD1* had a significant association with GN/spike at all three years, with a negative effect of allele 'B'. However, the marker effect in 2014 was almost three times greater than that of 2013 and 2015 (Table 7). Marker *Xgwm335* (5BL) showed significant marker by year interaction due to its association with GN/spike only in 2014 and 2015, with a similar magnitude (Table 7). No additional association was detected for *Xgwm540*, even though it had been described as a yield-related marker in a set of Serbian cultivars (Kobiljski *et al.* 2007).

Grain number per square meter. Seven markers on four regions showed a significant effect on GN m^{-2} (Table 6), notably those on chromosome 7AS which in turn were associated with SF. This is expected, given that these two traits are positively correlated (Acreche *et al.* 2008, Terrile *et al.* 2017; Lo Valvo *et al.* 2018; Alonso *et al.* 2018b). Three markers had a significant marker-by-year interaction effect. The *Rht-D1* gene (4DS) showed the strongest effect. In 2013 and 2014 the variation explained by the marker was notably higher than in 2015 (Table 7).

Grain yield. Grain yield was only associated with allelic variation at *Rht-D1* (4DS). A significant marker-by-year interaction was detected, similar to the one observed for GN m^{-2}. Similarly, grain yield variation due to this gene was far greater in 2013 and 2014 than it was in 2015 (Table 7).

Grain weight. Eight marker-trait associations were detected for grain weight on five regions, with no marker-by-year interaction. Markers with effect on SF on 7AS also showed effect for this trait, with the opposite effect. However, this is unsurprising, as a negative genetic correlation has been reported between SF and grain weight (Ferrante *et al.* 2012, 2015; Gonzalez-Navarro *et al.* 2016; Terrile *et al.* 2017; Alonso *et al.* 2018b). The variability explained by each marker ranged between 2.6 and 5.2% (Table 6). In this population, Alonso *et al.* (2018a, b) reported a negative correlation of SF with grain weight, which can lead to a tradeoff between SF and grain weight (Ferrante *et al.* 2015; Slafer *et al.* 2015; Gonzalez-Navarro *et al.* 2016; Terrile *et al.* 2017), but also to unbalances in the sink/source ratio (Alonso *et al.* 2018a). Besides, markers associated with grain weight were detected in several genomic regions, not linked to those associated with SF (Table 6).

Test weight. Three independent markers were associated with test weight, without marker-by-year interaction effect. The highest association was found with *Rht-D1* (4DS), which explained ~3% of the total variation (Table 6).

Plant height. Thirteen markers showed a significant effect on plant height; eleven of them, located on seven regions, showed no marker-by-year interaction. The remaining two markers, on chromosomes 4DS and 5AL, did show such interaction (Table 6). Marker effect ranged mainly between 3.2 and 6.7%, except for *Rht-D1*. It showed a significant marker–by-year interaction effect. Variation at this gene was associated with plant height at all three years with a positive effect of allele 'B', but it explained a different portion of total variation depending on the year (~18-24%; Table 7). Marker *Xgwm293* on chromosome 5AL showed a similar pattern, respectively, explaining 3.8 and 5.4% of plant height variation in 2014 and 2015, with a negative effect of allele 'B'. Regarding chromosome 5BL, marker *Xgwm213* was reported as associated with this trait by Wang *et al.* (2010) in a RIL population, and by Zhang *et al.* (2011) in a doubled haploid population. Although not associated with SF, these results give support for the marker analysis approach used in the present study.

In this study, a low genome coverage was reached, partly due to the lack of polymorphic microsatellites between the parents of the RIL population. This is also reflected in the phenotypic variability that was not explained by the available genotypic information. However, a few genomic regions associated with SF and related traits were detected, which were stable trough different years with different environmental conditions. Using the two markers that were most associated with SF in this study (located in chromosomes 5BS and 7AS, respectively), it was possible to classify lines into high, intermediate and low SF groups. Further studies with higher genome coverage and additional phenotypic evaluations are needed to validate the present results and to delimit genomic regions containing genes that control SF.

Table 2. Mean, standard deviation and coefficient of variation of spike fertility index, spike chaff dry weight, grain number per spike, grain number per m^2, grain yield, grain weight, test weight and plant height of a RIL population ('Baguette 10' x 'Klein Chajá') evaluated in 2013, 2014 and 2015 at Balcarce, Argentina. Partially published data (Alonso et al. 2018a,b).

	Mean			Standard deviation			Coefficient of variation		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
Spike fertility index (grains g^{-1})	98.3	91.9	89.4	9.2	11.7	9.5	9.4	12.8	10.6
Spike chaff dry weight (g $spike^{-1}$)	0.47	0.46	0.57	0.07	0.08	0.09	15.8	16.9	14.8
Grain number per spike (grains $spike^{-1}$)	45.5	42.3	50.6	5.8	6.9	6.7	12.8	16.4	13.1
Grain number per m^2 (GN m^{-2})	21916	9964	17824	6382	2687	3457	29.1	26.9	19.4
Grain yield (g m^{-2})	717.7	367.1	749.1	185.9	89.4	123.5	25.9	24.4	16.5
Grain weight (g 1000 $grains^{-1}$)	33.0	37.4	42.5	4.2	4.6	3.8	12.6	12.2	8.9
Test weight (kg hl^{-1})	79.4	73.8	80.3	2.3	2.2	1.8	2.8	3.0	2.3
Plant height (cm)	101.6	97.5	97.2	11.5	12.3	15.4	11.3	12.7	15.8

Table 3. Analysis of variance of spike fertility index, spike chaff dry weight, grain number per spike, grain number per m2, grain yield, grain weight, test weight and plant height of a RIL population ('Baguette 10' x 'Klein Chajá') evaluated in 2013, 2014 and 2015 at Balcarce, Argentina. Broad-sense heritability (H^2) values. Partially published data (Alonso et al. 2018a,b).

Trait	Factor	Degrees of freedom	Mean Square	P (>F)	H^2
Spike fertility index	Genotype (G)	145	344.7	<0.0001	0.86
	Year (Y)	2	6219.7	<0.0001	
	Block in Y	3	203.7	0.0047	
	G x Y	288	63.3	0.0021	
	Residuals	416	46.5		
Spike chaff dry weight	Genotype (G)	145	0.0169	<0.0001	0.70
	Year (Y)	2	1.05354	<0.0001	
	Block in Y	3	0.06167	<0.0001	
	G x Y	288	0.00481	<0.0001	
	Residuals	416	0.00316		
Grain number per spike	Genotype (G)	145	135	<0.0001	0.78
	Year (Y)	2	4999	<0.0001	
	Block in Y	3	262.6	<0.0001	
	G x Y	288	27.4	0.0001	
	Residuals	417	18.5		
Grain number per m^2	Genotype (G)	145	44584000	<0.0001	0.52
	Year (Y)	2	1.0376E+10	<0.0001	
	Block in Y	3	297900000	<0.0001	
	G x Y	288	20764000	<0.0001	
	Residuals	411	8.77E+06		
Grain yield	Genotype (G)	145	34708	<0.0001	0.42
	Year (Y)	2	12908156	<0.0001	
	Block in Y	3	455230	<0.0001	
	G x Y	288	21296	<0.0001	
	Residuals	422	9498		
Grain weight	Genotype (G)	145	56.2	<0.0001	0.67
	Year (Y)	2	6419.2	<0.0001	
	Block in Y	3	1.3	0.9153	
	G x Y	288	13.2	<0.0001	
	Residuals	420	7.7		
Test weight	Genotype (G)	145	18.3	<0.0001	0.63
	Year (Y)	2	3652.7	<0.0001	
	Block in Y	3	4.1	0.0063	
	G x Y	288	2.6	<0.0001	
	Residuals	421	1		
Plant height	Genotype (G)	145	831.32	<0.0001	0.93
	Year (Y)	2	1810.98	<0.0001	
	Block in Y	3	45.47	0.1732	
	G x Y	288	61.17	<0.0001	
	Residuals	429	27.26		

Table 4. Molecular markers associated with spike fertility index (grains /g chaff) in a RIL population ('Baguette 10' x 'Klein Chajá') evaluated in 2013, 2014 and 2015 at Balcarce, Argentina.

				Allelic difference [a]	
Marker	**Chromosome**	**p-value**	**R^2**	**grains /g chaff**	**%**
Xgwm540	5BS	<0.001	1.4	-2.44	-2.6
Xgwm282	7AS	<0.001	2.8	3.49	3.7
Xgwm332	7AS	<0.001	1.4	2.46	2.6
Xpsp3094.1	7AS	<0.001	2.3	3.21	3.4
Xwmc790	7AS	<0.001	3.7	4.09	4.4

[a] Average spike fertility index difference between lines with the 'B' vs. the 'K' allele.

Table 5. Spike fertility index (grains /g chaff) of haplotypes at the two markers most significantly associated with the trait in a RIL population ('Baguette 10' x 'Klein Chajá') evaluated in 2013, 2014 and 2015 at Balcarce, Argentina. The'B' and 'K' denote lines with the 'B' and the 'K' allele, respectively. Same letters within a column indicate non-significant differences (p>0.05).

Haplotype			**Spike fertility index**							
Xwmc790	***Xgwm540***	**N**	**Mean**		**2013**		**2014**		**2015**	
B	K	30	95.8	a	101.3	a	95.2	a	91.1	a
B	B	34	93.4	ab	99.0	ab	91.8	ab	89.4	ab
K	K	31	92.4	bc	96.8	b	91.6	ab	88.7	ab
K	B	22	89.7	c	95.5	b	87.0	b	86.5	b

Table 6. Molecular markers associated with spike chaff dry weight, grain number per spike, grain number per m^2, grain yield, grain weight, test weight and plant height of a RIL population ('Baguette 10' x 'Klein Chajá') evaluated in 2013, 2014 and 2015 at Balcarce, Argentina.

					Allelic difference[a]		
Trait	**Marker**	**Chromosome**	**p-value**	**R^2%**	**Trait units**	**%**	
Spike chaff dry weight	*Rht-D1*	4DS	<0.001	1.7	-0.026	-5.1	*
(g $spike^{-1}$)	*Xgwm194*	4DL	<0.001	1.4	-0.022	-4.4	
	Vrn-A1	5AL	<0.001	4	-0.039	-7.8	*
	Xbarc151	5AL	<0.001	1.3	-0.022	-4.3	
	Xgwm291	5AL	0.0014	0.9	0.018	3.6	
	Xgwm282	7AS	<0.001	1	0.018	3.7	
	Xgwm332	7AS	<0.001	1.1	0.020	4.0	
	Xpsp3050	7AS	<0.001	1	-0.019	-3.8	
Grain number per spike	*Xgwm314*	3DS	0.0018	1.1	1.56	3.4	
(grains $spike^{-1}$)	*Rht-D1*	4DS	<0.001	5.4	-3.49	-7.6	*
	Xgwm194	4DL	<0.001	1.9	-2.07	-4.5	
	Vrn-A1	5AL	<0.001	4.4	-3.20	-6.9	
	Xbarc151	5AL	<0.001	2.7	-2.42	-5.2	
	Xgwm335	5BL	<0.001	1.9	-2.06	-4.5	*
	Xgwm282	7AS	<0.001	5.6	3.40	7.4	
	Xgwm332	7AS	<0.001	4.4	3.08	6.7	
	Xpsp3094.1	7AS	<0.001	4.4	3.13	6.8	
	Xwmc790	7AS	<0.001	5.6	3.42	7.4	
Grain number m^{-2}	*Xwmc317*	2BL	0.0012	0.6	-979.8	-5.9	
	Xgwm495	4BL	0.0019	0.6	-1054.4	-6.4	*
	Rht-D1	4DS	<0.001	3.7	-2610.6	-15.8	*
	Xgwm282	7AS	<0.001	0.8	1103.4	6.7	
	Xgwm332	7AS	0.0016	0.6	930.5	5.6	
	Xpsp3094.1	7AS	<0.001	1.1	1355.4	8.2	
	Xwmc790	7AS	<0.001	1.2	1409.5	8.5	*
Grain yield (g m^{-2})	*Rht-D1*	4DS	<0.001	1.8	-59.93	-9.8	*
Grain weight	*Rht-D1*	4DS	<0.001	2.3	1.66	4.4	
(g 1000 $grains^{-1}$)	*Xgwm194*	4DL	0.0012	0.7	0.96	2.6	
	Xgwm626	6BL	<0.001	2.1	-1.62	-4.3	
	Xgwm282	7AS	<0.001	2.6	-1.84	-4.9	
	Xgwm332	7AS	<0.001	1.1	-1.15	-3.1	
	Xpsp3094.1	7AS	<0.001	3.1	-1.96	-5.2	
	Xwmc790	7AS	<0.001	2.4	-1.76	-4.7	
	Xgwm344	7BL	<0.001	1.0	1.18	3.1	
Test weight	*Rht-D1*	4DS	<0.001	3.0	1.27	1.6	
(kg hl^{-1})	*Xgwm194*	4DL	<0.001	0.6	0.54	0.7	
	Xgwm626	6BL	<0.001	1.8	-0.98	-1.3	
Plant height	*Xgwm372*	2AL	<0.001	2.4	-4.1	-4.2	
(cm)	*Xwmc63*	2AS	<0.001	1.3	3.0	3.1	
	Xwmc317	2BL	<0.001	1.5	3.2	3.2	
	Xgwm495	4BL	<0.001	2.2	3.9	4.0	
	Rht-D1	4DS	<0.001	20	12.1	12.2	*
	Xgwm293	5AL	<0.001	2.7	-4.4	-4.4	*
	Xgwm304	5AL	<0.001	1.8	-3.6	-3.6	
	Xgwm213	5BL	<0.001	6.1	6.6	6.7	
	Xgwm335	5BL	<0.001	1.7	3.5	3.5	
	Xgwm282	7AS	<0.001	1.8	-3.6	-3.7	

[a] Average trait difference between lines with the 'B' vs. the 'K' allele.

* significant GxE interaction.

Table 7. Molecular markers which showed significant GxE interaction, associated with spike chaff dry weight, grain number per spike, grain number per m^2, grain yield and plant height of a RIL population ('Baguette 10' x 'Klein Chajá') evaluated in 2013, 2014 and 2015 at Balcarce, Argentina.

					Allelic difference[a]	
Trait	**Marker**	**Year**	**p-value**	**R^2%**	**Trait units**	**%**
Spike chaff dry weight	*Rht-D1*	2013	0.108			
(g spike^{-1})		2014	<0.001 *	7.3	-0.042	-9.1
		2015	0.111			
	Vrn-A1	2013	0.042 *	1.8	-0.020	-4.3
		2014	0.002 *	3.8	-0.032	-6.8
		2015	<0.001 *	13	-0.063	-11.0
Grain number per spike	*Rht-D1*	2013	<0.001 *	4.6	-2.4	-5.3
(grains spike^{-1})		2014	<0.001 *	13	-5.1	-12.0
		2015	<0.001 *	4.2	-2.7	-5.4
	Xgwm335	2013	0.824			
		2014	0.001 *	4.3	-2.9	-6.9
		2015	<0.001 *	5.1	-3.0	-6.0
Grain number m^{-2}	*Rht-D1*	2013	<0.001 *	13	-4507.1	-20.6
		2014	<0.001 *	19	-2281.8	-22.9
		2015	0.023 *	1.7	-916.5	-5.1
	Xgwm495	2013	0.002 *	4.2	-2541.9	-11.6
		2014	0.260			
		2015	0.851			
	Xwmc790	2013	0.001 *	3.9	2547.8	11.6
		2014	<0.001 *	6.5	1362.9	13.7
		2015	0.165			
Grain yield	*Rht-D1*	2013	<0.001 *	8.1	-107.5	-15.0
(g m^{-2})		2014	<0.001 *	1.2	-62.8	-0.3
		2015	0.435			
Plant Height	*Rht-D1*	2013	<0.001 *	18	9.8	9.7
(cm)		2014	<0.001 *	22	11.5	34.8
		2015	<0.001 *	24	14.8	2.1
	Xgwm293	2013	0.408			
		2014	0.001 *	3.8	-4.8	-10.6
		2015	<0.001 *	5.4	-7.1	-8.9

[a] Average trait difference between lines with the 'B' vs. the 'K' allele.

* significant association ($p<0.05$).

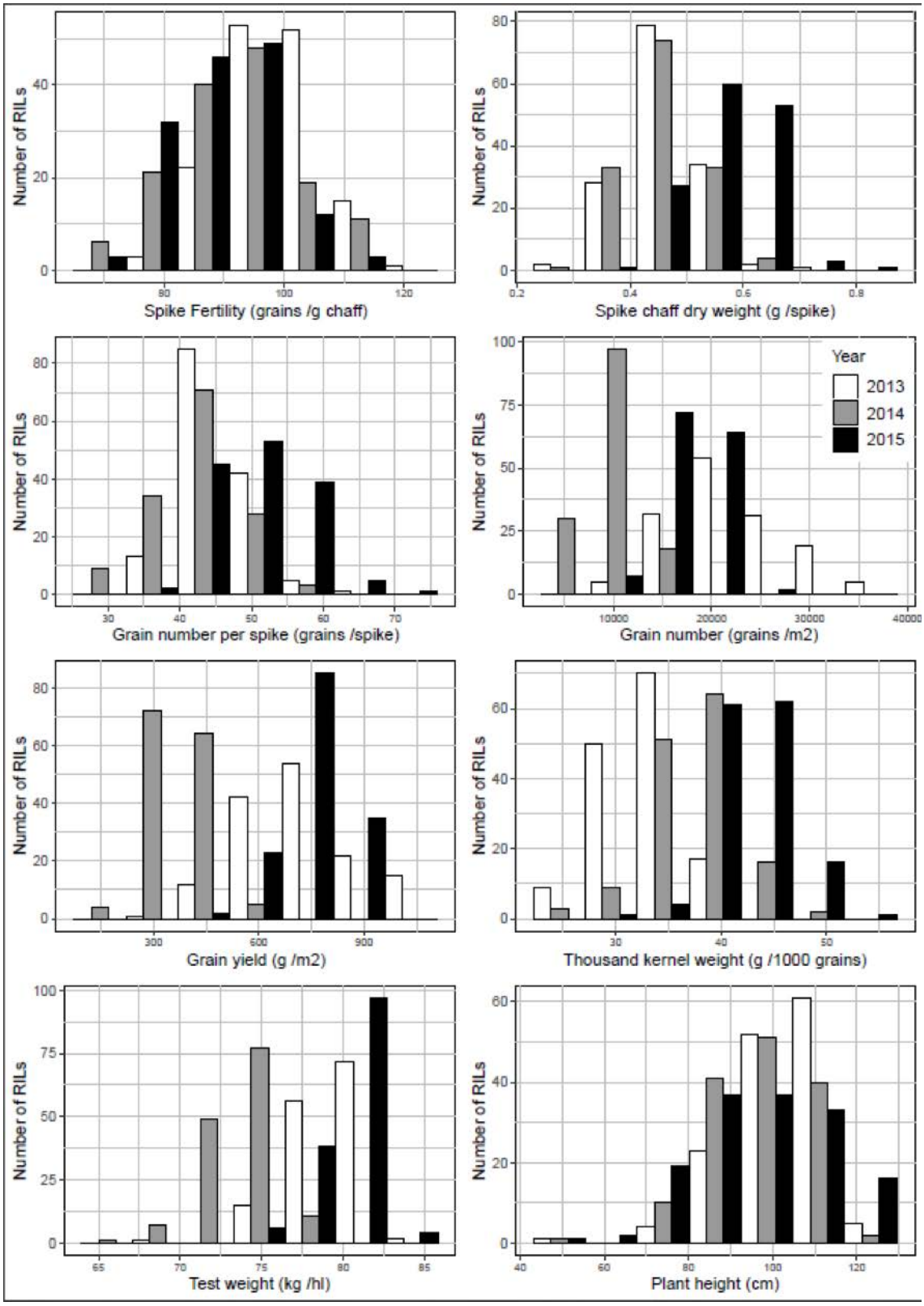

Figure S2. Histograms of (A) spike fertility index, (B) spike chaff dry weight, (C) grain number per spike, (D) grain number per m^2, (E) grain yield, (F) grain weight, (G) test weight and (H) plant height, in a RIL population ('Baguette 10' x 'Klein Chajá') evaluated in 2013 (white), 2014 (grey) and 2015 (black) at Balcarce, Argentina. Values for the parents are indicated with stars ('Baguette 10') and triangles ('Klein Chajá').

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