Journal of Agricultural Science, Page 1 of 9.
doi:10.1017/S0021859612000068

CROPS AND SOILS RESEARCH PAPER

A method of screening for spike fertility in wheat

P. E. ABBATE[1]*, A. C. PONTAROLI[1,2]*, L. LÁZARO[3] AND F. GUTHEIM[4]

[1] *Estación Experimental Agropecuaria (EEA) Balcarce, Instituto Nacional de Tecnología Agropecuaria (INTA), CC 276 (7620) Balcarce, Buenos Aires, Argentina*
[2] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*
[3] *Facultad de Agronomía, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNICEN), CC 178 (7300) Azul, Buenos Aires, Argentina*
[4] *Chacra Experimental Miramar, Ministerio de Asuntos Agrarios (MAA) de la Provincia de Buenos Aires, CC 35 (7607) Miramar, Buenos Aires, Argentina*

(*Received 26 July 2011; revised 16 November 2011; accepted 11 January 2012*)

SUMMARY

Wheat grain yield is often associated with grain number/m^2. Spike fertility (SF), i.e. the quotient between grain number and spike chaff dry weight, is a major component of grain number/m^2 determination. Several methodologies have been proposed in the literature for field determination of SF, but they are tedious and expensive. Also, no comparison between methodologies has been done. The feasibility of using wheat SF as a selection criterion in a breeding programme or as a variable of interest in crop physiology studies depends largely upon the availability of a simpler and faster method for collecting and processing samples. Thus, the objective of the present study was to determine: (1) the association between SF calculated with the non-grain spike dry weight at anthesis (reference method) or at crop maturity, (2) the association between SF evaluated at the plot level (i.e. both non-grain spike dry weight and grain number determined as per area unit) and at the individual spike level and (3) the minimum number of individual spikes that should be sampled for the development of a screening method that can be applied in wheat breeding programmes or in crop physiology studies. Associations between variables were determined by correlation analysis of treatment means, and by a test of agreement for categorical rating (low, medium and high SF) between individual data of each variable. Four experiments (BY95, BC96, BC97 and ML07) were performed with five, ten, eight and eight wheat cultivars, respectively, under no environmental limitations, except for experiment ML07 which was not irrigated. In the first three experiments, SF was determined both at the beginning of grain filling and at maturity, in plot-size samples (0·8 m^2/plot). In experiments BC96 and BC97, SF was determined both in plot-size samples and in individual spikes (five spikes per plot), at the beginning of grain filling. In experiment ML07, increasing numbers of individual spikes were sampled at maturity to assess SF. As a result: (1) a significant association ($R^2 = 0{\cdot}78$; $P < 0{\cdot}001$; D.F. = 20) was detected between SF determined at the beginning of grain filling and at maturity, and the test of agreement for categorical rating showed that the classification of data into categories of SF was equivalent between methods ($P > 0{\cdot}05$); (2) when comparing SF determined in large plot-size samples *v.* in small samples of individual spikes, a good adjustment ($R^2 = 0{\cdot}77$; $P < 0{\cdot}001$; D.F. = 6) was also observed, with no significant cultivar × experiment interaction and a good agreement between methods in the classification of data into categories of SF ($P > 0{\cdot}05$); and (3) increasing sample size from 5 to 40 spikes gradually decreased the average relative standard error of the mean (from 0·034 to 0·012, respectively). In conclusion, wheat SF can be determined in a fairly accurate way by sampling a small group of individual spikes at crop maturity, thereby allowing the evaluation of a large number of treatments in a timely fashion and the screening of breeding material from early generations.

* To whom all correspondence should be addressed. Email: pabbate@balcarce.inta.gov.ar, apontaroli@balcarce.inta.gov.ar

INTRODUCTION

In most wheat-producing countries including Argentina, increasing grain yields is, and will continue to be, one of the main goals of wheat breeding programmes. Genetic progress in wheat grain yield over the last 30–40 years has been attributed largely to an increase in grain number/m^2 (Fischer 2007). According to Fischer (1984), under non-limiting environmental conditions, wheat grain number/m^2 can be considered as the product of the duration of the spike growth phase (SGP), crop growth rate during SGP, dry matter partitioning to spikes during SGP and the number of grains per unit of spike dry weight, i.e. an indicator of spike fertility (SF). Yield increases accomplished in the 1970s were mainly due to greater dry matter partitioning to spikes, as a consequence of the introgression of dwarfing genes (Austin *et al*. 1980; Fischer & Stockman 1986; Brooking & Kirby 1981; Slafer *et al*. 1990; Youssefian *et al*. 1992; Reynolds *et al*. 1999). Manipulation of the length of SGP and crop growth rate during SGP has been attempted from a breeding standpoint, with variable results (see Slafer 2007; Fischer 2007). In contrast, SF has only been investigated with an ecophysiological approach (Abbate *et al*. 1995, 1997*b*, 1998; Abbate & Lázaro 2001; González *et al*. 2005; Lázaro *et al*. 2010, 2011; among others) but not used, at least intentionally, as a selectable trait in a yield breeding programme.

The information available strongly suggests that SF would be an attractive trait to screen and select for in a yield breeding programme. For instance, data from Stapper & Fischer (1990) revealed differences in number of grains/g spike dry weight at maturity among Australian wheat cultivars, which were associated with grain number/m^2. Abbate *et al*. (1998) reported that differences in SF explained most of the differences in grain yield among Argentine wheat cultivars across a wide range of yield variation. Shearman *et al*. (2005) also detected large differences in SF among British cultivars, whereas Acreche *et al*. (2008) concluded that genetic gain in wheat grain yield in Spain could be largely ascribed to an increase in SF (which they termed 'fruit efficiency'). Moreover, Abbate *et al*. (2007) observed that the ranking order in the SF of a group of Argentine cultivars was maintained across several environments, even under sub-potential environmental conditions, and Lázaro & Abbate (2011) found that variations of grain number among cultivars in response to the photothermal quotient were greatly associated with their SF. Recently, Fischer (2011), Foulkes *et al*. (2011) and González *et al*. (2011) discussed the idea of focusing on SF as one of the avenues for increasing yield potential.

The methodology typically used for field determining SF (Abbate *et al*. 1997*a*, *b*) involves three steps. First, a crop sample of 0·5–1 m^2 is drawn near the beginning of grain filling (*c*. 7 days after anthesis) for determining the spike dry weight per unit area, after discounting the weight of the developing grains (detached from the spikes with forceps). Then, a crop sample of similar size is drawn at maturity for determining the grain number per unit area. Finally, SF is calculated as the quotient between the grain number and the non-grain spike weight (hereafter called spike chaff dry weight). According to the present authors' experience, a person can hardly collect and process more than 15 crop samples per day, or detach grain from spikes in more than 15 samples per day. This means the procedure is slow, tedious and expensive, and hence not useful for screening a large number of treatments. Therefore, the feasibility of using wheat SF as a selection criterion in a breeding programme or as a variable of interest in crop physiology studies depends largely upon the availability of a simpler and faster method for collecting and processing samples. This would allow screening of a large number of individuals, families and/or other treatments (e.g. nutrient levels) simultaneously. The objective of the present study was to determine: (1) the correlation between SF calculated with the spike chaff dry weight at anthesis (as proposed by Abbate *et al*. 1997*b*) and at crop maturity, (2) the correlation between SF evaluated at the plot level (i.e. both spike chaff dry weight and grain number determined as per area unit) and at the individual spike level (i.e. in a small sample of spikes) and (3) the minimum number of individual spikes that should be sampled for the development of a screening method that can be applied in wheat breeding programmes or in crop physiology studies.

MATERIALS AND METHODS

Plant material, crop husbandry and experimental design

Four field experiments were carried out, termed BY95, BC96, BC97 and ML07. A randomized complete block design with four replications in BY95 and BC96, and three replications in BC97 and ML07, was used. Experiments BY95, BC96 and BC97 were

Table 1. *Wheat cultivars evaluated in experiments BY95, BC96, BC97 and ML07; sowing year and location of each experiment. Cultivars included in each experiment are marked with X*

Cultivar				Experiment, sowing year and location			
Denomination*	Origin†	Type	Cycle‡	*BY95* 1995 Balcarce	*BC96* 1996 Balcarce	*BC97* 1997 Balcarce	*ML07* 2007 Miramar
ACA 303	A	Bread	Long				X
B14994 line	A	Bread	Short			X	
Bacanora	M	Bread	Short	X	X		
Baguette Premium 11	A	Bread	Long				X
Baviacora	M	Bread	Short	X	X		
BF1776 line	A	Durum	Short	X			
BIOINTA 1001	A	Bread	Short				X
BIOINTA 1002	A	Bread	Short				X
BIOINTA 1003	A	Bread	Short				X
Buck Ámbar	A	Durum	Short	X		X	
Buck Charrúa	A	Bread	Long		X		
Buck Cristal	A	Durum	Short		X		
Granero INTA	A	Bread	Short	X	X	X	
Klein Chajá	A	Bread	Short				X
Klein Escorpión	A	Bread	Long				X
Klein Tauro	A	Bread	Short				X
PROINTA Cinco Cerros	A	Bread	Long		X		
PROINTA Bonaerense Redomón	A	Bread	Long			X	
PROINTA Elite	A	Bread	Short		X		
PROINTA Oasis	A	Bread	Long		X	X	
PROINTA Pigüé	A	Bread	Long			X	
PROINTA Puntal	A	Bread	Long		X	X	
PROINTA Quintal	A	Bread	Short		X		
PROINTA Súper	A	Bread	Long			X	

* In alphabetical order.
† A: Argentina; M: Mexico (CIMMYT).
‡ As determined in Balcarce.

conducted at Balcarce, Argentina (37°45′S, 58°18′W, 130 m asl); experiment ML07 was carried out at Miramar, Argentina (38°10′S, 58°00′W, 64 m asl). Wheat cultivars evaluated in these experiments are listed in Table 1. The experimental unit consisted of a plot of seven rows wide (inter-row distance 0·2 m) by *c*. 5·5 m long; no crop samples were drawn from the first or seventh row, which were discarded. Diseases, weeds and pests were chemically controlled in all experiments. Seeds were treated with a benomyl–thiram mixture before seeding and weeds were controlled with a commercial mixture (Misil II) of metsulfuron methyl (5 g a.i./ha) and dicamba (60 g a.i./ha). Fungal diseases were prevented with tebuconazole (Folicur, 187 g a.i./ha). With the exception of experiment ML07, which was not irrigated, none of the experiments experienced water or nutrient limitations. Cultivars were sown in batches according to the duration of their life cycle (Table 1), so that all would have a fairly similar anthesis date. At the individual spike level, anthesis date was defined as the day on which the spike showed at least one spikelet with an extruded anther. At the plot level, anthesis date was defined as the day on which half of the spikes showed at least one spikelet with an extruded anther. For this purpose, the number of spikes at anthesis was computed in 40–50 spikes per plot every 2–3 days, and the date on which half of the spikes were at anthesis was calculated by linear interpolation of anthesis proportion on calendar time.

Evaluation of SF at anthesis *v*. maturity

In experiments BY95, BC96 and BC97, biomass samples were collected 7 days after anthesis (A+7),

i.e. end of the non-grain spike growth period (Abbate *et al.* 1997*b*), by cutting plants from the five central rows of each plot (0·8 m^2) at ground level. A sub-sample of 40 tillers per sample was dissected to determine the dry weight proportion of stems, leaves and spikes. In a sub-sample of three to five spikes, immature grains were detached from the spike and weighed to determine their contribution to spike dry weight. These data were used to calculate spike chaff dry weight per area unit at A+7.

At maturity, another crop sample of 0·8 m^2 was taken from each plot. A sub-sample of 40 tillers per sample were cut at the lowest spikelet level, dried, weighed and threshed to determine grain proportion of spike dry weight. Then, the remaining spikes were threshed for assessing grain yield. Dry weight per grain was determined by counting and weighing two groups of 500 grains each from a manually cleaned subsample. This variable and grain yield were used to calculate grain number/m^2. Spike chaff dry weight/m^2 at maturity was calculated with the weight proportion of grain to spike and with grain yield. SF at A+7 (SF_{A+7}) was calculated as the quotient between grain number/m^2 at maturity and spike chaff dry weight/m^2 at A+7. This formula, proposed by Abbate *et al.* (1997*a*, *b*), was considered here as the reference method. SF at maturity (SF_M), on the other hand, was calculated as the quotient between grain number/m^2 and spike chaff dry weight/m^2, both determined at maturity.

Evaluation of SF at the plot *v.* individual spike level

In experiments BC96 and BC97, SF_{A+7} was determined at the plot level as described previously. This variable was then compared with SF evaluated on an individual spike basis. SF of individual spikes was assessed 15 days after anthesis (A+15) instead of A+7, because: (1) both the number of grains and spike weight had to be determined for each individual spike, which posed the need to determine grain number and spike weight in the same spike, (2) the immature grains were more easily distinguished at A+15 than at A+7; therefore, removing the grains from the spike at A+15 was much easier than it was at A+7 and (3) spike weight (devoid of grain) did not change significantly from A+7 to A+15 (Abbate *et al.* 1997*b*). Forty spikes per plot were harvested; length and spikelet number were determined in each spike, and five spikes of length and spikelet number similar to the plot average were drawn from the harvested sample. Grains were removed and counted, and spikes devoid of grain were dried and weighed. SF at the individual spike level (SF_{ind}) was calculated for each of these spikes as the quotient between grain number and spike chaff dry weight and averaged by plot.

Evaluation of SF at different sample sizes

In experiment ML07, 40 individual spikes were sampled per plot at maturity. Sampling was done by drawing five consecutive spikes in each of eight different spots within a plot. Each group of spikes was dried and weighed and the grains were then detached from the spikes, counted and weighed. SF was calculated for each group of spikes as the quotient between grain number and spike chaff dry weight.

Statistical analysis

An ANOVA was performed for each of experiments BY95, BC96 and BC97. An additional ANOVA was done according to Annicchiarico (2002) with combined data from experiments BC96 and BC97, comprising only those cultivars evaluated in both experiments. To ascertain the association between SF_{A+7} and SF_M, regression and correlation analysis were carried out with data from experiments BY95, BC96 and BC97, both individually and jointly. Data from cultivars included in both experiments BC96 and BC97 were used to calculate the regression and correlation between SF_{A+7} and SF_{ind}. All regressions were carried out with the treatment means.

In the context of a breeding programme, the feasibility of separating genetic materials into SF categories (e.g. low, medium and high) would be very useful. Thus, the McNemar test of agreement for categorical rating (Sun & Yang 2008) was performed for comparing all different methods of SF estimation, using the MH Program software (Uebersax 2006). Data were ordered and separated into three categories of equal number of observations (i.e. 1/3 each).

Data generated in experiment ML07 were used to determine the evolution of the standard error of the mean of each treatment upon changes in sample size (n). The method for defining the sample size was based on the 'confidence interval method' described by Montgomery (1997). The relative standard error of

Table 2. *Cultivar effects on grain number/m^2 at maturity (GN_M), spike fertility measured 7 days after anthesis (SF_{A+7}) and maturity (SF_M), in three experiments at Balcarce*

Cultivar	GN_M (grains 10^3/m^2)	SF_{A+7} (grains/g)	SF_M (grains/g)
Experiment BY95			
Bacanora	22·5	103	83
Baviacora	19·2	98	77
Granero INTA	17·7	78	58
BF1776 line	13·5	56	44
S.E.M. (9 D.F.)*	0·46	4·1	3·0
P (*F*>Fp)†	<0·001	<0·001	<0·001
Experiment BC96			
Buck Charrúa	15·1	76	54
PROINTA Cinco Cerros	16·9	66	52
PROINTA Oasis	18·7	79	66
PROINTA Puntal	24·6	112	85
Bacanora	22·1	113	70
Baviacora	16·8	84	67
Buck Cristal	11·7	52	35
PROINTA Elite	17·6	78	59
Granero INTA	16·1	68	60
PROINTA Quintal	16·2	83	68
S.E.M. (27 D.F.)*	0·70	4·4	5·2
P (*F*>Fp)†	<0·001	<0·001	<0·001
Experiment BC97			
PROINTA Puntal	22·0	107	87
PROINTA Súper	21·0	96	58
PROINTA Bonaerense Redomón	17·4	75	63
PROINTA Pigüé	15·0	81	53
B14994 line	17·3	80	58
Buck Ámbar	11·2	45	35
Granero INTA	16·3	65	60
PROINTA Oasis	14·4	76	54
S.E.M. (14 D.F.)*	0·81	5·7	3·4
P (*F*>Fp)†	<0·001	<0·001	<0·001
Mean of experiments BC96 and BC97			
Granero INTA	16·2	67	60
PROINTA Oasis	16·5	78	61
PROINTA Puntal	23·3	110	86
Durum‡	11·4	49	35
S.E.M. (15 D.F.)*	0·57	3·68	2·29
P (*F*>F_p)†			
Experiment	0·007	0·214	0·349
Cultivar	<0·001	<0·001	<0·001
Experiment×cultivar	0·148	0·977	0·208

* S.E.M., standard error of mean of cultivars; D.F., degrees of freedom.
† Probability of *F*-test (Fp) from ANOVA.
‡ Mean of durum wheats Buck Cristal and Buck Ámbar.

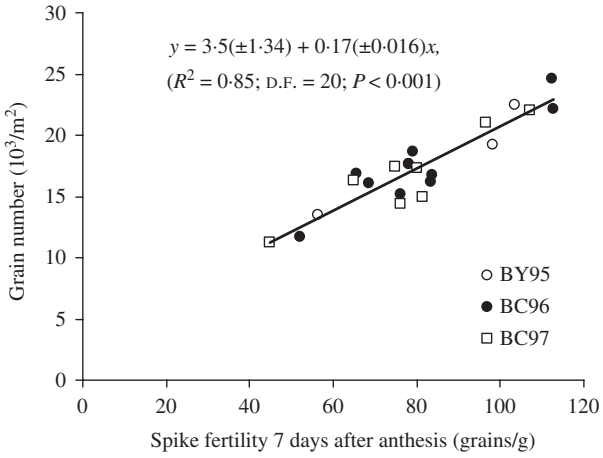

Fig. 1. Relationship between grain number/m^2 and SF 7 days after anthesis, in three experiments (BY95, BC96 and BC97) at Balcarce. One line per individual experiment did not produce a better fit. The S.E.M. are shown in Table 2.

the mean (RSEM) was calculated as a function of *n*, as follows:

$$S = \sqrt{\frac{\sum_{i=1}^{i=p} (X_i - \overline{X})^2}{p}} \quad (1)$$

$$\text{RSEM} = \frac{S/\sqrt{n}}{\overline{X}} \quad (2)$$

where *S* is the standard deviation of $_X$, *p* is the total number of observations, X_i is the value of the *i*th observation and $\overline{X}$ is the mean of *X*.

RESULTS

Differences between cultivars were observed for grain number/m^2, SF_{A+7} and SF_M in experiments BY95, BC96 and BC97 (Table 2), and an association between grain number/m^2 and SF_{A+7} was found (Fig. 1). Analysis of combined data from those cultivars in common between experiments BC96 and BC97 (Granero INTA, PROINTA Oasis, PROINTA Puntal and a durum wheat cultivar, Buck Cristal or Buck Ámbar) showed significant differences between cultivars with no cultivar×experiment interaction (Table 2).

Correlation analysis between SF_M and SF_{A+7} (as method of reference) was carried out for each experiment individually. Both variables were linearly associated in each of the experiments BY95, BC96

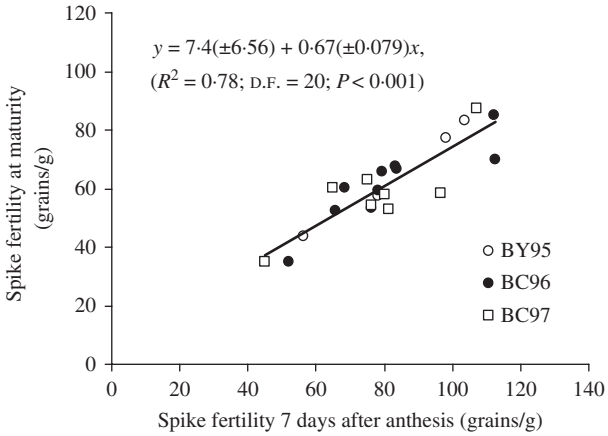

Fig. 2. Relationship between SF measured at maturity and 7 days after anthesis, both at plot level, in three experiments (BY95, BC96 and BC97) at Balcarce. One line per individual experiment did not produce a better fit. The S.E.M. are shown in Table 2.

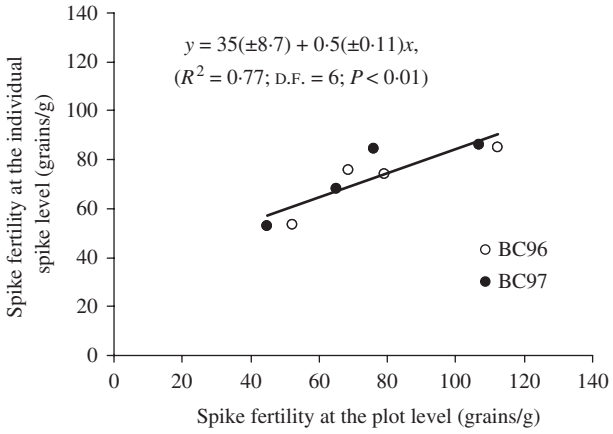

Fig. 3. Relationship between SF determined in large samples 7 days after anthesis (i.e. plot level) and in small samples 15 days after anthesis (i.e. individual spike level), for four cultivars in two experiments (BC96 and BC97) at Balcarce. One line per individual experiment did not produce a better fit. The S.E.M. are shown in Table 3.

and BC97, with $R^2 = 0{\cdot}99$, 0·78 and 0·68, respectively ($P < 0{\cdot}001$). As the fitted regression did not differ significantly among experiments, a line combining data from all three experiments is shown in Fig. 2. The linear fit was highly significant ($P < 0{\cdot}001$) with $R^2 = 0{\cdot}78$. The McNemar test showed complete agreement between SF_M and SF_{A+7} as methods of classification.

The relationship between SF_{ind} (individual spikes) and SF_{A+7} (i.e. plot samples) is shown in Fig. 3. For the four cultivars evaluated in both experiments BC96 and BC97, a linear, highly significant adjustment was detected ($P < 0{\cdot}001$; $R^2 = 0{\cdot}77$). Differences between cultivars were observed for SF_{A+7} as well as for SF_{ind} (Table 3). The McNemar test showed agreement between SF_{ind} and SF_{A+7} as methods for classification, with only a proportion of 0·20 of discrepancies. None of these discrepancies involved the most extreme categories (i.e. low and high SF).

The evolution of the RSEM, estimated by Eqn (2) and statistics (Table 4), as a function of increasing quantities of spikes sampled, is shown in Fig. 4. For the eight cultivars evaluated in experiment ML07, reducing the sample size from 15 to 10 or five spikes increased the RSEM, but its estimated value continued to be, on average, rather low: 0·019 for $n = 15$, 0·024 for $n = 10$ and 0·034 for $n = 5$ (maximum RSEM among cultivars was 0·024, 0·029 and 0·041, respectively). In addition, further increasing sample size from 15 to 40 spikes only slightly reduced the estimated RSEM, to an average of 0·012 for $n = 40$ (maximum RSEM between cultivars 0·015).

DISCUSSION

According to the approach originally proposed by Fischer (1984), SF is a component of potential grain number/m^2 in wheat. Subsequently, Abbate *et al.* (1998) found evidence of the importance of SF_{A+7} in grain number/m^2 determination in a group of Argentine cultivars. More recently, Acreche *et al.* (2008) confirmed this in Spanish cultivars. In the present study, a strong correlation between grain number/m^2 and SF_{A+7} was detected, providing additional support to the cited literature. However, the method used by the cited authors for SF determination is laborious, time-consuming, expensive and destructive. This makes it impracticable in the context of a breeding programme, in which a large number of entries have to be screened within a short time frame and the seed has to be conserved for further generations. The present results indicate that SF determination at maturity can satisfactorily substitute for SF determination around anthesis, thereby allowing the evaluation of SF after all the breeding material has been harvested.

Some studies (Abbate *et al.* 1997*b*; Fischer & Stockman 1980; Fischer 1985) have shown that spike chaff dry weight at maturity may not be a good estimator of spike chaff dry weight at the beginning of grain filling, which could constitute a potential caveat of the methodology proposed here. Abbate *et al.* (1997*b*) observed that the greater the availability of assimilates during grain filling, the greater the increase in spike dry weight: the increase was 38% under good

Table 3. *Cultivar effects on SF determined in large samples 7 days after anthesis (i.e. plot level; SF_{A+7}) and in small samples 15 days after anthesis (i.e. individual spike level; SF_{ind}), for four cultivars in two experiments at Balcarce*

Cultivar	SF_{A+7} (grains/g)	SF_{ind} (grains/g)
Experiment BC96		
Granero INTA	68	76
PROINTA Oasis	79	74
PROINTA Puntal	112	85
Buck Cristal	52	53
S.E.M. (15 D.F.)*	4·8	2·1
Experiment BC97		
Granero INTA	65	68
PROINTA Oasis	76	84
PROINTA Puntal	107	86
Buck Ámbar	45	53
S.E.M. (15 D.F.)*	5·6	2·7
Mean of experiments BC96 and BC97		
Granero INTA	67	72
PROINTA Oasis	78	79
PROINTA Puntal	110	85
Durum†	49	53
S.E.M. (15 D.F.)*	3·7	2·1
P(*F* > Fp)‡		
Experiment	0·214	0·728
Cultivar	< 0·001	< 0·001
Experiment × cultivar	0·977	0·269

* S.E.M., standard error of mean of cultivars; D.F., degrees of freedom.
† Mean of durum wheats Buck Cristal and Buck Ámbar.
‡ Probability of *F*-test (Fp) from ANOVA.

growing conditions, but as low as −8% under shading or the occurrence of lodging. In the experiments shown in Fig. 2, spike chaff dry weight increased, on average, 33% from anthesis to maturity. As a result, mean SF_{A+7} (80 grains/g) was greater than mean SF_M (61 grains/g), and thus the relationship between these variables exhibited a slope less than one (Fig. 2). However, the present study did not aim to evaluate the evolution of spike chaff dry weight under environmental changes, but focused on SF of different genotypes growing under the same environment. Thus, the SF variation during grain filling should not be relevant in this context, unless there was a significant genotype × environment interaction; however, the results presented here (Table 2) show that this interaction is very low and never statistically significant.

Table 4. *Parameters for estimating the RSEM of SF at maturity as a function of sample size (*n *in Eqn (2); Fig. 4), for eight cultivars in experiment ML07.* $\overline{X}$ *is the mean and* S *is the standard deviation of* X*, obtained with 24 observations (each one of 5-spike samples, i.e. parameter* p *of Eqn (1)). RSEM is expressed as relative to the mean, calculated for* n = *p*

	$\overline{X}$ (grains/g)	*S* (grains/g)	RSEM (1/100)
ACA 303	72	6·4	1·8
Baguette Premium 11	102	6·7	1·3
BIOINTA 1001	74	6·3	1·7
BIOINTA 1002	54	5·0	1·9
BIOINTA 3004	72	5·4	1·5
Klein Chajá	78	5·3	1·4
Klein Escorpión	72	4·3	1·2
Klein Tauro	70	4·7	1·4
General mean	74	5·5	1·5

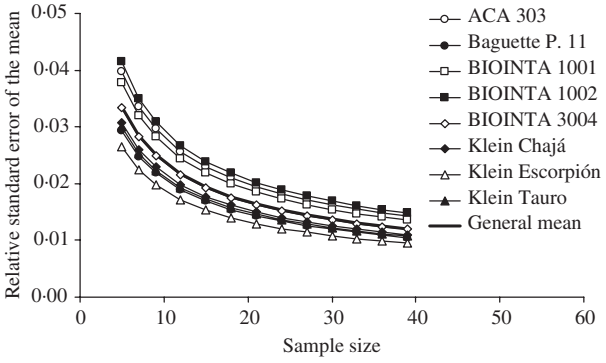

Fig. 4. Evolution of the RSEM, estimated by Eqn (2) and Table 4 statistics, as a function of increasing quantities of spikes sampled, for eight cultivars in experiment ML07 at Miramar. Symbols help to identify the curve for each cultivar, they do not represent data points.

The data discussed above were obtained with samples collected per area unit; that is, at the plot scale. From a breeding standpoint, this would only be applicable to advanced generations, which have enough seed for carrying out plot-size evaluations. Screening and selection in the initial stages of a breeding programme, on the other hand, have the obvious advantage of fixing a trait early during the breeding process, so long as it has mild to high heritability. SF appears to be genetically controlled, with a low genotype × environment interaction (calculated from Abbate *et al.* 1998, 2007; Lázaro & Abbate 2011 and Table 2), which merits attempting its

evaluation in early generations. The present results indicate that SF determination at the individual spike level appears to yield substantially similar results to SF determination at the plot level. Thus, the application of the latter method would be acceptable in the context of a breeding programme aiming to select for high SF.

In the experiment ML07 discussed above, a sample size of five individual spikes per plot was used for SF determination (i.e.15–20 spikes per treatment). But would increasing the sample size significantly reduce the RSEM? As shown in Fig. 4, increasing the sampling size to 10–15 spikes decreased the RSEM, but only a negligible decline in RSEM was achieved when further increasing the sample size. Therefore, drawing *c*. 10–15 spikes per treatment seems to yield satisfactory results, and it constitutes a fairly manageable amount of sampling and processing material in practical terms. Moreover, such a sample size would allow the process to be automated, i.e. a machine could be designed to process samples more quickly than manually.

In conclusion, wheat SF can be determined in a fairly accurate way by sampling a small group of individual spikes at crop maturity, thereby allowing the evaluation of a large number of treatments in a timely fashion and the screening of breeding material from early generations.

We thank María Castaño for her technical assistance in experiment ML07.

REFERENCES

Abbate, P. E., Andrade, F. H. & Culot, J. P. (1995). The effects of radiation and nitrogen on number of grains in wheat. *Journal of Agricultural Science, Cambridge* **124**, 351–360.

Abbate, P. E., Andrade, F. H., Culot, J. P. & Bindraban, P. S. (1997*b*). Grain yield in wheat: Effects of radiation during spike growth period. *Field Crops Research* **54**, 245–257 [Erratum, 1998, 56, 317–318].

Abbate, P. E., Andrade, F. H., Lázaro, L., Bariffi, J. H., Berardocco, H. G., Inza, V. H. & Marturano, F. (1998). Grain yield increase in recent Argentine wheat cultivars. *Crop Science* **38**, 1203–1209.

Abbate, P. E. & Lázaro, L. (2001). Ecofisiología del trigo candeal. In *Manual de Trigo Candeal* (Eds Chacra Experimental Integrada Barrow), pp. 23–29. Tres Arroyos, Argentina: Chacra Experimental Integrada Barrow.

Abbate, P. E., Lázaro, L. & Andrade, F. H. (1997*a*). ¿Es posible incrementar el número de granos por unidad de superficie en trigo? *Explorando Altos Rendimientos en Trigo. INIA La Estanzuela, Colonia, Uruguay, October 20–23, 1997* (Eds M. M. Kohli & D. Martino), pp. 71–90. Uruguay: CIMMYT-INIA.

Abbate, P. E., López, J. R., Brach, A. M., Gutheim, F. & Gonzalez, F. (2007). Fertilidad de las espigas de trigo en ambientes sub-potenciales. *Workshop Internacional: Ecofisiología vegetal aplicada al estudio de la determinación del rendimiento y la calidad de los cultivos de granos. Mar del Plata, Buenos Aires, Argentina, September 6–7, 2007* (Eds B. Kruk & R. Serrago), pp. 2–3. Buenos Aires, Argentina: FAUBA.

Acreche, M. M., Briceño-Félix, G., Sánchez, J. A. M. & Slafer, G. A. (2008). Physiological bases of genetic gains in Mediterranean bread wheat yield in Spain. *European Journal of Agronomy* **28**, 162–170.

Annicchiarico, P. (2002). *Genotype × Environment Interactions: Challenges and Opportunities for Plant Breeding and Cultivar Recommendations*. Plant Production and Protection Paper 174. Rome: FAO.

Austin, R. B., Bingham, J., Blackwell, R. D., Evans, L. T., Ford, M. A., Morgan, C. L. & Taylor, M. (1980). Genetic improvements in winter wheat yield since 1900 and associated physiological changes. *Journal of Agricultural Science, Cambridge* **94**, 675–689.

Brooking, I. R. & Kirby, E. J. M. (1981). Interrelationships between stem and ear development in winter wheat: the effects of a Norin 10 dwarfing gene Gai/Rht2. *Journal of Agricultural Science, Cambridge* **97**, 373–381.

Fischer, R. A. (1984). Growth and yield of wheat. In *Potential Productivity of Field Crops under Different Environments* (Eds W. H. Smith & S. J. Banta) pp. 129–154. Los Baños, Philippines: IRRI.

Fischer, R. A. (1985). Number of kernels in wheat crops and the influence of solar radiation and temperature. *Journal of Agricultural Science, Cambridge* **105**, 447–461.

Fischer, R. A. (2007). Understanding the physiological basis of yield potential in wheat. *Journal of Agricultural Science, Cambridge* **145**, 99–113.

Fischer, R. A. (2011). Wheat physiology: a review of recent developments. *Crop and Pasture Science* **62**, 95–114.

Fischer, R. A. & Stockman, Y. M. (1980). Kernel number per spike in wheat (Triticum aestivum L.). Responses to preanthesis shading. *Australian Journal of Plant Physiology* **7**, 169–180.

Fischer, R. A. & Stockman, Y. M. (1986). Increased kernel number in Norin 10-derived dwarf wheat: evaluation of the cause. *Australian Journal of Plant Physiology* **13**, 767–784.

Foulkes, M. J., Slafer, G. A., Davies, W. J., Berry, P. M., Sylvester-Bradley, R., Martre, P., Calderini, D. F., Griffiths, S. & Reynolds, M. P. (2011). Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. *Journal of Experimental Botany* **62**, 469–486.

González, F. G., Slafer, G. A. & Miralles, D. J. (2005). Floret development and survival in wheat plants exposed to contrasting photoperiod and radiation environments during stem elongation. *Functional Plant Biology* **32**, 189–197.

González, F. G., Terrile, I. I. & Falcón, M. O. (2011). Spike fertility and duration of stem elongation as promising traits to improve potential grain number (and yield): variation in modern Argentinean wheats. *Crop Science* **51**, 1693–1702.

Lázaro, L. & Abbate, P. E. (2011). Cultivar effects on relationship between grain number and photothermal quotient or spike dry weight in wheat. *Journal of Agricultural Science, Cambridge*, 14 September 2011. DOI:10.1017/S0021859611000736.

Lázaro, L., Abbate, P. E., Cogliati, D. H. & Andrade, F. H. (2010). Relationship between yield, growth and spike weight in wheat under phosphorus deficiency and shading. *Journal of Agricultural Science, Cambridge* **148**, 83–93.

Montgomery, D. C. (1997). Experiments with a single factor: the analysis of variance. *Design and Analysis of Experiments*, 5th edn (Ed. D. C. Montgomery), pp. 60–125. New York: John Wiley & Sons, Inc.

Reynolds, M. P., Rajaram, S. & Sayre, K. D. (1999). Physiological and genetic changes of irrigated wheat in the post-green revolution period and approaches for meeting projected global demand. *Crop Science* **39**, 1611–1621.

Shearman, V. J., Sylvester-Bradley, R., Scott, R. & Foulkes, M. (2005). Physiological processes associated with wheat yield progress in the UK. *Crop Science* **45**, 175–185.

Slafer, G. A. (2007). Physiology of determination of major wheat yield components. In *Wheat Production in Stressed Environments* (Eds H. T. Buck, J. E. Nisi & N. Salomón), pp. 557–565. Dordrecht, the Netherlands: Springer.

Slafer, G. A., Andrade, F. H. & Satorre, E. H. (1990). Genetic-improvement effects on pre-anthesis physiological attributes related to wheat grain-yield. *Field Crops Research* **23**, 255–263.

Stapper, M. & Fischer, R. A. (1990). Genotype, sowing date and plant spacing influence on high-yielding irrigated wheat in Southern New South Wales. II. Growth, yield and nitrogen use. *Australian Journal of Agricultural Research* **41**, 1021–1041.

Sun, X. & Yang, Z. (2008). Generalized McNemar's test for homogeneity of the marginal distributions. In *The SAS Global Forum 2008 Conference* (Eds SAS Users Group International), Paper 382–2008, pp. 1–10. Cary, NC: SAS Institute Inc. Available online at http://www2.sas.com/proceedings/forum2008/382–2008.pdf (verified 25 November 2011).

Uebersax, J. S. (2006). User Guide for the MH Program (version 1.2). Available online from the Statistical Methods for Rate Agreement http://john-uebersax.com/stat/mh.htm (verified 25 November 2011).

Youssefian, S., Kirby, E. J. M. & Gale, M. D. (1992). Pleiotropic effects of the GA-insensitive *Rht* dwarfing genes in wheat. 2. Effects on leaf, stem, ear and floret growth. *Field Crops Research* **28**, 191–210.