A quantitative approach to characterize sink–source relationships during grain filling in contrasting wheat genotypes

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A B S T R A C T

We present a simple generic framework to quantify source–sink relationships during grain filling, by using a determinate growth function which has a unique property, namely being able of explicitly describing the time for the end of a growth process. This model framework was applied to analyze these relationships in plants of six wheat (*Triticum aestivum* L.) genotypes grown in pots in climate-controlled greenhouses under two temperature regimes (day/night: 20/15 and 25/20 °C). The function accurately described the sigmoid pattern of grain growth (sink activity), as its modified form did for the reversed sigmoid shape of flag-leaf area (source capacity), during grain filling. The six genotypes differed significantly in grain number as well as in grain yield, ranging from 54 to 81 grains and from 2.67 to 4.52 g DM per culm, respectively, when grown at 20/15 °C. Biomass and grain yield were significantly reduced by a rise of 5 °C. Grain nitrogen contents raised from 2.1 to 2.6% as a consequence of less carbon accumulation resulting in lower grain weights at the high temperature. On average, a rise of 5 °C in temperature reduced the duration of grain growth by 12 days (>30%), and increased the growth rate from 1.32 to 1.67 mg grain^{-1} d^{-1} (20%). Genotypic differences in grain-filling duration were also larger than in rate of grain growth. The genetic variation in the flag-leaf area duration (a proxy for the capacity for intercepting radiation and photosynthesis) was positively associated with sink size. Model analysis showed that whether or not the timing for the cessation of grain filling and for the end of post-anthesis source activity was synchronized depended on temperature. The quantitative approach yielded parameters that characterize genotypic differences of post-anthesis source and sink capacity in responding to environmental variables.

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1. Introduction

Since wheat production should meet the increasing demand of the world for food and feed, raising the wheat yields under both optimum and stress conditions has been a global target for breeders and agronomists for decades (Evans and Fischer, 1999; Araus et al., 2008). One of the abiotic stresses that constrain wheat productivity in many wheat growing areas is heat during grain filling (Porter and Gawith, 1999; Yang et al., 2002; Barnabás et al., 2008). High temperatures shorten the duration and accelerate the rate of grain growth and as a consequence the supply of carbon (C) assimilates by photosynthesis may become limiting (Spiertz, 1977; Calderini et al., 2006). However, under temperate conditions grain yield of wheat is mainly sink limited. In general, the adaptation in

sink capacity to environmental conditions shows a greater plasticity than source capacity. This high plasticity applies especially for grain number per culm and less for seed size (Sadras, 2007). Analyzing the plasticity of genotypes in response to environmental variations in terms of source activity, sink capacity, and their relationships in critical yield-determining periods is the basis for a targeted selection of genotypes to improve yield potential (Araus et al., 2008). For example, Tewolde et al. (2006) reported that early-anthesis cultivars had a longer post-anthesis duration and yielded better than late-anthesis cultivars with yield reductions ranging from 35 to 91 kg ha^{-1} for every 1 day delay in anthesis. Thus, characterizing environmental influences on source and sink relationships during the post-anthesis period for diverse genotypes is important to assist breeders in designing wheat varieties for target environments.

Grain filling in cereals usually follows a sigmoid pattern from anthesis to maturity, comprising the initial lag phase, linear phase, and saturation phase. Numerous studies have been performed to quantify this pattern and to estimate grain-filling duration and rate in wheat cultivars in response to various

environmental conditions, using different approaches (e.g. Sofield et al., 1977; Jones et al., 1979; Panozzo and Eagles, 1999). In the linear approach (e.g. Sofield et al., 1977), a line of best fit to several data points in the middle region (i.e. the linear phase) of grain growth was estimated by the method of least squares. This middle region was then progressively extended by the inclusion of data points first at one end then the other and the least squares fit recalculated. This procedure was continued until inclusion of a data point no longer gave as good a least squares linear fit as the ones previously calculated. This data point was then discarded and the slope of the line of best fit was defined as the maximum growth rate, and the duration of grain growth was derived by extrapolation of the line to its intersection with zero and with maximum mature-grain weight (w_{max}). Obviously, this trial-and-error approach is laborious, and more importantly some data points (especially those in the lower region and in the region of transition from the linear to the maturation phase) are not used in the final estimation of parameters of the linear model. Other studies used classical growth functions or polynomials to fit the entire range of data points. For example, Darroch and Baker (1990) and Panozzo and Eagles (1999) used the Logistic function. Typically, these classical growth functions use w_{max} as an asymptotic parameter. To calculate grain-filling duration with the asymptotic growth functions an empirical assumption has to be made, e.g. assuming when 95% of w_{max} is reached (e.g. Panozzo and Eagles, 1999). A cubic polynomial model can adequately describe grain filling and give a definite mathematical solution to its duration and w_{max} (e.g. Jones et al., 1979). However, not only do the coefficients of cubic polynomial equations have no biological interpretation, but they (like the Logistic function) are symmetric, presenting a growth pattern with the maximum slope midway through the growth duration. The Richards growth function (also using w_{max} as an asymptotic parameter) can accommodate an asymmetric growth, but at the cost of using an additional parameter compared with the Logistic function. A relatively new, simple yet robust function for describing the determinate growth, presented by Yin et al. (2003), can overcome all the above problems of these existing approaches.

Unlike the sigmoid pattern of grain (sink) growth, the temporal course of source activity (e.g. green-leaf area, leaf photosynthetic capacity) during grain filling follows a reversed sigmoid pattern (e.g. Bertheloot et al., 2008). This time course of source activity is associated with the degradation of chlorophyll and the breakdown of photosynthetic enzymes (mainly Rubisco) into amino acids which are then exported as a source of nitrogen (N) to growing grains (Chiba et al., 2003). Thus, to analyze the sink–source relationships during grain filling, another model is needed to accommodate the temporal dynamics of source activity.

The objective of this study is twofold. We first outline a generic, robust empirical model approach to analyze sink–source relationships during grain filling, based on the determinate growth function of Yin et al. (2003). Secondly, we apply this approach to quantify the response of wheat genotypes in rate and duration of grain growth and yielding ability to temperature.

2. Materials and methods

2.1. Plant material and cultivation

Six wheat genotypes (*Triticum aestivum* L.) derived from CIMMYT-crosses with contrasting traits were selected:

G1: CMH79A.955/4/AGA/3/4*SN64/cno67/INIA66/5/NAC/6/ CMH83.2517);

G2: VEE/CMH77A.917//VEE/6/CMH79A.955/4/AGA/3/4*SN64/ CNO67//INIA66/5;

G3: Baviacora;

G4: ALTAR 84/AE.SQ//OPATA;

G5: ALTAR 84/AE.SQ//OPATA;

G6: SRMA/TUI.

where G4 and G5 are different selections from the same cross. G1, G2 and G3 are genotypes with "big spikes"; G4, G5 and G6 were selected for drought tolerance. These genotypes were chosen for this study because of their expected similar pre-anthesis duration, different post-anthesis durations, and different grain yield components.

Plants of the six genotypes were grown in naturally lit, climate-controlled glasshouses in Wageningen, the Netherlands. Supplemental light was provided by 400 W SON-T Agro Philips lamps, which were switched on during daytime when solar radiation dropped below 400 W m^{-2}, and switched off when the radiation exceeded 500 W m^{-2}. Seeds were sown on 19 February 2001 in 5-L pots with standard potting soil enriched with 4 g L^{-1} osmocoat (N–P–K content: 15–11–13%, 2% MgO and trace elements). The number of seeds per pot was corrected for differences in germination rate between genotypes to have 12 seedlings per pot. Pots were watered twice a day. The nutrient supply was kept optimal by adding nutrients at regular intervals. Plants were kept free from pests and diseases.

2.2. Temperature treatments

Plants were grown under one temperature regime until anthesis. From sowing until the seedlings emerged the temperature was kept at 15/10 °C with a day/night interval of 12 h. After seedling emergence the temperature was lowered to 4 °C in order to vernalize the plants. From the two-leaf stage until anthesis the temperature was again kept at around 15/10 °C.

There were only minor differences among the genotypes in the pre-anthesis duration. At anthesis the pots were divided into two groups and placed in two different glasshouse compartments at 14-h daylength. Two temperatures were imposed from anthesis until maturity: 20/15 °C (T1) and 25/20 °C (T2) for day/night (12 h for day/night temperature each). The four replicate pots per genotype were randomized within each temperature.

2.3. Sampling and measurements

From anthesis onwards two culms per pot were sampled weekly. The culms were divided into several parts: flag leaf, older leaves, peduncle, lower internodes, grains and rachis. The green area of the flag leaf was measured with a LI-Cor area meter (Li-Cor Inc., Lincoln, NE, USA). The organs were weighed after oven-drying at 70 °C to constant weight. Grain number per ear was counted at each sampling date. Per-grain weight was calculated from grain weight per ear and grain number per ear.

The nitrogen analyses of grains and vegetative plant parts were carried out for samples of G3, G4 and G6 at maturity. Analytical methods were based on the procedures described by Temminghoff et al. (2000).

2.4. Model analysis

Rate and duration of grain filling were quantified by fitting the data for the time course of grain weight (w) to a determinate sigmoid growth function (Yin et al., 2003):

$$w = \begin{cases} w_{max}\left(1 + \dfrac{t_e - t}{t_e - t_m}\right)\left(\dfrac{t}{t_e}\right)^{t_e/(t_e - t_m)} & \text{if } 0 \leq t \leq t_e \\ w_{max} & \text{if } t > t_e \end{cases} \tag{1}$$
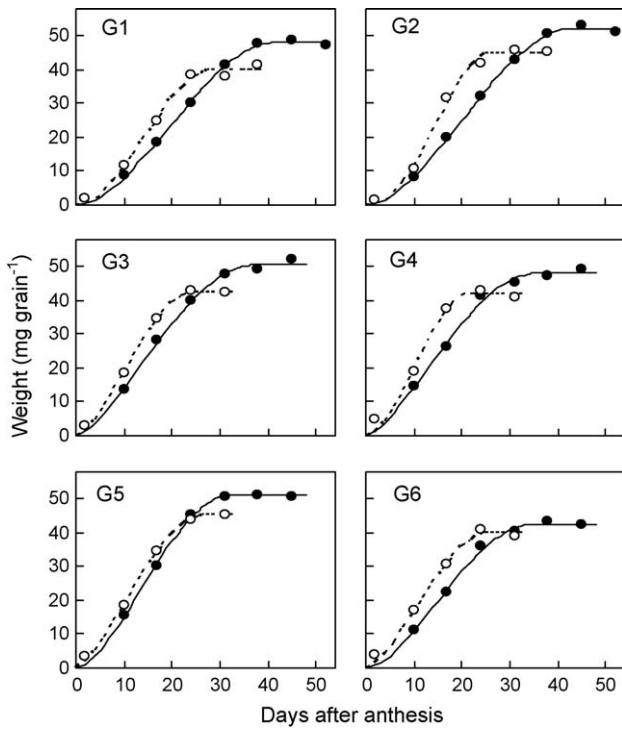
Fig. 1. Observed grain weights (points) and the time course of grain weight described by Eq. (1) (curve) of grain weight for six wheat genotypes grown at day/night temperatures 20/15 °C (filled circles and full curve) and 25/20 °C (unfilled circles and dashed curve). The curves are drawn using estimated parameter values as shown in Table 1. The first filled symbol at the lower left part of each panel is often not visible because it is overlapped by the corresponding unfilled circle.
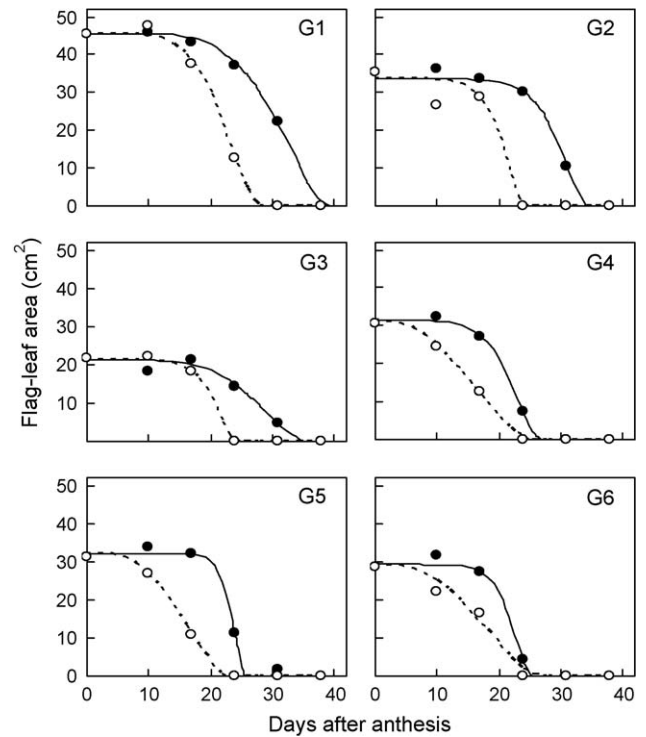

Fig. 2. Observed flag-leaf area (points) and time courses of post-anthesis flag-leaf area described by Eq. (3) (curve) during grain filling for six wheat genotypes grown at day/night temperatures 20/15 °C (filled circles and full curve) and 25/20 °C (unfilled circles and dashed curve). The curves are drawn using estimated parameter values as shown in Table 2. Some filled symbols are not visible because they are overlapped by corresponding unfilled circles.

where t is days after anthesis, w_{max} is the maximum value of w, which is reached at time t_e, and t_m is the time at which the maximum growth rate is achieved (so $0 \le t_m < t_e$). Eq. (1) obeys constraints that $w = 0$ at the start of growth (i.e. $t = 0$), and $w = w_{max}$ at and after the terminate point of growth (i.e. t_e). Compared with the commonly used Logistic equation (see Panozzo and Eagles, 1999), this model is preferred for many purposes (Müller et al., 2006), for example, to analyze the dynamics of grain filling because it produces an asymmetric curve (i.e. t_m can be at any point between 0 and t_e) and predicts an explicit value for growth duration (i.e. t_e) and gives a definite value of w_{max} if $t = t_e$ (Fig. 1). With this model, the average grain-filling rate ($\bar{c}$) during the period will be calculated simply as: $\bar{c} = w_{max}/t_e$. The maximum grain-filling rate c_m, which is obtained at time t_m, is given by Yin et al. (2003):

$$c_m = \frac{2t_e - t_m}{t_e(t_e - t_m)} \left(\frac{t_m}{t_e}\right)^{t_m/(t_e - t_m)} w_{max} \qquad (2)$$

In comparison with the dynamics of grain filling, the pattern of source activity (represented as flag-leaf area, in our case) during the grain-filling period followed a reversed sigmoid curve (Fig. 2), as a consequence of senescence. Based on Eq. (1), we formalize this reversed sigmoid pattern of source activity (s), as

$$s = \begin{cases} s_{max}\left[1 - \left(1 + \frac{t_e - t}{t_e - t_m}\right)\left(\frac{t}{t_e}\right)^{t_e/(t_e - t_m)}\right] & \text{if } 0 \le t \le t_e \\ 0 & \text{if } t > t_e \end{cases} \qquad (3)$$

where s_{max} is the maximum value of s at the onset of grain filling. At time t_m, senescence is fastest. Eq. (3) can be applied to quantify the

rate and duration of senescence within the time span of $0 \le t \le t_e$. The total source activity during this period (S) can be calculated as the area between the curve of Eq. (3) and the t-axis, which can be solved analytically:

$$S = \int_0^{t_e} s \, dt = \frac{s_{max}t_e^2}{(3t_e - 2t_m)} \qquad (4)$$

For the symmetric form of Eq. (3) (i.e. $t_m = t_e/2$), Eq. (4) becomes $s_{max}t_e/2$, the solution that can be expected for a symmetric sigmoid curve with a fixed end point.

Values of parameters in Eqs. (1) and (3) were estimated from iterative nonlinear least-square regression using the Gauss method, as implemented in the PROC NLIN of the SAS software package. For fitting Eq. (3), the initial flag-leaf area at anthesis (i.e. the onset of temperature treatment), s_{max}, is expected to be the same for both T1 and T2 treatments (see Fig. 2). To maintain the same value for s_{max} while allowing different values for t_m and t_e to be estimated from a single fitting to two curves of T1 and T2 treatments, we introduced dummy variables: $Z_1 = 1$ and $Z_2 = 0$ for T1, and $Z_1 = 0$ and $Z_2 = 1$ for T2 treatment, so

$$t_m = Z_1 t_{m1} + Z_2 t_{m2} \qquad (5a)$$

$$t_e = Z_1 t_{e1} + Z_2 t_{e2} \qquad (5b)$$

where t_{m1} and t_{e1} are the values of t_m and t_e, respectively, to be estimated for the T1 treatment, and t_{m2} and t_{e2} are the values of t_m and t_e to be estimated for the T2 treatment.

Table 1
Estimated parameter values (standard error in parenthesis) of Eq. (1), fitted to data for the time course of grain filling (see Fig. 1) in six wheat genotypes as tested at two temperatures.

Temperature (day/night)	G[a]	w_{max} (mg grain^{-1})	t_m (d)	t_e (d)	R^2	Rate (mg grain^{-1} d^{-1})	
						$\bar{c}$[b]	c_m[c]
T1: 20/15 °C	G1	48.09(0.94)	19.54(1.14)	41.37(2.13)	0.98	1.16	1.72
	G2	52.20(1.22)	19.92(1.40)	42.95(2.55)	0.97	1.22	1.79
	G3	50.53(1.08)	13.05(1.72)	37.27(2.42)	0.98	1.36	1.96
	G4	48.09(0.91)	12.53(1.51)	35.50(2.15)	0.98	1.35	1.95
	G5	50.98(0.70)	13.23(0.99)	31.96(1.53)	0.99	1.60	2.36
	G6	42.51(1.21)	14.58(1.81)	34.59(2.96)	0.96	1.23	1.79
	Mean	48.79	15.47	37.27		1.32	1.92
T2: 25/20 °C	G1	39.73(1.14)	14.47(1.16)	28.01(2.17)	0.96	1.42	2.15
	G2	44.96(1.53)	14.87(1.11)	25.94(2.50)	0.95	1.73	2.74
	G3	42.54(0.99)	9.79(1.40)	24.80(2.06)	0.98	1.72	2.48
	G4	41.98(0.74)	10.34(0.78)	21.60(1.19)	0.99	1.94	2.88
	G5	45.17(1.22)	9.75(1.49)	27.11(2.23)	0.98	1.67	2.40
	G6	39.90(1.50)	10.31(1.97)	25.77(3.14)	0.96	1.55	2.24
	Mean	42.38	11.59	25.54		1.67	2.48

[a] G: genotype; the code of genotypes is defined in the text.
[b] Average grain-filling rate = w_{max}/t_e.
[c] Maximum grain-filling rate at t_m, calculated from Eq. (2).

3. Results

3.1. Parameters to characterize grain filling

An accurate fit of grain filling for all six genotypes at both temperature regimes was provided by Eq. (1) (Fig. 1), accounting for ≥95% of the variation (Table 1). Classical sigmoid growth functions such the Logistic, Gompertz, or Richards functions, can also provide fairly good fits to the data (results not shown). The singular property of Eq. (1), in comparison with those classical growth functions, is that it predicts a definite value of final grain weight w_{max} reached at time t_e, in contrast with the classical growth functions that use w_{max} as an asymptotic parameter. Thus, using Eq. (1), the grain-filling duration t_e is estimated directly (Table 1). The estimated t_e differed among the genotypes, and not surprisingly, was greater at T1 than at T2. The 5 °C rise in temperature shortened grain-filling duration by 15–40%.

The estimated t_m, the time when maximum filling rate is obtained, was not equal to, but smaller than, $t_e/2$ in most cases (Table 1), in contrast to the Logistic function or a cubic polynomial that assumes a symmetric pattern. For G2 at T2, t_m was higher than $t_e/2$. So Eq. (1), having only three parameters, can generate various asymmetric sigmoid growths which can be obtained by some classical growth functions at the cost of using more parameters, e.g. the Richards function that has four parameters.

The estimated single-grain weights (w_{max}) differed among genotypes, and were consistently higher at T1 than at T2 (Table 1). However, the response differed among genotypes. The 5 °C increase in temperature reduced w_{max} to a lesser extent in drought-tolerant genotypes G4, G5 and G6 (by 6–12%) than in the others (by 14–17%).

While a higher temperature reduced both w_{max} and t_e, their reduction was not proportional; so the calculated average grain-filling rate $\bar{c}(= w_{max}/t_e)$ was higher at T2 than at T1 (Table 1), by 5–43%, which is visually apparent in Fig. 1. The maximum filling rate c_m, calculated by Eq. (2), ranged from 1.72 to 2.31 mg grain^{-1} d^{-1} for T1, and from 2.15 to 2.88 mg grain^{-1} d^{-1} for T2 (Table 1). The ratio of $\bar{c}$ to c_m was relatively conservative among genotypes and across temperatures, i.e. 0.68 on average.

The association between T1 and T2 for the estimated values in t_e, $\bar{c}$ and c_m was weak, indicating that genotype-by-temperature interaction existed for these parameters. However, the correlation coefficient was appreciable for the estimated w_{max} ($r = 0.66$) and high for t_m ($r = 0.98$), indicating that the

effects of genotype and temperature on these two parameters are more additive.

3.2. Parameters to characterize flag-leaf area and duration

The reversed sigmoid time course of flag-leaf area during the grain-filling period was accurately described by Eq. (3) (Fig. 2). By making use of the aforementioned dummy variable approach, Eq. (3) accounted for ≥98% of the variation in the combined data of T1 and T2 treatments (Table 2).

The estimated flag-leaf area at anthesis, s_{max}, differed by more than twofold among the genotypes (Table 2). As expected, the estimated t_e for leaf area generally had higher values at T1 than at T2, and differed among genotypes. The estimated t_m was consistently higher than $t_e/2$, resulting in skewed reversed sigmoid curves (Fig. 2). However, the extent of skew differed between the two temperatures: the ratio of t_m to t_e was, on average, 0.88 for T1 and 0.77 for T2 treatments, indicating that the fastest senescence moment was relatively earlier at the higher temperature. Because both t_e and the t_m:t_e ratio were lower at T2 than at T1, the total flag-leaf area duration, S, calculated using Eq. (4), was lower at T2 (Table 2), being 63–75% of the value at T1.

Like for the estimated t_e for grain weight, there was no significant correlation between T1 and T2 for the estimated t_e for flag-leaf area. However, the correlation coefficient was high between the two temperatures for other parameters: 0.96 for the estimated t_m and 0.99 for the calculated S.

The correlation between the estimated values of t_e for grain weight (Table 1) and those for flag-leaf area (Table 2) was high at T1 ($r = 0.8$), but the correlation generally was not obtained for T2. Grain filling at T1 continued for 2–10 days (depending on genotypes) after the complete senescence of the flag leaf, whereas at T2 grain filling stopped more or less at the same date as the flag leaf senesced (Fig. 3). Thus, during the prolonged grain-filling period at T1 (on average, accounting for 15% of total filling period) grain growth relied on the remobilization of assimilate reserves, or on any small non-foliar photosynthesis. Calculations using Eq. (1) showed that the grain growth during this prolonged period, on average, accounted for 7% of w_{max} at T1.

3.3. Other traits

The genotypes differed significantly in grain number per ear as well as in grain yield per culm (Table 3). As expected, grain number

Table 2
Estimated parameter values (standard error in parenthesis) of Eq. (3), fitted to data of flag-leaf area during grain filling (see Fig. 2) in six wheat genotypes as tested at two temperatures (T1: 20/15 °C; T2: 25/20 °C).

G[a]	s_{max} (cm^2)	t_m (d)		t_e (d)		R^2	S (cm^2 d)[b]	
		T1	T2	T1	T2		T1	T2
G1	45.65(0.60)	32.95(0.36)	23.46(0.27)	39.42(0.83)	28.14(0.65)	0.99	1355	964
G2	33.47(1.37)	31.10(0.69)	21.60(1.47)	34.35(1.88)	24.29(2.33)	0.98	967	665
G3	21.19(0.63)	29.43(0.72)	21.63(0.88)	35.70(1.63)	24.35(1.35)	0.99	560	422
G4	31.33(0.43)	23.29(0.20)	16.23(0.46)	26.99(0.41)	25.89(0.89)	0.99	664	465
G5	32.23(0.58)	24.26(0.22)	16.38(0.44)	25.80(2.08)	23.11(1.18)	0.99	743	471
G6	29.25(1.08)	23.06(0.55)	18.00(1.15)	25.48(0.75)	27.00(2.14)	0.99	626	474
Mean	32.19	27.35	19.55	31.29	25.46		819	577

[a] G: genotype; the code of genotypes is defined in the text.
[b] Total flag-leaf area duration during grain filling, calculated from Eq. (4).

did not differ significantly between T1 and T2 treatments as it is largely determined during the pre-anthesis period, i.e. before the temperature treatments were imposed. The average grain number ranged from 52 to 81 grains ear^{-1} among the genotypes. The spikes of G1 and G2, with grain numbers amounting to ca 80 per ear, were much larger than of the other genotypes. There was no significant relationship between grain weight per ear and single-grain weight among genotypes. So, the larger grain number was not obtained at the cost of reducing individual grain weight (see Table 1); as a consequence the grain yields per ear of these two genotypes were highest at both temperatures (Table 3). Overall, the correlation between grain number and per-ear grain yield was high ($r = 0.95$ and 0.96 for T1 and T2, respectively).

Grain yield was positively associated with biomass yield at maturity (Table 3). Biomass was only slightly lower at 25/20 °C than at 20/15 °C ($P > 0.05$). A rise of 5 °C decreased significantly harvest index (HI), on average from 0.53 to 0.48. A higher HI at T1 stemmed partly from longer post-anthesis duration of photosynthetic activity (Table 2) at T1 and partly from the aforementioned wider time window for possible remobilization of reserves from vegetative organs to the grains at T1.

Genotypes G3, G4 and G6, in general, did not differ significantly in N uptake and N concentration of organs at maturity (Table 4). A rise in temperature with 5 °C during the grain-filling period increased grain-N on average from 2.1 to 2.6%. The increased temperature also led to a slightly higher N-concentration of vegetative organs at maturity. Because of the decline in grain

weight, the nitrogen harvest index decreased slightly but significantly from 0.86 to 0.83 due to a rise in temperature despite the higher N-concentration of the grains.

3.4. Relationships between source and sink

Parameter S, calculated by Eq. (4), represents an overall source capacity for intercepting radiation and photosynthesis during the post-anthesis period. The genetic variation in S (integrating size and duration of the green area of the flag leaf in this analysis) was associated with sink size (represented by grain yield per culm), although the temperature effect on the calculated S was greater than the effect on grain yield (Fig. 4). In general, genotypes with larger S values produced higher biomass and grain yields per culm. Not surprisingly, the association of parameter S was stronger with grain yields than with biomass, as the latter is affected also by pre-anthesis source capacity. The large variation of scatter even for grain yields in Fig. 4 may be due to the fact that S was calculated from the flag-leaf area rather than the green area of the whole plant, which was not measured in the experiment. On average, the grain-filling duration of G1 and G2 exceeded the duration of the other genotypes (Table 1). This prolonged duration was associated with a delayed senescence of the leaves, at least for T1 (Fig. 3), indicating that genetic variation in the duration of grain filling is coupled, to some extent, with photosynthetic longevity of leaves.
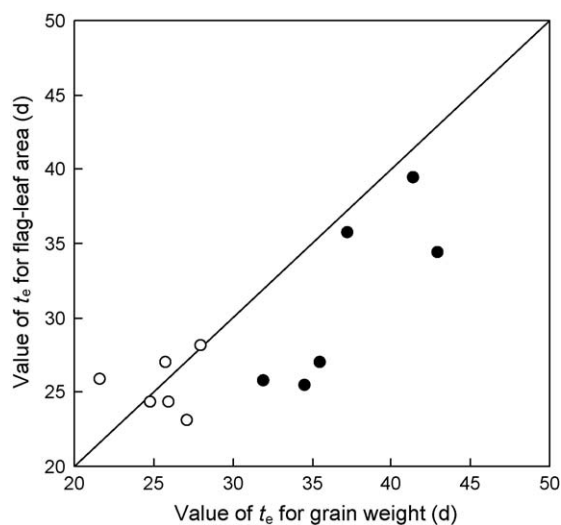

Fig. 3. Comparison between estimates of t_e for grain filling and estimates for flag-leaf area of six wheat genotypes at day/night temperatures of 20/15 °C (T1, filled circles) and 25/20 °C (T2, unfilled circles). The line represents the 1:1 relationship.
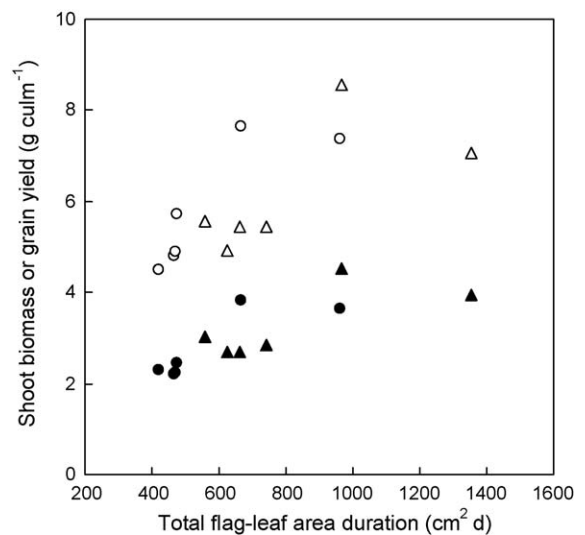

Fig. 4. The relationship between shoot biomass (unfilled symbols) or grain yield (filled symbols) per culm and cumulative post-anthesis flag-leaf area duration [calculated by Eq. (4)] across six wheat genotypes grown at day/night temperatures of 20/15 °C (T1, triangles) and 25/20 °C (T2, circles).

Table 3
Grain number per ear, biomass, grain yield, and harvest index (HI) of six wheat genotypes tested at 20/15 °C (T1) and 25/20 °C (T2).

	Grains ear^{-1}		Biomass (g culm^{-1})		Grain yield (g culm^{-1})		HI	
	T1	T2	T1	T2	T1	T2	T1	T2
G1	76.8a	83.7a	7.06b	7.37a	3.94ab	3.63a	0.56a	0.49ab
G2	81.1a	79.9a	8.56a	7.64a	4.52a	3.83a	0.53ab	0.50ab
G3	54.4b	53.9c	5.58c	4.50b	3.03bc	2.29b	0.54a	0.51a
G4	55.7b	54.5c	5.46c	4.81b	2.67c	2.21b	0.49b	0.46c
G5	55.3b	49.5c	5.44c	4.88b	2.85c	2.23b	0.52ab	0.46c
G6	59.1b	63.8b	4.93c	5.16b	2.68c	2.45b	0.54a	0.47bc
Mean	63.7a	64.2a	6.17a	5.73a	3.28a	2.77b	0.53a	0.48b

The same letter in a column means insignificant difference between the two of six genotypes, and the same letter in the last row for each trait indicates insignificant difference between the two temperatures in the mean value ($P > 0.05$), according to pairwise t-tests.

Table 4
Grain-N (%), N of the vegetative parts (%), the amount of grain-N and vegetative-part N, and N harvest index of three wheat genotypes at two temperature regimes: 20/15 °C (T1) and 25/20 °C (T2).

Genotype	Grain-N (%)		N of vegetative parts (%)		Grain-N (mg culm^{-1})		N in other parts (mg culm^{-1})		N harvest index	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
G3	1.87a	2.49a	0.37a	0.52a	56.8a	57.0a	9.4a	11.5a	0.86ab	0.83a
G4	2.10a	2.56a	0.39a	0.43a	56.3a	56.7a	10.8a	11.1a	0.84b	0.84a
G6	2.27a	2.65a	0.38a	0.46a	60.6a	65.0a	8.5a	12.4a	0.88a	0.84a
Mean	2.08b	2.57a	0.38b	0.47a	57.9a	59.6a	9.6b	11.7a	0.86a	0.84b

The same letter in a column means insignificant difference between the two of three genotypes, and the same letter in the last row for each trait indicates insignificant difference between the two temperatures in the mean value ($P > 0.05$), according to pairwise t-tests.

4. Discussion

The formation of sinks depends mainly on the availability of carbohydrates before and at anthesis (Fischer, 1985). When wheat plants grow and develop in a functional balanced way, C- and N-resources (source) will be sufficient to meet the assimilate requirements of the grains (sink). However, under conditions with water and N limitations and weather stress events (e.g. heat) the reproductive capacity of wheat plants will be reduced, resulting in a yield gap (Abeledo et al., 2008). The reduced capacity can be associated with fewer grains, lower grain weight or both, depending on the timing and magnitude of the stress event. Generally, heat will shorten growth duration more than it will accelerate growth rate; thus, resulting in smaller grains and a lower grain yield (Spiertz, 1977). A determinate growth function, which has advantages over the classical growth functions for evaluating differences between genotypes in rate and duration of growth processes, was provided by Yin et al. (2003). Based on this function, we presented a general framework to analyze the source and sink relationships during grain filling.

4.1. Methodology of analyzing source and sink relationships during grain filling

Many studies on estimating rate and duration of grain filling used a trial-and-error approach, in which a line of best fit for the linear phase of grain growth was repeatedly assessed by the method of least squares (e.g. Sofield et al., 1977). A clear drawback of this linear approach is that the part of data points are not used in the final-round parameter estimation. In other studies classical growth functions such as the Logistic, Richards or Gompertz functions were applied. In these classical growth functions the maximum value for grain weight (w_{max}) is expressed as an asymptotic parameter. Thus, to calculate grain-filling duration some empirical assumption has to be made, e.g. assuming when 95% of w_{max} is reached (e.g. Panozzo and Eagles, 1999). The growth function described by Yin et al. (2003), Eq. (1), on the other hand,

uses time of the end of growth (t_e) as its parameter in addition to w_{max} and time of maximum growth rate (t_m). Using Eq. (1), the grain-filling duration t_e can be fitted directly, and rate of filling can be obtained from the estimated values of w_{max}, t_m and t_e (Table 1). In addition to dealing with a terminate growth pattern, advantages of using Eq. (1) in describing growth processes include (Yin et al., 2003): (1) clear biological meaning of its three parameters (w_{max}, t_m and t_e), and (2) accommodation of any asymmetric growth pattern, depending on the ratio of t_m to t_e which can be any value between 0 and 1, and its symmetric form (i.e. when t_m: $t_e = 0.5$) is a cubic polynomial, which has also been used previously to analyze grain filling (e.g. Jones et al., 1979). When the three parameters of Eq. (1) are estimated by fitting to grain weight data, instantaneous sink demand by grain growth can be obtained as the first-order derivative of Eq. (1) with respect to time t:

$$\frac{dw}{dt} = \begin{cases} w_{max} \dfrac{(2t_e - t_m)(t_e - t)}{t_e(t_e - t_m)^2}\left(\dfrac{t}{t_e}\right)^{t_m/(t_e - t_m)} & \text{if } 0 \leq t \leq t_e \\ 0 & \text{if } t > t_e \end{cases} \quad (6)$$

Eq. (6) gives a bell-shaped trend of sink demand within time spell between 0 and t_e.

In contrast to this pattern of sink-demand dynamics, the dynamics of source activity during grain filling normally followed a reversed sigmoid pattern, also with a clear end point, at and beyond which source activity becomes zero (e.g. Fig. 2). We presented Eq. (3) as a general, flexible mathematical formula for this reversed sigmoid pattern. The merits discussed above for Eq. (1) for the normal growth pattern also applies to Eq. (3) for describing the reversed sigmoid pattern. Eq. (3) can be integrated to obtain the cumulative source activity during the period between 0 and t_e [see Eq. (4)]. If the source activity is indicated by available net crop photosynthetic assimilates daily produced, then comparison between w_{max} estimated by Eq. (1) and S calculated by Eq. (4) will allow to quantify the proportion of total grain weight that comes from post-anthesis photosynthesis and the proportion that comes from pre-anthesis reserves. Furthermore, comparison

between daily source activity [quantified by Eq. (3)] and daily sink demand [quantified by Eq. (6)] will allow to establish the temporal course of the net balance of C reserves. Finally, comparison in t_e estimated by Eq. (1) and by Eq. (3) will allow to quantify whether or not the timing for the cessation of grain filling and for the end of post-anthesis photosynthesis is synchronized.

4.2. Sink–source relationships during grain filling in six wheat genotypes

The determinate growth function, Eq. (1), proved to be accurate in fitting the sigmoid pattern (Fig. 1), and in determining the rate and duration of grain filling in six wheat genotypes grown at two contrasting temperatures (Table 1). Both the duration and the rate of grain filling in wheat can vary substantially depending on genotypes and on temperatures. The estimated maximum growth rates ranged from 1.72 for G1 at 20/15 °C to 2.88 mg grain^{-1} d^{-1} and G4 at 25/20 °C. A rise in temperature of 5 °C reduced the duration of grain filling in all genotypes, and this far outweighed the effect of any increase in grain-filling rate on final grain weight, confirming the previously published results (e.g. Sofield et al., 1977; Panozzo and Eagles, 1999). At each temperature, there was a genotype which had a high rate of grain growth with a shorter duration without little sacrificing of final grain weight. Thus, there is an opportunity to select genotypes with fast growing grains that may suffer less from terminal heat stress.

In our experiment we did not assess the whole-plant photosynthetic rate (source activity) of the tested genotypes during the grain-filling period. So we were unable to partition the total grain weight into the contributions between post-anthesis photosynthesis and pre-anthesis reserves, nor to establish the dynamics of the net balance of reserves during the grain filling. However, for the potential application of our methodology in a breeding program to screen a large number of genotypes, assessing leaf area would be a more feasible than measuring photosynthetic rate per se. In our experiment, we only measured the time course of flag-leaf area, which was considered as a proxy of the photosynthetic source activity for illustrating our methodology. The green area of the whole plant would have been a better proxy. Nevertheless, the timing for the end of the flag-leaf area duration might coincide with the end of the whole-plant green area, and the flag-leaf area dynamics was accurately described by the flexible reversed sigmoid function Eq. (3) (Fig. 2).

The general coincidence between t_e for grain filling (Table 1) and t_e for flag-leaf area (Table 2) was obtained only at T2 (Fig. 3), indicating that the source–sink balance during grain filling may be temperature dependent. At T1, grain filling continued well after the complete senescence of flag leaves, most likely utilizing the remaining mobile C assimilates stored in the reserves and contributing to an average of 7% of the final grain weight. As leaf senescence during grain filling is strongly a result of N remobilization from leaves to grains (Sinclair and de Wit, 1975; Chiba et al., 2003), the relative faster leaf senescence at the lower temperature (T1) may indicate that the speed of C and N filling during grain growth was not in parallel across temperatures. This suggests that C accumulation in and N flow to the grains are not entirely coupled (see also Panozzo and Eagles, 1999).

4.3. Yield component traits

We found in our pot experiment with six genotypes that grain yield was mainly associated with grain number. It has widely been reported (e.g. Peltonen-Sainio et al., 2007) that under favorable conditions, increasing grain number is the key to improve yielding ability of genotypes, although it has been argued that increased grain number may be considered more as a consequence of high

yield than a determinant (Sinclair and Jamieson, 2006). Grain number is related to the accumulated dry matter before and after anthesis. Sadras (2007) stated that grain number is plastic and highly responsive to resource availability; data from various sources illustrate that grain number in wheat is roughly consistent with the model of equitable resource allocation (Borras et al., 2004). The availability of pre-anthesis assimilates affects the development of spikelets and florets, which is one of the major factors limiting grain number. Post-anthesis accumulated assimilates, especially during embryo development and endosperm cell proliferation period, affect mainly the initial development of grains and the setting of grains (Guo et al., 1995). So, raising assimilate accumulation around anthesis is beneficial to enlarge sink size resulting in a higher grain yield.

Under environmental stress, e.g. high temperatures after anthesis, grain yield will be reduced due to a decline of single-grain weight (Table 3; see also Porter and Gawith, 1999; Spiertz, 1977). It is not only because of the decrease of available photosynthetic capacity, but also due to the reduced capacity of sinks to convert sugars into starch (Zhao et al., 2008). This phenomenon could even be more responsible for the decrease of grain weight because of a negative feedback of sinks to the source vigor (Reynolds et al., 2005). Our preliminary analysis (Fig. 4) showed a non-causal association between source and sink activities during grain filling, in line with some literature reports that high sink capacity may be associated with delayed leaf senescence (e.g. Spano et al., 2003). A better quantitative assessment of the causal relationship between C and N flows and grain growth can be carried out experimentally under well-controlled conditions (Spiertz, 1977; Spiertz and Ellen, 1978).

In conclusion, the quantitative approach in determining grain growth and flag leaf senescence yielded parameters that characterize genotypic differences and environmental responses in post-anthesis source and sink capacity. The yielding ability of the tested wheat genotypes was associated with grain number and leaf area duration as well as the response of grain growth to ambient temperature.

Acknowledgments

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