Early anthesis and delayed but fast leaf senescence contribute to individual grain dry matter and water accumulation in wheat

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***Abbreviations*:** chl, chlorophyll; Chl_{accum}, accumulated chlorophyll content; Chl_{loss}, duration of rapid chlorophyll loss; Chl_{per}, duration of chlorophyll persistence; Chl_{tot}, total duration of chlorophyll persistence and loss; ^{o}Cd, degree day; GA, green area; GA_{accum}, accumulated green area; GA_{loss}, duration of rapid green area loss; GA_{per}, duration of green area persistence; GA_{tot}, total duration of green area persistence and loss; GF, grain filling; GFR, grain filling rate; H^2, broad sense heritability; LOD, logarithm of the odds; Max chl, maximum chlorophyll content; Max CLR, maximum chlorophyll loss rate; Max GALR, maximum green area loss rate; MWC, maximum water content of grain; QTL, quantitative trait locus; RIL, recombinant inbred line; TGW, thousand grain weight; t_{max}, the time at maximum grain filling rate; t_{mwc}, the time at maximum water content; WAR, grain water absorption rate; WLR, grain water loss rate.

Abstract

The physiological process of how anthesis time and leaf senescence patterns affect individual grain weight of wheat has only been partially elucidated. In this study, a recombinant inbred line mapping population of bread wheat (*Triticum aestivum* L. 'Forno') and its relative spelt (*Triticum spelta* L. 'Oberkulmer'), contrasting for phasic development and leaf senescence kinetics, was used to understand the physiological and genetic relationships among anthesis time, leaf senescence, grain filling processes, and individual grain weight. Phenotypic measurements were taken in the field over two growing seasons. The results showed that earlier anthesis and delayed leaf senescence were associated with larger grains. Furthermore, early anthesis and delayed but fast leaf senescence promoted grain filling rate (but shortened its duration), grain water absorption rate and maximum grain water content, while individual grain dry matter and water accumulation displayed strong relationships with individual grain weight. A total of 118 significant quantitative trait loci (QTL) were identified in this mapping population, including six QTL for anthesis dates, 24 for flag leaf senescence, 69 for grain filling traits, and 19 for individual grain weight. Frequent QTL coincidences between these traits were observed on chromosomes 2A, 3B, 4A, 4DL, 5A, 5B, 5DL and 7B. Analysis of allelic effects confirmed the above physiological relationships. Therefore, anthesis time and leaf senescence affect individual grain weight at least partly through their effects on individual grain dry matter and water accumulation, resulting from pleiotropy or tight gene linkages. Slightly early anthesis, and delayed but fast leaf senescence, can be used to maximize individual grain weight and yield potential in wheat.

Keywords: Anthesis; grain filling; individual grain weight; leaf senescence; spelt; wheat

1. Introduction

Flowering in wheat (*Triticum aestivum* L.) is a key event during the plant life cycle, as it defines the beginning of the grain filling process for yield formation. Flowering time in wheat is flexible, which allows it to be cultivated in diverse environments around the world, from South America and southern Oceania to North America and northern Europe and Asia, and from sea level to *c*. 3000 m (Slafer and Whitechurch, 2001). For a given genotype, however, an appropriate anthesis date is needed to match its regional environment for adaption. In addition, fine-tuning of this time is also important to maximize grain yield. It has been found that the growth period immediately before anthesis, which coincides with rapid spike growth, determines floret fertility and in turn grain number at maturity (González et al., 2011; Slafer and Rawson, 1994). This critical period also overlaps with the ovary development within florets, and consequently affects individual grain weight (Calderini et al., 1999). Immediately after fertilization, endosperm cell division and enlargement take place (Shewry et al., 2012), an important process determining final individual grain weight (Brocklehurst, 1977). Therefore, optimizing the timing of anthesis can contribute to grain yield potential. On the other hand, wheat production is highly sensitive to environmental changes at and around anthesis. Drought and high temperature during this time, for example, reduce yield (8–30%) and yield components (grain number and grain size) (Lizana and Calderini, 2013; Semenov et al., 2014). These effects can be more common in global warming scenarios, where an increase in frequency of heat stress around anthesis has been predicted in Europe (Semenov et al., 2014). A potential strategy to adapt wheat for climate change is earlier anthesis, by escaping excessive temperature and drought through rapid development. Earliness may also work for wheat growing areas with terminal drought (Izanloo et al., 2008; Lopes and Reynolds, 2011), and with short growing seasons (Iqbal et al., 2007).

From anthesis onwards, grain growth commences, coinciding with leaf senescence. Leaves are the major sites for current photosynthesis, which, together with the preanthesis reserves, supplies assimilates for grain filling. Leaf senescence kinetics during grain filling can be divided into two phases: full functionality and rapid senescence (Wu et al., 2012). Delayed onset of senescence with longer functional photosynthesis (stay-green) may produce more assimilates for developing grains, and thus has potential to maximize grain yield. In fact, higher crop productivity has been well documented to be associated with delayed senescence, for example, in wheat (Bogard et al., 2011; Christopher et al., 2008; Derkx et al., 2012; Gaju et al., 2011; Verma et al., 2004), and other crops as reviewed by Gregersen et al. (2013). The stay-green phenotype is more advantageous when wheat plants grow under stressed conditions during the postanthesis period such as high temperature, drought, elevated ozone, nutrient deficiency and disease infections, where grain yield is more prone to be source-limited (Christopher et al., 2008; Gaju et al., 2011; Gelang et al., 2000; Joshi et al., 2007). Rapid senescence is the final stage of the leaf life cycle. Senesing leaves at this stage display yellowing and loss of photosynthetic capacity, proceeding from lamina tips to the bases close to the stems. This process has been considered as a form of programmed cell death (Gan and Amasino, 1997), and plays an important role in nutrient recycling. During senescence, chloroplasts are broken down; chlorophyll, proteins (e.g. Rubisco), membrane lipids and other macromolecules are then degraded, so that the resultant nutrients can be transported into growing grains. In particular, the remobilization of nitrogen in the forms of glutamate, aspartate, threonine, serine and glutamine from senesing leaves greatly contributes to grain protein concentration at maturity (Distelfeld et al., 2014; Gaju et al., 2014). It has been demonstrated that the functional *Gpc-1* (*NAM-1*) genes confer wheat cultivars or lines with earlier senescence, efficient nutrient remobilization from leaves, and, in turn, higher grain protein and micronutrient (iron and zinc) contents; however, they reduce grain yield under

some environments (Distelfeld et al., 2014; Uauy et al., 2006). Delayed leaf senescence favours grain yield improvement, but not nutrient use efficiency, a dilemma of senescence in wheat breeding (Gregersen et al., 2008). Therefore, optimizing leaf senescence kinetics is needed to make better use of both current photosynthetic capacity and degraded nutrients.

The present study aimed to understand how anthesis time and leaf senescence affect individual grain weight, the major determinant of yield during the postanthesis period. A mapping population of bread wheat and spelt with contrasting phasic development and leaf senescence kinetics was used, and then the variation in anthesis time, the onset and progression of leaf senescence, individual grain dry matter accumulation, grain water uptake and loss, and final individual grain weight, was quantified. Physiological and genetic relationships between these processes were established, resulting in a trait interaction model, which can be used to build a wheat ideotype with appropriate anthesis and leaf senescence patterns for breeding.

2. Materials and Methods

2.1. Plant materials and field experiments

A mapping population, consisting of 226 F_5 recombinant inbred lines (RILs) derived from the cross between a Swiss winter bread wheat cultivar 'Forno' (*Triticum aestivum* L.) and a Swiss winter spelt cultivar 'Oberkulmer' (*Triticum spelta* L.) (Messmer et al., 1999), was used in this study. Field experiments were carried out at the University of Nottingham Farm, Leicestershire, UK (52° 50' N, 1° 15' W, 50 m) in 2011–2012 and 2012–2013. The RILs and two parents were grown in a randomized complete block (RCB) design with three replicates. The soil was a sandy loam (pH 7.6), containing 78.2 (in 2012) and 70.4 (in 2013) kg N ha^{-1} in the top 90 cm. The seeds were sown at 250 seeds m^{-2} on 19 October 2011 (6 × 1.6 m plots) and on 31 October 2012 (12 × 1.6 m plots). A prophylactic program of crop management (disease, weed, pest, and fertilizer) was conducted according to standard agronomic practice. Two subsets (72 RILs in 2012 and 110 RILs in 2013; based on the significant variation in the traits of interest) were selected to quantify all the traits, with the exception of individual grain weight, which was measured on 226 RILs.

2.2. Anthesis time

A spike was judged as flowering when the first anthers were extruded from the middle spikelets. Anthesis date of a plot was recorded when 50% of the spikes started flowering. Evaluation was carried out every day until all plots finished flowering. Calendar dates of anthesis were then converted into accumulated thermal time (degree days, °Cd). Temperature data was obtained from the nearby meteorological station. Daily thermal time was calculated as the average of maximum and minimum air temperature (or the base temperature 0°C, whichever was higher).

2.3. Leaf senescence

Leaf senescence was assessed based on flag leaves, using two approaches: green area (GA) loss and chlorophyll (chl) loss, at a 5-day interval from anthesis onwards in both seasons. GA of the flag leaves in a plot was rated visually using a scale from 10 (0% yellowing) to 0 (100% yellowing) (Torres and Pietragalla, 2012). Meanwhile, the chl concentrations of flag leaves were non-destructively measured using a chlorophyll meter (SPAD 502, Minolta, USA). For each plot, measurements were taken on five healthy, clean leaves, three points along each leaf (one third, half and two thirds, avoiding the midrib and major veins). The average of 15 readings was recorded, and expressed as chlorophyll concentration index (CCI; ranging from 0 to 99.9).

Data of GA and chl loss of flag leaves were then fitted over the accumulated thermal time after anthesis using the Gompertz growth curve (Fig. 1) (Gooding et al., 2000).

$$G = A + C\mathrm{e}^{-\mathrm{e}^{-B(t-M)}}$$

where G is the visual score or SPAD reading; A and (A + C) are the lower and upper asymptotes, respectively; B is the relative senescence rate at the time M; M is the accumulated thermal time when senescence rate is at maximum and when visual scores or SPAD readings decline to (A + 0.37C); and t is the accumulated thermal time after anthesis.

Total duration of flag leaves (t_{total}; GA_{tot} or Chl_{tot}) was defined as the period from anthesis to the time at 90% senescence. t_{total} consisted of two components: persistence phase (GA_{per} or Chl_{per}), from anthesis to t_{onset} (the onset of senescence, 10% senescence), and rapid loss phase (GA_{loss} or Chl_{loss}), from t_{onset} to t_{total} (Fig. 1). When $t = M$, maximum senescence rate (MSR), i.e. maximum GA loss rate (Max GALR) or maximum chl loss rate (Max CLR), was reached, and calculated as $MSR = BC/\mathrm{e}$ (e = 2.718). Area under the Gompertz curve from anthesis to t_{total} was also calculated as a measure of total flag leaf greenness of a genotype, and termed

accumulated GA (GA_{accum}) or chl content (Chl_{accum}). In addition, maximum chl concentration (Max chl) was derived from the upper asymptote of the curve ($A + C$).

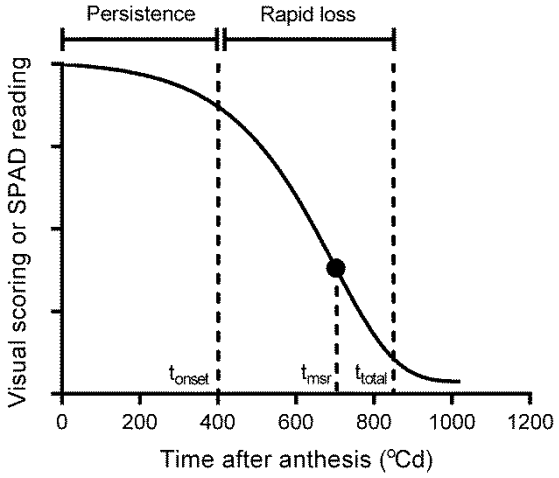

Fig. 1. Gompertz growth curve fitting for flag leaf senescence. Total duration of flag leaves (t_{total}) is defined as the period from anthesis to the time when 90% of the green area (visual scoring) or chlorophyll (SPAD meter) has been lost (90% senescence). t_{total} is then divided into two phases: persistence and rapid loss. Duration of leaf persistence is from anthesis to the time at 10% senescence (t_{onset}), while the duration of rapid loss is from t_{onset} to t_{total}. The closed circle on the curve indicates the maximum senescence rate (msr).

2.4. Individual grain dry matter and water accumulation

From anthesis onwards, young grains were sampled every five days to quantify the dynamics of individual grain dry matter and water accumulation. Five main spikes at anthesis and maturity, and two spikes during grain filling, were collected; two middle spikelets of each spike in 2012, and three spikelets of one side of each spike in 2013 (the third one from the base, the third one from the tip and the middle one between the two spikelets), were dissected using forceps. All grains were then weighed for fresh weight, and dried in an oven at 85^{o}C for 48 h, and weighed again for dry weight. Water content of individual grains was calculated as the difference between fresh and dry weight.

Data of grain dry weight was then fitted over the accumulated thermal time after anthesis using the logistic growth curve (Wang et al., 2009; Zahedi and Jenner, 2003).

$$W_{\mathrm{d}} = A + \frac{C}{1 + \mathrm{e}^{-B(t-M)}}$$

where W_{d} is the dry weight of individual grains, A is the lower asymptote, (A + C) is the upper asymptote (i.e. final individual grain weight), B is the doubled relative growth rate at the time M, M is the accumulated thermal time when grain filling rate (GFR) is at maximum and when grains grow at (A + 0.5C), and t is the accumulated thermal time after anthesis.

Grain filling (GF) duration (GFD) was defined as the period from anthesis to the time when grain dry weight reached (A + 0.99C), and calculated as: $GFD = M + 4.5951/B$. This duration was then divided equally into three phases: initial, rapid and late, which correspond to the timing of endosperm cell division and enlargement, rapid grain filling, and maturation, respectively (Shewry et al., 2012). Average GFR during each phase and across all three phases were calculated. Onset of GF (OGF), the time when grain dry weight reached (A + 0.05C), was calculated as: $OGF = M - 2.9444/B$. At the time M (t_{max}), maximum GFR (MGFR) was derived from $MGFR = BC/4$.

Water content of individual grains was fitted over the accumulated thermal time after anthesis using a cubic function.

$$W_{\mathrm{W}} = b_3 t^3 + b_2 t^2 + b_1 t + a$$

where W_{w} is the grain water content, t is the accumulated thermal time after anthesis, b_3, b_2, b_1 and a are coefficients.

When $\mathrm{d}W_{\mathrm{w}}/\mathrm{d}t = 0$, $W_{\mathrm{w}} = W_{\max}$ (maximum water content, MWC), $t = t_{\mathrm{mwc}}$ (the time at maximum water content),

$$W_{\max} = b_3 t_{\mathrm{mwc}}^3 + b_2 t_{\mathrm{mwc}}^2 + b_1 t_{\mathrm{mwc}} + a$$

$$t_{\mathrm{mwc}} = \frac{-b_2 - \sqrt{b_2^2 - 3b_1 b_3}}{3b_3}$$

Average water absorption rate (WAR) of grains from anthesis to t_{mwc}, and water loss rate (WLR) from t_{mwc} to the time for last measurement, were also calculated.

2.5. Individual grain weight at maturity

All the plots of 226 RILs and parents were combined at maturity using a harvester (2010, Sampo Rosenlew, Finland). Grain samples were then threshed by a thresher and completed by hand, because of low threshability derived from spelt. For each plot, 200 grains were dried in an oven at 85$^{\mathrm{o}}$C for 48 h to calculate thousand grain weight (TGW).

2.6. Statistical analysis of phenotypic data

Genotypic differences between the parental lines and between the RILs in anthesis dates, flag leaf senescence, grain filling traits, and individual grain weight, were tested according to analysis of variance and multiple comparisons (Fisher's unprotected least significant difference). Pearson correlations between different traits were computed using the average values across replicates in each environment. Phenotypic data were transformed to improve

the normality of trait distribution when necessary. Broad sense heritability (H^2) of each trait across years was calculated as: $H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/n + \sigma_e^2/rn)$, where σ_g^2 is the genotypic variance, σ_{ge}^2 is the genotype-by-environment interaction variance, σ_e^2 is the error variance, n and r are the environment (years) and replicate number, respectively. Variance components were estimated from a linear mixed model using the method of residual maximum likelihood (REML), by considering the environment (year) and replicate as fixed factors, and genotype and genotype-by-environment interaction as random factors. All the statistics and graphics, including curve fitting, were performed using Genstat v17 and GraphPad Prism v6.05.

2.7. Quantitative trait locus (QTL) analysis

The genetic map of Forno × Oberkulmer is available online in the GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml). This map contains 182 polymorphic markers (restriction fragment length polymorphism and simple sequence repeat), and 230 segregating loci. Linkage analysis was performed using JoinMap v4 (Van Ooijen, 2006), resulting in 23 linkage groups, and covering 2,469 cM with an average marker density of 13.6 cM (Messmer et al., 1999). QTL detection was performed in each environment using MapQTL v6 (Van Ooijen, 2009). Chromosomal locations of significant QTL, logarithm of the odds (LOD), additive effects and the percentages of phenotypic variation explained by individual QTL (R^2) were obtained by interval mapping. Co-factors, which were the markers nearest to QTL peaks, were tested for significance at $P < 0.02$, and then used for multiple-QTL model (MQM) mapping. A genome-wide significance threshold was computed for each trait in each environment through a permutation test with 1,000 iterations ($P < 0.05$). At each locus, the allele increasing the phenotypic value of the trait was defined as the increasing allele, and the allele from the other parent relatively decreasing the phenotypic value was defined as the decreasing allele. The genetic map and QTL locations were drawn using the MapChart v2.2 (Voorrips, 2002).

3. Results

3.1. Large phenotypic variation between the parents and between the RILs in anthesis dates, flag leaf senescence, grain filling traits, and individual grain weight

Anthesis was later in the spelt Oberkulmer than in the bread wheat Forno in both 2012 and 2013 seasons (Supplementary Table S1). Flag leaf senescence was assessed using two approaches: loss of green area and chlorophyll content estimation. Compared with Forno, Oberkulmer had shorter durations of green area (GA_{per}) and chlorophyll (Chl_{per}) persistence, longer durations of rapid green area loss (GA_{loss}) and chlorophyll (Chl_{loss}) loss, and longer total duration of senescence (GA_{tot} and Chl_{tot}) (Fig. 2 and Supplementary Table S1); that is, Oberkulmer started rapid senescence earlier but finished later, consistent across two years. Max GALR and Max CLR were lower in Oberkulmer than in Forno. GA_{accum} was higher in Oberkulmer; however, Chl_{accum} was comparable in both parents. In addition, significant differences between the parental lines in grain filling traits and individual grain weight were found. As expected, the contrasting parents resulted in substantial variation in all traits observed among the RILs (Supplementary Table S1), which enabled further physiological and genetic analyses. H^2 varied among different traits: being relatively higher in anthesis dates, Chl_{per}, rapid and average GFR, MWC, WAR, and TGW ($H^2 > 0.70$), but lower in Chl_{loss}, initial and late GFR, the onset and duration of GF, and t_{mwc} ($H^2 < 0.40$), suggesting different levels of genetic control and environmental influence and/or interaction (Supplementary Table S1).

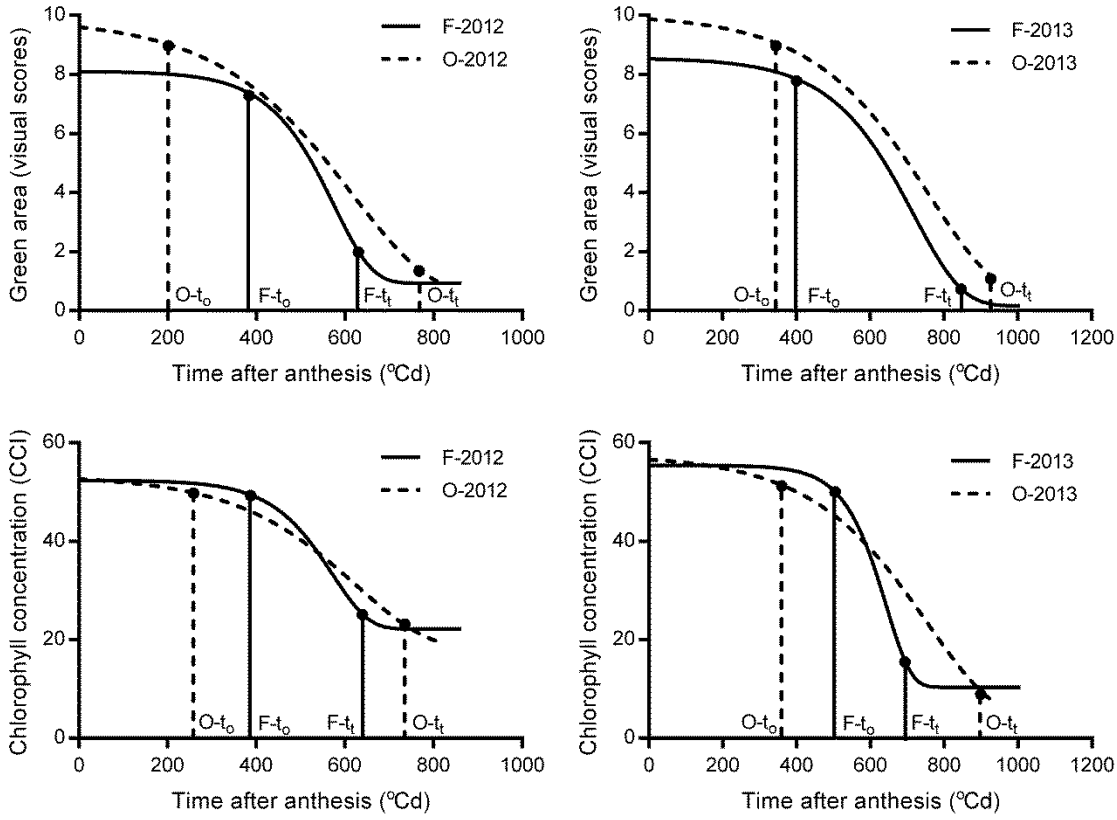

Fig. 2. Flag leaf senescence of bread wheat Forno and spelt Oberkulmer. Data of the green area and chlorophyll loss of flag leaves are fitted over the accumulated thermal time after anthesis in 2012 and 2013, using the Gompertz growth curve. Abbreviations: F, Forno; O, Oberkulmer; t_o (t_{onset}), the onset of leaf senescence; t_t (t_{total}), total duration of flag leaves; and CCI, chlorophyll concentration index.

3.2. Significant physiological relationships between anthesis dates and flag leaf senescence

Earlier anthesis was associated with longer GA_{per} and Chl_{per} (delayed onset of senescence), shorter GA_{loss} and Chl_{loss}, and faster Max GALR and Max CLR (Table 1). In 2012, the anthesis date was negatively associated with GA_{accum} and Chl_{accum}, but not in 2013. A positive relationship between anthesis dates and Max chl was found.

Table 1
Phenotypic correlations between anthesis dates and flag leaf senescence.

Trait[a]	Anthesis date		Trait	Anthesis date	
	2012	2013		2012	2013
GA_{per}	-0.40**[b]	-0.41**	Chl_{per}	-0.57**	-0.16
GA_{loss}	0.14	0.43**	Chl_{loss}	0.41**	0.26**
GA_{tot}	-0.18	0.29**	Chl_{tot}	-0.29*	0.23*
Max GALR	-0.25*	-0.39**	Max chl	0.17	0.35**
GA_{accum}	-0.51**	-0.04	Max CLR	-0.34**	-0.10
			Chl_{accum}	-0.55**	0.19*

[a] Trait abbreviations: GA_{per}, duration of green area persistence; GA_{loss}, duration of rapid green area loss; GA_{tot}, total duration of green area persistence and loss; Max GALR, maximum green area loss rate; GA_{accum}, accumulated green area; Chl_{per}, duration of chlorophyll persistence; Chl_{loss}, duration of rapid chlorophyll loss; Chl_{tot}, total duration of chlorophyll persistence and loss; Max chl, maximum chlorophyll content; Max CLR, maximum chlorophyll loss rate; Chl_{accum}, accumulated chlorophyll content.

[b] * Significant at $P < 0.05$, ** significant at $P < 0.01$.

3.3. Anthesis dates and flag leaf senescence show relationships with final individual grain weight

Correlation analysis revealed a negative relationship between anthesis dates and individual grain weight (Table 2), indicating that earlier anthesis contributed to larger grains. Longer GA_{per} and Chl_{per}, rather than GA_{loss} and Chl_{loss}, were significantly associated with larger grains. In 2012, final individual grain weight was negatively associated with Chl_{loss}, but positively associated with Max CLR. GA_{accum} and Chl_{accum} showed positive associations with final individual grain weight in both years.

Table 2
Phenotypic correlations between anthesis dates, flag leaf senescence, and final individual grain weight.

Trait[a]	TGW		Trait	TGW	
	2012	2013		2012	2013
Anthesis	-0.25*[b]	-0.32**	Chl_{per}	0.49**	0.21*
GA_{per}	0.33**	0.26**	Chl_{loss}	-0.44**	-0.08
GA_{loss}	0.02	-0.09	Chl_{tot}	0.10	0.03
GA_{tot}	0.32**	0.09	Max chl	0.03	0.12
Max GALR	0.08	0.00	Max CLR	0.39**	0.10
GA_{accum}	0.45**	0.37**	Chl_{accum}	0.34**	0.25**

[a] Trait abbreviations: GA_{per}, duration of green area persistence; GA_{loss}, duration of rapid green area loss; GA_{tot}, total duration of green area persistence and loss; Max GALR, maximum green area loss rate; GA_{accum}, accumulated green area; Chl_{per}, duration of chlorophyll persistence; Chl_{loss}, duration of rapid chlorophyll loss; Chl_{tot}, total duration of chlorophyll persistence and loss; Max chl, maximum chlorophyll content; Max CLR, maximum chlorophyll loss rate; Chl_{accum}, accumulated chlorophyll content; TGW, thousand grain weight.

[b] * Significant at $P < 0.05$, ** significant at $P < 0.01$.

3.4. Anthesis dates show relationships with individual grain dry matter and water accumulation

To understand how anthesis dates affected final individual grain weight, the relationships between anthesis dates and grain filling processes were analyzed (Table 3). As a result, anthesis dates were negatively associated with GFR (initial, rapid, late, average, and maximum), and with MWC and WAR, indicating that earlier anthesis contributed to individual grain dry matter and water accumulation. In 2013, there were positive relationships between anthesis dates, GF duration, t_{max} and t_{mwc}, indicating earlier anthesis accelerated the progress of grain filling and shortened its duration.

Table 3
Phenotypic correlations between anthesis dates and grain filling traits.

Grain filling[a]	Anthesis date		Grain filling	Anthesis date	
	2012	2013		2012	2013
Initial GFR	-0.16	0.00	GF duration	-0.05	0.35**
Rapid GFR	-0.18	-0.49**[b]	t_{max}	-0.09	0.21*
Late GFR	-0.16	-0.40**	MWC	-0.36**	-0.53**
Average GFR	-0.20	-0.50**	WAR	-0.41**	-0.60**
Max GFR	-0.18	-0.49**	WLR	0.22	-0.02
Onset of GF	-0.07	-0.19*	t_{mwc}	0.13	0.39**

[a] Trait abbreviations: GFR, grain filling rate; GF, grain filling; t_{max}, the time at maximum grain filling rate; MWC, maximum water content of grains; WAR, water absorption rate of grains; WLR, water loss rate of grains; t_{mwc}, the time at maximum water content.

[b] * Significant at $P < 0.05$, ** significant at $P < 0.01$.

3.5. Flag leaf senescence shows relationships with individual grain dry matter and water accumulation

To understand how flag leaf senescence affected final individual grain weight, the relationships between flag leaf senescence and grain filling processes were analyzed (Tables 4 and 5). It was found that GA_{per} and Chl_{per} were positively associated with GFR (rapid, late, average, and maximum), and with MWC and WAR, indicating that longer persistence of flag leaves (delayed onset of senescence) was associated with increased individual grain dry matter and water accumulation. Additionally, GA_{loss} and Chl_{loss} showed negative relationships with GFR and grain water accumulation, but the opposite was true for Max GALR and Max CLR, suggesting that shorter duration and accordingly faster rate of rapid senescence led to more effective dry matter synthesis and water uptake of grains. Meanwhile, shorter duration and faster rate of rapid senescence accelerated the progress of grain filling (t_{max} and t_{mwc}) and shortened its duration mainly in 2013. Max chl did not affect grain filling traits except the grain water loss rate (WLR), which was positively associated with Max chl. GA_{accum} and Chl_{accum} showed positive associations with GFR and grain water accumulation in 2012, but not in 2013.

Table 4

Phenotypic correlations between green area loss of flag leaves and grain filling traits.

Grain filling[a]	GA_{per}		GA_{loss}		GA_{tot}		Max GALR		GA_{accum}	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Initial GFR	0.06	0.01	-0.10	0.04	-0.07	0.07	0.23*	-0.14	0.06	0.14
Rapid GFR	0.34**[b]	0.34**	-0.02	-0.34**	0.28*	-0.21*	0.07	0.33**	0.46**	0.06
Late GFR	0.35**	0.30**	-0.02	-0.34**	0.27*	-0.25**	0.05	0.36**	0.44**	-0.06
Average GFR	0.34**	0.36**	-0.04	-0.36**	0.25*	-0.22*	0.12	0.31**	0.45**	0.07
Max GFR	0.35**	0.35**	-0.03	-0.37**	0.27*	-0.24**	0.08	0.36**	0.46**	0.01
Onset of GF	0.27*	0.09	0.12	-0.10	0.38**	-0.08	-0.16	0.19*	0.41**	0.00
GF duration	-0.09	-0.24**	0.08	0.41**	0.02	0.40**	-0.07	-0.40**	-0.10	0.28**
t_{max}	0.07	-0.18	0.15	0.35**	0.24*	0.38**	-0.17	-0.27**	0.13	0.31**
MWC	0.35**	0.31**	-0.04	-0.27**	0.26*	-0.13	0.13	0.19*	0.50**	0.20*
WAR	0.27*	0.36**	-0.16	-0.40**	0.04	-0.30**	0.27*	0.31**	0.34**	0.02
WLR	0.21	0.06	0.06	0.07	0.26*	0.15	-0.07	-0.09	0.27*	0.29**
t_{mwc}	0.15	-0.23*	0.22	0.45**	0.41**	0.48**	-0.27*	-0.37**	0.28*	0.38**

[a] Trait abbreviations: GFR, grain filling rate; GF, grain filling; t_{max}, the time at maximum grain filling rate; MWC, maximum water content of grains; WAR, water absorption rate of grains; WLR, water loss rate of grains; t_{mwc}, the time at maximum water content; GA_{per}, duration of green area persistence; GA_{loss}, duration of rapid green area loss; GA_{tot}, total duration of green area persistence and loss; Max GALR, maximum green area loss rate; GA_{accum}, accumulated green area.

[b] * Significant at $P < 0.05$, ** significant at $P < 0.01$.

Table 5

Phenotypic correlations between chlorophyll degradation of flag leaves and grain filling traits.

Grain filling[a]	Chl_{per}		Chl_{loss}		Chl_{tot}		Max chl		Max CLR		Chl_{accum}	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Initial GFR	0.12	0.06	-0.21	-0.06	-0.14	-0.03	-0.18	0.17	0.24*	0.02	-0.10	0.15
Rapid GFR	0.45**[b]	0.18	-0.37**	-0.25**	0.15	-0.21*	0.05	-0.08	0.32**	0.24**	0.36**	-0.02
Late GFR	0.42**	0.13	-0.33**	-0.22*	0.17	-0.19*	0.11	-0.12	0.26*	0.22*	0.40**	-0.12
Average GFR	0.45**	0.20*	-0.39**	-0.29**	0.12	-0.23*	0.02	-0.03	0.35**	0.27**	0.33**	0.00
Max GFR	0.45**	0.18	-0.37**	-0.26**	0.15	-0.22*	0.07	-0.09	0.32**	0.26**	0.37**	-0.06
Onset of GF	0.34**	0.00	-0.18	0.02	0.27*	0.03	0.17	-0.16	0.13	0.02	0.43**	-0.08
GF duration	-0.06	-0.12	0.08	0.36**	0.04	0.38**	0.10	0.22*	-0.02	-0.30**	0.02	0.28**
t_{max}	0.13	-0.13	-0.02	0.42**	0.20	0.45**	0.21	0.10	0.05	-0.31**	0.27*	0.23*
MWC	0.54**	0.18	-0.48**	-0.19*	0.12	-0.13	0.04	0.08	0.43**	0.15	0.41**	0.11
WAR	0.45**	0.17	-0.49**	-0.31**	-0.04	-0.29**	-0.06	0.00	0.44**	0.23*	0.25*	-0.06
WLR	0.22	0.08	-0.19	0.04	0.06	0.10	0.27*	0.24**	0.19	0.01	0.20	0.29**
t_{mwc}	0.14	-0.10	0.02	0.44**	0.28*	0.50**	0.22	0.13	0.00	-0.30**	0.29*	0.36**

[a] Trait abbreviations: GFR, grain filling rate; GF, grain filling; t_{max}, the time at maximum grain filling rate; MWC, maximum water content of grains; WAR, water absorption rate of grains; WLR, water loss rate of grains; t_{mwc}, the time at maximum water content; Chl_{per}, duration of chlorophyll persistence; Chl_{loss}, duration of rapid chlorophyll loss; Chl_{tot}, total duration of chlorophyll persistence and loss; Max chl, maximum chlorophyll content; Max CLR, maximum chlorophyll loss rate; Chl_{accum}, accumulated chlorophyll content.

[b] * Significant at $P < 0.05$, ** significant at $P < 0.01$.

3.6. Close relationships between grain filling processes and final individual grain weight

Individual grain weight at maturity was closely associated with GFR (initial, rapid, late, average, and maximum), rather than GF duration (Table 6), indicating the importance of dry matter synthesis efficiency. Similarly, there were strong relationships between final individual grain weight and grain water accumulation (MWC, WAR, and WLR) across years. Furthermore, correlation analysis also revealed a strong relationship between MWC and average GFR ($r = 0.91$, $P < 0.01$ in 2012; $r = 0.88$, $P < 0.01$ in 2013).

Table 6
Phenotypic correlations between grain filling traits and final individual grain weight.

Grain filling[a]	TGW		Grain filling	TGW	
	2012	2013		2012	2013
Initial GFR	0.53**[b]	0.44**	GF duration	0.18	0.11
Rapid GFR	0.77**	0.63**	t_{max}	0.42**	0.08
Late GFR	0.64**	0.32**	MWC	0.84**	0.83**
Average GFR	0.83**	0.76**	WAR	0.60**	0.66**
Max GFR	0.76**	0.58**	WLR	0.68**	0.75**
Onset of GF	0.40**	-0.03	t_{mwc}	0.47**	0.11

[a] Trait abbreviations: GFR, grain filling rate; GF, grain filling; t_{max}, the time at maximum grain filling rate; MWC, maximum water content of grains; WAR, water absorption rate of grains; WLR, water loss rate of grains; t_{mwc}, the time at maximum water content; TGW, thousand grain weight.

[b] * Significant at $P < 0.05$, ** significant at $P < 0.01$.

3.7. QTL coincidences reflect the physiological relationships between anthesis dates, flag leaf senescence, grain filling traits, and individual grain weight

A total of 118 significant QTL were identified in the mapping population of Forno × Oberkulmer in two years, including six QTL for anthesis dates, 24 for flag leaf senescence, 69 for grain filling traits, and 19 for individual grain weight (Fig. 3 and Supplementary Table S2). These QTL individually explained 6.5−37.0% of the phenotypic variation, and were located on 17 chromosomes.

QTL coincidences among anthesis dates, flag leaf senescence, grain filling traits, and individual grain weight occurred (Fig. 3). Four QTL for anthesis dates were coincident with those for Chl_{per}, Max CLR, average GFR, MWC, WAR, and individual grain weight on chromosomes 4DL, 5A, and 7B. These coincident QTL had increasing alleles (increasing the trait values) conferred by the opposite parents (either Forno or Oberkulmer), indicating that earlier anthesis (decreasing alleles) contributes to Chl_{per}, Max CLR, average GFR, MWC, WAR, and individual grain weight, because of pleiotropy or tight gene linkages. In contrast, three QTL for anthesis dates were coincident with two QTL for Chl_{loss}, with the increasing alleles from the same parents. Three of four QTL for GA_{per} were coincident with six QTL for rapid GFR, two for late GFR, six for average GFR, four for max GFR, six for MWC, three for WAR, four for WLR, and five for individual grain weight on chromosomes 2A, 4A, and 7B; their increasing alleles originated from the same parents. Similarly, Chl_{per} had two QTL coincident with the GFR (rapid, late, average, and max), grain water accumulation (MWC, WAR, and WLR), and final individual grain weight on chromosomes 4A (all increasing alleles derived from Oberkulmer) and 5A (increasing alleles from Forno). On the other hand, Chl_{loss} showed QTL coincidences with GFR, grain water accumulation, and individual grain weight on 4A and 7B, but had increasing alleles conferred by the opposite parents. Six QTL were identified for Max CLR, and five of them coincident with GFR, grain water

accumulation, and final individual grain weight on 3A, 4A and 7B, with the increasing alleles from the same parents except those on 3A. A total of 27 (84% of the total) QTL for GFR and 27 (82%) for grain water uptake and loss coincided with those for individual grain weight, and all the increasing alleles were conferred by the same parents, explaining the close relationships between grain filling processes and final individual grain weight. Analysis of the allelic effects of these coincident QTL has been summarized in Supplementary Tables S3−S7.

Taken together, earlier anthesis was associated with delayed onset of flag leaf senescence, shorter duration and faster rate of rapid senescence, which led to faster individual grain dry matter accumulation, faster grain water absorption, and higher maximum grain water content; as a consequence, larger grains were produced (Fig. 4). This physiological model is consistent with the genetic analysis showing a high level of QTL coincidences, suggesting pleiotropy or tight linkages of functionally related genes.

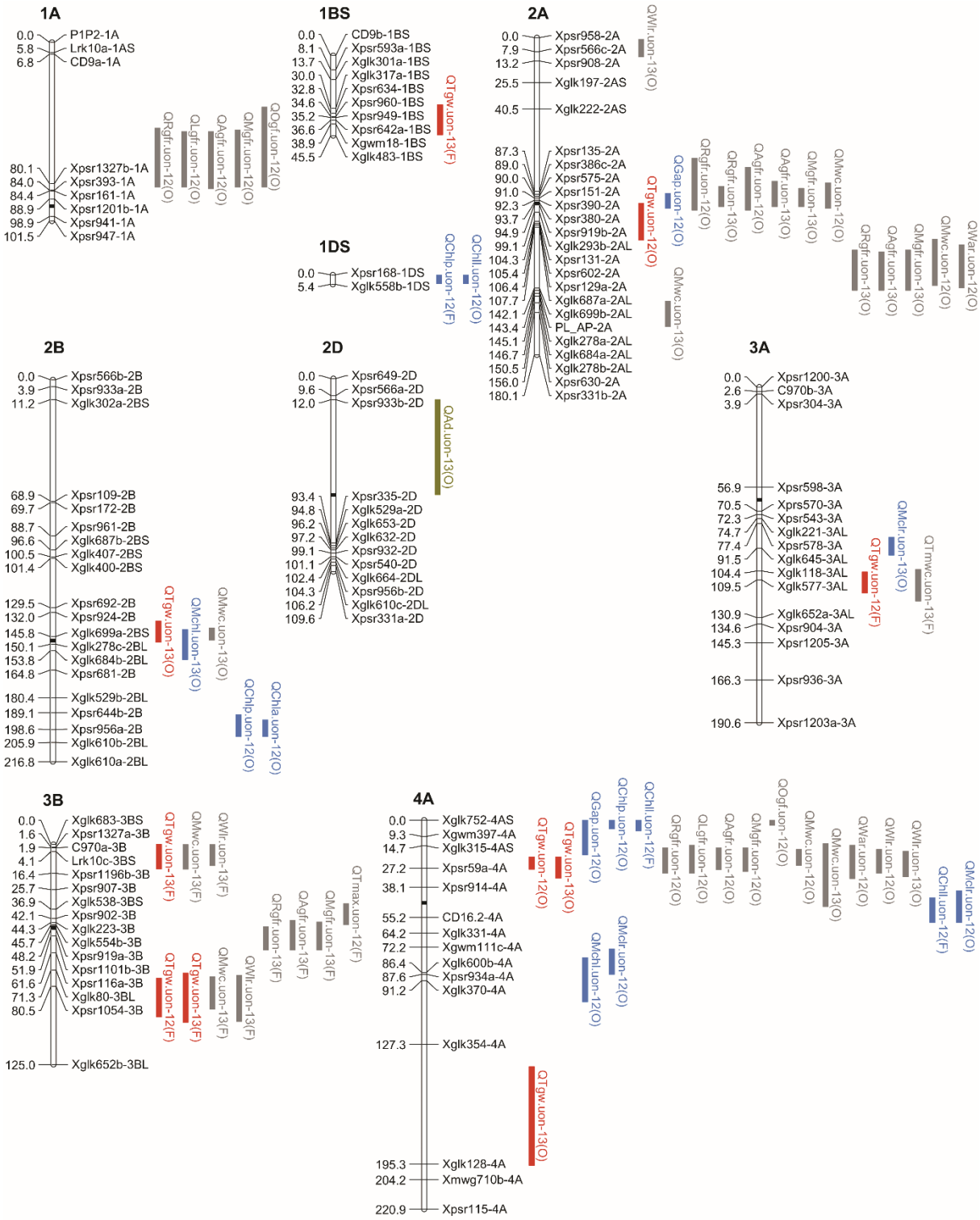

Fig. 3 (see the caption below)

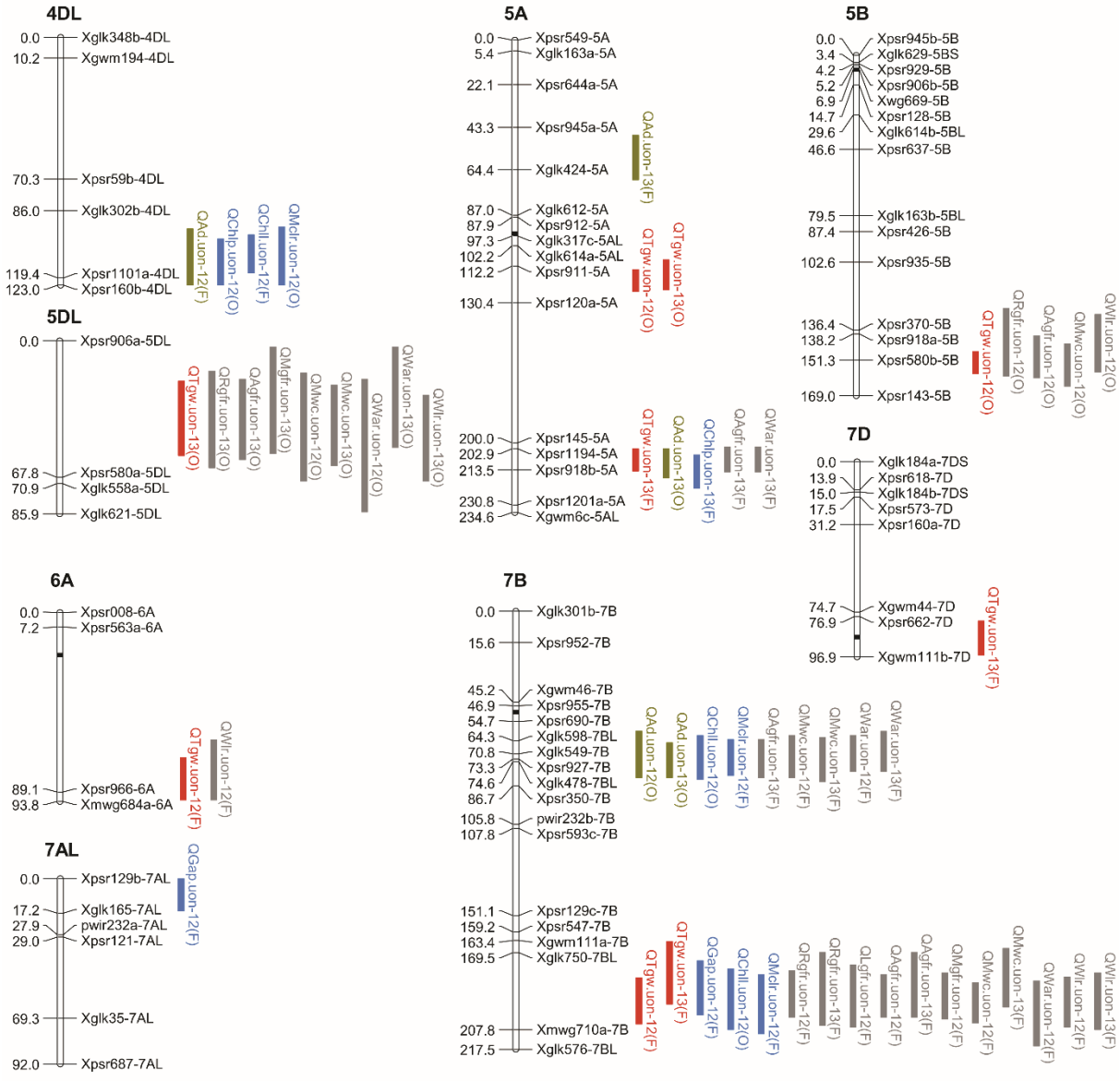

Fig. 3 (continued; see the caption below)

Fig. 3 (color in print). Quantitative trait locus (QTL) identification for anthesis dates, flag leaf senescence, grain filling traits, and final individual grain weight. The 1-LOD support intervals of significant QTL are indicated by red (individual grain weight), green (anthesis dates), blue (flag leaf senescence), and grey (grain filling traits) vertical bars. A QTL symbol includes a letter 'Q', trait abbreviation, laboratory name (*uon*), a suffix 12 or 13 indicating 2012 or 2013 in which the QTL was detected, and the parents in parentheses conferring the increasing alleles (increasing the values of the traits): F, bread wheat Forno; O, spelt Oberkulmer. Trait abbreviations: Tgw, thousand grain weight; Ad, anthesis date; Gap, duration of green area persistence; Chlp, duration of chlorophyll persistence; Chll, duration of rapid chlorophyll loss; Mchl, maximum chlorophyll content; Mclr, maximum chlorophyll loss rate; Chla, accumulated chlorophyll content; Rgfr, rapid grain filling rate; Lgfr, late grain filling rate; Agfr, average grain filling rate; Mgfr, maximum grain filling rate; Ogf, onset of grain filling; Tmax, the time at maximum grain filling rate; Mwc, maximum water content of grains; War, water absorption rate of grains; Wlr, water loss rate of grains; Tmwc, the time at maximum water content.

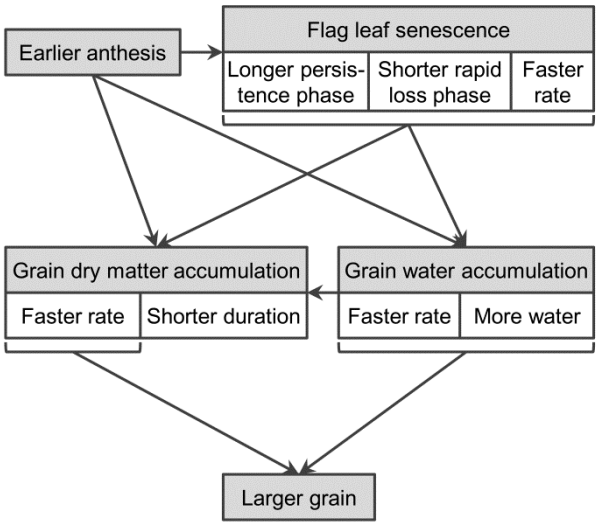

Fig. 4. A model showing the relationships between anthesis dates, flag leaf senescence, grain filling traits, and final individual grain weight

4. Discussion

4.1. Interactions among anthesis time, leaf senescence, grain filling and individual grain weight

In this study, earlier anthesis contributes to longer persistence phase of flag leaves, faster rate and shorter duration of rapid senescence. The negative relationship between anthesis time and the onset of leaf senescence is in line with the previous studies in wheat (Bogard et al., 2011; Kipp et al., 2014; Verma et al., 2004). There is also a negative association between heading time and green leaf duration calculated from heading (Naruoka et al., 2012). The QTL coincidences between anthesis time and leaf senescence are consistent with their physiological relationships. The QTL *QAd.uon-13* for anthesis date was identified on the short arm of chromosome 2D, and it may correspond to the photoperiod-response gene *Ppd-D1* (Hanocq et al., 2004; Snape et al., 2001). This gene has pleiotropic effects: the *Ppd-D1* allele (insensitivity to photoperiod) not only advances anthesis (*c*. 10 days), but also confers greater maintenance of green area after anthesis (Foulkes et al., 2004). Negative pleiotropic effects of *Ppd-D1* on anthesis dates and the onset of leaf senescence have been validated in a recent report (Bogard et al., 2011). Furthermore, Bogard et al. (2011) also reported the QTL coincidences between anthesis dates and the onset of leaf senescence on 2A, showing negative pleiotropic effects or closely linked genes. In this study, four QTL for anthesis dates were detected on 5A and 7B, consistent with the previous observation in the same population grown in Switzerland (Keller et al., 1999), suggesting stability of expression of these alleles. Of these QTL, the one coincident with the onset of leaf senescence on 5AL corresponds to a previous QTL for ear emergence time based on common marker analysis (Kato et al., 1999; Simonetti et al., 1999). This QTL is approximately 40 cM distal to *Vrn-A1*, the major homeologous gene controlling vernalization response in wheat; however, the two genes have comparable effects on ear emergence time (*c*. 4 days) (Kato et al., 1999). In diploid wheat

Triticum monococcum L., similarly, there is a second vernalization gene *Vrn-A^m2*, which is 50 cM distal to *Vrn-A^m1* on 5A^mL (Dubcovsky et al., 1998). Thus, the QTL in the present study could be *Vrn-A2* in hexaploid wheat. The *Vrn-2* series have been predicted existing distally on the long arms of 4B, 4D and 5A (because of the 4A/5A translocation) (Snape et al., 2001). Intriguingly, a QTL for anthesis time was mapped on the distal region of 4DL, and could be the *Vrn-D2* in hexaploid wheat. This QTL also showed coincidence with those for leaf senescence. On chromosome 7B, the coincidence of the QTL for anthesis dates, and the duration and rate of rapid senescence, were identified, but whether or not they are related to the *Vrn-B3* gene is unknown (Yan et al., 2006). Taken together, leaf senescence in wheat is partially dependent on the genetics of the flowering system. Interactions between them have also been found in other plants such as *Arabidopsis* (Wingler, 2011) and barley (*Hordeum vulgare* L.) (Lacerenza et al., 2010).

Earlier anthesis and delayed leaf senescence were found to contribute to larger grains at maturity. Many reports have demonstrated the phenotypic relationships between anthesis time, leaf senescence, and yield; however, only a few have presented their associations with individual grain weight, a major determinant of yield after anthesis (Gooding et al., 2000; McIntyre et al., 2010; Naruoka et al., 2012). In 2012, there was a significant association between leaf senescence rate and final individual grain weight, as suggested in durum wheat by Hafsi et al. (2000). A detailed analysis showed that early anthesis, delayed but short and fast leaf senescence tended to increase grain filling rates, grain water absorption rate and in turn maximum grain water content, but shorten the progression of individual grain dry matter and water accumulation. The latter observation does not follow the traditional notion that extending stay-green could increase grain filling duration. In fact, grain filling rate is more important than its duration for individual grain weight, and more responsive to the increased assimilate availability through stay-green. This responsiveness has also been seen under the

stressed conditions such as heat, drought and elevated CO_2 that lead to faster but shorter grain filling (Li et al., 2001; Yang et al., 2004; Zahedi and Jenner, 2003). Relationships between anthesis time, leaf senescence, grain filling rates, grain water uptake and loss, and final individual grain weight, are consistent with the genetic analysis showing considerable QTL coincidences between QTL for these traits. Thus, these processes may be coordinately regulated by the pleiotropic effects (either directly or through transcription factor action) or close linkages of functionally related genes. Potential genes may include the *Vrn* and *Ppd* loci responsible for flowering time. Among different vernalization gene combinations, for example, the genotypes with one or more dominant alleles display early flowering and higher individual grain weight than the combination of *vrn-A1/vrn-B1/vrn-D1* (Zhang et al., 2014).

4.2. Early anthesis, and delayed but fast leaf senescence as an ideotype for wheat breeding

Earlier anthesis increases final individual grain weight through improved grain filling rates and grain water accumulation, as discussed above. Thus, accelerating anthesis to some extent seems to be important for grain size improvement under the rainfed conditions. With predicted climate change and consequently more frequent heat stress at meiosis and anthesis, when wheat yield is most sensitive, early anthesis could confer summer drought escape (Semenov et al., 2014). An appropriate anthesis time is likely achieved by adjusting the major genes responding to vernalization and photoperiod. Examination of near isogenic lines has showed that the photoperiod insensitive allele *Ppd-D1* reduces anthesis time by 6–14 days, increases spikelet fertility, and produces larger grains and higher yield, depending on growing environment and summer conditions (Snape et al., 2001). Genotypes combining one or more dominant alleles of *Vrn* genes tend to flower earlier with high individual grain weight and yield (Iqbal et al., 2007; Zhang et al., 2014). In wheat breeding programs, selection for early anthesis should be efficient, as this trait has high heritability (Keller et al., 1999; McIntyre et

al., 2010). While designing wheat for local climatic conditions, care must be taken not to shorten the critical period for grain number generation, namely from the emergence of penultimate leaf to anthesis, during which the rapid growth of spikes occurs (González et al., 2011). Meanwhile, sowing time should be adjusted so that early genotypes are able to avoid frost damage in spring (Foulkes et al., 2004).

Recently, breeding for leaf senescence has thrown up a potential dilemma, with it being proposed that delayed senescence is associated with higher yield potential, but with lower nutrient use efficiency (Gregersen et al., 2008). To address this dilemma, Wu et al. (2012) suggested an ideotype for leaf senescence: delaying the onset, but speeding the rate, of senescence. Delayed senescence could extend the phase of functional photosynthesis so that more assimilates would be produced for grain filling. Once terminal leaf senescence is initiated, a faster rate would improve the efficiency of nutrient remobilization, leading to a higher harvest index (the ratio of grain yield to total above-ground plant biomass). In the present study, the results concur with this model. Indeed, delayed leaf senescence increased grain filling rate (but not its duration), grain water absorption rate and maximum grain water content, resulting in larger grain. Although delayed onset of leaf senescence reduced the duration of the rapid senescence phase, the senescence rate was accelerated greatly. Fast senescence allows the rapid degradation of macromolecules and subsequent remobilization of these nutrients to grains. As a result, leaf senescence rate was positively associated with grain filling rates. This type of stay-green has also been found in tobacco P_{SAG12}*:IPT* transformants, which display a dramatic delay of senescence, followed by a sudden death, resulting in significant increases in biomass (40%) and seed yield (52%) (Gregersen et al., 2013). Therefore, delayed, shorter but faster leaf senescence could lead to better utilization of current photosynthetic capacity and structural nutrients for larger grains. This ideotype is also beneficial to improve grain number, the other major component of grain yield of wheat. The

current study reveals a significant positive relationship between the onset of leaf senescence and grain number per m^2 in each year regardless of the assessment methods for senescence dynamics (data not shown). It suggests that more grains defined before anthesis require a longer phase of functional photosynthesis, and a similar result has been reported in a stay-green mutant (Derkx et al., 2012). Thus, it seems that delayed leaf senescence is a favorable trait for both grain size and grain number, giving rise to high yield potential. In addition, it could also be possible to increase grain protein concentration for improved grain quality through stay-green phenotype (Bogard et al., 2011). An example is overexpressing some particular NAC transcription factors (Zhao et al., 2015).

5. Conclusions

This work describes the relationships between anthesis time, leaf senescence and individual grain weight, and their underlying physiological and genetic mechanisms, using a mapping population of bread wheat crossed with spelt with contrasting phasic development and leaf senescence kinetics. Earlier anthesis, and delayed, shorter but faster leaf senescence, are associated with larger individual grain at maturity. These result from increased grain filling rate (but not its duration) and grain water accumulation, two major processes driving final individual grain weight. Genetic analysis revealed a high level of QTL coincidences between these traits, indicating pleiotropic effects or tight gene linkages, which confirm their physiological relationships. Therefore, slightly early anthesis, and delayed but fast leaf senescence, are desirable traits to improve individual grain weight and, in turn, yield potential in wheat breeding.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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