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# Variability of duration of pre-anthesis phases as a strategy for increasing wheat grain yield

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ABSTRACT

In wheat, stem elongation phase (SEP) duration is critical for grain number (GN) per unit of area determination, as it is the phase in which the spikes grow. Lengthening SEP, for instance by photoperiodic sensitivity, without altering the cycle to anthesis (AT) has been proposed as an alternative way to increase spike dry weight, and in turn GN. As most works supporting this idea have modified only SEP by artificial manipulation (e.g. photoperiod extensions), it is relevant to evaluate this hypothesis in populations segregating for this attribute in natural conditions. The aim of this work was to analyse the variability in SEP duration relative to AT in two F4 populations; in order to select contrasting phenotypes to evaluate the impact of this attribute on grain yield components and to analyse the selection response of this attribute. These segregating populations (Las Rosas INTA × Triguero 230 (A) and Klein Estrella × ProINTA B. Alazán (B)) were derived from parental lines with similar flowering time but different relative duration of their pre-anthesis phases. Two field experiments with previous vernalization treatment in cool chamber were carried out. In 2006, F4 populations were characterized and from one of them (population B, which presented higher variability) four groups were selected, which presented contrasting phenotypes in the attribute under study. Progenies of these groups (F5), together with remnant F4 full-sib of each one, were studied during 2007. Grain yield per plant was higher, due to GN increases, when duration of the SEP was lengthened. However, selection response to longer SEP with similar cycle to AT could not be found, possibly as the result of a high environmental influence on this attribute. The phenotypic variability evidenced in this attribute was not clearly associated with major adaptation genes evaluated (i.e. Ppd and/or Vrn), suggesting that other minor genes could be associated.



## 1. Introduction

Improving wheat (*Triticum aestivum* L.) grain yield potential is required to match expected population growth. From the beginning of the twentieth century, wheat grain yield was consistently increased, mainly after the introgression of dwarfing genes (*Rht*) into the background of adapted varieties (Siddique et al., 1989; Calderini et al., 1999). However, during the last decades, strategies of conventional breeding have been insufficient to keep improvement rate similar to the past (Calderini and Slafer, 1998; Miralles and Slafer, 2007), suggesting efficiency losses in breeding programs (Fischer, 2007). Therefore, since this cereal is an essential component of the global food security (Reynolds et al., 2011), increasing wheat production is necessary to follow the pace of population growth in the near future (Evans, 1998; Borlaug, 2007). In this scenario, progress in grain yield is still the factor of greatest impact on productivity growth, and the genetic gain in grain yield potential is still the main component of this progress (Fischer, 2007).

Genetic improvement in grain yield potential could be accelerated if ecophysiological attributes are used as selection criteria, especially in a crop such as wheat, which has already been subjected to an intense selection pressure (Shorter et al., 1991; Reynolds et al., 2001, 2011). For this purpose, it is necessary not only to identify

*Abbreviations:* TR, transplant; FN, first node detectable; SEP, stem elongation phase; SGP, spike growth period; AT, anthesis; GN, grain number; GW, grain weight; SD, standard deviation; ES, early flowering time and short SEP; EL, early flowering time and long SEP; LS, late flowering time and short SEP; LL, late flowering time and long SEP.

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ecophysiological attributes functionally linked to grain yield potential, but also to improve the understanding of the genetic basis controlling those traits for easy handling (Slafer, 2003). In this sense, interdisciplinary approaches that combine studies at different approximation levels (i.e. molecular, crop ecophysiology and genetic improvement) are required to raise this genetic gain in wheat grain yield potential, which will probably be harder to achieve than in the past (Slafer, 2003; Edmeades et al., 2004; Sinclair et al., 2004).

Considering a wide range of environmental conditions, grain number (GN) per unit of area is the main component that explains the variations in grain yield (Magrin et al., 1993; Calderini et al., 1999; Fischer, 2008). This component, although it is generated during the whole period, from sowing to immediately after anthesis (AT), is determined a few weeks before flowering (Fischer, 1983, 1985). The stem elongation phase (SEP) (i.e. from terminal spikelet initiation to AT), during which the spike growth period (SGP) is included, is critical to GN setting, as assimilates allocated in spikes at the end of this period are crucial for floret establishment (Kirby, 1988; Slafer et al., 1994; Miralles and Slafer, 1999). In fact, a strong relationship was found between the spike dry weight at flowering and the number of fertile florets (the majority set a grain) under a wide range of conditions (e.g. Siddique et al., 1989; Miralles et al., 2000; González et al., 2005c; Serrago et al., 2008). As future increases in biomass partitioning to spike through genetic reduction of height seem to be unlikely, it is necessary to find alternatives to raise spike growth maintaining high levels of partition as a way to improve GN (Miralles and Slafer, 2007).

Development manipulation during the pre-anthesis phases has been proposed as a way to reduce source competition between stems and spikes during SGP. Along this line, lengthening the duration of SEP (or more specifically SGP) without altering the flowering time has been hypothesized as an alternative way to increase spike dry weight, and GN in turn (Slafer et al., 1996; Miralles et al., 2000; Slafer, 2003). Following that hypothesis, previous evidence demonstrated: (i) the existence of variability in SEP duration in commercial wheat cultivars with similar flowering time (Whitechurch et al., 2007a,b); (ii) the sensitivity to actual photoperiod during SEP is physiologically independent of the response in previous development phases, using artificial manipulation of photoperiod both under controlled conditions (Miralles and Richards, 2000) and in field studies (Whitechurch and Slafer, 2002); and (iii) the number of fertile florets raised as spike dry weight was increased, due to a longer SEP (Miralles et al., 2000; González et al., 2005c; Serrago et al., 2008), suggesting that increased spike fertility was mediated by a greater accumulation of carbohydrates (Ghiglione et al., 2008).

The evidence described above supports the idea that any genetic factor (e.g. photoperiod sensitivity) lengthening SEP, independently of the total flowering time, will improve spike dry weight at AT and increase its fertility (Slafer et al., 2001). However, most of the experiments to test the hypothesis were carried out using different commercial cultivars. Besides, changes in SEP duration were made by artificial manipulation of environmental factors, such as photoperiod, focusing on particular phases. Those approaches were important to establish the physiological bases of the spike growth and floret survival response to SEP duration. Nevertheless, they cannot be extrapolated to real crops, as the environment manipulation described above cannot be artificially made in a commercial crop. Therefore, it is necessary to demonstrate the advantage of a longer SEP duration on grain yield in natural field conditions, employing genetic populations specifically designed for this purpose. This approach constitutes a starting point for new research assisted by molecular tools, which can be focused on a precise determination of these genetic bases.

The objective of this work was, firstly, to analyse the variability in SEP duration relative to AT in two F4 populations obtained from contrasting cultivars for this attribute. Secondly, to select divergent phenotypes to evaluate the impact of SEP duration relative to AT on the main grain yield components and to analyse the selection response of this attribute.

## 2. Materials and methods

### 2.1. Experimental conditions and plant material

Two field experiments were carried out in the experimental field of the Department of Plant Production of the University of Buenos Aires (34° 35′S, 58° 29′W, 25 m.a.s.l.) during 2006 and 2007 growing seasons. Trials were irrigated and adequate nutrients applied (i.e. fertilization was adequate to obtain more than 35 ppm of phosphorus and 200 kg of nitrogen per hectare). Plant pathogens and pests were prevented by chemical treatments, and weeds were removed by hand to avoid any negative effect of hormonal herbicides on crop development. Seedlings were vernalized before transplant (TR) to avoid any difference in vernalization requirement among materials. Thus, seeds were germinated in pots filled with an inert substrate (i.e. vermiculite) and, after 24 h, they were transferred to a cold chamber (4–6 °C, 8 h daylength) for 15 days. Vernalized seedlings were transplanted to the field on 19 July 2006 and 13 July 2007. Both dates were taken as the beginning of each experiment (indicated through the text as TR). Seeds of each material, previous to vernalization treatment, were equally distributed in strips of biodegradable paper which were finally transplanted ensuring a uniform seedling depth and distance distribution in the field.

In the first experiment (2006), two F4 populations, called A and B in this work, were evaluated. They derived from parental lines with similar flowering time but different SEP duration. Population A was obtained from cultivars Las Rosas INTA and Triguero 230, and population B was obtained from Klein Estrella and ProINTA Bonaerense Alazán. These cultivars were selected screening more than 60 Argentinean commercial cultivars (Whitechurch et al., 2007a). Population A consisted of a smaller number of F4 individuals than population B (73 and 173, respectively). Both F4 were derived from F2 by single seed descendant methodology (SSD) (Goulden, 1939; Brim, 1966). Both F4 populations (A and B) and parental lines were randomly distributed in the field (i.e. they were randomly distributed in the strips of biodegradable paper) and each individual was tagged in order to be identified.

In the second experiment (2007), four groups of F5 progenies, obtained from contrasting F4 (2006) phenotypes in SEP duration relative to AT were evaluated. Divergent selection was carried out to obtain contrasting groups for that attribute from data obtained only from population B. Firstly, two sets of ca. 20 individuals, classified as early or late flowering time, were selected considering those whose cycle to AT differed more than 1.5 standard deviation (SD) from the mean of the whole population. Within each set with similar flowering time, two groups of 6 individuals according to their SEP were selected. The selection of extreme phenotypes within each set (early and late) was carried out based on differences of at least one SD from its mean SEP, obtaining materials with long and short SEP within early and late flowering time. Therefore, the resulting groups combined the following phenology: (i) early flowering time with short (ES) and long (EL) SEP, and (ii) late flowering time with short (LS) and long (LL) SEP. Besides, this second experiment included remnant seeds of F4 full-sib of each individual selected in 2006 for the progeny test, which were disposed in contiguous rows to the corresponding F5 progeny. The experimental arrangement was a complete randomized design with 6 replicates

(i.e. six individual of each selecting group in 2006). Each replicate consisted of a single row of ten plants.

### 2.2. Measurements and analyses

In both experiments, all plants of each individual were observed every 2–3 days to determine the stages of first node detectable (FN) (GS31; Zadoks et al., 1974) and AT (GS65; Zadoks et al., 1974) in main stems. The non-destructive measurement of the FN stage was considered as a phenotypic marker to determine the onset of SEP which, under most agronomic conditions, coincides with terminal spikelet initiation in wheat (Slafer and Rawson, 1994; Kirby et al., 1999). Durations of phases were expressed in thermal time, using the average temperature recorded at the site (Vantage Pro 2, Davis Instruments Co. Inc.; San Francisco, USA) and assuming a base temperature of 0 °C (Kirby et al., 1985). Cycle to AT was divided in two phases, from TR to FN and from FN to AT, the last one corresponding to SEP. Although the beginning of the spike growth starts a short time after the onset of SEP, in this work the SGP was considered as the period from FN to AT because (i) the determination of the onset of active spike growth would require frequent sampling and plant dissection, and (ii) the methodology proposed in this work is, in fact, the realistic determination that could be performed in a breeding programme (Whitechurch et al., 2007a).

All plants were harvested ca. three weeks after physiological maturity to obtain grains with the lowest moisture percentage, in order to keep seeds for the next generation without the need to be oven-dried. Grain yield and its main components, i.e. GN and grain weight (GW) were determined at plant level. Physiological maturity was determined indirectly by the colour of peduncle as a morphological marker, assuming that this maturity stage coincides with the complete loss of green colour from the peduncle or the flag leaf (Hanft and Wych, 1982). It is the most common indirect method to detect this physiological stage in wheat (Calderini et al., 2000).

Based on previous evidence about the influence of photoperiodic response on the duration of pre-anthesis phases (Whitechurch et al., 2007b), molecular marker analysis was performed in parental lines of population B (Klein Estrella and ProINTA Bonaerense Alazán) in order to determine the existence of polymorphism between them. The genes analysed were the main controlling photoperiodic response *Ppd-D1*, *Ppd-B1* and *Ppd-A1* (Scarth and Law, 1984; Worland et al., 1998). For *Ppd-D1* and *Ppd-B1* primer sets used to screen parental lines were previously described (Beales et al., 2007; Blake et al., 2009; respectively) and polymerase chain reaction conditions followed published protocols. To develop markers for *Ppd-A1*, primers PPD-A1-LAL-F3: TCACAAGTGCCGGAAGGT and PPD-A1-LAL-R1: TCTTCTTGCTCGTTTCATCAG were designed exploiting A-genome specific mutations identified by sequence alignment of DQ885756 (*Ppd-A1*), DQ885763 (*Ppd-B1*) and DQ885768 (*Ppd-D1*) genomic sequences. These primers were used to amplify a 548-bp fragment of *Ppd-A1* from Klein Estrella and ProINTA Bonaerense Alazán which were directly sequenced. This allowed the development of a *Ppd-A1* locus-specific cleaved amplified polymorphic sequence marker. As obtained *Ppd-A1* sequences from both parental lines could be differentiated by an additional *Hae* III restriction site in ProINTA Bonaerense Alazán (positions 273 and 323 of the PCR fragment) that was absent in Klein Estrella (only position 323). Amplification with primers PPD-A1-LAL-F3 and PPD-A1-LAL-R1 was performed with a touchdown program: 94 °C for 3 min + 10 touchdown cycles (94 °C for 30 s, 58 °C for 30 s, decrease 0.5 °C per cycle, 72 °C for 45 s) + 39 cycles (94 °C for 30 s, 53 °C for 30 s, 72 °C for 45 s) + 72 °C for 5 min. After that, polymerase chain reaction products were directly digested with 5 units of restriction enzyme *Hae* III and the digested products were distinguished using 1.5% agarose gels. Primers and nucleotidic sequences used in this study were analysed using softwares Primer3 (Rozen and Skaletsky, 2000) and Clustal W (Thompson et al., 1994).

Different statistical parameters (i.e. mean, standard deviation, minimum and maximum) were calculated. Besides, regression analysis with test of similitude between slopes ($\alpha = 0.05$) were performed to characterize each population in 2006, based on phenotypic data. Statistical differences between contrasting groups during 2006 (phenology and grain yield) together with the corresponding progeny (F5) and F4 full-sib during 2007 (phenology) were tested through standard analyses of variance. When this analysis revealed significant differences, the mean values of each group were compared using Tukey test ($\alpha = 0.05$). The software used for statistical analysis was InfoStat (Di Rienzo et al., 2010).

## 3. Results

### 3.1. Phenology of F4 populations

Both populations (A and B) showed similar relative durations of pre-anthesis phases with a similar variability in SEP and in cycle to AT (Table 1). The F4 derived from Klein Estrella and ProINTA Bonaerense Alazán (population B) showed a longer cycle to AT (ca. 150 °Cd) than the F4 derived from Las Rosas INTA and Triguero 230 (population A). Each pre-anthesis phase (i.e. TR-FN and SEP) was ca. 75 °Cd longer in population B with respect to population A. On average, SEP represented ca. 57% of total flowering time in both populations. The range of variability in SEP (ca. 330 °Cd in both populations) was similar to the range observed in cycle to AT. However, the range of variability observed in the duration of first pre-anthesis phase (TR-FN) was smaller and different between populations (ca. 120 °Cd and 220 °Cd in populations A and B, respectively).

Population B showed a higher variability in the duration of pre-anthesis phases for a similar flowering time than population A (Fig. 1). A positive lineal relationship was observed between SEP duration and cycle to AT in both populations ($r^2 = 0.93$ and $r^2 = 0.66$, for A and B populations, respectively), without significant differences ($p > 0.05$) in the slopes of linear regressions in both populations. No relationship was found between TR-FN phase and cycle to AT (Fig. 1), and TR-FN phase and SEP (Fig. 2). Variability in the duration of pre-anthesis phases for a similar flowering time in population B was more than twice the one observed in the population A (ca. 70 °Cd and 150 °Cd for populations A and B, respectively) and virtually constant for every cycle to AT.

Transgressive segregation was observed in flowering time and in each pre-anthesis phase, registering individuals with longer and shorter durations with respect to the parental lines (Fig. 1). Considering the same flowering time, transgressive segregation was more evident in population B than in A. SEP relative duration to AT of individuals was in general shorter than that observed in their respective parental lines. Conversely, the relative duration of TR-FN phase of individuals was longer than that observed in the parental lines.

### 3.2. Relationship between stem elongation phase duration and grain yield

Based on the results of the first experiment (2006), four contrasting groups of individuals, considering the relative duration of pre-anthesis phases, were selected (Table 2). Taking into account the flowering time data, individuals from population B were separated in three different cycles to AT using iso-duration lines (Fig. 2). Differences in the extreme flowering times were ca. 200 °Cd (i.e. 1300 °Cd and 1500 °Cd for early and late flowering time, respectively). From the extreme divergent cycles to AT, individual phenotypes were selected considering SEP duration. Thus, individ-

**Table 1**
Duration of cycle to anthesis (AT) and pre-anthesis phases, from transplant to first node detectable (TR-FN) and stem elongation phase (SEP), in thermal time units (°Cd). Data from populations A (73 individuals) and B (173 individuals), and the corresponding parental lines that originated these populations. The standard deviation (SD) values and the range of variability (minimum and maximum values) of each population were included.

| | TR-FN (°Cd) | | | | SEP (°Cd) | | | | Cycle to AT (°Cd) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Range | | Mean | SD | Range | | Mean | SD | Range | |
| Population A | 537.9 | 27.7 | 491.9 | 610.8 | 716.6 | 90.7 | 583.2 | 915.7 | 1254.5 | 101.9 | 1129.7 | 1455.1 |
| Las Rosas INTA | 508.4 | | | | 800.1 | | | | 1308.5 | | | |
| Triguero 230 | 554.5 | | | | 701.3 | | | | 1255.8 | | | |
| Population B | 611.3 | 41.4 | 521.3 | 741.3 | 790.4 | 69.3 | 641.8 | 1005 | 1401.7 | 65.2 | 1273.7 | 1602.5 |
| Klein Estrella | 554.5 | | | | 885.9 | | | | 1440.4 | | | |
| ProINTA B. Alazán | 566.9 | | | | 840.3 | | | | 1407.2 | | | |

uals with early and late flowering time (i.e. E- and L-, first letter of the abbreviation), combining with shorter or longer (i.e. -S and -L, second letter of the abbreviation) SEP were obtained. The two selected groups for the same flowering time (i.e. -S or -L) showed significant differences ($p < 0.05$) in the duration of pre-anthesis phases (Table 2), which were of ca. 100 °Cd (i.e. 6 days considering the mean temperature of the phase).

Taking into account similar flowering time, grain yield per plant was higher in groups with extended SEP (Table 2). Although the increments were not significant in statistical terms they were ca. 30% and 50% higher than grain yield of groups with shorter SEP in early and late flowering time groups, respectively. Grain yield per plant was related to GN per plant ($r^2 = 0.87$, $p < 0.05$), without significant association with GW when all groups were considered (Fig. 3). The variations in GN were greater than those observed in GW (coef-

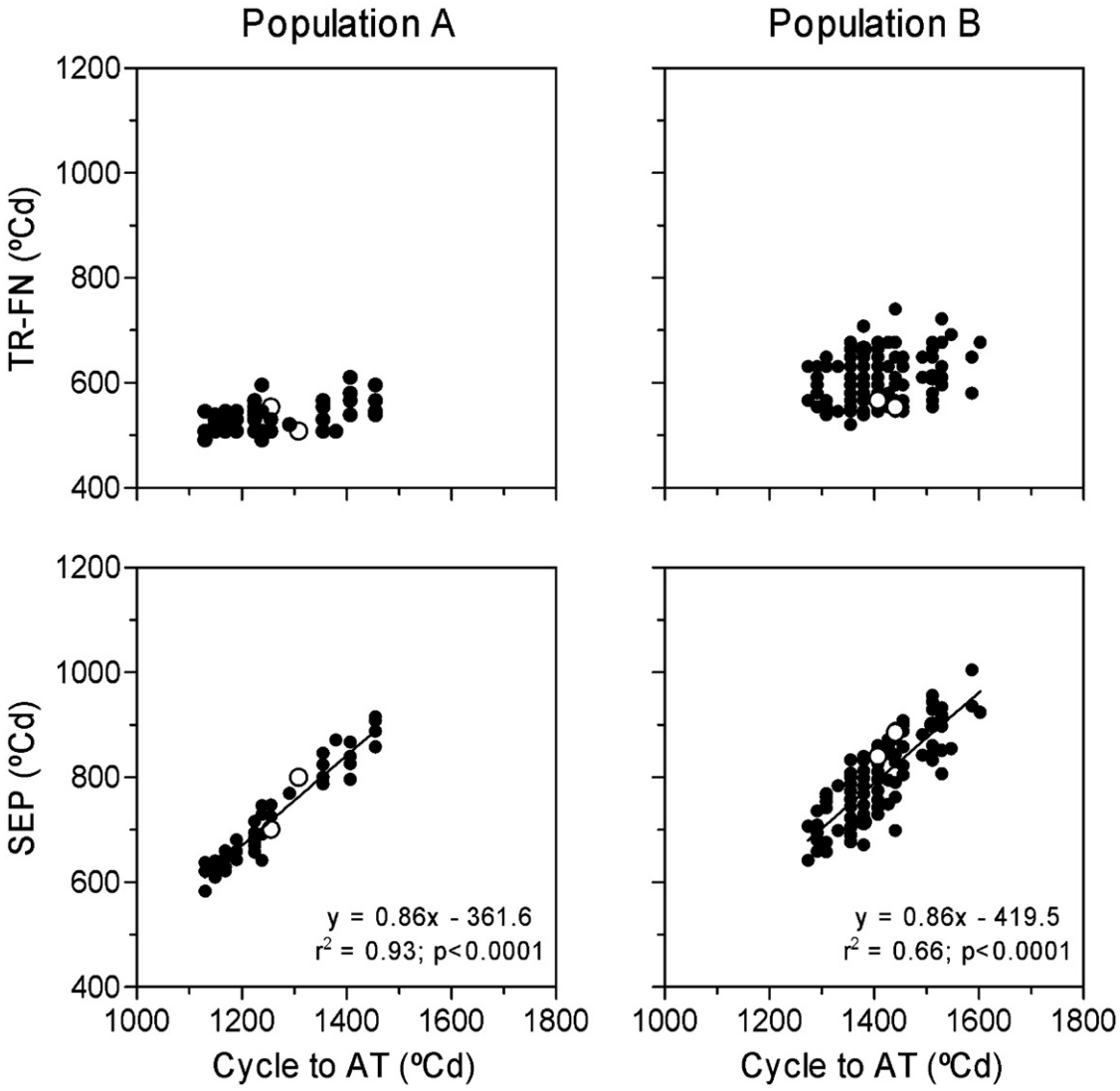


**Fig. 1.** Duration of pre-anthesis phases, from transplant to first node detectable (TR-FN) and stem elongation phase (SEP), in relation to cycle to anthesis (AT), in thermal time units (°Cd), of segregant individuals (closed circles) and parental lines (open circles) from populations A (left panel) and B (right panel). Lines corresponded to the linear regressions ($\alpha = 0.05$) with the corresponding equations and regression coefficients.

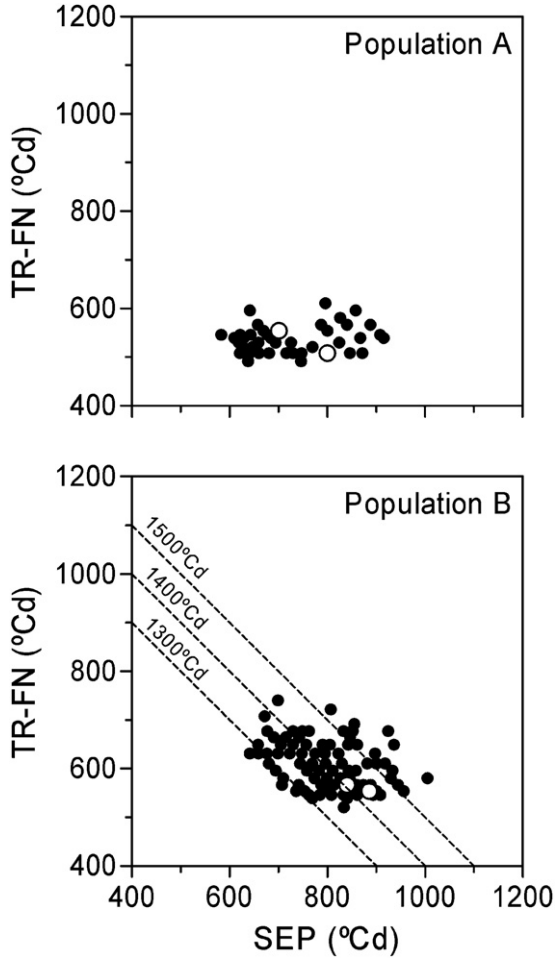


**Fig. 2.** Relationship between duration of pre-anthesis phases, from transplant to first node detectable (TR-FN) and stem elongation phase (SEP), in thermal time units (°Cd), of segregant individuals (closed circles) and parental lines (open circles) from populations A and B. Dotted lines representing iso-durations of flowering time delimited arbitrarily according to data dispersion (i.e. population mean ± 1.5 standard deviation; see Table 1).

ficient of variation: 36% and 14% for GN and GW, respectively). In fact, GW was similar between groups of individuals with the same flowering time ($p > 0.05$) presenting differences lower than 5% (Table 2). In line with the grain yield, GN per plant tended to increase when SEP was lengthened, although increments were not statistically significant (Table 2). Extensions of SEP increased GN per plant ca. 25% and 40% in early and late flowering time, respectively. In general, grain yield and GN were higher in those groups with lower cycle to AT (Fig. 3, inset). Those plants grouped within the EL and LS combination presented the higher and lower grain yield per plant ($p < 0.05$), respectively (Table 2).

### 3.3. Selection response of stem elongation phase duration relative to anthesis

Plants selected in 2006 grouped as late and early flowering time tended to produce an F5 progeny with long and short cycle to AT, respectively, in 2007 (Fig. 4). The same behaviour was observed in the four groups of F4 full-sib. However, opposite to the results observed in response to selection by flowering time, the relative duration of the pre-anthesis phases was not maintained among groups with the same cycle to AT both F5 and F4 in 2007 (Fig. 4). The mean difference in the duration of cycle to AT between late and early groups was ca. 200 °Cd in F4 2006, but only ca. 100 °Cd in both F4 full-sib and F5 during 2007. Neither the F4 full-sib nor F5 progeny showed any discrimination in the relative duration of phases as during 2006. Therefore, no significant differences were detected in the duration of both TR-FN phase and SEP (Fig. 4). In 2007, both F4 full-sib and F5 groups showed a longer duration (ca. 150 °Cd; $p < 0.05$) of TR-FN phase than that observed during 2006 (Fig. 4), while mean duration of SEP was statistically similar between years ($p > 0.05$).

The molecular marker analysis carried out in the parental lines and in the F5 progeny for the PPD1 loci showed different results. A diagnostic 414-bp amplification fragment associated with the recessive *ppd-D1* allele, which confers sensitivity to photoperiod (Beales et al., 2007), was detected in both parental lines and, thereby, no further analysis was done with this locus. Alternatively, both molecular markers amplifying *Ppd-A1* and *Ppd-B1 loci* were polymorphic for parental lines and then F5 individuals were analysed. Even though there was evidence of response to selection in F4 for flowering time suggesting underlying genetic factors, the anal-

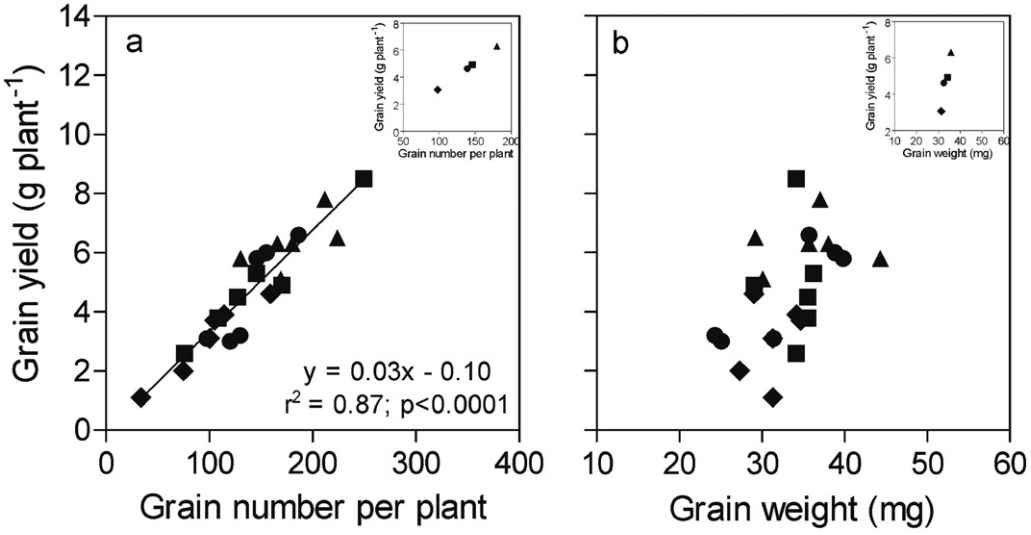


**Fig. 3.** Grain yield per plant in relation to (a) grain number per plant and (b) grain weight. Data includes all segregant individuals and mean (insets) of the F4 contrasting groups selected within population B in 2006, considering the early (E-) and late (L-) flowering time with short (-S) and long (-L) stem elongation phase duration. Symbols for the different groups selected are as follows: ES (square), EL (triangle), LS (diamond), and LL (circle). Line indicates the linear regression ($\alpha = 0.05$) with the corresponding equation and regression coefficient.

**Table 2**
Duration of cycle to anthesis (AT) and pre-anthesis phases, from transplant to first node detectable (TR-FN) and stem elongation phase (SEP), in thermal time units (°Cd), and grain yield per plant (GY), grain number (GN) per plant, and grain weight (GW). Data from the F4 contrasting groups selected within population B in 2006, considering the early (E-) and late (L-) flowering time with short (-S) and long (-L) stem elongation phase duration. Different letters indicate significant differences ($\alpha = 0.05$) among different groups of segregant individuals.

| F4 groups | Phenology | | | Yield | | |
|---|---|---|---|---|---|---|
| | TR-FN (°Cd) | SEP (°Cd) | Cycle to AT (°Cd) | GY (g plant$^{-1}$) | GN per plant | GW (mg) |
| ES | 634.9 b | 661.9 d | 1296.8 b | 4.9 ab | 145.8 ab | 34.1 a |
| EL | 552.8 d | 755.7 c | 1308.5 b | 6.3 a | 180.1 a | 35.7 a |
| LS | 673.8 a | 837.2 b | 1510.9 a | 3.1 b | 97.8 b | 31.3 a |
| LL | 584.4 c | 935.7 a | 1520.1 a | 4.6 ab | 139.0 ab | 32.5 a |

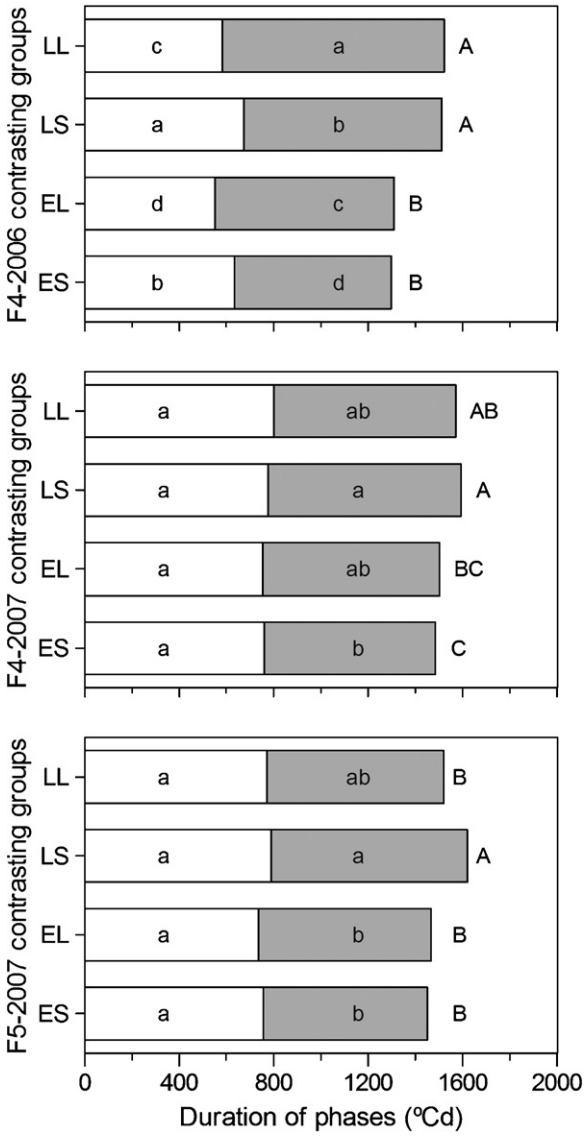


**Fig. 4.** Duration of cycle to anthesis (AT) and pre-anthesis phases, from transplant to first node detectable (TR-FN, open bars) and stem elongation phase (SEP, closed bars), in thermal time units (°Cd), of the F4 contrasting groups selected within population B in 2006, considering the early (E-) and late (L-) flowering time with short (-S) and long (-L) stem elongation phase duration of segregant generations, and their progenies (F5) and full-sibs (F4) in 2007. Different letters indicate significant differences ($\alpha = 0.05$) among cycle to AT (capital letters) and among pre-anthesis phases (lower-case letters) within each growing season.

ysis of the markers for both loci at F5 and the phenotypic data did not show any evidence of a clear association between *Ppd-A1* and *Ppd-B1* loci and flowering time (Table 3 and Fig. 4). Segregation of allelic variants was the one expected for independent characters (i.e. 1:1 for co-dominant and 0.53:0.47 for dominant markers; Table 3). On the other hand, considering the relative duration of pre-anthesis phases, differences in allelic frequency ($p < 0.05$) from those expected for an F5 marker were evidenced only between late flowering time groups, i.e. LS vs. LL- (Table 3). However, phenotypic correspondence with this allelic discrimination was not found (Fig. 4).

## 4. Discussion

Multiple previous evidence, where the duration of pre anthesis phases were artificially manipulated, demonstrated the importance of SEP duration (and in particular of SGP) for increasing GN (Miralles et al., 2000; González et al., 2003a,b, 2005a,b,c; Serrago et al., 2008). However, notwithstanding the extreme importance of those studies in order to comprehend the physiological bases of GN increases in response to SEP duration, they did not allow to understand the genetic bases of SEP duration relative to AT. As far as we know, the results of the present work are the first to demonstrate in a genetic population, growing in field conditions: (i) the value of SEP duration for promoting GN per plant, and thereby grain yield, and (ii) the relevance of the environmental influence on SEP which avoided finding selection response for this phenological attribute.

In relation to the first point, the results of the present study showed that grain yield per plant was higher when duration of SEP was longer, due to GN increases. Taking into account all contrasting selected groups, variations in grain yield were mostly associated with changes in GN rather than in GW, as widely reported in the literature (Calderini et al., 1999; Borrás et al., 2004). These results reinforce the importance of previous weeks to AT (especially SGP) for grain yield determination in wheat (Fischer, 1983, 1985; Kirby, 1988; Slafer et al., 1994; Miralles and Slafer, 1999). Even though results showed clear tendencies of GN improvement when SEP was lengthened, the variability registered in this component and in grain yield for a similar SEP duration suggests that other ecophysiological attributes (besides of SGP) are involved in the GN definition. Attributes like crop growth rate during this pre-anthesis period (SEP or SGP), partition to spike and spike fertility coefficient (i.e. grain per dry mass unit of spike) are involved in GN determi-

**Table 3**
Haplotypes of *Ppd A1* and *Ppd B1 loci*. Proportions of each allele corresponding to Klein Estrella and ProINTA B. Alazán, in the four F5 contrasting groups, considering the early (E-) and late (L-) flowering time with short (-S) and long (-L) stem elongation phase duration.

| F5 groups | Marks proportions (K. Estrella:P. B. Alazán) | |
|---|---|---|
| | *Ppd-A1* | *Ppd-B1* |
| ES | 2:4 | 1:5 |
| EL | 3:2 | 3:3 |
| LS | 2:3 | 5:0$^{*,**}$ |
| LL | 3:2 | 1:5$^{*,**}$ |

$^{*}$ Significant difference ($\alpha = 0.05$) from the expected proportion.
$^{**}$ Significant difference ($\alpha = 0.05$) from independence test.

nation in wheat (Fischer, 1983, 2008). Consequently, differences in any of these attributes among individuals of the same group could result in variations of grain yield for the same SEP. In this sense, the spike fertility coefficient could vary within a determinate selected group (with the same SPG) due to differences in the genetic background of individuals that integrate it, as demonstrated in previous works analysing different cultivars (Abbate et al., 1998; Acreche et al., 2008; Serrago et al., 2008). In spite of the fact that experiments of the present study were conducted under field condition, it is important to highlight that the results obtained were recorded at plant level and we have to be cautious to extrapolate to canopy level. Therefore, it is necessary to explore the correlation between the SGP and GN sets, together with grain yield, at canopy level to confirm those responses, as in many cases the attributes selected in individual plants are not reflected in the crop (Pedró et al., 2010).

To analyse the selection response of SEP duration relative to AT, the first step was to select contrasting phenotypes from a F4 genetic population. Similarly to findings reported by Borràs et al. (2009) in a barley population, our results showed that transgressive segregation was an important source of variability in phenology, which allowed to select contrasting phenotypes for the phenological attribute under study (i.e. similar flowering time but different SEP). The lack of association between both pre-anthesis phases, when contrasted with each other, agrees with the results reported not only in wheat (Halloran and Pennell, 1982; González et al., 2002; Whitechurch et al., 2007a,b), but also in other species such as barley (Kernich et al., 1997) and triticale (Estrada-Campuzano et al., 2008). This reinforces the idea that the length of one particular phase (e.g. period between emergence to SEP onset) is not necessary linked to the length of the subsequent phase (e.g. SEP). Therefore, it could be possible to select materials with different duration of the pre-anthesis phases for a similar cycle to AT (Miralles and Richards, 2000; González et al., 2003b). This speculation supports the idea of a possible manipulation of wheat development as a way to increase SGP (Halloran and Pennell, 1982; Slafer et al., 1996, 2001; Miralles et al., 2000; Slafer, 2003). However, if the impact of genetic factors that modulates the relative duration to AT of each pre-anthesis phase cannot be determined, this idea will remain as a theoretical rather than a practical approach.

As a second step, progeny of F4 contrasting selected phenotypes (i.e. F5) and their F4 full-sib were evaluated in the same environment (2007 experiment). Although F4 (2006) divergent phenotypes selected in the present study showed an important difference in SEP duration (ca. a week for a similar flowering time), F5 progenies derived from them and F4 full-sib did not show any differences in 2007 for that attribute. It suggests that SEP duration is under potentially strong environmental influence. The main difference between F4 (2006), F4 full-sib (2007) and F5 progenies was the longer duration of the first pre-anthesis phase (TR-FN) in the second year (2007). This delay in SEP onset (ca. 150 °Cd or 10 days considering the difference in date of transplant) exposed the next phase to more inductive photoperiods than in 2006 which as stated above, minimized the difference between progenies of selected contrasting groups and between their F4 full-sib.

Even though the transplanting date was advanced in 2007, this did not cause differences in the mean photoperiod explored during the first pre-anthesis phase in both years (ca. 11.8 h in all cases). The temperatures explored during 2007 which were higher than in 2006 (ca. 1 °C more), could determine different duration of TR-FN phase due to photoperiod × temperature interaction (Slafer and Rawson, 1995). The higher temperature explored during the second year could have increased the thermal units required for a given photoperiod as a consequence of the relatively short photoperiod explored in this environment (ca. 11.8 h). This behaviour could be attributed to an increase of the optimum photoperiod with the higher temperature (Slafer and Rawson, 1996), requiring more thermal time to complete the phase. Another possible explanation could be associated with vernalization requirements. The parental lines used in the present study were cultivars reported to have a low vernalization requirement, which was supported phenotypically by Whitechurch et al. (2007b), and genotypically in this work by the analysis of the set of *Vrn-1* genes (data not shown), which were scored using markers described in Yan et al. (2004) and Fu et al. (2005). Although it was assumed that vernalization response was saturated with the treatment applied, the lack of differences in the mean photoperiod explored in both growing seasons and the slightly higher temperature during 2007 suggests that vernalization requirements could not be fully satisfied by the treatment applied. Therefore, although seedlings were vernalized in the same form in both growing seasons, this requirement could have been completed more rapidly in 2006 growing season, shortening TR-FN phase with respect to 2007 one.

Flowering time in many species (Bäurle and Dean, 2006), and particularly in wheat (Slafer, 2003; González et al., 2005a), is determined by many genes. Both their recombination and assortment may generate not only different duration of cycle to AT but also different relative duration of the pre-anthesis phases. Whitechurch et al. (2007b) showed that the parental lines used to obtain population B (Klein Estrella and ProINTA B. Alazán) had different photoperiod sensitivity during SEP. Thus, the candidate factor determining the differences in the duration of phases was centered on photoperiod response. However, the analysis of molecular markers for genes controlling photoperiod did not provide any evidence of significant association between the tested allelic polymorphisms in *Ppd-A1* or *Ppd-B1* loci and the phenological pattern, except between the late flowering groups but without phenological correspondence. These results suggest that other photoperiod genes could be involved in the duration of the pre-anthesis phases, the expression of which depends on the environment that is explored by the crop (González et al., 2005a), explaining the variability observed under different growing seasons. Moreover, presence of an alternative *Ppd-D1* functional allele different from that described in Beales et al. (2007) cannot be discarded. On the other hand, minor genes associated to flowering time can be related to this attribute. As an example, in a recent study Griffiths et al. (2009) detected 19 mega-QTLs associated with variation in heading time, using four double-haploid populations derived from crosses involving European winter and photoperiod-sensitive wheat germplasm. Major QTLs affecting heading time were detected in 1BL, 1DL, 3A, 3B, 6B and 7D chromosomes, which are different *loci* from major genes affecting vernalization (*Vrn*) and photoperiod (*Ppd*) response.

Summarizing, the results of the present work support the hypothesis of lengthening SEP duration (or more specifically SGP) without altering cycle to AT as a way to increase GN and grain yield. However, selection response to longer SEP in individuals with similar flowering time could not be found. SEP duration was strongly influenced by environmental factors, although the registered variability in this physiological attribute does not seem to be associated with major genes that control the main development pattern in wheat (i.e. *Ppd* and/or *Vrn*). Factors to be taken into account are the influence of genes out of the ones molecularly marked in the present study as well as interactions among these genes. The lack of selection response in SEP duration relative to AT makes its introduction as a selection criterion in breeding programmes unlikely without a better comprehension of its genetic basis, which demands new research.

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