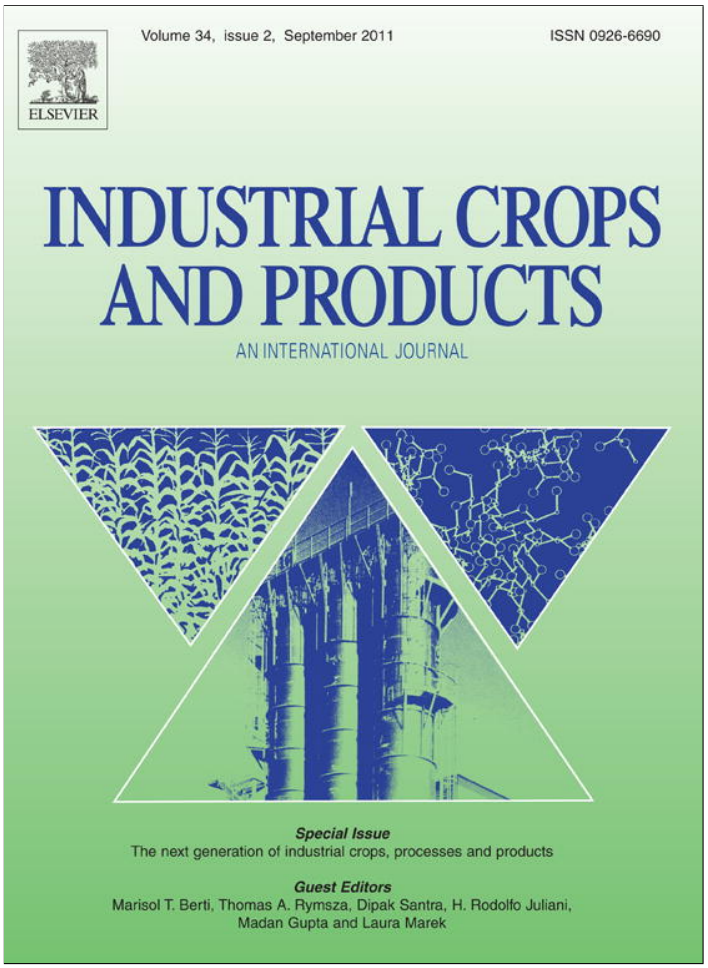

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Factors that modify early and late reproductive phases in oilseed rape (*Brassica napus* L.): Its impact on seed yield and oil content

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ABSTRACT

Oilseed rape yield potential could be improved lengthening the duration of the late reproductive phase by increasing the number of grains per unit area. Photoperiod sensitivity could be used as a tool to manipulate the reproductive phase and thereby the number of grains. The aim of this study was to assess (i) the effects of different combinations of photoperiod on the duration of different phases and (ii) analyze how the changes in that duration affect yield (and its components) as well as oil seed content in oilseed rape. Field experiments were conducted in a factorial combination of three cultivars and three photoperiod regimes: natural photoperiod (NP) which represents the control and extended photoperiod of 6 h over NP (NP + 6) during emergence (E)–flower buds visible (FBV) and FBV–maturity (M) arranged in a randomized complete block design with three replicates, during two years representing three environments. Results showed that oilseed rape evidenced photoperiod responses during vegetative and early reproductive phases. Due to the lack of correlation between the duration of the vegetative and reproductive phases, it is possible to speculate the vegetative period may be altered independently of the modification of the rest of the phases. The positive relationship between grain number per m^2 and the duration of the late reproductive phase suggests that yield could be increased by lengthening the duration of that phase. Thus, regardless of the effect on the previous phase, the photoperiod sensitivity found in the early reproductive phase opens the possibility to manipulate the relative durations of vegetative and reproductive phases. Therefore, the length of the reproductive phase will be increased at the expense of a reduction in the duration of the vegetative phase, but without changing the whole duration of the crop cycle. This strategy could increase yield in oilseed rape in the future. Variations in yield were mostly explained by changes in the grain number per unit area without significant correlation with grain weight. However, a negative relationship between grain weight and grain number was found, showing a slight counterbalance in yield, by decreasing the grain weight. Since oil concentration appeared to be a more conservative attribute, increases in crop yield through a higher grain number per unit area would be a suitable strategy for improving oil yield as no reductions in oil concentration can be expected.

1. Introduction

Crop cycle in oilseed rape is associated with sequences of phenological events controlled by environmental factors, i.e., temperature (including vernalization) and photoperiod (Mendham and Salisbury, 1995; Robertson et al., 2002) which determine changes in the duration of the phases. Although the modification of those environmental factors on the timing of flowering is widely characterized (Hogdson, 1978; Myers et al., 1982; Salisbury and Green, 1991; Robertson et al., 2002), less information is available about the impact of the photoperiod and vernalization on the duration of different sub-phases of the crop ontogeny. Diverse evidence found in literature has reported photoperiod sensitivity in different oilseed rape cultivars between emergence and floral bud visible (Hogdson, 1978; Myers et al., 1982; Nanda et al., 1996; Robertson et al., 2002). However, other authors have suggested that photoperiod sensitivity continues during the stem elongation phase (Thurling and Vijendra Das, 1979; Thurling and Kaveeta, 1992).

Knowledge related to the effects of photoperiod, temperature and vernalization on the duration of the crop cycle is important since it is not only restricted to the genotypic adaptability for a particular environment, but also for exposing the duration of

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the critical period for yield determination to better environmental conditions. The critical period for yield determination in oilseed rape was estimated around 300 °C d (base temperature = 0 °C) after mid-flowering (Habekotté, 1993; Mendham et al., 1981; Leterme, 1988), which represents about 19–25 days in most field conditions (Berry and Spink, 2006). Changes in the duration of the phenological phases preceding critical period, or even the duration of the critical period, could not only modify the yield and its components, but also the grain quality associated with the oil seed content. Different evidence collected about wheat and barley, using photoperiod responses to modify the duration of the late reproductive phase (LRP), demonstrated that the longer the LRP, the greater the number of grains established per spike (Miralles and Richards, 2000; González et al., 2003a,b). A similar approach was applied to study soybean (Kantolic and Slafer, 2007) demonstrating that sensitivity to photoperiod during the post-flowering phase, lengthening the duration of that phase, increased the number of pods per plant and thereby the number of grains per unit area.

The same line of reasoning that was applied to other species could be relevant in the case of oilseed rape, assuming that the longer the reproductive period, the higher the yield, associated with a larger number of grains per unit area. The hypothesis behind this speculation is that by extending the period during which oilseed rape is initiating floret primordia in the branches, more reproductive points could be initiated which would in turn increase the number of grains per plant. In order to test that hypothesis, the development phases of the crop need to be manipulated through environmental factors that regulate development at one or more stages of the crop. Thus, one approach could modify artificially the photoperiod which will alter the relative duration of vegetative and reproductive phases, and thereby determine the impact of this modification on yield and its components. The aim of this study was (I) to assess the effects of different combinations of photoperiods on the length of vegetative and reproductive phases and (ii), determine the impact of altering the duration of both phases on yield and its components in oilseed rape crop grown under field conditions.

2. Materials and methods

2.1. Culture, experimental design and treatments

A field study was carried out at the experimental field of the Department of Plant Production at the University of Buenos Aires (34°35′S, 58°29′W), during 1999 and 2000 growing seasons on a silty clay loam classified as Vertic Argiudol according to the USDA taxonomy.

Three commercial oilseed rape cultivars differing in flowering time were selected. The cultivars were: Zafiro, Impulse and Mistral, corresponding to early, medium and late flowering time, respectively. Seeds of the cultivars were hand-sown on early – 15 June (SD1) – and late – 12 August (SD2) – sowing dates in 1999 and on 3 July in 2000, in plots of 6 rows 0.20 m apart and 2.5 m long. Thus, the crop explored three different environments during the two growing seasons. When plants had expanded two or three leaves, plots were hand-thinned to obtain a uniform plant population of 100 plants per m^2.

Plots were irrigated throughout the crop cycle, supplementing natural rainfall, in order to avoid water stress. During both years, nitrogen application, using urea (46% Nitrogen), was split into two identical quantities (2×90 kg Urea ha^{-1}) and applied at sowing and when the crop expanded 2 or 3 leaves. As soil was 86 kg N ha^{-1} at sowing in the 0–0.40 m of the top soil, total nitrogen available for the crop after fertilization was 149 kg N ha^{-1}. Soil-available phosphorus concentration at sowing in the top 0.20 m soil layer was >20 g/g (Bray and Kurtz, 1945), then, phosphorus fertilizer was not applied. Information on meteorological conditions (temperature, solar radiation and rainfall) during the crop cycle was provided by Villa Ortuzar meteorological station, located 250 m away from the experimental site (Fig. 1).

The study consisted of the factorial combination of three cultivars and three photoperiod regimes, arranged within each sowing dates, in a randomized complete block design with three replicates per treatments. Photoperiod treatments consisted of (i) the natural photoperiod of the season (NP), and 6 h (NP + 6) daylength extensions beyond the natural photoperiod in two crop phases: (ii) emergence (E)–flower buds visible (FBV) and (iii) FBV–full maturity (M) (Fig. 2). Natural photoperiod was extended using portable lighting structures (0.5 m wide and 2 m long), placed over the plots, which combined incandescent and fluorescent lamps of extremely low intensity, connected to automatic timers which turned the lights on and off. The timers were reset once a week to maintain the differences between the photoperiod extension and the control (NP), due to the changes of the natural photoperiod throughout the crop cycle. Each portable lighting structure registered a photosynthetic photon flux density of 4.2 μmol m^{-2} s^{-1} measured at canopy surface at night with a LI-COR line quantum sensor (LI-COR Inc., Lincoln, NE), and a red:far red quantum ratio of 1.23 measured with a Sensor SKR 110 (Skye Instrument Ltd., Powys, UK). This value was similar than that reported by Smith (1982) who found values of red:far red quantum ratio of 1.19 for values of photon flux density of 1900 μmol m^{-2} seg^{-2}. The portable lighting structures did not produced light drift to other plots as the separation among the plots was ca. 2 m and as an additional security measurement, we included a stand black net (pierced to avoid negative wind effects) in the middle of the streets among the plots to prevent any light drift. It is important to highlight that the black nets did not produce any shadow to the neighboring plots. Additionally, the portable lighting structures and the black nets used to avoid light drift did not produce changes in temperature and incident radiation.

2.2. Data collection and analysis

The different phenological stages (Arnoud, 1989) were recorded from emergence to beginning of pod development. At each stage, the date of the event was defined when 50% of the plants in the plots reached the particular morphological stage described by the scale. Thus, the stages of emergence (E), inflorescence initiation (Ii), flower buds visible (FBV) and onset of fructification were determined. The duration of the phases was recorded in degree days (°C d) considering a base temperature of 0 °C (Gabrielle et al., 1998; Robertson et al., 2002).

The dynamics of leaf appearance on the main stem was measured every 3–4 days from seedling emergence to flowering in plants previously tagged. A leaf was considered to have appeared after unfolding (Morrison and McVetty, 1991). Additionally, the external morphology of leaves, i.e., petiole, semi-petiole and sessile, was recorded together with the dynamics of leaf emergence. From emergence, two plants per replicate were monitored and dissected twice weekly to determine timing of inflorescence initiation (Ii) at the apex level according to the description made by Moncur (1981).

The rate of leaf appearance was calculated as the slope of the relationship between the leaf number and thermal time from emergence to flowering. The phyllochron values of the emerged leaves for the different treatments were calculated as the reciprocal rate of leaf appearance. When appropriate, linear or bi-linear functions were used to fit the relationship between leaf appearance and thermal time from emergence (base temperature of 0 °C was used). The final leaf number (FLN) was also recorded in the tagged plants.

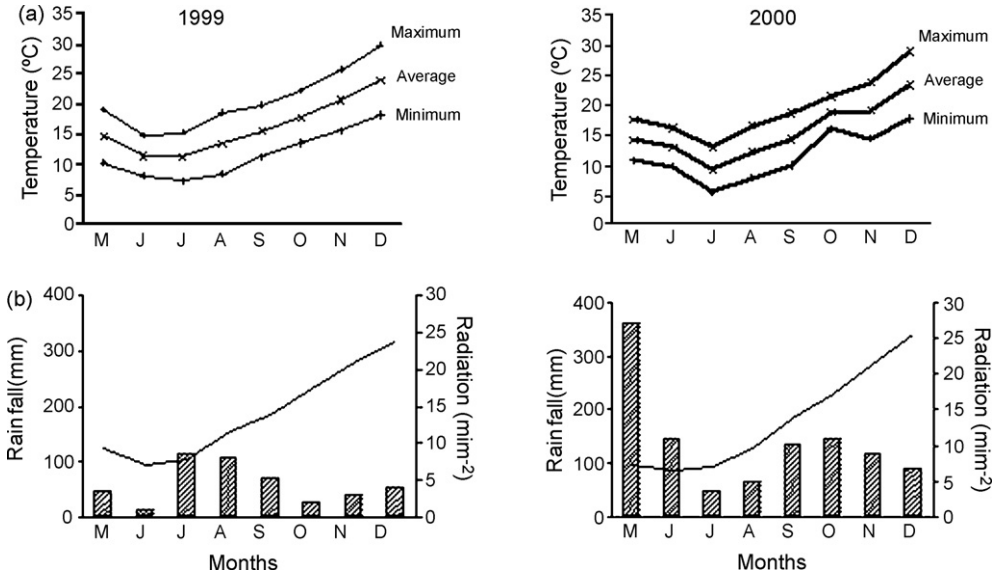

Fig. 1. Weather conditions during the experiments (a) maximum (top line), average (central line) and minimum (lower line) temperature and (b) rainfall (bars) and global radiation (full line) during the 1999 and 2000 growing seasons.

The numbers of flower buds, flowers and pods on the main stem during the crop cycle in the same tagged plants were recorded throughout the crop cycle in order to determine the dynamics of reproductive organs appearance. The dynamic progress of reproductive organs (RO, flower buds and flowers) was analyzed using a bi-linear model (Eq. (1)) (Jandel Scientific, 1991):

$$RO = a + bx(x \leq c) + bc(x > c) + d(x - c)(x > c) \quad (1)$$

where *RO* represents the number of reproductive organs (flower buds and flowers) per main stem, *a* is the number of reproductive organs at the initial point, *b* is the rate of reproductive organs appearance from seedling emergence (*x*), *c* is the day, measured from emergence, when maximum reproductive organs number was achieved, and *d* is the rate of reproductive organs mortality after the maximum number of reproductive primordia was reached. The dynamics of pods appearance (*P*) was described by a linear-plateau model fitting the data by optimization techniques (Eq. (2)) (Jandel Scientific, 1991):

$$P = a + bx(x \leq c) + bc(x > c) \quad (2)$$

where *P* is the number of pods on day × (from day seedling emergence), *a* is the ordinate value, *b* is the rate of pods appearance from seedling emergence and *c* is the time when maximum number of pods was achieved.

After the crop reached the time of physiological maturity, one linear meter from the central row of the plot was sampled and yield, together with its main components (number of grains per m^2—NG m^{-2}; number of pods per m^2—Pods m^{-2}; number of grains per pod—NG Pod^{-1}; average grain weight—AGW; and oil grain content (mg kg^{-1})), were measured. All data were analyzed using General Linear Models, and traditional ANOVA within each sowing date in each experimental years, as during the 1999 growing seasons two sowing dates were performed and only one sowing date was carried out in the 2000. Tukey's test, at 5% significant level, was used for the comparison of means within each environment.

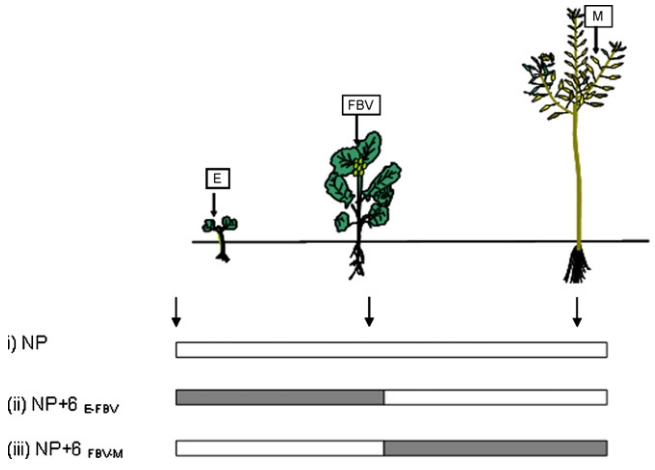

Fig. 2. Schematic representation of the application of photoperiodic treatments, indicating the time of the crop cycle when photoperiods were applied (i) natural photoperiod (NP) (ii) extended photoperiod 6 h over NP between emergence (E) and flower buds visible (FBV) (NP + 6_{E-FBV}) and (iii) extended photoperiod 6 h over NP between FBV and maturity (M) (NP + 6_{FBV-M}).

3. Results

3.1. Photoperiod effects on different developmental phases

As oilseed rape has a quantitative long day response to photoperiod, extension of photoperiod over the natural one produced a shortening in the duration of the phase in which photoperiod was applied. Thus, the duration of the phase emergence–flower buds visible (E–FBV) was modified by photoperiod. Extended photoperiod (NP + 6_{E-FBV}) during that phase reduced its duration between 3 and 40% in all cultivars (depending on the genotype) when compared to the natural photoperiod (NP). Mistral was the most sensitive genotype to photoperiod, shortening the E–FBV phase between 172 and 502 °C d, when photoperiod was extended over the NP. The second most sensitive genotype to photoperiod was Impulse (from 43 to 343 °C d) and thirdly Zafiro (from 26 to 236 °C d). The shortening observed in the E–FBV phase, due to extended photoperiod, was mainly explained by reductions in the duration of the Ii–FBV sub-phase (57%), as the E–Ii sub-phase was only reduced 20% compared to the NP, when photoperiod was extended during the E–FBV phase. Similarly, during the E–FBV phase, the genotypes Mistral and Impulse were the most sensitive cultivars to photoperiod during the E–Ii phase, showing reductions in the length of that phase of

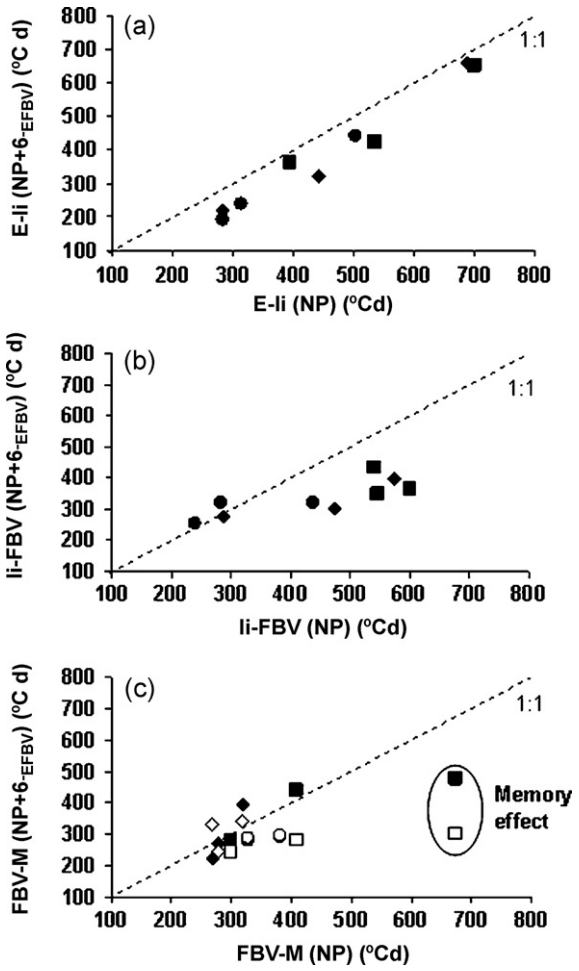

Fig. 3. Relationship between the duration of the phases (°C d). (a) Emergence (E)–Inflorescence initiation (Ii); (b) Ii–flower buds visible (FBV); (c) FBV–maturity (M) when plants were grown under natural (NP) and extended photoperiod (NP + 6) during each of the considered phases for the cultivars Zafiro (circles), Impulse (diamonds) and Mistral (squares) during the 1999 and 2000 growing seasons. The full line indicates the ratio 1:1. *Note*: The points represent the average duration of each stage for each of the sowing dates.

between 120 and 190 °C d in response to the extended photoperiod when compared to the NP, while Zafiro was almost insensitive to photoperiod during that phase (Fig. 3).

Extended photoperiod during the FBV–M phase promoted a shortening of this phase when compared with NP, which was evident only in Mistral in two of the three environments analyzed (SD2 1999: 351 °C d and 2000: 126 °C d). There was also a slight reduction of the duration of the phase in Zafiro in only one of the tested environments (i.e., 2000: 84.3 °C d), without significant effects on the other genotypes and environments (Fig. 3).

3.2. Final leaf number (FLN) and phyllochron

Final leaf number per main stem ranged between 10 and 17 leaves. Extended photoperiod over the natural one during the E–FBV phase significantly reduced the number of leaves (two leaves on average) ($p \leq 0.001$), while as expected, the extension of photoperiod during the FBV–M phase did not affect the FLN (Table 1).

With the exception of the later sowing date in the 1999 growing season, when the rate of leaves appearance was the same for all initiated leaves, in the other two environments, the rate of leaves appearance was different between early and late appeared leaves. When the rate of leaf appearance changed during the crop

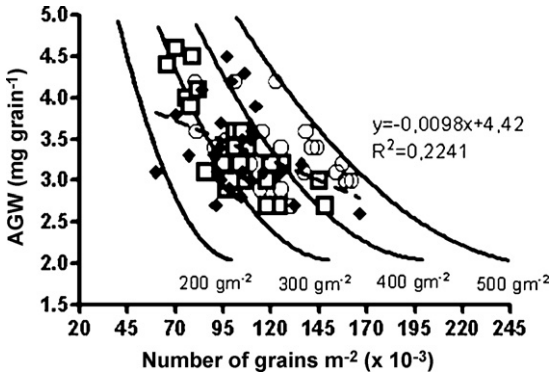

Fig. 4. Relationship between average grain weight (AGW) and number of grains per unit area for all cultivars and treatments (i) NP empty circles; (ii) NP + 6_{E-FBV} empty squares; and (iii) NP + 6_{FBV-M}: black diamonds, in 1999 (SD1 and SD2) and 2000. The full lines were drawn considering different combinations of number of grains and AGW for the same yield values (g m^{-2}).

ontogeny, it was lower in the early leaves when compared to the late ones, and thereby, the phyllochron values were higher in the early leaves compared to those which appeared later in the crop cycle (Table 1). While the phyllochron of early leaves ranged between 63 and 130 °C d per leaf, the range observed in the late appearance leaves was between 30 and 62 °C d per leaf (Table 1). When phyllochron changed with ontogeny, this change generally matched with the emergence of the 6th leaf in most of the treatments. Leaf morphology in which phyllochron changed was different depending on the genotype, e.g., appearance of sessile leaves in Zafiro and semi-petiolated in Impulse and Mistral.

3.3. Yield and its components: number and weight of seeds and oil content

Significant differences in yield among genotypes and environments were observed. Yield ranged between 267 g m^{-2} (for Zafiro, in the 1999 SD2, treatment NP + 6_{FBV-M}) and 478 g m^{-2} (for Mistral, 2000, in the NP treatment). On average for all genotypes, the yield for the treatments NP, NP + 6_{E-FBV} and NP + 6_{FBV-M} was 396, 336 and 342 g m^{-2}, respectively (Table 2).

Variations in yield were mainly explained by changes in the number of grains per unit area ($r^2 = 0.62$), rather than by changes in the AGW ($r^2 = 0.02$). The general trend was that the shorter the crop cycle up to maturity the lower the number of grains per unit area. In fact, averaging the genotypes, the NG m^{-2} was 119,638, 101,663, and 102,307 for the NP, NP + 6_{E-FBV} and NP + 6_{FBV-M} photoperiod treatments, respectively. In general, average grain weight showed low variation between genotypes and photoperiod treatments, ranging between 2.7 and 4.5 mg per grain (Table 3). AGW showed a slightly negative association with the number of grains per unit area. However, on average, there was not a full compensation between both yield components, as the slope showed that increases of 1000 grains per unit area determined a reduction of 10 mg in the AGW (Fig. 4).

When NG m^{-2} was divided into its two main sub-components (i.e., Pods m^{-2} and NG Pod^{-1}), results showed that variations in NG m^{-2} were accounted for by changes in the NG Pod^{-1} ($r^2 = 0.45$, $p < 0.001$) rather than by changes in Pods m^{-2} ($r^2 = 0.04$, $p < 0.10$). Regarding the genotype effects, Mistral had on average greater number of Pods m^{-2} when compared to the other materials tested in the three environments. Mistral explored a range between 6533 and 8579 Pods m^{-2}, Zafiro ranged between 5481 and 7070 Pods m^{-2} and Impulse ranged between 5440 and 6432 Pods m^{-2} (Table 3). Averaging all genotypes, the photoperiod extension, especially during the E–FBV phase, slightly reduced

Table 1
Phyllochron (with the corresponding standard errors) for leaves appearing early or late, coefficient of determination for the relationship between leaf appearance and time from emergence (R^2), number of leaves when phyllochron changes (PC), and final number of leaves (FLN) in the 1999 (SD1 and SD2) and 2000 from Zafiro, Impulse and Mistral in natural (NP) and extended photoperiod: NP + 6 $h_{E–FBV\ and\ FBV–M}$.

Cultivars	Phyllochron (°C d $leaf^{-1}$)					
	Photoperiod	Early	Late	R^2	PC	FLN
1999 SD1						
Zafiro	NP	108.4 ± 8.1a	30.2 ± 8.7a	0.98	7	15a
	NP + $6_{E–FBV}$	118.6 ± 10.6a	39.5 ± 8.3a	0.97	6	12b
	NP + $6_{FBV–M}$	119.9 ± 10.2a	45.1 ± 14.7a	0.97	6	12b
Impulse	NP	125.3 ± 5.6a	50.4 ± 8.6a	0.99	6	13a
	NP + $6_{E–FBV}$	114.6 ± 5.4a	48.2 ± 11.6a	0.99	6	12ab
	NP + $6_{FBV–M}$	130.2 ± 7.8a	49.1 ± 7.3a	0.99	6	10b
Mistral	NP	114.9 ± 8.5a	55.8 ± 3.4a	0.99	5	15a
	NP + $6_{E–FBV}$	109.1 ± 4.5a	43.8 ± 4.0b	0.99	6	12b
	NP + $6_{FBV–M}$	111.1 ± 8.2a	50.6 ± 6.0a	0.98	6	11c
1999 SD2						
Zafiro	NP	67.4 ± 4.7a	–	0.95	–	15a
	NP + $6_{E–FBV}$	62.8 ± 2.9a	–	0.98	–	12a
	NP + $6_{FBV–M}$	66.5 ± 3.8a	–	0.97	–	12a
Impulse	NP	93.5 ± 4.0a	–	0.98	–	13a
	NP + $6_{E–FBV}$	76.3 ± 2.7b	–	0.99	–	11a
	NP + $6_{FBV–M}$	82.4 ± 4.2ab	–	0.97	–	11a
Mistral	NP	82.1 ± 4.8a	–	0.97	–	15a
	NP + $6_{E–FBV}$	72.3 ± 2.3a	–	0.99	–	13b
	NP + $6_{FBV–M}$	80.5 ± 4.9a	–	0.95	–	12c
2000						
Zafiro	NP	89.2 ± 4.8a	41.2 ± 5.6a	0.98	6	11a
	NP + $6_{E–FBV}$	79.3 ± 7.4a	45.1 ± 8.4a	0.98	6	11a
	NP + $6_{FBV–M}$	84.5 ± 6.9a	44.4 ± 6.3a	0.98	6	11a
Impulse	NP	101.9 ± 4.2a	62.1 ± 8.3a	0.99	5	11a
	NP + $6_{E–FBV}$	92.8 ± 5.7a	43.5 ± 7.9b	0.98	5	11a
	NP + $6_{FBV–M}$	101.9 ± 4.2a	52.3 ± 8.9a	0.99	6	10a
Mistral	NP	98.5 ± 4.5a	35.1 ± 7.9a	0.98	7	11a
	NP + $6_{E–FBV}$	87.5 ± 4.5a	45.2 ± 7.7a	0.98	6	10a
	NP + $6_{FBV–M}$	90.3 ± 4.9a	55.3 ± 6.8a	0.99	7	10a

Different letters within each column indicate significant differences ($\alpha = 0.05$) among photoperiod treatments when compare within each cultivar into the same growing season.

Pods m^{-2} in the three environments analyzed, i.e., 6506, 6340 and 6409 Pods m^{-2} for NP, NP + $6_{E–FBV}$ and NP + $6_{FBV–M}$ photoperiod treatments, respectively (Table 3). However, the NG Pod^{-1} decreased proportionally more (in average 16%) than the number of Pods m^{-2} as a consequence of photoperiod extension when compared to the natural photoperiod (Table 3).

The oil content in grains ranged between 430 g kg^{-1} (Zafiro, 2000) and 500 g kg^{-1} (Impulse 1999 SD2). Considering the genotype effects, i.e., averaging environments and photoperiod treatments, no significant differences in oil content were found among genotypes, as this trait registered values of 480, 470 and 450 g kg^{-1} for Impulse, Mistral and Zafiro, respectively (Table 3). With the exception of Zafiro 1999 SD1, photoperiod treatments did not significantly affect oil content (Table 3), and no significant correlation was observed between oil grain content and yield and its components ($p > 0.1$).

3.4. Dynamics of reproductive organs

Photoperiod extension modified the dynamics of primordia appearance and mortality, affecting thereby the maximum and final number of reproductive organs (i.e., flower buds, flowers and pods), depending on cultivar and photoperiod treatments. Exposures to extended photoperiods increased the rates of flower buds appearance without a clear trend as to the duration of effective period of appearance of flower buds (Table 4). However, the association between maximum number of flower buds and the rate of flower bud appearance was not significant in statistical terms ($p > 0.1$), although the trend was consistent throughout the experiments. The magnitude of variation observed in the rates was substantially higher (ca. 3 orders of magnitude), compared with changes in the maximum number of flower buds (ca. 20%), leading to a low correlation coefficient between both attributes.

The extension of photoperiod during both ontogeny phases (E–FBV and FBV–M), increased the rates of appearance of flower buds, without differences between the photoperiod treatments applied in both phases, i.e., 1.7 and 1.6 buds per day for the extended photoperiod during E–FBV and FBV–M, respectively. However, when crop was grown under natural photoperiod (NP), the rate of bud appearance was substantially reduced, i.e., 1.3 buds per day, compared to those in the extended photoperiod. The period of appearance of buds was similar for the three photoperiod treatments (i.e., 46 days). However, as extended photoperiod increased the rates of bud appearance, under that treatment there appeared between 4 and 5 more buds on the main stem (i.e., 31 buttons) than on those plants grown under natural photoperiod (i.e., 27 buttons).

The mortality rate of flower buds (MR) was reduced ca. 16% in the extended photoperiod treatment when measured between FBV and fruiting stage (−1.07 buttons per day), while no significant differences were found in MR between natural and extended photoperiod E–FBV for all cultivars and sowing dates (Table 4).

4. Discussion

Oilseed rape development has been widely studied to understand environmental adaptation and its consequences on grain yield (Thurling and Vijendra Das, 1979; Hogdson, 1978; Thurling and Kaveeta, 1992; Myers et al., 1982; Nanda et al., 1996; Mendham and Salisbury, 1995; Robertson et al., 2002). Nevertheless, understanding the responsiveness to photoperiod throughout different

Table 2
Yield of the cultivars during 1999 (in early: SD1 and late: SD2, sowing dates) and 2000 grown under conditions of natural (NP) and extended photoperiods before and after flower bud visible (NP + 6_{E-FBV} and NP + 6_{FBV-M}).

Cultivars	Photoperiods	Yield ($g\,m^{-2}$)
1999 SD1		
Zafiro	NP	459.9a
	NP + 6_{E-FBV}	340.7b
	NP + 6_{FBV-M}	433.6a
Impulse	NP	424.9a
	NP + 6_{E-FBV}	322.6a
	NP + 6_{FBV-M}	390.3a
Mistral	NP	399.2a
	NP + 6_{E-FBV}	380.5a
	NP + 6_{FBV-M}	349.8a
1999 SD2		
Zafiro	NP	340.3a
	NP + 6_{E-FBV}	339.6a
	NP + 6_{FBV-M}	267.0a
Impulse	NP	424.6a
	NP + 6_{E-FBV}	335.8b
	NP + 6_{FBV-M}	383.7ab
Mistral	NP	347.5a
	NP + 6_{E-FBV}	318.4b
	NP + 6_{FBV-M}	289.8c
2000		
Zafiro	NP	373.8a
	NP + 6_{E-FBV}	320.7a
	NP + 6_{FBV-M}	326.4a
Impulse	NP	312.1a
	NP + 6_{E-FBV}	300.7a
	NP + 6_{FBV-M}	286.1a
Mistral	NP	477.8a
	NP + 6_{E-FBV}	365.2a
	NP + 6_{FBV-M}	346.7a

Different letters within each column indicate that mean values were significantly different ($\alpha = 0.05$) between photoperiod treatments when compared within each cultivar into the same growing season.

Table 3
Pods per unit area, number of grains (NG) per pod, average weight of grain (AWG) and oil content ($g\,kg^{-1}$) of different cultivars during 1999 (SD1 and SD2) and 2000 grown under natural photoperiod (NP) and extended (NP + $6_{E-FBV\ and\ FBV-M}$) photoperiod treatments.

Cultivars	Photoperiods	Pods m^{-2}	NG Pod^{-1}	AWG (mg)	Oil Content ($g\,kg^{-1}$)
1999 SD1					
Zafiro	NP	7363a	18a	3.5b	460a
	NP + 6_{E-FBV}	6710a	15a	3.4b	440b
	NP + 6_{FBV-M}	7138a	15a	4.1a	450a
Impulse	NP	6503a	15a	4.2a	470a
	NP + 6_{E-FBV}	6390a	11a	4.5a	470a
	NP + 6_{FBV-M}	6403a	15a	4.1a	470a
Mistral	NP	9363a	13b	3.2a	470a
	NP + 6_{E-FBV}	7168b	19a	2.9a	460a
	NP + 6_{FBV-M}	9205a	12b	3.3a	470a
1999 SD2					
Zafiro	NP	4783a	21a	3.3a	480a
	NP + 6_{E-FBV}	5833a	17b	3.7a	460a
	NP + 6_{FBV-M}	5542a	14c	3.6a	470a
Impulse	NP	7468a	17ab	3.3a	460a
	NP + 6_{E-FBV}	6363b	14a	3.8a	470a
	NP + 6_{FBV-M}	5138b	23b	3.2a	500a
Mistral	NP	6143a	19a	2.9a	500a
	NP + 6_{E-FBV}	7173a	15a	3.0a	470a
	NP + 6_{FBV-M}	6600a	14a	3.0a	470a
2000					
Zafiro	NP	5950b	20b	3.1a	450a
	NP + 6_{E-FBV}	6003a	17c	3.1a	430a
	NP + 6_{FBV-M}	4490c	23a	3.1a	430a
Impulse	NP	5638a	17b	3.3a	490a
	NP + 6_{E-FBV}	5115a	19a	3.2a	470a
	NP + 6_{FBV-M}	5568a	16b	3.2a	470a
Mistral	NP	5348b	30a	3.0a	460a
	NP + 6_{E-FBV}	6305b	19b	3.0a	470a
	NP + 6_{FBV-M}	7945a	16b	2.7b	470a

Different letters within each column indicate that mean values were significantly different ($\alpha = 0.05$) between photoperiod treatments when compared within each cultivar into the same growing season.

Table 4
Rate of appearance (RA) and mortality (MR) of flower buds (flower buds per day) with the corresponding standard errors, effective period of appearance of flower buds (EPAB-days) and maximum number of flower buds (MNFB) for different cultivars in 1999 (SD1 and SD2) and 2000 grown under conditions of natural (NP) and extended ($NP + 6_{E-FBV \text{ and } FBV-M}$) photoperiods before and after flower buds visible.

Cultivars	Photoperiods	RA	MR	EPAB	MNFB	R^2
1999 SD1						
Zafiro	NP	2.2 ± 0.3a	−0.7 ± 0.1a	48a	33a	0.99
	$NP + 6_{E-FBV}$	0.6 ± 0.2c	−1.1 ± 0.2a	41a	31a	0.97
	$NP + 6_{FBV-M}$	1.3 ± 0.1b	−1.0 ± 0.1a	47a	28a	0.95
Impulse	NP	1.0 ± 0.2b	−0.4 ± 0.2b	45b	32a	0.81
	$NP + 6_{E-FBV}$	1.9 ± 0.4a	−1.1 ± 0.1a	44b	25a	0.95
	$NP + 6_{FBV-M}$	0.6 ± 0.2b	−0.5 ± 0.2b	59a	24a	0.77
Mistral	NP	1.1 ± 0.1b	−1.1 ± 0.1ab	46b	29b	0.95
	$NP + 6_{E-FBV}$	2.0 ± 0.1a	−0.8 ± 0.1b	42b	25b	0.92
	$NP + 6_{FBV-M}$	0.5 ± 0.1c	−2.0 ± 0.4a	64a	25a	0.95
1999 SD2						
Zafiro	NP	0.7 ± 0.2a	−08 ± 0.1b	41a	34a	0.96
	$NP + 6_{E-FBV}$	1.2 ± 0.2a	−20 ± 0.3a	38a	25ab	0.96
	$NP + 6_{FBV-M}$	1.3 ± 0.3a	−10 ± 0.2b	43a	21b	0.89
Impulse	NP	1.1 ± 0.7a	−14 ± 0.1a	37a	33a	0.99
	$NP + 6_{E-FBV}$	1.8 ± 0.2a	−12 ± 0.1a	45a	29a	0.97
	$NP + 6_{FBV-M}$	2.2 ± 0.7a	−09 ± 1.1a	48a	23a	0.94
Mistral	NP	0.6 ± 0.1b	−12 ± 0.7a	49b	33a	0.97
	$NP + 6_{E-FBV}$	0.9 ± 0.2b	−10 ± 0.1a	53a	29a	0.92
	$NP + 6_{FBV-M}$	3.0 ± 1.1a	−11 ± 0.3a	39b	24a	0.87
2000						
Zafiro	NP	2.1 ± 0.2a	−1.2 ± 0.1b	41a	29b	0.98
	$NP + 6_{E-FBV}$	1.7 ± 0.2a	−2.4 ± 0.2a	48a	29a	0.98
	$NP + 6_{FBV-M}$	1.9 ± 0.5a	−1.5 ± 0.1b	23b	16a	0.93
Impulse	NP	2.1 ± 0.7a	−1.3 ± 0.2a	43b	39a	0.93
	$NP + 6_{E-FBV}$	2.1 ± 0.2a	−0.8 ± 0.1a	56a	37b	0.98
	$NP + 6_{FBV-M}$	2.0 ± 0.7a	−0.4 ± 0.5a	54a	35b	0.82
Mistral	NP	0.8 ± 0.1b	−1.4 ± 0.5a	53a	37a	0.97
	$NP + 6_{E-FBV}$	2.3 ± 0.6a	−1.1 ± 0.2b	51a	33a	0.92
	$NP + 6_{FBV-M}$	2.0 ± 0.5a	−1.2 ± 0.2b	46b	33a	0.91

Different letters within each column indicate significant differences ($\alpha = 0.05$) among photoperiod treatments when compare within each cultivar into the same growing season.

phenophases, as a strategy to improve oilseed rape yield potential and determine the impact on grain quality is a new aspect not previously explored under field conditions.

Some studies have previously reported (Robertson et al., 2002) that oilseed rape plants responded to photoperiod from emergence (E) to FBV, in agreement with previous evidence (Nanda et al., 1996; Hogdson, 1978; Myers et al., 1982). However, the sensitivity to photoperiod during that phase appears to be different when two sub-phases (into the whole vegetative period) were considered, as the Ii–FBV phase was even more photoperiod sensitive than the previous E–Ii phase, suggesting that different genes (or the interaction between them) are involved in the photoperiod responses and/or other genes (e.g., those related to earliness per se, Appendino and Slafer, 2003) are differentially affecting the sub-phase of the crop cycle.

The results of this study showed that oilseed rape presents photoperiod sensitivity, even in advanced phenological stages of the crop cycle, such as the late reproductive phase, defined here as the period from FBV to maturity. In controlled environment, Thurling and Vijendra Das (1979) and Thurling and Kaveeta (1992), found important effects of photoperiod on the duration of the stem elongation phase, which could roughly correspond to the results observed in the present study when plants were exposed to long photoperiod after the FBV stage.

It is important to highlight that one of the cultivars (i.e., Mistral), showed a "memory effect", as evidenced by certain residual effect on the duration of the FBV–maturity phase, due to extending the photoperiod on the earlier phase (i.e., E–FBV). Although this memory effect was evident in only one genotype (i.e., the most sensitive to photoperiod), similar effects were found in other species such as wheat (Slafer and Rawson, 1995; Miralles and Richards, 2000) and soybean (Kantolic and Slafer, 2007).

In this present study, there was a lack of correlation between the vegetative and reproductive phases, similar to that described in other species such as wheat (González et al., 2002), barley (Miralles and Richards, 2000) and soybean (Kantolic and Slafer, 2005), suggesting that it is possible to alter the vegetative period independently of the modification of the rest of the phases. Thus, knowing the effect of the genes controlling the sensitivity to photoperiod, it could be possible to design cultivars with the same cycle to flowering and maturity but with different combination of vegetative and reproductive length of the phases.

The duration of the phases up to the appearance of last leaf initiated in the apex is related to (i) the FNL produced during the vegetative phase and to (ii) the rate of leaf appearance during the crop cycle (phyllochron). Frequently, phyllochron is assumed as a unique value for the particular genotype throughout the crop ontogeny, with values around 50 °C d per appeared leaf (Morrison and McVetty, 1991; Nanda et al., 1995). However, in two of the three environments explored in the present study phyllochron changed during the crop ontogeny, and those changes were associated with the modification of the morphology of the leaves rather than with any particular development stage.

As it was expected, according to what was described in many other crops as wheat (Slafer and Whitechurch, 2001; González et al., 2003a); and soybean (Kantolic and Slafer, 2007), the changes in yield associated with photoperiod manipulations, and genotypes, were explained by variations in the number of grains per unit area confirming the importance of this component of yield in oilseed rape. In fact, the number of grains per unit area has frequently been demonstrated to be the most important yield component in oilseed rape accounting for 85% of yield variation (Mendham et al., 1981; Berry and Spink, 2006).

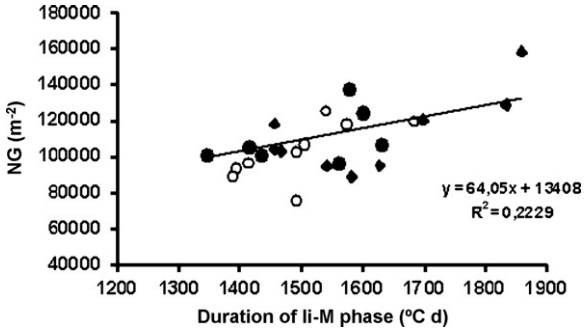

Fig. 5. Relationship between the duration of the reproductive phase (i.e., from Inflorescence initiation to maturity) measured in thermal time (°C d) and the number of grains per unit area (NG m^{-2}) for all cultivars and treatments evaluated in three environments: (i) 1999 (SD1): black circles; (ii) 1999 (SD2): white circles, and (iii) 2000: black rhombi.

The number of grain is mostly determined during a critical phase (lasting about 300 °C d after mid-flowering onwards), as pods and floret survival have been shown to be related to the amount of radiation intercepted by photosynthetic tissue per flower and per pod, respectively during that critical period (Mendham et al., 1981; Leterme, 1988; Habekotté, 1993). In the present study, the positive association between NG per unit area and the length of the Ii–M phase (Fig. 5) suggest that the lengthening of the phase, during which floral buds are developing, promote the establishment of reproductive organs by increases in the number of flower buds on main stems. The photoperiod extension, reducing the length of the E–FBV phase did not reduce the number of pods per unit area although it diminished the number of grains per pod in seven of the nine situations analyzed. Thus, it seems that the main negative impact of shortening that phase was on grain establishment into the pods. This behavior observed in oilseed rape, appears to be different from other oil seed crops such as soybean. In this way, Kantolic and Slafer (2005), by extending the late reproductive phase in soybean through photoperiod manipulation, showed increases in the number of grains per plant due to an enhanced number of pods per plant rather than by changes in the number of grains per pod, which appear to be a more stable crop attribute, probably due to an important degree of genetic control on the number of pods per node (Egli, 1998).

Variations in AGW associated with the photoperiodic treatments were much smaller than those observed in the number of grains per unit area. However, the negative relationship between grain weight and grain number, suggests that yield gains associated with the extended early reproductive phases could be partially counterbalanced by reductions in grain weight. This negative relationship could suggest limitations in source to fill the grains previously formed.

In the range of environmental conditions to which the crop was exposed during the grain filling period, as a consequence of different growing seasons and photoperiod treatments, the concentration of oil in the grain resulted to be relatively stable between cultivars and treatments. Si and Walton (2003) showed that oil content was reduced as post-anthesis temperature increased. In the present study, and despite the shortening in the length of the phases previous to the grain filling period, the temperature during that phase was not extremely different among treatments and thereby the oil content in the seed was similar among treatments (i.e., 470 g kg^{-1}).

5. Conclusions

Summarizing the results of the present study, it is possible to suggest that extending the duration of the post-flowering period could be a way to promote a higher number of grains per unit area. Photoperiod sensitivity could be used to modify the relative duration of the vegetative and reproductive period promoting the latter at the expense of the vegetative period, without altering the total length of the crop cycle. The independence, in terms of duration, of the vegetative and reproductive phase promotes the possibility to modify one of the phases without significantly altering the other. In this particular study, artificial photoperiod treatments were used to modify the length of the phases, but it is undoubtedly impossible to use in commercial production or breeding. Thereby, it is necessary explore genetic combinations (for example genes that confer different photoperiod sensitivity in the different phases) to alter the relative duration of the pre- and post-flowering phases to promote the duration of the last phase and promote the number of grains. As oil concentration was stable, at least for the range of environments explored in the present study, it could be possible to increase grain yield, maintaining the quality of the grains, whenever the extension of the late reproductive phase avoids exposing the grain filling period to high temperatures.

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References

Appendino, M.L., Slafer, G.A., 2003. Earliness per se and its dependence upon temperature in diploid wheat lines differing in the major gene Eps-Am1 alleles. J. Agric. Sci. 141 (2), 149–154, Cambridge University Press.
Arnoud, F., 1989. Colza, sélection, varietés, Cahier technique. CETIOM, París, Francia, 28 pp.
Berry, P.M., Spink, J.H., 2006. A physiological analysis of oilseed rape yields: past and future. J. Agric. Sci. 144, 381–392.
Bray, R.H., Kurtz, L.T., 1945. Determination of total organic and available forms of phosphorus in soils. Soil Sci. 59, 39–45.
Egli, D.B., 1998. Seed Biology and Yield of Grain Crops. CAB International, UK, 178 pp.
Gabrielle, B., Denoroy, P., Gosse, G., Justes, E., Andersen, M.N., 1998. Development and evaluation of a CERES-type model for winter oilseed rape. Field Crops Res. 57, 95–111.
González, F.G., Slafer, G.A., Miralles, D.J., 2003a. Grain and floret number in response to photoperiod during stem elongation in fully and slightly vernalized wheats. Field Crops Res. 81, 17–27.
González, F.G., Slafer, G.A., Miralles, D.J., 2003b. Floret development and spike growth as affected by photoperiod during stem elongation in wheat. Field Crops Res. 81, 29–38.
González, F.G., Slafer, G.A., Miralles, D.J., 2002. Vernalization and photoperiod responses in wheat pre-flowering reproductive phases. Field Crops Res. 74, 183–195.
Habekotté, B., 1993. Quantitative analysis of pod formation, seed set and seed filling in winter oilseed rape (*Brassica napus* L.) under field conditions. Field Crops Res. 35, 21–33.
Hogdson, A.S., 1978. Rapeseed adaptation in Northern New South Wales II. Predicting plant development of *Brassica campestris* L. and *Brassica napus* L. and its implications for planting time. Aust. J. Res. 29, 711–726.
Jandel Scientific, 1991. TableCurve Userĭs Manual V.3.0 Sofware. AISN. Sofware, Corte Madera, CA, 393 pp.
Kantolic, A.G., Slafer, G.A., 2007. Development and seed number in indeterminate soybean as affected by timing and duration of exposure to long photoperiods after flowering. Ann. Bot. 99, 925–933.
Kantolic, A.G., Slafer, G.A., 2005. Reproductive development and yields components in indeterminate soybean as affected by post-flowering photoperiod. Field Crops Res. 93, 212–222.
Leterme, P., 1988. Croissance et développement du colza dĭhiver: Les principales étapes. In: CETIOM (Eds.), Colza-Physiologie et élaboration du rendement du colza d'hiver. Supplément à Informations Techniques. CETIOM 103, pp. 23–33.
Mendham, N.J., Salisbury, P.A., 1995. Physiology: crop development, growth and yield. In: Kimber, D., McGregor, D.I. (Eds.), Brassica Oilseed, Production, Utilization, Camb., pp. 11–64.
Mendham, N.J., Shypway, P., Scott, R., 1981. The effects of delayed sowing and weather on growth, development and yield of winter oil-seed rape (*Brassica napus* L.). J. Agric. Sci., Camb. 96, 389–416.
Miralles, D.J., Richards, R., 2000. Response of leaf and tiller emergence and primordium initiation in wheat and barley to interchange photoperiod. Ann. Bot. 85, 655–663.

Moncur, M.W., 1981. Floral Initiation in Field Crops. An Atlas of Scanning Electron Micrographs. CSIRO, Camberra, Australia, pp. 94–95.
Morrison, M.J., McVetty, B.E., 1991. Leaf appeareance rate of summer rape. Can. J. Plant Sci. 71, 405–412.
Myers, L.F., Christian, K.R., Kichner, R.J., 1982. Flowering responses of 48 lines of oilseed rape (*Brassica* spp.) to vernalization and daylength. Field Crps Res. 46, 93–103.
Nanda, R., Bhargava, S., Rawson, H.M., 1995. Effect of sowing date on rate of leaf appearence, final leaf numbers and areas in *Brassica campestris*, *B. Juncea*, *B. napus* and *B. carinata*. Field Crops Res. 42, 125–134.
Nanda, R., Bhargava, S., Tomar, D., Rawson, H.M., 1996. Phenological development of *Brassica campestris*, *B. juncea*, *B. napus* and *B. carinata* grown in controlled environments and from 14 sowing dates in the field. Field Crops Res. 46, 93–103.
Robertson, M.J., Watkinson, A.R., Kirkegaard, J.A., Holland, J.F., Potter, T.D., Burton, W., Walton, G.H., Moot, D.J., Farre, I., Asseng, S., 2002. Environmental and genotypic control of time to flowering in canola and Indian mustard. Aust. J. Agric. Res. 53, 793–809.
Salisbury, P.A., Green, A., 1991. Development responses in spring canola cultivars. In: Mc Gregor, D.I. (Ed.), Proceedings of the Eight International Rapeseed Congress. Saskatoon, Saskatchewan, Canada, pp. 1769–1774.
Si, P., Walton, G.H., 2003. Determinants of oil concentration and seed yield in canola and Indian mustard in the lower rainfall areas of Western Australia. Aust. J. Agric. Res. 55, 367–377.
Slafer, G.A., Whitechurch, E.M., 2001. Manipulating wheat development to improve adaptation and to search for alternative opportunities to increase yield potential. In: Reynolds, M.P., Ortiz-Monasterio, I., McNab, A. (Eds.), Application of Physiology in Wheat Breeding. CIMMYT, Mexico, DF, pp. 160–170.
Slafer, G.A., Rawson, H.M., 1995. Development in wheat as affected by timing and length of exposure to long photoperiod. J. Exp. Bot. 46, 1877–1886.
Smith, H., 1982. Light quality photoperception and plant strategy. Annu. Rev. Plant Physiol. 33, 481–518.
Thurling, N., Kaveeta, R., 1992. Yield improvement of oilseed rape (*Brassica napus* L.) in low rainfall environments. I. Utilization of genes for early flowering in primary and secondary gene pools. Aust. J. Agric. Res. 43, 609–622.
Thurling, N., Vijendra Das, D., 1979. The relationship between pre-anthesis development and seed yield of spring rape (*Brassica napus* L.). Aust. J. Res. 31, 25–36.