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Seed number responses to extended photoperiod and shading during reproductive stages in indeterminate soybean

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ABSTRACT

The number of seeds per unit land area, the major yield component in soybean (*Glycine max* (L.) Merrill) is largely determined after the beginning of flowering, particularly from R3 to R6. Environmental factors increasing crop growth rate (e.g. radiation) or extending the duration of the reproductive phases (e.g. photoperiod) increase the number of seeds. We aimed to compare the mechanisms by which photoperiod and radiation affect the definition of final seed number during the critical period of R3–R6. Two field experiments were conducted with indeterminate soybeans at intermediate maturity group. All plots in each experiment were grown under natural conditions until the beginning-pod stage (R3); and from then onwards different treatments were imposed. Treatments consisted of the factorial combination of two levels of radiation (natural or shading) and two photoperiod regimes (natural or extended). Extended photoperiod increased the duration of reproductive phases, the number of nodes and the number of pods produced on the nodes that flowered during or after the applications of the treatments. Shading had negligible effects on development and node number, but reduced crop growth rate and also reduced the number of pods produced on most nodes of the plants. The number of seeds was positively related to the crop growth rate during R3–R6, but photoperiod increased the number of seeds produced per unit of crop growth rate, due to the lengthening of the phase. The number of seeds was therefore even better related to accumulated growth during R3–R6, irrespective of the factor that increased the accumulated biomass (higher daily radiation or longer duration of the phase) suggesting that long photoperiods increased the number of pods and seeds established per unit land area, mainly through increasing the total resource availability during a phase that is critical for the determination of seed number in soybean. However, photoperiod regulation involved additional changes in the development, evidenced by changes in the pod distribution pattern within the canopy.

1. Introduction

Pod and seed number are critical determinants of seed yield in soybean (Egli, 1998), as well as in other grain crops (Slafer et al., 2006). Most variations in these components are related to changes in assimilate availability after the beginning of flowering (Jiang and Egli, 1993, 1995; Board and Tan, 1995; Board et al., 1995). Thus, although components of seed number are being developed throughout most of the growing season, the reproductive phases are critical for the determination of seed number per land area (e.g. Slafer et al., 2009 and several references quoted therein). For instance, in studies with soybean shaded at different phases, it became clear that the critical period for seed number determination is between the beginning of flowering (R1 stage; Fehr and Caviness, 1977) and the onset of seed filling (Jiang and Egli, 1993, 1995; Egli, 1997, 2010a). During this period, the final number of seeds and pods per unit land area can be also modified by defoliations (e.g. Board and Tan, 1995), canopy opening (Mathew et al., 2000) or carbon dioxide enrichment (Hardman and Brun, 1971; Nakamoto et al., 2004). As crop growth rate during the critical period is positively related with seed number (Egli and Zhen-Wen, 1991), soybean yield is related to crop ability to capture radiation immediately after flowering (Board et al., 1992; Lee et al., 2008). The functional model commonly accepted to interpret seed number responses to changes in photoassimilate production, proposes that seed number results from the relationship between daily net photosynthesis, its partitioning to seeds and a minimum flux of assimilates per seed that allows seed development: if photosynthesis decreases or seed number increases, the flux of assimilates per seed may decrease

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below a threshold that triggers grain abortion (Charles-Edwards et al., 1986; Egli, 1998).

Seed number may be also increased by lengthening the critical phase, as it has been shown when exposing the crop to long photoperiod after the beginning of podset (Kantolic and Slafer, 2001, 2005) even if the extension lasts only a proportion of reproductive phases (Kantolic and Slafer, 2007). However, it is not clear if photoperiod effects on pod and seed production are similar to those produced by modifying daily assimilate production. The consistent positive relationship that has been found between seed number and accumulated intercepted radiation during the critical period (Kantolic and Slafer, 2001, 2005, 2007) suggests that a high daily growth rate during a relatively short period should be, in terms of final seed number, equivalent to a reduced growth rate sustained a period of increased duration. However, as photoperiod also modifies developmental pattern before and after flowering (Guiamet and Nakayama, 1984; Kantolic and Slafer, 2001, 2005, 2007; Han et al., 2006) its effects may be related to other mechanisms not directly involved in crop growth (i.e. differentiation and development of flowers and pods). Moreover, considering that interferences among pods of different ages are mainly intra-nodal phenomena (Egli and Bruening, 2006b) the effects of photoperiod on changing the number of nodes per plant (Caffaro et al., 1988; Kantolic and Slafer, 2001, 2005, 2007) cannot be disregarded. As the relevance of understanding the photoperiodic responses of soybean to photoperiod during the whole cycle is related to the likelihood of further rising yield potential by fine-tuning the duration of critical phases (Kantolic et al., 2007), understanding whether the photoperiodic effect is mediated through the growth accumulated during a longer reproductive phase or if it includes additional direct effects on pod development is crucial. In order to look more closely at the mechanisms underlying photoperiodic control of pod set, the objective of this paper is to compare the effects of photoperiod and radiation on growth and development and their final effects on the determination of yield in soybean, analyzing the production of seeds per unit area and the distribution of pods on different nodes of the plants.

2. Materials and methods

2.1. Culture, treatments and experimental design

Two field experiments were carried out in a silty clay loam soil (classified as Vertic Argiudol) at the experimental site of the Faculty of Agronomy, University of Buenos Aires (34°35′S, 58°29′W). In both experiments, we evaluated the responses of indeterminate soybean to the combination of photoperiod and shading treatments. In the first experiment (Exp. 1) we evaluated the response of a single cultivar (A-5409RG, Maturity Group V), which in previous studies had exhibited sensitivity to photoperiod after R3 independently of responses in pre-flowering phases (Kantolic and Slafer, 2001). In Exp. 2 we expanded the analysis to three other cultivars, one of the Maturity Group V as well (NA5009RG) and two of Maturity Group IV (A4910RG, NA4990RG). In previous papers (Kantolic and Slafer, 2001, 2005) we have shown that cultivars of these two maturity groups showed photoperiod sensitivity after R3, and that this late developmental sensitivity might not follow the rank of sensitivity in which the maturity group classification is strongly based (Kantolic and Slafer, 2005). Sowing date in Exp. 1 was 9 November 2001, whilst in Exp. 2 it was later (January 12, 2007) to achieve R3 under shorter natural photoperiods. In all cases, plots consisted of 6 rows, 0.35 m apart and 2.5 m long.

All plots were grown under natural photoperiod and radiation until beginning-pod stage (R3, as described by Fehr and Caviness, 1977). When the plants achieved the R3 stage a factorial combination of two levels of radiation (natural or shading) and two photoperiod regimes (natural or extended), was applied following a completely randomized block design with 3 replicates. Treatments were applied independently to each experimental unit, considering its phenological stage; however, within experiments, all the plots achieved the desired stage at the same time within each experiment (82 days after sowing in Exp. 1 and 50 days after sowing in Exp. 2). The extension of photoperiod and shading were maintained until maturity. Shading was imposed by means of a black mesh transmitting a 60% (Exp. 1) or 33% (Exp. 2) of incident radiation. Air temperature above the canopy was measured in shaded and not shaded plots with temperature loggers (HOBO-Temp H01-001-01, Onset Computer Corp., MA); these measurements revealed that the effects of the mesh over daily mean temperature were negligible. The photoperiod extension treatment consisted in lengthening the day by 2 h (Exp. 1) or 3 h (Exp. 2) in the field through portable lighting placed individually in designated plots (see details in Kantolic and Slafer, 2005). Each structure combined incandescent and fluorescent lamps that provided a mean photosynthetic photon flux density of 4.2 μmol m^{-2} s^{-1} and a red:far red quantum ratio of 1:2:1; considering that the light compensation point of soybean is about 37 μmol m^{-2} s^{-1} (Valle et al., 1985) and the red:far red of sunlight reaching bare soil is approximately 1:1 during most part of the day (Smith and Holmes, 1977), the effects of the additional lights on photosynthesis, photomorphogenic responses and growth were considered negligible. Light intensity and quality were measured at the canopy surface at night with a LI-COR line quantum sensor (LI-COR Inc., Lincoln, NE) and a 660/730 Sensor SKR 110 (Skye Instrument Ltd., Powys, UK), respectively. During the treatment imposition, lights were automatically switched on before sunset and off at the required time depending on the length of extension. To calculate photoperiod, both civil twilights were taken into account.

In both experiments, seeds were inoculated with Bradyrhizobium liquid inoculant and hand sown at a high planting rate. When the unifoliate leaves were expanded, the plots were hand-thinned to obtain a uniform plant population of 45 plants per m^2, a population capable of attaining 95% of radiation interception at flowering. To minimize stresses as much as possible, fungal diseases were prevented by spraying recommended fungicides, while weeds and insect pests were controlled with conventional herbicides and insecticides. Plots were irrigated whenever natural rainfall had to be supplemented to prevent water limitations.

2.2. Measurements and data analysis

Phenological stages (following the scale proposed by Fehr and Caviness, 1977) were recorded at 1–3-days intervals in a previously defined area, included in the two central rows of each plot. The date of occurrence of each stage was that when 50% of the plants of the area had reached it. When the plants achieved the R1 stage, the node in the stem where the first flower was opened was identified and labeled in 5 plants per plot. The uppermost node, whose flowers were opened on the day when the treatments were applied (at R3), was also identified in the same plants. These labels allowed the later division of the nodes in three sections of the mainstem: (i) basal nodes, comprising from the cotyledonal to the node below that labeled at R1, (ii) central nodes, from the node labeled at R1 to the node labeled at R3, and (iii) upper nodes, from the node labeled at R3 to the apex.

The duration of reproductive phases was estimated separately for each plot. By virtue of the delay provoked by exposure to long days (Kantolic and Slafer, 2001) and due to the fact that reproductive phases occur with decreasing natural temperatures, phases of development occurring after R3 tended to be exposed to lower temperatures, which might delay development. To avoid any

Table 1

Duration of post-R3 phases in soybean plants of Exp. 1, cultivated under natural (P0) or extended (P + 2 h) photoperiod, under natural radiation (R0) or shading (R−), both treatments imposed from R3 onwards. Durations of R3–R6 are shown both in calendar days and in days corrected by temperature (*T*). The average photoperiod (*P*) for R3–R6 is also shown.

		Duration of developmental phases (*d*)					*P* (h)
		R3–R4	R4–R5	R5–R6	R3–R6	R3–R6 *T*	R3–R6
R0	Pnat	15.7	6.3	13.7	35.7	32.7	14.14
R0	P + 2 h	40.7	13.0	22.0	75.7	67.7	15.40
R-	Pnat	17.3	5.0	13.7	36.0	33.0	14.13
R-	P + 2 h	50.0	13.0	21.3	84.3	73.7	15.35
MS P[a]		2491.0***	161.3**	192.0**	5852.0***	4294.5***	
MS R		90.8*	1.3 ns	0.3 ns	60.8**	30.1***	
MS P × R		44.1*	0.3 ns	0.3 ns	52.1**	24.1**	
LSD$_{0.05}$		6.7	5.7	5.4	3.2	4.3	

[a] Mean square for the effects of radiation (R), photoperiod (P) and their interaction.

* Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

*** Significant at $P \leq 0.001$.

ns: Not significant at $P \leq 0.05$.

overestimation of true photoperiod effects on rate of development, duration of the critical phase was also corrected by temperature using a linear three-segmented function (Piper et al., 1996) as it has been done in previous studies (e.g. Kantolic and Slafer, 2005).

At maturity, two adjacent rows of 1 m (Exp. 1) or 0.5 m (Exp. 2) long were harvested and pods and seeds were counted; seed yield per unit land area was obtained from the whole samples while average weight per seed was estimated from two samples of 100 seeds per sample. In Exp. 2 two additional samples were taken in R3 and R6 to measure total aboveground biomass; crop growth rate was estimated as the ratio between the increment in weight from R3 to R6 and the duration of the phase. The number of nodes in both the mainstem and the branches, and the number of pods in each node, were recorded in five plants per experimental unit. Pods were also counted separately for each group of mainstem nodes (upper, central and basal nodes).

Daily global solar radiation and maximum and minimum temperatures were obtained from a standard meteorological station located c. 300 m away from the plots, estimating daily mean temperature as the average between maximum and minimum. The integral of solar radiation during the period for seed formation was calculated as the sum of daily incident global radiation between R3 and R6 stages of each plot, affected by shading treatments when corresponded.

3. Results

3.1. Development and growth

Long photoperiods imposed after R3 effectively reduced the rate of reproductive development causing the lengthening of the post-R3 phases, both in Exp. 1 (Table 1) and in Exp. 2 (Table 2). A

Table 2

Duration of post-R3 phases in soybean plants of Exp. 2, cultivated under natural (P0) or extended (P + 3 h) photoperiod, under natural radiation (R0) or shading (R−), both treatments imposed from R3 onwards. Durations of R3–R6 are shown both in calendar days and in days corrected by temperature (*T*). The average photoperiod (*P*) for R3–R6 is also shown.

		Duration of developmental phases (*d*)					*P* (h)
		R3–R4	R4–R5	R5–R6	R3–R6	R3–R6 *T*	R3–R6
A4910 RG							
R0	Pnat	7.7	9.0	34.0	50.7	44.9	12.8
R0	P + 3 h	6.3	11.7	54.3	72.3	63.2	15.4
R−	Pnat	7.7	9.0	34.0	50.7	45.2	12.8
R−	P + 3 h	6.3	11.7	54.3	72.3	63.2	15.4
NA4990RG							
R0	Pnat	11.3	7.0	31.7	50.0	44.5	13.0
R0	P + 3 h	12.3	9.3	50.3	72.0	62.6	15.6
R−	Pnat	11.3	7.0	31.7	50.0	44.5	13.0
R−	P + 3 h	12.3	9.3	50.3	72.0	62.6	15.6
NA5009RG							
R0	Pnat	11.7	6.0	30.0	47.7	42.5	13.0
R0	P + 3 h	12.0	15.0	47.3	74.3	64.2	15.7
R−	Pnat	11.7	6.0	30.0	47.7	42.5	13.0
R−	P + 3 h	13.7	13.3	47.3	74.3	64.2	15.6
MS R		0.7 ns	0.7 ns	0.0 ns	0.0 ns	0.0 ns	
MS P[a]		0.7 ns	173.4***	3173.4***	4946.8***	3355.7***	
MS C		102.2***	16.8 ns	91.4*	1.0 ns	2.0 ns	
LSD$_{0.05}$		4.7	7.6	8.7	4.5	4.1	

[a] Mean square for the effects of radiation (R), photoperiod (P) and cultivar (C). Interactions were not significant.

* Significant at $P \leq 0.05$.

*** Significant at $P \leq 0.001$.

ns: Not significant at $P \leq 0.05$.

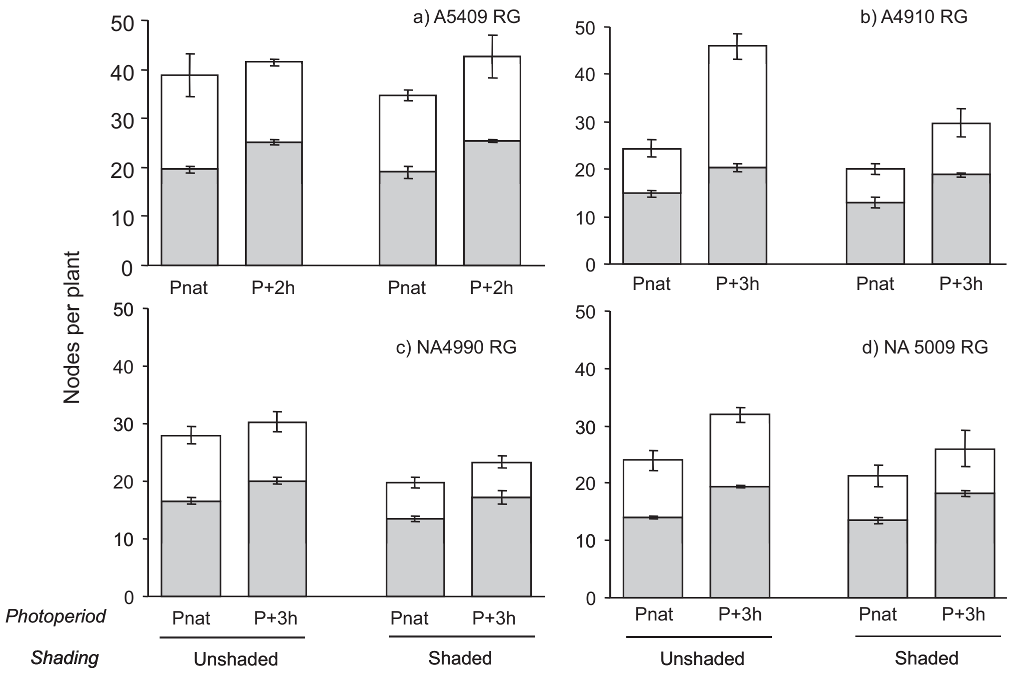

Fig. 1. Average number of nodes in the mainstem (gray bars) or branches (white bars) in soybean plants grown under natural photoperiod (P0) and natural radiation (unshaded) during the whole cycle or exposed to extended photoperiod (P + 2 h or P + 3 h) and/or shading (shaded) from R3 onwards. Vertical bars show the standard error of the means.

significant interaction was found between photoperiod and radiation in Exp. 1, where the R3–R6 phase was lengthened by exposure to extended photoperiod significantly more in shaded than in unshaded plots (Table 1); under natural photoperiod, shading did not affect the duration of the developmental phases. Modifying incident radiation had no effect, either direct or interactively with photoperiod, on the duration of the phases in Exp. 2 (Table 2). In addition, the three cultivars had similar responses to photoperiod, disregarding that they belong to two different maturity groups (Table 2).

Photoperiod did not affect crop growth rate during R3–R6 in Exp. 2 (data not shown), while shading strongly reduced it ($p < 0.001$). Crop growth rate dropped from $13.7 \pm 3.2\,\mathrm{g\,m^{-2}\,d^{-1}}$ under natural radiation to $4.4 \pm 0.9\,\mathrm{g\,m^{-2}\,d^{-1}}$ under shading, in parallel with the severity of the shading imposed.

3.2. *Nodes and pods per plant*

The number of nodes in the mainstem of the plants was significantly increased ($p < 0.01$) by exposure to extended photoperiod. On average, mainstems of plants grown under extended photoperiod had 6.0 ± 0.4 (Exp. 1) and 4.7 ± 0.4 (Exp. 2) additional nodes than those growing under natural daylength (Fig. 1). Photoperiod effects on the number of nodes in branches were significant only in one case (A4910RG under natural radiation). Shading did not significantly reduce the number of nodes per plant with the exception of NA4990.

Pod distribution was not uniform along the mainstem and photoperiod and shading modified the number of pods produced on some nodes (Fig. 2, Table 3). Besides increasing the number of nodes formed in the mainstem, extended photoperiod increased the number of pods on the nodes whose flowers were opening after the treatment had been imposed (upper nodes, Table 4). In general, shading the canopy from R3 onwards reduced the number of pods on the central and upper nodes, respect to the unshaded control (Fig. 2, Table 4). The number of pods in the basal nodes was, in general, very low and it was not affected by the treatments. In A5409 RG (Exp. 1) the change in the pattern of pod distribution by extending photoperiod was larger than in the rest of the cultivars (Exp. 2), likely due to the fact that it was sown earlier and produced, in the control, more nodes; in this experiment, there was a wider difference between the node in which the first flower opened and the node in which the flowers were opening at R3. In contrast, shading effect was stronger in Exp. 2, as expected due to the stronger reduction of radiation by the mesh used in this experiment. There were not significant effects of cultivars or the interactions between cultivars, treatments or positions in Exp. 2. All in all and disregarding the differences between experiments, it seemed generalized that lengthening the duration of R3–R6 increased the number of pods on the nodes of the upper nodes of the mainstem, while shading reduced the number of pods along most the nodal positions of the mainstem, respect to the unshaded control. In most cases, the average number of pods per node in the branches was much lower under the combination of shading and natural photoperiod than in unshaded plots with extended photoperiod (Fig. 2, inset).

3.3. *Yield components*

As a consequence of the effects of the treatments on the number of nodes and pods per node, the overall number of pods per unit land area resulted consistently increased by lengthening the R3–R6 period due to the exposure to long photoperiod, and consistently reduced by diminishing the growth rate of the crop during that period due to shading (Table 5). The main factors (photoperiod and radiation) affected the number of pods per m^2 highly significantly, whilst their interaction resulted mostly insignificant, with only one exception (and even in that case, cultivar A4910RG, the magnitude of the interaction effect was negligible compared to that of both main factors; Table 5).

As a consequence of the responses to the treatments of the number of nodes and pods, seed number per unit land area was strongly affected by the length of photoperiod and the level of radiation (Table 5). In both experiments, shading reduced and lengthening photoperiod increased seeds per m^2. Changes in seed

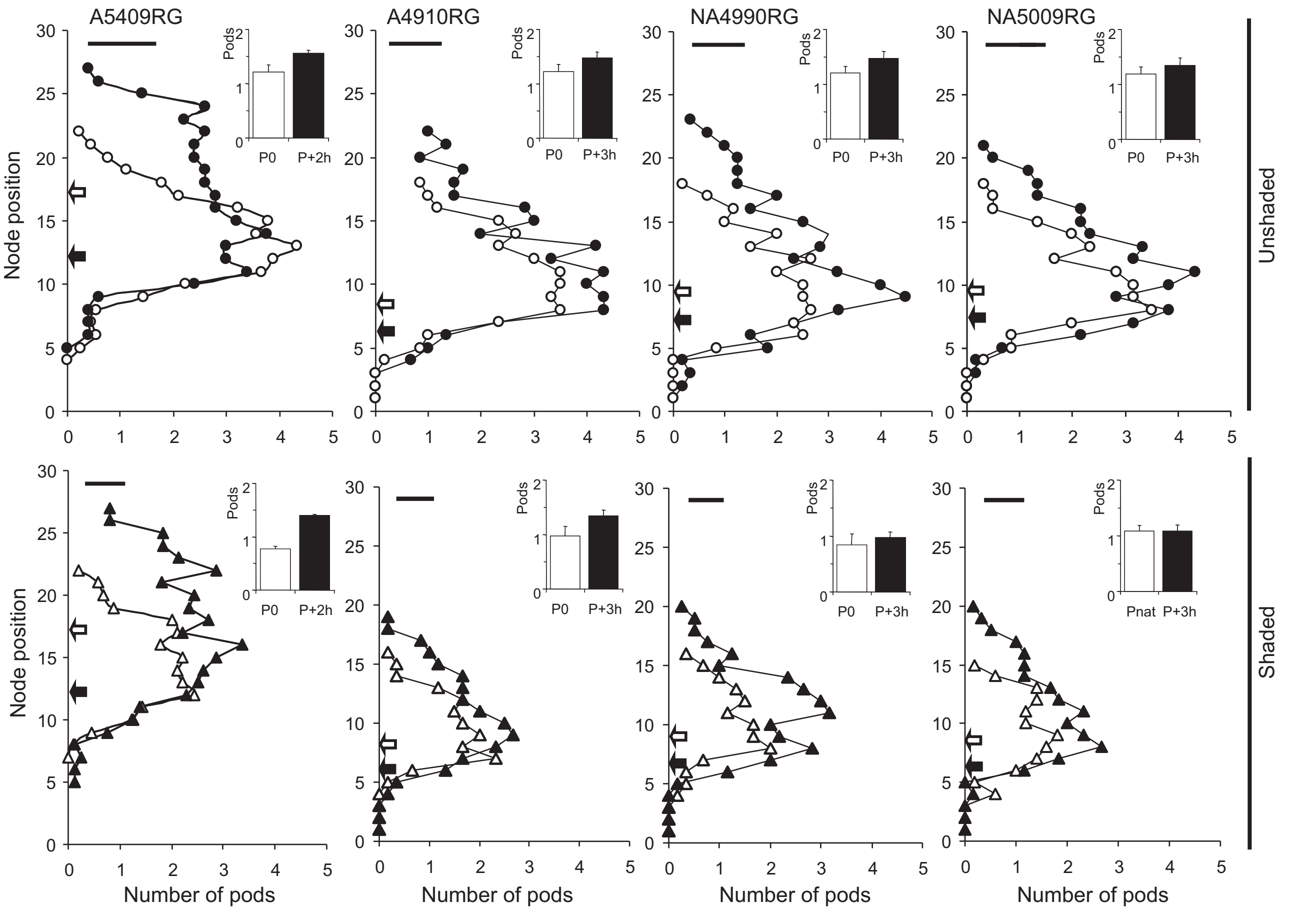

Fig. 2. Number of pods established in different positions along the mainstem of soybean plants cultivated under field conditions until R3 and from then on either maintained under natural (open symbols) or extended photoperiod (closed symbols) and left unshaded (circles, upper panels) or shaded (triangles, lower panels). Node number reflects its position, from the cotyledonal node (node 1) to the uppermost node developed in each case. The arrows indicate the first node when one open flower was registered (closed arrow) and the node that was flowering when treatments were applied (open arrow). The horizontal segments are the $LSD_{0.05}$ for comparisons across nodes and treatments for each cultivar and radiation level. The average number of pods per node in the branches is shown in the insets for natural (open bars) and extended photoperiods (closed bars).

number were closely related to variations in pod number ($r^2 = 0.97$; $p < 0.001$), as the average number of seeds per pod remained virtually unchanged (about 2 seeds per pod). The number of seeds was positively related to crop growth rate during the critical period from R3 to R6. Although a linear adjustment considering all the cultivars and treatments was highly significant ($r^2 = 0.57$; $p < 0.001$), the analysis of residuals showed that positive and negative residuals corresponded to lengthened and natural photoperiod, respectively. Then, stronger linear relationships were found between seeds per land area and crop growth rate when the levels of photoperiod were analyzed separately (Fig. 3), these models suggested that a higher number of seeds was produced per unit of crop growth rate under extended photoperiod.

To evaluate if the different responses between levels of photoperiod were just a consequence of the different duration of the phase, we analyzed the relationship between seed number and the accumulated growth during the critical period. This relationship significantly explained the variations in seed number across

Table 3

Results from statistical analysis of the number of pods in different nodes on the mainstem (N) of soybean plants cultivated under different photoperiod regimes (P: natural or extended) and different radiation regimes (R: natural radiation or shading) both treatments imposed from R3 onwards.

	Exp. 1	Exp. 2		
	A5409	A4910RG	NA4990RG	NA5009RG
MS R[a]	52.43***	135.90***	38.73***	114.08***
MS P	4.60***	38.80***	35.59***	27.08***
MS N	29.47***	27.42***	23.09***	21.65***
MS P × R	2.56 ns	2.69 ns	0.02 ns	4.80*
MS R × N	2.35*	2.31***	1.52*	3.29***
MS P × N	6.48***	0.84 ns	1.08 ns	1.22*
MS P × R × N	0.96 ns	0.86 ns	1.49*	0.56 ns

[a] Mean square for the effects of radiation (R), photoperiod (P) and nodal position (N) and their interactions.
* Significant at $P \leq 0.05$.
*** Significant at $P \leq 0.001$.
ns: Not significant at $P \leq 0.05$.

Table 4
Number of pods at maturity, located in different groups on nodes in the mainstem of soybean plants cultivated under natural (P0) or extended (P+) photoperiod, under natural (R0) radiation or shading (R−), both treatments imposed from R3 onwards.

	Exp. 1	Exp. 2		
	A5409	A4910RG	NA4990RG	NA5009RG
Upper nodes				
R0 P0	7.5	17.2	14.3	14.2
R0 P+	25.8	30.8	26.7	26.3
R− P0	3.5	7.0	7.2	4.2
R− P+	16.9	11.8	16.5	13.3
Central nodes				
R0 P0	19.0	12.3	8.7	9.2
R0 P+	19.5	12.7	9.8	10.5
R− P0	11.8	6.2	5.2	4.3
R− P+	13.8	8.0	7.7	6.5
Basal nodes				
R0 P0	5.5	1.5	1.3	2.0
R0 P+	2.8	2.0	2.0	2.7
R− P0	3.4	0.5	0.5	2.7
R− P+	3.6	1.2	0.2	1.8
MS[a] P	666.8***	238.4***	329.4***	298.4***
MS R	490.5***	875.0***	329.4***	504.7***
MS GN	1319.0***	1431.1***	1384.7***	880.3***
MS P × R	0.1ns	25.7ns	3.6ns	6.7ns
MS P × GN	679.3***	142.1***	197.6***	196.3***
MS R × GN	91.1*	291.1***	90.1 ns	194.8***
MS P × R × GN	35.0 ns	47.4 ns	7.1 ns	5.6 ns
LSD[b]	4.8	4.8	4.1	4.3

[a] Mean square for the effects of radiation (R), photoperiod (P) and group of node (GN) and their interactions.
[b] Least significant difference for comparisons within a column.
* Significant at $P \leq 0.05$.
*** Significant at $P \leq 0.001$.
ns: Not significant at $P \leq 0.05$.

cultivars, radiation levels and photoperiod treatments (Fig. 4a). A detailed analysis of the data, showed that the ratio between seed number and the accumulated growth during the critical period (a proxy of the efficiency of pod setting per unit of biomass) was slightly higher ($p < 0.05$) in shaded treatments (ca. 6.6 seeds per gram of biomass) than under natural radiation (4.2 seeds per gram of biomass), without significant effects of photoperiod. As growth was not measured in Exp. 1, we assumed that the radiation accumulated during this period should be a good estimate of crop growth (as all plots maximized radiation interception well before R3). The relationship between the number of seeds and intercepted radiation accumulated during R3–R6 was evaluated, using the data of both experiments and including also results from previous studies. This relationship, that included data from different cultivars, years, sowing dates and photoperiods, was highly significant (Fig. 4b) without evident differences associated with the nature of the treatments that modified the accumulated radiation.

Final weight per seed was reduced under lengthened photoperiod (Table 6). However, in Exp. 1 the reduction in weight per seed was proportionally lower (ca. 11%) than the increase in seed number (ca. 40%); and yield was therefore significantly increased. In Exp. 2, weight per seed decreased in a higher proportion (a range from 24% to 31% among cultivars) and yield increments were only significant in one case (NA4990RG). The effects of shading on weight per

Table 5
Number of pods and seeds per unit land area at maturity in soybean plants cultivated under natural (P0) or extended (P+) photoperiod, under natural radiation (R0) or shading (R−), both treatments imposed from R3 onwards.

	Number of pods (10^{-3} m^{-2})				Number of seeds (10^{-3} m^{-2})			
	Exp. 1	Exp. 2			Exp. 1	Exp. 2		
	A5409	A4910RG	NA4990RG	NA5009RG	A5409	A4910 RG	NA4990RG	NA5009RG
R0 P0	1.51	1.51	0.98	1.09	3.04	2.87	1.94	2.03
R0 P+	2.15	2.32	1.45	1.67	4.49	4.54	2.91	2.89
R− P0	1.07	0.78	0.63	0.54	2.19	1.55	1.32	1.05
R− P+	1.64	1.07	1.10	0.87	2.91	2.14	2.23	1.87
MS R[a]	0.67***	2.93***	0.37***	1.37***	4.44***	10.44***	1.25***	2.99***
MS P	1.11***	0.92***	0.67***	0.63***	3.57**	3.82***	2.64***	2.112***
MS P × R	0.00 ns	0.20*	0.00 ns	0.05 ns	0.39 ns	0.87*	0.00 ns	0.00 ns
LSD$_{0.05}$	0.29	0.65	0.18	0.52	0.75	0.67	0.36	0.18

[a] Mean square for the effects of radiation (R), photoperiod (P) and their interaction (P × R).
* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.
ns: Not significant at $P \leq 0.05$.

Table 6
Average seed weight and yield in soybean plants cultivated under natural (P0) or extended (P+) photoperiod, under natural radiation (R0) or shading (R−), both treatments imposed from R3 onwards.

	Seed weight (mg seed^{-1})				Yield (g m^{-2})			
	Exp. 1	Exp. 2			Exp. 1	Exp. 2		
	A5409	A4910 RG	NA4990RG	NA5009RG	A5409	A4910 RG	NA4990RG	NA5009RG
R0 P0	164.8	159.0	184.0	185.0	472.6	442.7	336.7	331.9
R0 P+	147.4	120.5	128.0	127.0	634.5	499.3	352.5	346.2
R− P0	163.6	166.5	180.4	181.2	337.9	237.3	207.9	188.7
R− P+	144.1	126.0	126.3	126.4	405.1	249.3	258.6	224.1
MS R[a]	15.4 ns	125.4*	21.3 ns	14.9 ns	99,468.9**	155,655.7**	37,149.7**	52,748.3**
MS P	1015.7**	4688.7**	9108.0**	9554.1**	39,352.4*	3526.0 ns	3316.0*	1855.0 ns
MS P × R	3.2 ns	2.8 ns	2.4 ns	7.7 ns	6719.3 ns	1489.6 ns	909.7*	334.9 ns
LSD$_{0.05}$	10.6	6.6	6.9	6.8	109.3	109.3	23.3	35.5

[a] Mean square for the effects of radiation (R), photoperiod (P) and interaction (P × R).
* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
ns: Not significant at $P \leq 0.05$.

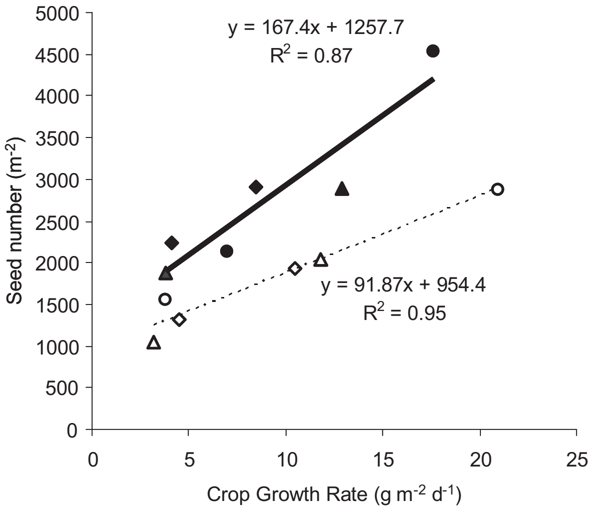

Fig. 3. Relationships between the number of seeds per unit area and crop growth rate during R3–R6, for soybean cultivars A4910 (circles), NA4990 (diamonds) and NA5009 (triangles) grown under natural (open symbols) or extended photoperiod (closed symbols). Symbols within boxes correspond to shaded treatments. Data from experiment 2.

seed were negligible; consequently, shading effects on yield were in parallel with those on seed number.

4. Discussion

Reducing incident radiation through shading applied from R3 onwards decreased the number of pods and seeds per unit land area. This was an expected result, given the strong relationship that exists between seed number and growth during the critical period in soybean (Egli, 1998), and in this paper the relevance of crop growth rate during the critical period from R3 to R6 was further confirmed for cultivars of Argentina of two different maturity groups. Only small effects of radiation on development were found in one of the two experiments, as plants grown under shading in Exp. 1 slightly delayed the occurrence of the R6 stage when photoperiod was extended. As this stage is defined considering seed size (seed filling the pod cavity, Fehr and Caviness, 1977), assimilation effects on seed growth rate (Egli, 1997; Egli and Bruening, 2002a), rather than direct effects of radiation on true developmental processes, may be the cause of that exceptional and minor delay. However, the shading treatment applied in the current study failed to reduce final weight per seed, suggesting that the mechanisms behind the combined effects of photoperiod and radiation on the dynamics of individual seed growth should be still evaluated.

Extending photoperiod, in contrast, did not modify growth rate, but delayed development, in coincidence with previous results

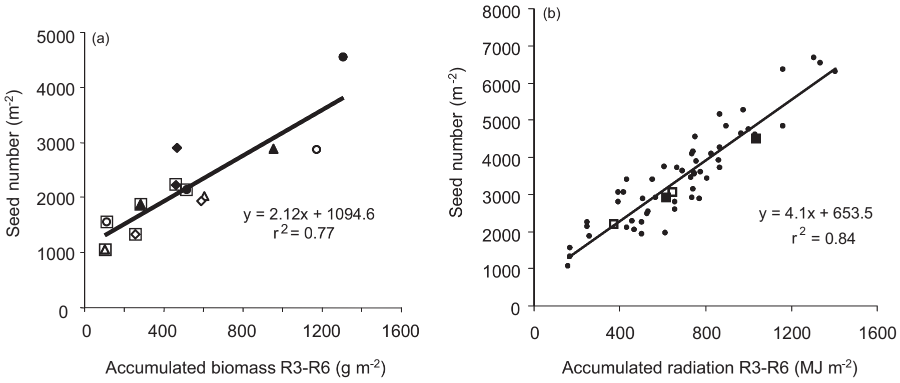

Fig. 4. (a) Relationship between the number of seeds per unit area and aboveground growth during R3–R6, for soybean cultivars A4910 (circles), NA4990 (diamonds) and NA5009 (triangles) grown under natural (open symbols) or extended photoperiod (closed symbols); symbols within boxes correspond to shaded treatments. (b) Relationship between the number of seeds and accumulated incident radiation during R3–R6 for data of A5409 in Exp. 1 under natural (open squares) or extended photoperiod (closed squares) together with data from Exp. 2 and previous experiments (small circles). Cultivars, sowing dates and photoperiod regimes from previous experiments are described in Kantolic and Slafer (2001, 2005, 2007).

(Kantolic and Slafer, 2001, 2005, 2007). The sensitivity to photoperiod (defined as the increase in the duration of R3–R6 per h of increment of photoperiod) was greater in Exp. 1 than in Exp. 2 and this effect may be attributable to the late sowing of this latter experiment. This sort of interaction between response to photoperiod and sowing date had been previously found (Kantolic and Slafer, 2001), and can be due to the natural changing of photoperiod and temperature as the season progresses. In Exp. 1 natural photoperiod during R3–R6 was longer (14.1 h) than in Exp. 2 (13 h), being closer to the threshold for photoperiodic sensitivity (Piper et al., 1996); temperature during R3–R6 was lower in this latter experiment and it has been proposed that low temperatures may reduce photoperiod sensitivity (Cober et al., 2001). However, the sensitivity to photoperiod of the R3–R6 period, in absence of any response of previous phases (that could have implied a sort of memorized effect associated with longer vegetative phases, see Slafer and Rawson, 1995; Miralles et al., 2003), was confirmed in the present study for cultivars of soybean so far untested for this responsiveness before and even in a background condition that minimizes the responsiveness.

In parallel with the increase in duration of the critical period, plants exposed to extended photoperiods produced more seeds per unit land area. The number of additional seeds produced per day of increase of the duration of R3–R6 was not constant across experiments, genotypes or radiation conditions. From the results obtained in experiments that did not include radiation manipulation but included different sowing dates (Kantolic and Slafer, 2001) and degrees of extension of photoperiod (Kantolic and Slafer, 2005) it has been proposed that the improvement of seed number by lengthening the duration of R3–R6 would be conditioned to a concomitant improvement in accumulated crop growth. The strong relationship between seed number and growth or accumulated intercepted radiation from R3 to R6 (Fig. 4) reinforces this idea and also extends its validity to lower ranges of radiation respect to those explored in previous results. Close relationships between the number of grains produced per unit of land area and the amount of radiation accumulated during the critical periods have been also found in other crops (e.g. Andrade et al., 1993; Cantagallo et al., 1997; Otegui and Bonhomme, 1998). As intercepted radiation is the main environmental factor regulating crop growth rate, the amount of radiation accumulated may be a simple variable for combining time and growth during the period when seeds are produced. Therefore, it seems that longer reproductive phases are, in terms of the number of pods and seeds established per unit land area, equivalent to increased daily photosynthesis of the crop during a shorter period. Both factors seem to operate through changes in resource availability during a phase that is critical for the determination of seed number in soybean: radiation, increasing daily growth, and photoperiod increasing the time available for radiation interception and growth. Interestingly, although the number of seeds produced per unit accumulated growth tended to increase when plants were shaded, photoperiod did not have significant effects on this ratio, reinforcing the suggestion that photoperiod main effects are related with an increase in total biomass accumulation due to the extended period of growth.

Plants exposed to extended photoperiod increased the number of seeds through the formation of a higher number of nodes and more pods established per node. Photoperiod treatments have been found to affect the number of nodes in indeterminate soybeans even when applied as late as 30 d after flowering (Kantolic and Slafer, 2007); this increment in node number may be an effect of long photoperiod promoting the vegetative activity of shoot apex and branches (Caffaro et al., 1988) or even reverting it from a reproductive stage (Wu et al., 2006). The increment in seed number per node in response to extended photoperiod has been previously estimated, as an average, at a whole plant or crop level (Kantolic and Slafer, 2001, 2005). In the present study we presented a nodal analysis that gives detailed evidence that extending photoperiod increases the number of pods that can be established on the different nodes of a soybean plant.

Although the general picture of the overall results of the present study showed similar responses of seed number to radiation and photoperiod, changes in the distribution of pods along the mainstem in response to treatments show some specific differences. Shading may have reduced the number of flowers (Jiang and Egli, 1993), or it could have stimulated flower and small-pod abortion (Hansen and Shibles, 1978; Huff and Dybing, 1980; Heitholt et al., 1986), being the effects evident in a broad range of nodes. In coincidence, responses to light enrichment and shading imposed at R1 occurred proportionately across the mainstem nodes in three soybean cultivars with determinate growth habit grown under different plant population (Liu et al., 2010). Considering that pods are sensitive to assimilate supply until the pod reaches its maximum size (Egli and Bruening, 2006b; Egli, 2010b) it seems clear that there were sensitive pods on most of the nodes of the plants, even when shading was imposed after some of them had flowered. Extended photoperiod, instead, increased the number of pods on the nodes that began to flower during or after the applications of the treatments, suggesting that those which flowered before the imposition of the treatments were less sensitive to photoperiod effects. Considering the mechanisms that control seed number (Egli, 2005), it may be argued that photoperiod, somehow, increased the availability of assimilates for pods located on those upper nodes. Our results suggests that photoperiod may have either (i) increased the total production of assimilates through increasing the period of growth or (ii) reduced the daily use of assimilates of the most hierarchical pods within a node, reducing their interference with late-appearing ones. A slowing down of development by a less inductive photoperiodic condition may favor both alternatives. The strong relationships between seed number and crop growth, or the accumulated radiation, during the critical period support the first alternative. The delay in the early development of pods, evidenced with the more time elapsed until mid-length pods were present (R4 stage) of or until seeds began to grow (R5 stage) supports the second alternative. The changes in development in response to photoperiods may be also the cause of the increase of productivity in uppermost nodes of the plants. In natural and non-stressed environments it has been found that the production of small pods and surviving pods declined rapidly as the plants approached growth stage R5 (Egli and Bruening, 2006a). As extended photoperiods delayed R5, it is possible that more time was available to form new pods on these nodes.

The exposure to extended photoperiods did not modify the number of pods in mid or mid-low node positions. This apparent insensitivity to photoperiod may reflect that, at the moment of applying the treatment of photoperiod extension, these nodes had ceased their flower production and then the treatment could not affect this process. The number of flowers has been suggested to be the driving force that shapes the pod production and survival profiles in soybean (Egli and Bruening, 2006a). Another possibility is that some kind of regulation among pods of different ages should define the maximum number of pods per node before the end of flowering of these nodes (Egli and Bruening, 2002b). In the present studies, the positions where the number of pods did not increase in response to extended photoperiod had already flowered before the treatments were imposed; thus, some hierarchical relationships between pods would be established around the time of flowering of particular nodes.

Increasing seed number by lengthening reproductive phases increased yield in Exp. 1 but not in Exp. 2. In previous experiments using a similar approach, differences in seed number per unit land area due to exposures to long photoperiods during pod formation, resulted in concomitant changes in yield, although small decreases in weight per seed were found in some treatments (Kantolic and Slafer, 2005, 2007). Those experiments were sown as early as Exp. 1 of present study. It seems possible that the strategy of increasing seed number by lengthening the reproductive phases may be an attractive alternative to improve yields only in early sowing. On the other hand, extremely late sowings may exacerbate source limitations during the seed-filling phase and simultaneously expose growing seeds to low temperature which reduce seed growth (Calviño et al., 2003). However, in normal sowing dates, photoperiod effects did in fact increased yield consistently.

Most genetic improvement in soybean has been mainly associated with traits that increase crop growth rate, mainly during reproductive phases (Specht et al., 1999; Morrison et al., 1999; Kumudini et al., 2001, 2002; De Bruin and Pedersen, 2009). Although genetic variability in the length of reproductive phases has been found among genotypes of similar maturity group (Metz et al., 1984; Hanson, 1992; Kantolic and Slafer, 2001), few attempts have been made to manipulate the durations of reproductive phases (Hanson, 1985; Metz et al., 1985; Smith and Nelson, 1986). Based in previous research, we have proposed that increasing photoperiod sensitivity during reproductive phases should lengthen the pod addition phase and may increase potential yield (Kantolic and Slafer, 2001, 2005). That suggestion was based on experiments manipulating photoperiod, and for it to be possible increasing sensitivity to photoperiod should be equivalent to exposing the crop to longer days. The results of the present research give support to the speculation that breeding for increasing the length of reproductive phases, should increase potential yields similarly to what was achieved by breeding for increasing crop growth rate during this reproductive period. Breeding for enhancing crop growth should result in plants with more pods per node, while improvement for increased photoperiod sensitivity after flowering would bring about plants with more nodes and a rather homogeneous distribution of pods along the nodes. Given the fact that age-related interferences among pods within a node seem to be stronger than those among pods produced on different nodes (Egli and Bruening, 2006b), this latter strategy might also confer higher yield stability. In coincidence, results from an exercise with a simulation model suggest that the mechanism of advancing flowering without changing the duration of the whole cycle should increase yields in a broad range of latitudes and environmental conditions (Kantolic et al., 2007).

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