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# Seed production determines the entrance to dormancy of the inflorescence meristem of *Pisum sativum* and the end of the flowering period

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## Abstract

Flowering plants adjust their reproductive period to maximize the success of the offspring. Monocarpic plants, those with a single reproductive cycle that precedes plant senescence and death, tightly regulate both flowering initiation and flowering cessation. The end of the flowering period involves the arrest of the inflorescence meristem activity, known as proliferative arrest, in what has been interpreted as an evolutionary adaptation to maximize the allocation of resources to seed production and the viability of the progeny. Factors influencing proliferative arrest were described for several monocarpic plant species many decades ago, but only in the last few years studies performed in Arabidopsis have allowed to approach proliferative arrest regulation in a comprehensive manner by studying the physiology, hormone dynamics, and genetic factors involved in its regulation. However, these studies remain restricted to Arabidopsis and there is a need to expand our knowledge to other monocarpic species to propose general mechanisms controlling the process. In this work, we have characterized proliferative arrest in *Pisum sativum*, trying to parallel available studies in Arabidopsis to maximize this comparative framework. We have assessed quantitatively the role of fruits/seeds in the process, the influence of the positional effect of these fruits/seeds in the behavior of the inflorescence meristem, and the transcriptomic changes in the inflorescence associated with the arrested state of the meristem. Our results support a high conservation of the factors triggering arrest in pea and Arabidopsis, but also reveal differences reinforcing the need to perform similar studies in other species.

## 1 | INTRODUCTION

A great number of flowering plants have adopted semelparity as their reproductive strategy, characterized by a single reproductive cycle in which fruits and seeds are produced for a discrete period of time, after which the plants stop producing flowers and enter a senescent state followed by plant death (Stearns, 1976). The end of flowering and subsequent plant senescence has been studied in several semelparous (a.k.a. monocarpic) plant species, including legumes, solanaceae, grasses, and other important crops. These studies, in general,

describe the end of the reproductive phase as a cessation in the activity of the shoot apical meristems (SAM), a "proliferative arrest", coupled with the onset of plant senescence, as well as identifying the production of fruits/seeds as the major factor triggering this arrest (Murneek, 1926; Whaley, 1939; Leopold et al. 1959; Lockhart and Gottschall 1961; Malik and Davies 1976; Biswas and Choudhuri 1980; Hensel et al. 1994). Some even propose the existence of a mobile signal emitted by the fruits, a "death hormone", that would reach the meristem to repress its activity and promote plant death (Kelly and Davies 1988; Noodén and Leopold 1988; Engvild 1989; Wilson 1997).

Following the adoption of Arabidopsis as the most widely used model system for plant biology, Hensel et al. (1994) carried out a systematic study of factors that influence the mode and timing of the arrest in flower production in this species. The major conclusions of this work were that seeds are the major factor triggering the end of flowering, and that when they are produced to a certain threshold, cause the inflorescence meristem to stop producing flowers and to acquire a typical morphology with a cluster of unpollinated floral buds that do not grow further. Sterile plants, or those producing less than around a 30% of the seeds of a fully fertile plant, extend the flowering period and the meristem terminates into a differentiated floral structure instead of this cluster of arrested floral buds. The study also reported that flowering arrest occurs coordinately in all aerial meristems of the plant, prompting the authors to name the process as Global Proliferative Arrest. It also reported that arrested meristems can be reactivated by removing the already developed fruits, strongly supporting the hypothesis of a long-distance repressive signal produced by the fruits/seeds to stop the production of new flowers (Hensel et al. 1994). Following this work, and somehow surprisingly, proliferative arrest (PA) received little attention and became an almost forgotten topic. Only in the last few years several works have expanded these physiological studies in Arabidopsis and have incorporated a wealth of genetic and molecular data, including the characterization of transcriptomic signatures associated with the inflorescence meristems in different stages of the reproductive phase, the characterization of plant hormone roles and plant hormone dynamics in proliferative arrest or the identification of a gene network that controls the end of the flowering phase (Wuest et al. 2016; Balanzà et al. 2018, 2023; Goetz et al. 2021; González-Suárez et al. 2020; Martínez-Fernández et al. 2020; Ware et al. 2020; Walker et al. 2022).

The transcriptomic changes associated with proliferative arrest in Arabidopsis have been determined by comparing inflorescence meristems actively producing flowers with arrested meristems and meristems reactivated by fruit removal. Results indicate that the end of flowering is characterized by low mitotic activity, the upregulation of abscisic acid (ABA) responses, the repression of cytokinin (CK) signaling, the activation of senescence and stress-related programs, and a reduction of reactive oxygen species (ROS), all of which have previously been associated with axillary bud dormancy, thus suggesting that the SAM at the end of flowering enters into a similar dormant state (Wuest et al. 2016; Martínez-Fernández et al. 2020). Other studies have delved into physiological aspects, describing how the meristems need to acquire competence to perceive the fruit/seed derived signals triggering the arrest. They have also challenged the idea of a coordinated "global" nature of the proliferative arrest, proposing instead a strong local component, with control mainly exerted by fruits proximal to the apex (Ware et al. 2020). The presence of the typical cluster of undeveloped floral buds in arrested inflorescences has also been uncoupled from the actual cessation of inflorescence meristem activity, thus dividing the end of flowering into two sequential steps: a block to floral development, and the arrest of the inflorescence meristem (Walker et al. 2022; Sánchez-Gerschon et al. 2024). Auxins produced in the developing fruits and redistributed in the plant have been proposed to disrupt auxin signaling in the inflorescence apex once it has acquired the competence to arrest, and are suggested to be a component of the still elusive death hormone (Ware et al. 2020; Goetz et al. 2021). However, a recent study suggests that their role may be restricted to promoting the developmental block of floral buds rather than inflorescence meristem arrest (Walker et al. 2022). CK signaling, on the other hand, needs to be repressed locally in the inflorescence meristem to allow the cessation of cell division and of meristem activity, and, in fact, CK decline is an early event that marks the onset of meristem arrest (Goetz et al. 2021; Merelo et al. 2022; Walker et al. 2022). Genetically, APETALA2 (AP2) family members control the activity of the meristem by promoting the activity of WUSCHEL (WUS), a transcription factor essential to maintain stem cell activity. AP2-like factors are progressively repressed in the inflorescence by FRUITFULL (FUL) and miR172, and this repression eventually leads to WUS extinction in the meristem and the concomitant cessation of cell division and stem cell maintenance (Balanzà et al. 2018). Interestingly, *AP2* induction in arrested meristems can reactivate the production of flowers in these arrested meristems in a manner similar to fruit removal, and the transcriptomic response to *AP2* induction in the inflorescence strikingly resembles the effect of eliminating the fruits, suggesting that AP2-like factors could be integrating the signals coming from the seeds and the age-related cues (Martínez-Fernández et al. 2020).

The recent advances in our knowledge of proliferative arrest in Arabidopsis have not been paralleled with comparative studies in other species, and it is not yet clear whether it can be extended as a general model for duration of flowering in other monocarpic plants. In this work we aim to contribute to fill this gap by studying the end of flowering in *Pisum sativum* (pea). We have chosen pea because it was one of the first species where proliferative arrest was described (Leopold et al. 1959; Lockhart and Gottschall 1961; Proebsting et al. 1977; Davies and Gan 2012); it has an indeterminate growth habit but a different inflorescence architecture than Arabidopsis, a compound raceme instead of a single raceme (Benlloch et al. 2007); and there is a wealth of genetic resources, including a high-quality genome and pan-genome, that can be used in further research (Dalmais et al. 2008; Alves-Carvalho et al. 2015; Kreplak et al. 2019; Pandey et al. 2021; Yang et al. 2022). Several studies have already addressed the control of the end of flowering in pea and provide a consensus view that developing fruits have a direct role in the induction of proliferative arrest. However, many of these studies are relatively old, are not consistent in approaches and methodology, and

reach controversial conclusions, making it difficult to build a clear understanding of how the process is regulated. On one hand, some authors support the existence of the "death hormone," with a direct role for seeds proposed in modulation of proliferative arrest (Leopold et al. 1959; Lockhart and Gottschall 1961; Yang et al. 2022), whereas other authors argue that the nutritional imbalance caused by fruit/seed development is the trigger for arrest (Gifford and Evans 1981; Kelly and Davies 1986; Kelly and Davies 1988). Finally, there are those who propose a combined effect of these two factors in promoting flowering cessation (Proebsting et al. 1977; Zhu and Davies 1997). These studies nevertheless agree on describing that fully developed fruits are not implicated in the process, pointing to seeds or fruits in early developmental stages as major promoting forces (Lockhart and Gottschall 1961; Malik and Davies 1976; Gianfagna and Davies 1981; Yang et al. 2001). Furthermore, similar to *Arabidopsis thaliana*, some authors have hypothesized that only fruits proximal to the SAM play a primary role in the modulation of PA in pea (Malik and Berrie 1975; Sklensky and Davies 2011; Ware et al. 2020).

Here, we have performed physiological experiments in pea that complement the most recent studies in Arabidopsis to establish a comparative framework useful for further work. Moreover, we have characterized the transcriptomic changes in pea inflorescence apexes at different stages during the reproductive phase. Our results point to seed and not fruit production as the major factor controlling proliferative arrest and suggest that the seeds signal the meristem in a systemic and quantitative manner. Finally, we show that the transcriptomic responses in the inflorescence apex throughout the reproductive cycle are quite similar between Arabidopsis and pea, despite the difference in inflorescence architecture and physiological responses.

## 2 | MATERIALS AND METHODS

### 2.1 | Plant material and growth conditions

Plants were grown in the greenhouse under a 16-hour light and 8-hour dark photoperiod, with natural light supplemented by 400 W sodium vapor lamps. Plants grew in vermiculite irrigated with Hoagland Solution No.1 enriched with trace elements (Hewitt 1966). The temperature ranged from 21–22°C (day) to 15°C (night). The cultivars used have been Cameor, CDC Amarillo and NGB5839.

### 2.2 | Physiological characterization

Several parameters were annotated to characterize the different aspects related with the meristematic activity. The moment of arrest was determined when the first senescent flower was visible. The number of reproductive nodes was scored by counting all nodes from the first flowering node to the last node producing open flowers. The number of fruits was determined by counting fruits producing at least one viable seed. For statistical analysis, a two-tailed Student's t-test was performed whenever two groups were compared. In all cases, size sample was n ≥ 10.

### 2.3 | RNAseq of inflorescence apexes

Apical inflorescence apexes were collected at different stages of pea plant development. (1) Proliferative: SAM from plants after the production of the first flower during anthesis. (2) Arrested: SAM from plants with arrested meristems (PA). (3) Pruned: SAM from plants collected on the same day as stage 2, but that had been continuously deflowered since the production of the first flower. (4) Reactivated: SAM from plants 24 hours after reactivation (plants with previously arrested meristems, from which all flowers and fruits were removed to reinitiate flowering).

The inflorescence apexes were carefully dissected under a microscope, eliminating all visible leaves and floral primordia. Four biological replicates were sampled for each timepoint. For RNA extraction, the commercial RNeasy Mini Kit by Qiagen was used. RNA integrity was assessed using a 2100 Bioanalyzer (Agilent). RNA sequencing was performed by Novogen Company United, and yielded an average of 20 million raw reads, totaling approximately 6 gigabytes of raw data per sample in FASTQ format. For bioinformatic analysis, in the first place, the raw reads were cleaned to remove adapters and eliminate low-quality regions using *cutadapt*. Subsequently, the clean reads were aligned to the *Pisum sativum* genome using *HISAT2*. Once the alignment was complete, per-gene read counting was performed using *htseq-count*. Finally, the differential expression analysis was executed using *DESeq2*, utilizing default parameters. All the processing was carried out using Python. To identify genes with differential expressions, we computed the LOG Fold Change (logFC) by dividing the RPKM or TPM values of one sample by those of another. To simplify the interpretation of differential gene expression, the FC values were then converted to a $\log_2$ scale ($LOG_2FC$), where a ± 1 value of log2FC indicates a doubling or halving of expression between the samples. Furthermore, statistical assessments, such as the p-value and adjusted p-value (p adjusted), were employed to assess the significance of these expression alterations.

Gene Ontology (GO) Term Enrichment Analysis was performed with R Studio and followed two different strategies. The first strategy involved conducting an enrichment analysis of Biological Process (BP) using the existing GO categories for peas (https://urgi.versailles.inra.fr/Data/Genome/Genome-data-access). However, the still partial annotation of the pea genome precluded obtaining conclusive results. Therefore, we decided to pursue a second strategy. In this approach, we performed a homology analysis of all pea genes with respect to Arabidopsis genes and assigned each pea gene its "potential" ortholog in *Arabidopsis thaliana*. Given that a simple homology alignment does not definitively establish orthology, we referred to them as genes with the highest sequence score. After transforming all "Psat" genes in peas into "AtXg" genes of Arabidopsis, we proceeded to perform the enrichment analysis using the more robust *Arabidopsis thaliana* genome information.

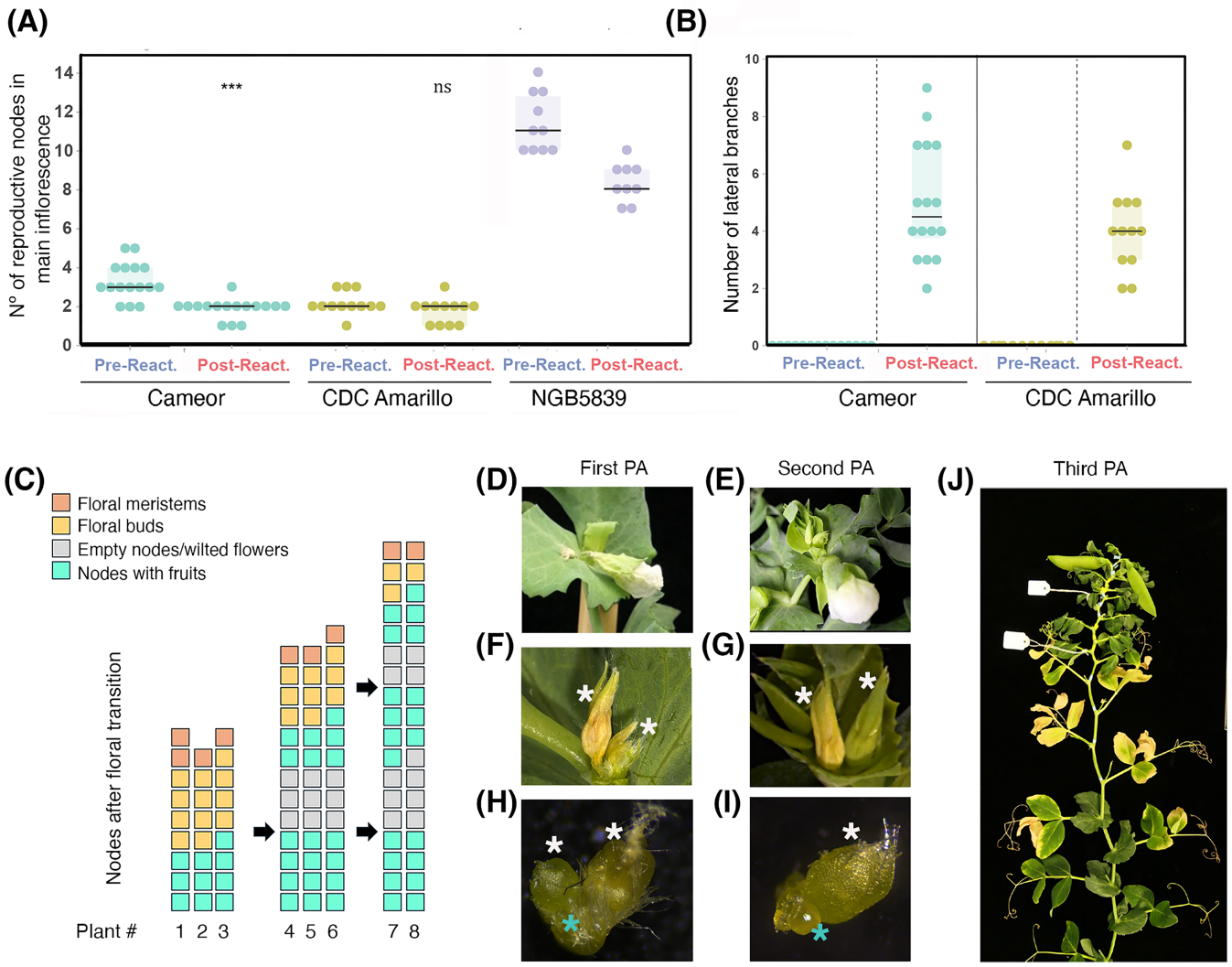


**FIGURE 1** **Reactivation capacity of the SAM by removing secondary inflorescences with flowers and fruits from plants that had undergone proliferative arrest**. **(A)** Number of reproductive nodes produced before arrest (pre-react) and additional reproductive nodes with new flowers produced after reactivation by pruning of all flowers and fruits present at the moment of arrest (post-react) in three different genetic backgrounds. **(B)** Number of lateral branches that grow after the reactivation treatment (pruning of flowers/fruits) in positions corresponding to vegetative nodes in two genetic backgrounds. Data corresponding to individual plants for each treatment are represented as dots in all graphs. Asterisks indicate a significant difference from the corresponding reproductive node number at first proliferative arrest (pre-react) plants according to Student's t-test: ***: p-value <0.001; n.s., not significant. **(C)**. Diagram representing the nodes produced after floral transition by eight different plants (Cameor). Plants 1–3 were left untreated and the nodes with fruits at the moment of PA were scored. The cluster formed above these nodes was inspected **(D)** and visible floral buds (marked with white asterisks in **F**) and floral meristems (marked with blue asterisk in **H**) were scored and represented in the diagram in (c) as additional nodes. After PA was evident in plants 4–6, fruits already formed were removed (all below black arrow) and additional nodes were produced. After a second PA was evident, empty nodes (those that formed wilted flowers that fell with no further development), nodes with fruits, and floral buds and floral meristems in the second cluster (E-I) were scored and represented in (C). Plants 7 and 8 were subjected to fruit removal after first PA, and additional empty nodes and nodes with fruits produced when second PA was evident were scored. A subsequent pruning of these additional fruits was done. When a third PA was observed, empty nodes, nodes with fruits and the floral buds and meristems in the cluster were scored and represented similarly in (C). **(J)** Picture of plant #7 in the experiment in (C). Tags mark the last node producing fruits before the first and second PA.

For the enrichment analysis, we used the *TopGO* package in R Studio (Alexa et al. 2006). As input for the GO categories, we used the file "ATH_GO_GOSLIM.txt.gz" available on The Arabidopsis Information Resource (TAIR) webpage which receives periodic updates. Regarding the details of the enrichment analysis, we used the *weight01* algorithm, which is described as the best available for this process, as it takes into account aspects such as the hierarchical structure of GO terms and helps to avoid false positives (reference weight). Additionally, we used the Fisher's exact test, the best one in combination with the *weight01* algorithm (Alexa et al. 2006). The remaining packages used for data processing are listed below: *readr*, *tidyr*, *dplyr*, *Hmisc*, *ggsci*, *gridExtra*, *ggpubr*, *car*, *stringr*, *topGO*, *forcats*, *viridis*, *tidyverse*.

To visualize the entire dataset comprehensively, we utilized R Studio. For heatmaps, the *pheatmap* package was used; for visualizing the GO categories, the *rrvgo* package, which allowed us to generate the "treemap" and "scatterplot" representations; and for generating the Venn Diagrams, the *VennDiagram* package was used. For the rest of the diagrams and plots, we used the package *ggplot2*.

The libraries employed for data processing and plot generation also included:

*readr*, *tidyr*, *dplyr*, *RColorBrewer*, *Hmisc*, *ggsci*, *gridExtra*, *ggpubr*, *car*, *and stringr*.

All the plots generated using R Studio were saved in PDF format and subsequently underwent post-processing using Adobe Illustrator and Photoshop (https://www.adobe.com/).

## 3 | RESULTS

### 3.1 | Pea inflorescences in proliferative arrest can be reactivated by defruiting

Pea plants flower after the production of several vegetative nodes, where leaves subtend axillary buds that usually remain dormant. After floral transition, the SAM continues to grow indeterminately and the new axillary meristems develop into secondary inflorescences, that each typically produce one or two flowers before the secondary meristem is consumed forming a reduced pin-like residual organ. After the production of a certain number of reproductive nodes, the SAM also ceases producing new nodes and enters a proliferative arrest; the nodes that bear unpollinated floral buds remain in a cluster and do not progress in development, while in the nodes where the flowers have self-pollinated, fruit filling is usually completed (Suppl Figure 1A).

The number of vegetative nodes before floral transition is dependent on environmental factors such as photoperiod as well as genetic factors that control this developmental phase change (Weller and Ortega 2015). To test whether the number of reproductive nodes produced before proliferative arrest is also controlled genetically, we quantified this trait in three different genetic backgrounds: Cameor, an early-flowering cultivar that was used to produce the first reference genome sequence and to generate a widely used TILLING platform; CDC Amarillo, a commercial variety of high agronomic performance developed by the University of Saskatchewan (Canada) and the NGB5839 line, a dwarf isogenic line derived from the Swedish Torsdag cultivar (Lester et al. 1999; Warkentin et al. 2014; Kreplak et al. 2019). In the greenhouse conditions when the experiment was conducted, NGB5839 plants produced 11.4 ± 0.5 reproductive nodes on average before proliferative arrest, while Cameor only produced an average of 3.3 ± 0.2 and CDC Amarillo of 2.2 ± 0.2 (Figure 1A), supporting the idea of proliferative arrest being under genetic control. It is well established that developing fruits/seeds signal the SAM to arrest, ending the flowering period, not only in pea but also in many other species. To assess the effect of eliminating this signal on meristem activity, we removed simultaneously all developed fruits produced by the plant once proliferative arrest was morphologically evident. In all three backgrounds the SAM reactivated and produced new reproductive nodes with fruits, before entering a second arrest. The first 3–4 nodes above the last node with pruned fruits expanded their associated leaf, but the secondary inflorescence wilted, and no flowers opened. After these empty nodes, new nodes with flowers were observed (Figure 1C, J; Suppl Figure 1B). NGB5839 produced 8.3 ± 0.3 new nodes with flowers, again more than Cameor (1.9 ± 0.1) or CDC Amarillo (1.8 ± 0.2; Figure 1A). To assess whether these new formed nodes were produced by the reactivated meristem and not merely by the release of the development block in nodes already present in the arrested cluster, we inspected the size of these clusters at the first PA (before reactivation) and the second PA (after reactivation) in Cameor plants, and quantified the number of nodes produced after the reactivation and before the second cluster was evident (Figure 1C-I). The second PA clusters were only slightly smaller in bud number and size, and the number of nodes produced before the second arrest was higher than the number of buds in the cluster at the first PA, supporting the production of new primordia by the SAM after reactivation (Figure 1 C-I). Moreover, when fruits formed post-reactivation were subsequently eliminated, flower production was restarted again, before a third event of PA was observed (Figure 1C, J). Altogether, these observations indicate that the SAM reinitiates growth when the putative fruit-derived signal is eliminated. Interestingly, the pruning of developing fruits also released the dormancy of secondary branches in the axils of vegetative nodes. In this experiment, no branching was observed in any of the three genotypes before proliferative arrest. After reactivation of the SAM, axillary branches were also activated in most positions and produced flowers and fruits (Figure 1B).

### 3.2 | The timing of proliferative arrest in pea correlates with the total weight of seeds produced

It has been suggested that the proliferative arrest of the SAM is triggered mainly by the seeds and not the fruits. In Arabidopsis, Hensel et al. (1994) reported that when seed set is lower than 30% of the wild type, proliferative arrest does not take place and the inflorescence meristem instead turns into a terminal floral structure, after producing flowers for an extended period of time. Likewise, Lockhart and Gottschalk (1961) determined that complete deflowering of pea plants extended the activity of the SAM and the production of reproductive nodes. They also described the morphology of the apex of deflowered plants as a single terminal flower, produced in the last flowering node, but their description and illustration left this interpretation open to debate. We decided to revisit these questions by deflowering plants in different proportions and inspecting the effect of these treatments on the number of reproductive nodes produced before proliferative arrest and on the morphology of the meristem.

Cameor plants produce 1–2 flowers per secondary inflorescence, and, in our growing conditions, usually only one develops into a seed-filled fruit. To simplify the approach, the entire secondary inflorescence of the reproductive node was removed instead of removing

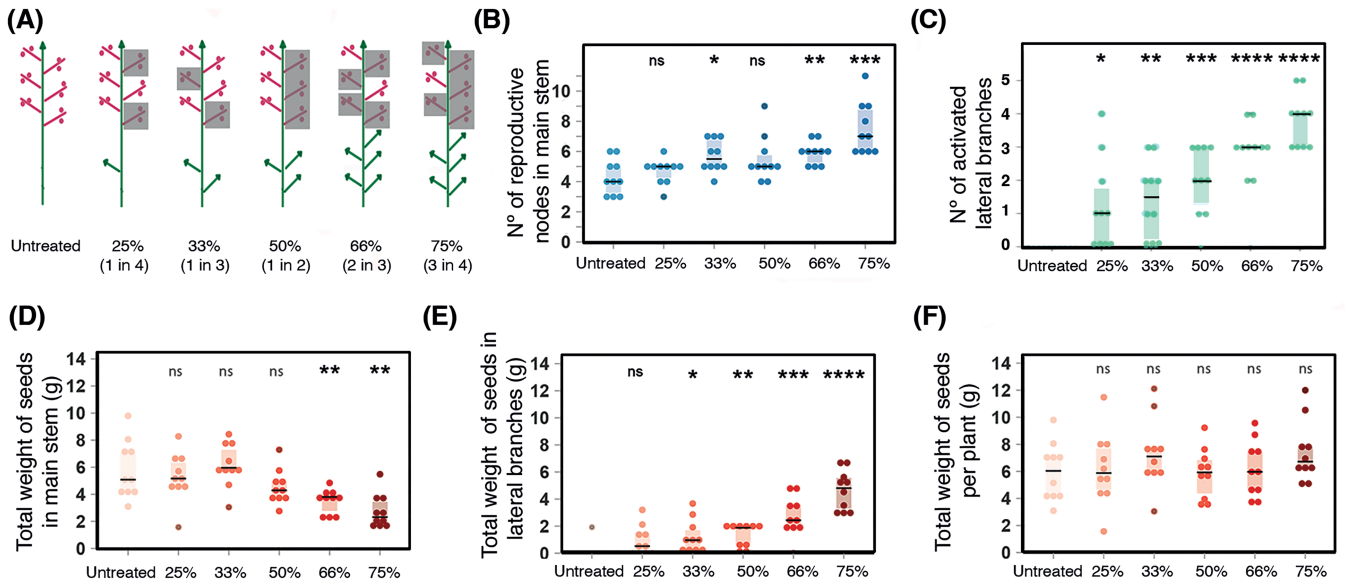


**FIGURE 2** **Proliferative arrest is gradually influenced by the total seed production.** (**A**) Scheme of treatments performed in plants of the Cameor cultivar. Secondary inflorescences are represented in magenta, and, when eliminated, it has been indicated with a grey box. The activation of lateral branches is represented by green arrows forming at basal positions in the plant. These branches produced fruits and seeds (not shown for clarity). (**B**) Reproductive nodes produced by the main stem at proliferative arrest in plant subjected to the described treatments. (**C**) Activation of lateral branches in response to the treatments. (**D**) Combined weight of seeds produced in the fruits borne in the secondary inflorescences of the main axis. (**E**) Combined weight of seeds produced in the fruits borne in lateral branches. (**F**) Combined weight of all the seeds produced by the plant. Data corresponding to individual plants for each treatment are represented as dots in all graphs (n = 10). Asterisks indicate a significant difference from the corresponding untreated plants according to Student's t-test: *: p-value <0.05; **: p-value <0.01; ***: p-value <0.001; ****: p-value <0.0001; n.s., not significant.

individual flowers, given that we defined the reproductive period by the number of reproductive nodes that formed before proliferative arrest. Secondary inflorescences were removed as soon as flowers opened, in the following proportions: 25% (first one eliminated, three following nodes left, and so on), 33%, 50%, 66% and 75% (Figure 2A) and compared to an untreated control in both genetic backgrounds. These treatments had two conspicuous effects. First, the activity of the SAM was gradually extended in time with the progressive removal of secondary inflorescences, although only in the 66% and 75% treatments the differences were statistically significant (Figure 2B). Second, the lateral branches were activated also gradually with secondary inflorescence elimination (Figure 2C). When we quantified the total weight of seeds produced by the plant, both in the fruits borne in the main axis and in those produced by lateral branches, we observed a clear compensation effect, where the total weight of seeds produced at the time of proliferative arrest was comparable in all the plants regardless of the treatments they were subjected (Figure 2D-F). An additional experiment of gradual pruning of secondary inflorescences was performed in the CDC Amarillo background, with similar outcome (Suppl Figure 2). These results supported the idea of seed production as the major factor triggering arrest, and revealed that it is unlikely that an all-or-nothing threshold similar to that proposed in Arabidopsis (below/above 30% seed production) exists in pea.

A second set of experiments was designed to evaluate the importance of a possible positional effect of the flowers/fruits with respect to the SAM to trigger proliferative arrest. In our growing conditions the number of reproductive nodes in untreated plants was usually low. For this reason, we first assessed whether the fruits produced in the main inflorescence and those produced in the more distant lateral branches of vegetative nodes had equivalent capacities to trigger SAM arrest.

As in previous experiments, untreated Cameor plants did not branch, and thus, all fruits produced by untreated plants were born in the secondary inflorescences of the main axis. In this experiment, these plants produced an average of 3.56 ± 0.34 reproductive nodes before proliferative arrest (Figure 3 A-C). When all secondary inflorescences were continuously removed as the flowers opened, the SAM extended its activity producing more reproductive nodes, and lateral branches were activated, producing fruits (Figure 3 A-C). Although PA was delayed by this treatment, the total amount of seeds produced by the plant did not show a significant difference between plants that only had fruits in the main axis and those that only had fruits in the lateral branches, suggesting that the position of the fruits was less important to trigger arrest than the amount of seeds produced (Figure 3D). The extended activity of the SAM could be explained by the delay in the activation of the lateral branches, that only grew after the secondary inflorescences were pruned and took some time to produce flowers and fruits. In plants where all fruit production was prevented, by elimination of secondary inflorescences and lateral branches, the SAM remained active for longer, producing 14.5 ± 1.29 reproductive nodes before arrest (Figure 3B). The morphology of the arrested apexes of untreated plants and of plants where fruit

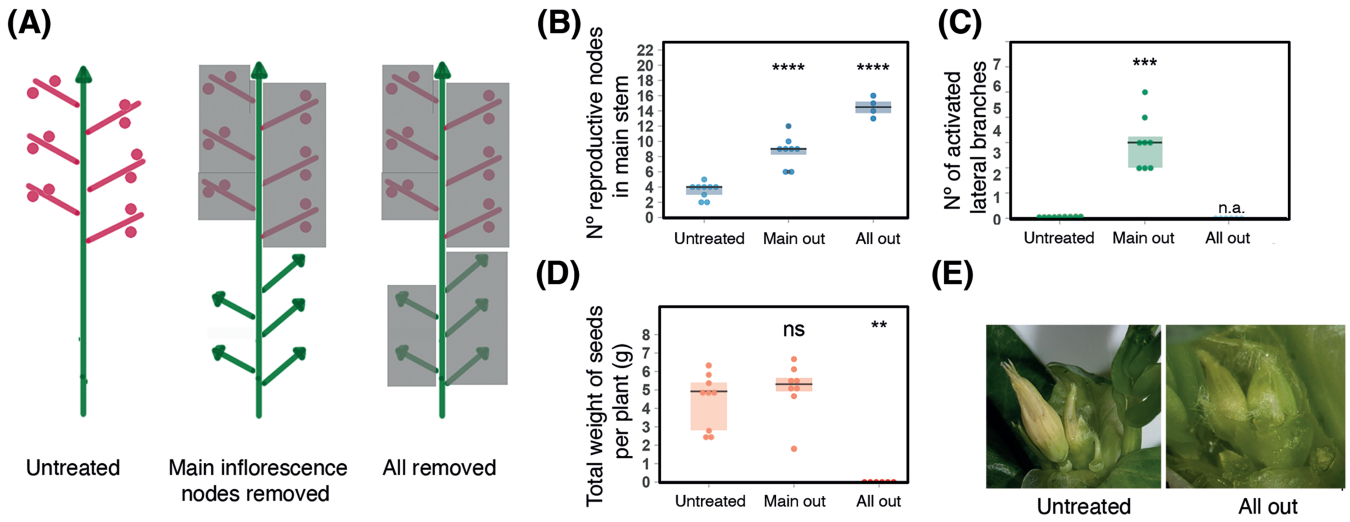


**FIGURE 3** **Fruits in lateral branches are able to long-distance signal the main inflorescence to arrest in a seed-weight dependent manner.** **(A)** Scheme of treatments performed in Cameor plants. Secondary inflorescences are represented in magenta, and, when eliminated, they have been shaded in grey. The activation of lateral branches is represented by green arrows forming at basal positions in the plant. These branches produced fruits and seeds (not shown for clarity). **(B)** Reproductive nodes produced by the main stem at proliferative arrest in plant subjected to the described treatments. **(C)** Activation of lateral branches in response to the treatments. These branches were not quantified if removed (n.a. in All-out treatment). **(D)** Combined weight of all the seeds produced by the plant. **(E)** morphology of the cluster of floral buds observed in plants that had undergone proliferative arrest. On the left, untreated plants; on the right, plants where fruit production was completely prevented by pruning of lateral branches and secondary inflorescences in the main axis. Data corresponding to individual plants for each treatment are represented as dots in all graphs. Asterisks indicate a significant difference from the corresponding untreated plants according to Student's t-test: *: p-value <0.05; **: p-value <0.01; ***: p-value <0.001; ****: p-value <0.0001; n.s., not significant; n.a., does not apply.

production was avoided was very similar, with a cluster of senescent buds and no signs of terminal differentiation (Figure 3E).

We also assessed the potential positional effect of the fruits in the main axis on the arrest of the shoot apical meristem. Both Cameor and CDC Amarillo backgrounds were tested, and in all experiments, lateral branches were eliminated (Figure 4). Untreated Cameor plants produced an average of 4.1 ± 0.4 reproductive nodes (Figure 4A). The elimination of secondary inflorescences at either the two first nodes or at the third and fourth nodes delayed proliferative arrest by one-two nodes, but, again, the total weight of seeds produced by the plant was similar for the three treatments (Figure 4A-C). In CDC Amarillo, the average number of reproductive nodes in untreated plants or those where secondary inflorescences at either the 1st-4th nodes or the 5th-8th nodes were removed was the same, as was the weight of seed produced by the plants (Figure 4D-F). For CDC Amarillo an additional treatment where all flowers and lateral branches were removed was included. In this case, the plants had not experienced proliferative arrest when we decided to stop scoring, after they had produced an average of 47.25 ± 1.06 reproductive nodes (Figure 4D). These experiments argued against the relevance of the position of the developing flowers/fruits with respect to the SAM in two different genetic backgrounds and confirmed the importance of seeds as major contributing factors to the end of flowering.

To further confirm the role of seeds and to uncouple it from pod development, NGB5839 plants were deseeded by inserting a needle into young pods and separating the developing seeds from the pod approximately nine days after anthesis, and compared with plants completely deflowered. This treatment allowed pod elongation and maturation, and, therefore, the putative sink effect of the developing pod was present. Untreated plants underwent proliferative arrest after producing an average of 11.4 ± 0.5 reproductive nodes, approximately one month after flowering initiation. The shoot apical meristem of both deseeded and deflowered plants remained active, with no signs of arrest, three months after floral transition, when the experiment was stopped (Figure 5A). At this time point, deseeded plants had produced an average of 23.4 ± 0.5 reproductive nodes, while deflowered plants had produced an average of 29.1 ± 0.7 reproductive nodes, suggesting that developing pods could be acting as sinks for plant resources, reducing the activity of the SAM, but not influencing the proliferative arrest per se (Figure 5B). This experiment also revealed that the shortening of the internodes after floral transition in pea is not determined by the arrest of the meristem, since it was similarly observed in fertile, deseeded and deflowered plants.

## 3.3 | The arrested inflorescence apexes have the transcriptomic signature of dormant axillary buds

To understand the molecular changes associated with proliferative arrest, the transcriptomes of apexes at four different stages were analyzed: (1) proliferative apexes, collected after the first flower opened; (2) arrested apexes, collected at the moment of visible cluster formation; (3) apexes of plants of the same age than those arrested, but where proliferative arrest was prevented by pruning of secondary

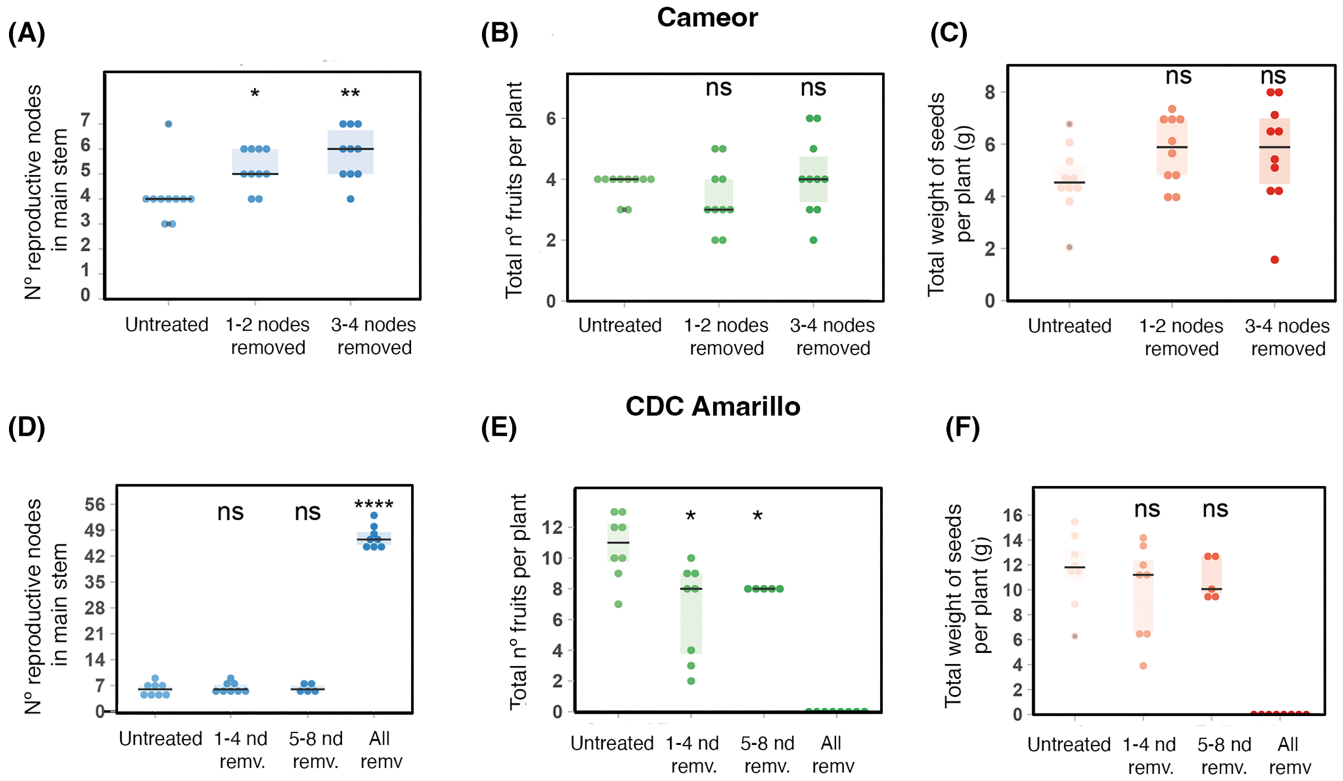


**FIGURE 4** **The position of the flowers/fruits with respect to the inflorescence apex does not influence proliferative arrest as much as total seed production. (A-C)** Scoring of Cameor plants. (**D-F**) Scoring of CDC Amarillo plants. In all cases, lateral branches were eliminated, and the scored data refers to the main inflorescence axis. (**A, D**) Reproductive nodes produced by the main stem at proliferative arrest in plants subjected to the described treatments. (**B, E**) Total number of fertile fruits produced per plant. (**C, F**) Total weight of seeds produced in plants subjected to the described treatments. Data corresponding to individual plants for each treatment are represented as dots in all graphs. Asterisks indicate a significant difference from the corresponding untreated plants according to Student's t-test: *: p-value <0.05; **: p-value <0.01; ***: p-value <0.001; ****: p-value <0.0001; n.s., not significant.

inflorescences, and (4) apexes of reactivated inflorescences, 24 h after the elimination of all flowers and fruits. The rationale of this sampling was similar to that followed by Wuest et al. (2016) when analyzing the transcriptomic changes associated to proliferative arrest in Arabidopsis. By comparing these samples, we could, on one hand, define the specific characteristics of arrested apexes regardless of the age of the meristem and of the presence of seeds, and on the other hand, compare the signatures of the proliferative arrest of Arabidopsis and pea inflorescences.

Transcripts with a log2 fold change (FC) ≥3 and ≤3, and a p-adjusted value <0.05 were considered as differentially expressed genes (DEG). Under these restrictive conditions, 1813 DEGs were identified in proliferative (1) vs. arrested (2) apexes (730 and 1085 up-regulated genes in active and arrested inflorescences respectively; 2272 in deflowered (3) vs. arrested (2) (1043 and 1229 up-regulated genes in active and arrested inflorescences respectively); and 2482 in reactivated (4) vs. arrested (2; 1283 and 1199 up-regulated genes in active and arrested inflorescences respectively; Suppl. Table S1). To better understand the processes affected at each developmental stage, we conducted a Gene Ontology (GO) analysis. As the GO terms for pea transcripts are still scarce, we decided to check for the putative ortholog genes in Arabidopsis to perform the analysis, which involved discarding some of the original pea DEGs if no clear equivalence was found or different pea genes corresponded to a single Arabidopsis gene. For DEGs in proliferative (1) vs. arrested (2) apexes the list was reduced to 472 and 750 up-regulated genes in active and arrested inflorescences respectively; for DEGs in deflowered (pruned) (3) vs. arrested (2) inflorescences the list was reduced to 672 and 840 up-regulated genes in active and arrested inflorescences respectively; finally, for DEGs in reactivated (4) vs. arrested (2) apexes, the reduced list consisted of 799 and 866 up-regulated genes in active and arrested inflorescences respectively (Suppl. Table S2). With these new lists of ortholog DEGs we conducted a Gene Ontology (GO) enrichment analysis for each comparison, first with the up-regulated genes in each developmental stage compared (Suppl. Table S3). Then, the different enriched GO categories obtained with the lists of up-regulated in active or up-regulated in arrested apexes were compared. Most of the categories obtained in each group were common for the three comparisons, with 129 GO terms shared in the "up-regulated in active apexes" group (Figure 6A) and 96 in the "up-regulated in arrested apexes" group (Figure 6B). The enriched GO terms in the up-regulated DEGs in active inflorescences were diverse, but in general the terms could be related to the promotion of cell division, cell wall dynamics, and other aspects related to flower development (Figure 6C). On the other hand, the enriched GO terms in the

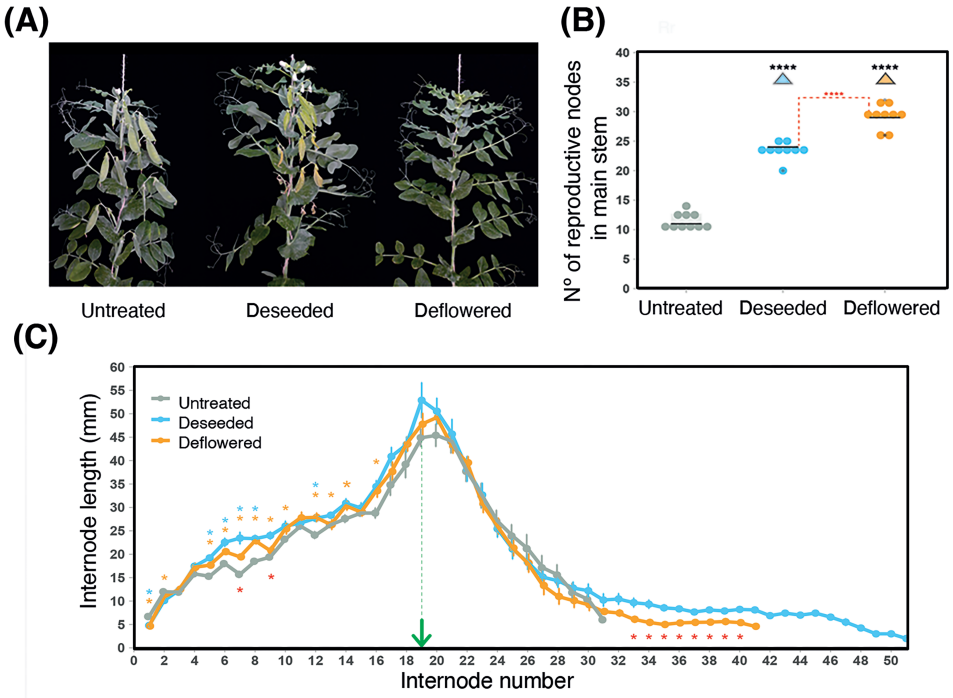


**FIGURE 5** **Differential effect of deflowering and deseeding on proliferative arrest. (A)** Inflorescences of untreated plants, plants where seeds have been excised shortly after pollination and plants where all secondary inflorescences were eliminated as flowers appeared. Untreated plants are already at proliferative arrest, while deseeded and deflowered plants are still active. All lateral branches were removed in all cases. **(B)** Number of reproductive nodes produced by untreated plants at the moment of arrest, and of deseeded and deflowered plants three months after the arrest of untreated plants, when the experiment was terminated. Deseeded plants produced new reproductive nodes at lower pace than deflowered plants, but both deseeded and deflowered plants had not experienced proliferative arrest and were actively growing (indicated by arrow pointing upwards). **(C)** The average length of all internodes for plants subjected to each treatment is represented. Green arrow indicates the average node of floral initiation. Asterisks indicate a significant difference ($P < 0.05$) from untreated plants according to Student's tests (parametric data). Red asterisks indicate significant difference ($p < 0.05$) between deseeded and deflowered plants according to Student's t-test. Cultivar NGB5839, $n = 10$.

up-regulated DEGs in arrested apexes comprised different aspects of abscisic acid responses, as it has been described for Arabidopsis proliferative arrest, as well as the regulation of transcription, which suggests a thorough reprogramming of gene expression associated with the change of the state of the meristem (Figure 6D). All together, these results were consistent with the suggestion that the inflorescence apex undergoing proliferative arrest effectively enters a dormant state.

Although this analysis indicated that most of the transcriptomic changes observed in the three active/arrested comparisons were similar, the analysis performed also allowed us to identify unique GO terms enriched in specific comparisons (Suppl. Table S4; Suppl. Figure 3). For proliferative inflorescences, additional categories related to cell cycle were enriched in active apexes, as well as additional categories related to ABA signaling and sucrose transport in arrested inflorescences. In reactivated inflorescences, ribosomal assembly and translation and glycolytic processes were enriched categories, while the auxin responses were preferentially altered in the arrested inflorescence. Lastly, in deflowered inflorescences, the category jasmonic acid biosynthesis seemed to be a specific enriched category in the active inflorescence.

A second analysis was carried out comparing the transcriptomic changes associated with arrest in pea and in Arabidopsis, using the available data published by Wuest et al. (2016), which only comprised proliferative, arrested and reactivated apexes. For this comparison, we considered DEGs all transcripts that had padj ≤0.05 regardless of log2FC. The behavior of the inflorescence apexes in both species was remarkably similar, with more that 86% of the equivalent transcripts being up- or down-regulated in the same manner when active and arrested apexes were compared (Figure 7).This strong correlation suggested that at the molecular level, the changes induced in the meristem by the entrance in proliferative arrest could be widely conserved, even in distantly related species with different inflorescence architecture, and despite the different physiological and developmental responses of pea and Arabidopsis (influence of position of the fruit respect to the apex, effect of total seed production, mode of meristem termination in the absence of seeds, etc.). We also performed a new GO enrichment analysis with the genes that behaved similarly in both species, as well as with those that showed opposite behavior (Suppl. Table S5). As expected, with the genes that shared similar expression pattern, the enriched terms associated with active (73 GO terms) and arrested (172 GO terms) inflorescences where similar to the obtained when only pea inflorescences were analyzed (Figure 6C,D; Figure 7B,C; Suppl. Table S5), reinforcing

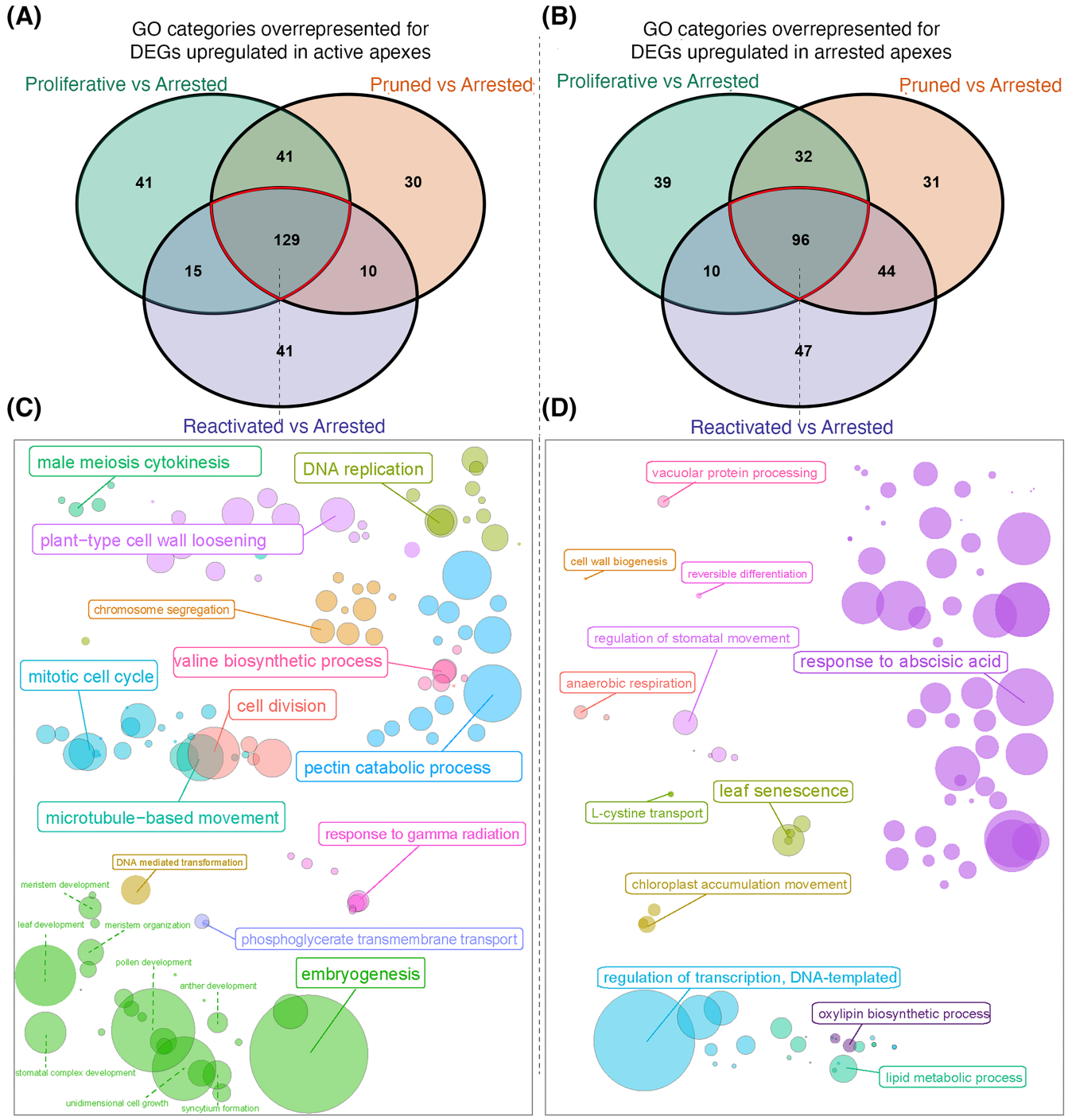


**FIGURE 6** **Differentially expressed genes (DEGs) of active and arrested apexes.** **(A)** Venn diagram representing GO categories enriched in DEGs upregulated in active apexes (equivalent to downregulated at arrest). **(B)** Venn diagram representing GO categories enriched in DEGs upregulated in arrested apexes. Significant DEGs are defined as those with Padj ≤0.05 and − 3 ≤ log2FC ≤3 when active (proliferative, pruned and reactivated) were compared with arrested apexes. **C.** Scatter plot representation of GO categories enriched in upregulated DEGs common to all active states (circled in red in **A**). **(D)** Scatter plot representation of GO categories enriched in upregulated DEGs in arrested apexes common to all comparisons with active states (circled in red in **B**). GO terms derived from the same parental GO term (labelled) are grouped together and represented in the same color. The size of the circles is proportional to the size of the GO term (number of genes annotated in the category).

the idea that molecular changes associated with the end of the reproductive phase could be conserved between these two species. On the other hand, the number of GO terms obtained with the genes presenting opposite patterns were low and represented by just one or two genes per category (Suppl. table S5), with probably limited significance.

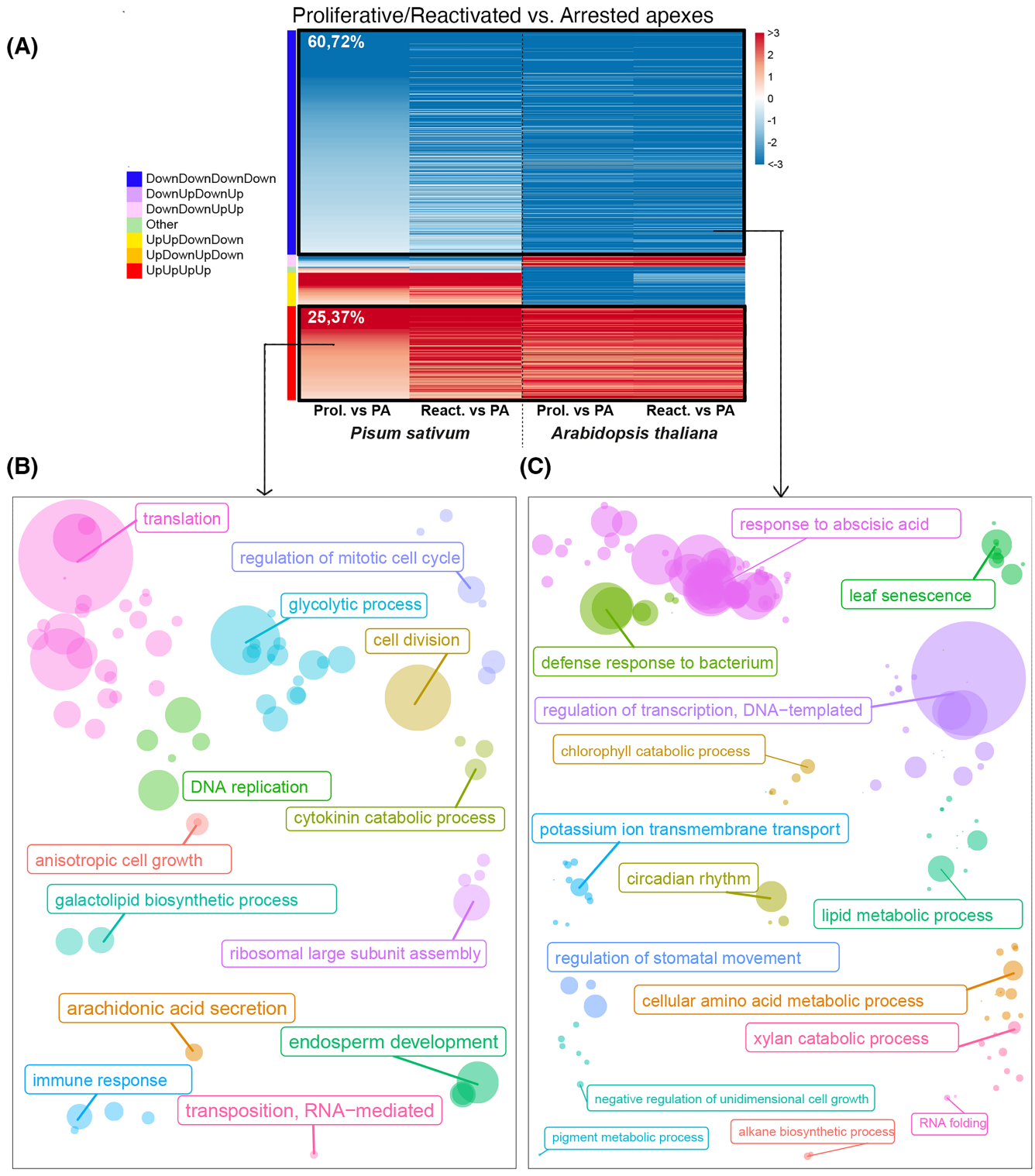


**FIGURE 7** **The transcriptomic changes associated to proliferative arrest are similar between *Arabidopsis thaliana* and *Pisum sativum*.** (**A**) Heatmap representing the expression profiles of Proliferative and Reactivated vs Arrested apexes in *Pisum sativum* and *Arabidopsis thaliana*. TPMs of *Pisum sativum* and TMMs of Arabidopsis for each transcript in each biological replicated have been normalized against arrested apex samples for representation. Significant DEGs are defined as those with Padj ≤0.05. In blue, upregulated genes in arrested apexes, in red, upregulated genes in active (Proliferative/Reactivated) apexes. **(B)** Scatter plot representation of GO categories enriched in the zones delimited by boxes. GO terms derived from the same parental GO term (labelled) are grouped together and represented in the same color. The size of the circles is proportional to the size of the GO term (number of genes annotated in the category).

## 4 | DISCUSSION

The study of the regulation of the end of flowering in monocarpic plants has been rekindled in the last few years, mostly in the model species *Arabidopsis thaliana*. Physiological, molecular and genetic studies are being integrated into an increasingly complex framework to understand the process (González-Suárez et al. 2020; Balanzà et al. 2023; Sadka et al. 2023). However, these studies still need to be extended to other monocarpic species in order to reveal common and divergent regulatory elements, propose general models, and help to develop biotechnological strategies of wide applicability. In this work we have approached this goal by carrying out a comparative study of the end of flowering in pea, evaluating some factors controlling the process previously described in Arabidopsis.

A main conclusion of our work is the unequivocal identification of seeds as the major factor promoting proliferative arrest in pea, confirming previous studies that supported this idea (Lockhart and Gottschall 1961; Malik and Davies 1976; Gianfagna and Davies 1981; Kelly and Davies 1988; Davies and Gan 2012). We have shown how the meristems are instructed to arrest when a certain weight of seeds is produced, which can be interpreted as a required threshold of seed production to switch meristem state. In Arabidopsis a threshold of seed production influencing the timing and mode of arrest has also been proposed (Hensel et al. 1994), but the two species differ on the response of the inflorescence meristem. Our experiments in pea show how the gradual elimination of fruits and therefore seeds proportionally delays the arrest of the meristem: whenever the number of seeds reaches the required level, the meristem stops producing new nodes and terminates forming a typical bud cluster associated to PA. Even in the absence of seeds, the SAM eventually arrests forming this cluster, at least in the genetic backgrounds tested in this work. However, in Arabidopsis, the meristem arrest takes place after the production of a similar number of flowering nodes in plants that produce seed in quite a wide range (from about 35% to 100% of the fully fertile plants). In other words, eliminating up to 60–70% of seed production does not affect the timing of proliferative arrest and only plants below this threshold of seed production extend the reproductive period, ending flower production when the SAM develops into a floral structure instead of a PA-associated bud cluster (Hensel et al. 1994). This observation suggests that in Arabidopsis seed production is relevant but other factors, such as age or other still unknown components, have also a strong influence in promoting the end of the reproductive cycle, while in pea, seeds play a more determinant role in the process. The mode of termination of the meristem is also strikingly different in both species when seed formation is avoided (cluster in pea, terminal structure in Arabidopsis; Hensel et al. 1994; Balanzà et al. 2019). Different hypotheses can be proposed to explain this different behavior, related with the idea of "meristem maturation" proposed by several authors (Murfet. 1985; Lifschitz et al. 2014; Park et al. 2014). According to this, the SAM experiences a progressive reduction in vegetativeness and an increase in a floral promoting program, controlled by the antagonistic action of factors such as florigen/antiflorigens, and the dynamics of this maturation process in different species result in strikingly different inflorescence architectures and determinate/indeterminate growth habits (Lifschitz et al. 2014; Park et al. 2014). Both Arabidopsis and pea have indeterminate inflorescences, and thus the meristem maturation program does not reach its final stage in form of full floral identity, but Arabidopsis forms a simple raceme while pea forms a compound raceme where flowers are not produced directly by the SAM. It is possible that this different inflorescence architecture prevents the maturation of the SAM into a floral structure in pea more effectively than in Arabidopsis. Alternatively, the genetic networks that prevent SAM differentiation in both species might be different. For example, in Arabidopsis, terminal differentiation in sterile *ful* mutants does not occur (Balanzà et al. 2019; Merelo et al. 2022), indicating that there are factors that promote the final stages of meristem maturation that might not exist in pea or could have different activities. In this work we have used three different pea cultivars, and no terminal differentiation was observed in any of them when seed production was avoided, but other authors had described otherwise for other cultivars, so it is possible that these putative factors have variable activities in cultivars adapted to different climatic conditions, as is likely to occur in a crop subjected to extensive breeding programs.

A second relevant conclusion of this study is the assessment of the positional and cumulative effect of developing seeds with respect to the meristem to promote arrest. In Arabidopsis it has been proposed that fruits proximal to the SAM are most effective in promoting inflorescence arrest, and only when the plant has acquired the competence to respond (Ware et al. 2020). Gradual removal of increasing proportions of fruits in the inflorescence does not have a great impact on the duration of flowering unless it is restricted to the later developed apical nodes (Ware et al. 2020). Lateral branches also are able to influence arrest, and in Arabidopsis plants where all lateral branches are removed, the main inflorescence produces many more fruits than untreated plants, but the total fruit production in treated vs. untreated plants is strikingly different, with untreated plants forming 3–4 times more fruits than those where all branches are eliminated, suggesting that distant branches are less effective in triggering arrest because they need to produce many more fruits before arrest takes place (Hensel et al. 1994). In contrast, we have found that in pea distant lateral branches producing fruits are able to trigger the arrest of the main inflorescence meristem when the required amount of seed are produced regardless of the position of the fruits respect to the meristem. This observation also reinforces the more prominent role of the seeds in this species, and also supports strongly the existence of a long-distance mobile signal, the idea of the "death hormone", that would instruct the SAM and would come from the seeds.

Finally, the transcriptomic analysis comparing inflorescence apexes at different stages related to proliferative arrest provides three major outcomes of this work. First, it describes the transcriptional changes associated with arrest, which are clear indicators of the dormancy-like nature of this state, with high ABA signaling, and repression of cell division processes. The strong similarities in gene expression between young proliferative and reactivated apexes are consistent with the reversibility of the state of the arrested apexes, which only 24 h after the seeds have been eliminated, restore the transcriptional program of proliferating inflorescences. Second, the

comparative analysis with Arabidopsis (Wuest et al. 2016) shows how similar the changes in the meristem in these two distant species with different inflorescence architectures are, which supports the general conservation of the events leading to proliferative arrest in monocarpic plants. This conservation is further supported by the recently uncovered role of pea *FRUITFULL* genes in promoting the end of flowering in pea, similar to their ortholog in Arabidopsis (Martínez-Fernandez et al. 2024). Lastly, we describe a simple procedure to improve GO annotation in pea and for cross-species comparative analyses by finding equivalences between pea and Arabidopsis genes based on highest sequence similarity score, a procedure instrumental for this study.

In conclusion, our work reveals strong points of coincidence between the process of proliferative arrest in pea and Arabidopsis, both at the physiological and the molecular level, but also finds important differences in the relative importance of factors triggering the end of flowering in both species. These findings stress the need to characterize the process in more species to establish general models that support and fuel translational biotechnological approaches to crop improvement.

## AUTHOR CONTRIBUTIONS

E.B., V.B., and C.F. conceived the original research plan; C.F., V.B. and J.L.W. supervised the experiments; E.B. performed most of the experiments and analyzed the data, with help of R.O., J.K.V. and I.M.-F.; E.B. and A.B. analyzed the RNAseq experiments; C.F. wrote the article together with E.B. and V.B.

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## DATA AVAILABILITY STATEMENT

The RNA-seq data discussed in this article have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus and are accessible through GEO Series accession number GSE247124.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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