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# Lonely at the top? Regulation of shoot apical meristem activity by intrinsic and extrinsic factors.

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## Abstract

All the above-ground organs of a plant are derived from stem cells that reside in shoot apical meristems (SAM). Over the last 25 years, the genetic pathways that control the proliferation of stem cells within the SAM, and the differentiation of their progenitors into lateral organs, have been described in great detail. However, longstanding questions regarding the importance of communication between cells within the SAM and lateral organs have, until recently, remained unanswered. In this review, we describe recent investigations into the extent, nature and significance of signaling both to and from the SAM.

### Keywords

SAM; protein mobility; intercellular communication; developmental transitions; miRNA mobility

## Introduction

The growth and architecture of a plant shoot depend on the activity of shoot apical meristems (SAMs). These structures are stably maintained by the precisely controlled balance between cell proliferation and differentiation, but are also capable of responding to endogenous and environmental cues that influence their growth and the types of organs they produce. The degree to which the SAM regulates shoot development autonomously, or acts in response to extrinsic factors that originate outside the SAM, is a classic question in plant biology. Half a century ago, Ian Sussex [1] asked: ‘are … meristems to be considered as organizer regions whose functional changes represent changes initiated within the meristem itself, or are they simply plastic regions in which new cells are molded into organs and tissues in response to stimuli proceeding from other sources?’ At the time, data from microsurgical, shoot culture, and hormone treatment studies suggested that the SAM was largely autonomous of neighboring tissues and organs. However, with the benefit of modern

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Conflict of Interest

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molecular tools, it has become apparent that apical meristems typically function as signaling integrators, coordinating cues from elsewhere in the plant and from the environment.

The structure and activity of the SAM, and the regulatory networks that determine these features, have been reviewed extensively recently [2,3]. SAMs possess several distinct functional domains (Figure 1). Stem cells—which divide to produce additional stem cells as well as cells that will differentiate—reside within the central zone (CZ). Cells derived from the CZ are displaced laterally to the peripheral zone (PZ), where they differentiate into lateral organs, or basally to the rib zone (RZ), where they differentiate into cells of the stem. Stem cell proliferation within the SAM is maintained by the activity of members of two distinct families of homeobox gene: *WUSCHEL-LIKE* (*WOX*) and *KNOTTED-LIKE* (*KNOX*). In *Arabidopsis*, *WUSCHEL* (*WUS*) is expressed in the 'Organizing Center' of the CZ (Figure 1), where it promotes the division of stem cells. The expression domain of *WUS* is restricted by the activity of the signaling peptide *CLAVATA3* (*CLV3*), which regulates *WUS* in a negative feedback loop. Therefore loss-of *CLV3* function leads to an expansion of the SAM, whereas loss-of *WUS* function leads to meristem termination. The *Arabidopsis KNOX* gene *SHOOT MERISTEMLESS* (*STM*) is expressed more broadly in the SAM, and acts to maintain stem cells in an undifferentiated state. Both *WUS* and *STM* are necessary for meristem maintenance throughout the ontogeny of individual meristems, and within different developmental contexts, i.e. SAMs in the vegetative and reproductive phases of a plant life cycle. In this review we detail how core developmental processes in the SAM are influenced by extrinsic genetic and metabolic factors, describe examples of environmental signals that are perceived both within and without the SAM, and provide an update on a longstanding hypothesis regarding extrinsic signaling by the SAM on lateral organs.

## Regulation of developmental transitions by extrinsic factors

The best described example of an extrinsically regulated change in the activity of the SAM is floral induction (Figure 2A). It has been known for many years that inductive photoperiods trigger production of a mobile 'florigen' in leaves, which moves to the SAM to initiate flowering [4]. This signal is now known to be a small protein encoded by the *FLOWERING LOCUS T* (*FT*) gene. FT moves from leaves to the SAM via the phloem [5–8], where it interacts with the locally expressed bZIP transcription factor FLOWERING LOCUS D (FD) to induce the transition from a vegetative to an inflorescence meristem [9,10]. Recently it was shown that FT enhances, but is not necessary, for the binding of FD to its targets and that phosphorylation of FD, presumably by two calcium-dependent kinases, is required for this interaction [11,12]. It has also been suggested that leaves regulate floral induction at the SAM via the hormone, gibberellin (GA) [13,14], a hypothesis that is supported by the identification of GA-transporters and mobile forms of GA [15,16]. Although there is considerable evidence that GA regulates flowering time, whether GA acts in the leaves or in the SAM, as well as the functional significance of mobile GA, is still unclear because GA synthesized within the SAM can also affect flowering time [17].

Communication between leaves and the SAM is also important for the regulation of the transition between the juvenile and adult phases of vegetative growth (vegetative phase change) [18]. Traditionally, it was thought that the phase identity of the vegetative shoot (i.e.

juvenile versus adult) was determined by factors operating endogenously within the SAM [19]. However, more recent work has shown that this transition is promoted by signaling from pre-existing leaves, via repression of the master regulator of vegetative phase change, miR156 [20]. This leaf-derived signal consists, at least in part, of carbohydrates because exogenous application of glucose delays vegetative phase change, represses miR156 expression, and compensates for the loss of leaf primordia [21,22] (Figure 2A). Evidence that leaves regulate the activity of the SAM is also provided by the finding that a leaf-expressed miR156-resistant version of SPL9 is able to regulate the rate of leaf initiation in the SAM [23], although whether the endogenous SPL9 protein moves intercellularly within the shoot apex remains to be demonstrated.

Conversely, recent work has shown that the SAM is important for the specification of leaf identity early in shoot development [24**]. In *Arabidopsis*, constitutive expression of miR156 within the SAM regulates leaf identity and represses its direct targets in leaf primordia. These observations suggest that this is because miR156 is able to diffuse from the SAM into leaf primordia [24**]. These and the results of other region-specific gene expression experiments suggest that the control of vegetative identity shifts spatially from the SAM to leaves over time. More generally, while miRNA movement within the SAM regulates core genetic networks [25*,26], the extent of miRNA mobility across the shoot apex appears strictly controlled [27**].

## Regulation of SAM size and maintenance by extrinsic factors

### Short-range signaling

As described in the introduction, the SAM is initiated and maintained by transcription factors (WUS and STM) that are expressed exclusively within the SAM. The distribution and activity of these transcription factors is regulated by molecules that are produced locally within the SAM, and by molecules produced by surrounding organs and tissues. Several of these molecules act to repress the growth of the SAM. For example, although a basal level of auxin signaling is required for the maintenance of the SAM [28*], a combination of experimental approaches and mathematically modelling suggest that the growth of the meristem is repressed by auxin produced by lateral organs [28**] (Figure 2B). As lateral organs are stronger auxin sources than the SAM, it is predicted that basipetal auxin flow from leaves competitively inhibits the export of auxin from the SAM, and restricts meristem size [29**]. This model is supported by analyses of *yabby* (*yab*) mutants, which non-autonomously increase meristem size [30], possibly because they reduce the export of auxin from leaves [29**]. In maize, there is evidence that leaf primordia non-autonomously repress the growth of the SAM through their production of the CLV3-like peptide, FCP1. This conclusion is based on *in situ* data suggesting that *FCP1* is expressed in leaf primordia, and the observation that expressing *FCP1* specifically in leaf primordia reduces *WUS* expression and represses the growth of the SAM [31]. However, a transcriptomic survey of the maize SAM has found evidence for *FCP1* expression within the CZ, indicating it likely functions at least in part SAM-autonomously [32*]. A third example is provided by the diffusible miRNAs, miR165/miR166, which repress the expression of a group of HD-ZIP III transcription factors essential for the specification of the apical domain of the embryo and

the initiation and maintenance of the SAM [33,34]. miR165/miR166 are expressed in cells surrounding the SAM, but are capable of moving into the SAM and repressing HD-ZIP III expression in this domain [35,36]. They are prevented from doing so by ARGONAUTE10 (AGO10) [34], which is expressed in the SAM and provasculature [37,38] and specifically sequesters miR165/miR166 [39], causing them to become hyper-susceptible to degradation by SMALL RNA DEGRADING NUCLEASE family members [40]. Thus, meristem activity is exogenously maintained by the coordinated degradation of specific miRNAs.

In contrast to these examples of negative regulation of the SAM by lateral organs and tissues, a role for lateral organs in promoting meristem maintenance has recently been described in the liverwort *Marchantia polymorpha* [41**]. Naramoto *et al* identified the gene *LATERAL ORGAN SUPRESSOR1* (*MpLOS1*) in a mutant screen for regulators of organogenesis. *Mplos1* mutants produce unusual green outgrowths in place of transparent scales and, in addition, fail to maintain an apical meristem. *MpLOS1* expression is restricted to lateral organs and the MpLOS1 protein does not appear to move into the apical meristem, suggesting that MpLOS1 promotes meristem maintenance non-cell autonomously. Whether it does so indirectly—by promoting the differentiation of lateral organs—or in a more direct fashion, remains to be determined. Interestingly, homologs of *MpLOS1* in angiosperms are also important for the differentiation of lateral organs and, in eudicots at least, their expression domain is restricted to the flanks of the SAM [42–44]. Although it is beyond the scope of this review, it should be noted that meristem boundaries are critical for isolating proliferating stem cells from differentiation programs in lateral organs, and are required for SAM establishment and axillary meristem formation [reviewed by 45,46].

## Long range signaling

The plant hormone cytokinin (CK) maintains the growth of the SAM by promoting the expression of the transcription factor, *WUS*, via *CLV*-dependent and independent mechanisms. Although mathematical models suggest that CK biosynthesis and signaling within the SAM are sufficient for its function [47–49], the transport of CK precursors from the root has also been shown to affect *WUS* expression and SAM size [50**,51] (Figure 2B). In *Arabidopsis*, acropetal transport of CK-precursors requires the activity of the ATP-binding cassette transporter *ABCG14* in the root [52,53]. Root-derived *trans*-zeatin and *trans*-zeatin riboside both affect leaf development, but only *trans*-zeatin riboside is able to regulate the activity of the SAM [51]. Movement of CKs up the shoot is dependent on nitrate levels in the soil, providing a potential mechanism by which shoot growth can be regulated by nutrient availability [50**,54].

Additional evidence that root signals regulate the activity of the SAM is provided by mutations in the gene *BYPASS1* (*BPS1*), which terminate the growth of the SAM by diminishing CK signaling in the SAM and repressing *WUS* expression [55]. The exact mechanism of this effect is still unknown, but it has been attributed to a mobile, root-synthesized signal [56] that is dependent on carotenoid biosynthesis [57]. Unknown seed-derived signals, in combination with an endogenous age-dependent pathway, also lead to the termination of apical growth through repression of *WUS* activity [58*,59].

## Intrinsic and extrinsic environmental regulation of SAM activity

In nature, plants modify their growth and development to adapt to varying environmental conditions. Although many of these responses involve changes in the activity of the SAM (e.g. the timing of developmental transitions, the rate of leaf initiation, branching patterns), it is usually unclear if the environmental stimulus is perceived directly by the SAM, or perceived elsewhere in the plant and transmitted to the SAM. For example, cold-induced repression of the floral suppressor *FLC* occurs in both the SAM and leaves [60], while the mechanism for cold-induced SAM-termination in *Brassica oleracea* seedlings is unknown [61].

The best characterized examples of exogenous regulation of SAM activity concern the effects of light. The effects of light on the activity of the SAM can be partitioned into direct light-signaling effects and those mediated by the derivatives of photosynthesis [62]. The proliferation of the SAM, as well as changes in its pattern of differentiation, require both and —when deprived of light and sugar—meristematic growth, organogenesis, and developmental transitions are severely affected. As noted above, light (specifically, photoperiod) regulates flowering through its effect on the synthesis of FT in leaves. In addition, low red:far red light ratios, which recapitulate shade conditions, promote the expression of *FT* [63]. More generally light, and metabolic pathways dependent on light, regulate the activity of the SAM through their effects on cytokinin signaling and the TARGET OF RAP (TOR) kinase complex, which promote *WUS* expression [62,64–66] (Figure 2B). Light perception appears to take place in the leaves, rather than the SAM, as transgenic activation of light signaling, or exposure to high light in leaves (but not the SAM), promotes cell proliferation in the shoot apex [62,65]. However, the identity of the putative light-induced factor that non-cell autonomously regulates meristem development is unknown. In terms of sugar signaling, *WUS* activity requires metabolizable sugars, suggesting that sugars function as an energy source rather than as signaling molecules [62]. Although the SAM does not appear to be photosynthetically active [62,67], the sucrose-signaling regulator *TREHALOSE-6-PHOSPHATE SYNTHASE1* is expressed within the SAM [68,69]. Therefore, despite depending on extrinsic sources of sugar, the SAM can regulate sugar-signaling networks intrinsically.

It has recently been demonstrated that SAM-activity is dependent on the endogenous perception and regulation of its own oxygen environment. Oxygen is excluded from the CZ, which limits the oxygen-dependent proteolysis of the HD-ZIP III interactor LITTLE ZIPPER 2 [70**]. A general role for oxygen derivatives in the regulation of the SAM is supported by the mutant phenotypes of SAM-expressed redox regulators [71–73], and the non-uniform accumulation and effects of reactive oxygen species within the SAM [74]. Localized temperature manipulation experiments in cucumber suggest the SAM may also interpret temperature signals to regulate leaf initiation [75].

## Extrinsic regulation by the SAM?

A classic hypothesis in plant biology is that the SAM non-cell autonomously regulates dorsoventral patterning in leaves [76]. Based on microsurgical experiments in potato and

tomato, it has been proposed that a SAM-derived signal is required to correctly induce leaf dorsoventrality [76,77]. However, evidence from laser-ablation of cells in the SAM has recently led to an alternative interpretation of these microsurgical experiments [78**]. Caggiano *et al* demonstrated that wounding disrupts the adjacent expression domains of dorsoventral specificity factors *REVOLUTA* (*REV*) and *KANADI1* (*KAN*) in the SAM, and the boundary of auxin signaling that separates them. In accordance with previous hypotheses [79,80], the authors propose that dorsoventral patterning of leaves does not require a mobile signal from the SAM, but instead maintenance of boundaries between key regulators during leaf initiation. It has also been suggested that dorsoventral patterning is dependent on the flow of auxin from the dorsal side of leaf primordia to the SAM [81,82]. Whether or not asymmetric auxin signaling establishes leaf polarity, and how to test this experimentally (particularly with regard to genetic auxin sensors), have recently been of some debate [83,84].

## Conclusion and outlook

Although the function of the SAM depends on integrating extrinsic signals, the core regulatory networks that coordinate stem cell proliferation appear to operate with minimal external input. The relative independence of these networks is demonstrated by the limited range of signals to which they are susceptible (Table 1). Plant hormones, microRNAs, sugars and small proteins are all able to extrinsically regulate SAM activity, whereas there is little support for extrinsic regulation by larger proteins. The extent to which external factors can regulate the activity of the SAM may therefore depend on their size, which could determine their ability to enter the SAM. Smaller molecules may be able to travel symplastically from the vasculature to the SAM, but this may be impossible for larger proteins. On the other hand, transcription factors such as WUS and STM move readily within the SAM [85,86], suggesting that once inside the SAM intercellular movement of larger proteins is less restricted.

Regardless of how transport into the SAM is regulated, the exclusion of sRNAs and proteins that could lead to the mis-expression of SAM regulators is critical to ensuring balanced meristematic growth. Understanding the cellular and spatial mechanisms by which access to the SAM, and to subdomains within the SAM, is regulated is an important avenue of future research. Recent transcriptome [32*] and translatome [87*] maps of domains in the shoot apex make it easier to identify localized patterns of gene expression, and facilitate predictions about whether genes function non-cell autonomously across the shoot apex. These predictions can be tested using domain specific-expression approaches [24**,27**]. Such techniques will also be useful in elucidating the contribution of the SAM to the perception and interpretation of environmental signals.

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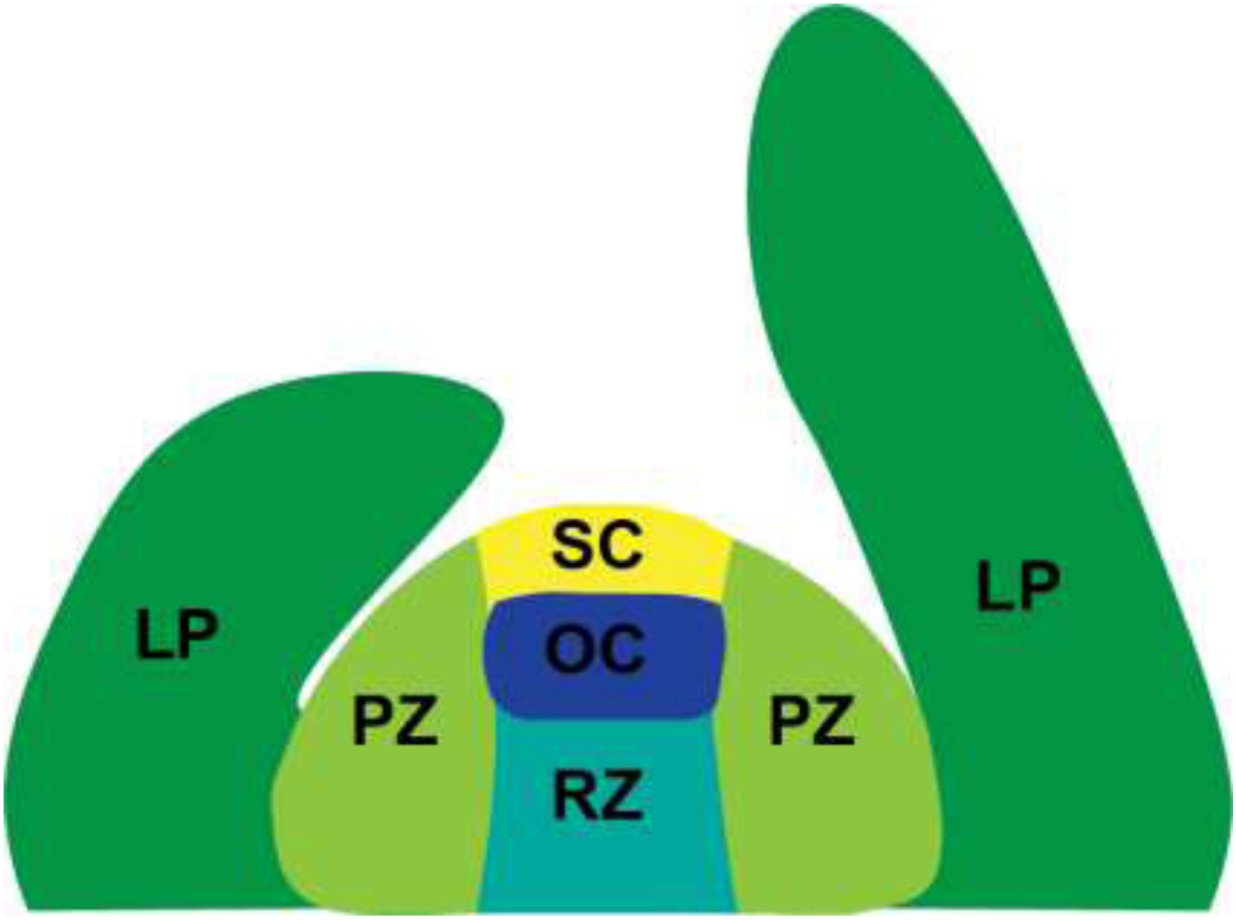


**Figure 1. Functional domains of the SAM**

LP – leaf primordia, PZ – peripheral zone, RZ – rib zone, OC – organizing center, SC – stem cells. The SC and OC together define the SAM central zone (CZ). *WOX* gene family expression (e.g. *WUS*) is associated with the OC, whereas *CLE* family expression (e.g. *CLV3*) is associated with SC.

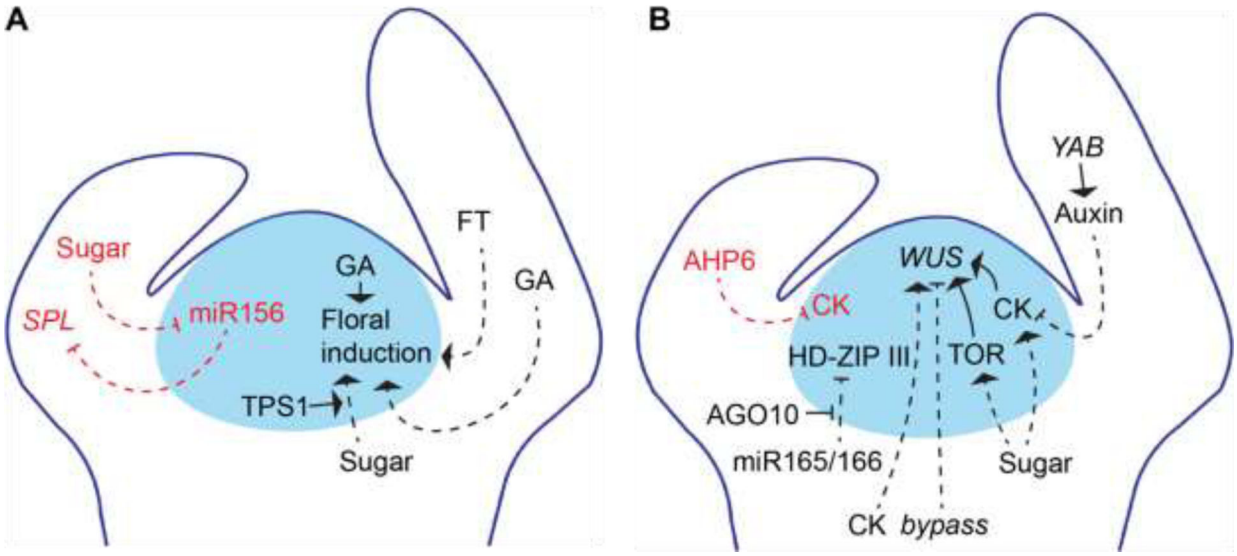


**Figure 2. Mobile regulators in the shoot apex**

Simplified genetic networks regulating A) Vegetative phase change (red) and floral induction (black), B) Phyllotaxy (red) and meristem maintenance (black). Arrows represent positive regulation, flat lines represent negative regulation, blue circle represents the SAM (vegetative in A, vegetative or inflorescence in B), dashed lines depict movement into the SAM.

## Table 1

A summary of the plant-derived and environmental signals that regulate SAM growth. Unless specified, gene products encoded by *Arabidopsis* loci.

| **Molecular signal** | **Direction** | **Effect** | **References** |
|---|---|---|---|
| *Protein* FT | From leaves to the SAM | Induction of flowering | [5–8] |
| SPL9 | From leaves to the SAM | Repress leaf initiation | [23] |
| AHP6 | From leaves to the SAM | Reinforce phyllotactic patterning | [88] |
| MpLOSI | From lateral organs to the apical meristem | Promotes meristem maintenance | [41] |
| ZmFCPI | From lateral organs to the apical meristem | Limit SAM size | [30] |
| *miRNA* miR156 | From the SAM to leaves | Repress adult identity | [24] |
| miR165/miR166 | From provasculature into the SAM | Repress SAM growth (butnormally excluded) | [35,36,39] |
| *Hormone* GA | From leaves to the SAM | Induction of flowering | [13,14] |
| Auxin | From leaves to the SAM | Induce dorsoventral patterning in leaves | [78] |
| | From leaves to the SAM | Limit SAM size | [29] |
| CK | From roots to the SAM | Promote SAM growth in response to nitrate | [50,51] |
| *Nutritional* Sugar | From leaves to the SAM | Induction of vegetative phase change | [21,22] |
| | Into the SAM | Sugar is required for SAM proliferation | [62,65] |
| *Unknown bypass* | From roots to the SAM | Represses SAM growth | [55,56] |
| Seed-derived | From seed to the SAM | Terminate flower production | [58,59] |
| *AhPHAN*- dependent | From leaves to the SAM | Promote SAM maintenance | [89] |
| **Environmental signal** | **Site of perception** | **Effect** | **References** |
| Light (quantity) | Leaves | Low light delays production of adult traits | [90,91] |
| | Leaves | Light is required for SAM proliferation | [62,64,65] |
| Light (quality) | Leaves | Shade promotes flowering and represses leaf initiation | [63,92,93] |
| | Leaves | Shade promotes SAM size | [94] |
| Temperature | Shoot apex | Leaf initiation rate increases with (sub-maximal) temperature | [75,95] |
| | Unknown | Cold temperatures inhibit SAM growth | [61] |
| | Leaves and SAM | Induction of flowering by vernalization | [60] |
| Oxygen | SAM | Low oxygen concentrations promote SAM activity | [70] |
| | SAM | Reactive oxygen species regulate stem cell maintenance | [74] |