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REVIEW

SPATULA as a Versatile Tool in Plant: The Progress and Perspectives of SPATULA (SPT) Transcriptional Factor

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ABSTRACT

With the rapid development of modern molecular biology and bioinformatics, many studies have proved that transcription factors play an important role in regulating the growth and development of plants. SPATULA (SPT) belongs to the bHLH transcription family and participates in many processes of regulating plant growth and development. This review systemically summarizes the multiple roles of SPT in plant growth, development, and stress response, including seed germination, flowering, leaf size, carpel development, and root elongation, which is helpful for us to better understand the functions of SPT.

KEYWORDS

SPT; bHLH; plant growth; plant development; *Arabidopsis thaliana*

1 Introduction

SPATULA, as an important class of bHLH transcription factors, can combine light signals, auxin signals, and gibberellin (GA) signals to participate in various stress responses and thus regulate many processes of plant growth and development [1–3]. In addition to regulating pistil development, the Arabidopsis *SPT* gene has roles in organs such as cotyledons, leaves, roots, and fruits, and also plays a role in seed germination [4–9]. Thus, the *SPT* is involved throughout the plant life cycle. With global warming and the constant destruction of the earth, the outer environment has been altered, which thereby affects the growth and development of plants. Therefore, it is very essential to study the role of transcription factor SPT in plant growth and development.

SPT is a transcription factor encoding a family of bHLH proteins that play an essential role in plant growth and development [10]. Transcription factors, also called trans-acting factors, contain many functional structural domains, among which the DNA binding domain plays a vital role in regulating the transcription activity of genes [11]. The activity and function of transcription factors are also regulated by these functional domains. A transcription factor contains at least one DNA recognition site, also known as a motif, the transcription factor binds to a specific DNA sequence of the target gene through this site, thereby improving or repressing the transcriptional activity of the gene [12,13]. In turn, the expression of a gene is often co-regulated by multiple transcription factors.

The bHLH transcription factor contains a basic region and a helix-loop-helix region. The basic region is rich in basic amino acids and binds to the E-box (CANNTG), and the helix-loop-helix region contains two amphiphilic a-helices and a hydrophobic loop [14–16]. The hydrophobic loop is the key factor in this process that the bHLH transcription factors function by forming homo-or heterodimers with other proteins. The bHLH transcription factor was first identified in the E4 and E12 transcription factors of the mouse, whereas in plants, the study of bHLH transcription factor has been slower, and a total of more than 500 bHLH proteins, which are classified into 26–32 subfamilies, and members of the same family of transcription factors are often functionally redundant [17,18]. In addition, bHLH proteins play essential roles in signal transduction, hormone synthesis, as well as the plant's resistance to abiotic stresses [19–21].

2 *SPT* Is Involved in the Regulation of Seed Germination

Seed dormancy and germination are significant stages in the process of plant growth and development, it was shown that *SPT* is also involved in regulating the germination of newly harvested seeds [1]. In *Arabidopsis*, the germination of dormant seeds is controlled by light and low temperatures [22]. This signaling pathway is regulated by the gibberellin oxidase GA3ox. GA3ox regulates gibberellin biosynthesis and it plays a vital role in the process of gibberellin biosynthesis [23]. Seed dormancy is the inability of viable seeds to germinate for intrinsic reasons despite suitable ecological conditions [24,25]. GA is necessary for seed germination and its application breaks seed dormancy. Bioactive GA content in seeds is also regulated by brightness and cold [26–28]. SPT, a multifunctional transcription factor, plays a vital role in light-and temperature-controlled seed germination by regulating the transcription of GA oxidase GA3ox and controlling the seed response to cold stratification. In the absence of light and low-temperature stratification, newly harvested wild-type Ler seeds exhibited dormancy, but the mutant *spt-10*, in contrast to Ler, showed higher germination. These researches suggest that *SPT* is involved in the regulation of seed germination. By RT-qPCR analysis of the expression level of *GA3ox1* and *GA3ox2* in the Ler ecotype and *spt-10* mutant, it was found that there was a remarkable increase in the expression level of *GA3ox1* and *GA3ox2* in the *spt-10* mutant as compared to the Ler ecotype [1]. These results suggest that *SPT* negatively regulates seed germination by negatively regulating the expression of *GA3ox1* and *GA3ox2*.

Freshly harvested seeds usually exhibit initial dormancy, which severely affects seed germination, which is co-regulated by a balance of phytohormone ratios and environmental factors, including abscisic acid (ABA) and gibberellin (GA) [29]. ABA transcription level is regulated by the ABA transcription factors ABI3, ABI4, and ABI5 [30–33]. *SPT* plays a vital role in seed germination, however, it has been proved that *SPT* exhibits opposite regulatory effects on seed germination in two different ecotypes, Col-0 and Ler. By observing the phenotypes of seed germination of the *spt* mutant and overexpression materials in the Col-0 and Ler ecotypes, it was found that the *SPT* overexpression plants promoted seed dormancy in the Col-0 ecotype, but *SPT* inhibited seed dormancy in the Ler ecotype. The results showed that the mutant *spt-2* in the Ler ecotype exhibited less seed dormancy than the wild type under conditions of unstratified low-temperature stratification, but the mutant *spt-11* had higher germination rates than the Col-0 ecotype [34]. These results illustrate the importance of different ecotypes for *SPT* regulation of the seed germination process. This physiological phenomenon can be explained by the regulation of gene expression by *SPT*. Evaluation of the abundance of transcripts of genes related to ABA and GA biosynthesis during seed germination proved that the expression level of ABA1 was notably increased in *spt-2* (Ler), but the *ABA1* expression level of the mutant *spt-12* was lower than that of Col-0. This also well explains the contrary regulatory effects of *SPT* on seed germination in Col-0 and Ler. It has been proved that SPT can bind to the G-box (CACGTG) on the promoter sequences of target genes and thus regulate their expression level [35–37]. Further analysis by ChIp and RT-q-PCR to confirm whether SPT binds specifically to the promoters of the target genes proved that only *ABI5*, *REPRESSOR OF GA*

(*RGA*), and *MOTHER OF FT AND TFL1* (*MFT*) were enriched in G-Box specific amplicons, on which suggests that SPT could bind to the promoter of *MFT*, *ABI5*, and *RGA*. Moreover, SPT promotes the expression of the *ABI5* gene, while the expression of the *RGA* and *MFT* gene was suppressed by *SPT*. This physiological process takes place in two different ecotypes, Col-0 and Ler, resulting in converse seed dormancy phenomena in Col-0 and Ler.

In addition, *MFT*, as a phosphatidylethanolamine-binding protein, has been shown to play a vital role in multiple growth signaling pathways in plants. In wheat, cold-induced up-regulation of *MFT* results in strong seed dormancy [38,39]. Similarly, in *Arabidopsis, MFT* also promotes seed dormancy. It was shown that under far-red light conditions, the expression of *SOM* was promoted by PIF1, which led to the increase of GA. The ABA level was notably decreased in the *mft* mutant compared with the wild type under far-red light conditions, suggesting that the inhibitory effect of *MFT* on seed germination acts downstream of ABA, which also implies that *MFT* inhibits seed germination under far-red light by positively regulating ABA signaling. The transcription level of *MFT* was notably increased under far-red light conditions and was lower in the *pif1-1* mutant than in the wild type, which also implies that far-red light promotes *MFT* expression through the PIF1 signaling pathway. However, the expression level of the *MFT* gene was found to be up-regulated in the *spt-2*, which indicated that *SPT* repressed the expression of *MFT*. Meanwhile, under far-red light conditions, the expression level of *SPT* was extremely reduced in the Col-0 ecotype, but the expression level of *SPT* was remarkably increased in the *pif1-1* mutant, which implies that far-red light inhibits the expression of *SPT* through the PIF1 pathway [22].

3 *SPT* Is Involved in the Regulation of Flowering Time in *Arabidopsis thaliana*

The transition of plants from nutrient growth to reproductive growth is co-regulated by both external and internal factors, such as light, temperature, and plant hormones [10,40]. *CONSTANS* (*CO*) and *Flowering Locus C* (*FLC*) were found to be involved in the regulation of flowering time in Arabidopsis [41,42]. *CO* regulates photoperiodic flowering by binding to the promoter of *FLOWERING LOCUS T* (*FLT*) through the TGTG (N2-3) ATG motif. *FLC* inhibits flowering by binding to the promoter of the *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) and inhibiting its transcriptional activity [43–45].

In addition, photoperiod is also involved in regulating the flowering process of plants [46]. At least three classes of photoreceptors are known to exist: photoreceptors: sensing red and far-red light, cryptochromes and phototropins as well as proteins such as the ZTL family, which sense blue and near-ultraviolet light, and UVR8, which belongs to the ultraviolet B (UV-B) receptor and senses the UV-B region [47–49]. Among them, phytochromes play a vital role in regulating plant growth [50]. *PHYB* is one of the phytochromes family and is involved in regulating plant's response to light [51,52]. SPT, as a multifunctional transcription factor, also plays a vital role in regulating the flowering process in *Arabidopsis thaliana* [7]. It was shown that the *SPT* overexpression plant has the phenotype of long hypocotyls, whereas *phyb* mutants likewise exhibit long hypocotyls, suggesting that *SPT* also plays a part in the regulation of photomorphogenesis by *PHYB* [1]. It was shown that *PHYB* participates in the process of *SPT* regulating flowering time and *PHYB* is also involved in the process of *SPT* response to light [7]. Under long-day conditions, the *SPT* overexpression plant promoted flowering compared with the Ler ecotype, and conversely, the *spt* mutant suppressed flowering, and the *phyb* mutant promoted flowering. However, in the *spt/phyb* mutant, flowering time was intermediate between the *spt* mutant and the *phyb* mutant. This implies that the regulation of the flowering process by *SPT* is dependent on *PHYB*. By the RT-qPCR analysis of the transcription level of key flowering-related genes (*FLC*/*SHORT VEGETATIVE PHASE* (*SVP*)/*FT*/*SOC1*), among which *FT* and *SOC1* promoted flowering, while *FLC* and *SVP* inhibited flowering. The results showed that in the *spt-2* mutant, the transcription level of the *FLC* and *SVP* were both remarkably higher than those of the Ler ecotype, whereas those of the *FT* and *SOC1* were notably lower than those of the Ler ecotype. Conversely, in the *phyb* mutant, the transcription level

of the *FLC* and *SVP* was extremely lower than those of the Ler ecotype, whereas the transcription level of the *FT* and *SOC1* was remarkably higher than those of the Ler ecotype. These results suggest that *SPT* regulates the flowering process of plants by modulating the expression level of key flowering-related genes, which is dependent on *PHYB*.

4 *SPT* Is Involved in the Regulation of Carpel Development

The morphogenesis of plants is regulated by a cascade of different genes. Pistils are determined by the transcription factors AGAMOUS (AG) and SEPALLATA (SEP) [53]. The development of specific tissues at the marginal placentation is regulated by specific genes such as *LEUNIG* (*LUG*), *AINTEGUMENTA* (*ANT*), *NO TRANSMITTING TRACT* (*NTT*), *SHI RELATED SEQUENCE 1* (*STY1*) and *SHI RELATED SEQUENCE 2* (*STY2*) [54–56]. In addition, *SPT* is also involved in the regulation of carpel development in *Arabidopsis*, and its expression was observed in both margin and pollen tract tissues throughout *Arabidopsis* pistil development, implying that SPT promotes the growth of tissues at the margins of the carpels, such as septa, styles, and stigmas [5]. When *SPT* is mutated, it leads to disruption of the septa and transmission bundles of the style, resulting in impaired development of the carpels [4]. The SPT protein contains two structural domains: an acidic structural domain and an amphipathic helix. SPT requires an acidic structural domain to mediate carpel development [57,58]. To investigate the function of *SPT* in pistil and fruit development, structural domains were truncated or deleted from transgenes inserted into the *spt* mutant. The results showed that the fruit pods of the *spt-2* mutant were notably shorter and set few seeds compared with Ler, but in the *SPT* overexpression plant, the fruit pods were almost as same as those of the Ler ecotype. This implies the importance of *SPT* in pistil development [59]. For example, *STY2* is one of the genes promoted by *SPT*, but when *SPT* was mutated, *STY2* was still expressed in the style, suggesting that it may be regulated in conjunction with other proteins [56].

The function of *SPT* is also regulated by the level of auxin inside the pistil. It was shown that in *Arabidopsis*, the formation of stigma, style, ovary, and the short stem from the tip to the base of the pistil is regulated by the concentration of auxin [60]. When the auxin transport at the top of the growing pistil is damaged, the number of styles and ovaries will be reduced. However, in contrast to Col-0, all but the apical portion of the pistil was not affected by the damaged polar transport of auxin in the *spt* mutant, suggesting that *SPT* is also involved in the polar transport of auxin from the apical to the basal portion of the pistil.

It was shown that *SPT* is regulated by the *AUXIN RESPONSE TRANSCRIPTION FACTOR 3* (*ETT*), which senses the concentration of auxin in the pistil, and that the marginals between the style and ovary as well as between the ovary and pistil has been determined by *ETT* [61]. When *ETT* is mutated, gynoecium development is also affected; however, in *spt/ett* double mutant plants, gynoecium development is significantly less affected compared with Col-0, implying that *ETT* function is *SPT*-dependent [62].

In addition, the fusion of marginal tissues during gynoecium development is also influenced by gene interactions. It has been shown that *SPT* is required for carpel development, but the promotion of carpel development by *SPT* is hampered by *CUC1* and *CUC2*, *CUP-SHAPED COTYLEDON* genes encoding NAC transcription factors [63,64]. *SPT* promotes carpel fusion at the pistil tip by negatively regulating *CUC1* and *CUC2* [65]. By constructing *CUC1* and *CUC2* promoter-driven GUS vectors and observing their GUS expression positions, it was found that in wild pistils, strong GUS activity was detected on the adaxial side of the medial region, but in *spt* mutants, strong GUS activity was not detected on the adaxial side, implying that the *SPT* is involved in the modulation of *CUC2* promoter activity, which leads to accumulation of its mRNAs on the abaxial side through the modulation of the transcription of *CUC2*.

In addition, *SPT*, *CUC1*, and *CUC2* are involved in ovule formation in the ovary. This also implies a synergistic role of *SPT* and *CUC1*/*CUC2*, and *SPT* expression was not observed in *CUC1/CUC2* mutants,

which also implies that SPT plays a role in the downstream of *CUC1*/*CUC2* [4,66]. The effect of *SPT* on *CUC1*/*CUC2* expression was also ectopic, in the apical region of the pistil, the GUS staining activity of *CUC1* and *CUC2* was inhibited by SPT, but in the basal region, *SPT* did not modulate the GUS staining of *CUC1*/*CUC2*, instead *SPT* and *CUC1*/*CUC2* acted synergistically to promote the formation of carpel margin-derived organs.

SPT could also interact with red/far-red light reversible photo-receptors and participate in photo-regulatory processes [10]. It was shown that *SPT* is also closely related to photo-regulatory processes regulated by *PHYB* and *PIF*s. For example, the phenotype of the *spt* mutant in pistils was inhibited in the *spt-11*/*phyb-9* double mutant [67]. In contrast, the *SPT* overexpression plant disrupted light signaling in seedlings and promoted hypocotyl elongation, and the *phyb* mutant also exhibited longer hypocotyls than the Ler ecotype [1]. *SPT* inhibits seed germination by suppressing the transcription of the gibberellin oxidase GA3ox, which is consistent with *PIF1*. These growth responses are caused by low red/far-red light ratios, which characterize plant shading [68]. SPT was shown to be a photo-regulatory transcription factor and shares several specific target genes with other photo-regulatory transcription factors such as the PIF transcription factor, which are involved in the regulation of carpel development [69]. For example, *SPT* plays a vital role in carpel development in *Arabidopsis* through the activation of genes associated with shade avoidance, such as *ATHB2* and *ATHB4*, which promote cell elongation and are mainly involved in the regulation of shade response and carpel development [4,70,71]. Further studies revealed that the original pistil-deficient phenotypes of the *spt-2* mutant and *spt-11* mutant were restored under low-red/far-red light conditions compared with the wild type, which also implies that the process of *SPT* regulation of carpel development was related to the plant shade avoidance response. In addition, there is also a genetic relationship between *SPT* and *PHYB*, and by observing the pistil development of the *spt-2* and the *phyb* mutant, it was found that the *spt-2* mutant exhibited a significant defect in carpel development, but this defect was significantly ameliorated in the *spt-2*/*phyb* mutants. Similarly, by analyzing the pistil development phenotypes of the *spt-11* mutant, the *spt/pif4-101* mutant, the *spt/pif5* mutant, and the *spt/pif4-101/pif5* mutant, it was found that under low-red/far-red light conditions, the *spt-11* mutant and the *spt/pif4-101* mutant, as well as the *spt/pif5* mutant, could be recovered to the normal gynoecium developmental phenotype, but the gynoecium developmental phenotype of the *spt/pif4-101/pif5* mutant did not, which implies that the PIF4 and PIF5 are also involved in the regulation of *SPT* on the development of the carpel and that there is functional redundancy between PIF4 and PIF5 [69].

In addition, SPT is also involved in regulating the carpel and fruit development of strawberries [72]. *FaSPT* is the homologous gene of Arabidopsis *SPT* in strawberry. After the *SPT* gene was mutated in strawberries, it was found that the shape and size of the mutant fruit changed. Moreover, strawberry fruit is developed from many separated carpels, which also implies that *SPT* plays a vital role in the carpel and fruit development of strawberries.

5 *SPT* Is Involved in the Regulation of Organ Size and Leaf Size in *Arabidopsis thaliana*

In plants, the leaves are the basic organs of the lateral, leaves possess intrinsic information that determines the ultimate size of the plant, and the cell proliferation is maintained by the chloroplast, which starts at the periphery of the phloem tissue at the stem tip [73,74]. The number and size of leaf pulp cells determine the leaf size. Past studies on leaf size have identified several inhibitors of leaf cell proliferation, e.g., *AUXIN RESPONSE TRANSCRIPTION FACTOR 2* (*ARF2*), which negatively regulates *ANT* expression [75]. There is also *BIG BROTHER* (*BB*), and two ubiquitin receptors, *DA1* and *DAR* [76,77]. Previous studies have also identified several cell proliferation-promoting factors, such as *ANGUSTIFOLIA3* (*AN3*), and *GROWTH-REGULATING FACTOR 5* (*AtGRF5*), that facilitate cell proliferation and enlarge final leaf size [78,79]. In a past study, transcription factor SPT has also been proven to play a vital role in the regulation of leaf size. *SPT* was shown to inhibit leaf size by limiting the

size of meristematic tissues in the leaf primordium [3]. RT-qPCR analysis of *SPT* transcription revealed that *SPT* was found to be strongly expressed in young leaf primordia and lightly expressed in mature leaves. This also implies the role of *SPT* in regulating the process of young leaf primordia.

Observation of the leaf phenotypes of the *spt* mutant revealed that the *spt* mutant leaves were significantly larger and had longer petioles, and the total cell number in the petiole was notably higher than that of the Col-0 ecotype, suggesting that *SPT* regulates *Arabidopsis* leaf size by inhibiting leaf proliferation. The number of cells has been increased by the loss of *SPT* function. In conclusion, *SPT* functions throughout the life cycle of the plant, and the differences between leaf and flower phenotypes of *spt* mutants are significant.

It has also been proved that *SPT* is a growth inhibitory factor that inhibits the size of root meristematic tissue by regulating the transport of auxin, thereby inhibiting root size, *spt* mutants exhibit larger cotyledons and leaves [2,3,80]. In cotyledons, *SPT* co-represses cell expansion through GA-inhibitory DELLA, and *SPT* also receives negative regulation from GA, and it has been shown that mutant plants tend to exhibit less accumulation of PIN auxin transporter proteins compared to the Col-0 ecotype [81]. In contrast, in leaves, *SPT* inhibited cell proliferation by regulating auxin accumulation. *SPT* expression was also found in the proliferative zone. Moreover, in the *spt* mutant, there were also significantly more meristematic tissues in the leaf primordia compared with the wild type [8,82]. It also implies that the expansion of meristematic tissues leads to larger leaves in *spt* mutants. To sum up, *SPT* negatively regulates cell proliferation in *Arabidopsis thaliana*.

Higher expression of *SPT* was found in regions with high auxin concentrations, which also implies that the expression of *SPT* is correlated with auxin distribution [5,83]. By applying the auxin inhibitor NPA to the *spt-11* mutant, it was found that the phenotype of the *spt-11* mutant with defective carpel development was restored, which also implies that *SPT* may also be involved in the regulation of auxin transport [60,67]. In addition, *SPT* is also involved in regulating the auxin redirection pathway [84].

6 *SPT* Is Involved in the Regulation of Stomata, Epidermal Hairs, and Anthocyanin Biosynthesis in *Arabidopsis*

Many new functions of the transcription factor SPT remain to be discovered. Transcriptome analysis of *SPT* revealed the existence of target genes for stomatal development, epidermal hair formation, and anthocyanin synthesis. *SPT* was shown to negatively regulate stomatal development and positively regulate epidermal hair formation as well as anthocyanin biosynthesis [9]. Stomatal development requires the combined action of multiple transcription factors, such as the bHLH transcription factor, the transcription factor FAMA, and MUTE, their helix-loop-helix regions can form heterodimers with SCREAM1 (SCRM1) and SCRM2 [85–88]. Three polypeptide receptor kinases (EPF1, FPF2, and EPFL9) are involved in the regulation of stomatal pattern formation in *Arabidopsis*, where EPF1 is involved in the negative regulation of the MUTE-SCRM module, EPF2 in the negative regulation of the SPCH-SCRM module, and EPFL9 in the positive regulation of the SPCH-SCRM module [89,90].

Plant epidermal hairs are hair-like appendages extending from the epidermal tissue of the above-ground part of the plant. Epidermal hairs increase the thickness of the epidermal layer of the plant, construct a natural physical barrier between the epidermis and the environment, reduce the loss of water and excessive accumulation and consumption of heat in the plant body, and to a certain extent mitigate pests, freezing, ultraviolet rays, and mechanical damage; in addition, the epidermal hairs with glands can secrete alkaloids, aromatic oils, and resins, and other chemical substances to defend against biotic and abiotic stresses and signaling [91,92]. On the other hand, the light-leafed, light-shelled phenotype of crop rice with missing epidermal trichomes (light-leaved rice) facilitates crop harvesting and subsequent processing

and thus has been widely utilized in breeding practices. Therefore, the study of trichome formation is of great importance for agricultural breeding.

Trichome formation begins with the internal replication of the protoplast, after which the trichome begins to expand and branch outwards, finally forming a mature trichome [93]. The formation of trichomes of stomata helps the plant to exchange gases with the outside world for better photosynthesis [94]. In addition, *SPT* plays a vital role in the biosynthesis of anthocyanins in *Arabidopsis thaliana*. Anthocyanins are glycosylated polyphenolic compounds, also known as anthocyanidin glycosides, which are an important class of plant flavonoid compounds. Anthocyanins are widely found in plant organs such as flowers, fruits, and leaves and are the basis for the colorful appearance of plants [95]. Anthocyanin biosynthesis is regulated by temperature, light, hormones, sugars, and so on [96,97]. Several structural genes are involved in the regulation of anthocyanin biosynthesis, which is categorized as early biosynthetic genes (EBG): *CHS*, *CHI*, *F3H*, and F3H: late biosynthetic genes (LBG): *F3*, *S'H*, *DFR*, *ANS*, and *UFGT*, etc. The expression of these structural genes is controlled by environmental factors and transcription factors, which mainly include the three major categories of MYB, bHLH, and WD40 [98,99]. They could regulate structural genes individually or combine to form MBW complexes to co-regulate structural genes.

Transcriptomic analysis of *SPT* revealed a high enrichment of genes associated with leaf tissue, and leaf structure, implying the role played by *SPT* in regulating leaf structure. Moreover, genes related to defense cells were also enriched, implying the role of *SPT* in regulating stomatal development [9]. In addition, the transcription factors GLABRA 2 (GL2) and TESTA 8 (TT8), which are associated with trichome formation, and PAP1, which is involved in anthocyanin production, were also identified. This also implies a role for *SPT* in regulating trichome formation and anthocyanin synthesis. Observation of the stomatal number of *spt* mutants revealed that the number of stomata was notably increased in the *spt-12* mutant compared with the Col-0 ecotype, whereas the *SPT* overexpression plant was not remarkably different from the Col-0 ecotype. This suggests that *SPT* inhibits stomatal production in *Arabidopsis thaliana*. This process may be regulated by *STOMGEN*, which is involved in stomatal development and is also the target gene of *SPT* [100]. In the *spt-12* mutant, however, the number of trichomes was notably decreased compared with the Col-0 ecotype, which also implies that *SPT* is a positive regulator of *Arabidopsis* trichomes. This may be related to *GL2*, which has been shown to be involved in the regulation of trichome development [101].

SPT also plays a vital role in sucrose-mediated anthocyanin biosynthesis. Previous studies have revealed the association between *SPT* and anthocyanin synthesis and genes related to anthocyanin synthesis [102]. By observing anthocyanin synthesis in *SPT* overexpression plant and *spt-2* mutant distributed in 1% sucrose and 5% sucrose, no obvious differences were found between *SPT* overexpression plants and *spt-2* and Ler in 1% sucrose, but in 5% sucrose, it was found that the color intensity of anthocyanin in *SPT* overexpression plants was remarkably higher than that of Ler, and that of the *spt-2* mutant had the notably lower color intensity of anthocyanins than Ler. This process may be regulated by *DFR* or *UF3GT*, which are involved in regulating anthocyanin biosynthesis and are also the target genes of *SPT* [103,104]. This demonstrates that *SPT* promotes sucrose-mediated anthocyanin biosynthesis.

7 *SPT* Is Involved in Regulating the Auxin-Cytokinin Signaling Pathway in the Pistil

Auxins and cytokinins are involved in plant growth, and the interaction of auxins and cytokinins plays a vital role in many physiological processes in plants, such as stem cell maintenance, vascularisation, and root development [105–110]. It was shown that in the medial region of the pistil, the auxin biosynthesis gene *TAA1* and the auxin efflux transporter protein gene *PIN3* are promoted by cytokinin signaling. A recent study has shown that TIR1 and AFB signals of auxin also participate in regulating the growth of root

acid growth [111]. At the same time, some studies have proved that auxin regulates its functions in flowers and fruits by integrating the processes of biosynthesis, and signal transduction [112]. Auxin also regulates the formation of plant lateral roots. For example, the formation of plant lateral roots also requires the activation of auxin-dependent transcription factors ARF7, ARF19, and LBD16 [113]. Auxin signaling is also involved in regulating cambium, the stem cell niche that mediates wood formation [114]. In addition, the bHLH family member *SPT* promotes cytokinin signaling in the medial region of the pistil by regulating the *TYPE-B ARABIDOPSIS RESPONSE REGULATOR* (*ARR*) [5,115]. By crossing the auxin response reporter gene line *DR5*::GFP with *SPT* overexpressing plants and *spt* mutant plants, respectively, to observe the expression of *DR5* signal, it was shown that in the stigma region of the style of the Ler ecotype, *DR5* signal was expressed predominantly in the form of a "ring" around the styles at stage 9, but this ring-shaped pattern of *DR5* signal was not observed in the *spt* mutant. In contrast, a notably enhanced *DR5* signal was found in the stigma of pistil styles in *SPT* overexpression plants compared with Ler, suggesting that *SPT* is involved in the regulation of *Arabidopsis* auxin signaling. Auxins and cytokinins act in complex ways [115,116]. By treating the cytokinin reporter gene line *TCS*::GFP with the auxin IAA, it was found that in the wild type, the application of IAA enhanced *TCS* signaling. However, *TCS* signaling was not observed in the *spt* mutant. It implies that auxin enhances cytokinin expression in an *SPT*-dependent pathway. Similarly, the application of IAA also enhanced *DR5* signaling in plants with the *spt* mutant, suggesting that exogenous auxin acts independently of SPT in the gynoecium [117].

8 *SPT* Is Involved in Regulating Plant Response to Abiotic Stress in Cucumber

Abiotic stress is an abiotic environmental condition that is not conducive to the survival, and growth of plants and even leads to injury, destruction, and death [118–120]. In nature, plants often face different kinds of abiotic stresses, such as alkali stress, salt stress, high-temperature stress, and so on [121]. Abiotic stress has seriously endangered plant growth and crop yield, so we must pay attention to the influence of abiotic stress on plant growth [122–124]. High-temperature stress also affects the growth of plants [125,126]. It has been indicated that bHLH transcription factors are involved in regulating the process of plants responding to high-temperature stress [127,128]. Recently, *SPT* was found to be involved in regulating plant responses to high ambient temperature stress in cucumbers [129]. Through RNA sequencing technology, it has been found that the expression of 75 genes changed after high-temperature treatment. At the same time, after high-temperature treatment, the leaves of the *spt* mutant became withered and the photosystem and chloroplast activity of the *spt* mutant were also seriously damaged, which proved that *SPT* positively regulated the heat resistance of cucumber. In addition, GO enrichment analysis showed that compared with wild-type plants, high temperature induced more photosynthesis and chloroplast-related genes in *Csspt* mutant plants, which proved that *SPT* regulated plant heat tolerance by regulating photosystem heat sensitivity.

9 Conclusion

In conclusion, this paper reviews the multiple roles of the bHLH family transcription factor SPT in the growth and development of *Arabidopsis thaliana*. SPT is a versatile transcription factor, and the helix-loop-helix region of *SPT* can form protein complexes with its family members as well as with members of other proteins. This paper mainly reviews the important roles of *SPT* in regulating flowering, seed germination, carpel development, leaf size, stomatal development, trichome formation, anthocyanin synthesis, and auxin-cytokinin interactions in *Arabidopsis thaliana*, which is helpful for us to deeply understand the role of transcription factor SPT in plant growth (summarized in Fig. 1). In addition, we also summarized the role of *SPT* in regulating the response of cucumbers to high-temperature stress, which also suggested the potential application of *SPT* in regulating the response of plants to abiotic stress.

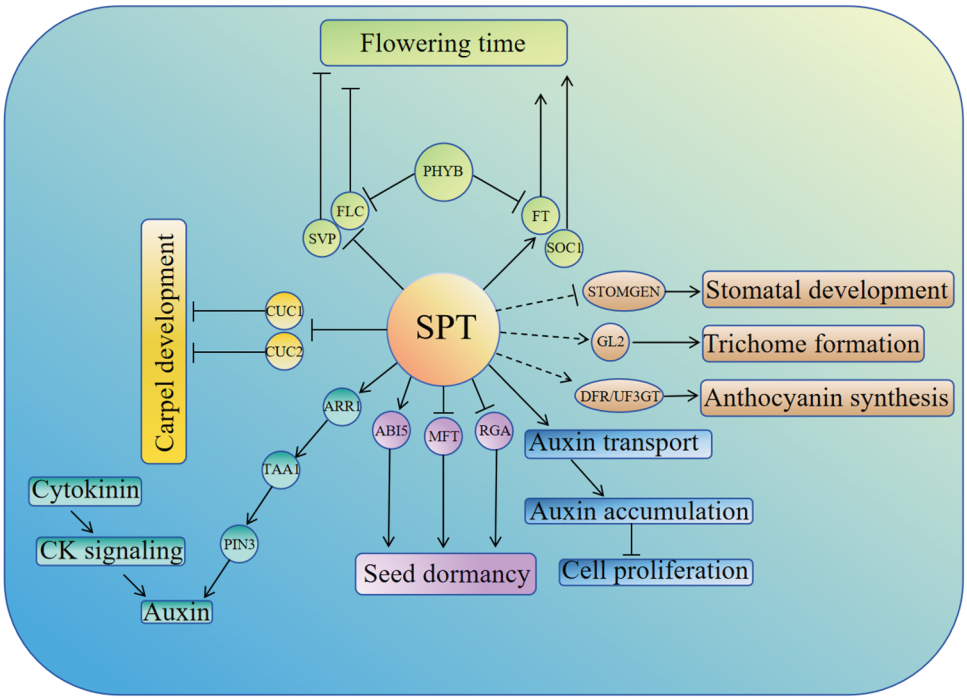

Figure 1: Schematic representation of *SPT* regulation of the growth and development processes in *Arabidopsis*, including flowering time, seed germination, leaf size, carpel development, stomatal development, epidermal hair synthesis, anthocyanin biosynthesis, etc. Dashed lines indicate targets that are not direct targets

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