Basic Helix-Loop-Helix (bHLH) Transcription Factors Regulate a Wide Range of Functions in Arabidopsis

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Abstract: The basic helix-loop-helix (bHLH) transcription factor family is one of the largest transcription factor gene families in Arabidopsis thaliana, and contains a bHLH motif that is highly conserved throughout eukaryotic organisms. Members of this family have two conserved motifs, a basic DNA binding region and a helix-loop-helix (HLH) region. These proteins containing bHLH domain usually act as homo- or heterodimers to regulate the expression of their target genes, which are involved in many physiological processes and have a broad range of functions in biosynthesis, metabolism and transduction of plant hormones. Although there are a number of articles on different aspects to provide an overview of insights into the pleiotropic regulatory roles of these transcription factors in plant growth and development, stress response, biochemical functions and the web of signaling networks. We then provide an overview of the functional profile of the bHLH family and the regulatory mechanisms of other proteins.

Keywords: basic helix-loop-helix (bHLH); transcription factor; cross-talk; plant growth and development

1. Introduction

Plants frequently encounter a wide array of environmental stresses, including drought, cold, salinity, UV radiation, ozone and other abiotic stresses through their lifecycle, which severely affect plant growth, development and crop yield. To cope with those problems, plants adapt to the different harsh conditions through transcription factors that regulate the expression of a series of target genes. The basic helix-loop-helix (bHLH) protein family is one of the largest transcription factor gene families in Arabidopsis thaliana, and contains a highly conserved amino acid motif that can be found throughout eukaryotic organisms. They were originally identified in animals, and found to play important roles in diverse regulatory networks, via the functions of DNA binding and dimerization [1,2]. The binding and dimerization networks of those proteins also have diverse functions in plants, including in multiple aspects of plant growth and development, which were characterized in recent years.

The bHLH superfamily, named after its highly conserved alkaline/helix-loop-helix domain, is the second largest transcription factor family and is widely present in animals and plants [3,4]. The bHLH domain is composed of approximately 60 conserved amino acid residues and consists of two conserved motifs, namely, a basic region and helix-loop-helix region (HLH region). The basic region participates in DNA binding to E-box (usually CANNTG) or G-box (CACGTG) motif in their target genes, and the HLH region, which is composed of two alpha helices containing hydrophobic residues, is required for dimerization to change the expression of target genes involved in various signaling pathways. Based on their phylogenetic relationship and DNA binding function, the bHLH members in eukaryotes have been classified into six main groups (named A, B, C, D, E and F) [5].
In brief, Group A can specifically bind to the E-box core sequence, Group B prefers to bind the G-box, and Group C binds to the ACTTG or GCGTG sequences [6,7]. Group D lacks a typical basic region and mainly participates in heterodimerization with other bHLH family proteins [8]. Group E can preferentially bind to the CACGNG sequence, and the Group F members can bind to specific DNA target sequences [9–11]. At least 162 members of bHLH proteins have been identified in Arabidopsis [12–14], which could be clustered into 12 distinctive subfamilies, namely bHLH I to XII [13,14]. Though bHLH proteins in Arabidopsis are quite divergent, the most abundant type of Arabidopsis bHLHs are more closely related to the phylogenetic group B of eukaryotic bHLHs, which is proposed to be the ancestral bHLH type [14,15]. Arabidopsis also contains some atypical bHLH proteins, which lack the basic region and are referred as AtHLHs. However, they do not share a close sequence similarity to the group D proteins in animals [4,14].

As a superfamily, bHLHs have been characterized in Arabidopsis over the years, and members of this family members play vital roles in plant growth, development, light signal transduction and stress responses [16–20]. They are also involved in the crosstalk of hormones signaling, including abscisic acid (ABA), jasmonic acid (JA), brassinosteroid (BR), salicylic acid (SA) and ethylene (ET) [1,4,13,21], and they are pivotal for plant growth and survival in the environment.

To date, many excellent reviews have covered the roles of bHLH transcription factors in plants in different contexts, but an overall summary is not available. Thus, we focused mainly on summarizing the knowledge on bHLH functions in various essential processes, such as how bHLH proteins mediate plant growth and development, including flowering time, seed germination and cell fate determination; how they are involved in maintaining iron homeostasis in plants, how they respond to light, stress, phytohormones and the crosstalk among these factors; and how they perform the functions in other processes, such as senescence and anthocyanin biosynthesis (Figure 1 and Table 1).

**Figure 1.** bHLHs functions in various signaling pathways.
| Pathway         | AGI Gene Code | Generic Name | Synonym         | Functions Characterized                                                                 | Group | Reference |
|-----------------|---------------|--------------|-----------------|----------------------------------------------------------------------------------------|-------|-----------|
| Seed germination| At4g01460     | bHLH57       |                 | Involved in seed dormancy process                                                        | Ia    | [22,23]  |
|                 | At2g20180     | bHLH15       | PIF1/PIF5       | Negative regulator of phytochrome-mediated seed germination                             | VIIa  | [24–26]  |
|                 | At4g36930     | bHLH24       | SPT             | Reduced seed dormancy                                                                   | VIIb  | [27,28]  |
| Flowering       | At1g35460     | bHLH80       | FDB1            | Regulate the effect of CO flowering time                                                | IX    | [29]     |
|                 | At4g34530     | bHLH63       | CIB1            | Promote CRY2-dependent floral initiation                                               | XII   | [30–32]  |
|                 | At5g65640     | bHLH93       | NFL             | Involved in GA mediated control of flowering time                                        | IIIb  | [33]     |
|                 | At1g32640     | bHLH6       | MYC2            | Required in the JA pathway for regulating flowering time                                | IIle  | [34]     |
|                 | At5g46760     | bHLH5       | MYC3            | Required in the JA pathway for regulating flowering time                                | IIle  | [34]     |
|                 | At4g17880     | bHLH4       | MYC4            | Required in the JA pathway for regulating flowering time                                | IIle  | [34]     |
|                 | At1g18400     | bHLH44      | BEF1            | Regulate photoperiodic flowering                                                        | XII   | [35]     |
|                 | At2g43010     | bHLH9       | PIF4            | Accelerate the flowering by activating FT at high temperature                          | XII   | [36]     |
|                 | At4g36930     | bHLH24      | SPT             | Play a role in floral morphogenesis processes                                          | VIIa  | [37]     |
|                 | At1g66470     | bHLH83      | RHD6            | ROOT HAIR DEFECTIVE6                                                                   | VIIIb | [13,38,39]|
|                 | At5g37800     | bHLH86      | RSL1            | Partially redundant and involved in root hair development                                | VIIIc | [40]     |
|                 | At4g33880     | bHLH85      | RSL2            | Partially redundant and involved in root hair development                                | VIIIc | [40]     |
|                 | At2g14760     | bHLH84      | RSL3            | RHD6-LIKE 3, required for root-hair growth                                              | VIIIc | [40–42]  |
|                 | At1g27740     | bHLH54      | RSL4            | Promote postmitotic cell growth in root-hair cells                                      | VIIIc | [40–42]  |
|                 | At5g58010     | bHLH82      | LRL3            | Regulate root hair development                                                         | XI    | [40–42]  |
| Cell fate       | At5g41315     | bHLH1       | GL3             | Trigger the trichome initiation pathways                                               | IIIf  | [41,43,44]|
|                 | At1g63650     | bHLH2       | EGL3            | Regulate trichome and root hair development                                            | IIIf  | [45,46]  |
|                 | At5g46830     | bHLH28      | MYC5            | Calcium-binding transcription factor involved in salt stress signaling                  | IIle  | [47]     |
|                 | At1g32640     | bHLH6       | MYC2            | Positive regulator of lateral root formation                                            | IIle  | [47]     |
|                 | At5g46760     | bHLH5       | MYC3            | Form the bHLH-MYB complex to regulate the stamen development                           | IIle  | [47]     |
|                 | At4g17880     | bHLH4       | MYC4            | Form the bHLH-MYB complex to regulate the stamen development                           | IIle  | [47]     |
|                 | At3g24140     | bHLH97      | FAMA            | Promote differentiation of stomatal guard cells                                         | Ia    | [48]     |
### Table 1. Cont.

| Pathway                                      | AGI Gene Code | Generic Name | Synonym | Functions Characterized                                                                 | Characterized Group | Reference |
|----------------------------------------------|---------------|--------------|---------|-----------------------------------------------------------------------------------------|---------------------|-----------|
| **Plant mineral nutrition and abiotic stress** | At3g56970     | bHLH38       | ORG2    | Regulate the Fe-deficiency response                                                       | Ib                   | [49]      |
|                                              | At3g56980     | bHLH39       | ORG3    | Regulate the Fe-deficiency response                                                       | Ib                   | [49]      |
|                                              | At2g41240     | bHLH100      |         | A key regulator of iron-deficiency responses                                              | Ib                   | [50]      |
|                                              | At5g04150     | bHLH101      |         | A key regulator of iron-deficiency responses                                              | Ib                   | [50]      |
|                                              | At2g28160     | bHLH29       | FIT     | Regulate iron uptake responses                                                            | IIIa                 | [49–53]  |
|                                              | At3g19860     | bHLH121      | URI     | Act as an essential part of the iron deficiency signaling pathway                         | IVb                  | [54]      |
|                                              | At3g23210     | bHLH34       | IDT1    | Involved in Fe regulation.                                                                | IVc                  | [55–58]  |
|                                              | At4g14410     | bHLH104      |         | Positively regulate Fe deficiency response                                                | IVc                  | [55–58]  |
|                                              | At5g54680     | bHLH105      | ILR3    | Plays an important role in Fe homeostasis                                                | IVc                  | [55–58]  |
|                                              | At1g51070     | bHLH115      |         | Involved in response to Fe                                                                | IVc                  | [55–58]  |
|                                              | At3g47640     | bHLH47       | PYE     | Regulate response to iron deficiency in Arabidopsis roots                                | IVb                  | [59–62]  |
|                                              | At4g36060     | bHLH11       |         | Basic helix-loop-helix (bHLH) DNA-binding superfamily protein                            | IVb                  | [63]      |
| **Stress response**                          | At3g26744     | bHLH116      | ICE1    | Improve cold tolerance through an ABA independent pathway                                | IIIb                 | [64]      |
|                                              | At3g06590     | bHLH148      | AIF2/RITF1 | Involved in the detoxification of ROS which generated by salt stress                   | Orphans              | [65]      |
|                                              | At1g61660     | bHLH122      |         | Mediate multiple response to improve stress tolerance                                     | IX                   | [66,67]  |
|                                              | At2g43010     | bHLH9        | PIF4    | Accelerate the flowering by activating FT at high temperature                           | XII                  | [68–70]  |
| **Light signaling**                         | At2g46970     | bHLH124      | PIL1    | Associated with APRR1/TOC1 and is a member of PIF3 family                                 | VIIa                 | [24,25]  |
|                                              | At3g59060     | bHLH65       | PIF3/PIL6 | Involved in shade avoidance                                                              | VIIa                 | [24,25]  |
|                                              | At3g62990     | bHLH132      | PIF6/PIL2 | Associated with APRR1/TOC1 and is a member of PIF3 family                                 | VIIa                 | [24,25]  |
|                                              | At1g09350     | bHLH8        | PIF3    | Interact with photoreceptors phyA and phyB.                                              | VIIa                 | [24,71,72] |
|                                              | At2g43010     | bHLH9        | PIF4    | Interact with active PhyB protein                                                         | VIIa                 | [24,25,73,74] |
|                                              | At1g02340     | bHLH26       | HFR1    | Involved in phytochrome signaling                                                        | VIIb                 | [75]      |
|                                              | At2g42870     | bHLH165      | PAR1    | Control plant development and as a negative regulator of SAS                            | Orphans              | [76–78]  |
|                                              | At3g58850     | bHLH166      | PAR2    | Control plant development and as a negative regulator of SAS                            | Orphans              | [76–78]  |
|                                              | At2g20180     | bHLH15       | PIF1/PIL5 | A key negative regulator of phytochrome-mediated response                               | VIIa                 | [79–82]  |
|                                              | At5g61270     | bHLH72       | PIF7    | Interacts specifically with Pfr form of phyB                                             | VIIb                 | [83–85]  |
| Pathway                        | AGI Gene Code | Generic Name | Synonym | Functions Characterized                                                                 | Group | Reference          |
|-------------------------------|---------------|--------------|---------|-----------------------------------------------------------------------------------------|-------|-------------------|
| JA signaling                  | At1g32640     | bHLH6        | MYC2    | Regulates diverse JA-dependent functions                                                | Ille  | [86,87]           |
|                               | At5g46760     | bHLH5        | MYC3    | Act together with MYC2 and MYC4 to activate JA-responses                                 | Ille  | [86,87]           |
|                               | At4g17880     | bHLH4        | MYC4    | Act together with MYC2 and MYC3 to activate JA-responses                                 | Ille  | [86,87]           |
|                               | At5g41315     | bHLH1        | GL3     | Repressed by JAZs                                                                       | Ilif  | [88–91]          |
|                               | At2g22750     | bHLH18       |         | Induced by JA and inhibit the transcription of the FIT                                   | IvA   | [92]              |
|                               | At2g22760     | bHLH19       |         | Induced by JA and inhibit the transcription of the FIT                                   | IvA   | [92]              |
|                               | At2g22770     | bHLH20       | NA1     | Induced by JA and inhibit the transcription of the FIT                                   | IvA   | [92]              |
|                               | At4g37850     | bHLH25       |         | Induced by JA and inhibit the transcription of the FIT                                   | IvA   | [92]              |
|                               | At1g59640     | bHLH31       | BPEp    | Involved in the control of petal size                                                   | XII   | [93,94]           |
|                               | At1g26945     | bHLH163      | PRE6    | Involved in ABA and salt responses                                                       | XV    | [93,95]           |
| IAA signaling                 | At5g39860     | bHLH136      | PRE1/BNQ1 | Mediate multiple response to improve stress tolerance                                   | X     | [96,97]           |
|                               | At5g15160     | bHLH134      | PRE2/BNQ3 | Involved in response to ABA, repress MYC2-activated leaf senescence                     | Ilid  | [98,99]           |
|                               | At1g32640     | bHLH6        | MYC2    | Induced by dehydration stress, ABA and blue light                                       | Ille  | [17]              |
|                               | At2g43140     | bHLH129      |         | Regulate root elongation and ABA response.                                               | IX    | [100]             |
|                               | At1g26945     | bHLH163      | PRE6    | Involved in the regulation of ABA and salt responses                                     | XV    | [101]             |
|                               | At2g43060     | bHLH9        | PIF4    | Repressed by ABA and responses to abiotic stress                                        | Va    | [102]             |
|                               | At5g08130     | bHLH50       | BEE3    | Involved in brassinosteroid signaling and modulated SAS                                 | Va    | [111,112]         |
|                               | At5g38860     | bHLH141      | BIM3    | Involved in brassinosteroid signaling and modulated SAS                                 | Va    | [111,112]         |
| ABA signaling                 | At1g61660     | bHLH112      |         | Mediate multiple response to improve stress tolerance                                   | X     | [96,97]           |
|                               | At2g46510     | bHLH17       | AIB     | Involved in response to ABA, repress MYC2-activated leaf senescence                     | Ilid  | [98,99]           |
|                               | At1g32640     | bHLH6        | MYC2    | Induced by dehydration stress, ABA and blue light                                       | Ille  | [17]              |
|                               | At2g43010     | bHLH9        | PIF4    | Negatively regulate phyB mediated responses and involved in SAS                         | VIIa  | [25,104]          |
|                               | At1g18400     | bHLH44       | BEE1    | Function in the early response to BRs                                                   | XII   | [18,109]          |
|                               | At4g36540     | bHLH58       | BEE2    | Function redundant with BEE1/3                                                         | XII   | [18,110]          |
|                               | At1g73830     | bHLH50       | BEE3    | Function redundant with BEE1/2                                                          | XII   | [18]              |
|                               | At5g08130     | bHLH46       | BIM1    | BES1-INTERACTING MYC-LIKE 1, involved in BRs signaling                                   | Va    | [111,112]         |
|                               | At1g69010     | bHLH102      | BIM2    | Involved in brassinosteroid signaling and modulated SAS                                 | Va    | [111,112]         |
|                               | At5g38860     | bHLH141      | BIM3    | Involved in brassinosteroid signaling and modulated SAS                                 | Va    | [111,112]         |
Table 1. Cont.

| Pathway     | AGI Gene Code | Generic Name | Synonym  | Functions Characterized                                      | Group | Reference       |
|-------------|---------------|--------------|----------|--------------------------------------------------------------|-------|----------------|
| Other aspects | At4g16430     | bHLH3        | JAM3     | Repress MYC2-activated leaf senescence, negatively regulate JA response | IlId  | [47]           |
|             | At1g01260     | bHLH13       | JAM2/MYC7E | Repress MYC2-activated leaf senescence, negatively regulate JA response | IlId  | [47]           |
|             | At4g00870     | bHLH14       |          | Repress MYC2-activated leaf senescence, negatively regulate JA responses | IlId  | [47]           |
|             | At2g46510     | bHLH17       | AIB      | Involved in response to ABA, repress MYC2-activated leaf senescence | IlId  | [47]           |
|             | PIFs          |              |          | Promoted leaf senescence                                    | VIIa  | [113]          |
|             | MYCs          |              |          | Positive regulator of Positively regulates flavonoid biosynthesis | IIIe  | [67,114–117]   |
2. Functions of bHLH Factors in Plant Growth and Development in Arabidopsis

2.1. Roles in Seed Germination

Seed dormancy and germination are critical processes in the lifespan of plants, which are mediated by various external environmental factors such as temperature, light and humidity, and internal factors, such as phytohormones. Two type of phytohormones, ABA and gibberellin (GA), are crucial regulators of seed dormancy and germination. ABA enhances seed dormancy, whereas GA breaks dormancy and promotes germination. The transcription factor bHLH57 is involved in ABA-regulated the seed dormancy process. NCED (9-cis-epoxycarotenoid dioxygenase) is considered to catalyze the first dedicated step in ABA biosynthesis [22], ODR1 (reversal of rdo5, an ortholog of the rice seed dormancy 4 (Sdr4)) protein interact with bHLH57 in a complex to inhibit NCED gene transcription [23,24].

In addition to phytohormones, photoreceptors also regulate seed dormancy and germination. Phytochrome interacting factors (PIFs), a group of typical of bHLH proteins, could interact with diverse groups of factors to integrate external environmental and internal signals, and further control seed germination, shade avoidance and crosstalk of plant hormones and the clock-derived signaling pathway [25,26]. The phytochrome-interacting bHLH protein PIL5 (PIF3-like 5 PIF1/bHLH15) is a repressor of seed germination, acting by reducing GA level in the dark; phytochromes accelerate the PIL5 degradation and increase the level of bioactive GA in seeds upon exposure to light. In addition, PIL5 activates the expression of the ABA synthesis gene and maintains a high level of ABA in the dark to inhibit seed germination [27]. Another light stable bHLH transcription factor SPATULA (SPT) also mediates seed germination, by interacting with PIL5 in response to light and temperature [28,29].

2.2. Functions in the Flowering Time Control

It is critical for plants to manage their flowering time, which is regulated by an intricate network of molecular signaling and controlled by various environmental factors such as photoperiod and temperature. The CONSTANS (CO) protein functions as an essential component for transforming biological clock signals into flowering signals to initiate plant flowering. Four bHLH related proteins (flowing bHLHs), namely, FBH1, FBH2, FBH3 and FBH4, are transcriptional activators of the CO gene; they bind to the E-box cis-elements in the promoter of CO and then positively regulate the CO-mediated flowering time [30]. Cryptochromes (CRY1/2) are blue light receptors that inhibit hypocotyl elongation and control floral initiation [31]. CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP-HELIX 1 (CIB1) is a CRY2-interacting bHLH protein that promotes the FLOWERING LOCUS T (FT) transcription; CRY2, CIB1 and CO can form a protein complex in response to blue light and then promote floral initiation [32,33].

Recently, a bHLH transcription factor, named as NO FLOWERING IN SHORT DAY (NFL) was shown to be necessary for the promotion of flowering specifically under short-day (SD) conditions, and nfl mutants did not flower under SD conditions but were similar to wild type under long-day (LD) conditions [34]. Additionally, three myelocytomatosis (MYC) proteins redundantly regulate flowering under both LD and SD conditions. Although MYC4 (bHLH4) is more important in mediating flowering than MYC3 (bHLH5), MYC2/3/4 are required in the JA pathway for regulating the flowering time by inhibiting FT transcription in Arabidopsis [35]. Moreover, the Brassinosteroid Enhanced Expression1 (BEE1, bHLH44) protein is stabilized under blue light; this protein is an integrator regulating photoperiodic flowering [36].

In Arabidopsis, temperature also affects the flowering time, and high temperature (29 °C) not only induces rapid hypocotyl elongation but also results in early flowering. In addition, the central integrator bHLH transcription factor phytochrome-interacting factor 4 (PIF4) can accelerate the flowering process by activating FT at high temperature conditions [37]. In addition to regulating the flowering time, bHLH family members are involved in the flower organ development. SPATULA (SPT) encodes a bHLH transcription factor and plays a role in floral morphogenesis processes as previously described [38].
In summary, the flowering time is strictly controlled by an intricate network, and bHLH family members act together with many other proteins to allow plants to flower at suitable time and under favorable environments.

2.3. Functions on Cell Fate Determination

Plants process a cell determination mechanism for formatting specific cell types, and this system relies on the expression of different genes in a proper spatiotemporal manner. Many transcription factors participate in this critical process, including a number of bHLH transcription factors, which play essential roles in the root and shoot cell fate determination [39]. During the root development, two types of cells arise from the epidermis: root hair cells and non-hair cells. Root hair cells produce an outside long tubule from a specialized root surface area and have functions in absorbing nutrition and water, and also in interacting with microbes.

The bHLH VIIIc subfamily member, bHLH83 (root hair defective 6, RHD6), is a transcription factor that has fewer root hairs and functions in the inhibition root hair formation in cortical cells [13,39], and another bHLH protein, ROOT HAIR DEFECTIVE 6-Like 1 (RLS1), functions redundantly with RHD6 to modulate the root hair development [40]. RSL2, a homolog of RSL1, is also required for the normal root hair formation, which is controlled by RHD6 and RSL1 [41]. Additional genes, such as RSL3/4 and LRL3 (Lj-RHL1-LIKE3) also act as downstream of RHD6 and RSL1 to promote the root hair and cell differentiation [41–43]. Moreover, RSL4 is suppressed by GLABRA2 (GL2) through ZINC FINGER PROTEIN1 (ZP1) [44]. GL2, a member of homeodomain-leucine zipper (HD-ZIP) protein, affects the epidermal cell fate including in trichomes, root hair and the seed coat. It represses the transcription of RHD6 to inhibit hair formation in N-cells (non-root hair cells), leading to the expression of non-hair genes [42,45]. The bHLH transcription factor GL3 (GLABRA3, bHLH1) has similar functions, regulating its own transcription and downstream target genes to trigger the trichome initiation pathways, ENHANCER OF GLABRA3 (EGL3, bHLH2) plays partially redundant roles with GL3 in root hair [46,118].

Furthermore, ET is involved in root hair initiation and elongation, and ETHYLENE INSENSITIVE 3 (EIN3) and its homolog EIN3-like1 (EIL1) can coordinate with RHD6/RLS1 to upregulate RSL4 and then participate in root epidermis development [119]. Another bHLH complex named MYB-bHLH-WD40 was proposed to regulate the guard cell and root hair differentiation [47].

In addition to their roles in the root epidermis formation, the bHLH family is also involved in other cell fate related processes, including stamen and stomatal development. The Illb bHLH transcription factor MYC5 (bHLH28) has redundant functions with MYC2 (bHLH6), MYC3 (bHLH5) and MYC4 (bHLH4), which interact with MYB21 and MYB24 to form the bHLH-MYB complex to regulate the stamen development [48]. In addition, FAMA (bHLH97) is specifically expressed in the stomata and has functions in halting proliferative division and promoting guard cells [120].

Overall, the establishment of this specification mechanism is complicated and also influenced by several plant growth regulators, including hormones such auxin (indole-3-acetic acid, IAA), ET, JA [55,119].

3. Functions in Environmental Response

3.1. Functions in Plant Mineral Nutrition and Abiotic Stress

Iron is an indispensable mineral micronutrient for photosynthesis and respiration in plants. Low concentration of effective iron in soil leads to the iron deficiency in plants, conversely, too much iron can have a toxic effect. To cope with this problem, plants have developed a series of sophisticated regulatory systems to control the iron uptake and maintain the Fe homeostasis. Among the regulatory mechanisms, bHLH transcription factors play important roles in this complicated process [49,92].

Four closely related clade lb bHLH genes, namely, bHLH38, bHLH39 [50], bHLH100 and bHLH101 [121], have been characterized as key transcription factors regulating the
Fe-deficiency response in Arabidopsis, and their transcript levels were shown to be up-regulated under Fe deficiency [50,121]. A single loss-of-function mutant of these genes did not show an obvious change in phenotype compared with wild-type because of functional redundancy in this subfamily. A triple loss-off function mutant of bHLH39, bHLH100 and bHLH101 showed severe leaf chlorosis only under Fe-deficient conditions [51]. bHLH29 (FER-like iron deficiency-induced transcription factor, FIT), an ortholog of FER (from the T3238FER line), acts as a central regulator to iron deficiency inducible genes in iron uptake and homeostasis [52–54]. Research has indicated that FIT interacts with bHLH38, bHLH39 [50], bHLH100 and bHLH101 [121] to form heterodimers that activate the transcription of genes involved in the Fe uptake system. Recent research has shown that bHLH121 can form homo or heterodimers and directly bind the FIT promoter to regulate its expression [122]. In addition, cadmium (Cd) stress is a severe heavy metal stress that limits plant growth, and FIT/bHLH38 or FIT/bHLH39 heterodimers can respond to Cd tolerance by affecting the transcriptional expression of downstream genes [56].

Four IVc bHLH transcription factors (bHLH34, bHLH104, bHLH105/ILR3 - IAA-LEUCINE RESISTANT3, and bHLH115) form homo- and heterodimers to regulate the Fe deficiency response and the Fe homeostasis [57–59,92]. Knockout of bHLH104 reduced Arabidopsis tolerance to Fe deficiency and suppressed the activation of Fe deficiency-inducible genes, whereas overexpression had the opposite effect. The bHLH034, homolog of bHLH104, also positively regulated the Fe deficiency response. The bHLH105, also named IAA-LEUCINE RESISTANT3 (ILR3), interacted with bHLH104, which plays an important role in maintaining the Fe homeostasis. Notably, a triple mutant deficient of bHLH34/104/105 is sensitive to Fe deficiency [59].

In the case of FIT, its function is required to regulate Fe uptake responses; bHLH47 (POPEYE, PYE) is another bHLH transcription factor that plays a major role in the Fe homeostasis. PYE interacts with bHLH104, bHLH105 and bHLH115 to bind to the promoters of several Fe response genes and repress their activity [60–62,123]. Interestingly, the bHLH104, bHLH105 and bHLH115 protein levels are posttranslationally controlled by BRUTUS (BTS), a hemerythrin E3 ligase, via the proteosomal degradation to negatively regulate the bHLH protein stability [61,124]. It was reported that the BTS paralogs, BTS LIKE1 (BTSL1) and BTSLIKE2 (BTSL2) act redundantly as negative regulators of the Fe starvation response, and the transcript levels are also controlled by FIT [125].

In most plants, Fe(II) is taken up by IRON REGULATED TRANSPORTER 1 (IRT1) in the root plasma membrane from soil under iron deficiency [63,126,127]. Moreover, ferric reduction oxidase 2 (FRO2) transfers electrons across the plasma membrane for iron reduction at the root surface. These are key genes regulating ferric reduction and iron transport in plants, and their transcription levels are upregulated by FIT and bHLH Ib heterodimers [50,121]. Furthermore, bHLH11 acts as a negative regulator to modulate Fe levels in Arabidopsis, and bHLH11 is highly expressed in roots and its expression is upregulated after plants are transferred to Fe sufficient conditions [64].

In addition to regulating the iron response, it was reported that bHLH104 loss-of-function mutants were sensitive to Cd stress, and the Cd tolerance was enhanced upon overexpression of bHLH104 [20]. In other words, bHLH104 positively regulates both Cd tolerance and Fe deficiency tolerance in Arabidopsis.

3.2. Functions in Stress Responses

Inducer of CBF Expression (ICE1, bHLH116) encodes an MYC-type bHLH transcription factors that directly binds the promoter of the C-Repeat Binding Factor/Dehydration-Responsive-Element-Binding protein (CBF/DREB1) protein-encoding gene and activates its transcription to improve cold tolerance through an ABA independent pathway [64]. The regulation is controlled by the binding of various downstream target cold and dehydration response genes that contain specific binding sites [65,128–130].
Other transcription factors, such as AIF2 (also named RITF1) and its partner RSA1 (SHORT ROOT IN SALT MEDIUM 1) regulate several genes for detoxification of reactive oxygen species (ROS), which are triggered by the salt stress [66].

Some proteins, such as bHLH122, can directly bind to the CYP707A3 promoter to repress its expression and enhance the ABA content, but these proteins were not induced by ABA treatment, indicating that they may function in ABA-independent or ABA-dependent ways [22,67].

As direct target genes of PIF4, JUNGBRUNNEEN1 (JUB1) and ORESARA1 (ORE1) have vital roles in salt stress response. PIF4 directly regulates ORE1 and JUB1, and is involved in regulating salt stress response [68–70].

4. Functions in the Response to Light and Phytohormones

Under specific conditions, multiple phytohormones and environmental factors are in constant crosstalk with each other to affect the plant growth and development. Based on previous studies, GA, IAA, BR, ET and light are usually considered to promote cell expansion in plant growth, while ABA, JA and SA are normally involved in the response to biotic and abiotic stresses. bHLH proteins that respond to plant hormones and environmental factors participate in various processes. They are able to interact with each other to cooperatively or antagonistically modulate the plant growth.

4.1. Response to Light Signaling

Light is an important factor that influences the plant growth and development, and many bHLH transcription factors are reported to participate in this process by inducing the expression of related downstream genes and various light-mediated effects.

PIFs interact with the active form (Pfr) of phytochrome to modulate growth, including in response to environmental signals such as light and stress and via other signaling pathways [73]. The PIF family belongs to the bHLH superfamily VII of transcription factors, which play central roles in the regulation of light signaling. To date, 15 PIF members have already been identified, and 7 PIF members have been shown to bind the Pfr form of phyB in Arabidopsis, while other members do not interact with the light-activated phytochrome [13,14,131–133]. Each PIF has individualized or redundant biological functions with other PIF proteins during various responses.

In the dark, four PIFs (PIF1/PIL5, PIF3, PIF4 and PIF5/PIL6) directly interact with Pfr to promote skotomorphogenesis by repressing photomorphogenesis. Loss-of-function of pif mutant showed phenotypes of reduced hypocotyl elongation. PIFs also interact with diverse groups of transcription factors to integrate external environmental and internal signals, including in seed germination, shade avoidance and crosstalk among plant hormones and clock-related signaling pathways [24,25]. A number of genes have been confirmed to be direct targets of PIF, thus mediating downstream light signaling networks through the PIFs.

PIF3 (bHLH8), the first identified member in the PIF family, acts as a regulator in the seedling de-etiolation and modulates both positive and negative response to phytochrome-mediated signaling [24,71]. PIF3 can also heterodimerize with the atypical basic bHLH protein HFR1 (long hypocotyl in far-red, bHLH26) to modulate phyA signaling [75]. In addition, PIF3 is involved in plant freezing response as a negatively regulator which can interact with EBF1 (EIN1-BINDING F-BOX1) through the CBF (C-REPEAT BINDING FACTOR) pathway [72].

PIF4 is a key transcription factor in the light signaling pathway; it interacts selectively with Pfr and negatively regulates phyB signaling in Arabidopsis [74]. Phytochrome Rapidly Regulated 1 (PAR1, bHLH165) and its homolog PAR2 (bHLH166) lack the ability to directly interact with phytochromes that are rapidly induced by shade [76,77], while PAR1 directly interacts with PIF4 to form a heterodimer to inhibit PIF4 function in cell elongation [78]. Moreover, growing evidence indicates that PIF4 acts as central regulator, coordinating plant response to multiple environmental signals [134].
The PIL (PIF3-like proteins, PILs) family has six members, designated as PIL1, PIL2, PIL3, PIL4, PIL5 and PIL6. PIF1/PIL5 (bHLH15) is a component that negatively regulates the chlorophyll biosynthetic pathway, seeds germination and inhibit hypocotyl elongation in the dark. The activity of PIF1 was repressed by phyA and phyB in light, and regulated ABA signaling [79–82]. In addition, other PIFs, such as PIF5 (PIL6), PIF6 (PIL2) and PIF7, have been shown to interact with the Pfr form of phyB or phyA, which are involved in phytochrome signaling [83–85].

Taken together, the bHLH proteins PILs/PIFs are proposed to form heterodimers to regulate bHLH network activity and are central components that integrate multiple signals in response to light.

4.2. Functions in JA Signaling Pathway

The phytohormone jasmonate acid (JA) plays a vital role in the plant development and the response to various stresses. The presence of JA triggers the key protein jasmonate ZIM-domain (JAZ) to interact with CoronatineInsensitive 1 (COI1), part of the SCF^{COI1} ubiquitin E3 ligase complex. Then, JAZ proteins were degraded by the 26S protease, resulting in multiple transcription factors free from JAZ-mediated repression and further activating downstream JA-mediated responsive genes. The bHLH transcription factor MYC2 forms a signaling module COI1/JAZs/MYC2 to participate in the JA-induced signaling pathway. The homologs of MYC2, MYC3 (bHLH5) and MYC4 (bHLH4) are known to form homodimers/heterodimers, and they can also bind to the G-box involved in the JA signaling pathway but exhibit gene redundancy with MYC2 [86,87].

The bHLH transcription factor GLABRA3 (GL3, bHLH1) can form a WD-repeat/bHLH/MYB complex with TRANSPARENT TESTA GLABRA1 (TTG1) and the R2R3-MYB transcription factor GLABRA1 (GL1), which is repressed by JAZs and DELLA, is responsible for trichome initiation [88–91].

Four subgroup IVa bHLH transcription factors (bHLH18, bHLH19, bHLH20 and bHLH25) can be induced by JA and inhibit the transcription of the FIT and Ib bHLH genes, which have been suggested to function redundantly in JA-mediated FIT protein degradation in the presence of JA or under iron deficient conditions via the 26S proteasome pathway [92].

The jasmonate-activated transcription factor MYC2 has also been found to interact with the key component in the ET signaling pathway EIN3 and its close homolog EIL1 to repressed its DNA binding activity and affect hook formation [135], suggesting that jasmonate and ET have antagonistic functions during apical hook development.

4.3. Functions in IAA Signaling Pathway

The plant hormone IAA has multiple roles in the plant growth and development, such as in cell division, cell elongation and cell differentiation, which are affected by the regulation of IAA response genes [93]. BIGPETALp (BPEp, bHLH31) is a bHLH transcription factor that can interact with AUXIN RESPONSE FACTOR8 (ARF8) to influence cell expansion and petal growth [94], PRE6 is a target of ARF5 and ARF8 that negatively regulates auxin response genes in Arabidopsis [95].

4.4. Roles in ABA Signaling Pathway

Plant are constantly under extrinsic abiotic/biotic environmental stresses, including cold, drought, high salinity, pathogen and extreme temperature. In response to these diverse stresses, plants have evolved sophisticated adaptation mechanisms. To date, several bHLH transcription factors have been reported to mediate abiotic and biotic stress signaling pathways to regulate plant responses in Arabidopsis in different ways.

The plant hormone ABA plays a central role in a variety of physiological processes and environmental response involved in plant growth, including responses to drought, cold, heat and salinity stresses [136–138]. Several bHLH transcription factors, such as bHLH112,
MYC2, AIB, AtAIG1, bHLH129 and bHLH92, have been reported to be involved in the regulation of ABA signaling via these processes [96,98–100].

A loss-of-function mutant of bHLH112, a transcriptional activator, displayed a late-flowering phenotype under long day conditions in Arabidopsis, and its transcript level was correlated with salt and drought tolerance. bHLH112 regulates gene expression by binding to E-box and GCG-box motifs in the gene promoters and then mediate multiple physiological response to improve stress tolerance [96,97].

ABA-inducible bHLH-type transcription factor (AIB) and ABA-inducible gene (AIG1) are ABA-induced genes, the proteins contain a bHLH type DNA binding domain and play a positive role in ABA signaling in Arabidopsis [98,99]. MYC2 and MYB2 have been shown to be transcription activators that function in the ABA signal transduction pathway by directly regulating the expression of ABA response genes [17]. In addition, MYC2 can also be involved in JA [139–141].

The expression level of bHLH129 is reduced upon exogenously application of ABA, and bHLH129 regulates the expression of several ABA signaling component genes [100] to promote root elongation.

PREs are involved in plant growth and development and are also involved in the regulation of ABA mediated salt responses in Arabidopsis. Some PREs are ABA responsive genes; their expression levels are decreased under ABA treatment, and this response has functions in regulating plant growth and environmental stimuli [101].

ABA induces stomatal closure and changes the expression of numerous genes to adapt to drought stress. ABA-responsive kinase substrate (AKS1), also a bHLH transcription activator, is inhibited by ABA through phosphorylated to form monomer by SNF1-related protein kinase 2 (SnRK2) in Arabidopsis guard cells [142,143].

In addition, the BEE transcription factor family members (BEE1/2/3) regulate plant responses to abiotic stress. BEE genes are strongly repressed by ABA and are redundant negative regulators of physiological responses to abiotic stress, whereas the BEE2 dimerized protein IBH1 is a positive modulator that improves salt and drought tolerance [102]. NaCl-induced expression of bHLH92 confers tolerance to salt and osmotic stress which is partially dependent on ABA and SALT OVERLY SENSITIVE 2 (SOS2) [103].

4.5. The Cross-Talk between Light and Phytohormones

Some bHLH transcription factors are involved in signal transduction networks mediated by plant hormones. For example, several genes are involved in light and GA signaling pathways, such as PIL5 and SPT, which also belong to the bHLH transcription factor family [27,80]. Moreover, many bHLH transcription factors have been reported to be involved in the regulation of BRs, ABA and IAA signaling pathways. Paclobutrazol Resistance (PREs) proteins are atypical bHLH transcription factors that lack the DNA binding domain but can dimerize with bHLH factors to inhibit DNA binding [107,144]. PREs participate in various hormone-, temperature- and light-responsive signaling pathways to regulate plant growth and development in many ways [104,105,107,145]. Briefly, PRE1 (bHLH136), PRE5 (bHLH164) and PRE6 (bHLH163) are direct targets of PIF4 and are involved in the regulation of the light, GA and BR signaling pathways [25,104]. PRE1 regulates cell elongation in Arabidopsis together with ARF6 [105]. PRE3 (bHLH135), also named Activation-Tagged BRI1 Suppressors 1 (ATBS1), can suppress the BR insensitive 1 (bri1) phenotype, and its mutant shows an auxin-related phenotype [16]. PRE4 (bHLH161, BNQ3) mutant shows a light-related phenotype, including pale-green sepals, decreased chlorophyll levels and late flowering [106]. PRE2 (bHLH134) and PRE6 (bHLH163) are ABA response genes that affect plant sensitivity to ABA, indicating that some PREs are involved in ABA and salts responses [95].

Several atypical bHLH proteins, such as Arabidopsis ILI1 binding bHLH1 (IBH1) and ATBS1 Interacting Factors (AIFs), negatively regulate cell elongation in Arabidopsis [107,108]. Activators for cell elongation (ACEs) can promote cell elongation, while IBH1 interacts with ACEs to inhibit their functions in the induction of cell elongation. Another bHLH
protein, homolog of BEE2 interacting with IBH1 (HBI1), is involved in BR-mediated growth to promote cell elongation [104]. Functional analysis showed that bHLH proteins have related functions but with different mechanisms to regulate cell elongation. In these processes, bHLHs proteins are mostly dependent on multiple phytohormones including BR, GA, IAA and light signaling [108,146].

BEE1 (bHLH44), BEE2 (bHLH58) and BEE3 (bHLH50) are functionally redundant bHLH transcription factors for which expression is induced by BL treatment, indicating that they function in the early response to BRs and their expression is required by ABA [18]. In brief, the BEE1 protein is stabilized under blue light, which is an integrator to regulate photoperiodic flowering [109]. BEE2 and CIB1 negatively regulate immunity and are functionally redundant with HBI1 in plant immunity [104,110]. Moreover, BEEs positive regulate shade avoidance syndrome with BES1-INTERACTING MYC-LIKE protein (BIMs) [111,112].

5. Functions in Other Aspects of Plant Biology

In Arabidopsis, bHLH members are also involved in other aspects of plant growth and development, such as senescence and the anthocyanin biosynthesis pathway. These aspects are only briefly discussed here.

In plants, senescence is triggered by developmental and environmental factors. The IIIId bHLH transcription repressors (bHLH3, bHLH13, bHLH14 and bHLH17) can bind to the promoter of senescence-associated gene 29 (SAG29) to repress MYC2-activated leaf senescence [47]. PIFs can promote leaf senescence during age-triggered and dark-induced processes [113].

bHLH transcription factors have been identified as key regulators of anthocyanin biosynthesis in various plant species [147]. MYC-like bHLH proteins can physically interact with MYB transcription factor and WD40-repeat protein genes to form complexes, known as MBW complex, that control the transcript levels of genes by binding to their promoter regions, thereby regulating anthocyanin in biosynthesis [22,114], and these complexes are also crucial for the regulation of flavonoid pathway [115–117].

6. Conclusions and Future Prospects

As the second largest superfamily in Arabidopsis, most bHLH transcription factors are characterized by a signature domain, which consists of approximately 60 amino acids, with an N-terminal basic DNA binding domain and a C-terminal protein interaction domain. Although more than 160 genes have already been predicted to belong to the bHLHs family and are classified into 12 subfamilies [14], relatively few have been characterized according to current studies [13,148]. Recent research studies have demonstrated that bHLHs transcription factors are characterized by their roles in a broad range of plant growth and various developmental processes.

Some transcription factors play the more crucial roles in many aspects of plant development, such as MYCs, PREs and PIFs, and these bHLHs are key transcriptional regulators in the phytohormone crosstalk pathway and multiple biosynthesis pathways. As JAZ targets, MYCs are involved in JA-mediated gene expression and the ABA signaling pathway; they also mediate ET biosynthesis and regulate stamen development and flowering time. PREs respond to GA, BR, temperature and light signaling to positively regulate cell elongation. PIFs are also involved in many other signaling pathways, such as in mediating pathways responsive to light, GA, IAA, ABA, ET and abiotic/biotic stress [73,149,150] (Figure 2).
Figure 2. Simplified model for bHLH PREs, MYCs and PIFs, which play central roles in many pathways in Arabidopsis. PIFs bind to their target genes to regulate plant growth and development via manipulation of signaling pathways. PREs and MYCs also participate in core transcription networks together with PIFs to control plant responses to the environment.

Overall, in this review, we have compiled the current research on bHLH function and provided a relatively complete overview of the bHLH transcription factor family. This review enriches our understanding of this family and provides new insight into the mechanism by which bHLHs regulate various biological processes. However, there remain many aspects that have not been described because of the complicated crosstalk with other transcription factors. Our understanding of function the bHLH proteins have improved tremendously during the past several years, and the research in this area is well characterized. Future studies will further elucidate the mechanism of how bHLH proteins coordinate the multiple internal and external environments to regulate plant growth.

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References
1. Murre, C.; McCaw, P.S.; Baltimore, D. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. Cell 1989, 56, 777–783. [CrossRef]
2. Massari, M.E.; Murre, C. Helix-loop-helix proteins: Regulators of transcription in eucaryotic organisms. Mol. Cell. Biol. 2000, 20, 429–440. [CrossRef]
3. Duek, P.D.; Fankhauser, C. bHLH class transcription factors take centre stage in phytochrome signalling. Trends Plant Sci. 2005, 10, 51–54. [CrossRef]
4. Feller, A.; Machemer, K.; Braun, E.L.; Grotewold, E. Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. Plant J. 2011, 66, 94–116. [CrossRef] [PubMed]
5. Atchley, W.R.; Fitch, W.M. A natural classification of the basic helix-loop-helix class of transcription factors. *Proc. Natl. Acad. Sci. USA* 1997, 94, 5172–5176. [CrossRef]

6. Henriksson, M.; Lüscher, B. Proteins of the Myc network: Essential regulators of cell growth and differentiation. *Adv. Cancer Res.* 1996, 68, 109–182. [CrossRef]

7. Godin, C.R. Motif from neural crest to melanoma: Signal transduction and transcription in the melanocyte lineage. *Genes Dev.* 2000, 14, 1712–1728. [CrossRef] [PubMed]

8. Sun, X.H.; Copeland, N.G.; Jenkins, N.A.; Baltimore, D. Id proteins Id1 and Id2 selectively inhibit DNA binding by one class of helix-loop-helix proteins. *Mol. Cell. Biol.* 1991, 11, 5603–5611. [CrossRef] [PubMed]

9. Ledent, V.; Vervoort, M. The basic helix-loop-helix protein family: Comparative genomics and phylogenetic analysis. *Genome Res.* 2001, 11, 754–770. [CrossRef] [PubMed]

10. Fisher, A.; Caudy, M. The function of hairy-related bHLH repressor proteins in cell fate decisions. *Bioessays* 1998, 20, 298–306. [CrossRef]

11. Crozatier, M.; Valle, D.; Dubois, L.; Ibnsouda, S.; Vincent, A. Collier, a novel regulator of Drosophila head development, is expressed in a single mitotic domain. *Curr. Biol.* 1996, 6, 707–718. [CrossRef]

12. Bailey, P.C.; Martin, C.; Toledo-Ortiz, G.; Quail, P.H.; Huq, E.; Heim, M.A.; Jakoby, M.; Werber, M.; Weissshaar, B. Update on the basic helix-loop-helix transcription factor gene family in Arabidopsis thaliana. *Plant Cell* 2003, 15, 2497–2501. [CrossRef] [PubMed]

13. Heim, M.A.; Jakoby, M.; Werber, M.; Marti, C.; Weissshaar, B.; Bailey, P.C. The basic Helix-Loop-Helix transcription factor family in plants: A genome-wide study of Protein structure and functional diversity. *Mol. Biol. Evol.* 2003, 20, 735–747. [CrossRef] [PubMed]

14. Toledo-Ortiz, G.; Huq, E.; Quail, P.H. The Arabidopsis basic/helix-loop-helix transcription factor family. *Plant Cell* 2003, 15, 1749–1770. [CrossRef] [PubMed]

15. Buck, M.J.; Atchley, W.R. Phylogenetic Analysis of Plant Basic Helix-Loop-Helix Proteins. *J. Mol. Evol.* 2003, 56, 742–750. [CrossRef]

16. Castelain, M.; Hir, R.L.; Bellini, C. The non-DNA-binding bHLH transcription factor PRE3/bHLH135/ATBS1/TMO7 is involved in the regulation of light signaling pathway in Arabidopsis. *Physiol. Plantarum* 2012, 145, 450–460. [CrossRef]

17. Abe, H.; Urao, T.; Ito, T.; Seki, M.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *Plant Cell* 2003, 15, 63–78. [CrossRef]

18. Friedrichsen, D.M.; Nemhauser, J.; Muramitsu, T.; Maloof, J.N.; Alonso, J.; Ecker, J.R.; Furuya, M.; Chory, J. Three redundant brassinosteroid early response genes encode putative bHLH transcription factors required for normal growth. *Genetics* 2002, 162, 1445–1456. [CrossRef]

19. Liu, Y.; Li, X.; Li, K.; Liu, H.; Lin, C. Multiple bHLH Proteins form Heterodimers to Mediate CRY2-Dependent Regulation of Flowering-Time in Arabidopsis. *PLoS Genet.* 2013, 9, e1003861. [CrossRef] [PubMed]

20. Yao, X.; Cai, Y.; Yu, D.; Liang, G. bHLH104 confers tolerance to cadmium stress in Arabidopsis thaliana. *J. Integr. Plant Biol.* 2018, 60, 691–702. [CrossRef]

21. Pires, N.; Dolan, L. Origin and diversification of Basic-helix-loop-helix proteins in plants. *Mol. Biol. Evol.* 2010, 27, 862–874. [CrossRef]

22. Nambara, E.; Marion-Poll, A. Abscisic acid biosynthesis and catabolism. *Annu. Rev. Plant Biol.* 2005, 56, 165–185. [CrossRef] [PubMed]

23. Lefebvre, V.; North, H.; Frey, A.; Sotta, B.; Seo, M.; Okamoto, M.; Nambara, E.; Marion-Poll, A. Functional analysis of Arabidopsis NCED6 and NCED9 genes indicates that ABA synthesized in the endosperm is involved in the induction of seed dormancy. *Plant J.* 2006, 45, 309–319. [CrossRef] [PubMed]

24. Liu, F.; Zhang, H.; Ding, L.; Soppe, W.J.J.; Xiang, Y. REVERSAL OF RDO5 1, a homolog of rice seed dormancy 4, interacts with bHLH5 and controls ABA biosynthesis and seed dormancy in Arabidopsis. *Plant Cell* 2020, 32, 1933–1948. [CrossRef] [PubMed]

25. Ni, M.; Pepperman, J.I.; Quail, P.H. PIF3, a Phytocrome-interacting factor necessary for normal photoinduced signal transduction, as a novel basic helix-loop-helix protein. *Cell* 1998, 95, 657–667. [CrossRef]

26. Oh, E.; Zhu, J.; Wang, Z. Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. *Nat. Cell Biol.* 2012, 14, 802–809. [CrossRef]

27. Oh, E.; Yamaguchi, S.; Kamiya, Y.; Bae, G.; Chung, WI.; Choi, G. Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. *Plant J.* 2006, 47, 124–139. [CrossRef]

28. Penfield, S.; Josse, E.; Kannangara, R.; Gilday, A.D.; Halliday, K.J.; Graham, I.A. Cold and light control of seed germination through the bHLH transcription factor SPATULA. *Curr. Biol.* 2005, 15, 1998–2006. [CrossRef] [PubMed]

29. Grossmann, M.; Bylstra, Y.; Lampugnani, E.R.; Smyth, D.R. Regulation of tissue-specific expression of SPATULA, a bHLH gene involved in carpel development, seedling germination, and lateral organ growth in Arabidopsis. *J. Exp. Bot.* 2010, 61, 1495–1508. [CrossRef] [PubMed]

30. Ito, S.; Song, Y.H.; Josephson-Day, A.R.; Miller, R.J.; Breton, G.; Olmstead, R.G.; Imaizumi, T. FLOWERING BHLH transcriptional activators control expression of the photoperiodic flowering regulator CONSTANS in Arabidopsis. *Proc. Natl. Acad. Sci. USA* 2012, 109, 3582–3587. [CrossRef]
31. Guo, H.; Yang, H.; Mockler, T.; Lin, C. Regulation of flowering time by Arabidopsis photoreceptors. Science 1998, 279, 1360–1363. [CrossRef]

32. Liu, H.; Yu, X.; Li, K.; Klejnot, J.; Yang, H.; Lisiero, D.; Lin, C. Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in Arabidopsis. Science 2008, 322, 1535–1539. [CrossRef] [PubMed]

33. Liu, H.; Yu, Z.; Ma, D.; Chen, Z.; Wang, J.; Liu, H. CIB1 and CO interact to mediate CRY2-dependent regulation of flowering. EMBO Rep. 2018, 19, e45762. [CrossRef] [PubMed]

34. Sharma, N.; Xin, R.; Kim, D.; Sung, S.; Lange, T.; Huq, E. NO FLOWERING IN SHORT DAY (NFL) is a bHLH transcription factor that promotes flowering specifically under short-day conditions in Arabidopsis. Development 2016, 143, 682–690. [CrossRef] [PubMed]

35. Wang, H.; Li, Y.; Pan, J.; Lou, D.; Hu, Y.; Yu, D. The bHLH transcription factors MYC2, MYC3, and MYC4 are required for jasmonate-mediated inhibition of flowering in Arabidopsis. Mol. Plant 2017, 10, 1461–1464. [CrossRef]

36. Wang, Z.; Yang, Z.; Li, F. Updates on molecular mechanisms in the development of branched trichome in Arabidopsis and nonbranched in cotton. Plant Biotechnol. J. 2019, 17, 1706–1722. [CrossRef] [PubMed]

37. Kumar, S.V.; Lucyshyn, D.; Jaeger, K.E.; Alvey, E.; Harberd, N.P.; Wigge, P.A. Transcription factor PIF4 controls the thermosensory activation of flowering. Nature 2012, 484, 242–245. [CrossRef] [PubMed]

38. Heisler, M.G.; Atkinson, A.; Bylstra, Y.H.; Walsh, R.; Smyth, D.R. SPATULA, a gene that controls development of carpel margin tissues in Arabidopsis, encodes a bHLH protein. Development 2001, 128, 1089–1098. [CrossRef]

39. Zhao, H.; Li, X.; Ma, L. Basic helix-loop-helix transcription factors and epidermal cell fate determination in Arabidopsis. Plant Signal. Behav. 2012, 7, 1556–1560. [CrossRef]

40. Menand, B.; Yi, K.; Jouannic, S.; Hoffmann, L.; Ryan, E.; Schaefer, D.G.; Dolan, L. An ancient mechanism controls the development of cells with a rooting function on land plants. Science 2007, 316, 1477–1480. [CrossRef]

41. Li, K.; Menand, B.; Bell, E.; Dolan, L. A basic helix-loop-helix transcription factor controls cell growth and size in root hairs. Nat. Genet. 2010, 42, 264–267. [CrossRef]

42. Bruex, A.; Kainkaryam, R.M.; Wiekowski, Y.; Kang, Y.H.; Bernhardt, C.; Xia, Y.; Zheng, X.; Wang, J.Y.; Lee, M.M.; Benfey, P.; et al. A gene regulatory network for root epidermis cell differentiation in Arabidopsis. PLoS Genet. 2012, 8, e1002446. [CrossRef]

43. Han, X.; Zhang, M.; Yang, M.; Hu, Y. Arabidopsis JAZ proteins interact with and suppress RHD6 transcription factor to regulate jasmonate-stimulated root hair development. Plant Cell 2020, 32, 1049–1062. [CrossRef]

44. Lin, Q.; Ohashi, Y.; Kato, M.; Tsuge, T.; Gu, H.; Qu, L.J.; Aoyama, T. GLABRA2 directly suppresses basic helix-loop-helix transcription factor genes with diverse functions in root hair development. Plant Cell 2015, 27, 2894–2906. [CrossRef] [PubMed]

45. Masucci, J.D.; Rerie, W.G.; Foreman, D.R.; Zhang, M.; Galway, M.E.; Marks, M.D.; Schiefelbein, J.W. The homeobox gene GLABRA2 is required for position-dependent cell differentiation in the root epidermis of Arabidopsis thaliana. Development 1996, 122, 1253–1260. [CrossRef] [PubMed]

46. Bernhardt, C.; Lee, M.M.; Gonzalez, A.; Zhang, F.; Lloyd, A.; Schiefelbein, J. The bHLH genes GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3) specify epidermal cell fate in the Arabidopsis root. Development 2003, 130, 6431–6439. [CrossRef]

47. Ramsay, N.A.; Glover, B.J. MYB-bHLH-WD40 protein complex and the evolution of cellular diversity. Trends Plant Sci. 2005, 10, 63–70. [CrossRef]

48. Qi, T.; Huang, H.; Song, S.; Xie, D. Regulation of jasmonate-mediated stamen development and seed production by a bHLH-MYB complex in Arabidopsis. Plant Cell 2015, 27, 1620–1633. [CrossRef]

49. Cui, Y.; Chen, C.L.; Cui, M.; Zhou, W.J.; Wu, H.L.; Ling, H.Q. Four IvA bHLH transcription factors are novel interactors of FIT and mediate JA inhibition of iron uptake in Arabidopsis. Mol. Plant 2018, 11, 1166–1183. [CrossRef] [PubMed]

50. Yuan, Y.; Wu, H.; Wang, N.; Li, J.; Zhao, W.; Du, J.; Wang, D.; Ling, H.Q. FIT interacts with AtbHLH38 and AtbHLH39 in regulating iron uptake gene expression for iron homeostasis in Arabidopsis. Cell Res. 2008, 18, 385–397. [CrossRef] [PubMed]

51. Wang, H.Y.; Klatte, M.; Jakoby, M.; Bäumlein, H.; Weisshaar, B.; Bauer, P. Iron deficiency-mediated stress regulation of four Fru-like bHLH transcription factors in Arabidopsis. Plant Cell 2015, 27, 787–805. [CrossRef] [PubMed]

52. Colangelo, E.P.; Guerinot, M.L. The essential basic helix-loop-helix protein FIT1 is required for the iron deficiency response. Plant Cell 2004, 16, 3400–3412. [CrossRef]

53. Jakoby, M.; Wang, Y.; Reidt, W.; Weisshaar, B.; Bauer, P. FRU (BHLH029) is required for induction of iron mobilization genes in Arabidopsis thaliana. FEBS Lett. 2004, 577, 528–534. [CrossRef] [PubMed]

54. Yuan, Y.X.; Zhang, J.; Wang, D.W.; Ling, H.Q. AtbHLH29 of Arabidopsis thaliana is a functional ortholog of tomato FER involved in controlling iron acquisition in strategy I plants. Cell Res. 2005, 15, 613–621. [CrossRef]

55. Pitts, R.J.; Cernac, A.; Estelle, M. Auxin and ethylene promote root hair elongation in Arabidopsis. Plant J. 1999, 16, 553–560. [CrossRef] [PubMed]

56. Wu, H.; Chen, C.; Du, J.; Liu, H.; Cui, Y.; Zhang, Y.; He, Y.; Wang, Y.; Chu, C.; Feng, Z.; et al. Co-overexpression FIT with AtbHLH38 or AtbHLH39 in Arabidopsis-enhanced cadmium tolerance via increased cadmium sequestration in roots and improved iron homeostasis of shoots. Plant Physiol. 2012, 158, 790–800. [CrossRef] [PubMed]

57. Zhang, J.; Liu, B.; Li, M.; Jin, H.; Wang, P.; Liu, J.; Xiong, F.; Wang, J.; Wang, H. The bHLH transcription factor bHLH104 interacts with IAA-LEUCINE RESISTANT3 and modulates iron homeostasis in Arabidopsis. Plant Cell 2015, 27, 787–805. [CrossRef] [PubMed]

58. Liang, G.; Zhang, H.; Li, X.; Ai, Q.; Yu, D. bHLH transcription factor bHLH115 regulates iron homeostasis in Arabidopsis thaliana. J. Exp. Bot. 2017, 68, 1743–1755. [CrossRef] [PubMed]
59. Tissot, N.; Robe, K.; Gao, F.; Grant-Grant, S.; Boucherez, J.; Bellegarde, F.; Maghairiou, A.; Marcelin, R.; Izquierdo, E.; Benhamed, M.; et al. Transcriptional integration of the responses to iron availability in Arabidopsis by the bHLH factor ILR3. *N. Phytol.* 2019, 223, 1433–1446. [CrossRef]

60. Long, T.A.; Tsukagoshi, H.; Busch, W.; Lahner, B.; Salt, D.E.; Benfey, P.N. The bHLH transcription factor POPEYE regulates response to iron deficiency in Arabidopsis roots. *Plant Cell* 2010, 22, 2219–2226. [CrossRef] [PubMed]

61. Selote, D.; Samura, R.; Matthiassid, A.; Gillikin, J.W.; Long, T.A. Iron-binding E3 ligase mediates iron response in plants by targeting basic helix-loop-helix transcription factors. *Plant Physiol.* 2015, 167, 273–286. [CrossRef] [PubMed]

62. Gao, F.; Dubos, C. Transcriptional integration of plant responses to iron availability. *J. Exp. Bot.* 2017, 72, 2056–2070. [CrossRef] [PubMed]

63. Martin-Barranco, A.; Spielmann, J.; Dubeaux, G.; Vert, G.; Enri Zelazny, E. Dynamic control of high-affinity iron uptake complex in root epidermal cells. *Plant Physiol.* 2020, 184, 1236–1250. [CrossRef]

64. Tanabe, N.; Noshi, M.; Morii, D.; Nozawa, K.; Tamoi, M.; Shigeoka, S. The basic helix-loop-helix transcription factor, bHLH11 functions in the iron-uptake system in Arabidopsis thaliana. *J. Plant Res.* 2018, 132, 93–105. [CrossRef] [PubMed]

65. Kurbidaeva, A.; Ezhova, T.; Novokreshchenova, M. Arabidopsis thaliana ICE2 gene: Phylogeny, structural evolution and functional diversification from ICE1. *Plant Sci.* 2014, 229, 10–22. [CrossRef]

66. Guan, Q.; Wu, J.; Yue, X.; Zhang, Y.; Zhu, J. A Nuclear calcium-sensing pathway is critical for gene regulation and salt stress tolerance in Arabidopsis. *PLoS Genet.* 2017, 9, e1003755. [CrossRef]

67. Liu, Q.; Tai, H.; Li, S.; Gao, W.; Zhao, M.; Xie, C.; Li, W. bHLH112 is important for drought and osmotic stress resistance in Arabidopsis and in the repression of ABA catabolism. *N. Phytol.* 2014, 201, 1192–1204. [CrossRef]

68. Balazadeh, S.; Siddiqui, H.; Allu, A.D.; Matallana-Ramirez, L.P.; Caldana, C.; Mehrnia, M.; Zanor, M.; Köhler, B.; Mueller-Roeber, B. A gene regulatory network controlled by the NAC transcription factor member ANAC092/ANAC2/ORE1 during salt-promoted senescence. *Plant J.* 2010, 62, 250–264. [CrossRef] [PubMed]

69. Wu, A.; Allu, A.D.; Garapati, P.; Siddiqui, H.; Dortay, H.; Zanor, M.; Munne-Bosch, S.; Antonio, C.; Tohege, T.; et al. JUNGBRUNNEN1, a reactive oxygen species-responsive NAC transcription factor, regulates longevity in Arabidopsis. *Plant Cell* 2012, 24, 482–506. [CrossRef] [PubMed]

70. Sakuraba, Y.; Bülbül, S.; Piao, W.; Choi, G.; Paek, N. Arabidopsis EARLY FLOWERING3 increases salt tolerance by suppressing salt stress response pathways. *Plant J.* 2017, 92, 1106–1120. [CrossRef]

71. Kim, J.; Yi, H.; Choi, G.; Shin, B.; Song, P.S.; Choi, G. Functional characterization of phytochrome interacting factor 3 in phytochrome-mediated light signal transduction. *Plant Cell* 2003, 15, 2399–2407. [CrossRef]

72. Jiang, B.; Shi, Y.; Zhang, X.; Xin, X.; Qi, L.; Guo, H.; Li, J.; Yang, S. PIF3 is a negative regulator of the CBF pathway and freezing tolerance in Arabidopsis. *Proc. Natl. Acad. Sci. USA* 2017, 114, E6695–E6702. [CrossRef] [PubMed]

73. Paik, I.; Kathare, P.K.; Kim, J.; Huq, E. Expanding roles of PIFs in signal integration from multiple processes. *Mol. Plant* 2017, 10, 1035–1046. [CrossRef] [PubMed]

74. Huq, E.; Quail, P.H. PIF4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in Arabidopsis. *EMBO J.* 2002, 21, 2441–2450. [CrossRef]

75. Fairchild, C.D.; Schumaker, M.A.; Quail, P.H. HFR1 encodes an atypical bHLH protein that acts in phytochrome A signal transduction. *Genes Dev.* 2000, 14, 2377–2388. [CrossRef] [PubMed]

76. Roig-Villanova, I.; Bou, J.; Sorin, C.; Devlin, P.F.; Martínez-García, J.F. Identification of primary target genes of phytochrome signaling: Early transcriptional control during shade avoidance responses in Arabidopsis. *Plant Physiol.* 2006, 141, 85–96. [CrossRef]

77. Roig-Villanova, I.; Bou-Torrent, J.; Galstyan, A.; Carretero-paulet, L.; Portoles, S.; Rodriguez-Concepción, M.; Martínez-García, J. Interaction of shade avoidance and auxin response: A role for two novel atypical bHLH proteins. *EMBO J.* 2007, 26, 4756–4767. [CrossRef]

78. Hao, Y.; Oh, E.; Choi, G.; Liang, Z.; Wang, Z.Y. Interactions between HLH and bHLH factors mediate light-regulated plant development. *Mol. Plant* 2012, 5, 688–697. [CrossRef]

79. Oh, E.; Kim, J.; Park, E.; Kim, J.; Kang, C.; Choi, G. PIL5, a phytochrome-interacting basic helix-loop-helix protein, is a key negative regulator of seed germination in Arabidopsis thaliana. *Plant Cell* 2004, 16, 3045–3058. [CrossRef]

80. Oh, E.; Yamaguchi, S.; Hu, J.; Yusuube, J.; Jung, B.; Paik, I.; Lee, H.; Sun, T.; Kamiya, Y.; Choi, G. PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GA1 and RGA promoters in Arabidopsis Seeds. *Plant Cell* 2007, 19, 1192–1208. [CrossRef]

81. Huq, E.; Al-Sady, B.; Hudson, M.; Kim, C.; Apel, K.; Quail, P.H. PHYTOCHROME-INTERACTING FACTOR 1 is a critical HLH regulator of chlorophyll biosynthesis. *Science* 2004, 305, 1937–1941. [CrossRef] [PubMed]

82. Moon, J.; Zhu, L.; Shen, H.; Huq, E. PIF1 directly and indirectly regulates chlorophyll biosynthesis to optimize the greening process in Arabidopsis. *Proc. Natl. Acad. Sci. USA* 2008, 105, 9433–9438. [CrossRef]

83. Shen, Y.; Khanna, R.; Carle, C.M.; Quail, P.H. Phytochrome induces rapid PIF5 phosphorylation and degradation in response to red-light activation. *Plant Physiol.* 2007, 145, 1043–1051. [CrossRef] [PubMed]

84. Khanna, R.; Huq, E.; Kikis, E.A.; Al-Sady, B.; Lanzatella, C.; Quail, P.H. A novel molecular recognition motif necessary for targeting photoactivated phytochrome signaling to specific basic helix-loop-helix transcription factors. *Plant Cell* 2004, 16, 3033–3044. [CrossRef] [PubMed]
85. Leivar, P.; Monte, E.; Al-Sady, B.; Carle, C.; Storer, A.; Alonso, J.M.; Ecker, J.R.; Quail, P.H. The Arabidopsis phytochrome-interacting factor PIF7, together with PIF3 and PIF4, regulates responses to prolonged red light by modulating phyB levels. *Plant Cell* 2008, 20, 337–352. [CrossRef]

86. Fernández-Calvo, P.; Chini, A.; Fernández-Barbero, G.; Chico, J.M.; Gimenez-Ibanez, S.; Geerinck, J.; Eeckhout, D.; Schweizer, F.; Godoy, M.; José Manuel Franco-Zorrilla, J.M.; et al. The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. *Plant Cell* 2011, 23, 701–715. [CrossRef]

87. Schweizer, F.; Fernández-Calvo, P.; Zander, M.; Diez-Diaz, M.; Fonseca, S.; Glauser, G.; Lewsey, M.G.; Ecker, J.R.; Solano, R.; Reymond, P. Arabidopsis basic helix-loop-helix transcription factors MYC2, MYC3, and MYC4 regulate glucosinolate biosynthesis, insect performance, and feeding behavior. *Plant Cell* 2013, 25, 3117–3132. [CrossRef] [PubMed]

88. Gonzalez, A.; Zhao, M.; Leavitt, J.M.; Lloyd, A.M. Regulation of the anthocyanin biosynthetic pathway by the TTG1/bHLH/Myb transcriptional complex in Arabidopsis seedlings. *Plant J.* 2008, 53, 814–827. [CrossRef]

89. Zhao, M.; Morohashi, K.; Hatlestad, G.; Grotewold, E.; Lloyd, A. The TTG1-bHLH-MYB complex controls trichome cell fate and patterning through direct targeting of regulatory loci. *Development* 2008, 135, 1991–1999. [CrossRef]

90. Qi, T.; Song, S.; Ren, Q.; Wu, D.; Huang, H.; Chen, Y.; Fan, M.; Peng, W.; Ren, C.; Xie, D. The Jasmonate-ZIM-Domain proteins interact with the WD-Repeat/bHLH/MYB complexes to regulate jasmonate-mediated anthocyanin accumulation and trichome initiation in Arabidopsis thaliana. *Plant Cell* 2011, 23, 1795–1814. [CrossRef]

91. Qi, T.; Huang, H.; Wu, D.; Yan, J.; Qi, Y.; Song, S.; Xie, D. Arabidopsis DELLA and JAZ proteins bind the WD-Repeat/bHLH/MYB complex to modulate gibberellin and jasmonate signaling synergy. *Plant Cell* 2014, 26, 1118–1133. [CrossRef]

92. Li, X.; Zhang, H.; Ai, Q.; Liang, G.; Yu, D. Two bHLH transcription factors, bHLH34 and bHLH104, regulate iron homeostasis in Arabidopsis thaliana. *Plant Physiol.* 2016, 170, 2478–2493. [CrossRef]

93. Teale, W.D.; Paponov, I.A.; Palme, K. Auxin in action: Signalling, transport and the control of plant growth and development. *Nat. Rev. Mol. Cell Biol.* 2006, 7, 847–859. [CrossRef] [PubMed]

94. Varaud, E.; Brioudes, F.; Szécsi, J.; Leroux, J.; Brown, S.; Perrot-Rechenmann, C.; Bendahmane, M. AUXIN RESPONSE FACTOR8 regulates Arabidopsis petal growth by interacting with the bHLH transcription factor BIGPetalAp. *Plant Cell* 2011, 23, 973–983. [CrossRef] [PubMed]

95. Zheng, K.; Wang, Y.; Zhang, N.; Jia, Q.; Wang, X.; Hou, C.; Chen, J.; Wang, S. Involvement of PACLOBUTRAZOL RESISTANCE6/KIDARI, an atypical bHLH transcription factor, in auxin responses in Arabidopsis. *Front. Plant Sci.* 2017, 8, 1813. [CrossRef] [PubMed]

96. Liu, Y.; Ji, X.; Nie, X.; Qu, M.; Zheng, L.; Tan, Z.; Zhao, H.; Huo, L.; Liu, S.; Zhang, B.; et al. Arabidopsis AtbHLH112 regulates root elongation and ABA response when overexpressed in Arabidopsis. *Sci. Rep.* 2015, 5, 17587. [CrossRef] [PubMed]

97. Moreno, J.E.; Moreno-Piovano, G.; Chan, R.L. Functional characterization of the Arabidopsis bHLH92 transcription factor in abiotic stress. *Plant Sci.* 2018, 257, 143–150. [CrossRef]

98. Jiang, Y.; Yang, B.; Deyholos, M.K. The antagonistic basic helix-loop-helix partners BEE and IBH1 contribute to control plant tolerance to abiotic stress. *Plant Sci.* 2021, 223, 41, 342–352. [CrossRef]

99. Kim, J.; Kim, H.Y. Molecular characterization of a bHLH transcription factor involved in Arabidopsis abscisic acid-mediated response. *BBA Gene Struct. Expr.* 2006, 1759, 191–194. [CrossRef]

100. Li, H.; Sun, J.; Xu, Y.; Jiang, H.; Wu, X.; Li, C. The bHLH-type transcription factor AtA1B positively regulates ABA response in Arabidopsis. *Plant Mol. Biol.* 2007, 65, 655–665. [CrossRef] [PubMed]

101. Tian, H.; Guo, H.; Dai, X.; Cheng, Y.; Zheng, K.; Wang, X.; Wang, S. An ABA down-regulated bHLH transcription repressor gene, bHLH129 regulates root elongation and ABA response when overexpressed in Arabidopsis. *Sci. Rep.* 2015, 5, 17587. [CrossRef] [PubMed]

102. Zhou, Q.; Chen, Y.; Wang, X.; Zhang, X.; Zhao, J.; Sun, X.; et al. Antibiotic bHLH/bHLH transcription factors mediate brassinosteroid regulation of cell elongation and plant development in rice and Arabidopsis. *Plant Cell* 2019, 239–245. [CrossRef] [PubMed]

103. Ikeda, M.; Mitsuda, N.; Ohme-Takagi, M. ATBS1 INTERACTING FACTORS negatively regulate Arabidopsis cell elongation in the triantagonistic bHLH system. *Plant Signal. Behav.* 2013, 8, e23448. [CrossRef]

104. Wang, F.; Gao, Y.; Liu, Y.; Zhang, X.; Gu, X.; Ma, D.; Zhao, Z.; Yuan, Z.; Xue, H.; Liu, H. BEN1-regulated BEE1 controls photoperiodic flowering downstream of blue light signaling pathway in Arabidopsis. *N. Phytol.* 2019, 223, 1407–1419. [CrossRef]
110. Malinovsky, F.G.; Batoux, M.; Schwessinger, B.; Youn, J.H.; Stransfeld, L.; Win, J.; Kim, S.K.; Zipfel, C. Antagonistic regulation of growth and immunity by the arabidopsis basic helix-loop-helix transcription factor HOMOLOG OF BRASSINOSTEROID ENHANCED EXPRESSION2 INTERACTING WITH INCREASED LEAF INCLINATION1 BINDING bHLH1. Plant Physiol. 2014, 164, 1443–1455. [CrossRef] [PubMed]

111. Liu, Y.; Vafeados, D.; Tao, Y.; Yoshida, S.; Asami, T.; Chory, J. A new class of transcription factors mediates brassinosteroid-regulated gene expression in Arabidopsis. Cell 2005, 120, 249–259. [CrossRef]

112. Cifuentes-Esquivel, N.; Akmakjian, G.Z.; Pivarski, K.L.; Punshon, T.; Baxter, I.; Salt, D.E.; Guerinot, M.L. BRUTUS and its paralogs, BTS LIKE1 and BTS LIKE2, encode important negative regulators of the iron deficiency response in Arabidopsis thaliana. [CrossRef]

113. Feng, Y.; Xu, P.; Li, B.; Li, P.; Wen, X.; An, F.; Gong, Y.; Xin, Y.; Zhu, Z.; Wang, Y.; et al. Ethylene promoters root hair growth and freezing tolerance in Arabidopsis. Mol. Plant 2010, 13, 634–649. [CrossRef] [PubMed]

114. Leung, J.; Giraudat, J. Abscisic acid signal transduction. Annu. Rev. Plant Phys. 2005, 56, 497–519. [CrossRef] [PubMed]

115. Malinovsky, F.G.; Batoux, M.; Schwessinger, B.; Youn, J.H.; Stransfeld, L.; Win, J.; Kim, S.K.; Zipfel, C. Antagonistic regulation of growth and immunity by the arabidopsis basic helix-loop-helix transcription factor HOMOLOG OF BRASSINOSTEROID ENHANCED EXPRESSION2 INTERACTING WITH INCREASED LEAF INCLINATION1 BINDING bHLH1. Plant Physiol. 2014, 164, 1443–1455. [CrossRef] [PubMed]

116. Liu, Y.; Vafeados, D.; Tao, Y.; Yoshida, S.; Asami, T.; Chory, J. A new class of transcription factors mediates brassinosteroid-regulated gene expression in Arabidopsis. Cell 2005, 120, 249–259. [CrossRef]

117. Zhang, X.; Zhu, Z.; An, F.; Hao, D.; Li, P.; Song, J.; Yi, C.; Guo, H. Jasmonate-activated MYC2 represses ETHYLENE INSENSITIVE3 activity to antagonize ethylene-promoted apical hook formation in Arabidopsis. Plant Cell 2014, 26, 1105–1117. [CrossRef] [PubMed]

118. Morohashi, K.; Zhao, M.; Yang, M.; Read, B.; Lloyd, A.; Lamb, R.; Grotewold, E. Participation of the Arabidopsis bHLH factor ENHANCED EXPRESSION2 INTERACTING WITH INCREASED LEAF INCLINATION1 BINDING bHLH1. Plant Physiol. 2007, 145, 736–746. [CrossRef]

119. Feng, Y.; Xu, P.; Li, B.; Li, P.; Wen, X.; An, F.; Gong, Y.; Xin, Y.; Zhu, Z.; Wang, Y.; et al. Ethylene promoters root hair growth and freezing tolerance in Arabidopsis. Mol. Plant 2010, 13, 634–649. [CrossRef] [PubMed]

120. Ohashi-Ito, K.; Bergmann, D.C. Arabidopsis FAMA controls the final proliferation/differentiation switch during stomatal development. Plant Cell 2006, 18, 2493–2505. [CrossRef] [PubMed]

121. Wang, N.; Cui, Y.; Liu, Y.; Fan, H.; Du, J.; Huang, Z.; Yuan, Y.; Wu, H.; Ling, H. Requirement and functional redundancy of Ib subgroup bHLH proteins for iron deficiency responses and uptake in Arabidopsis thaliana. Mol. Plant 2013, 6, 503–513. [CrossRef]

122. Lei, R.; Li, Y.; Cai, Y.; Li, C.; Pu, M.; Lu, C.; Yang, Y.; Liang, G. bHLH121 functions as a direct link that facilitates the activation of FIT by bHLH IVc transcription factors for maintaining Fe homeostasis in Arabidopsis. Mol. Plant 2020, 13, 274–289. [CrossRef] [PubMed]

123. Matthiandis, A.; Long, T.A. Further insight into BRUTUS domain composition and functionality. Plant Signal. Behav. 2016, 11, 8. [CrossRef]

124. Hindt, M.N.; Akmakjian, G.Z.; Pivarski, K.L.; Punshon, T.; Baxter, I.; Salt, D.E.; Guerinot, M.L. BRUTUS and its paralogs, BTS LIKE1 and BTS LIKE2, encode important negative regulators of the iron deficiency response in Arabidopsis thaliana. Metallomics 2017, 9, 876–890. [CrossRef] [PubMed]

125. Eide, D.; Broderius, M.; Fett, J.; Guerinot, M.L. A novel iron-regulated metal transporter from plants identified by functional expression in yeast. Proc. Natl. Acad. Sci. USA. 1996, 93, 5624–5628. [CrossRef] [PubMed]

126. Leivar, P.; Monte, E. PIFs: Systems integrators in plant development. Plant Cell 2014, 26, 56–78. [CrossRef] [PubMed]

127. Brumbarova, T.; Ivanov, R. The nutrient response transcriptional regulome of Arabidopsis. Iscience 2019, 19, 358–368. [CrossRef]

128. Zhang, X.; Zhu, Z.; An, F.; Hao, D.; Li, P.; Song, J.; Yi, C.; Guo, H. Jasmonate-activated MYC2 represses ETHYLENE INSENSITIVE3 activity to antagonize ethylene-promoted apical hook formation in Arabidopsis. Plant Cell 2014, 26, 1105–1117. [CrossRef] [PubMed]

129. Leivar, P.; Monte, E. PIFs: Systems integrators in plant development. Plant Cell 2014, 26, 56–78. [CrossRef] [PubMed]

130. Brumbarova, T.; Ivanov, R. The nutrient response transcriptional regulome of Arabidopsis. Iscience 2019, 19, 358–368. [CrossRef]

131. Castillon, A.; Shen, H.; Huq, E. Phytochrome Interacting Factors: Central players in phytochrome-mediated light signaling networks. Trends Plant Sci. 2007, 12, 514–521. [CrossRef] [PubMed]

132. Leivar, P.; Monte, E. PIFs: Systems integrators in plant development. Plant Cell 2014, 26, 56–78. [CrossRef] [PubMed]

133. Brumbarova, T.; Ivanov, R. The nutrient response transcriptional regulome of Arabidopsis. Iscience 2019, 19, 358–368. [CrossRef]

134. Zhao, L.; Gao, L.; Wang, H.; Chen, X.; Wang, Y.; Yang, H.; Wei, C.; Wan, X.; Xia, T. The R2R3-MYB, bHLH, WD40, and related transcription factors in flavonoid biosynthesis. Funct. Integr. Genomic 2013, 13, 75–98. [CrossRef]

135. Zhang, X.; Zhu, Z.; An, F.; Hao, D.; Li, P.; Song, J.; Yi, C.; Guo, H. Jasmonate-activated MYC2 represses ETHYLENE INSENSITIVE3 activity to antagonize ethylene-promoted apical hook formation in Arabidopsis. Plant Cell 2014, 26, 1105–1117. [CrossRef] [PubMed]

136. Leung, J.; Giraudat, J. Abscisic acid signal transduction. Annu. Rev. Plant Phys. 1998, 49, 199–222. [CrossRef] [PubMed]

137. McCourt, P. Genetic analysis of hormone signaling. Annu. Rev. Plant Phys. 1999, 50, 219–243. [CrossRef] [PubMed]
138. Finkelstein, R.R.; Gampala, S.S.; Rock, C.D. Abscisic acid signaling in seeds and seedlings. *Plant Cell* **2002**, *14*, S15–S45. [CrossRef] [PubMed]

139. Lorenzo, O.; Chico, J.M.; Saénchez-Serrano, J.J.; Solano, R. JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in Arabidopsis. *Plant Cell* **2004**, *16*, 1938–1950. [CrossRef]

140. Anderson, J.P.; Badruzsaufari, E.; Schenk, P.M.; Manners, J.M.; Desmond, O.J.; Ehler, C.; Maclean, D.J.; Ebert, P.R.; Kazan, K. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *Plant Cell* **2004**, *16*, 3460–3479. [CrossRef]

141. Yadav, V.; Mallappa, C.; Gangappa, S.N.; Bhatia, S.; Chattopadhyay, S. A basic helix-loop-helix transcription factor in Arabidopsis, MYC2, acts as a repressor of blue light-mediated photomorphogenic growth. *Plant Cell* **2004**, *16*, 1938–1950. [CrossRef]

142. Anderson, J.P.; Badruzsaufari, E.; Schenk, P.M.; Manners, J.M.; Desmond, O.J.; Ehler, C.; Maclean, D.J.; Ebert, P.R.; Kazan, K. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *Plant Cell* **2004**, *16*, 3460–3479. [CrossRef]

143. Yadav, V.; Mallappa, C.; Gangappa, S.N.; Bhatia, S.; Chattopadhyay, S. A basic helix-loop-helix transcription factor in Arabidopsis, MYC2, acts as a repressor of blue light-mediated photomorphogenic growth. *Plant Cell* **2004**, *16*, 1938–1950. [CrossRef]

144. Anderson, J.P.; Badruzsaufari, E.; Schenk, P.M.; Manners, J.M.; Desmond, O.J.; Ehler, C.; Maclean, D.J.; Ebert, P.R.; Kazan, K. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *Plant Cell* **2004**, *16*, 3460–3479. [CrossRef]

145. Anderson, J.P.; Badruzsaufari, E.; Schenk, P.M.; Manners, J.M.; Desmond, O.J.; Ehler, C.; Maclean, D.J.; Ebert, P.R.; Kazan, K. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *Plant Cell* **2004**, *16*, 3460–3479. [CrossRef]

146. Anderson, J.P.; Badruzsaufari, E.; Schenk, P.M.; Manners, J.M.; Desmond, O.J.; Ehler, C.; Maclean, D.J.; Ebert, P.R.; Kazan, K. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *Plant Cell* **2004**, *16*, 3460–3479. [CrossRef] [PubMed]

147. Anderson, J.P.; Badruzsaufari, E.; Schenk, P.M.; Manners, J.M.; Desmond, O.J.; Ehler, C.; Maclean, D.J.; Ebert, P.R.; Kazan, K. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *Plant Cell* **2004**, *16*, 3460–3479. [CrossRef] [PubMed]

148. Anderson, J.P.; Badruzsaufari, E.; Schenk, P.M.; Manners, J.M.; Desmond, O.J.; Ehler, C.; Maclean, D.J.; Ebert, P.R.; Kazan, K. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *Plant Cell* **2004**, *16*, 3460–3479. [CrossRef] [PubMed]

149. Anderson, J.P.; Badruzsaufari, E.; Schenk, P.M.; Manners, J.M.; Desmond, O.J.; Ehler, C.; Maclean, D.J.; Ebert, P.R.; Kazan, K. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *Plant Cell* **2004**, *16*, 3460–3479. [CrossRef] [PubMed]

150. Jeong, J.; Kim, K.; Kim, M.E.; Kim, H.G.; Heo, G.S.; Park, O.K.; Park, Y.I.; Choi, G.; Oh, E. Phytochrome and ethylene signaling integration in Arabidopsis occurs via the transcriptional regulation of genes co-targeted by PIFs and EIN3. *Front. Plant Sci.* **2016**, *7*, 1055. [CrossRef] [PubMed]