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Diverse role of basic Helix-Loop-Helix (bHLH) transcription factor superfamily genes in the fleshy fruit-bearing plant species

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Abstract: The basic Helix-Loop-Helix (bHLH) superfamily is the most widespread family of transcription factors in eukaryotic organisms, which can activate the expression of genes by interacting with specific promoters in the genes. The bHLH transcription factors direct the development and metabolic process of plants, including flowering initiation and secondary metabolite production, by attaching to specific sites on their promoters. These transcription factors are essential for encouraging plant tolerance or the adjustment to harsh environmental conditions. The involvement of *bHLH* genes in anthocyanin formation in fleshy fruit-bearing plants, as well as the role of these genes in response to stimuli including drought, salt, and cold stress, are discussed in this article. New concepts and goals for the production of stress-tolerant fruit species are suggested. Furthermore, solid evidence for the critical role of *bHLH* genes in the growth and development, as well as anthocyanin biosynthesis in fleshy fruit plants, are also presented in this article. This review identifies several future research directions that can shed light on the roles of *bHLH* genes in fruit-bearing plants and will assist the use of these genes in efforts to breed fruit crop varieties that are more resistant to stress. Generally, there has been little research carried out on the role of *bHLHs* transcription factor family genes in fleshy fruit-bearing plant species and more in-depth studies are required to fully understand the diverse role of *bHLH* genes in these species.

Keywords: anthocyanin; bHLH TFs; fruit crops; growth and development; stress response

The bHLH (basic Helix-Loop-Helix) transcription factor family is the largest gene family in plants that regulates the growth and development, anthocyanin biosynthesis, etc. (Mao et al. 2017; Li et al. 2021; Shen et al. 2021). The *bHLH* gene family gets its name from the bHLH domain it contains. This domain is comprised of 50–60 amino acids which can be divided into basic amino acids at the N-terminus (10–15 amino acids) and the Helix-Loop-Helix (HLH) at the C-terminus (about 40 amino acids) (Li et al. 2006; Miao et al. 2020; Zhang et al. 2020). Furthermore, this region contains six basic residues and a highly conserved HER motif (His

5-Glu 9-Arg 13), both of which are believed to bind certain DNA sequences (Kavas et al. 2016; Kurt & Filiz 2018; Sun et al. 2020).

The bHLH motif was discovered in the murine transcription factors E12 and E47 for the first time (Murre et al. 1989; Atchley & Fitch 1997). Following the discovery of multiple novel bHLH proteins, understanding their interrelations has become increasingly challenging, and a natural classification scheme is desperately required to bring order to this enormous and crucial set of proteins. For this purpose Atchley and co-workers presented an evolutionary categorisation of the bHLH motif based on 242 dif-

ferent amino acid sequences; a subset of 122 divergent sequences was used for the final phylogenetic analysis (Atchley & Fitch 1997).

As a result of the completion of the genome sequencing for multiple species, various plant bHLH proteins have been found and classified (Mao et al. 2017; Zhou et al. 2020). However, in comparison to animal species research, such studies are quite limited (Mao et al. 2017; Kavas et al. 2016). Based on the evolutionary relationships, DNA-binding specificity, and the conservation of particular amino acids and domains (other than the bHLH domain), animal bHLH proteins have been categorised into six groups, A through F (Atchley & Fitch 1997). After that, these bHLH proteins are subdivided into smaller subfamilies.

The number of groups in plant species, on the other hand, has not been conclusively defined, but it is considered to be between 15 and 26 (Pires & Dolan 2010; Mao et al. 2017; Zhang et al. 2018b). Phylogenetic investigations of several atypical bHLH proteins have brought the total number of bHLH proteins to 32 (Carretero-Paulet et al. 2010). Several additional motifs and amino acids are shared by different bHLH subgroups (Pires & Dolan 2010; Mao et al. 2017; Feller et al. 2011).

According to the current research, plant bHLH-coding genes are involved in a variety of physiological and biological activities like light signalling, stomata and root development, hormone signalling, and abiotic stress responses (Petroni & Tonelli 2011). This review describes the current findings on the *bHLH* TFs gene family role in the response to environmental stresses, growth and development, and anthocyanin accumulation in fleshy fruit-bearing plants.

Role of *bHLH* family genes in anthocyanin biosynthesis

Anthocyanins are powerful antioxidants as well as water-soluble vacuolar pigments. They also have a number of biological activities, such as protection from solar exposure and ultraviolet radiation, free radical scavenging and antioxidant potential, defensive performance against various pathogens, and signalling pathway regulation (Kayesh et al. 2013; Jezek et al. 2018). Fruit plants that contain anthocyanins include, for example, blackberries, raspberries, blueberries, cherries, currants, oranges, plums, peaches, pears, and grapes (Kayesh et al. 2013). Anthocyanins are involved in the red, purple,

and blue colouration of different organs in a variety of plant species. Anthocyanins also play many different roles in stress tolerance, herbivore and pathogen resistance, pollinator, and seed disperser attraction (Shang et al. 2011; Xie et al. 2012). Furthermore, anthocyanins have several other characteristics that improve human nutritional health, including their ability to operate as potent antioxidants and free radical scavengers (Xie et al. 2012). The vivid organ colours that result from anthocyanin deposition affect the ornamental, dietary, and market value of cereal, ornamental, and fresh fruits (Xie et al. 2012). Two types of genetic regulation play a role in the synthesis of anthocyanin in plants. Among them, the first is the biosynthetic genes, which encode the enzymes required for anthocyanin syntheses (Feng et al. 2016; Khan et al. 2022), including chalcone synthase (CHS), chalcone isomerase (CHI), dihydroflavonol-4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX), and UDP-glyucose (Khan et al. 2022), and the second are regulatory genes that regulate the magnitude and structure of the biosynthetic genes (Feng et al. 2016). With this type, anthocyanin regulation studies focus on R2R3- myeloblastosis (MYB), a basic helix-loop-helix (bHLH), and WD40 groups (Feng et al. 2013). These three regulators can combine to form a protein complex called MYB-bHLH-WD40, which ties to promoters and then stimulates transcription of the anthocyanin biosynthetic genes (Feng et al. 2013).

The flavonoid pathway, in which biosynthetic genes are regulated by the MYB-bHLH-WD40/WDR (MBW) regulatory network, is used to synthesise anthocyanins (Xie et al. 2012). Members of this transcriptional network, such as the bHLH and myeloblastosis (MYB) transcription factors, have been widely studied in herbaceous model plants like *Arabidopsis*, petunias, maize, snapdragons, tomatoes, and others (Koes et al. 2005; Ramsay & Glover 2005; Ballester et al. 2010; Xie et al. 2012) and in fruit species like apples, *Ziziphus jujuba*, pears, the kiwifruit, etc., for the past three decades (Li et al. 2021; Shi et al. 2019; Wang et al. 2019). The importance of this complex in regulating the anthocyanin production in higher plants has been thoroughly documented in a variety of species. Furthermore, locus mutation and gene modification of the *bHLH* and *MYB* genes have increased many red or purple cereal, fruit, and aesthetic crops high in anthocyanins, offering consumers several health benefits (Xie et al. 2012). As a result, there is a lot of excitement about uncovering new regulatory genes

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in commercially and economically beneficial plants. The discovery of an increasing amount of MYB Transcription Factors (TFs) in woody fruit trees suggests that the MBW model is also functional in perennial deciduous plants. Nevertheless, most plants' bHLH interaction partners have not been functionally characterised, especially in deciduous ornamental and fresh fruit trees.

Regarding the role of the *bHLH* genes in anthocyanin biosynthesis in fleshy fruit-bearing species like apples, Xie et al. (2012) demonstrated that *MdbHLH3* regulates low temperature-induced anthocyanin biosynthesis and fruit colouration in apples. Similarly, Starkevič et al. (2015) presented the transcription level of the variant *PaMYB10.1-1* corresponds with the fruit hue in the cherry, although another form, *PaMYB10.1-3*, is abstemiously expressed in fruits, inducing anthocyanin synthesis in *Nicotiana tabacum*. The *PabHLH3* increases the MYB-induced anthocyanin synthesis, while *PabHLH33* inhibits it, as was shown in the analysis of two fruit-expressed *bHLH* genes (Starkevič et al. 2015). In the eggplants, there are two *SmbHLH* (*SmbHLH1* and *SmbHLH117*) genes identified that are related to the production of anthocyanins (Tian et al. 2019).

bHLH genes have a major role in the production of anthocyanin and hormone transduction in strawberry fruits also. The seven *FabHLH* genes (*FabHLH17*, *FabHLH25*, *FabHLH27*, *FabHLH29*, *FabHLH40*, *FabHLH80*, and *FabHLH98*) affect the fruit anthocyanin biosynthesis and hormone transduction (Zhao et al. 2018). Furthermore, according to the protein interaction network prediction, four *bHLHs* (*FabHLH25*, *FabHLH29*, *FabHLH80*, and *FabHLH98*) are implicated in the fruit anthocyanin synthesis and hormone signalling cascades (Zhao et al. 2018). On the other hand, the findings of Li et al. (2020) pointed to a possible mechanism by which *FvbHLH9* controls the anthocyanin production in strawberry fruits in an *HY5-bHLH9* heterodimer-dependent fashion that coordinates and boosts the anthocyanin synthesis.

Similarly, the genes of this family (*bHLH*) also have a vital role in the regulation of the fruit colour in kiwis. *AcMYB123* and *AcbHLH42* interact with *Arabidopsis thaliana* TT2 and TT8, respectively, and thus play critical roles in the anthocyanin biosynthesis through regulating *AcANS* and *AcF3GT1* (Wang et al. 2019). *AcbHLH42* also interacts directly with *AcMYBF110*, *A. thaliana* PAP1's most genetically similar ortholog, to regulate the synthesis of some

anthocyanins (Wang et al. 2019). As transcription factors regulate the gene expression in many tissues and developmental stages of plants, primarily by inhibiting or stimulating the associated downstream genes, affecting plant growth, development, and stress response, among them bHLH is the second-largest transcription factor family (Zhang et al. 2018a). The peach (family Rosaceae) is a diploid model plant ($2n = 2x = 16$) with a small genome size of 265 Mb. The colour of the flesh is an attractive and crucial quality for the commercial value of peaches. Peaches come in four various flesh colours: white, green, yellow, and red in general (Zhang et al. 2018a). Anthocyanin is thought to have a role in the production of colouration in some fruit species, and *bHLH* genes have been implicated in this process. For this purpose, Zhang et al. (2018a) investigated and discovered 48 pairs of paralogous *bHLH* genes on the 8 chromosomes of the peach genome, as well as a few *bHLH* genes that may be implicated in fruit ripening and anthocyanin biosynthesis, by combining the relative expression assessment of the *bHLH* genes in red-fleshed peach fruit at five stages of the fruit's development.

bHLH genes play a significant role in pear anthocyanin biosynthesis regulation. In one of the studies, two *bHLHs* with MIR domains were discovered as positive regulators of anthocyanin production in pear fruits (Li et al. 2021). Furthermore, Li et al. (2021) evaluated the functions of *PbbHLH2* in the anthocyanin biosynthesis in the pear fruit peel and found that the role of the MIR-domain was important in the interactions between *PbbHLH2* and *PbMYB* (*PbMYB 9*, *PbMYB 10*, and *PbMYB10b*). The MIR-domain of *PbbHLHs* coupled with *PbMYBs* make complexes that boosted the expression of *PbDFR*, *PbANS*, and *PbUFGT* in pear fruits, accelerating the anthocyanin production (Li et al. 2021). Moreover, the MIR-domain of *PbbHLH2* increased the anthocyanin accumulation and triggered the regulation of anthocyanin biosynthesis genes on its own (Li et al. 2021).

In addition, bHLH and MYB proteins play a key role in the fine adjustment of the anthocyanin biosynthesis in tomatoes. Furthermore, Gao et al. (2018), checked the expression of *bHLH* (*SITT8*) and *MYB* (*SIANT1* and *SIAN2*) genes in transgenic lines. The expression of *MYB* (*SIANT1* and *SIAN2*) was stable, but the expression of *bHLH* (*SITT8*) was very low. They suggest that the low expression of *bHLH* (*SITT8*) may cause low levels of anthocyanins in tomatoes.

The Chinese jujube (*Ziziphus jujuba* Mill.) species is in high demand in many Asian countries due to its good flavour and nutritious benefits (Wang et al. 2016; Liu et al. 2020). *ZjGL3a*, *ZjGL3b*, and *ZjTT8* share a significant degree of homology with bHLH transcription factors implicated in the anthocyanin production in other fruit-bearing plants (Shi et al. 2019). Thus, the authors reveal that the three potential *bHLH* genes (*ZjGL3a*, *ZjGL3b*, and *ZjTT8*) may have an important role in anthocyanin biosynthesis in the jujube fruit. The differences in the anthocyanin content in the jujubes were commensurate with the relative expression levels of *ZjGL3a*, *ZjGL3b*, and *ZjTT8* (Shi et al. 2019). Furthermore, they found that the expression patterns of the jujube *ZjbHLH* genes were changed during fruit development.

The *bHLH*'s function in growth and development

The *bHLH*s have an essential role in the growth and development of the fleshy fruit species (Wang et al. 2020). *MabHLH6* was shown to be upregulated in bananas at both the transcript and protein levels, and it had a favourable impact on the expression of the genes responsible for starch degradation during fruit maturation (Xiao et al. 2018; Wang et al. 2020). *MabHLH6* was suppressed by *MaMYB3*, which slowed down the ripening of banana fruits (Fan et al. 2018). During banana fruit ripening, the *MabHLH7* transcript levels increased, which was ethylene inducible and nuclear-localised (Wang et al. 2020; Song et al. 2020). Simultaneously, Wang et al. (2020) discovered that at 0 days post-harvest (DPH), 8 DPH, and 14 DPH, *MabHLH229*, *MabHLH035*, *MabHLH152*, *MabHLH263*, *MabHLH155*, and *MabHLH157* were significantly expressed and gradually decreased, indicating that these genes may have a negative critical role in the regulation of banana fruits at the time of post-harvest ripening.

Similarly in the pineapple, the cryptochrome-interacting *bHLH2*, *AcCIB2* (*AcbHLH8*), is involved in the flowering time and also participates in the regulation and response to abiotic stress (Aslam et al. 2020).

SIAN11, a tomato WD40 protein that works in tandem with the bHLH and MYB proteins to fine-tune flavonoid synthesis and seed dormancy, is critical for tomato seed dormancy (Gao et al. 2018).

In the Chinese jujube (*Z. jujuba*) many *ZjbHLH* genes have been recognised as being implicated in the floral and fruit development (Li et al. 2019). Further-

more, *ZjbHLH83* was found to be highly expressed during the early stages of the floral development (Li et al. 2019). Furthermore, in the jujube, *ZjbHLH65* (homologs of *CIB1*), *ZjbHLH74*, and *ZjbHLH 75* (homologs of *CIB3*), *ZjbHLH68* (homologs of *CIB4*), and *ZjbHLH69* (homologs of *CIL1*) play a significant role in the floral development (Li et al. 2019).

Role of bHLH transcription factor (TF) family genes in stress tolerance

In addition to the functions in normal plant growth, development, flowering, and anthocyanin accumulation, numerous *bHLH* genes participate in the signal transduction and resistance to biotic or abiotic stresses, such as salinity, drought, low temperature, and nutrient deficiencies (Qi et al. 2020; Guo et al. 2021).

Response of *bHLH* genes to abiotic stress

In apples, most of the predicted proteins interacted with *MdbHLH24* involved in the abscisic acid (ABA) signalling pathway, which is important for the abiotic stress response (Agarwal et al. 2006). Furthermore, the *MdbHLH24* transcription factor gene may regulate the stress tolerance by regulating the expression of key genes in the ABA signalling pathway in apples (Mao et al. 2017).

Some bHLH TFs are activated under stress environments and attach themselves to the promoters of key genes engaged in multiple signalling cascades, regulating plant stress tolerance by regulating the transcription level of the target genes. For example, in *A. thaliana*, the bHLH domain of *ICE1* is highly similar in the amino acid sequence to that of a known MYC-related bHLH transcription factor (Chinnusamy et al. 2003). Furthermore, *ICE1* encodes an MYC-like bHLH transcriptional activator. The *AtICE1* specifically attaches to the MYC recognition sequence (CANNTG; a kind of E-box) in the promoter region of the *CBF3* gene in response to low temperatures and induces *CBF3* transcription, increasing the cold stress tolerance in the model plant *A. thaliana* (Chinnusamy et al. 2003; Mao et al. 2017). Plants' reactions to abiotic stress, comprising drought, low temperature, salt, ABA, and mechanical injury, are thought to be mostly regulated by the *bHLH* genes. For example, in the model plant *A. thaliana*, the *bHLH* genes (*AtbHLH006*, *AtbHLH17*, *AtbHLH32*, and so on) are implicated in the ABA signalling pathway

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to enhance drought resistance (Le et al. 2017; Dong et al. 2021). However, these types of studies are very rarely conducted in fleshy fruit-bearing plant species. Therefore, special emphasis will be paid on the role of bHLH TFs in some important fleshy fruit crops in the subsequent chapters.

The function of bHLH genes in drought stress tolerance. Drought stress is one of the most important environmental factors that can affect the plant physiology, particularly in the areas of development and photosynthesis, hormone metabolism, enzyme mechanism, productivity, and so on, resulting in irreparable plant damage (Chen et al. 2012; Wang et al. 2015, 2016a, 2020; Sun et al. 2018; Guo et al. 2021). bHLH genes are also involved in the plant's response to drought stress. The response of bHLH TF genes to drought stress in fruit species is systematically described in the studied fruit-bearing species, the *MdbHLHs* in apples likely interact with proteins and regulatory networks for the genes involved in drought or salt stress responses (Mao et al. 2017). In one of the studies, *MdCIB1*, an apple bHLH transcription factor, was found to be a positive activator of abscisic acid (ABA) sensitivity and with the ABA-mediated stomatal closure in apples (Ren et al. 2021). Furthermore, *MdCIB1* (a bHLH transcription factor, cryptochrome-interacting bHLH 1) transgenic *Arabidopsis* had a higher proline content, a reduced MDA content, and a decreased H₂O₂ and O₂ deposition, as well as an improved antioxidant enzyme activity under drought stress, protecting plants from osmotic and oxidative damage or harm (Ren et al. 2021). Thus, the *MdCIB1* gene has a crucial role to play in drought stress tolerance.

Similarly, in another study, Wang et al. (2016a) chemically synthesised the codon-optimised *VvbHLH1* gene from grapes. The *VvbHLH1*-overexpressing *A. thaliana* plants produced more flavonoids and were more resistant to salt and drought stress. The *VvbHLH1* gene can improve the abiotic stress tolerance and raise the concentration of important flavonoids in *A. thaliana*, as well as in other plants (Wang et al. 2016a). The schematic model of drought stress tolerance in apples and grapes, as an example, is shown in Figure 1.

The function of bHLH genes in cold stress tolerance. A low temperature is an important environmental element that has a negative impact on the plant growth, development, yield, quality, and fruit crop distribution. Plants must control a variety of physiological and biochemical processes to respond to cold

stress, which frequently necessitates bHLH TF involvement (Guo et al. 2021).

In apples, only a few studies have been published on the control of *MdbHLH* genes regarding cold stress tolerance. *MdCIBHLH1*, an *AtICE1*-like protein from apples, was discovered by Feng et al. (2012) and later on by Mao et al. (2017), and concluded that it may attach to the MYC recognition site in the promoter region of the *DREB* family gene *MdCBF2*, and up-regulate the *MdCBF2* expression to modulate the apple plant resilience to cold temperatures.

On the other side, the five *MabHLHs* were discovered to contribute to methyl jasmonate (MeJA)-induced chilling tolerance in banana fruits, and *MaMYC2* from the bHLH gene family can connect with *MaICE1* to govern induced chilling tolerance in fruit (Zhao et al. 2013; Wang et al. 2020).

Grapes (*Vitis vinifera* L.) are one of the world's most frequently produced fruit crop. Abiotic factors like low temperatures and winter droughts, on the other hand, often significantly reduce the grape output. Due to the extreme continental climate, only a limited group of grape cultivars survive under natural conditions of low temperatures and low air humidity in the winter in the northern coldest key grape-producing zones (Wang et al. 2018). For this

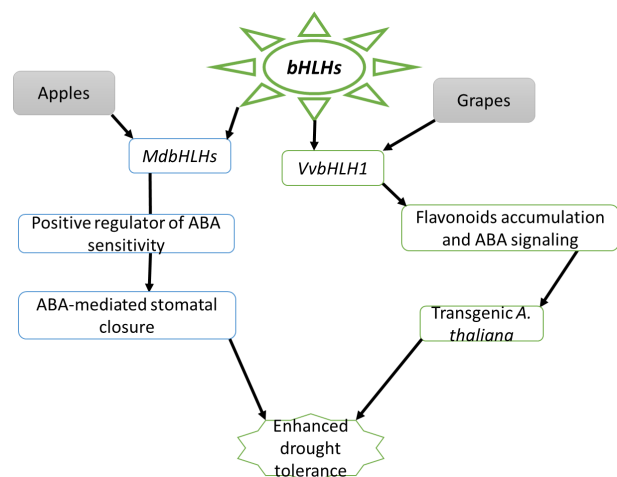


Figure 1. The role of the bHLH genes in apples and grapes in response to drought stress; in apples, the *MdbHLHs* interact like proteins and regulatory networks for the genes involved in drought stress tolerance; *MdbHLHs* work as a positive activator of abscisic acid (ABA) sensitivity and ABA-mediated stomatal closure in apples; on the other side, in grapes, *VvbHLH1* boosts the flavonoid accumulation and ABA signalling in transgenic *A. thaliana*, thus conferring drought resistance

purpose, Wang et al. (2018) sought to find *bHLH* TFs in grapes to investigate their possible significance in cold and drought tolerance, as the *bHLH* family has been linked to abiotic stress tolerance in other several species.

Furthermore, Chen et al. (2012) and later Wang et al. (2018) concluded that ABRE elements were found in the promoters of *VvbHLH003* and *VvbHLH007* that are actively engaged in ABA-dependent or independent stress tolerance, as a result, these genes may play an essential part in the grape's response to various stresses. Moreover, Wang et al. (2018) claimed that based on the GO and Nr function annotation, the three genes (*VvbHLH003*, *VvbHLH007*, and *VvbHLH10*) that are likely associated with anthocyanin or flavonol production are also anticipated to have other activities involved in response to abiotic stresses, especially cold stress tolerance.

The transgenic expression of *VabHLH1* from the wild Amur grape (*Vitis amurensis* Rupr.) improves the transgenic *A. thaliana* cold tolerance (Xu et al. 2014). Furthermore, wild Amur grape genes *ICE1* and *ICE2* encode the MYC-Type *bHLH* transcription activators that regulate cold tolerance in *Arabidopsis* (Xu et al. 2014). *VaICE1* and *VaICE2* (both genes serve as *bHLH* genes) also regulate cold stress-related variables, such as electrolyte leakage, proline, and MDA amounts, reducing the reactive oxygen species (ROS) destruction and improving the osmotic defence (Xu et al. 2014; Guo et al. 2021).

Some potential *CpbHLH* genes may also be involved in papaya abiotic stress tolerance. *CpbHLH027* and *CpbHLH062*, for example, have been reported to participate under chilling stress (Yang et al. 2020). In this fashion, the transcripts of *CpbHLH027* and *CpbHLH062* were elevated during cold stress, showing that *CpbHLH027* and *CpbHLH062* may also be engaged in the chilling stress response (Yang et al. 2020).

Dong et al. (2021) recognised the *bHLH* genes in the Chinese white pear for the first time, and demonstrated that *PbrbHLH195* is actively engaged in the production of reactive oxygen species in response to cold stress, implying that members of the *PbrbHLH* family play an important role in pear cold stress tolerance. By interacting with *PuHHP1*, the *bHLH* transcript factor *ICE1* may improve the *Pyrus ussuriensis* cold tolerance by increasing the *PuDREBa* transcription levels (Huang et al. 2015). The model of cold stress tolerance in apples and pears, as an example, is shown in Figure 2.

The function of *bHLH* genes in salt and nutrient stress tolerance. The salinisation of soil is a main environmental issue globally. Plant development, metabolism, and reproduction can all be affected by salt stress, including photosynthesis (Sui et al. 2018), ion homeostasis, seed germination, membrane permeability (Sun et al. 2018), and so on. More and more research has shown that *bHLH* transcription factors are implicated in the plant response to abiotic stress and enhance the plant stress tolerance. The role of *bHLH* genes in response to salt and nutrient stress in the studied fruit species is discussed as follows:

In apples, *MdbHLH104*, a Fe-responsive *bHLH* gene, was discovered to encode a member of the *IVCbHLH* subgroup and was triggered by Fe deficiency (Zhao et al. 2016). *MdbHLH104*, which has been genetically engineered into the apple and calli, has been discovered to play a key role in the Fe acquirement and tolerance to Fe deprivation by attaching directly to the promoter sites of the *MdAHA8*, *MdbHLH38*, *MdbHLH39*, and *MdPYE* genes, affecting the PM H^+ -ATPase functioning (Zhao et al. 2016). Thus,

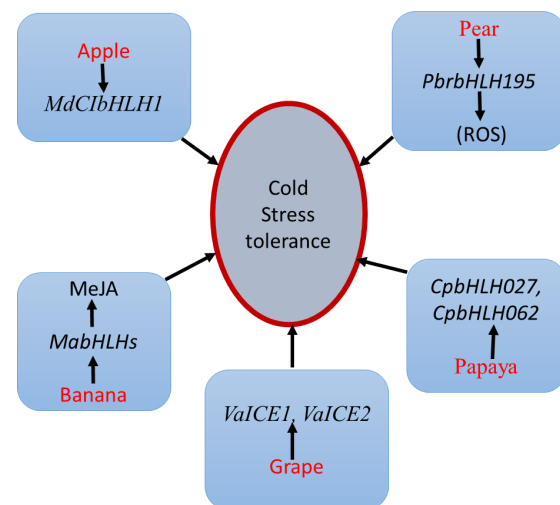


Figure 2. The regulatory role of *bHLH* genes during cold stress tolerance in apples, bananas, grapes, papayas, and pears; the *MdCibHLH1*, from apples, regulates the apple plant resistance to cold temperature; similarly, *MabHLHs* contribute to methyl jasmonate (MeJA)-induced chilling tolerance in banana fruits; in grapes, *VaICE1* and *VaICE2* *bHLH* TFs regulate the cold stress tolerance; the papaya's *CpbHLH027* and *CpbHLH062* genes participate in response to chilling stress; *PbrbHLH195* is actively engaged in the production of reactive oxygen species (ROS) in response to cold stress, implying that members of the *PbrbHLH* family play an important role in pear cold stress tolerance

<https://doi.org/10.17221/2/2022-CJGPB>Table 1. The regulatory role of *bHLH* genes in different developmental processes and stress resistance in fleshy fruit-bearing plants

Fruit species	Gene	Functions	References
Anthocyanin biosynthesis			
Apple	<i>MdbHLH3</i>	low temperature-induced anthocyanin production and fruit colouration	Xie et al. (2012)
Sweet cherry	<i>PabHLH3</i>	induce anthocyanin synthesis	Starkevič et al. (2015)
	<i>PabHLH33</i>	inhibits anthocyanin synthesis	Starkevič et al. (2015)
Eggplant	<i>SmbHLH1, SmbHLH117</i>	anthocyanin production	Tian et al. (2019)
	<i>FabHLH25</i>	fruit anthocyanin production and hormone signalling	Zhao et al. (2018)
Strawberry	<i>FabHLH29</i>	fruit anthocyanin production and hormone signalling	Zhao et al. (2018)
	<i>FabHLH80</i>	fruit anthocyanin production and hormone signalling	Zhao et al. (2018)
	<i>FabHLH98</i>	fruit anthocyanin production and hormone signalling	Zhao et al. (2018)
	<i>FvbHLH9</i>	boosting anthocyanin synthesis	Li et al. (2020)
Kiwi fruit	<i>AcMYB123</i>	anthocyanin biosynthesis by regulating <i>AcANS</i> and <i>AcF3GT1</i>	Wang et al. (2019)
	<i>AcbHLH42</i>	anthocyanin biosynthesis by regulating <i>AcANS</i> and <i>AcF3GT1</i>	Wang et al. (2019)
	<i>AcbHLH42</i>	interacts with <i>AcMYB110</i> regulate particular anthocyanin production	Wang et al. (2019)
Pear fruit	<i>PbbHLH2</i>	increased anthocyanin accumulation and triggered the regulation of anthocyanin biosynthesis genes	Li et al. (2021)
Jujube (<i>Ziziphus jujuba</i> Mill.)	<i>ZjbHLH</i>	anthocyanin production	Shi et al. (2019)
Growth and development			
Banana	<i>MabHLH6</i>	fruit ripening	Xiao et al. (2018); Wang et al. (2020)
Pineapple	<i>MabHLH7</i>	banana fruit ripening	Xiao et al. (2018)
	<i>AcCIB2 (AcbHLH8)</i>	regulates flowering time in pineapple	Aslam et al. (2020)
Jujube (<i>Z. jujuba</i>)	<i>ZjbHLH83</i>	floral and fruit development	Li et al. (2019)
	<i>ZjbHLH65</i>	plays a comparable role in floral development	Liu et al. (2013); Li et al. (2019)
	<i>ZjbHLH74</i>	floral development	Liu et al. (2013); Li et al. (2019)
	<i>ZjbHLH68</i>	floral development	Liu et al. (2013); Li et al. (2019)
Drought stress tolerance	<i>ZjbHLH69</i>	floral development	Liu et al. (2013); Li et al. (2019)
	<i>VvbHLH1</i>	flavonoids accumulation and ABA signalling	Wang et al. (2016a)
Cold stress tolerance			
Apple	<i>MdC1bHLH1</i>	resilience to cold temperatures	Feng et al. (2012)
Banana	<i>MabHLHs</i>	<i>MabHLHs</i> were discovered to contribute to methyl jasmonate (MeJA)-induced chilling tolerance in banana fruits	Zhao et al. (2013); Wang et al. (2020)

8 Table 1 to be continued

Fruit species	Gene	Functions	References
Cold stress tolerance			
Grapes	<i>VabHLH1</i>	cold tolerance	Xu et al. (2014)
Grapes	<i>VaICE1</i> , <i>VaICE2</i>	regulate cold stress-related variables	Xu et al. (2014); Guo et al. (2021)
Papaya	<i>CpbHLH027</i> , <i>CpbHLH062</i>	participate under chilling stress	Yang et al. (2020)
Pear	<i>PbrbHLH195</i>	play an important role in pear cold stress tolerance	Dong et al. (2021)
Salt stress tolerance			
Apple	<i>MdbHLH104</i>	plays an important role in Fe acquisition and tolerance to Fe deficiency	Zhao et al. (2016)
Cucumber	<i>CsbHLH041</i>	increased salt and ABA tolerance in transgenic <i>Arabidopsis</i> and cucumber seedlings	Li et al. (2020a)
Tomato	<i>LeFER</i> , a bHLH protein encoded by <i>Solyc06g051550.2.1</i> , <i>SlybHLH083</i>	<i>LeFER</i> plays a key function in tomato Fe-deficiency response	Ling et al. (2002); Wang et al. (2015)
Biotic stress tolerance			
Banana	<i>MabHLH062</i> , <i>MabHLH067</i> , <i>MabHLH153</i> , <i>MabHLH185</i>	involved in banana resistance to Foc TR4 infection	Wang et al. (2020)
Tomato	<i>SlybHLH131</i>	involved in the infection of TYLCV tolerance by VIGS	Wang et al. (2015)
Jujube (<i>Z. jujuba</i>)	<i>ZjbHLH</i> genes	many <i>ZjbHLH</i> genes have been identified as being responsive to phytoplasma stress	Li et al. (2019)

ABA – abscisic acid; TYLCV – Tomato yellow leaf curl virus; VIGS – virus-induced gene silencing

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MdbHLH104 has also been shown to improve the iron deficiency tolerance by boosting the *MdAHA8* transcription (Zhao et al. 2016; Mao et al. 2017).

Furthermore, the cucumber (*Cucumis sativus* L.) is a commercially agricultural commodity cultivated all over the world. Li et al. (2020) discovered the CsbHLH proteins' activities and regulatory processes, and as prospective genes for stress tolerance in cucumbers. The *35S:CsbHLH041* construct increased the salt and ABA tolerance in the transgenic *A. thaliana* and cucumber seedlings, indicating that *CsbHLH041* is a significant regulator in response to abiotic stimuli (Li et al. 2020).

The *bHLH* genes were also found to have an important role in response to the Fe-deficiency in tomatoes. The first discovered activator of iron intake in plants was LeFER, a bHLH protein encoded by *Soly-c06g051550.2.1*, *SlybHLH083*. LeFER plays a key role in the tomato Fe-deficiency response (Ling et al. 2002; Sun et al. 2015; Wang et al. 2015).

Role of *bHLH* genes in biotic stress tolerance

Little is known about the role of *bHLH* genes in biotic stress tolerance in fleshy fruits. In the banana, Wang et al. (2020) concluded that *MabHLH062*, *MabHLH067*, *MabHLH153*, and *MabHLH185* are involved in the banana resistance to the Foc TR4 infection (Foc tropical race 4), which is a destructive disease of commercial banana cultivars. Similarly, in tomatoes, the findings of Wang and colleagues suggested that *SlybHLH131* may be involved in the infection of TYLCV tolerance by VIGS (Wang et al. 2015). In *Z. jujuba* many *ZjbHLH* genes have been identified as being responsive to phytoplasma stress (Li et al. 2019). Various *bHLH* genes involved in various developmental and stress tolerance are shown in Table 1.

CONCLUSION AND FUTURE PERSPECTIVES

As a whole, we summarised the most recent research on *bHLH*'s role in the fruit species and provided a comprehensive overview of the bHLH transcription factor family genes in this review. The significance of *bHLH* genes in the anthocyanin formation in fleshy fruit-bearing plants, as well as its sensitivity to stimuli including drought, salt, and cold stress, are discussed in this article. Furthermore, the role of *bHLH* genes in response to biotic stress is also discussed. For

example, *MabHLH062*, *MabHLH067*, *MabHLH153*, and *MabHLH185* are important genes in bananas that play a key role in resistance to the Foc TR4 disease (Foc tropical race 4), which is a destructive disease of the commercial banana cultivars, and so on. This review contributes to a better understanding of this family and sheds light on how *bHLHs* regulate various physiological processes. Even so, many facets have not been described yet due to the complex intermodulation with other transcription factors.

The bHLH TF family has been widely investigated with increased emphasis placed on their involvement in response to environmental factors like cold temperatures, salt, drought, and iron shortage. For example, in grapes, three genes (*VvbHLH003*, *VvbHLH007*, and *VvbHLH10*) are associated with anthocyanin or flavonol production and also have a key role in response to cold stress (Wang et al. 2018). Moreover, *MdCIB1* (a bHLH transcription factor) triggered a higher proline content, a reduced MDA content, and decreased H₂O₂ and O₂ deposition in transgenic *A. thaliana*, as well as improved antioxidant enzyme activity under drought stress, thus protecting plants from osmotic and oxidative damage (Ren et al. 2021). However, publications on the subject have been limited to the model plant *A. thaliana*, and the unique roles of the bHLH TFs family in other species need to be investigated further.

Furthermore, most reports to date have mostly focused on a restricted sample of crops. Although several researchers applied a range of molecular biological approaches to investigate the involvement of *bHLH* genes in the growth, development, and stress responses in various plant species, a greater explanation of their activities and regulatory roles is still needed. Nowadays, bHLH TFs have received a great deal of attention as a crucial regulatory protein in plant stress signal networks. Since the identification of the first bHLH TFs in maize, researchers have steadily uncovered its critical role in plant stress response utilising a variety of biological approaches, such as genetic engineering, bioinformatics, transcriptomics, and metabolomics. However, due to the complexity of the bHLH regulatory network, the current knowledge of the framework and regulatory mechanism implicated in *bHLH* genes is still narrow. As regulatory genes, it is not fully clear which structural genes are regulated by bHLH TFs in the fruit colouration of various species. Furthermore, in recent years, research of *bHLH* genes in fleshy fruits has primarily focused on fruit colouring, but sufficient

research work should be carried out on other aspects of fruit tree crops. This presented review of studies focused on bHLH TF family members may provide hints for future research. An in-depth investigation into the mechanism of bHLH TFs in plant stress-tolerant regulatory networks will be an essential future research topic as well. Simultaneously, elucidating the *bHLH* stress-tolerant regulatory mechanism will have significant theoretical and practical implications for producing resilient crop varieties.

This is the first time comprehensive review report on the role of the *bHLH* genes on fleshy fruit-bearing plant species. This review highlights several future research directions for the identification of the roles of *bHLH* genes in fleshy fruit and other commercially important unexplored plants, mainly, in an effort to breed fruit crop varieties with improved stress tolerance and a higher anthocyanin content in the fruits. However, the regulatory mechanisms of *bHLH* genes must still be thoroughly investigated in fruit-bearing plants.

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