REVIEW

The role and pathway of VQ family in plant growth, immunity, and stress response

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Abstract

Main conclusion **This review provides a detailed description of the function and mechanism of *VQ* family gene, which is helpful for further research and application of *VQ* gene resources to improve crops.**

Abstract Valine-glutamine (VQ) motif-containing proteins are a large class of transcriptional regulatory cofactors. VQ proteins have their own unique molecular characteristics. Amino acids are highly conserved only in the VQ domain, while other positions vary greatly. Most *VQ* genes do not contain introns and the length of their proteins is less than 300 amino acids. A majority of VQ proteins are predicted to be localized in the nucleus. The promoter of many *VQ* genes contains stress or growth related elements. Segment duplication and tandem duplication are the main amplification mechanisms of the *VQ* gene family in angiosperms and gymnosperms, respectively. Purification selection plays a crucial role in the evolution of many *VQ* genes. By interacting with WRKY, MAPK, and other proteins, VQ proteins participate in the multiple signaling pathways to regulate plant growth and development, as well as defense responses to biotic and abiotic stresses. Although there have been some reports on the *VQ* gene family in plants, most of them only identify family members, with little functional verification, and there is also a lack of complete, detailed, and up-to-date review of research progress. Here, we comprehensively summarized the research progress of *VQ* genes that have been published so far, mainly including their molecular characteristics, biological functions, importance of VQ motif, and working mechanisms. Finally, the regulatory network and model of *VQ* genes were drawn, a precise molecular breeding strategy based on *VQ* genes was proposed, and the current problems and future prospects were pointed out, providing a powerful reference for further research and utilization of *VQ* genes in plant improvement.

Keywords VQ proteins · Molecular characteristics · Growth and development · Biotic and abiotic stresses · Importance of VQ motif · Working mechanisms · Precise molecular breeding

Abbreviations

Ka   The nonsynonymous substitution rate
Ks   The synonymous substitution rate

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Introduction

Gene expression is usually regulated by the interactions between cis-acting elements and transcription factors, and the latter often require some cofactors to co-regulate gene expression. Valine-glutamine (VQ) motif-containing protein is a specific class of transcriptional cofactor widely found in plants, containing the highly conserved VQ motif Fxxh-VQxhTG (x represents arbitrary amino acid, and h represents hydrophobic amino acid) (Jiang et al. 2018). In 2002, *AtVQ23/AtSIB1* was first identified in *Arabidopsis thaliana* (Morikawa et al. 2002). Subsequently, the *VQ* genes were successively identified in various plant species (Yuan et al. 2021). Many *VQ* genes are up- or down-regulated when exposed to environmental stress, pathogen invasion, and phytohormone treatment, and participate in various life

processes and responses to biotic or abiotic stress (Kim et al. 2013; Zhang et al. 2015; Song et al. 2016; Jiang et al. 2018). By mainly interacting with WRKY transcription factor (WRKY), mitogen-activated protein kinase (MAPK), and other proteins, VQ proteins function as important transcription regulators (Cheng et al. 2012; Li et al. 2014a; Ali et al. 2019; Yuan et al. 2021). Although there have been some studies on the *VQ* family genes, they are scattered, and the overall regulatory context and application prospects are not clear enough. In this study, we reviewed the detailed molecular characteristics, biological functions, and working mechanisms of VQ proteins in plants, and emphasized their importance in regulation of transcriptional activity. Meanwhile, we also pointed out the problems faced in current research and proposed corresponding solutions and future prospects, which will provide sufficient reference and guidance for researchers.

Structural features of *VQ* genes and proteins

The *VQ* family genes have been individually identified and systematically analyzed from different plants via bioinformatics and experimental methods, such as *Arabidopsis*, soybean, apple, tomato, cucumber, potato, tobacco, cotton, rice, maize, wheat (Table 1). Some studies have also performed large-scale analysis and comparison of *VQ* gene families in multiple plant species, including angiosperms and gymnosperms, to elucidate their characteristics and patterns (Jiang et al. 2018; Cai et al. 2019; Ma et al. 2023b; Tian et al. 2023). VQ proteins have their own specific molecular features. The conserved VQ motif is FxxhVQxhTG, with three main terminal amino acids, namely LTG, FTG, and VTG, but occasionally, there are other types in some plants, such as ITG, YTG, and LTR (Zhang et al. 2022a) (Fig. 1). The core element 'VQ' in FxxhVQxhTG is slightly changed in some plants, such as FxxhVHxhTG and FxxhVExhTG (Wang et al. 2017; Liu et al. 2022a; Zhang et al. 2022a; Tian et al. 2023). In addition to the VQ motif, although the amino acid sequences at other positions vary greatly, there are still some relatively conserved motifs in some VQ proteins (Weyhe et al. 2014; Tian et al. 2023). The top 5, top 10, and top 20 conserved motifs of VQ proteins have been identified in different plants (Cai et al. 2019; Tian et al. 2023) (Fig. 1), which may be related to protein localization and interaction. The homologous sequence of VQ proteins in different plants can be preliminarily determined by comparing the composition and quantity of motifs. Studies have shown that many VQ proteins contain single- or dual-component nuclear localization signals, and some also contain chloroplast targeting signals. Most VQ proteins are expected to be located in the nucleus, with a few in the cytoplasm, chloroplast, and mitochondria (Cheng et al. 2012; Kim et al.

2013; Jing and Lin 2015; Guo et al. 2018). Moreover, the N-terminal sequence of *Arabidopsis* AtVQ15 and AtVQ22 contains a calmodulin (CaM) binding motif, which is essential for their interaction with CaM (Perruc et al. 2004; Yan et al. 2018). In addition to interacting with CaM, this motif is also speculated to be related to the nuclear localization of proteins (Tian et al. 2023). Some VQ proteins also contain the predicted MAPK docking sites, which are necessary for their interaction with MAPK (Pecher et al. 2014). Most *VQ* genes in higher plants are intron-free and encode relatively small proteins with less than 300 amino acids (Jiang et al. 2018; Cai et al. 2019; Tian et al. 2023). The lack of introns in these *VQ* genes leads to more effective transcription and translation, thus generating these small proteins. Interestingly, Poaceae plants, such as rice, corn, and wheat, have a high GC content (up to 70%) in their *VQ* genes, which is prominent among many plants and may be related to their genetics and evolution (Tian et al. 2023). These basic characteristics of the *VQ* genes help us to better understand and identify this family of plants.

Based on structural and sequence similarity, the phylogenetic tree divided VQ proteins from different species into 7 groups (Kim et al. 2013; Wang et al. 2017), 8 groups (Zhang et al. 2022a), 9 groups (Dong et al. 2018; Tian et al. 2023), and 10 groups (Pecher et al. 2014; Cai et al. 2019). Among them, gymnosperms and angiosperms, as well as monocotyledons and dicotyledons, were found to have their own independent and intersecting branches, which provides evidence for the evolutionary history of *VQ* genes (Jiang et al. 2018; Cai et al. 2019; Tian et al. 2023). Segmental duplication and tandem duplication are considered to be the main mechanisms for the expansion of the *VQ* gene family in angiosperms and gymnosperms, respectively, and there is no necessary relationship between *VQ* numbers and genome size (Jiang et al. 2018; Xu and Wang 2022; Zhang et al. 2022a; Tian et al. 2023). The estimated number of *VQ* genes based on the times of genome replication events is inconsistent with the actual number, indicating that there may be gene loss events after genome replication (Wang et al. 2019). The substitution rates of Ka and Ks are the basis of analyzing the selection pressure in gene duplication events (Wang et al. 2010a). The Ka/Ks values of most duplicated *VQ* gene pairs are < 1, indicating that they mainly evolved under purification selection in plants (Wang et al. 2017; Cao et al. 2018; Jiang et al. 2018; Zhang and Wei 2019; Zhang et al. 2022a; Tian et al. 2023). These findings provide important references for evolutionary comparison and biological function of *VQ* genes in different plants.

In addition, cis-acting elements such as W-box (WRKY binding site), and SA-, JA-, or ABA-related elements were enriched in the promoter region of *VQ* genes in different plants. These cis-acting elements are mainly classified into four categories: hormone, stress, growth, and photo-reactive

Table 1 Identification of *VQ* family genes in different plants

Species	No. of total *VQ* genes	References
Arabidopsis thaliana	34	Cheng et al. (2012)
Arabidopsis thaliana	34	Jing and Lin (2015)
Brassica juncea	120	Zheng et al. (2022)
Brassica napus	118	Zou et al. (2021)
Brassica oleracea	64	Yang et al. (2023)
Brassica rapa	57	Zhang et al. (2015)
Camellia sinensis	25	Guo et al. (2018)
Cicer arietinum	19	Ling et al. (2020)
Coix lacryma-jobi	31	Wang et al. (2023b)
Cucumis melo	30	Zhang and Wei (2019)
Cucumis sativus	32	Shan et al. (2021)
Cucurbita pepo	44	Xu and Wang (2022)
Eucalyptus grandis	27	Yan et al. (2019)
Fragaria	19 (*F. nipponica*)	Zhong et al. (2018)
	21 (*F. iinumae*)	
	23 (*F. orientalis*)	
	23 (*F. vesca*)	
	23 (*F. nubicola*)	
	25 (*F. x ananassa*)	
Fragaria	25	Garrido-Gala et al. (2019)
Glycine max	74	Wang et al. (2014)
Glycine max	74	Zhou et al. (2016)
Glycine max	75	Wang et al. (2019)
Gossypium	89 (*G. hirsutum*)	Chen et al. (2020)
	89 (*G. barbadense*)	
	45 (*G. raimondii*)	
	45 (*G. arboretum*)	
Helianthus annuus	20	Ma et al. (2021)
Ipomoea	55 (*I. batatas*)	Si et al. (2023)
	58 (*I. triflida*)	
	50 (*I. triloba*)	
	47 (*I. nil*)	
Malus domestica	49	Dong et al. (2018)
Medicago truncatula	32	Ling et al. (2020)
Nicotiana tabacum	59	Liu et al. (2020)
Nicotiana tabacum	61	Yan et al. (2023)
Oryza sativa	39	Kim et al. (2013)
Oryza sativa	40	Li et al. (2014b)
Phyllostachys edulis	29	Wang et al. (2017)
Populus trichocarpa	51	Chu et al. (2016)
Prunus	55 (*P. yedoensis*)	Zhong et al. (2021)
	70 (*P. domestica*)	
	25 (*P. avium*)	
	23 (*P. dulcis*)	
	26 (*P. persica*)	
	23 (*P. yedoensis* var. *nudiflora*)	
Pyrus	41 (*Pyrus bretschneideri*)	Cao et al. (2018)
	28 (*Pyrus communis*)	
Saccharum spontaneum	78	Liu et al. (2022a)
Setaria italica	32	Liu et al. (2023)
Solanum lycopersicum	26	Ding et al. (2019)

Table 1 (continued)

Species	No. of total *VQ* genes	References
Triticum aestivum	113	Zhang et al. (2022a)
Triticum aestivum	65	Cheng et al. (2022a)
Vitis vinifera	18	Wang et al. (2015a)
Zea mays	61	Song et al. (2016)
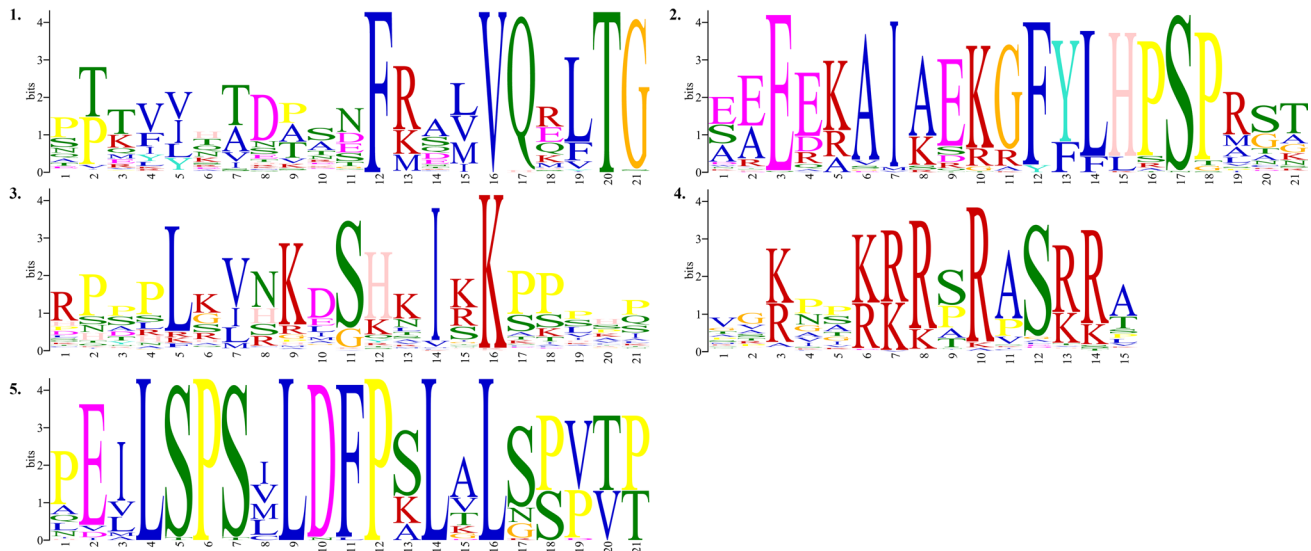
Fig. 1 The top 5 conserved motifs of VQ proteins. MEME online program (https://meme-suite.org) (Bailey et al. 2015) was used to identify motifs of VQ proteins from *Arabidopsis*, soybean, grape, tomato, Chinese cabbage, cotton, rice, maize, and wheat. Here, the top 5 motifs were listed. Motif analysis can help us to better understand protein structure and determine homologous sequences, and these motifs may play important roles in subcellular localization and interactions with different proteins

related elements (Wang et al. 2015a; Song et al. 2016; Wang et al. 2017; Zhang and Wei 2019; Zhang et al. 2022a). These findings suggest that *VQ* genes may be associated with responses to biotic and abiotic stresses, as well as growth and development. Additionally, *VQ* genes were reported to be regulated by microRNA (Guo et al. 2018; Zhang et al. 2022a). For example, 38 of 113 wheat *VQ* genes were predicted to be targeted by 15 putative miRNA. The latter belong to different miRNA families, such as miR160, miR395, miR1130, and miR9657, which play key roles in various biotic and abiotic stresses (Zhang et al. 2022a). These studies have expanded our understanding of the structure, function, and regulation of *VQ* genes, indicating that there may be more complex regulatory networks in plants.

Biological function of VQ protein

The role of VQ protein in plant growth and development

The growth and development process of plants is controlled by gene expression and its regulatory networks

(Scanlon and Timmermans 2013). The diverse spatiotemporal expression levels of *VQ* genes in different tissues and developmental stages indicate their widespread involvement in regulating plant growth and development (Wang et al. 2014, 2017; Zhou et al. 2016; Cao et al. 2018; Guo et al. 2018; Cai et al. 2019; Ling et al. 2020; Zhang et al. 2022a). Some studies have shown that knocking out or overexpressing certain *VQ* genes can significantly affect plant growth and development.

VQ proteins play important roles in regulating seed size, plant fertility, and growth. For example, the *AtVQ14/IKU1* is strongly expressed in early endosperm development, and the *vq14/iku1* mutant only produces small seeds, indicating that this gene may regulate endosperm development and thus affect seed size (Wang et al. 2010b). The loss-of-function mutants of *AtVQ8* showed yellow-green leaves and delayed growth, while plants overexpressing *AtVQ17*, *AtVQ18*, or *AtVQ22* showed a stunted phenotype with severely inhibited growth (Cheng et al. 2012). *AtVQ18* and *AtVQ26* were identified as two key VQ members involved in seed germination and early seedling establishment in *Arabidopsis*. The overexpression of *AtVQ18* or *AtVQ26* reduces the ABA response during seed germination, and

simultaneously reducing the expression of *AtVQ18* and *AtVQ26* can make the germinated seeds more sensitive to ABA, indicating *AtVQ18* and *AtVQ26* are functionally redundant (Pan et al. 2018). The growth and development of *AtVQ21/MKS1* overexpressing plants are inhibited, but the phenotype of RNAi plants is consistent with that of wild-type plants (Andreasson et al. 2005). Heterologous overexpression of *AtVQ21* in long-lived flowers (*Kalanchoë blossfeldiana*) and petunia (*Petunia hybrida*) promotes plant dwarfing and delayed flowering (Gargul et al. 2015). Compared with wild-type plants, the overexpression lines of *AtVQ23/SIB1* showed increased resistance to *Pseudomonas syringae*, but was accompanied by varying degrees of growth retardation (Xie et al. 2010). *AtVQ29* plays an important role in the photomorphogenesis of *Arabidopsis* seedlings. The expression level of *AtVQ29* is relatively higher in stem, and the length of the hypocotyl in overexpression plants is significantly longer than that in wild-type plants, while it is opposite in *vq29* mutants, indicating that *AtVQ29* regulates the elongation of the hypocotyl in seedlings under far red or weak light conditions (Li et al. 2014a). Meanwhile, *AtVQ29* overexpression plants also exhibited a delayed flowering phenotype without altering vegetative growth (Cheng et al. 2012). Leaf senescence is another developmental process that may be regulated by VQ proteins. AtVQ23/SIB1 and AtVQ16/SIB2 can interact with WRKY75 to negatively regulate ABA-mediated leaf senescence and seed germination (Zhang et al. 2022b).

In addition to *Arabidopsis*, the *VQ* genes in other plants were also found to affect plant growth and development. The overexpression of soybean *GmVQ43* and *GmVQ62* in *Arabidopsis* promotes flowering, and *GmVQ37* is associated with plant fertility (Zhou et al. 2016). Furthermore, the overexpression of apple *MdVQ37*, *MdVQ25*, or *MdVQ15* in *Arabidopsis* and tobacco affected vegetative and reproductive growth of plants (Dong et al. 2018). Cabbage *BoVQ25-1* is preferentially expressed in pollen and plays an important role in pollen germination (Yang et al. 2023). The overexpression of *OsVQ13* can increase grain size, and OsVQ13 can associate with OsMPK6 to affect grain development in rice (Uji et al. 2019). *ZmVQ52* is mainly expressed in maize leaves, and the overexpression of *ZmVQ52* in *Arabidopsis* can accelerate leaf senescence (Yu et al. 2019). The overexpression of wheat *TaVQ25* accelerates leaf senescence in *Arabidopsis* and leads to hypersensitivity reaction in ABA-induced leaf senescence, which is a positive regulatory factor for ABA-related leaf senescence (Meng et al. 2023).

The above results indicate that VQ proteins play an important role in balancing, promoting, or inhibiting plant growth. Plant hormones affect the growth and development of plants by influencing the expression of genes, and gene expression can also indirectly affect the secretion of plant hormones, thus forming interactive relationships (Oliva et al. 2013). Reactive oxygen species (ROS) and oxidative signals generated by metabolic or phytohormone pathways control almost all aspects of plant development (Considine and Foyer 2021). In addition, the absorption and transportation of plant nutrients also have an important impact on plant growth and development (Kopriva et al. 2019; Ahmad et al. 2023). Accordingly, we speculate that the changes in plant growth and development caused by VQ protein may be related to hormones, oxidation, or nutrients. In fact, VQ proteins can interact with transcription factors to regulate the expression of plant hormone synthesis and signal transduction related genes, leading to metabolic changes and ultimately affecting plant growth, development, and resistance (Cheng et al. 2012; Chen et al. 2018, 2022a; Yan et al. 2018; Hao et al. 2022; Gayubas et al. 2023; Meng et al. 2023). Therefore, the complex regulatory relationships between *VQ* genes, transcription factors, hormones, and the environment ensure the normal growth and development of plants.

The role of VQ protein in response to biotic stress

In general, plants resist pathogen infections through two different immune systems: ETI (effector-triggered immunity) and PTI (PAMP-triggered immunity) (Jones and Dangl 2006; Coll et al. 2011). The recognition of PAMP (pathogen-associated molecular patterns) activates a series of cellular signaling events, triggering changes in gene expression, cell biochemistry, and metabolism, ultimately leading to plant resistance to pathogen attacks (Senthil-Kumar and Mysore 2013; Pecher et al. 2014). Salicylic acid (SA) and jasmonic acid (JA) are two important hormone signaling molecules that mediate plant immunity and also participate in plant growth, development, and stress responses (Bari and Jones 2009; Meldau et al. 2012; Caarls et al. 2017; Myers et al. 2023). Studies have shown that the expression of many *VQ* genes in different plants is induced or inhibited by JA and SA hormones or different pathogen treatments (Cheng et al. 2012; Kim et al. 2013; Zhou et al. 2016; Jiang et al. 2018; Zhang et al. 2022a; Si et al. 2023). These results indicate that *VQ* genes play an important role in responding to complex signaling pathways mediated by hormones such as JA and SA.

The overexpression of *AtVQ4/MVQ1* in *Arabidopsis* reduces Flg22-induced resistance, thus AtVQ4/MVQ1 negatively regulates PAMP-induced pathogen resistance (Pecher et al. 2014). *AtVQ10*, as a positive regulator, is involved in JA-mediated signaling pathways to resist infection by *Botrytis cinerea* (Chen et al. 2018). *AtVQ16/SIB2* is strongly induced by pathogenic *B. cinerea*, and the disease resistance of its functional deficient mutant is reduced (Lai et al. 2011). The overexpression of *AtVQ20* in *Arabidopsis*

reduces plant resistance to *P. syringae*, indicating that *AtVQ20* negatively regulates the defense response (Cheng et al. 2012). *AtVQ21/MKS1* overexpression plants showed significantly increased resistance to *P. syringae* through SA signaling pathway (Andreasson et al. 2005), and decreased resistance to *B. cinerea* through negative regulation of the JA signaling pathway (Petersen et al. 2010; Fiil and Petersen 2011). The expression level of *AtVQ22/JAV1* significantly increased after JA treatment, and rapidly accumulated after mechanical injury. Its functional deficient mutants not only enhanced the resistance to necrotic pathogen *B. cinerea*, but also enhanced the resistance to herbivorous insects. It is a negative regulatory factor that controls plant defense without detected adverse effects on plant growth and development (Hu et al. 2013a). *AtVQ23/SIB1* can be significantly induced by SA, JA, or pathogens *P. syringae* and *B. cinerea*, and its overexpression in plants can observably enhance disease resistance. *AtVQ23/SIB1* can mediate the cross-talk between SA and JA signaling pathways in disease resistance (Narusaka et al. 2008; Xie et al. 2010). The overexpression of *AtVQ28* in *Arabidopsis* reduced resistance to *Phytophthora sojae* and *Phytophthora infestans*, and promoted infection of *Phytophthora parasitica*. *AtVQ28* negatively regulates non-host resistance (NHR) of plants to *Phytophthora* (Lan et al. 2022). Both *AtVQ12* and *AtVQ29* were strongly induced by JA and *B. cinerea*, and its overexpression plants showed a significant susceptible phenotype, whereas the single and double mutants showed significantly improved resistance to *B. cinerea* (Wang et al. 2015b).

Moreover, the overexpression of soybean *GmVQ35* or *GmVQ47* in *Arabidopsis* makes plants more susceptible to *B. cinerea*, indicating that these two *VQ* genes act as negative regulator in the response to necrotrophic pathogens (Zhou et al. 2016). Silencing *GmVQ58* can improve the resistance of soybean to common cutworm (Li et al. 2020a). The overexpression of *BnVQ7/BnMKS1* in rapeseed showed enhanced resistance to *Leptosphaeria maculans* in adulthood stage (Zou et al. 2021). Knocking out rapeseed *BnVQ12* enhanced plant resistance to *Sclerotinia sclerotiorum*, while overexpressing *BnVQ12* in plants showed the opposite effect (Zhang et al. 2022c). Tomato *SlVQ15* mutants showed reduced resistance to *B. cinerea*, while its overexpression plants showed increased resistance to pathogens (Huang et al. 2022a). Cucumber VQ protein CsSIB1 positively regulates cucumber defense response to downy mildew through the SA pathway (Tan et al. 2022). The overexpression of *OsVQ14* or *OsVQ32* in rice enhanced plant resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Li et al. 2021). It is worth noting that rice *OsVQ25* knockout mutants showed significantly enhanced resistance to rice blast and bacterial blight, without penalty of plant growth and development. The explanation of the function and mechanism of *OsVQ25* is of great significance for the study of *VQ* genes in

food crops (Hao et al. 2022). These results reflect the functional importance and diversity of VQ proteins in response to biotic stress.

The SA signaling pathway generally plays a role in defense against biotrophic pathogens; the JA-dependent signaling pathway usually exerts effect in defense against necrotrophic pathogens and insects; these two signaling pathways are sometimes antagonistic and sometimes synergistic (Halim et al. 2006; Bari and Jones 2009; Lai and Mengiste 2013; Ke et al. 2017). The above-mentioned results indicate that the *VQ* genes are widely involved in the JA- and SA-regulated disease resistance pathways and play a crucial role. The mechanism of plant disease resistance is the existence of resistance genes in plants, which can resist the invasion and spread of pathogens. These disease resistance genes are divided into physical resistance and chemical resistance factors (Miller et al. 2017; Parisi et al. 2019; Wang et al. 2020; Ninkuu et al. 2022). Therefore, the *VQ* gene should ultimately trigger at least one of these two disease resistance mechanisms, resulting in changes in plant immunity. In addition, there are many reports on the relationship between *VQ* gene and plant pathogen resistance, but relatively few reports on insect resistance, which requires more experiments to investigate this function.

The role of VQ protein in response to abiotic stress

Salt, drought, and low/high temperature are common abiotic stresses that impair the normal growth of plants and increase endogenous hormones such as ABA, which is a key hormone that helps plants to adapt and survive in these extreme environments (Tuteja 2007; Roychoudhury et al. 2013; Verma et al. 2016; Yu et al. 2020). Studies have found that the expression of a large number of *VQ* genes in many plants is induced or inhibited by salt, drought, temperature, low nitrogen stress, and abscisic acid (ABA), indicating that *VQ* genes also play important roles in regulating plant responses to abiotic stress (Kim et al. 2013; Wang et al. 2014; Wang et al. 2015a; Zhang et al. 2015; Song et al. 2016; Wang et al. 2017; Dong et al. 2018; Zhang and Wei 2019; Liu et al. 2020; Liu et al. 2022a; Zhang et al. 2022a; Si et al. 2023).

AtVQ9 is strongly induced by NaCl treatment, and its mutants exhibit higher seed germination rate and better seedling growth under NaCl treatment, while the overexpression lines exhibit the opposite effect, indicating that AtVQ9 negatively regulates the *Arabidopsis* resistance to NaCl stress (Hu et al. 2013b). AtVQ15 belongs to the calmodulin binding protein (AtCaMBP25), and its overexpression lines are highly sensitive to NaCl and osmotic stress during seed germination and seedling growth, while the mutants exhibit significant resistance, indicating that *AtVQ15* negatively regulates *Arabidopsis* tolerance to NaCl and osmotic stress (Perruc et al.

2004). The overexpression of poplar *VQ1* in *Arabidopsis* enhances resistance to salt stress and *P. syringae*, and confers resistance to multiple biotic and abiotic stresses by mediating ABA and SA signaling pathways (Liu et al. 2022b). The expression of bamboo *PeVQ28* is induced by salt and ABA treatment; the overexpression of *PeVQ28* in *Arabidopsis* shows increased tolerance to salt stress and is more sensitive to ABA; under salt stress, *PeVQ28* overexpression plants have low malondialdehyde, high proline, and increased expression levels of ABA signaling and ABA synthesis related genes. These results suggest that *PeVQ28* can mediate the positive regulation of salt tolerance through ABA-dependent signaling pathways (Cheng et al. 2020). The overexpression of sweet potato *IbWRKY2* in *Arabidopsis* exhibits strong drought and salt tolerance. IbWRKY2 can interact with IbVQ4, which can be induced by PEG or NaCl treatment, indicating that *IbVQ4* may play an important role in drought and salt tolerance of sweet potato (Zhu et al. 2020). Recent research has shown that *SlVQ16* positively regulates tomato resistance to salt stress, while *SlVQ21* negatively regulates tomato resistance to salt stress (Ma et al. 2023a). In addition, the overexpression of wheat *TaVQ4*-D in *Arabidopsis* and wheat plants increased their tolerance to drought stress. Under drought stress conditions, compared to the wild-type plants, transgenic wheat plants overexpressing *TaVQ4*-D showed increased levels of superoxide dismutase and proline, decreased levels of malondialdehyde, and up-regulated expression of genes related to reactive oxygen clearance and stress response (Zhang et al. 2023a). Similarly, under drought and salt stress, *TaVQ14* is up-regulated in wheat seeds, and heterologous expression of *TaVQ14* increases the resistance of *Arabidopsis* seeds to salt and drought stress (Cheng et al. 2022b). The overexpression of tomato *SlVQ6* in *Arabidopsis* showed a high sensitivity to high-temperature stress, indicating that *SlVQ6* negatively regulates plant thermotolerance (Ding et al. 2019). The overexpression of soybean *GmVQ47* in *Arabidopsis* displayed reduced heat tolerance in transgenic plants (Zhou et al. 2016). Under high-temperature stress, the relative transcription level of apple *MdVQ37* was significantly down-regulated; transgenic apple plants overexpressing *MdVQ37* exhibited a thermo-sensitive phenotype, resulting in a significant decrease in endogenous SA content and disruption of SA-dependent signaling pathways; exogenous SA could partially improve the survival rate of transgenic lines. These results suggest that the regulation of apple *MdVQ37* under high-temperature stress is related to the changes in transcription factor activity and SA homeostasis (Dong et al. 2021). RsVQ4, as a positive regulator of plant thermotolerance in radish, its overexpression can improve heat tolerance, while its RNAi plants have the opposite phenotype (He et al. 2023).

In addition, the analysis of the dynamic expression patterns of rice *VQ* genes under NO treatment found that 45% (14/31) of *VQ* genes showed significantly differential expression, indicating that *VQ* genes may play an important role in NO-mediated physiological processes (Peng et al. 2020). Transcriptome data of oxygen-related processes in *Arabidopsis* revealed that 56% (19/34) of *VQ* genes and 64% (48/75) of WRKY genes were up-regulated after ozone treatment (León et al. 2021). These data suggest that some VQ proteins have potential regulatory roles in response to NO and O_2. *COX6B-3* and *COA6-L* can be induced by osmotic stress, and their encoded proteins can interact with AtVQ27 in the presence of NO. The mitochondrial biogenesis was impaired in *vq27* mutant (Kumari et al. 2023). A hypermorphic *vq10-H* mutant of *AtVQ10* (T-DNA inserted into its 3'-UTR region) exhibits enhanced meristem development and increased tolerance to oxidative stress, as well as less sensitive to NO than wild-type plants (Gayubas et al. 2023).

The molecular mechanisms of plant responses to abiotic stress involve multiple processes, including sensing, signal transduction, transcription, transcript processing, translation, and post-translational protein modification (Waadt et al. 2022; Zhang et al. 2022d). The above findings suggest that the *VQ* genes regulate plant adaptation and resistance to abiotic stress through multiple pathways, such as ABA signaling, transcription factors, and ROS metabolism. Overall, *VQ* genes play important roles in both independent pathways and multi-factor cross-talk in the input stimuli of salt, drought, cold/heat, and ABA signals.

Working mechanisms of VQ protein

Interactions between VQ and WRKY

The precise regulation of gene expression usually requires transcription factors and transcription cofactors (Buscaill and Rivas 2014; Lai et al. 2019). As one of the largest transcription factor families in the plant kingdom, the WRKY family is involved in a variety of biological processes, including plant signal transduction, growth and development, and response to biotic and abiotic stresses (Jiang et al. 2017; Li et al. 2020b ; Wani et al. 2021; Goyal et al. 2023; Khoso et al. 2022). Many VQ proteins interact with WRKY transcription factors through conserved 'VQ' residues to regulate various biological activities, and WRKY proteins are also the main interacting proteins of the VQ family (Lai et al. 2011; Cheng et al. 2012; Weyhe et al. 2014). The interaction between VQ protein and WRKY protein depends on the WRKY domain, which is mainly associated with DNA binding (Bakshi and Oelmüller 2014). Therefore, their interaction can affect the DNA binding activity of WRKY, thereby regulating the expression of downstream genes.

WRKY33 has been reported to positively regulate plant defense response to *B. cinerea* (Zheng et al. 2006). AtVQ23/SIB1 and AtVQ16/SIB2 can bind to the C-terminal WRKY domain of WRKY33, stimulate its DNA binding activity, enhance its binding ability to the W-box, and positively regulate plant defense against *B. cinerea* (Lai et al. 2011; Cheng et al. 2012). Further research found that AtVQ23/SIB1 and AtVQ16/SIB2 can interact with WRKY33 and WRKY57. The presence of AtVQ23 and AtVQ16 further enhances the competition between WRKY57 and WRKY33, and their fine regulation of *B. cinerea* resistance will be more conducive to plant defense (Jiang and Yu 2016). In addition, AtVQ23/SIB1 and AtVQ16/SIB2 can interact with WRKY75 to inhibit its transcriptional repression function and regulate the expression of downstream *GLKs* genes, thereby regulating ABA-mediated leaf senescence and seed germination (Zhang et al. 2022b). By interacting with WRKY8 and inhibiting the binding activity of WRKY8 to the W-box, AtVQ9 regulates the expression of the salt stress resistance gene *AtRD29A*, thereby negatively regulating plant salt tolerance (Hu et al. 2013b). The interaction between AtVQ10 and WRKY8 in the nucleus activates the transcriptional activity of WRKY8, increases the expression of downstream defense-related gene *PDF1.2*, and positively regulates the basic defense of *Arabidopsis* against *B. cinerea* (Chen et al. 2018). AtVQ10 also interacts with WRKY25 and WRKY33, and F1 hybrids overexpressing AtVQ10 and WRKY25 or WRKY33 exhibit significantly slow and weakened growth, indicating that AtVQ10 and WRKY proteins synergistically inhibit plant growth and development (Cheng et al. 2012). AtVQ14/IKU1 can interact with WRKY10/MINI3 to promote the expression of *IKU2*, which synergistically regulates the development of endosperm, and then affects the size of seeds (Luo et al. 2005; Wang et al. 2010b). AtVQ15 can interact with CaM, WRKY25, and WRKY51, and may link Ca^{2+} signals with transcriptional regulation of downstream targets of osmotic stress signaling pathways, regulating plant tolerance to osmotic stress (Perruc et al. 2004; Cheng et al. 2012). Pollen-specifically expressed AtVQ20 can interact with WRKY2 or WRKY34 to form complexes, enhance the transcriptional repression activity of WRKY2 and WRKY34 to regulate the expression of downstream genes related to pollen development and pollen tube germination (such as *AtMYB97*, *AtMYB101*, and *AtMYB120*), thereby affecting male fertility (Lei et al. 2017, 2018). AtVQ22/JAV1 interacts with CaMs, JAZ8, and WRKY51 to form a JAV1-JAZ8-WRKY51 complex, which jointly regulates JA synthesis. Normally, the JAV1-JAZ8-WRKY51 complex can inhibit the expression of JA biosynthesis genes; when plants are subjected to insect feeding or mechanical damage, extracellular Ca^{2+} flows in and activates intracellular CaMs. CaMs bind to JAV1 in a Ca^{2+}-dependent manner and induce phosphorylation of JAV1. Subsequently, JAV1 is degraded

through the ubiquitin-26S proteasome pathway, thereby disrupting the JAV1-JAZ8-WRKY51 complex, alleviating the inhibition of JA signaling, promoting the expression of JA synthesis genes (such as *AtAOS*), leading to rapid outbreak of JA and activating plant defense (Hu et al. 2013a; Yan et al. 2018; Ali et al. 2019).

In addition, soybean GmVQ58 can interact with GmWRKY32, and silencing *GmVQ58* can significantly increase the expression levels of defense-related genes downstream of GmWRKY32 (*GmVSPβ* and *GmN:IFR*), thereby enhancing soybean resistance to common cutworm (Li et al. 2020a). Rapeseed BnVQ12 can interact with BnWRKY28 and promote the competitive binding of BnWRKY28 and BnWRKY33 to the promoter of BnWRKY33 to regulate the expression of downstream phytoalexin synthesis-related genes, thereby helping plants to effectively resist *S. sclerotiorum* (Zhang et al. 2022c). Banana *MaWRKY26* is induced by cold stress or methyl jasmonate (MeJA), enhancing the cold tolerance of banana fruit. MaVQ5 can interact with MaWRKY26, weakening the transcriptional activation of JA biosynthesis genes (*MaLOX2*, *MaAOS3*, and *MaOPR3*), indicating that MaVQ5 may act as an inhibitor of MaWRKY26 and participate in JA-mediated cold stress response (Ye et al. 2016). The interaction between RsVQ4 and RsWRKY26 promotes the expression of *RsHSP70-20*, thereby positively regulating the heat stress response in radish (He et al. 2023). Apple MdVQ10 and MdVQ15 were found to interact with MdWRKY52 to regulate defense and development (Dong et al. 2018). Apple MdVQ10 can also interact with MdWRKY75 to promote the expression of downstream senescence-related genes, thereby accelerating injury-induced leaf senescence, and is regulated by MdCML15 and MdJAZs (Zhang et al. 2023b). Moso bamboo PeVQ28 and PeWRKY83 interact in the nucleus, and the overexpression of *PeVQ28* promotes the expression of ABA-related genes downstream of PeWRKY83 and positively regulates salt tolerance in plants (Wu et al. 2017; Cheng et al. 2020). Tomato SlWRKY37 interacts with SlVQ7 to promote the expression of downstream *SlWRKY53* and *SlSGR1*, positively regulating JA- and dark-induced leaf senescence (Wang et al. 2022a). Tomato SlVQ15 interacts with SlJAZ and SlWRKY31, and participates in JA-mediated plant defense response to *B. cinerea* (Huang et al. 2022a). The latest research found that tomato SlWRKY57 plays a negative regulatory role in salt stress response. SlVQ16 and SlVQ21 competitively interact with SlWRKY57 and antagonize its transcriptional repression activity, thereby regulating the expression of downstream salt stress-related genes *SlRD29B*, *SlDREB2*, and *SlSOS1*. In addition, the SlWRKY57-SlVQ21/SlVQ16 module also interacts with the SlJAZ proteins and regulates the expression of JA-induced salt stress-related genes, suggesting that this module may be involved in the JA pathway to regulate tomato resistance

to salt stress (Ma et al. 2023a). TcJAV3 is a VQ protein in *Taxus chinensis*, and the TcJAV3-TcWRKY26 complex is involved in JA-mediated regulation of the taxol biosynthesis gene *DBAT* and plant defense response (Chen et al. 2022b). Rice OsVQ8 interacts with OsWRKY10 to inhibit its DNA binding and transcriptional activity. OsWRKY10 negatively regulates heat tolerance in rice by regulating ROS balance and allergic reaction, and its interacting protein OsVQ8 plays a antagonistic role. This functional module provides a safe and effective regulatory mechanism for rice response to heat stress (Chen et al. 2022a). Rice OsVQ25 balances the broad-spectrum disease resistance and growth of plants by interacting with U-Box E3 ubiquitin ligase OsPUB73 and the transcription factor OsWRKY53. OsVQ25 interacts with OsPUB73 and promotes the degradation of OsVQ25 through the 26S proteasome pathway, negatively regulating rice resistance to *Magnaporthe oryzae* and *Xoo.* Moreover, OsVQ25 interacts with OsWRKY53, a positive regulator of plant immunity, and inhibits its transcriptional activity. Defense-related genes downstream of OsWRKY53 are upregulated in the *OsVQ25* mutants. These results reveal a E3-VQ-WRKY module that can control plant immunity and growth at the transcriptional and post-translational levels (Hao et al. 2022). Recently, wheat TaVQ25 is reported to interact with TaWRKY133 to regulate ABA-mediated leaf senescence (Meng et al. 2023).

Based on the above studies, the WRKY transcription factors play a critical role in the function of VQ proteins. VQ proteins mainly regulate the expression level of downstream genes by regulating the transcriptional activation or repression activity of different WRKY transcription factors. Some WRKY transcription factors are known to have multiple effects and play a certain role in different aspects (Zheng et al. 2006; Jiang and Deyholos 2009; Li et al. 2011; Chi et al. 2013). VQ protein can form complexes with one or more WRKY proteins, while a WRKY protein can also interact with multiple VQ proteins. Therefore, the intricate interactions endow VQ proteins and WRKY proteins with a wide range of biological functions.

Interactions between VQ and MAPK

The immune response triggered by PAMP includes the changes in various phosphorylation cascades, among which one of the earliest and key phosphorylation-mediated signaling events is the activation of MAPK (Meng and Zhang 2013; Pecher et al. 2014; Yamada et al. 2016; Bi et al. 2018). Activated MAPKs phosphorylate downstream substrates to post-translationally regulate the function of many proteins, including changes in protein–protein interaction, protein activity, protein stability, thus promoting signal transduction in various environmental stresses and development processes (Taj et al. 2010; Pecher et al. 2014; Bigeard and Hirt

2018). Phosphorylation usually leads to targeted degradation of proteins through the ubiquitin–proteasome pathway (Henriques et al. 2009). Some VQ proteins can be targeted and phosphorylated by MPK3, MPK4, or MPK6, leading to their degradation, thereby affecting proteins that interact with VQ proteins, such as WRKY, and regulating plant immune responses (Pecher et al. 2014; Weyhe et al. 2014).

A total of 10 VQ proteins (MPK3/6 targeted VQ proteins, MVQ1-10) in *Arabidopsis* have been identified as phosphorylation substrates of MPK3 and MPK6, and these VQ proteins can also interact with a specific subset of WRKYs (Pecher et al. 2014). Normally, AtVQ4/MVQ1 can interact with WRKYs such as WRKY33 to suppress their transcriptional activity, thereby inhibiting the expression of downstream defense genes such as *NHL10*. However, when invaded by bacterial pathogens, AtVQ4/MVQ1 is phosphorylated by MPK3/6, which promotes its instability and degradation, leading to weakened or even ineffective activity inhibition of WRKYs. The expression level of downstream disease resistance genes increases along with the increase of phosphorylation level. MAPK3/6 participates in the interaction between AtVQ4/MVQ1 and multiple WRKYs to finely regulate the immune response in *Arabidopsis* (Pecher et al. 2014). AtVQ21/MKS1 was identified as a downstream substrate of MPK4. In the absence of pathogen infection, AtVQ21 can bridge MPK4 and WRKY33 to form a ternary complex, and physical interactions constrain the activity of WRKY33. When infected with pathogens, activated MPK4 phosphorylates AtVQ21, leading to denaturation and disassociation of the MPK4-AtVQ21-WRKY33 complex, completely releasing WRKY33 and targeting the promoter of antitoxin gene (*PAD3*) to regulate its expression, enhancing plant resistance to pathogens (Andreasson et al. 2005; Qiu et al. 2008; Fiil and Petersen 2011).

In addition, tomato SlVQ6 has been identified as a phosphorylation substrate of SIMPK1 and plays an important role in the response to some abiotic stresses such as drought, high temperature and salt; the overexpression of *SlVQ6* can reduce high-temperature tolerance of plants (Ding et al. 2019). It is reported that OsMPK6 positively regulates rice resistance to *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) and grain size/weight (Liu et al. 2015; Ma et al. 2017; Xu et al. 2018). Rice OsVQ1 interacts with OsMPK6, and the *OsVQ1* knockout mutant exhibit stronger resistance to *Xoo*, accumulate high levels of hydrogen peroxide, and exhibit a delayed flowering phenotype under natural long-term conditions (Wang et al. 2021). OsVQ13 can interact with OsMPK6 and activate the OsMPK6-OsWRKY45 component, positively regulating the JA signaling pathway and mediating rice resistance to bacterial blight. The overexpression of *OsVQ13* can also increase grain size, and OsVQ13 and OsMPK6 work together to regulate rice grain development (Uji et al. 2019). Both OsVQ14 and OsVQ32 can positively regulate

rice resistance to *Xoo*. OsVQ14 and OsVQ32 can interact with and be phosphorylated by OsMPK4. In *OsMPKK6* transgenic plants, OsMPK4 is highly phosphorylated after pathogen infection, enhancing plant resistance to *Xoo*, and meantime, phosphorylated OsVQ14 and OsVQ32 also accumulate significantly before and after infection. The results indicate that OsVQ14 and OsVQ32 serve as the substrate of the OsMPKK6-OsMPK4 signaling cascade, enhancing rice resistance to *Xoo*, thus defining a more complete signal transduction pathway in plant induced defense (Li et al. 2021). Recent research has found that wheat TaVQ4 interacts with MPK3 and MPK6 and plays a role as a phosphorylation substrate for MPK3 and MPK6 in plant drought stress resistance (Zhang et al. 2023a).

Moreover, MAPK can also directly interact with WRKY (Chi et al. 2013). It was shown that WRKY34 is phosphorylated by MPK3 and MPK6 during the early stages of male gametogenesis, and the MAPK-WRKY signaling module plays a crucial role in early pollen development (Guan et al. 2014). When inoculating *Arabidopsis* with *B. cinerea*, the MPK3/6 signaling pathway is activated, which in turn phosphorylates and activates WRKY33, and promotes the expression of *PAD3* to synthesize a large amount of phytoalexin, ultimately leading to a defense response in plant (Zhou et al. 2020). Rice OsWRKY45 has also been identified as a downstream target of OsMPK6 to regulate defense response of rice (Shimono et al. 2007; Ueno et al. 2013). OsWRKY53 can interact with and be phosphorylated by OsMAPK6 to regulate BR signaling and plant architecture (Tian et al. 2017), as well as herbivore-induced defense responses (Hu et al. 2015). A recent study showed that MPK3/6 interacts with and phosphorylates WRKY18 to regulate the expression of two protein phosphatases AP2C1 and PP2C5, and through the MPK3/6-WRKYs-PP2Cs module, PTI triggers and inhibits ETI responses to balance plant growth and defense (Wang et al. 2023a). Moreover, both barley MPK4 and WRKY1 negatively regulate powdery mildew resistance, and MPK4 phosphorylates WRKY1, enhancing its DNA binding ability and transcriptional repression activity. The MKK1-MPK4-WRKY1 module can regulate powdery mildew resistance in plant (Xue et al. 2023).

MAPK is one of the important signaling proteins in plants and plays an crucial role in many biological processes. The above results suggest that VQ protein can be phosphorylated by MAPK, affecting its activity and degradation, thereby regulating plant growth, immune and stress response. At the same time, MAPK can also directly phosphorylate WRKY transcription factors, affecting their activity and regulatory ability. Therefore, there is an interactive and interdependent relationship between VQ protein, WRKY, and MAPK. VQ proteins play an important role in balancing and buffering the interaction between MAPK and WRKY, which is of great significance for maintaining plant survival.

Interactions between VQ and other proteins

Except for WRKY and MAPK, VQ protein can also interact with other proteins, such as E3 ubiquitin ligase (E3), Calmodulin (CaM), JASMONATE-ZIM DOMAIN (JAZ), ABA Insensitive 5 (ABI5), and PHYTOCHROME INTERACTING FACTOR 1 (PIF1). These proteins have been reported to play important roles in plant growth, development, and response to external stimuli (Yang and Poovaiah 2003; Shen et al. 2005; Pauwels and Goossens 2011; Skubacz et al. 2016; Ban and Estelle 2021). VQ proteins expand their functions and participate in the regulation of various life processes by interacting with these proteins.

AtVQ15/AtCaMBP25 binds to typical CaM in a calcium-dependent manner, functions as a negative effector of osmotic stress tolerance, and may participate in stress signal transduction pathways (Perruc et al. 2004). AtVQ18 and AtVQ26 can interact with the ABI5 transcription factor to inhibit the transcriptional activation ability of ABI5, reducing the expression levels of *AtEM1* and *AtEM6*, and negatively regulate ABA response during seed germination and seedling establishment (Pan et al. 2018). AtVQ29 interacts with PIF1 transcription factor during the early stages of seedling development to enhance its transcriptional activation activity on downstream genes such as *PIL1* and *XTR7*, thereby regulating the elongation of hypocotyls in *Arabidopsis* under different light spectra (Li et al. 2014a). AtVQ22/JAV1 can interact with CaMs, JAZ8, and WRKY51 to regulate JA-mediated plant defense (Yan et al. 2018). Meanwhile, AtVQ22/JAV1 also interacts with RING-type E3 ubiquitin ligase JUL1, which ubiquitinates JAV1 and causes its degradation through ubiquitin-26S proteasome system, triggering the up-regulated expression of *PDF1.2* and the activation of the JA pathway, thereby conferring resistance to biotic stress in *Arabidopsis*. This study provides insights into the mechanisms by which the JAV1/JUL1 system specifically coordinates plant defense responses without interfering with plant growth and development (Ali et al. 2019). Similarly, rice OsVQ25 balances the broad-spectrum disease resistance and growth of plants by interacting with U-Box E3 ubiquitin ligase OsPUB73 and transcription factor OsWRKY53 (Hao et al. 2022). Tomato SlVQ15 can interact with SlJAZ2, SlJAZ5, SlJAZ6, SlJAZ7, and SlJAZ11 to regulate JA-mediated plant defense against *B. cinerea* (Huang et al. 2022a). In addition, VQ proteins can also form homodimers or heterodimers. For example, AtVQ12 can strongly interact with AtVQ3, AtVQ8, AtVQ10, AtVQ12, AtVQ17, AtVQ18, AtVQ29, and AtVQ32. AtVQ12 and AtVQ29 physically interact to form homodimers and heterodimers, negatively regulating plant defense against *B. cinerea* (Wang et al. 2015b). Apple MdVQ1, MdVQ10, MdVQ15, and MdVQ36 can interact with multiple MdVQ proteins to form heterodimers, while MdVQ15 can form homodimers with itself

through the C-terminal fragment (Dong et al. 2018). These results provide more important information for studying the interactions and functional pathways of the VQ family.

In summary, there is a complex and subtle triangular relationship between MAPK, VQ, and WRKY, which work together to achieve precise regulation of various physiological processes in complex environments. Meanwhile, the interaction between VQ protein and other types of proteins may also affect the interaction between VQ protein and transcription factors such as WRKY, thereby altering gene expression. Based on the above results, we comprehensively summarized the functions and interacting proteins of *VQ* genes in different plants (Table 2), and finally plotted their intracellular regulatory patterns (Fig. 2). When subjected to external environmental pressures, the transduction of phosphorylation signals, calcium signals, hormone signals, and photoelectric signals will occur in plant cell. This subsequently alters the interaction and modification between the VQ protein and one or more proteins in MAPK/CaM/E3/VQ/WRKY/JAZ8/ABI5/SIG1/PIF1, thereby activating or inhibiting relevant transcription factors and further regulating the expression levels of different downstream genes, including genes related to hormones, nutrients, and metabolites. Finally, plants will respond to external biotic or abiotic stresses, such as pathogens, insects, drought, salt, and high/low temperatures. Here, we proposed a typical signal transduction model, the MAPK-VQ-E3-WRKY cascade, based on these previous studies. When plants encounter external stimuli, MAPK is strongly activated, interacts with VQ proteins, and phosphorylates them, leading to their ubiquitination by E3 ubiquitin ligase and subsequent degradation by 26S proteasome pathway, ultimately triggering the disintegration of VQ-WRKY complex, releasing WRKY to regulate downstream genes (Fig. 2). The overexpression or mutation of some *VQ* genes, in addition to altering the plant's response to external stimuli, may also be accompanied by changes in plant growth and development, which is likely due to the sustained expression or suppression of certain downstream genes. These results not only provide a complete molecular perspective for the regulatory network of *VQ* genes, but also provide a reference for the cultivation of new materials through manipulating these related genes.

The importance of VQ domain

The VQ motif is the most conserved and important feature of VQ proteins, and multiple studies have shown that the VQ motif has a significant impact on the function of VQ proteins. For example, the mutation of IVQQ to EDLE in the VQ domain of AtVQ14 results in smaller seeds, while mutations at other positions do not have this phenotype (Wang et al. 2010b). Further study found that the role of

the VQ domain may be achieved through the following two mechanisms. Firstly, the VQ domain can affect the subcellular localization of the VQ protein. For example, the fusion protein of AtVQ9-GFP is localized in the nucleus, but when VVQK is mutated to EDLE in the VQ domain of AtVQ9, it is distributed in both the nucleus and cytoplasm (Hu et al. 2013b). The VQ motif of AtVQ21/MKS1 is not only involved in the interaction with WRKY33/WRKY25, but also participates in the nuclear localization of AtVQ21 (Petersen et al. 2010). However, not all VQ domains are related to subcellular localization. For instance, the mutation of VQ to AA in the VQ domain of AtVQ23 does not affect its localization, possibly because AtVQ23 contains nucleus and chloroplast targeting signal peptides in addition to the VQ motif (Lai et al. 2011). Secondly, the VQ domain can affect the interaction between VQ protein and other proteins. For example, mutations in the VQ domain can cause failed interactions of AtVQ9-WRKY8 (Hu et al. 2013b), AtVQ14-WRKY10 (Wang et al. 2010b), AtVQ20-WRKR2/34 (Lei et al. 2017), and AtVQ23-WRKY33 (Lai et al. 2011), respectively, indicating that the VQ motif is the core element for interaction with WRKY. In addition, an amino acid substitution (V70A or V70D) in the VQ motif of AtVQ29 eliminates its inhibitory activity, indicating that the VQ motif is essential for mediating AtVQ29-regulated transcriptional activity. Interestingly, the double substitution (V70D and Q71L) leads to significant induction of reporter gene *LUCIFERASE* (Li et al. 2014a), indicating that transcriptional regulatory activity can be transformed from inhibition to activation by modifying the VQ motif.

However, the VQ motif is not the only critical domain for the interaction between VQ protein and other proteins. Mutations at other sites can also lead to the loss of the original function of VQ protein. For example, the VQ motif is not essential for interaction or phosphorylation with MPK3/MPK6 (Pecher et al. 2014). Mutations in the VQ domain affect the transcriptional regulatory activity of AtVQ29, but do not alter its ability to interact with PIF1 (Li et al. 2014a). Studies have shown that the amino acid sequences on both sides of the VQ motif are highly diverse in VQ protein (Tian et al. 2023). The variable regions and sub-motifs of VQ protein can also regulate the interaction with WRKY, and affect the binding affinity and specificity of WRKY-VQ complex by interacting with other proteins (Cheng et al. 2012; Chi et al. 2013). Mutating the amino acid residues LVQR to EDLE in the VQ motif does not affect the interaction between AtVQ12 and AtVQ29, but mutating the C-terminal region eliminates their interaction. Therefore, AtVQ12 and AtVQ29 may interact with each other through their C-terminal segment, and interact with WRKY33 through their VQ motif, forming a large protein complex to mediate plant defense against *B. cinerea* (Wang et al. 2015b). In addition, when the two lysine residues K52 and K179 in AtVQ22/

Table 2 Biological functions and interacting factors of VQ proteins in different plants

Species	Gene ID	Name	Other name	Interacting proteins	Functions	References
Arabidopsis thaliana	AT1G28280	AtVQ4	MVQ1	MPK3/6/10; WRKY33/68	Negatively regulate disease resistance	Pecher et al. (2014); Weyhe et al. (2014)
	AT1G32585	AtVQ5			Negatively regulate disease resistance	Cheng et al. (2012)
	AT1G68450	AtVQ8			Regulate plant growth and development	Cheng et al. (2012)
	AT1G78310	AtVQ9	MVQ10	WRKY8/20; MPK3/4/6/10/11	Negatively regulate salt stress responses	Cheng et al. (2012); Hu et al. (2013b); Pecher et al. (2014)
	AT1G78410	AtVQ10		WRKY8; WRKY25/26/33	Positively regulate disease resistance Regulate vegetative growth Regulate meristem development and tolerance to oxidative stress and NO	Cheng et al. (2012); Chen et al. (2018); Gayubas et al. (2023)
	AT2G22880	AtVQ12		VQ proteins; WRKY20/23/24	Negatively regulate disease resistance	Cheng et al. (2012); Wang et al. (2015b)
	AT2G35230	AtVQ14	IKU1/MVQ9	WRKY10 (MINI3);	Influence endosperm growth and seed size	Wang et al. (2010b); Cheng et al. (2012)
	AT2G41010	AtVQ15	CAMBP25	CaM; WRKY25/51	Negatively regulate osmotic stress tolerance	Perruc et al. (2004); Cheng et al. (2012)
	AT2G41180	AtVQ16	SIB2	WRKY25/33; WRKY57/75; MPK1-18/20	Positively regulate disease resistance Regulate leaf senescence and seed germination	Lai et al. (2011); Cheng et al. (2012); Pecher et al. (2014); Jiang and Yu (2016); Zhang et al. (2022b)
	AT2G42140	AtVQ17			Regulate growth and development	Cheng et al. (2012)
	AT3G44340	AtVQ18		ABI5	Regulate seed germination Regulate growth and development	Cheng et al. (2012); Pan et al. (2018)
	AT3G18360	AtVQ20		WRKY2/34; WRKY20/75	Negatively regulate disease resistance Modulate pollen development and function	Cheng et al. (2012); Lei et al. (2017)
	AT3G18690	AtVQ21	MKS1	MPK4/11; WRKY25/33	Regulate plant growth and disease resistance	Andreasson et al. (2005); Qiu et al. (2008); Petersen et al. (2010); Cheng et al. (2012); Pecher et al. (2014); Gargul et al. (2015)
	AT3G22160	AtVQ22	JAV1	WRKY28/51; JAZ8; JUL1	Negatively regulate disease resistance Coordinate growth and defense	Cheng et al. (2012); Hu et al. (2013a); Ali et al. (2019)
	AT3G56710	AtVQ23	SIB1	WRKY33/57/75; SIG1; MPK6/14/16; WRKY3/4/20/25	Positively regulate disease resistance Regulate growth and development Regulate leaf senescence and seed germination	Morikawa et al. (2002); Narusaka et al. (2008); Xie et al. (2010); Lai et al. (2011); Pecher et al. (2014); Jiang and Yu 2016; Zhang et al. (2022b)
	AT3G58000	AtVQ25			Regulate plant disease resistance	Cheng et al. (2012)
	AT3G60090	AtVQ26		ABI5	Regulate seed germination	Pan et al. (2018)
	AT4G15120	AtVQ27		COX6b-3 and COA6-L	Osmotic stress and mitochondrial biogenesis	Kumari et al. (2023)
	AT4G20000	AtVQ28			Negatively regulate disease resistance	Lan et al. (2022)
	AT4G37710	AtVQ29		VQ proteins; PIF1	Negatively regulate disease resistance Regulate photomorphogenesis and flowering	Wang et al. (2015b); Li et al. (2014a)

Table 2 (continued)

Species	Gene ID	Name	Interacting proteins	Functions	References
Oryza sativa	Os01g17050	OsVQ1	OsMPK6	Negatively regulate disease resistance	Wang et al. (2021)
				Regulate flowering time	
	Os02g33600	OsVQ8	WRKY10	Regulates plant thermotolerance	Chen et al. (2022)
	Os03g47280	OsVQ13	OsMPK6	Positively regulate disease resistance	Uji et al. (2019)
				Positively regulate seed size	
	Os03g57520	OsVQ14	OsMPK4	Positively regulate disease resistance	Li et al. (2021)
	Os06g45570	OsVQ25	OsPUB73; OsWRKY53	Negatively regulate disease resistance	Hao et al. (2022)
				Coordinate growth and defense	
	Os08g01260	OsVQ32	OsMPK4	Positively regulate disease resistance	Li et al. (2021)
Solanum lycopersicum	Solyc02g078030	SlVQ6	SlMPK1	Negatively regulate thermotolerance	Ding et al. (2019)
	Solyc04g074520	SlVQ7	SlWRKY37	Regulate leaf senescence	Wang et al. (2022a)
	Solyc07g043250	SlVQ15	SlWRKY31; SlJAZ	Positively regulate disease resistance	Huang et al. (2022a)
	Solyc07g056600	SlVQ16	SlWRKY57; SlJAZ	Positively regulate salt stress	Ma et al. (2023a)
	Solyc10g007580	SlVQ21	SlWRKY57; SlJAZ	Negatively regulate salt stress	Ma et al. (2023a)
Malus domestica	MDP000193206	MdVQ10	VQ proteins; MdWRKY52; MdWRKY75	Regulate plant defense and development; Regulate leaf senescence caused by wound	Dong et al. (2018); Zhang et al. (2023b)
	MDP000182830	MdVQ15	VQ proteins; MdWRKY52	Regulate plant defense and development	Dong et al. (2018)
	MDP000312336	MdVQ25		Regulate growth and development	Dong et al. (2018)
	MDP000248043	MdVQ37		Regulate growth and development	Dong et al. (2018); Dong et al. (2021)
				Negatively regulate thermotolerance	

Species	Gene ID	Name	Other name	Interacting proteins	Functions	References
Musa acuminata	–	MaVQ5		MaWRKY26	Regulate JA biosynthesis and cold tolerance	Ye et al. (2016)
Glycine max	Glyma08g15620	GmVQ35			Regulate disease resistance	Zhou et al. (2016)
	Glyma08g18820	GmVQ37			Regulate development and seed setting	Zhou et al. (2016)
	Glyma09g05700	GmVQ43			Regulate flowering time	Zhou et al. (2016)
	Glyma11g04970	GmVQ47			Regulate disease resistance	Zhou et al. (2016)
					Regulate plant thermotolerance	
	Glyma.14g002800	GmVQ58		GmWRKY32	Negatively regulate pest resistance	Li et al. (2020a)
	Glyma15g16990	GmVQ62			Regulate flowering time	Zhou et al. (2016)
Ipomoea batatas	–	IbVQ4		IbWRKY2	Regulate drought and salt tolerance	Zhu et al. (2020)
Phyllostachys edulis	PH01007611G0010	PeVQ28		WRKY83	Positively regulate plant salt tolerance	Cheng et al. (2020)
Raphanus sativus	LOC108556901	RsVQ4		RsWRKY26	Regulate plant thermotolerance	He et al. (2023)
Zea mays	GRMZM2G122447	ZmVQ52		ZmWRKY20/36/50/71	Regulate leaf senescence	Yu et al. (2019)
Brassica napus	BnaA01g36880D	BnVQ7	BnMKS1		Positively regulate disease resistance	Zou et al. (2021)
	BnaA09g42280D	BnVQ12		BnWRKY28	Negatively regulate disease resistance	Zhang et al. (2022c)
Populus trichocarpa	Potri.001G029700	PtVQ1			Positively regulate salt tolerance/disease resistance	Liu et al. (2022b)

Table 2 (continued)

Species	Gene ID	Name	Other name	Interacting proteins	Functions	References
Taxus chinensis	WKE92269	TcJAV3		TcWRKY26	Regulate JA-mediated taxol biosynthesis and defense	Chen et al. (2022b)
Triticum aestivum	TraesCS1D02G340900	TaVQ4		TaMPK3/6	Positively regulate drought tolerance	Zhang et al. (2023a)
	TraesCS3B01G219100	TaVQ14			Regulate salt and drought tolerance	Cheng et al. (2022b)
	TraesCS4A02G290800	TaVQ25		TaWRKY133	Regulate leaf senescence	Meng et al. (2023)

JAV1 are mutated to threonine (T), the interaction between AtVQ22/JAV1 and the E3 ubiquitin ligase JUL1 is significantly reduced (Ali et al. 2019). The interaction between apple MdVQ10/MdVQ15 and MdWRKY52 requires not only the VQ motif, but also several amino acid residues flanking the VQ motif (Dong et al. 2018). Therefore, in addition to the VQ motif, some motifs or sites of VQ proteins also have specific functions, and their functions in protein stability, subcellular localization, and interactions with different proteins need further analysis.

With the advancement of technology, genome editing, including gene knockout, gene replacement, base editing, fragment insertion and deletion, has been applied in plants (Gao 2021; Molla et al. 2021). Therefore, the modification of the designated sequence of genes has received sufficient technical support. In addition, molecular design breeding based on the genes related to certain important agronomic traits is a promising crop breeding strategy (Zhang et al. 2018; Xing et al. 2020; Xu et al. 2021; Huang et al. 2022b; Song et al. 2022; Claeys et al. 2023; Li et al. 2023; Zhou et al. 2023). Given the regulatory functions of VQ proteins in plant disease resistance, stress resistance, and growth and development, as well as the presence of multiple functional motifs in their structure (Tian et al. 2023), *VQ* genes exhibit great potential for precise manipulation to study the functions of different motifs and achieve modern molecular breeding. Therefore, we proposed a new molecular design breeding strategy based on *VQ* genes (Fig. 3). Firstly, through big-data analysis, including genomics, transcriptomics, metabolomics, proteomics, and phenomics, candidate *VQ* genes associated with important traits in plants can be identified. These *VQ* genes may be related to plant responses to biotic or abiotic stress, growth and development, yield and quality, and on the other hand, may be related to plant hormones, metabolites, and nutrients. Secondly, based on the structure, interactions, and pathways of *VQ* genes, key active sites and motifs on *VQ* genes, as well as core regulatory elements on their promoter sequence, can be identified. According to this information, we can knock out one or more *VQ* genes (to study gene function), replace key loci (to alter protein activity through homologous recombination, base editing, or primer editing), delete specific fragment (to study the function of a certain region), insert specific fragment (to fuse with other proteins to generate new functional protein, or insert tags to observe gene real-time dynamics, including protein interactions and changes in gene expression levels), and edit promoter (to delete, replace, or insert certain sequences to alter gene expression). Thirdly, strategies such as manual design, artificial intelligence (AI) design, genome editing, transgene, and mutant library screening can be utilized to achieve the modification of *VQ* genes. Especially in AI design, it has begun to be highly recognized and increasingly applied in protein structure
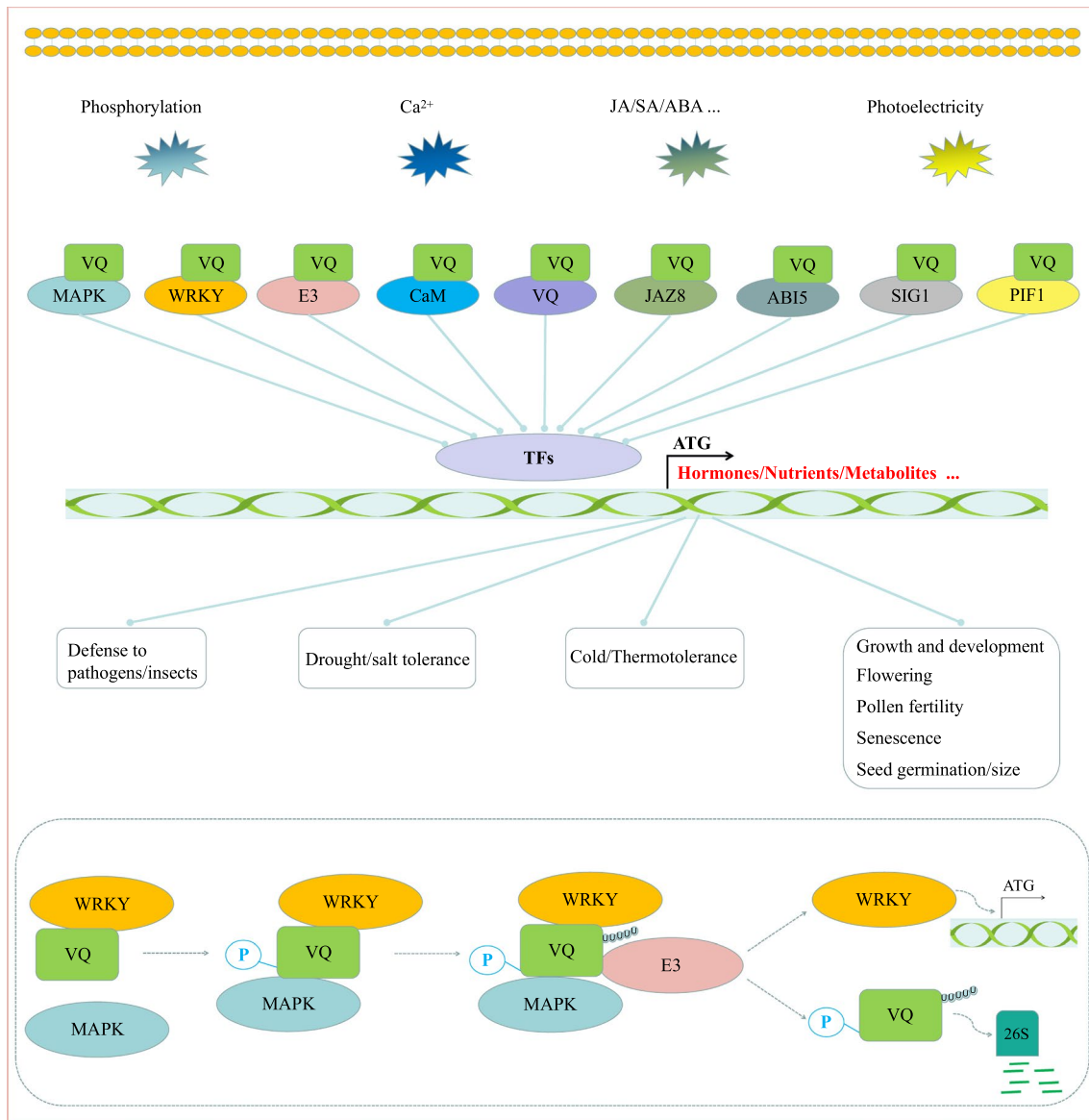
Fig. 2 The functional model and interaction network of *VQ* family genes. When faced with pathogen invasion or adversity stress, plants intracellular signal transduction will occur. Subsequently, the interaction between VQ protein and different proteins such as MAPK, CaM, E3, and transcription factors will be strengthened, which in turn affects the transcriptional activation or inhibition activity of these transcription factors, causing changes in downstream gene expression, ultimately affecting plant response and phenotype. The bottom frame displays a typical signal transduction mode, namely the 'MAPK-VQ-E3-WRKY' cascade. When exposed to external stimuli, the MAPK phosphorylation cascades are activated, MAPK phosphorylates VQ protein. Then, E3 ubiquitin ligase recognizes and ubiquitinates the phosphorylated VQ protein, leading to its degradation through the 26S proteasome pathway, thereby disassembling the VQ-WRKY complex and completely releasing WRKY transcription factor to regulate the expression of downstream genes

prediction and new functional protein development (Baek et al. 2021; Tunyasuvunakool et al. 2021; Bryant et al. 2022; Dauparas et al. 2022; Wang et al. 2022b; Huang et al. 2023; Lutz et al. 2023; Madani et al. 2023; Schuster et al. 2023; Watson et al. 2023). Fourthly, the characteristics of *VQ* gene molecular design materials will be clarified through molecular level evaluation, phenotype identification, and functional verification. Finally, the working mechanisms of the *VQ* gene can be revealed, and a series of new materials with specific or comprehensive traits improved can also be obtained. This provides clear research ideas and implementation approaches for further utilizing genetic resources to achieve molecular design breeding, intelligent breeding, and precision breeding.
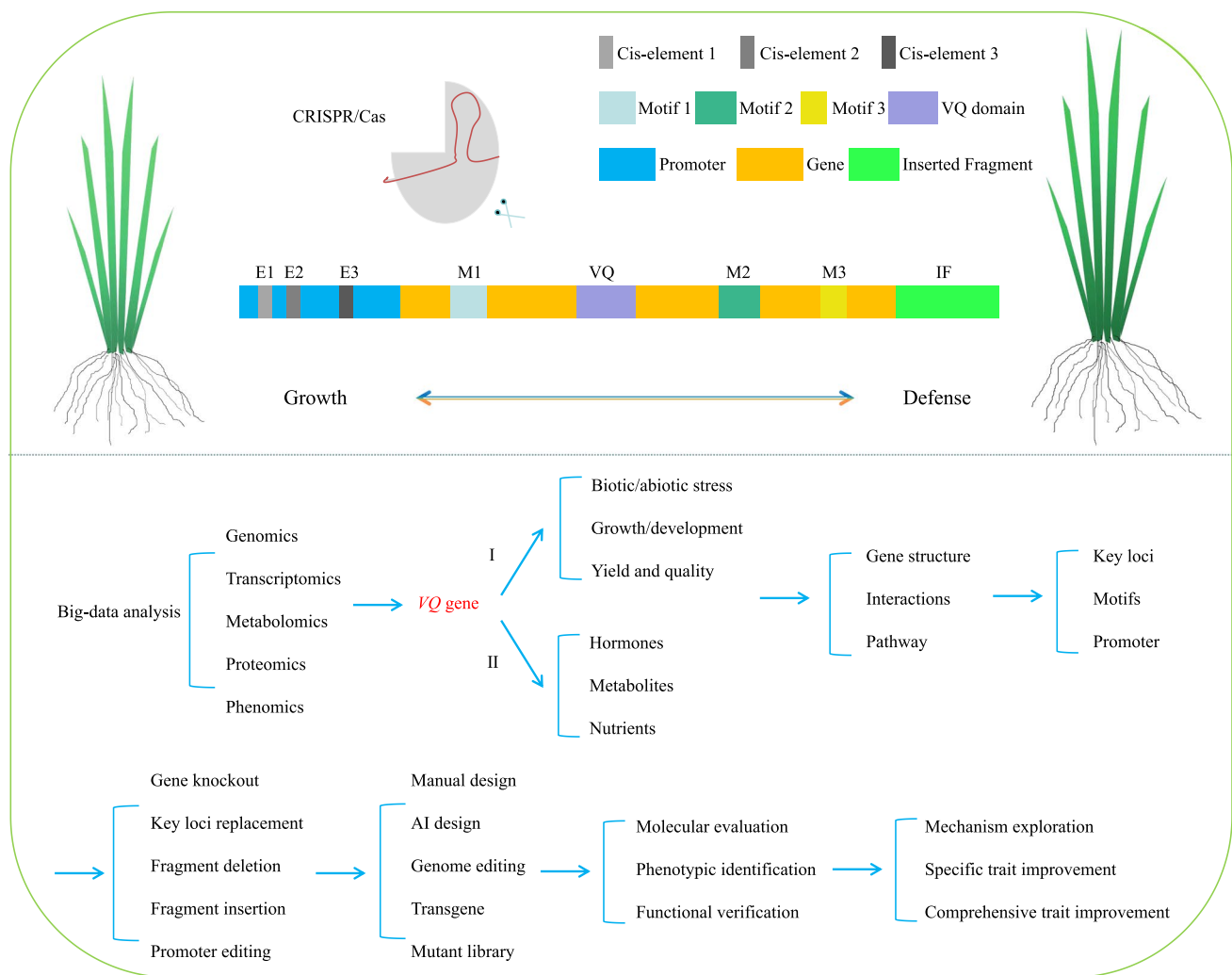
Fig. 3 Molecular design breeding strategy based on *VQ* gene. In the structure of *VQ* genes, blocks with different colors represent different functional units. Three different elements are exemplified on the promoter and gene sequences, respectively. E1 represents cis-element 1, E2 represents cis-element 2, and E3 represents cis-element 3; M1 represents motif 1, M2 represents motif 2, and M3 represents motif 3; VQ represents VQ domain; IF represents the inserted fragment. Mining candidate *VQ* genes through big-data analysis, identifying key loci, and applying genome editing tools for modification. The modification of cis-elements in the promoter and motifs in the coding region will further promote the working mechanism analysis of VQ proteins and create a series of new materials with different phenotypes

Conclusion and prospects

VQ proteins are a class of VQ motif-containing proteins that widely exist in plants as a multigene family. Early studies suggested that VQ family is a plant-specific transcription regulatory factor (Jing and Lin 2015), but afterwards, single or multiple *VQ* genes were identified in some fungi, lower animals, and bacteria, proving that it is not unique to plants, but an ancient gene family (Jiang et al. 2018). The *VQ* gene family has been widely identified and characterized in various plants, and they have unique structural and evolutionary features. They are not only involved in the response to biotic and abiotic stresses, but also involved in the regulation of various life processes in plants. By interacting with

many proteins such as MAPK and WRKY, VQ proteins play a very important role in balancing plant stress resistance, immunity, and growth and development. In this paper, we comprehensively summarized all research on the *VQ* gene to date, including member identification, molecular characteristics, biological functions, interacting proteins, and working mechanisms, and further depicted the functional model and regulatory network of VQ proteins, as well as its application in molecular breeding. These results can help us to gain a deeper understanding of the VQ family and the interactions between plants and the environment, and further promote their research and application.

However, there are still many limitations and urgent problems in the current research on *VQ* genes. Here, we

elucidated these limitations and proposed corresponding solutions and future prospects. Firstly, although the number, structure, and expression pattern of *VQ* genes have been preliminarily analyzed in some plants, published studies on their functions and mechanisms are mainly focused on *Arabidopsis*, with little research on other higher plants, especially crops. Therefore, we need to further explore *VQ* functional genes in more plants with higher application value, extensively and deeply reveal their functions and working mechanisms, and fully utilize their molecular and evolutionary characteristics. In addition, research on *VQ* family genes mostly focuses on angiosperms, and the existence status, specific functions, and evolutionary relationships of *VQ* genes in gymnosperms and other plants also require further exploration and utilization. Secondly, the VQ family contains a large number of members with significant sequence variations, so it remains to be determined whether the VQ motif plays an important role only in certain VQ proteins. This means that although some proteins contain VQ motif, this motif or even the entire protein actually does not play any role. Therefore, it is of great value to identify the VQ proteins with key regulatory capabilities and discover their structural and functional patterns. Moreover, mutations in the VQ motif can switch its transcriptional regulatory activity from inhibition to activation (Li et al. 2014a), reminding us to manipulate its activity through gene replacement or base editing, which may produce diverse plant phenotypes. Thirdly, from the perspective of sequence similarity, interacting proteins, and gene expression patterns, VQ proteins exhibit functional diversity or redundancy, which still needs further experimental verification. For example, both AtVQ12 and AtVQ29 can negatively regulate plant defense against *B. cinerea*, and *vq12/vq29* double-mutant plants displayed greater resistance than *vq29* single-mutant plants and wild-type plants (Wang et al. 2015b). But once these genes mutate simultaneously, although it may greatly improve plant resistance, it may also have a significant impact on plant development. Therefore, it will be of great significance for crop breeding if knocking out some *VQ* genes can observably improve plant resistance or agronomic traits without affecting growth and development, such as *OsVQ25* (Hao et al. 2022). Fourthly, the working mechanism of VQ proteins mainly focuses on some known interaction patterns, such as MAPK-VQ, WRKY-VQ, CaM-VQ, and E3-VQ. However, the multiple roles of VQ proteins in plant growth, development, defense, and stress response indicate that the signal transduction of these processes requires strict regulation and fine-tuning. Therefore, more interaction combinations (such as MYB-VQ, MADS-VQ, AP2/ERF-VQ, and bHLH-VQ), more specific pathways or mechanisms (such as regulatory mechanisms of plant development), and even more upstream and downstream networks need to be further uncovered. Fifthly, the application of *VQ* genes in

breeding is still lacking. Although *VQ* genes play a very important regulatory role and have been extensively studied, they are rarely used in breeding practices and *VQ* gene resources have not been fully utilized. At present, big-data platform breeding, molecular design breeding, and smart agriculture have gradually become the trend and theme of agricultural development. They associate genes with agronomic traits, decrypt and manipulate plant life codes from a molecular perspective, and obtain controllable new materials for crop improvement. Therefore, studying the characteristics and functions of multiple motifs in the *VQ* gene, utilizing genome editing and other technologies to explore their important sites, and creating new materials with different phenotypes and agronomic traits will greatly promote the application of the *VQ* gene family in crop breeding.

In conclusion, this study systematically reviewed the structural and functional characteristics of *VQ* genes in plants, as well as their working mechanisms and regulatory networks. However, despite many achievements, the current research on *VQ* family genes is still limited. Our study pointed out five limitations of current *VQ* gene research and proposed corresponding strategies and directions. With the advancement of technology and the deepening of research, we believe that exploring the specific functions and mechanisms of *VQ* genes in more plants will become a reality, and molecular breeding strategies based on *VQ* genes will also further promote the development and application of genetic resources.

Declarations

Conflict of interest The authors declare no conflict of interest.

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