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# Genome-wide characterization of DELLA gene family in blueberry (*Vaccinium darrowii*) and their expression profiles in development and response to abiotic stress

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## Abstract

**Background** The DELLA proteins, a class of GA signaling repressors, belong to the GRAS family of plant-specific nuclear proteins. Members of *DELLA* gene family encode transcriptional regulators with diverse functions in plant development and abiotic stress responses. To date, DELLAs have been identified in various plant species, such as *Arabidopsis thaliana*, *Malus domestica*, *Populus trichocarpa*, and other land plants. Most information of *DELLA* family genes was obtained from *A. thaliana*, whereas little is known about the *DELLA* gene family in blueberry.

**Results** In this study, we identified three *DELLA* genes in blueberry (*Vaccinium darrowii*, *VdDELLA*) and provided a complete overview of *VdDELLA* gene family, describing chromosome localization, protein properties, conserved domain, motif organization, and phylogenetic analysis. Three *VdDELLA* members, containing two highly conserved DELLA domain and GRAS domain, were distributed across three chromosomes. Additionally, *cis*-acting elements analysis indicated that *VdDELLA* genes might play a critical role in blueberry developmental processes, hormone, and stress responses. Expression analysis using quantitative real-time PCR (qRT-PCR) revealed that all of three *VdDELLA* genes were differentially expressed across various tissues. *VdDELLA2* was the most highly expressed *VdDELLA* in all denoted tissues, with a highest expression in mature fruits. In addition, all of the three *VdDELLA* genes actively responded to diverse abiotic stresses. Based on qRT-PCR analysis, *VdDELLA2* might act as a key regulator in *V. darrowii* in response to salt stress, whereas *VdDELLA1* and *VdDELLA2* might play an essential role in cold stress response. Under drought stress, all of three *VdDELLA* genes were involved in mediating drought response. Furthermore, their transiently co-localization with nuclear markers in *A. thaliana* protoplasts demonstrated their transcriptional regulator roles.

**Conclusions** In this study, three *VdDELLA* genes were identified in *V. darrowii* genome. Three *VdDELLA* genes were closely related to the *C. moschata* *DELLA* genes, *S. lycopersicum* *DELLA* genes, and *M. domestica* *DELLA* genes,

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respectively, indicating their similar biological functions. Expression analysis indicated that *VdDELLA* genes were highly efficient in blueberry fruit development. Expression patterns under different stress conditions revealed the differentially expressed *VdDELLA* genes responding to salt, drought, and cold stress. Overall, these results enrich our understanding of evolutionary relationship and potential functions of *VdDELLA* genes, which provide valuable information for further studies on genetic improvement of the plant yield and plant resistance.

**Keywords** Blueberry, Expression profiles, Gene family, DELLA, Development, Abiotic stress

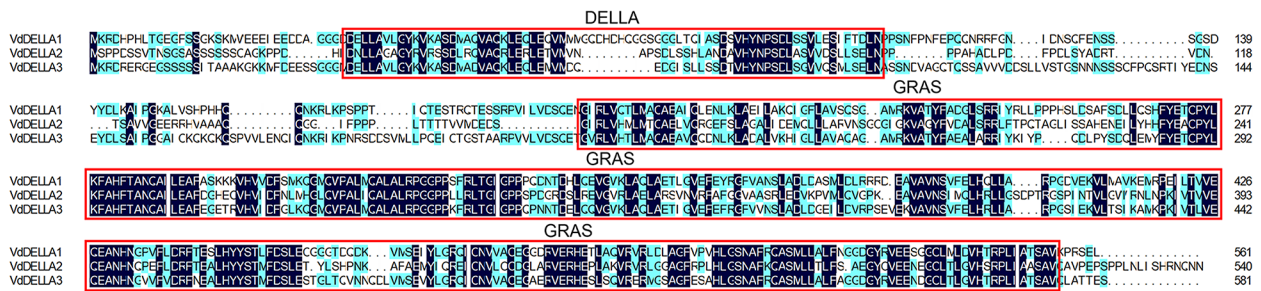
## Background

DELLA proteins are plant-specific transcriptional regulators belonging to a subfamily of the GRAS family [1]. The name was coined based on the basis of a short stretch of amino acids, named D-E-L-L-A, which is highly conserved in its N-terminal region [2]. DELLA and VHYNP domains, and polymeric Ser/Thr/Val (poly S/T/V) motifs constitute the unique N-terminal “DELLA” domain [3], which is tightly conserved among all plant species and is required for gibberellic acid (GA) responses [1]. GA, an extremely important endogenous phytohormone, promotes diverse growth and developmental processes by overcoming growth restraint mediated by DELLAs [1]. The C-terminal region is conserved among all GRAS family members, containing two leucine heptad repeats (LHR1 and LHR2), a putative nuclear localization signals (NLS), and three conserved motifs: VHIID, Src-homology 2 like (SH2-like), and SAW [1]. The LHRs are essential for protein homodimerization, DELLA activity, and GA-dependent DELLA degradation [1]. Specifically, the HLR1 motif mediates the interaction between DELLAs and TFs [1], VHIID and SAW are necessary for maintaining the functions of DELLA [4]. To date, DELLAs have been identified in various land plant species, such as five genes in *Arabidopsis thaliana* [5], seven genes in *Glycine max* [6], four genes in *Populus trichocarpa* [7], one gene in *Solanum lycopersicum* [8], one gene in *Vitis vinifera* [9], six genes in *Malus domestica* [10], two genes in *Lactuca sativa* [11], one gene in *Capsicum annuum* [12], and seven genes in *Cucurbita moschata* [13]. DELLA proteins are well-known key negative regulators of the GA signaling pathway [1]. All the information encoded by GAs is canalized through DELLAs, which modulate the activity of many transcription factors and transcriptional regulators through protein-protein interactions [14]. Therefore, DELLAs participate not only in plant growth and development, but also in abiotic stress responses. Several studies have demonstrated that DELLAs regulate plant height, stem elongation, flowering, root meristem, axillary bud formation, fruit development, seed germination, hypocotyl elongation, apical hook development, and abiotic stress responses [1].

As one of the five major healthy foods, blueberry is a highly attractive emerging crop species due to their enriched antioxidant phytochemical content, and is recognized as the “king of the world fruit” [15]. To date,

global blueberry cultivation zones, cultivated areas, and yields have increased significantly. The northern and southern highbush constitute the majority of blueberry production worldwide [16]. However, blueberry production also presents many challenges, as it varies by variety or genotype, regional adaptability, and environmental conditions. For example, the oxygen radical absorbance capacity (ORAC) and phenolics content in highbush blueberry leaf tissues are significantly higher than those in fruit tissue [17]. In addition, adverse abiotic environmental conditions such as salt, drought, cold, heat, and nutrient deficiencies limit the worldwide utilization of arable lands and negatively affect productivity [18]. In recent years, salt stress, drought stress, extreme temperatures, and flooding have limited the blueberry growth and survival. Therefore, it is importance to elucidate the stress response mechanism of blueberry for enhancing the stress resistance of *Vaccinium* horticultural crops. Most information of DELLA family genes was obtained from the studies in *A. thaliana*, *S. lycopersicum*, and *Oryza sativa*. In *A. thaliana*, DELLA proteins interact with SPL9 and attenuate the repressing activity of SPL9, promoting the initiation of axillary buds [19]. Fruit development can be regulated by DELLA proteins. In *S. lycopersicum*, SIDELLA interacted with SIARF7/SIIAA9 to regulate fruit initiation [20], and silencing of DELLA resulted in parthenocarpic fruits, which were smaller with a distinctive elongated shape when mature [21]. In *O. sativa*, plants overexpressing *SLR1* exhibited increased tiller number, whereas *SLR1* knock-down plants showed decreased tiller number [22]. Previously, studies have shown that DELLA proteins participate in abiotic stress response and improve the plant survival by regulating reactive oxygen species (ROS) levels during adverse environments [23, 24]. However, little is known about the *DELLA* gene family in blueberry fruit development and stress response. To investigate the role of DELLA proteins in these processes, we have identified DELLA-encoding genes from blueberry. There is potential to improve berry size, yield, and stress resistance in commercial blueberry production by introducing genetic variants of *VdDELLAs* into new cultivars.

In this study, three members of *VdDELLA* genes were identified and analyzed by phylogenetic relationship, sub-cellular localization, protein structure, and *cis*-elements in the *V. darrowii* genome. Additionally, we characterized



**Fig. 1** Multiple sequence alignment of amino acid sequences of VdDELLA proteins

**Table 1** Characterization of the VdDELLA gene family

Genes	Gene ID	CDS Length(bp)	Protein Length(aa)	MW (Da)	pI	Subcellular Location
VdDELLA1	Vadar_g43345	1686	562	62357.35	5.32	Nucleus
VdDELLA2	Vadar_g19556	1746	582	63767.06	4.99	Nucleus
VdDELLA3	Vadar_g25664	1626	542	59236.21	5.70	Nucleus

expression patterns of *VdDELLA* genes in six different tissues, leaves, stems, flowers, small fruits, big fruits, and mature fruits by qRT-PCR. Expression patterns under different stress conditions such as salt, drought, and cold were also analyzed using qRT-PCR. These results will provide valuable information for further studies on the multiple functions of the DELLA proteins in *V. darrowii* and genetic modification toward developing *V. darrowii* variants with increased yield and stress tolerance.

**Results**

**Genome-wide identification and chromosomal localization of DELLA family members in *V. darrowii***

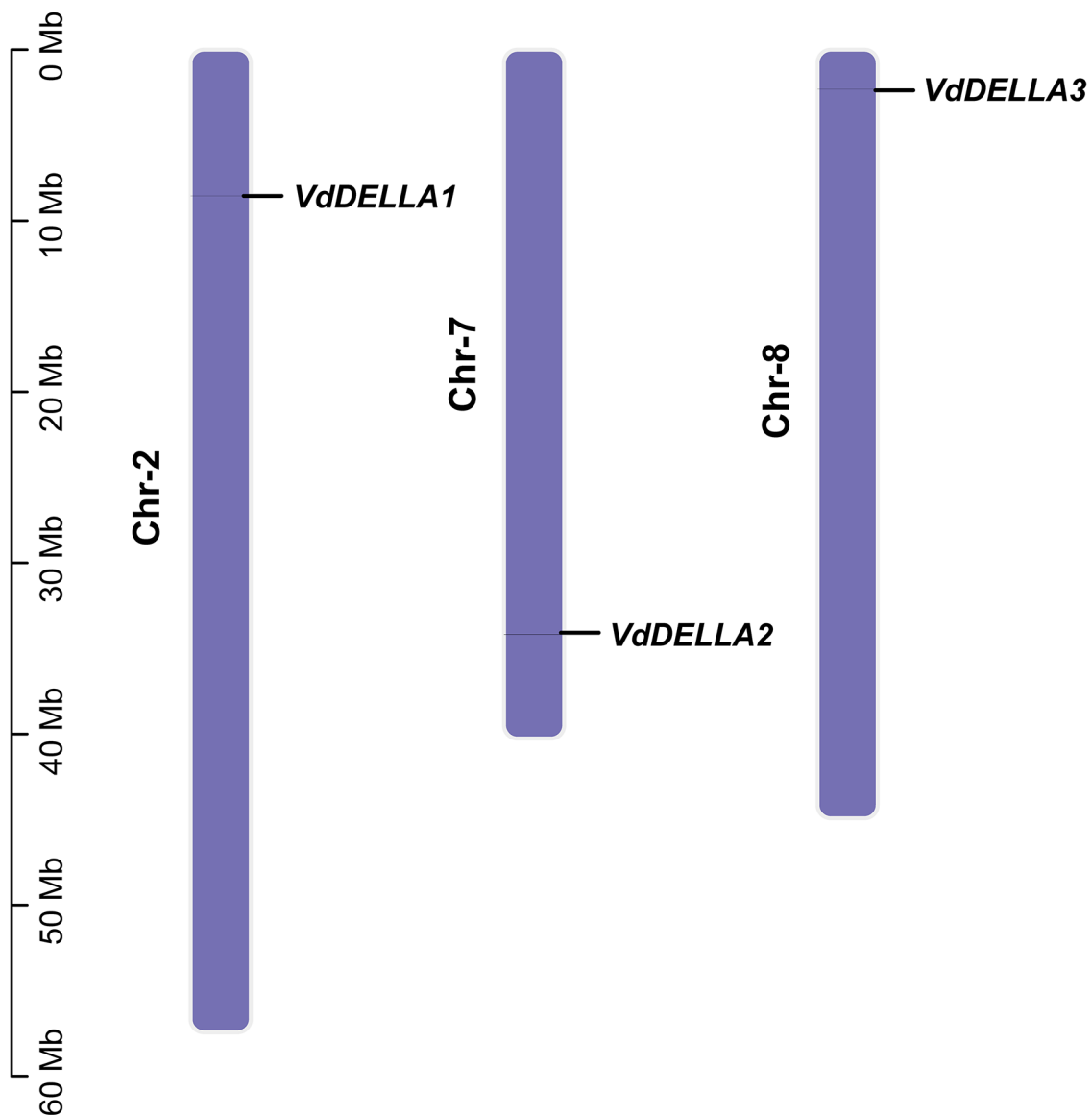
Based on the recently sequenced *V. darrowii* genome [16], three VdDELLA members harboring DELLA and GRAS domains were identified from the *V. darrowii* genome and named VdDELLA1 to VdDELLA3 (Fig. 1). The coding sequence (CDS) lengths of *VdDELLA* genes ranged from 1626 bp (*VdDELLA3*) to 1746 bp (*VdDELLA2*) (Table 1). Their corresponding proteins ranged from 541 to 581 aa, with a molecular weight varying from 59.24 kDa (*VdDELLA2*) to 63.77 kDa (*VdDELLA3*) (Table 1). Moreover, the isoelectric point (pI) values of VdDELLA proteins ranged from 4.99 (*VdDELLA2*) to 5.7 (*VdDELLA3*), which showed that these proteins were highly acidic. Subcellular localization analysis of the VdDELLA proteins showed that they were localized to the nucleus, which revealed their transcriptional regulator role. (Table 1). We further analyzed the chromosomal location of *VdDELLA* genes. Three *VdDELLA* genes were distributed on 2, 7, and 8 chromosomes of *V. darrowii*, respectively (Fig. 2), suggesting that they underwent duplication events.

**Conserved domains, motifs, and phylogenetic analysis of VdDELLA family**

All VdDELLA proteins contained an N-terminal DELLA domain and a C-terminal GRAS domain (Fig. 3A and B). The DELLA domain consisted of two motifs (motif 1 and motif 2), whereas eight motifs (motif 3 to motif 10) constituted the GRAS domain (Fig. 3C). To investigate the evolutionary relationship among *DELLAs* from *V. darrowii*, *A. thaliana*, *G. max*, *P. trichocarpa*, *S. lycopersicum*, *V. vinifera*, *M. domestica*, *L. sativa*, *C. annuum*, and *C. moschata*, a phylogenetic tree was constructed. All of *DELLA* genes were divided into four groups (Fig. 4). Group I consisted of fifteen members, Group II consisted of ten members, Group III consisted of five members, and Group IV contained eight members. *VdDELLA1*, *VdDELLA2*, and *VdDELLA3* were belonged to Groups I, II, and IV, respectively. As shown in phylogenetic tree, DELLA proteins from *V. darrowii* exhibited the conserved evolutionary relationship with those from *S. lycopersicum*, *L. sativa*, and *M. domestica*. In Group I, *VdDELLA1* clustered with *LsDELLA1* and *LsDELLA2*. In Group II, *VdDELLA2* clustered with *SIDE*. In Group IV, *VdDELLA3* clustered with *MdRGL3a* and *MdRGL3b*. The phylogenetic tree can provide some indications for the putative roles of *VdDELLA* genes in blueberry. For instance, the evolutionary relationship of *VdDELLA2* with *SIDE* revealed the functional prediction of *VdDELLA2* in fruit development [20].

**Cis-acting regulatory elements in the promoter region of VdDELLA genes**

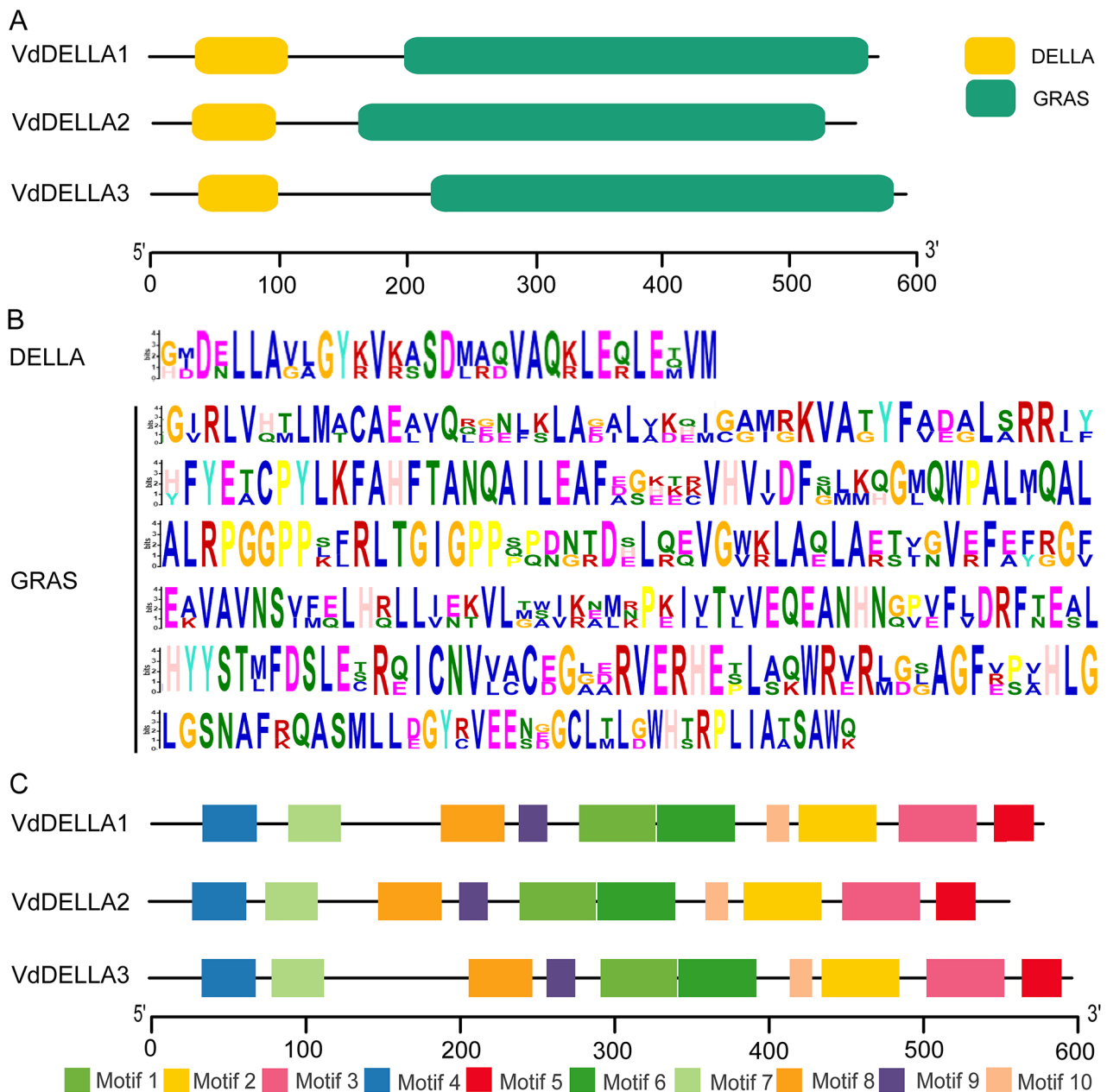
Transcriptional regulation plays an important role in the activation and suppression of gene expression [25]. *Cis*-acting elements in gene promoters are specific binding sites for proteins involved in the initiation and regulation



**Fig. 2** Distribution of *VdDELLA* genes on the *V. darrowii* chromosomes

of transcription [26]. To explore *VdDELLA* genes transcriptional regulation networks in blueberry, we analyzed the promoter sequences of *VdDELLA* genes. The analysis showed that *cis*-acting elements were mainly divided into hormone response, stress response, and developmental elements (Fig. 5). Among them, stress response elements were the most abundant, whereas developmental elements had the lowest abundance. Stress response elements containing ARE (for anoxic specific inducibility), LTR (for low temperature responsiveness), MBS and MYC (for drought-inducibility), STRE (for heat inducibility), TC-rich repeats (for defense and stress responsiveness) and WUN-motif (for wound responsiveness). Notably, *VdDELLA1* promoter contained all the response elements. There are several known hormone

response elements in the *VdDELLA* gene promoter, including ABRE, which is involved in the response to ABA; CGTCA-motif and TGACG-motif, which are known to respond to methyl jasmonic acid (MeJA); ERE, which is well known as the ethylene (ETH) response element; P-box, which is required for involvement in the response to GA; TCA-element, which is involved in the response to salicylic acid (SA); and TGA-element, which is required for auxin-responsiveness. Only four developmental elements (A-box, AAGAA-motif, CAT-box, and circadian) were distributed in the *VdDELLA* gene promoter. Interestingly, there was no developmental element in the promoter region of *VdDELLA1*, whereas the promoter region of *VdDELLA2* contained only one developmental element.



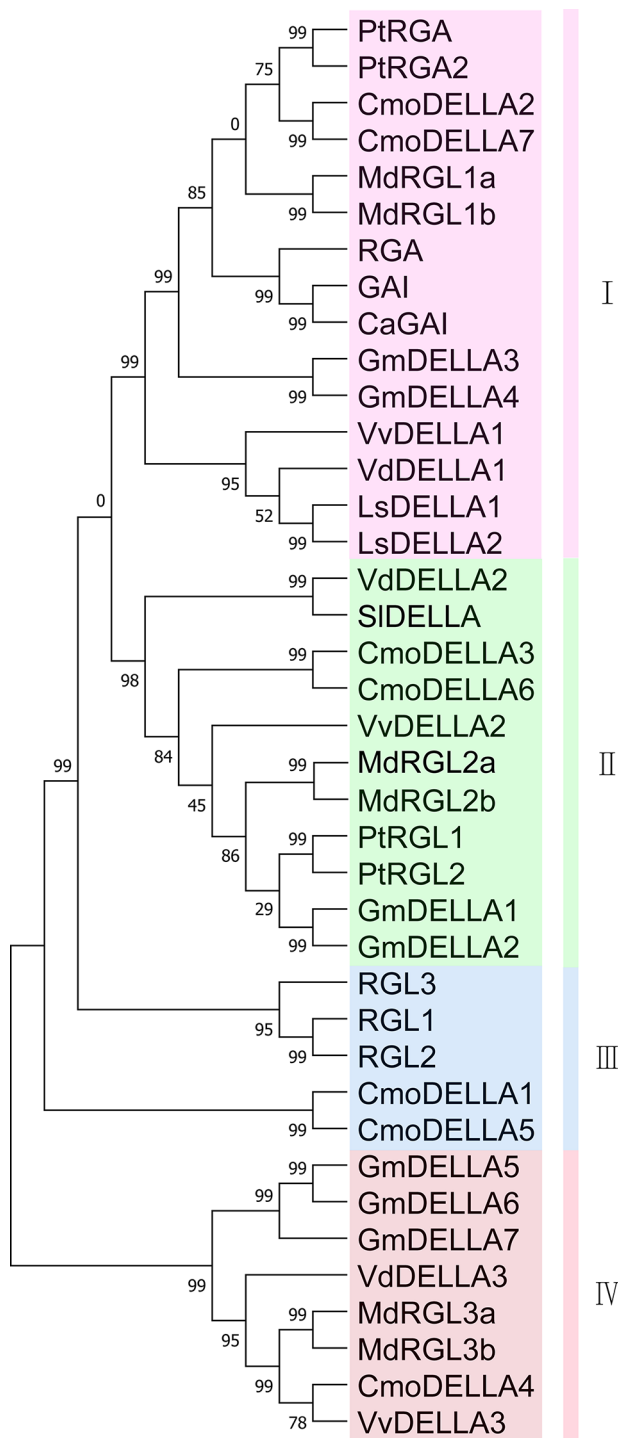
**Fig. 3** Conserved domains and motifs in VdDELLA proteins. **(A)** The conserved domains of VdDELLA proteins. DELLA domain and GRAS domain are represented by yellow and green box, respectively. **(B)** Sequence logos of conserved domains in VdDELLA protein sequences. **(C)** The conserved motifs of VdDELLA proteins. Colored boxes indicate different motifs

**Expression patterns of VdDELLA genes in different tissues**

The DELLA family is involved in multiple aspects of plant growth and development [1]. To explore the role of VdDELLA genes in blueberry growth and development, we evaluate the contribution of VdDELLA genes in leaves, stems, flowers, small fruits, big fruits, and mature fruits using qRT-PCR. According to these findings, the expression levels of VdDELLA genes were predominantly high in blueberry fruits (Fig. 6). VdDELLA2 and VdDELLA3 expression in mature fruits was significantly

higher than in the other five tissues, and VdDELLA1 was highly expressed in small fruits. In contrast, the stems and flowers exhibited relatively low expression levels. Noticeably, VdDELLA2 expressions in all denoted tissues were the most pronounced among the VdDELLAs. These results showed the tissue-specific expression patterns of the DELLA genes in multiple blueberry tissues, indicating the functional divergence of DELLA genes in the growth and development of blueberry.





**Fig. 4** Phylogenetic analysis of DELLA proteins from *V. darrowii* (Vd), *A. thaliana* (At), *G. max* (Gm), *P. trichocarpa* (Pt), *S. lycopersicum* (Sl), *V. vinifera* (Vv), *M. domestica* (Md), *L. sativa* (Ls), *C. annuum* (Ca) and *C. moschata* (Cmo). Different colors represent different subfamilies of DELLAs

#### Expression profiles of *VdDELLA* genes under abiotic stress

*Arabidopsis* DELLA proteins integrate responses to independent hormonal and environmental signals under adverse conditions [27]. To explore the role of

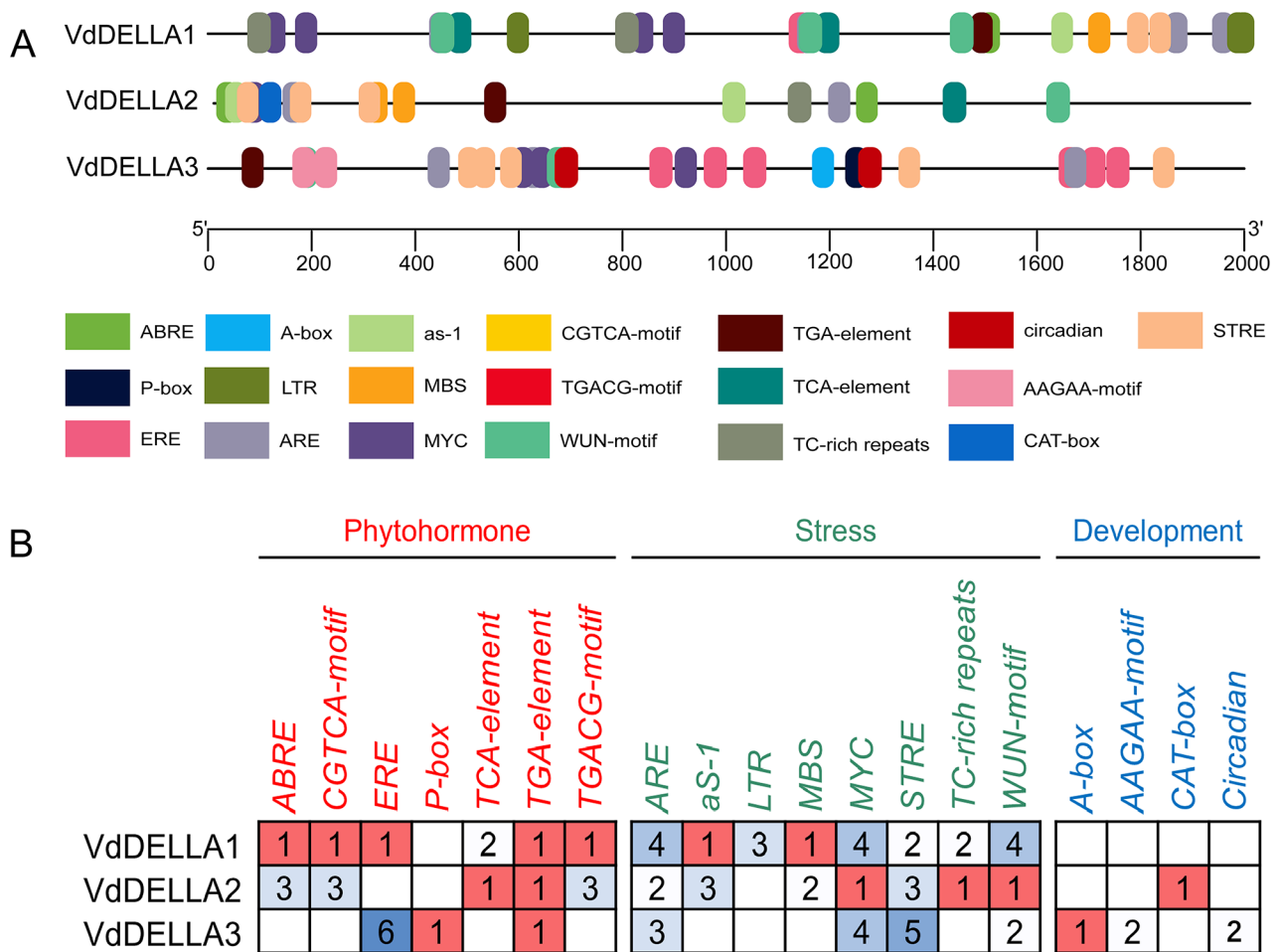
*VdDELLA* genes in the blueberry response to abiotic stress, we first evaluated the changes in their expression patterns under salt (200 mM NaCl), drought, and cold (4 °C) stress, respectively. *VdDELLA2* gene showed the higher expression under all tested stresses, whereas *VdDELLA3* gene exhibited the lowest expression. The expressions of all three *VdDELLA* genes showed an initial downregulation trend with salt, drought, and cold treatments, respectively (Fig. 7). However, the expression level of *VdDELLA2* gene showed a trend of first decreased, then increased and then decreased expression after treatment with 200 mM NaCl (Fig. 7A). At 5 d post-treatment, *VdDELLA2* gene had the highest expression levels, compared with normal condition, suggesting *VdDELLA2* gene play a critical role in response to salinity stress. Under drought stress, all three *VdDELLA* genes exhibited an initial downregulation followed by almost stable expression (Fig. 7B), suggesting that *VdDELLA* genes play an important role in the negative regulation in response to drought stress. Additionally, *VdDELLA1* and *VdDELLA2* expressions were increased by 4.2- and 2.7-fold, respectively, in response to cold stress after treatment for 48 h (Fig. 7C). Specifically, *VdDELLA3* exhibited a continuous downward trend over time under salt, drought, and cold stress, implying that *VdDELLA3* may function as a negative regulator in response to salt, drought, and cold stress, respectively. These results suggest the involvement of *DELLA* genes in the response of blueberry to salt, drought, and cold stress.

#### Subcellular localization of *VdDELLA* proteins

According to subcellular prediction analysis, *VdDELLA* proteins were found to be localized in the nucleus. To validate subcellular localization, 35S: *VdDELLA1*-GFP, 35S: *VdDELLA2*-GFP, and 35S: *VdDELLA3*-GFP vectors were constructed. Additionally, NLS-RFP was used as a nuclear marker. The co-localization of *VdDELLA1*-GFP, *VdDELLA2*-GFP, and *VdDELLA3*-GFP with NLS-RFP in *Arabidopsis* protoplasts was analyzed, respectively. We found that *VdDELLA1*-GFP, *VdDELLA2*-GFP, and *VdDELLA3*-GFP exhibited obvious co-localization with NLS-RFP in the nucleus (Fig. 8).

#### Discussion

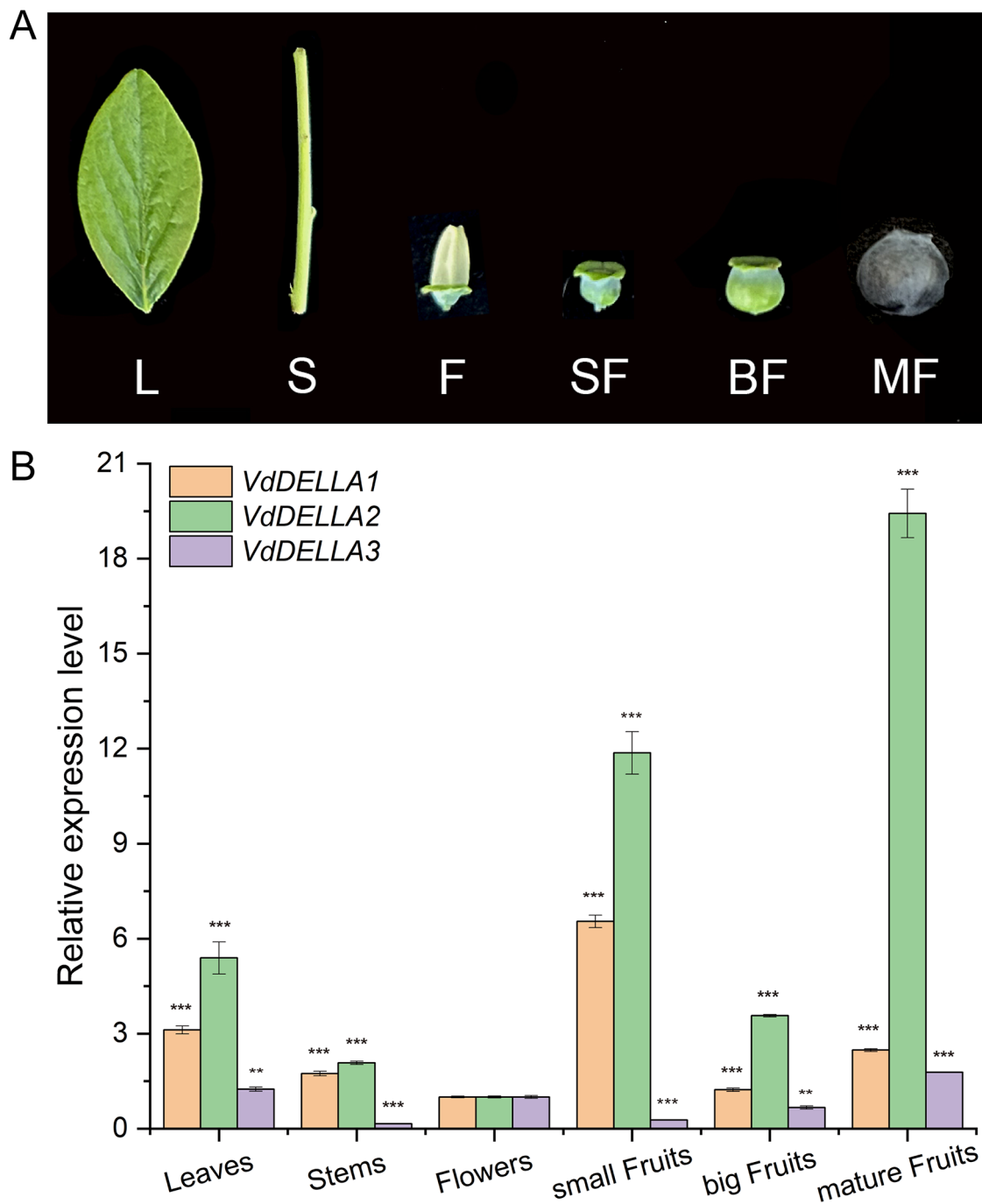
DELLA proteins are a well-known class of GA signaling repressors. They also regulate plant growth and development as well as stress response. DELLAs are widely distributed in dicots and monocots. To date, *DELLA* genes have been identified and characterized in crops, horticultural plants, and woody plants. Nevertheless, the DELLAs of *V. darrowii* are unknown. Based on the recently sequenced *V. darrowii* genome [16], three non-redundant DELLA proteins distributed on three chromosomes were identified from *V. darrowii* genome (Figs. 1



**Fig. 5** Cis-acting elements in the promoters of *VdDELLA* genes. **(A)** The types of promoter *cis*-acting elements of *VdDELLA* genes. The different colored boxes represent the different types and positions of *cis*-acting elements in each *VdDELLA* genes. **(B)** The numbers of promoter *cis*-acting elements of *VdDELLA* genes

and 2). Several species possess a single *DELLA* gene, such as *PROCERA* in *S. lycopersicum* [8], *L1* in *V. vinifera* [9], *LsDELLA1* in *L. sativa* [11], and *CaGAI* in *C. annuum* [12], whereas the *DELLA* genes in other plants have undergone multiplication [2]. For instance, *DELLA*s are encoded by four genes in *P. trichocarpa* [7], *A. thaliana* has five *DELLA*-encoding genes [5], ten *DELLA* genes are distributed in *B. napus* genome [28], and seven *DELLA* genes exist in the *C. moschata* genome [13]. Although the *V. darrowii* genome is 4.5-fold larger than the *A. thaliana* genome (560.5 Mb and 125 Mb, respectively), the gene number in *V. darrowii* is only three fifths that of *A. thaliana*, suggesting that there might be gene loss during genome duplication. All of *VdDELLA* proteins consist of N-terminal *DELLA* domain and C-terminal GRAS domain, which are two highly conserved domains of the *DELLA* family in plants and shared ten conserved motifs (Fig. 3). Interestingly, motif numbers and their composition between *VdDELLA*s were evenly distributed, which were inconsistent with the studies had been

found in *BnDELLA* and *CmoDELLA* proteins, whereas motif numbers between *BnDELLA* and *CmoDELLA* proteins were unevenly distributed [13, 28], implying the conserved functions of *VdDELLA* proteins in blueberry. Moreover, the prediction of subcellular localization showed that *VdDELLA* proteins were mainly localized in the nucleus. Subsequently, their transiently co-localization with nuclear markers in *Arabidopsis* protoplasts demonstrated the subcellular location of *VdDELLA* proteins. All of three *VdDELLA* proteins apparently co-localized with the nuclear marker (Fig. 8), indicating their transcriptional regulator role. Homologous genes in different species are presumed to perform similar biological functions [29]. Phylogenetic analysis showed that *VdDELLA* proteins of blueberry shared higher similarity with those of *S. lycopersicum*, *L. sativa*, and *M. domestica* (Fig. 4), providing some indications for the putative roles of *VdDELLA* genes in blueberry. For instance, the evolutionary relationship of *VdDELLA2* with *SIDE*



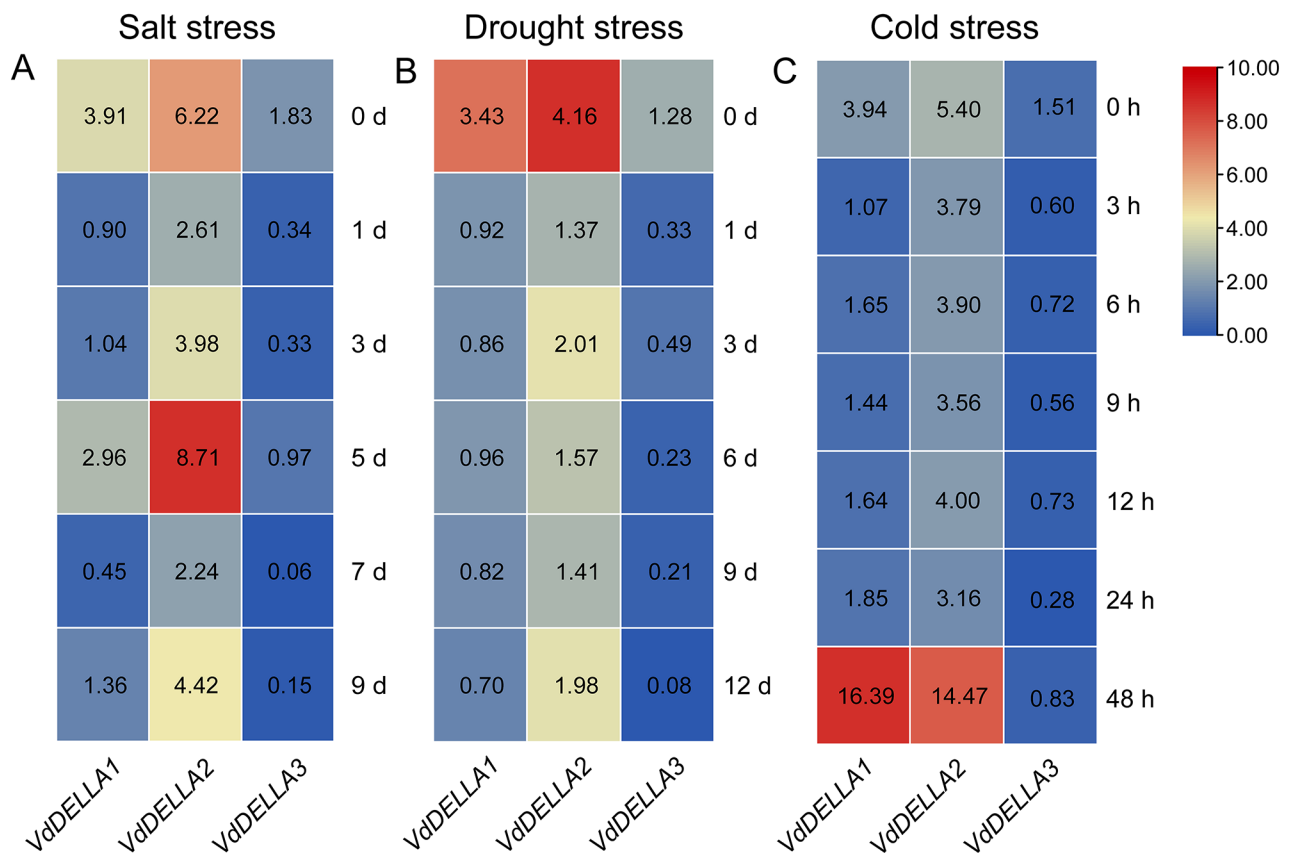
**Fig. 6** Expression patterns of the *VdDELLA* genes in the different tissues. **(A)** Samples of leaves, stems, flowers, small fruits, big fruits and mature fruits were collected from three-year-old 'O'Neal' plants. **(B)** *VdDELLA* gene expression patterns in leaves, stems, flowers, small fruits, big fruits, and mature fruits. Data are means of three replicates  $\pm$  SD. Relative expression of *VdDELLA1*, *VdDELLA2*, and *VdDELLA3* in flowers was set to 1.00. Asterisks indicate significant differences compared with flowers (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , two-tailed Student's *t*-test)

revealed the functional prediction of *VdDELLA2* in fruit development [20].

Various interactions between *cis*-acting elements and transcription factors function as molecular switches for transcription to determine transcription initiation events [30]. We found five phytohormone-related *cis*-acting

elements, seven types of *cis*-acting elements related to stress response, and four types of developmental elements in *VdDELLA* genes promoter regions (Fig. 5). Previous reports have shown that DELLA proteins act as modulators of plant growth, by interacting with a diversity of regulatory proteins that function in various





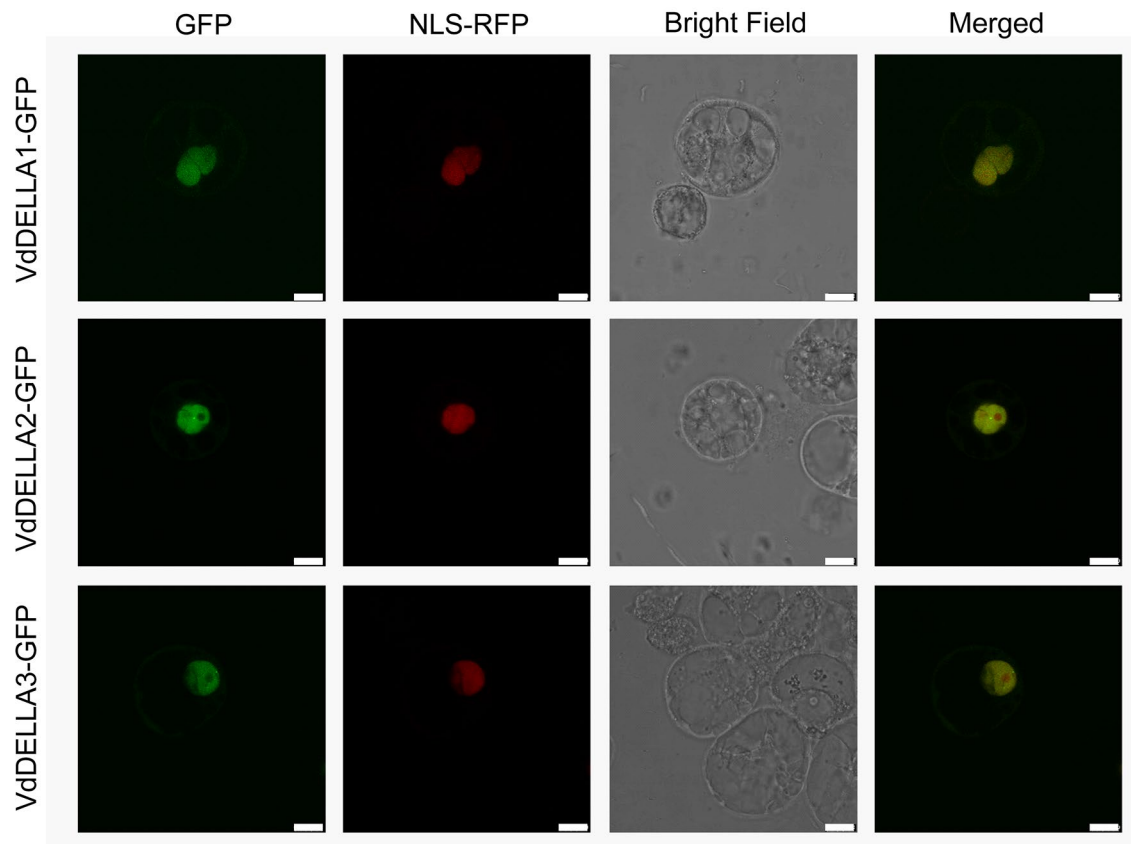
**Fig. 7** Expression profiles of *VdDELLA* genes under salt, drought and cold stress. **(A)** *VdDELLA* gene expression patterns under salt stress. **(B)** *VdDELLA* gene expression patterns under drought stress. **(C)** *VdDELLA* gene expression patterns under cold stress. Heat map was created by TBtools using the relative expression values. Data are means of three replicates  $\pm$  SD. The red and blue cells represent the highest and lowest expression levels, respectively

signaling pathways [31], such as GA, ABA, MeJA, SA, ETH, brassinosteroids (BRs), and auxin [1]. *VdDELLA* proteins contained ABRE, ERE, P-box, CGTCA-motif, TGACG-motif, TCA-element, and TGA-element, suggesting that *VdDELLA* genes might be responsible for plant development and stress response in blueberry by hormone signal pathway. Similar findings were also reported in *M. domestica* [10], *G. max* [6], *B. napus* [28], and *C. moschata* [13]. In *B. napus*, the *BnaDELLA* gene family contains a wide variety of stress and defense-related *cis*-elements compared to development, light, and hormone-responsive *cis*-elements [28]. Consistently, we also observed that both the types and the amount of stress-related *cis*-acting elements were the highest, suggesting the *VdDELLA* genes diverse function in response to various abiotic stresses. Accordingly, we speculate that *VdDELLA* proteins might be responsible for multiple environmental and hormonal signals in blueberry development.

Subsequently, we investigated *VdDELLA* gene expression patterns in different tissues under normal condition to understand their contribution to blueberry development. The results showed that *VdDELLA* genes were all

expressed in leaves, stems, flowers, small fruits, big fruits, and mature fruits, and exhibited the highest expression levels in fruits. In particular, *VdDELLA2* were abundantly expressed in small fruits and mature fruits, indicating that *VdDELLA2* might play a significant role in fruits development and ripening. Recently, the study had reported that four of the seven *C. moschata* *DELLA* genes (*CmoDELLA1*, *CmoDELLA4*, *CmoDELLA5*, and *CmoDELLA6*) were found to be mainly expressed in fruits and associated with fruits development [13]. Furthermore, *CmoDELLA1* exhibited significantly up-regulated in the pollinated fruits than that in the ovaries without pollination [32]. *DELLA* proteins have reported to interact with *CUC2* transcription factor to promote ovule initiation [33]. Based on the expression pattern of *VdDELLA* genes, we speculate that *VdDELLA* genes are highly efficient in blueberry fruit development. Gene overexpression and gene knockout of individual *VdDELLA* will be essential to verify their functions.

*DELLA* proteins are also involved in abiotic and biotic stress responses [13, 28]. The growth restraint conferred by *DELLA* proteins is beneficial and promotes survival [27]. Previous studies have shown that overaccumulation



**Fig. 8** The subcellular localization of VdDELLA proteins. Bar = 10  $\mu$ m

of DELLA proteins enhances not only salt tolerance but also cold tolerance, which causes plant fitness improvements [34]. In *Camellia sinensis*, the transcriptome data analysis showed that *CsDELLA* genes participated in response to NaCl, drought, and cold stress [35]. In *B. napus*, the expression patterns of *BnaDELLAs* varied upon salt, drought, and 4 °C treatments, and were associated with stress tolerance improvements [28]. In *C. moschata*, *CmoDELLA* genes mediate the stress response of pumpkin to NaCl, waterlogging, and cold [13]. Consistently, we also found *VdDELLA* genes were involved in response to salt, drought, and cold stress, and exhibited distinct expression patterns (Fig. 7). Induced expression was observed during salt and cold treatment. As shown in Fig. 7, *VdDELLA2* showed the highly increased expression after 5 d of salt treatment (Fig. 7A), suggesting *VdDELLA2* might be crucial in response to salt stress. *VdDELLA1* and *VdDELLA2* were significantly induced under cold stress after 48 h (Fig. 7C), indicating *VdDELLA1* and *VdDELLA2* might played critical roles in response to cold stress. In contrast, *VdDELLA* genes exhibited its reduced expression in response to drought treatment (Fig. 7B), which were consistent with studies that have been found in *BnaRGL3* [28]. Taken together, *VdDELLA* genes might participate in the stress response

of blueberry to salt, drought, and cold. However, further studies are required to explore the function of *VdDELLA* genes under stress condition. Moreover, it is very important to understand the molecular mechanism underlying the crosstalk between *VdDELLA* genes and phytohormone signal transduction pathways under stress condition.

## Conclusions

In this study, three *VdDELLA* genes were identified in *V. darrowii* genome. Three *VdDELLA* genes were closely related to the *C. moschata* *DELLA* genes, *S. lycopersicum* *DELLA* genes, and *M. domestica* *DELLA* genes, respectively, indicating their similar biological functions. Expression analysis indicated that *VdDELLA* genes were highly efficient in blueberry fruit development. Expression patterns under different stress conditions revealed the differentially expressed *VdDELLA* genes responding to salt, drought, and cold stress. Overall, these results enrich our understanding of evolutionary relationship and potential functions of *VdDELLA* genes, which provide valuable information for further studies on genetic improvement of the plant yield and plant resistance.

## Methods

### Identification of VdDELLA gene family members from blueberry genome

Five AtDELLA proteins from *A. thaliana* were downloaded from the TAIR database (<https://www.arabidopsis.org/>) [36]. To identify DELLA gene family members in *V. darrowii*, AtDELLA proteins were used as query sequences to perform online BLAST search against *V. darrowii* database (<https://phytozome-next.jgi.doe.gov/blast-search>) [16]. Then, the protein sequences of *V. darrowii* were downloaded from *V. darrowii* database (<https://phytozome-next.jgi.doe.gov/>) [16]. The Hidden Markov Model (HMM) profile of the DELLA domain (PF12041) was downloaded from the Pfam database (<http://pfam.xfam.org/>). HMMER3.0 program (<http://hmmmer.org/>) [37] was used to perform hidden Markov model searches for candidate proteins containing the DELLA domain. All the candidate protein sequences gained with the above two methods were submitted to CDD (<http://ncbi.nlm.nih.gov/cdd>) [38] in NCBI database (<https://www.ncbi.nlm.nih.gov/>) [38] to reconfirm the VdDELLA proteins, and redundant proteins were manually removed. Finally, molecular weight (Mw) and theoretical isoelectric point (pI) of VdDELLAs were predicted utilizing the online software ExPASy (<https://web.expasy.org/protparam/>) [39]. The subcellular location information was predicted using the online software WoLF PSORT (<https://wolfpsort.hgc.jp/>) [40].

### Structure of VdDELLA proteins, phylogenetic and chromosome location analysis

The conserved domains were visualized with TBtools software [41]. The protein sequences of *A. thaliana* [5], *G. max* [6], *P. trichocarpa* [7], *S. lycopersicum* [8], *V. vinifera* [9], *M. domestica* [10], *L. sativa* [11], *C. annuum* [12] and *C. moschata* [13] were obtained from TAIR database, Soybase database (<https://www.soybase.org/>), Phytozome13 database (<https://phytozome-next.jgi.doe.gov/>), NCBI database (<https://www.ncbi.nlm.nih.gov/>), Ensembl database (<http://plants.ensembl.org/index.html>), and cucurbit genomics database (<http://cucurbitgenomics.org/>), respectively. Based on multiple sequence alignment, a neighbor-joining phylogenetic tree was generated using MEGA 7.0 software [42] with 1000 bootstrap replicates. The resulting phylogenetic tree was beautified with the online tool Evolview v3 [43] (<https://evolgenius.info/>). MEME program (<http://meme-suite.org>) [44] was used to detect the conserved motifs of VdDELLA proteins, and the protein structures of them were visualized with TBtools software [41]. The chromosomal distribution of VdDELLA genes was determined from the annotation file (GFF3), and visualized with using TBtools software [41].

### Cis-acting element analysis

The promoter sequences of the VdDELLA genes (2000 bp DNA sequence upstream of the start codon ATG) were downloaded from Phytozome database (<https://phytozome.jgi.doe.gov/pz/portal.html>). Cis-acting elements in the promoter region were analyzed using online PLANT-CARE database (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>), and visualized using TBtools software [41].

### Plant materials and stress treatments

'O'Neal' plants were obtained from the Ludong University, Yantai, China by Prof. Hongxia Zhang. Seedlings from cuttings of a single 'O'Neal' clone were used for this study. Plants were grown in a growth chamber at 25 °C under a photoperiod of 16 h light and 8 h darkness. Leaves, stems, flowers, small fruits, big fruits, and mature fruits samples were collected from three-year-old 'O'Neal' plants. One-year-old 'O'Neal' seedlings were subjected to 200 mM NaCl, drought, and 4 °C stress, respectively. Leaves were sampled at a set time points after treatments, immediately frozen in liquid nitrogen and stored at -80 °C for further analyses.

### Quantitative Real-Time PCR analysis

Total RNA was extracted from all samples using the FastPure Universal Plant Total RNA Isolation Kit (Vazyme, Nanjing, China) according to the manufacturer's protocol. First-strand cDNA was synthesized using the HiScript III 1st Strand cDNA Synthesis Kit (+gDNA wiper) (Vazyme, Nanjing, China). RT-qPCR was performed on a CFX Connect Real-Time System (Bio-Rad) using Cham Q Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) with 10 µl PCR products. Relative gene expression values were analyzed using the  $2^{-\Delta\Delta C_t}$  method (each sample has three biological replicates, and each biological replicate includes three technical replicates). *Tubulin beta 8* (Vda04G014670.1) [16] was used as a reference gene. Heat maps of gene expression levels were visualized with TBtools software [41]. All primer sequences were listed in Table S1.

### Subcellular location analysis

Primers containing HindIII restriction sites were designed according to the CDS sequence of VdDELLA genes (removing the termination codon), and the fusion expression vectors were constructed using the Clone Express<sup>®</sup>II One Step Cloning Kit (Vazyme, Nanjing, China). Finally, the recombinant constructs (35S: VdDELLA1-GFP, 35S: VdDELLA2-GFP, and 35S: VdDELLA3-GFP) and nuclear marker plasmids (NLS-RFP) were then co-transformed into *Arabidopsis* protoplasts, isolating from *Arabidopsis* suspension culture cells. *Arabidopsis* suspension culture cells were obtained

from the Chinese University of Hong Kong, China by Prof. Liwen Jiang. Images were taken using an LSM-710 confocal microscope (Zeiss) equipped with an argon/krypton laser.

### Statistical analysis

All data were analyzed using Origin 8. A two-tailed Student's *t*-test was used for statistical analysis.

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12864-024-10721-4>.

Supplementary Material 1: Table S1 Protein sequences used for Phylogenetic analysis. Table S2 Primer sequences used for analyses.

Supplementary Material 2: Table S3 Expression levels of VdDELLAs genes in the different tissues. Table S4 Expression levels of VdDELLAs genes under salt stress. Table S5 Expression levels of VdDELLAs genes under drought stress. Table S6 Expression levels of VdDELLAs genes under cold stress

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### Author contributions

Conception: Houjun Zhou and Lei Yang. Interpretation or analysis of data: Houjun Zhou, Yanwen Wang, Xinyu Wang, and Rui Cheng. Preparation of the manuscript: Houjun Zhou, Yanwen Wang, Xinyu Wang, and Rui Cheng. Revision for important intellectual content: Houjun Zhou, Hongxia Zhang, and Lei Yang. Supervision: Houjun Zhou and Lei Yang. All authors reviewed the manuscript.

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### Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. *A. thaliana* gene sequence data from this article can be found in the TAIR database (<https://www.arabidopsis.org/>) under the following accession numbers: AtGAI (At1g14920), AtRGA (At2g01570), AtRGL1 (At1g66350), AtRGL2 (At3g03450), and AtRGL3 (At5g17490); *G. max* gene sequence data from this article can be found in the Soybase database (<https://www.soybase.org/>) under the following accession numbers: GmDELLA1 (Glyma.11G216500.1), GmDELLA2 (Glyma.18G040000.1), GmDELLA3 (Glyma.08G095800.1), GmDELLA4 (Glyma.05G140400.1), GmDELLA5 (Glyma.04G150500.1), GmDELLA6 (Glyma.06G213100.1), and GmDELLA7 (Glyma.10G190200.1); *C. moschata* gene sequence data from this article can be found in the cucurbit genomics database (<http://cucurbitgenomics.org/>) under the following accession numbers: CmoDELLA1 (CmoCh01G003940.1), CmoDELLA2 (CmoCh04G022200.1), CmoDELLA3 (CmoCh04G023970.1), CmoDELLA4 (CmoCh11G005830.1), CmoDELLA5 (CmoCh14G008330.1), CmoDELLA6 (CmoCh15G007670.1), and CmoDELLA7 (CmoCh15G010000.1); *P. trichocarpa* and *V. vinifera* gene sequence data from this article can be found in the Phytozome13 database (<https://phytozome-next.jgi.doe.gov/>) under the following accession numbers: PtRGA (Potri.008G131700), PtRGA2 (Potri.010G110700), PtRGL1 (Potri.004G089800), PtRGL2 (Potri.017G125200), VvDELLA1 (VIT\_201s0011g05260.1), VvDELLA2 (VIT\_214s0006g00640.1), and VvDELLA3 (VIT\_211s0016g04630.1); *M. domestica* and *L. sativa* gene sequence data from this article can be found in the NCBI database (<https://www.ncbi.nlm.nih.gov/>) under the following accession numbers: MdRGL1a (DQ007885), MdRGL1b (DQ007886), MdRGL2a (DQ007883), MdRGL2b (DQ007884), MdRGL3a (DQ007887), MdRGL3b (DQ007887), LsDELLA1 (XM\_023877838.3), and LsDELLA2 (XM\_023886497.3); *S. lycopersicum* and *C. annuum* gene sequence data can be found in the Ensembl database (<http://plants.ensembl.org/index.html>) under the accession numbers: SIDElla (Solyc11g011260.1) and CaGAI (PHT64689).

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### Declarations

#### Ethics approval and consent to participate

Not applicable. The sampling of plant material was performed in compliance with institutional guidelines. The research conducted in this study required neither approval from an ethics committee, nor involved any human or animal subjects.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

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