Genome-wide Identification and Analysis of VQ Gene Family in Cucumber

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Abstract  VQ protein highly conserved in plants, plays an important role in plant growth and development. In recent years, VQ protein has received extensive attention due to its interaction with WRKY transcription factors. However, there are few reports on the VQ gene family in cucumber, and its function is not clear. In this study, we systematically analyzed the VQ gene of cucumber by bioinformatics and their structures, subcellular localizations, evolutionary relationships, and tissue expression patterns were explored. The results showed that the VQ gene family of cucumber contained 26 members, and most of the CsVQs coding proteins were neutral or basic proteins, encoded by a single exon gene, and most of them were located on the nucleus. All 26 VQ genes had conserved valine (V) and glutamine (Q) motifs, and the core motif was FxxxVQx (L/V/F) TG, which was not unevenly distributed on the 7 chromosomes of cucumber. Gene expression analysis in various tissues demonstrated that most CsVQs genes showed tissue-specific expression in cucumber, uncovering their potential function in cucumber growth and development. Our research will benefit the functional study of cucumber VQ genes in the future.

Keywords  Cucumber; VQ gene family; Bioinformatics analysis

The VQ protein family is a highly conserved plant-specific protein family in plants, whose members include a conserved VQ motif (FxxxVQxLTG, F for phenylalanine, L for leucine, T for threonine, G for glycine, and x for any amino acid) (Cheng et al., 2012). VQ motif plays a key role in various biological reactions in which VQ protein is involved (Cao et al., 2018). With the development of bioinformatics, researchers have successively identified 34, 29, 74, 18 and 18 members of the VQ family in Arabidopsis, Chili, Rice, Soybean, Maize, Grape and other plants (Kim et al., 2013; Wang et al., 2014; Li et al., 2014; Wang et al., 2015; Zhang et al., 2016; Song et al., 2016).

Studies have shown that VQ protein is involved in the regulation of plant growth and development. In Arabidopsis, IKU1 (AT2G35230) encodes a protein containing the VQ motif, which is expressed in the early endosperm and central cells and is involved in the regulation of endosperm development, further affecting seed size (Garcia et al., 2003; Wang et al., 2010). In addition, IKU2 has been shown to regulate the size of Arabidopsis seeds by interacting with ATWRKY10 protein (Luo et al., 2005). Li et al. (2014) found that VQ29 is a negative transcriptional regulator that photomediates inhibition of hypocotyl elongation, and overexpression of VQ29 can reduce hypocotyl sensitivity to far-red and low-light, while mutants with loss of VQ29 function show inhibition of hypocotyl elongation under low-intensity far-red and white light (Li et al., 2014; Cheng et al. (2012) showed that in Arabidopsis, ATVQ8 gene affects leaf development, and the lack of ATVQ8 function makes the leaves appear yellow-green in the whole growth period, and the plants with overexpression of VQ17, VQ18 and VQ22 have poor growth and smaller yellows. VQ10 interacts with WRKY25 and WRKY33, which can weaken the growth of plants when overexpressed together VQ10 interacts with WRKY25 and WRKY33, while overexpression can make plant growth weak (Cheng et al., 2012). In addition, studies have shown that VQ protein plays an important role in the response of plants to biological and abiotic stresses such as pathogenic bacteria. It was found that, ATVQ21/MKS1 interacts with MAP4 transcription factor and enhances plant resistance to pseudomonas Eugenica by participating in SA signal transduction pathway in Arabidopsis. In addition, ATVQ21/MKS1 can also negatively regulate the JA signal transduction pathway to weaken the resistance of plants to pathogenic Botrytis cinerea (Petersen et al., 2010;...
Xie et al., 2010; Fill and Petersen, 2011). Studies on AtVQ23 (SIB1) and AtVQ16 (SIB2) found that transcriptional expression of SIB1 and SIB2 was strongly induced by Botrytis cinerea, and through interaction with WRKY33, resistance of plants to grey mildews was enhanced (Lai et al., 2011; Hu et al., 2013). Other studies have reported that the VQ gene can respond to drought and salt stress. For example, Hu et al. (2013) found that AtVQ9 inhibited the binding activity of WRKY8 and w-box by interacting with WRKY8 transcription factor, and negatively regulated the resistance of Arabidopsis to salt stress.

Cucumber is one of the ten most widely cultivated vegetable crops in the world. With the development of the protected cultivation, cucumber has gradually become an important ingredient for the annual food supply on the table. With a delicate flavor and crisp taste, cucumber is rich in protein, various vitamins, calabash and other nutrients, and has health care, anti-aging, weight loss and other effects, and is deeply loved by consumers in various countries (Xu et al., 2018). The completion of whole genome sequencing of cucumber provides conditions for the identification and functional prediction of gene family members (Yang et al., 2012). In the process of cucumber cultivation, cucumber is susceptible to various biological and abiotic stress, which will affect the fruit yield and quality. VQ protein may play an important role in promoting fruit development and improving plant resistance. However, the genome-wide identification and analysis of the VQ family in cucumber has not been carried out. This study used bioinformatics technology to identify 26 cucumber VQ genes at the genome-wide level, and analyzed their gene structure, protein structure, evolutionary relationship and studied their distribution on chromosome and tissue expression specificity. To further understand the structural characteristics of the members of the VQ family in cucumber, and to analyze the molecular mechanisms of the cucumber VQ gene to regulate plant growth and development, resist biological and abiotic stresses, and provide data support.

1 Results and Analysis

1.1 Identification of VQ gene in cucumber

In order to identify members of the VQ family in cucumbers, this study used 34 Arabidopsis VQ protein sequences to BLASTP search in the cucumber genome database. After HMMER and SMART analysis, 26 VQ genes were finally identified and named according to their positions on chromosomes. As shown in Table 1, the length of VQ gene in cucumber was between 246 and 10083 bp, and the length of CDS sequence was between 246 and 1395 bp. In addition, CsaV3_1G003880 (CsVQ1), CsaV3_2G003870 (CsVQ4), CsaV3_4G007280 (CsVQ12), CsaV3_4G029660 (CsVQ14), CsaV3_4G03003 (CsVQ15), CsaV3_4G032360 (CsVQ16), CsaV3_4G036400 (CsVQ18), CsaV3_6G030890 (CsVQ21) and CsaV3_6G045520 (CsVQ23) contains two exons, other CsaVQs contain only one exon (Figure 1). The 26 cucumber VQ genes can be divided into 3 branches. Branch 1 contains 5 genes, branch 2 contains 3 genes, and the remaining 18 genes are closely related and belong to branch 3 (Figure 1).

1.2 Structure analysis of cucumber VQ protein

Most of the cucumber VQ proteins were small proteins with less than 400 amino acids, but the length of the protein sequence varied greatly, ranging from 81 aa (CsaV3_4G007830) to 464 aa (CsaV3_1G003880). The molecular weight (MWs) of cucumber VQ protein ranged from 9.28 kD (CsaV3_4G007830) to 51.9 kD (CsaV3_1G003880). Isoelectric point (pI) is distributed between 4.09 (CsaV3_3G040160) and 11.29 (CsaV3_4G032360); The results of subcellular localization showed that most of the cucumber VQ proteins were located in the nucleus, except CsaV3_1G003880 (CsVQ1), CsaV3_2G003870 (CsVQ4), CsaV3_3G039860 (CsVQ8) and csav3_3g032360 (CsVQ16). The VQ motif of 26 VQ genes in cucumber was compared, and the WEBLOGO was generated (Figure 2). The results showed that there were conserved valine (V) and glutamine (Q) in the VQ motif of all genes, and the core motif was FxxxVQx (L/V/F) TG. Among them, LTG proteins accounted for 84.6% (22), VTG proteins accounted for 3.8% (1), and FTG proteins accounted for 11.5% (3) (Figure 3).
Table 1 Information of VQ gene family in cucumber

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<th>Genes</th>
<th>ID</th>
<th>No. of intron</th>
<th>Chromosome location</th>
<th>Length (gene)</th>
<th>Length (CDS)</th>
<th>Length (aa)</th>
<th>Molecular weight (kD)</th>
<th>Isoelectric point</th>
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Figure 1 Gene structure of the CsVQs
1.3 Distribution of VQ gene on chromosome in cucumber

Use Map Inspect software, this paper analyzes the cucumber VQ genes in the distribution of chromosomes (Figure 4), the results showed that 26 VQ genes are distributed on the cucumber seven chromosomes, chromosome including four distribution of most genes (8), the second is the chromosome 2 and chromosome 6 (5), the least amount of chromosome 7 (1); In addition, the VQ gene of cucumber is mostly distributed at the end of chromosome.

Figure 2 Conserved domain of cucumber VQ proteins

Figure 3 Alignment of the conserved amino acid sequence of the CsVQ domains

Figure 4 Chromosome locations of cucumber VQ genes
1.4 Specific analysis of VQ gene expression in cucumber tissue

For parsing VQ family gene function in the cucumber growth and development process, from the Cucurbit Genomics Database (http://cucurbitgenomics.org/organism/20) to download the gene transcription of 26 cucumber VQ set of data, analysis of the members of the family of VQ gene in leaf, stem, male and female flowers, expanded ovary (unfertilized), expanded ovary (fertilized), root fertilization, tendril of expression. As shown in Figure 5, CsaV3_3G040160 (CsVQ9) and CsaV3_3G047610 (CsVQ10) were highly expressed in leaves, stems, flowers, ovaries, roots and tendrils, especially in flowers, ovaries and tendrils, suggesting that they may play a role in the growth and development of multiple plant tissues. CsaV3_4G007830 (CsVQ13) was highly expressed in leaves and ovary, while CsaV3_4G012380 (CsVQ2) and CsaV3_4G03650 (CsVQ17) were highly expressed in ovary and root. CsaV3_1G003880 (CsVQ1), CsaV3_2G016320 (CsVQ5), CsaV3_4G030030 (CsVQ15), CsaV3_5G034400 (CsVQ19), CsaV3_6G044250 (CsVQ22), CsaV3_6G051770 (CsVQ25) and CsaV3_7G032250 (CsVQ26) were all low or no expression in leaves, stems, flowers and ovary.

![Figure 5 Expression of VQ genes in different tissues of cucumber](image)

Note:  R: root;  S: stem;  L: leaf;  FF: female flower;  MF: male flower;  O: ovary;  ex-o-unfer: expanded ovary (unfertilized);  ex-o-fer: expanded ovary (fertilized);  T: tendril

2 Discussion

VQ gene is widely found in higher plants and plays an important role in organ development and resistance to biological and abiotic stress (Perruc et al., 2004; Jing and Lin, 2015). At present, VQ protein family members have been identified in Arabidopsis, Rice, Maize, Grape and other plants, providing data support for further analysis of their functions (Li et al., 2014; Kim et al., 2013; Wang et al., 2014; Wang et al., 2015). In recent years, with the completion of cucumber genome sequencing, it has become faster and more convenient to discover and analyze gene functions at the whole genome level, greatly accelerating the pace of research on cucumber functional genomics (Yang et al., 2012). In this study, bioinformatics technology was used to identify the VQ gene family members of cucumber, and a total of 26 VQ genes were obtained. The protein structure analysis showed that the VQ proteins of cucumber all contained FxxVQx (L/V/F) TG domain, in which the core motif of the VQ domain of CsaV3_3G039860 (CsVQ8) was FxxxxVHxVTG, and the core motif of the VQ domain of CsaV3_4G004780 (CsVQ11), CsaV3_4G007280 (CsVQ12) and CsaV3_6G045520 (CsVQ23) was FxxxxVHxFTG. The core motif of the VQ domain of the remaining genes is FxxxxVHxLTG (Figure 2). This is consistent with the results of a large proportion of LTG-like VQ proteins, a small proportion of V/FGT proteins, and no GTG-like proteins in wheat. The results of subcellular localization showed that the VQ gene of cucumber was mostly located in the nucleus (Table 1), which was consistent with the experimental results obtained by the previous transient expression system of tobacco leaves (Wang et al., 2017).
In this study, tissue expression specificity of VQ gene in cucumber was analyzed, and the results showed that CsaV3_3G040160 (CsVQ9) and CsaV3_3G047610 (CsVQ10) were highly expressed in leaves, stems, flowers, ovaries, roots and tendrils (Figure 5), indicating that these two genes may be involved in the growth and development of multiple tissues and organs. This is consistent with the high expression of Capana00g004669 gene in multiple tissues (Zhang et al., 2016). In Arabidopsis, its homologous genes AtVQ23 and AtVQ9 play an important role in responding to biological and abiotic stress (Lai et al., 2011; Hu et al., 2013), suggesting that both CsVQ9 and CsVQ10 may play an important role in regulating plant organ development and stress response. CsaV3_4G033650 (CsVQ17) and CsaV3_1G012380 (CsVQ2) were closely related, and were highly expressed in female flowers and ovary, indicating that CsaV3_4G033650 was closely related to early fruit growth and development (Figure 5). In Arabidopsis, its homologous gene AtVQ4 interacts with the specific WRKY transcription factor, and the VQ motif mutation weakens the binding with WRKY factor and causes the expression error of the defense gene (Jing and Lin, 2015). CsaV3_1G003880 (CsVQ1), CsaV3_2G016320 (CsVQ5), CsaV3_4G030030 (CsVQ15), CsaV3_5G034400 (CsVQ19), CsaV3_6G044250 (CsVQ22), CsaV3_6G051770 (CsVQ25) and CsaV3_7G032250 (CsVQ26) were all low or no expression in leaves, stems, flowers and ovary. These genes are not involved in the development of leaves, stems, flowers and ovaries in cucumbers (Figure 5).

In summary, this study used bioinformatics methods to identify the VQ gene family members at the genome-wide level, and analyzed their gene structure, conserved structural domain, chromosome distribution and tissue expression specificity, which can provide data support for the exploration of the function of VQ gene in cucumber.

3 Materials and Methods

3.1 Identification of VQ gene in cucumber

Download 34 VQ protein sequences on the Arabidopsis genome website TAIR (https://www.Arabidopsis.org/), using AtVQ protein sequences as probes, respectively in the NCBI (https://www.ncbi.nlm.nih.gov) and the Cucurbit Genomics Database (http://cucurbitgenomics.org/) to BLAST (Ge et al., 2019), retrieval and output of the optimal ratio on the results, determine the members of the family of cucumber VQ candidate genes. Using the online software HMMER and SMART (http://smart.embl-heidelberg.de), the VQ protein domain of the candidate gene was analyzed, and finally 26 VQ gene family members were identified in cucumber. To use online software GSDS 2.0 (http://gsds.cbi.pku.edu.cn/) to analyze the cucumber VQ gene structure, and establish the phylogenetic tree.

3.2 Analysis of VQ protein domain in cucumber

ExPASy online analysis software was used to obtain VQ motif, theoretical molecular weight and isoelectric points of cucumber. Using Plant-mPLoc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/) for cucumber VQ protein subcellular localization prediction. Clustal X2.1 was used to analyze the structure of cucumber VQ protein, and the core motif of the conserved sequence of CsVQ protein was extracted. GENEDOC was used for multiple comparison. To use online software WEBLOGO (http:// WEBLOGO.berkeley.edu/logo.cgi) to make cucumber VQ conservative sequence diagrams (Ge et al., 2019).

3.3 Chromosome localization analysis

Respectively from the NCBI (https://www.ncbi.nlm.nih.gov) and Cucurbit Genomics Database (http://cucurbitgenomics.org/) for cucumber VQ the physical location of genes, using Map Inspect drawing tool to visualize it.

3.4 Tissue expression hotspot mapping

From the cucumber genome database (http://cucurbitgenomics.org/m/20) root, stem and leaf, male and female flowers, ovary, and tendril 9 organization transcriptome data FPKM (Fragments could per kilo base of exon per m letters reads mapped) data, using TBtools software, draw CsVQs gene expression heat maps.
Authors' contributions
Dong Xiaojing was the first author of the paper and the executor of the experimental research. She completed the writing of the first draft of the paper. Han Ni and Hu Yajing participated in the experimental design and the analysis of the experimental results. Professor Ren Zhonghai and associate professor Wang Lina participated in the design, conception and revision of the paper. All authors read and approved the final manuscript.

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