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Analysis of the *CRK* expressions in bottle gourd (*Lagenaria siceraria*) under *Fusarium oxysporum* f. sp. lagenariae stress by using genome-wide identification strategy

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Abstract

Background The cysteine-rich receptor-like kinases (CRKs) family in plants have been reported to perform multiple functions against various stresses. However, the CRK family in bottle gourd (*Lagenaria siceraria*) has not been well-explored. Herein, a comprehensive genome-wide identification and characterization of the CRK gene family has been carried out in bottle gourd under Genome-wide characterization of CRK genes in bottle gourds under *Fusarium oxysporum* f. sp. lagenariae infection.

Results A stringent set of bioinformatic analyses identified 18 *LsCRK*s in the bottle gourd genome. Chromosomal mapping of the identified *LsCRK*s revealed that the *LsCRK*s were distributed in 4 chromosomes in the bottle gourd genome. The phylogenetic analysis of *LsCRK*s divided them into two subgroups on the tree. The synteny and collinearity analysis of the *LsCRK*s among themselves and other plant CRKs provided insights into their conservancy and expansion. Gene ontology analysis of the identified *LsCRK*s suggested their possible roles in regulating different physiological processes and stress responses in bottle gourd. To assess the involvement of the *LsCRK*s under *F. oxysporum* f. sp. lagenariae infection, bottle gourd seedlings were transplanted into the pots with *F. oxysporum*-infected soil. The expression analysis revealed that multiple *LsCRK*s exhibited induced expression, suggesting their involvement in bottle gourd-*F. oxysporum* interactions. Additionally, the protein-protein interaction analysis suggested some important interacting partners of *LsCRK*s crucial to different physiological processes in bottle gourd.

Conclusions The present work explored and analyzed the *LsCRK*s in bottle gourd. Functional predictions and interaction network analysis suggested the roles of *LsCRK*s in modulating multiple physiological processes in bottle gourd. The expression dynamics of *LsCKR*s under fungal pathogen infection suggest their involvement in stress

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response in bottle gourds. Overall, the results of the study provide basic information about the CRK family in bottle gourds and their involvement in fungal pathogen response.

Keywords Cysteine-rich receptor-like kinases, Bottle gourd, Fungal pathogen, Resistance response, Differential expression

Background

Plants are exposed to biotic and abiotic stresses threatening their growth and productivity [1, 2]. Among these, fungal pathogens can cause significant damage or even plant death. Plants have evolved complex mechanisms to defend against such threats, including activating specific genes regulated by transcription factors (TFs). These TFs play a crucial role in modulating gene expression in response to environmental and pathogenic stimuli, initiating protective responses that enhance plant survival and resilience [3]. Receptor-like protein kinases (RLKs) are central to plant signalling networks, detecting external and internal signals and triggering downstream responses that activate specific genes [4, 5]. Among RLK sub-families, cysteine-rich receptor-like kinases (CRKs) are particularly noteworthy due to their conserved C-X8-C-X2-C motifs, which are involved in thiol redox regulation and the sensing of reactive oxygen species (ROS), critical elements in the plant stress response [6].

The CRK gene family is widespread across various plant species and is integral to numerous biological processes, including growth, development, hormone signalling, and responses to both biotic and abiotic stresses [4, 7]. In *Arabidopsis thaliana*, 44 CRK members have been identified, with similar families found in other species such as cotton, tomato, rice, and common bean [8–10]. Similarly, in cucumber, 15 CRK genes were identified in the *Cucumis sativus* genome. Multiple *CsCRK*s were reported to exhibit induced expressions under the fungal pathogen, *Sclerotium rolfsii*, infection [11]. In *A. thaliana*, CRKs like *AtCRK5* and *AtCRK13* are rapidly induced by pathogen attack, promoting programmed cell death (PCD) and enhancing resistance by activating pathogenesis-related genes [12]. Additionally, overexpression studies of multiple *AtCRKs* have highlighted their roles in PCD, ROS production, and pathogen resistance, with some CRKs influencing flowering and developmental processes [13]. *SlCRK1* showed higher expression in tomatoes in floral tissues, particularly in pollen than in other organs. Moreover, several *SlCRKs* were downregulated in response to heat stress, with co-expressed genes exhibiting similar patterns under heat and disease stress conditions [1, 11]. Additionally, in the cucurbits such as cucumber, the CRK genes have been reported to confer resistance against fungal stress. For instance, a major quantitative trait locus *Pm1.1* contributes to conferring resistance against powdery mildew infection. The Pm1.1 locus consists of two *CsCRK* genes in tandem which regulate the fungal resistance response in cucumbers [14].

Bottle gourd (*Lagenaria siceraria*), belonging to the Cucurbitaceae family, is extensively grown in tropical and subtropical areas [15, 16]. Despite genome-wide studies, including graft-responsive mRNA and miRNA identification, this species remains underexplored in the cysteine-rich receptor-like kinase (CRK) transcription factor family [17–19]. On the other hand, *Fusarium oxysporum* f. sp. lagenariae poses a significant threat to bottle gourd, severely affecting its growth and yield. While fungicides are commonly used for control, ongoing molecular breeding programs aim to develop resistant varieties [11, 20]. CRK transcription factors are critical regulators in plant defense and plant-pathogen interactions, making their characterization in bottle gourd crucial for understanding *F. oxysporum* f. sp. lagenariae resistance mechanisms. This study presents the first comprehensive genome-wide analysis of the CRK gene family in *L. siceraria*. The present work identifies 18 CRK genes (*LsCRKs*) in the bottle gourd genome. Further detailed characterizations, including protein property prediction, motif analysis, phylogenetic relationships, chromosomal distribution, intron-exon organization, gene ontology, synteny, collinearity, and protein-protein interactions, provided valuable insights. Furthermore, the expression patterns of *LsCRKs* were analyzed under *F. oxysporum* f. sp. lagenariae stress, providing insights into their possible roles in bottle gourd-*Fusarium* interactions.

Results

Identification of CRKs in bottle gourd

Rigorous computational analysis revealed the identification of 18 CRKs in the bottle gourd genome as identified *LsCRK1* to *LsCRK18*. The identified genes had stress-antifung and Pkinase domains and other canonical conserved domains (Fig. 1A). The distribution of introns and exons in the *LsCRK*s genes was checked, and interestingly, 18 *LsCRK*s showed a diverse distribution of introns and exons. The overall distribution range of exons in the *LsCRK*s ranged from 6 to 14. Out of the 18 *LsCRK*, *LsCRK1*, *2*, *5*, *6*, *10*, *14*, *15*, *16*, and *17* had similar intron-exon organization with an equal number of seven exons. While in *LsCRK7*, *8*, *11*, and *13*, six exons were present (Fig. 1B; Table 1).

Fig. 1 **(A)** Different conserved domains in the *L. siceraria* CRKs (*LsCRK*) sequences. **(B)** The intron-exon organization in *LsCRKs*. CDS: coding DNA sequence; UTR: untranslated region

Chromosomal distribution, conserved motif, and phylogenetic analysis

All 18 *LsCRK*s were mapped on the bottle gourd chromosomes to check their distribution. Results of chromosomal distribution revealed that *LsCRK*s were not distributed equally. Ten *LsCRK*s were mapped onto chromosome number 1, followed by chromosome 2, wherein six CRKs were mapped. On the other hand, chromosomes 3 and 8 have one each CRK (Fig. 2A). Further, a total of 15 canonical conserved motifs were predicted in the *LsCRK*s. All *LsCRK*s have shown the presence of the signature C-X_8-C-X_2-C consensus motif (Fig. 2B). Moreover, to find the evolutionary relationship of LsCRKs, a phylogenetic tree was constructed along with the AtCRKs in which both species CRKs were divided into seven clusters as represented (I-VII). Phylogeny clade I possessed the maximum number of CRKs, while clade VII had the minimum CRKs. LsCRKs were distributed in two clades, i.e., clade I had 15 LsCRKs while clade II had 3 LsCRKs, as depicted in Fig. 3.

Dene duplication, synteny, and collinearity analysis

Evaluation of the Ka/Ks ratios and the comparison of the *LsCRKs* ranged between 0.36 and 0.65 (Ka/Ks

Table 1 Analysis of the physicochemical properties of the *LsCRK*s

Name	Transcript ID	Chrom no.	Start Position	End Position	Strand	Exons	Amino acids	MW (KDa)	pI	GRAVY Score	Localization
LsCRK1	Lsi02G006340.1	2	5,659,371	5,663,881	+ve	8	617	69.13	6.39	-0.015	Nucleus
LsCRK2	Lsi02G006400	2	5,724,632	5,728,792	+ve	7	638	72.47	8.18	-0.165	Nucleus
LsCRK3	Lsi01G003960.1	1	3,401,529	3,409,258	-ve	14	1295	143.42	6.49	-0.145	Nucleus
LsCRK4	Lsi02G006480.1	2	5,801,730	5,805,104	+ve	9	710	78.72	8	-0.041	Cell Membrane
LsCRK5	Lsi01G003930.1	1	3,391,639	3,394,994	-ve	7	592	66.07	7.8	-0.163	Cell Membrane
LsCRK6	Lsi01G003920.1	1	3,385,104	3,389,918	-ve	7	676	75.63	6.41	-0.156	Nucleus
LsCRK7	Lsi01G003830.1	1	3,344,927	3,348,061	-ve	6	620	69.45	5.33	-0.132	Nucleus
LsCRK8	Lsi01G005960.1	1	4,755,811	4,759,088	+ve	6	618	69.97	8.61	-0.193	Cell Membrane
LsCRK9	Lsi02G006540.1	2	5,844,675	5,847,836	+ve	8	614	69.31	6.85	-0.135	Nucleus
LsCRK10	Lsi01G003840.1	1	3,350,839	3,353,605	+ve	7	659	73.51	5.78	-0.16	Cell Membrane
LsCRK11	Lsi01G003870.1	1	3,364,300	3,367,626	+ve	6	683	76.2	6.23	-0.188	Cell Membrane
LsCRK12	Lsi01G003910	1	3,378,840	3,384,830	-ve	9	715	80.12	8.7	-0.237	Nucleus
LsCRK13	Lsi03G013670.1	3	24,601,731	24,605,898	-ve	6	660	73.37	8.55	-0.148	Nucleus
LsCRK14	Lsi02G006550.1	2	5,852,260	5,854,901	+ve	7	661	73.82	7.84	-0.189	Cell Membrane
LsCRK15	Lsi01G003900.1	1	3,374,309	3,376,948	-ve	7	655	72.99	5.74	-0.267	Cell Membrane
LsCRK16	Lsi02G006440.1	2	5,766,332	5,769,392	+ve	7	741	83.63	6.27	-0.139	Cell Membrane
LsCRK17	Lsi01G003860.1	1	3,360,457	3,363,106	+ve	7	620	69.24	5.61	-0.238	Cell Membrane
LsCRK18	Lsi08G016570.1	8	24,371,565	4,383,094	+ve	14	1394	154.45	8.87	-0.188	Nucleus

MW: Molecular weight; KDa: kilo Dalton; pI: Isoelectric point; GRAVY: grand average of hydropathicity

ratio) with a possible 6 gene duplication pairs, including *LsCRK10-LsCRK17*, *LsCRK13-LsCRK18*, *LsCRK1-LsCRK2*, *LsCRK3-LsCRK15*, *LsCRK6-LsCRK12*, and *LsCRK9-LsCRK14* (Table S1). Among these, *LsCRK10-LsCRK17* and *LsCRK13-LsCRK18* gene pairs were predicted to result from tandem duplication, while the other gene pairs might be from segmental duplication. All these gene pairs were predicted to be generated from the purifying (negative) selection process. Synteny and collinearity analyses were performed to obtain better insights into the *LsCRK* evolution (Fig. 4). The synteny analysis showed the synteny blocks and duplicated gene pairs. Conversely, the collinearity analysis revealed the orthologs of the *LsCRKs* in *Arabidopsis* and watermelon genomes.

Gene ontology and *cis*-regulatory element analysis

Gene ontology of 18 *LsCRKs* was performed in the current study to predict their possible roles in bottle gourd. For example, the most enriched biological processes of the *LsCRKs* were responses to stimulus, stress, and metabolic processes. In contrast, the most enriched molecular functions were kinase activity, catalytic activity and binding (Fig. S1). Similarly, the cis-regulatory elements (CRE) analysis results illustrated that *LsCRKs* contained different CREs, including EIRE (elicitor-responsive element), TCA (tricarboxylic acid), JERE (jasmonate ethylene responsive elements) and LTR (long terminal repeats) having roles in various physiological processes (Fig. 5). Moreover, JERE, ABRE (abscisic acid-responsive element), AuxRE (auxin response element), ERE (ethylene-responsive elements), and EIRE reported to be involved in phytohormone signalling. Similarly, TCA, WUN-motif (wound-responsive element), MYB, MYC, W-box, LTR (long terminal repeat), and DRE (dehydration-responsive element) are reported to be involved in stress response regulations. Further, the CREs, including box-E and HSE (heat-shock element) as involved in regulating gene expressions during development and stress response.

Analysis of *LsCRK* expressions by using transcriptome data

The prediction of multiple key CREs in the promoter regions of the identified *LsCRKs* suggested their involvement in different physiological processes in bottle gourd. To verify this hypothesis, the *LsCRK* expressions were evaluated by using the transcriptome data obtained from NCBI. To start with, the tissue-specific expression of the *LsCRKs* was checked, and the results revealed that the *LsCRKs* were differentially expressed in different tissues of bottle gourd (Fig. 6A). Similarly, the expression dynamics of the *LsCRKs* were analyzed by using the transcriptome data under powdery mildew infection. The results revealed that the *LsCRKs* exhibited differential expressions in the control and powdery mildew bottle gourd plants in both resistant (J83) and susceptible (J73) varieties (Fig. 6B).

Expressions analysis of *LsCRKs* under *F. oxysporum* f. sp. lagenariae infection

The expression of all 18 *LsCRKs* was analyzed under the infection of *F. oxysporum* f. sp. lagenariae by performing qRT-PCR (Fig. S2). The results displayed the differential expression of the.

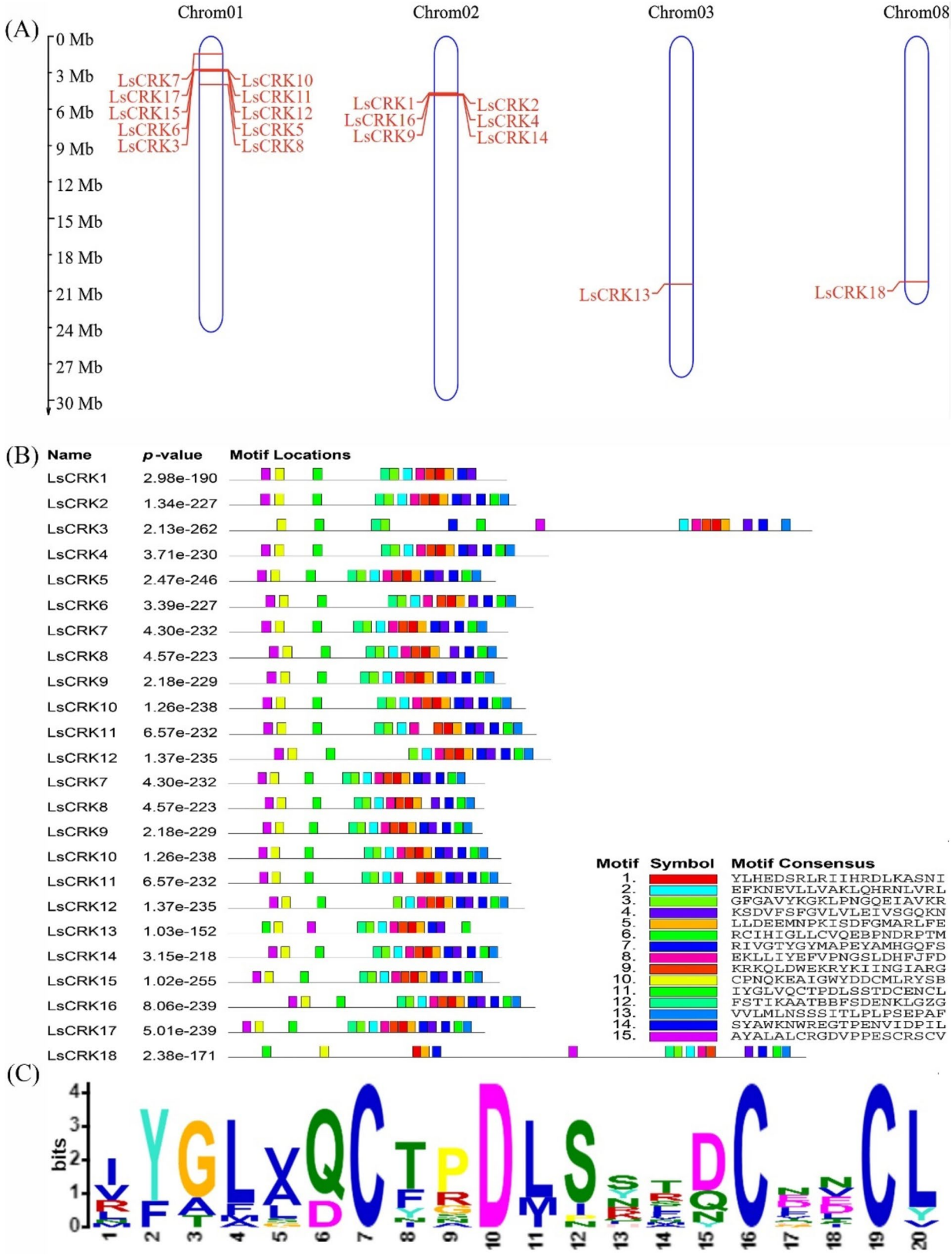

Fig. 2 **(A)** Chromosomal distribution of the *LsCRKs*. Individual chromosome sizes are indicated by the side ruler. Chrom: chromosome. **(B)** De novo prediction and distribution of motifs in the *LsCRK* sequences. Individual protein sequence lengths and the different identified motifs are represented by the solid lines and the coloured boxes, respectively. The motif consensus sequences are provided at the bottom side. **(C)** The signature conserved C-X_8-C-X_2-C motif possessed by the *LsCRKs*

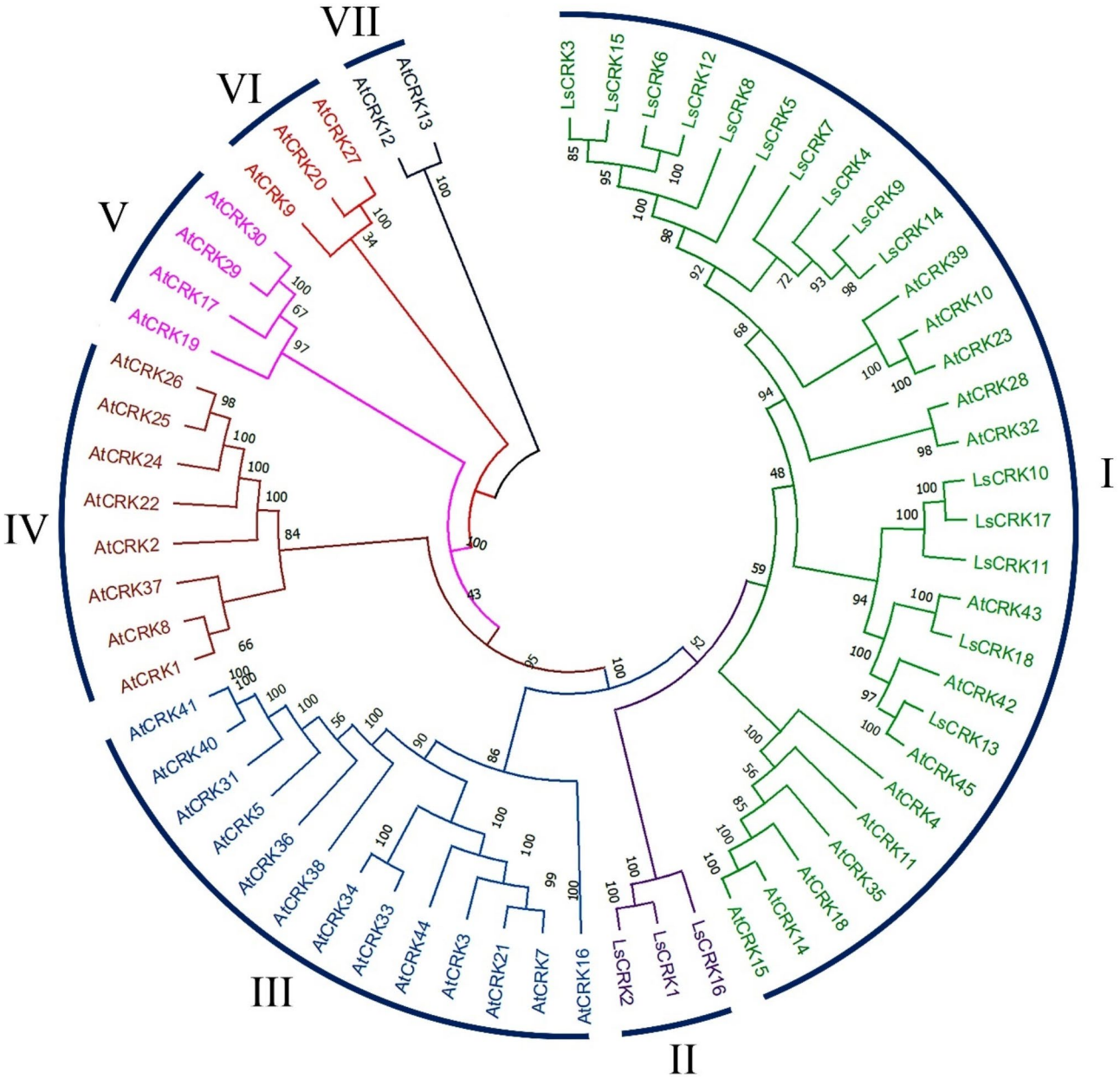

Fig. 3 The phylogenetic tree was constructed using the LsCRKs and AtCRKs using the neighbour-joining algorithm with 1000 bootstraps using MEGA v11. The Roman numeric represents the individual sub-groups on the phylogenetic tree

LsCRKs in the Arka Bahar variety of the bottle gourd. In addition, early (on 1-day post-inoculation, DPI) and late (on 5 DPI) expression dynamics can be seen in the *LsCRKs*. For instance, *LsCRK1*, *LsCRK3*, *LsCRK6*, and *LsCRK17* exhibited an early differential expression compared to the control. In contrast, *LsCRK4* exhibited a late induced expression (Fig. 7). Moreover, 17 out of the 18 *LsCRKs* showed significant expression dynamics compared to the control either at early, late, or both stages.

Protein-protein interaction network analysis of LsCRKs

CRK is a vital protein class that has the potential to interact with several other proteins and, hence, increase cell functions. The protein-protein interaction network of CRKs in bottle gourd predicted the interactions of the LsCRKs with other cellular proteins involved in some crucial physiological processes. For instance, multiple CRKs were expected to interact with the interacting partner P2C19_ARATH, which is a protein phosphatase 2 C and cyclic nucleotide-binding/kinase domain-containing protein (Fig. 8). Similarly, one more interacting partner, the MLP329 protein which regulates seed germination

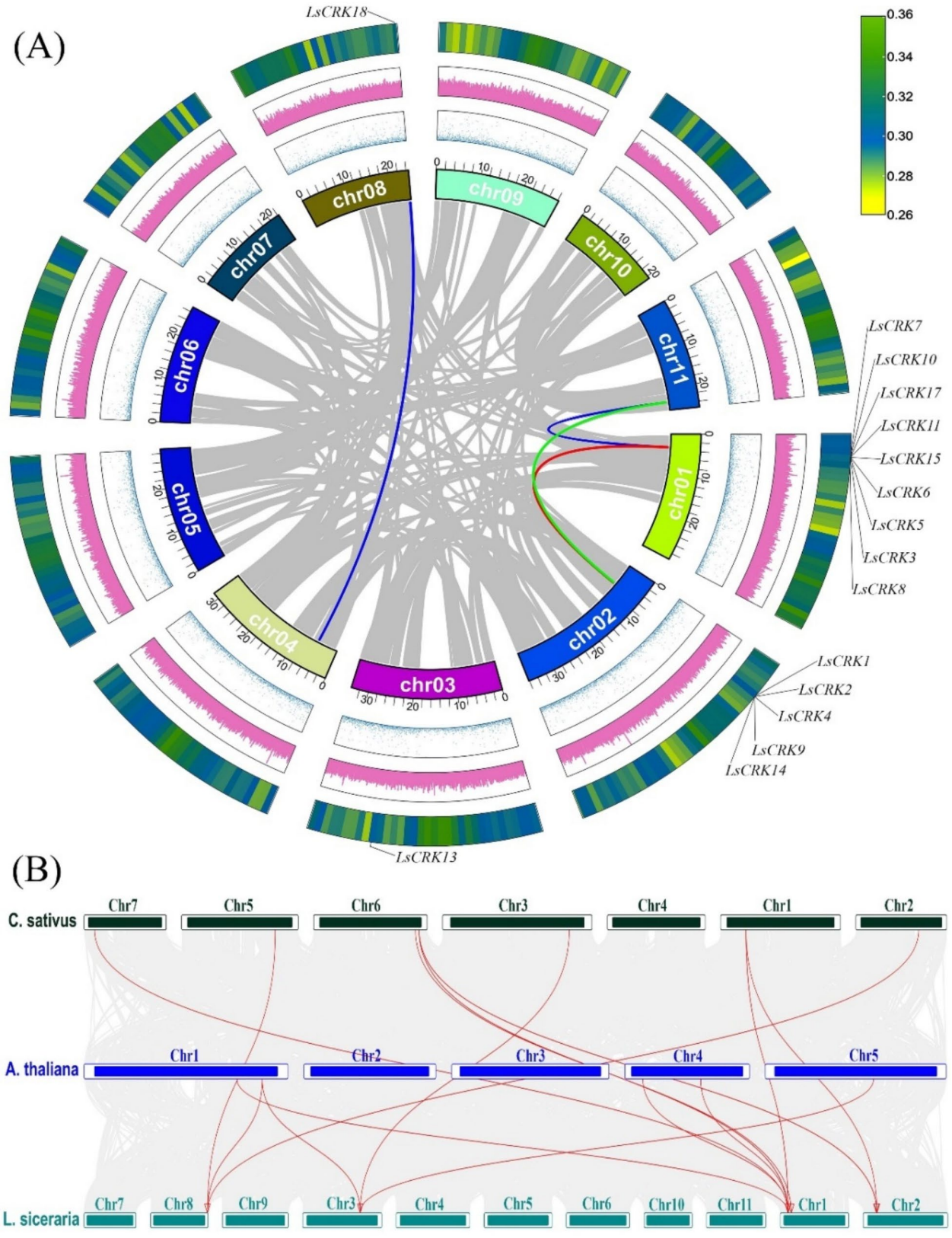

Fig. 4 **(A)** Synteny analysis of the *LsCRKs*–the coloured lines indicate the duplicated *LsCRK* pairs, while the background grey lines indicate the synteny blocks in the bottle gourd genome. The outer circle's lines, dots, and heat map represent the nucleotide numbers, GC content, and gene density, respectively, on the individual chromosome. **(B)** The collinearity analysis of the *LsCRKs* among the CRKs in *Arabidopsis* and cucumber genomes. The red lines represent the collinear CRK pairs, whereas the grey back-ground represents the genome-wide collinear pairs

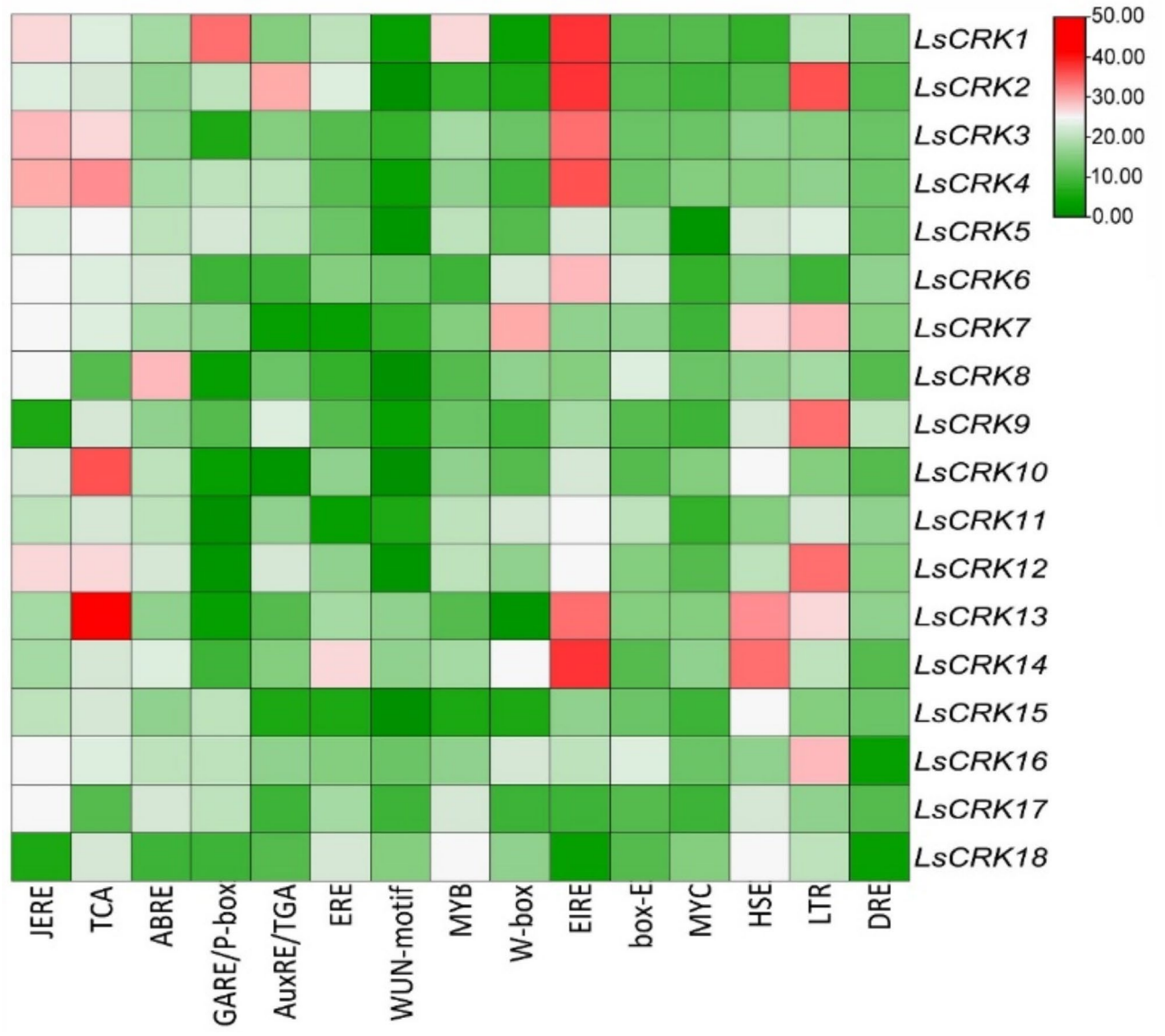

Fig. 5 The prediction and distribution of the *cis*-regulatory elements (CREs) on the promoter regions of the *LsCRKs*. The colour gradient represents the number of the CREs in increasing order from green to red for each of the identified *LsCRKs*

and dormancy. Likewise, other important interacting partners involved F22G5.6 and F9K23.7, which are annotated to be LRR receptor-like serine/threonine-protein kinase, NB-LRR family protein, and TIR-NBS class protein, involved in regulating processes like signal transduction, stress response, and disease resistance, respectively.

Discussion

The receptor-like kinases (RLKs) are one of the plant proteins superfamily that perform diverse roles in perceiving stimuli and signals [21–23]. The CRKs family belongs to the RLK superfamily, having three distinct domains, i.e., extracellular, transmembrane, and intracellular kinase domains [7, 21]. It has been documented that in plants, the CRKs have diverse roles in regulating several physiological processes [1, 24]. The CRKs reportedly produce plant stress responses against abiotic and biotic stresses [3, 4, 25]. The plant pattern recognition receptors (PRRs) are crucial in recognizing external pathogens and activating plant immune responses via different downstream pathways [26, 27]. The CRKs have been reported to participate in the plant-pathogen interactions and regulate the plant immune response [7, 11, 17]. *Fusarium* wilt (FW) is a soil-borne disease caused by *F. oxysporum* f. sp. lagenariae that adversely affects bottle gourd yield and production. To strengthen FW resistance in bottle gourds, a GWAS study was done based on thousands of SNPs in diverse bottle gourd accessions [28]. Though earlier research on bottle gourd is carried out, i.e., their genome is sequenced, the number of CRK

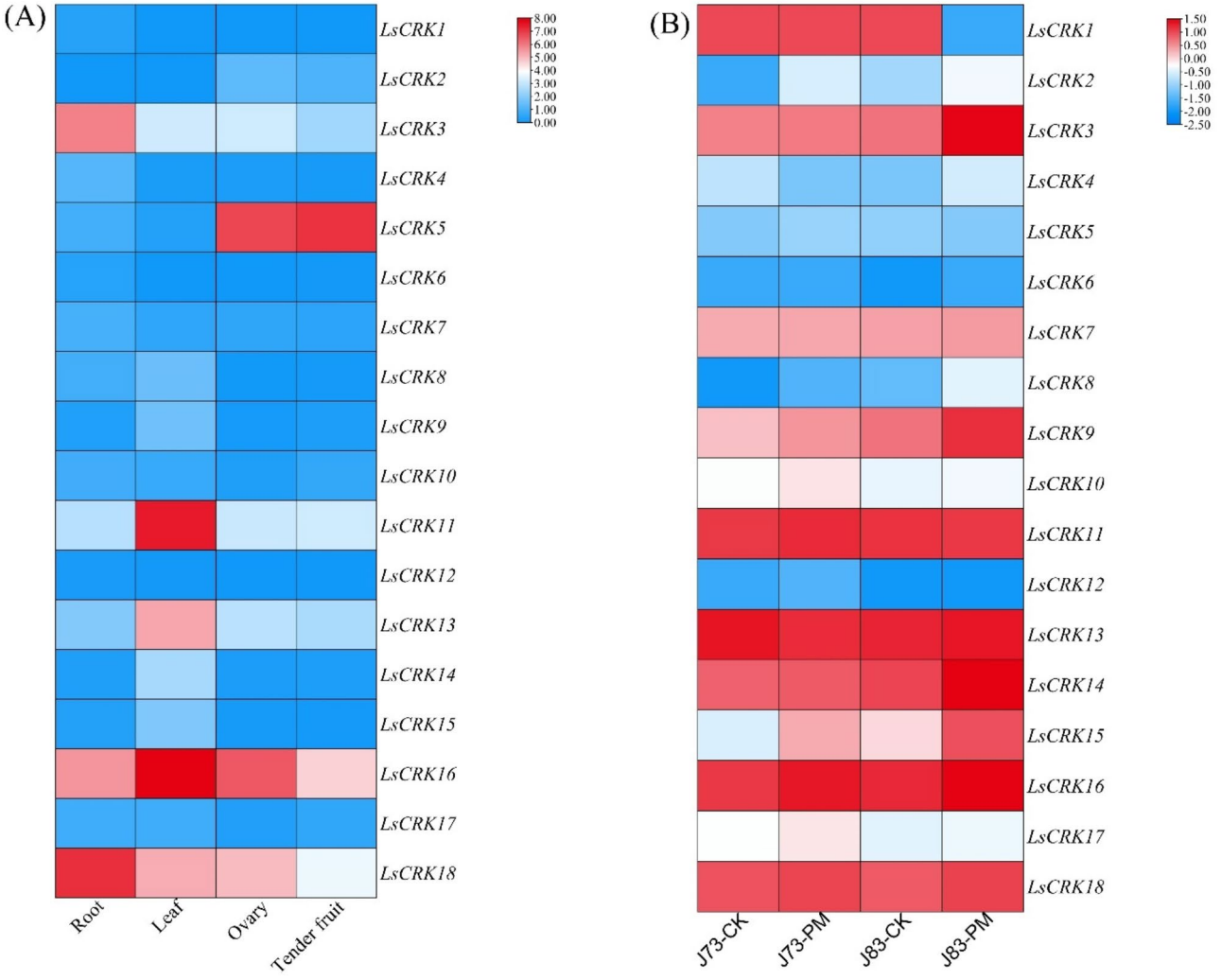

Fig. 6 **(A)** The tissue-specific expression profiles of the *LsCRKs*. The colour gradient from red to blue represents the upregulated to the downregulated expression of the *LsCRKs*, respectively. **(B)** The differential expression of the *LsCRKs* under the powdery mildew infection in two contrasting varieties, J73 (susceptible) and J83 (resistant). PM- powdery mildew infected, CK- control (uninfected). The colour gradient from red to blue represents the upregulated to the downregulated expression of the *LsCRKs*, respectively

genes and their functions in response to biotic stress with primary emphasis on *F. oxysporum* is unclear. Before this, the CRK gene family is well characterized through available genome sequences and by stringent bioinformatics analysis in crops like pepper, cotton, phaseolus, tomato, and soybean [1, 10, 29, 30]. In the current study, we have identified 18 *LsCRKs* using bioinformatics tools, and they were thoroughly characterized as possessing signature conserved domains and motifs. Moreover, phylogenetic analysis has distributed LsCRKs and AtCRKs into seven clades, wherein LsCRKs were only present in groups 1 and 2, respectively. In accordance with our evolutionary findings of LsCRKs in terms of a total of seven groups and their presence in only two sub-groups, the previous studies have reported the same pattern in their dendrogram of CRKs in pepper and cucumber [7, 13]. The results of *cis*-regulatory elements suggested that the *LsCRKs* were probably involved in stress response in bottle gourds due to the presence of important CRES, such as TCA, JERE, and EIRE [34–37]. Gene ontology results depicted that the *LsCRKs* primarily respond to stimulus, stress, membrane, and kinase activities in bottle gourd. Similar to our results, other reports show CRK's role in stress response, membrane, and kinase activities in different plants, such as pepper, wheat, cotton, cucumber, and *Arabidopsis* [11, 17, 21, 30–32].

The expression analysis of all identified *LsCRKs* revealed that they are differentially expressed in different tissues during the growth and development of bottle gourds. The CRKs in plants have been reported to regulate several vital processes, including plant growth and development [21, 33, 34]. The differential expression of the *LsCRKs* in tissues like leaf, stem, root, flower, and fruit suggested their possible involvement in the different

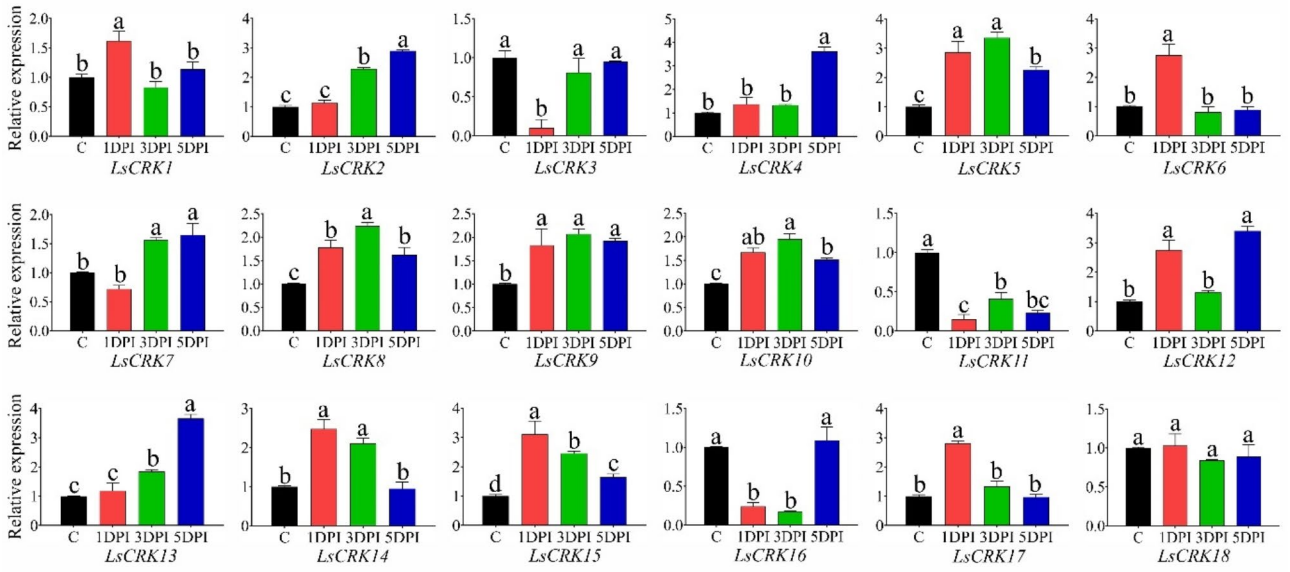

Fig. 7 The relative expression of the *LsCRKs* under *F. oxysporum* f. sp. lagenariae infection. The expression data (fold change) are represented as mean ± SE, and the statistical significance is indicated by the lowercase alphabets (a-e) analyzed at $P < 0.05$

organ growth and development. In addition, the RNA-seq data-based expression analysis of the *LsCRKs* under powdery mildew infection in two contrasting varieties of bottle gourds revealed that the *LsCRKs* are involved in the powdery mildew-bottle gourd interactions and the nature and number of *LsCRK* expressions may depend on the type of interactions (compatible or incompatible). For instance, multiple cotton CRKs, including *GhCRK057*, *GhCRK059*, *GhCRK058*, and *GhCRK081*, exhibited their importance in the resistant *Gossypium spp*. compared to the susceptible one [17]. Furthermore, the involvement of *LsCRKs* in response to *F. oxysporum* infection was investigated using qRT-PCR analysis. Interestingly, 17 out of 18 *LsCRKs* exhibited significant differential expressions compared to the control. Several *LsCRK*s were upregulated and then downregulated after infection with powdery mildew and *F. oxysporum*. The possible reason for this might be because of the transient nature of the CRK gene expressions. Briefly, several stress-responsive genes exhibit expressions at different time points and the expression can also dips down afterwards, classifying them into early and late responsive gene expressions [35]. Thus, a similar pattern of gene expression might be followed by the *LsCRK*s under the fungal infections causing some of them to get induced at the early time points (1 DPI), whereas dipping down at the late time points (3–5 DPI). However, in-depth analysis, especially at the protein levels is needed to confirm this hypothesis. These results suggest that multiple *LsCRKs* could regulate the defense response in bottle gourd during the bottle gourd-*F. oxysporum* interactions. Moreover, deeper functional analysis, including loss of function and overexpression studies, can provide further insights into the individual roles of the *LsCRKs*. Additionally, the protein-protein interaction network of the *LsCRKs* was predicted to get additional insights into the interacting partners and the processes in which the *LsCRKs* were possibly involved. The results indicated that multiple interacting partners of the *LsCRKs* had roles in crucial physiological processes. For instance, RLP, one of the interacting partners, has a role in resistance to biotic stress in plants [36, 37]. Similarly, EXO (EXORDIUM), MLP (primary latex-like protein), and DAR (DA1-related protein) proteins are mainly involved in the signalling processes, defense mechanisms, and plant development, respectively [38, 39]. These findings further strengthen the crucial roles of the *LsCRKs* in bottle gourds in regulating vital physiological processes, including plant defense.

Conclusion

In total, 18 *LsCRKs* were identified in the bottle gourd genome. A set of stringent bioinformatic analysis further characterized the *LsCRK*s, including their gene structure, chromosomal location, duplication events, synteny, and evolutionary relationship. Furthermore, the expression profiling of *LsCRKs* from publicly available transcriptome data and through qRT-PCR analysis revealed that multiple *LsCRKs* are involved in defense responses under biotic stress in bottle gourd. Protein interaction predictions of the *LsCRKs* hinted at their functions in regulating bottle gourd development and defense responses. In addition, the antioxidant enzyme assays, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione-s-transferase (GST) could further strengthen the obtained results. Overall, the findings of this study provide a basic understanding of the CRK

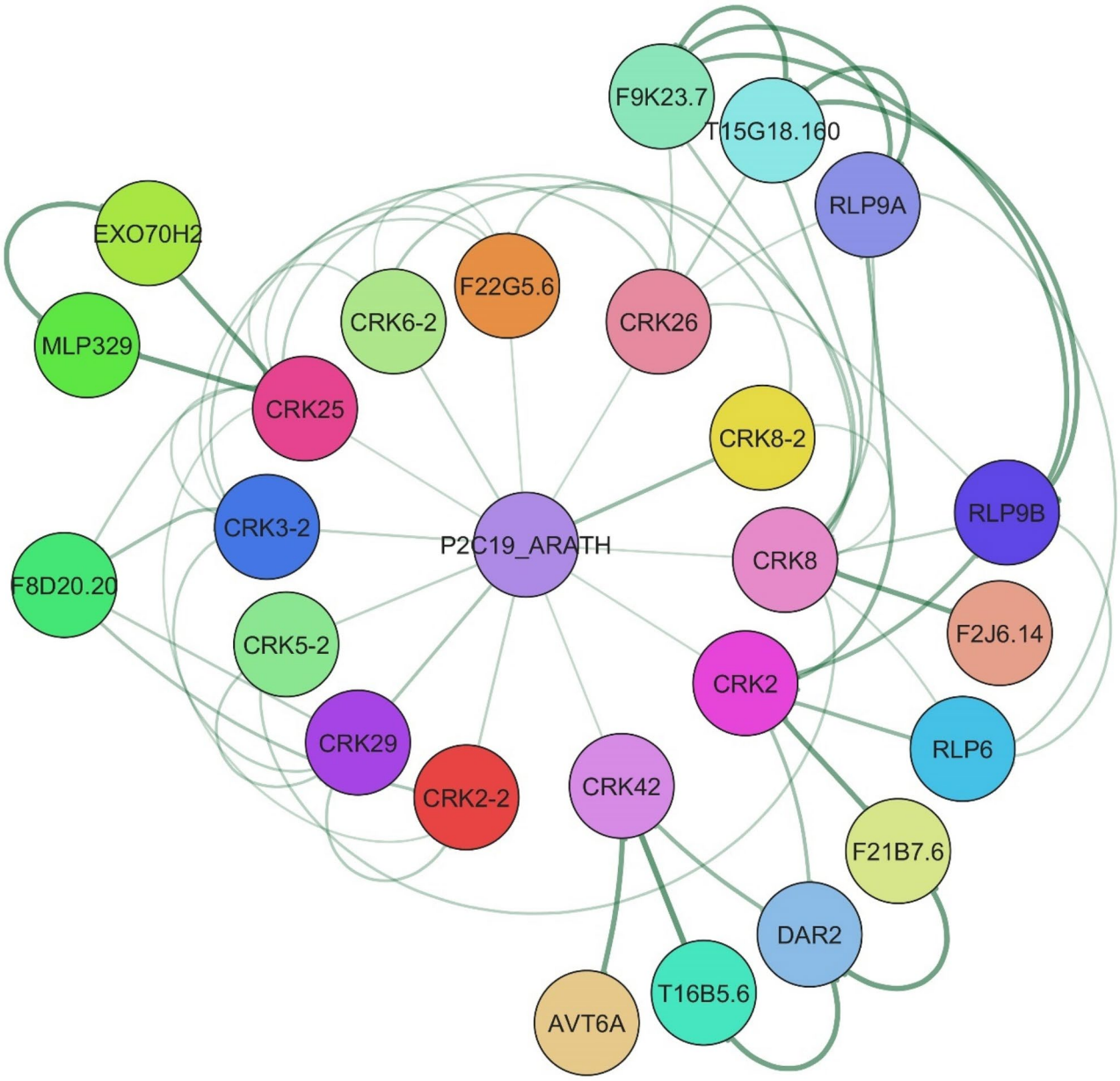

Fig. 8 The protein-protein interaction analysis. The coloured nodes are the interacting partners of the LsCRKs, and the coloured lines represent protein-protein associations. The thickness of the lines indicates the confidence levels for the specific interactions

gene family distribution and putative functions in bottle gourds.

Materials and methods

Identification of the CRK genes in bottle gourd

Homologous CRK sequences were identified using BLASTp searches on the CuGenDBv2 web portal [40] using the AtCRKs as queries. The obtained hits were then checked for the signature domains, namely the Pkinase (PF00069) and Stress-antifung (PF01657) domains, using Pfam and HMMER tools with an E-value threshold of <0.001 [41]. To further validate these candidates, they were analyzed using the Conserved Domain Database (CDD) and Simple Modular Architecture Research Tool (SMART) to ensure the accuracy of domain annotations [42, 43]. Subsequently, the DNA and coding sequences (CDS) were retrieved from the bottle gourd genome v2. Lastly, the ProtParam tool was used to compute the various peptide properties of the identified CRKs in bottle gourd [44].

Sequence alignment, motif prediction, and phylogenetic analysis of *LsCRKs*

De novo motif analysis of *LsCRKs* was conducted using the MEME tool. For multiple sequence alignments, *LsCRKs* were compared with CRKs from *Arabidopsis* and rice using Clustal Omega [45]. To assess the evolutionary relationship of the LsCRKs, the phylogenetic analysis was carried out on MEGA v11 by employing the neighbour-joining algorithm, with 1000 bootstraps to assess the robustness of the tree [46].

Intron-exon organization, *cis*-regulatory element analysis, and subcellular localization prediction of *LsCRKs*

The intron-exon organizations of *LsCRKs* were estimated using the Gene Structure Display Server. For *cis*-regulatory element analysis, we examined 2Kb upstream sequences of *LsCRKs* using the PlantCARE tool [47]. Subcellular localization predictions for *LsCRKs* were performed using the mGOASVM server for plants [48].

Chromosomal mapping, gene duplication, and synteny analysis

The chromosomal distribution of *LsCRKs* was mapped using MapGene2Chrom (MG2C) [49]. Homologous gene pairs were identified using the Ka/Ks Calculator 2.0 to assess gene duplication [50]. Genome sequences and annotations in FASTA and GFF/GTF formats were retrieved from bottle gourd genome version 3 and *Arabidopsis* (TAIR) for synteny analysis. The one-step MCScanX tool in TBtools was used to evaluate the synteny relationship [51].

Gene ontology, protein-protein interaction, and structural analysis of *LsCRKs*

Gene ontology (GO) analysis was conducted using Blast2GO and WEGO2.0 tools [52]. Protein-protein interactions of *LsCRKs* were analyzed with the String database using default parameters, and the results were visualized with Cytoscape [53]. The three-dimensional structure of the *LsCRKs* was modelled through homology using SWISS-Model with default settings [54].

Plant and pathogen materials

The bottle gourd variety "Arka Bahar" was selected for this study. Seeds were sterilized following a standard protocol and then sown in plastic pots (15 cm diameter) filled with a sterile mixture of soil and manure. The pots were maintained in a greenhouse with controlled conditions of 28 ± 2 °C and 80 ± 5% relative humidity to ensure optimal seedling growth. The pathogen was a virulent strain of *F. oxysporum* f. sp. lagenariae obtained from the ICAR-National Bureau of Agriculturally Important Micro-organisms. A 12-day-old culture of *F. oxysporum* on potato dextrose agar (PDA) was utilized to prepare the inoculum. Mycelial plugs from this culture were transferred to 500 g of pre-cooked, autoclaved sorghum seeds. The inoculated sorghum seeds were incubated at 28 ± 2 °C for one week to allow fungal colonization. Following incubation, the sorghum seeds were mixed with autoclaved soil at 10 g of seeds per 1 kg of soil. This soil-sorghum mixture was filled into individual pots (12 cm dia) and incubated under natural greenhouse conditions for ten days. Twenty-day-old Arka Bahar bottle gourd seedlings were transplanted into these pots to facilitate *F. oxysporum* infection. Control seedlings were grown in pots containing only sterilized soil. The experiment was replicated three times to ensure the reliability of the results.

Isolation of total RNA and synthesis of first-strand cDNA

Leaves of Arka Bahar bottle gourd were harvested from plants at various time points (0-, 1-, 3-, and 5 days post-inoculation) after they were transplanted into *F. oxysporum*-infected soil. The harvested tissues were immediately frozen in liquid nitrogen and stored at -70 °C. Total RNA was extracted from the frozen tissues using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). RNA quality and concentration were assessed using a QIAxpert System (Qiagen, Hilden, Germany) and by electrophoresis on a 0.8% agarose gel. For cDNA synthesis, the first-strand cDNA was generated using a Verso cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. The synthesized cDNA was then stored at -70 °C for subsequent analysis.

Expression analysis of *LsCRKs*

The expression of *LsCRK* genes in different tissues, including leaf, stem, root, flower, and fruit, was evaluated using the transcriptome data obtained from the project PRJNA387615. Similarly, the expression of *LsCRKs* under powdery mildew infection was deduced from the transcriptome data from bioproject PRJNA793252. The expression profiles were potted on a heatmap with TBtools software.

For the estimation of the *LsCRK* expressions under *F. oxysporum* infection, real-time PCR (qRT-PCR) was performed using a Roche Light Cycler (Basel, Switzerland). Each 10 µL reaction contained 5 µL of SYBR Green master mix, 2 µL of gene-specific primers (Table S2), 2 µL of ultrapure water, and 1 µL of 10-fold diluted cDNA. The bottle gourd *glyceraldehyde 3-phosphate dehydrogenase* gene (*LsGAPDH*) served as the internal control for normalization, and expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method [55, 56]. Each biological replicate was performed in triplicate, and the experiment included three independent biological replicates. Statistical significance was determined using one-way ANOVA with a

significance level of $P \leq 0.05$ and analyzed using Data Processing System software [57].

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11349-8.

Supplementary Material 1

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Author contributions
Conceptualization, S.N., and S.A.; methodology, P.S., I.U., D.S., and G.K.; data curation, C.B, N.A., and M.A.Z.; writing—original draft preparation, P.S., M.A.Z., I.U., S.A., S.S.L., M.F.S. and N.A.; writing—review and editing, S.N., C.B., G.K., and S.A.; supervision, S.N; funding acquisition, M.F.S., and S.A. All authors have read and agreed to the published version of the manuscript.

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Data availability
All data related to this study are present in this article (Table 1) and the corresponding supplementary material. The accession numbers of the 18 CRKs in bottle gourd (LsCRK1-18) are Lsi02G006340.1, Lsi02G006400, Lsi01G003960.1, Lsi02G006480.1, Lsi01G003930.1, Lsi01G003920.1, Lsi01G003830.1, Lsi01G005960.1, Lsi02G006540.1, Lsi01G003840.1, Lsi01G003870.1, Lsi01G003910, Lsi03G013670.1, Lsi02G006550.1, Lsi01G003900.1, Lsi02G006440.1, Lsi01G003860.1, and Lsi08G016570.1, respectively. The biological datasets used for analysis in this study are available in the Cu-GenDBv2 platform (http://cucurbitgenomics.org/, accessed on 08 June 2024).

Declarations

Ethics approval and consent to participate
No experiments were performed on animals or humans. The experiments conducted on plants, including plant stress subjection and sample collection were done in compliance with the institutional, national, and international guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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