

# Identification of a *cp-2* gene controlling compact plant architecture in cucumber

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## Introduction

Cucumber plants have indeterminate, long, trailing stems that require a large area for planting and high labor inputs for management. Identifying genes responsible for compact growth is, therefore, highly valuable in cucumber breeding programs focused on improving plant growth architecture to improve yields (Wang et al. 2022). Compact or semi-dwarf growth habits offer several agronomic advantages, such as higher planting density, reduced water consumption, and easier harvesting, contributing to higher yields or cost savings (Ferrero et al. 2019). Here, we report research progress on the cucumber line L504, which is characterized by a compact growth habit controlled by a single recessive gene *cp-2*, described by Kubicki et al. (1986) (Fig.1).

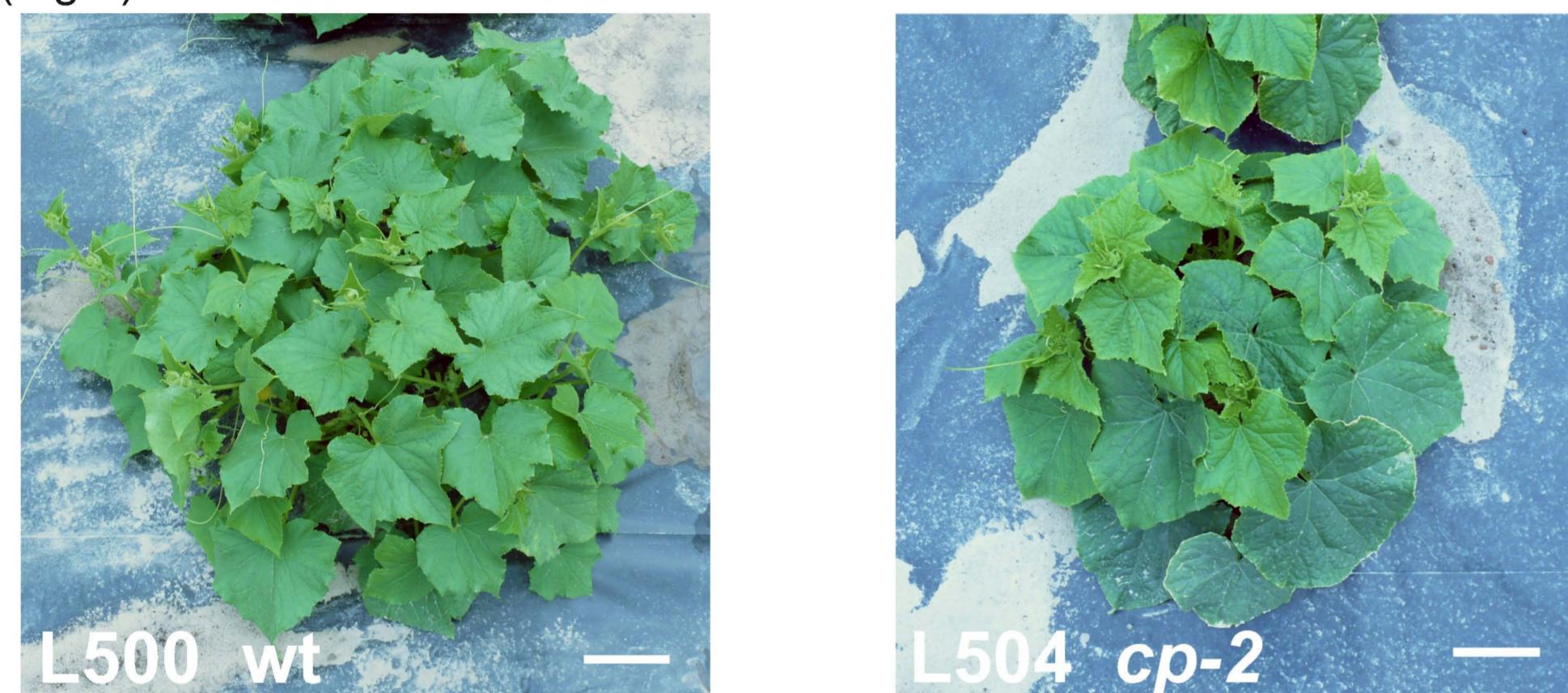


Figure 1. The control inbred line L500, with normal growth and inbred line L504 characterized by compact growth. Four week old plants under field conditions. Scale bars 10 cm

## Materials & Methods

- For fine-mapping, a set of molecular markers and a segregating F2 population (n=780) were developed from a cross between the wild-type line L500 and the compact line L504
- RNA-seq analysis of the L500 and L504 lines was performed using Illumina Novaseq (Illumina, CA, USA) for shoots of plants with 2-3 leaves grown under phytotron conditions
- Candidate gene expression analysis was performed using organspecific RT-qPCR
- Phylogenetic analysis of amino acid sequences was performed in CLUSTAL and MEGA11 software using the maximum likelihood algorithm

## Results

On the basis of phenotyping and high-throughput genotyping, we identified a genomic region corresponding to the *cp-2* locus. Then, using fine-mapping and bioinformatic analyses, we narrowed this region and identified a candidate gene, encoding a protein of unknown function with a serine/threonine kinase domain, as responsive for compact growth habit (Fig. 2).

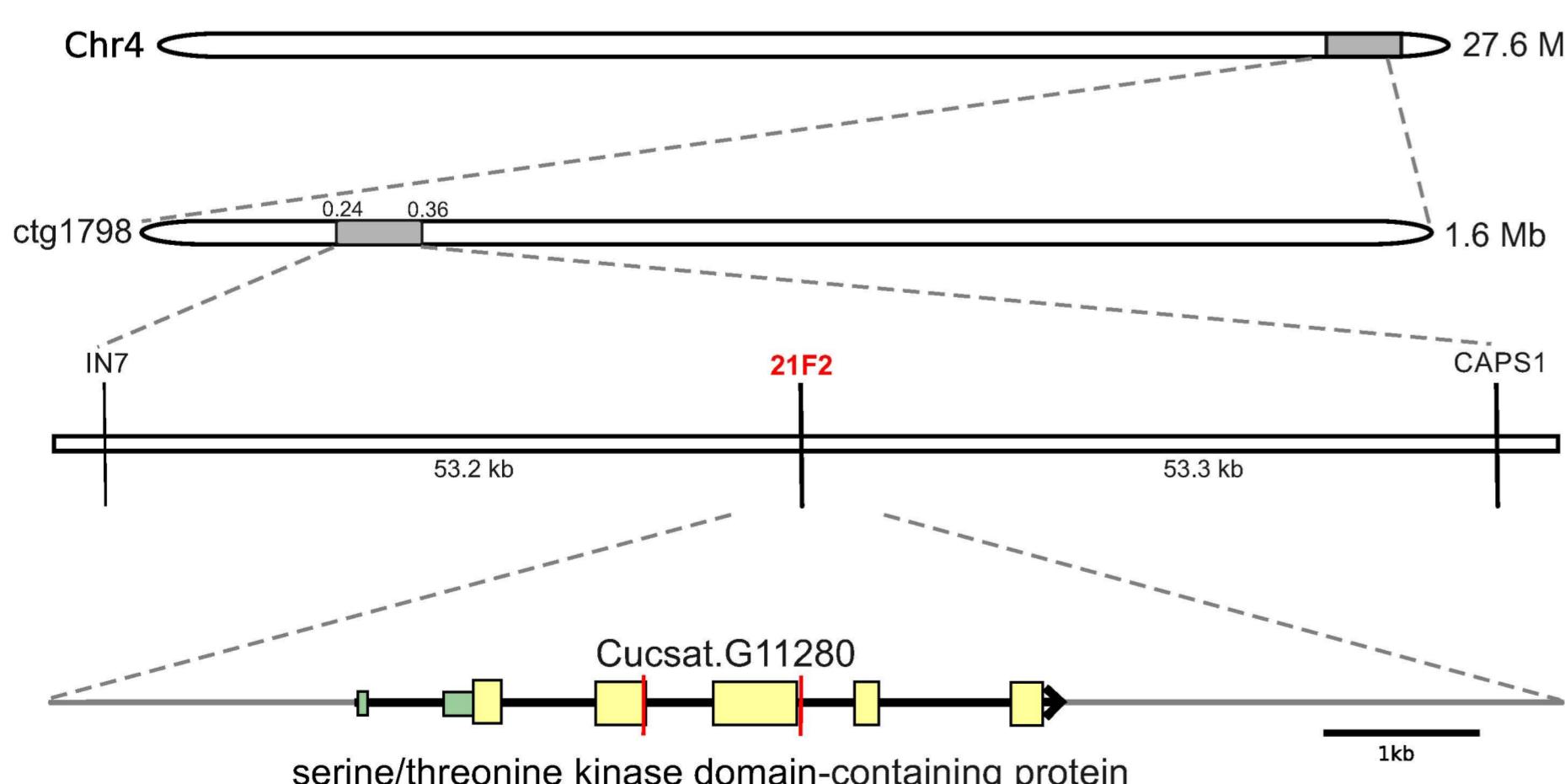


Figure 2. Fine-mapping of *cp-2* locus located on cucumber chromosome 4. Two SNPs detected in the candidate gene are marked with red lines

## Acknowledgements

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## Literature

Ferrero-Serrano A, Cantos C, Assmann SM 2019 The role of dwarfing traits in historical and modern agriculture with a focus on rice. *Cold Spring Harb Perspect Biol*, 11:a034645

Kubicki B, Sołtysiak U, Korzeniewska A 1986 Induced mutation in cucumber (*Cucumis sativus* L.). V. Compact type of growth. *Genet Pol* 27: 289-298

Wang S, Wang K, Li Z, Li Y, He J, et al 2022 Architecture design of cucurbit crops for enhanced productivity by a natural allele. *Nat Plants* 8:1394-1407

## Results

RNA-seq analysis revealed 15,590 genes co-expressed in both lines. In total, there were 641 DEGs in the compact L504 line compared with the control line L500, including 267 genes upregulated and 374 downregulated. KEGG analysis revealed that in compact line L504 genes related with phenylpropanoid biosynthesis and alpha-linolenic acid metabolism were downregulated. Other downregulated genes were associated with phenylalanine, glutathione and fatty acid metabolism (Fig 3).

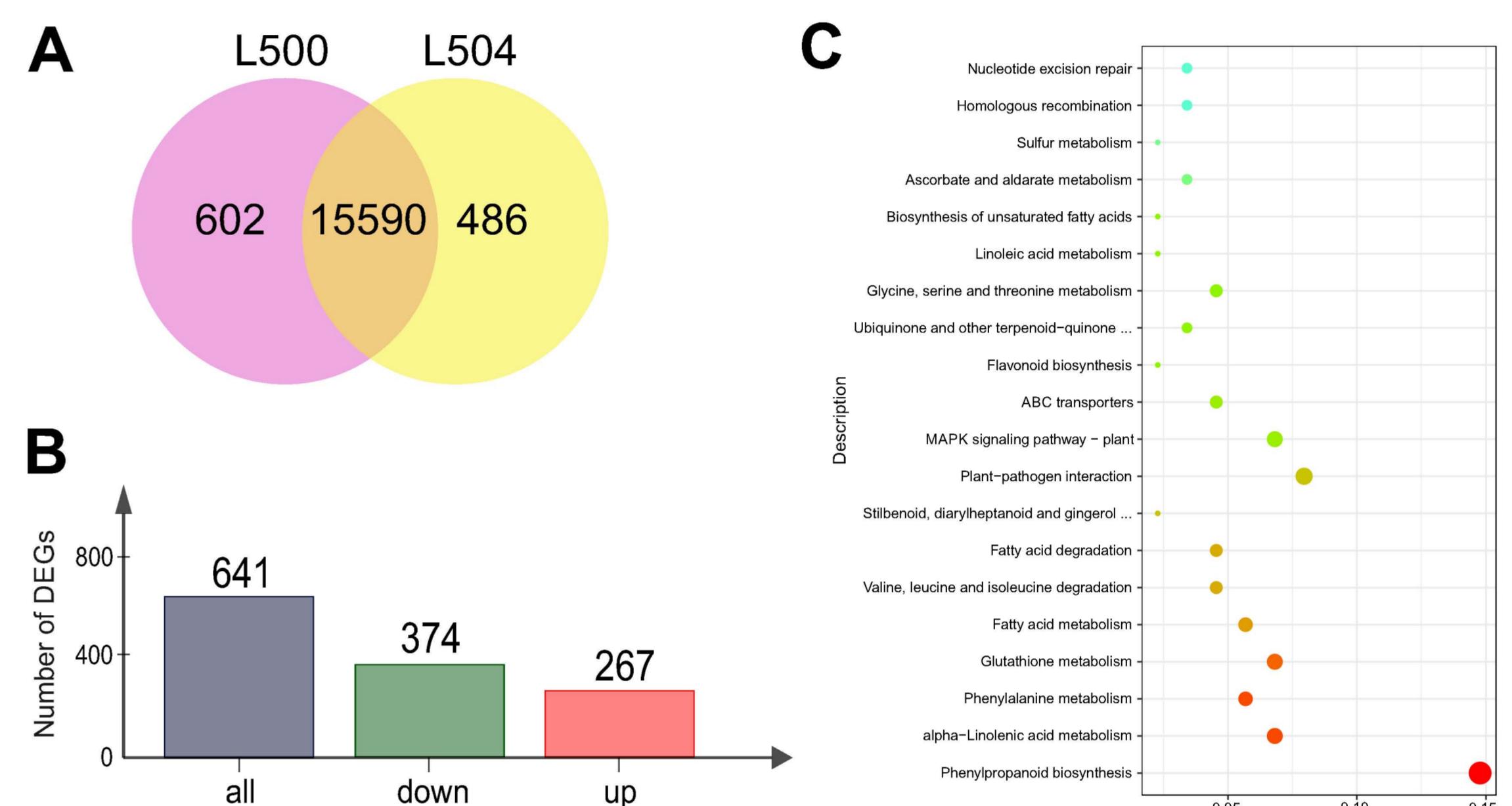


Figure 3. (A) Venn diagram representing the uniquely expressed genes for each line. The overlapping regions show the number of genes that are co-expressed in both lines. (B) Summary of DEGs identified for the L504 line compared with the L500 control line. Red and green represent up- and downregulated genes, respectively. (C) Summary of KEGG pathway enrichment analysis results for the L504 line

RT-qPCR revealed that the candidate gene was upregulated in the roots, leaves, stems, petioles and female flowers (Fig. 4A). Amino acid sequence analysis of the candidate and its orthologs revealed similarities of cucumber protein to poorly known proteins with a serine/threonine kinase domain. This domain was identified in the C-terminal region of the protein. Interestingly, no ortholog of this protein has been identified in *Arabidopsis*, although such orthologs have been found in the alga *Micromonas* and the liverwort *Marchantia* (Fig 4B).

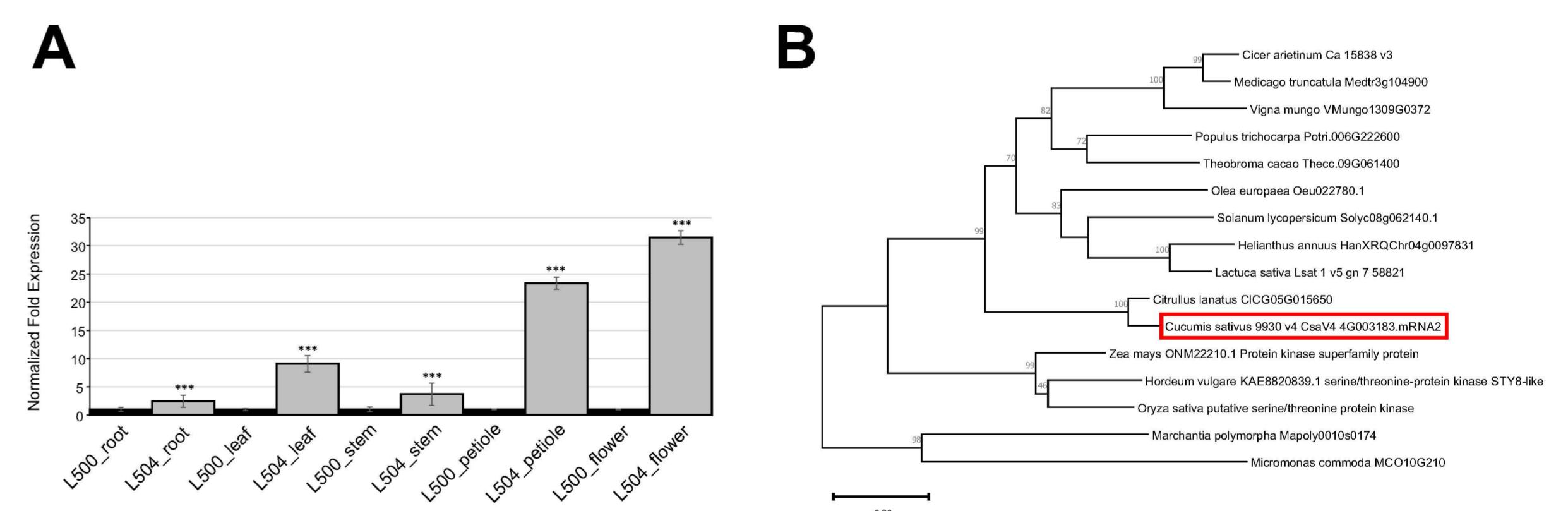


Figure 4. (A) Gene expression analysis of candidate gene for *cp-2* revealed via RT-qPCR. For data normalization, three references *CACS*, *UBI*ep, and *TIP41*, were used. Significance levels were calculated with Student's t-test  $p < 0.001$  (\*\*\*) (B) Phylogenetic analysis of amino acid sequences of proteins with a serine/threonine kinase domain

## Conclusions

- Fine-mapping and subsequent bioinformatic analyses allowed the identification of a candidate gene for *compact-2* which encodes an unknown protein with a serine/threonine kinase domain
- RNA-seq revealed that many downregulated genes are related to phenylpropanoid biosynthesis and alpha-linolenic acid metabolism
- The expression of the candidate gene in the L504 line was upregulated in various organs compared with that in the control L500 line
- These findings provide new insights into the molecular mechanisms underlying the compact growth of cucumber and offer potential targets for the genetic improvement of plant architecture