Identification of a *scp* gene controlling super compact plant architecture in cucumber

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Introduction

Plant architecture is important in plant breeding, influencing crop growth, yield, and stress tolerance. The altered plant architecture affects light perception, carbon assimilation, and dry matter accumulation, which can ultimately result in increased yields per cultivation area. It directly determines the workload in crop management and harvesting. Here, we report the research progress on cucumber line L505, characterized by super compact phenotype controlled by a single recessive gene *scp*, previously described by Niemirowicz-Szczyt et al. (1996) (Fig.1).

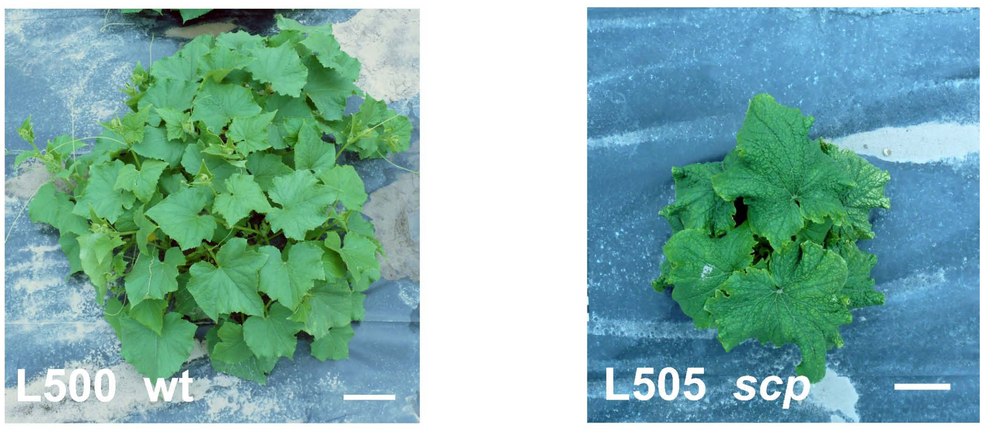

Figure 1. Control inbred line L500 with normal growth and inbred line L505 characterized by altered growth grown under field conditions. Scale bars, 10 cm

Results

Based on phenotyping and high-throughput genotyping, we identified a genomic region corresponding to the *scp-2* locus. Then, using fine-mapping and bioinformatic analyses, we narrowed down this region and identified a candidate gene G12021, encoding a protein with P450 cytochrome domain (Fig. 2A). Variome analysis revealed that line L505 possesses a unique T->C SNP at position 229 CDS, which does not occur naturally in other accessions available in databases (Fig. 2B).

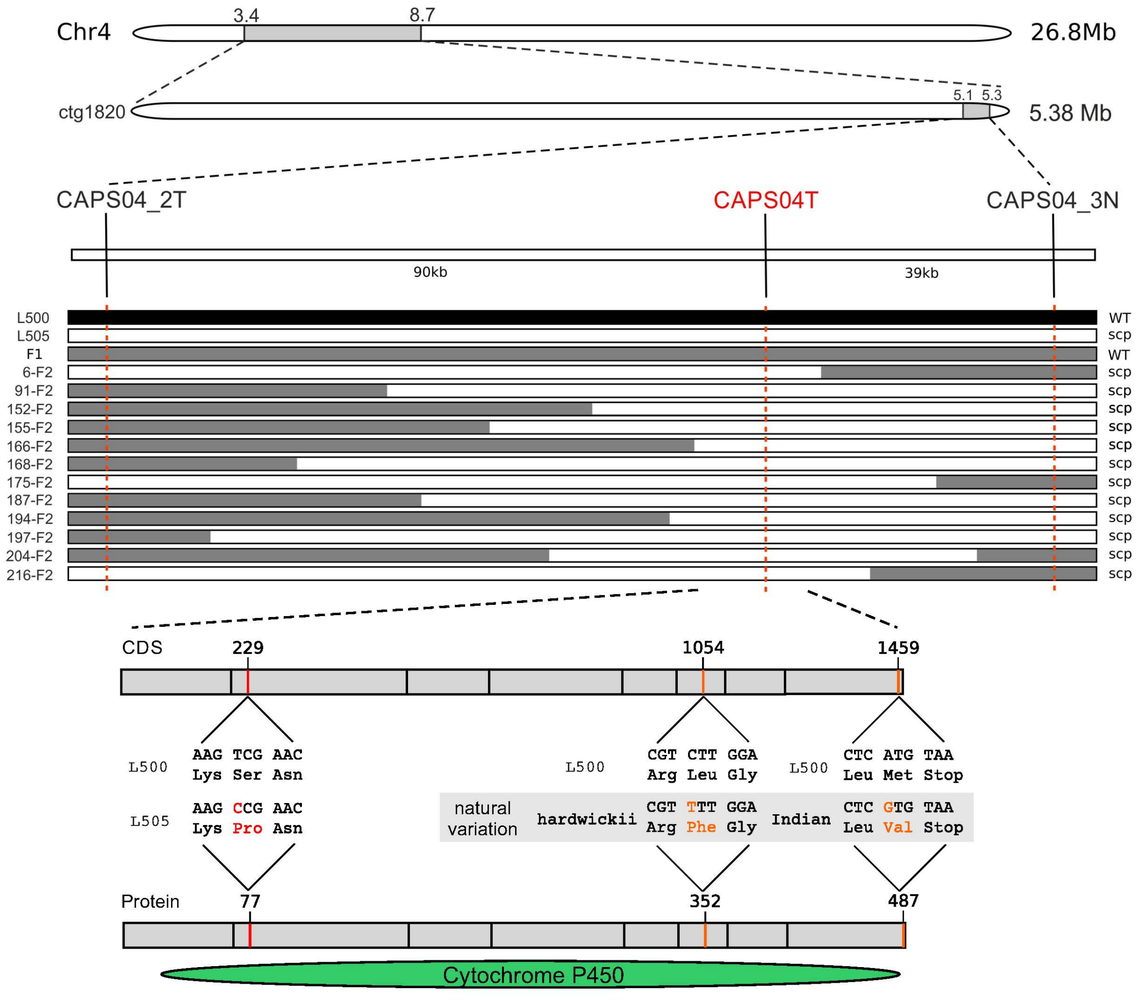

Figure 2. (A) Fine-mapping of *scp* locus located on cucumber chromosome 4. (B) Schematic presentation of CDS and protein structure of candidate gene G12021. The unique substitution detected in G12021 in line L505 is marked in red. Other substitutions occuring in wild-type cucumber hardwickii and Indian lines are marked in orange.

Acknowledgements

The study was supported by a research program of the Polish Ministry of Agriculture and Rural Development "Basic research for biological progress in crop production 2021-2025", specifically task 33 "Identification of genes associated with altered growth type in cucumber plants (*Cucumis sativus* L.)"

Literature

1. Guan et al.(2024) A near-complete cucumber reference genome assembly and Cucumber-DB, a multi-omics database. Mol Plant 17:1178–1182
2. Niemirowicz-Szczytt et al. (1996) An induced mutation in cucumber: super compact. Cucurbit Genet Coop Rep 19:1–3
3. Yu et al. (2023) CuGenDBv2: an updated database for cucurbit genomics. Nucleic Acids Res 51:D1457–D1464

Materials & Methods

- **Fine-mapping:** A set of molecular markers and a segregating F2 population (n=877) were developed from a cross between the wild-type line L500 and the super compact line L505
- **Variome analysis:** The analysis of natural variability in the candidate gene was performed by comparing the sequence of this gene to the sequences of 115 and 388 accessions available in the Cucumber DB (Guan et al. 2024) and CucurbitGenomic v2 (Yu et al. 2023) databases
- **RNA-seq:** Analysis of the L500 and L505 lines was performed using Illumina Novaseq (Illumina, USA) for above-ground part of the plant with 1-2 leaves grown under phytotron conditions
- **RT-qPCR:** Candidate gene expression analysis was performed using organspecific RT-qPCR
- **Phylogenetic analysis:** Amino acid sequences was used and phylogenetic tree was performed in CLUSTAL and MEGA 11 software using the Maximum Likelihood algorithm

Results

RNA-seq analysis identified 1290 DEGs in the super compact L505 line as compared to control, including 941 up- and 349 down-regulated genes. KEGG analysis revealed that the highest number of DEGs in L505 line were linked to phenylopropanoid biosynthesis, phenyloalanine metabolism, plant-pathogen interaction and MAPK signalling pathway. Some genes associated with brassinosteroids biosynthesis were also up- or down-regulated in L505 line (Fig 3).

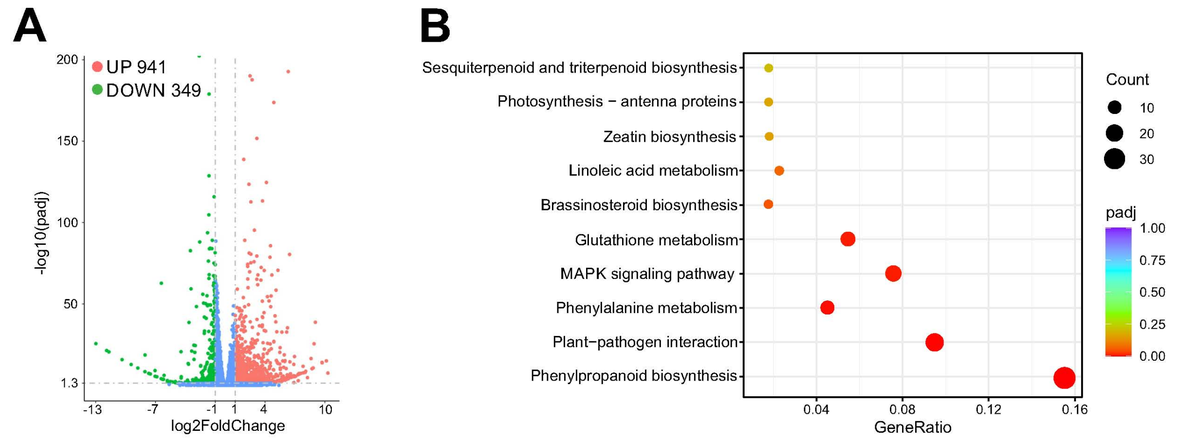

Figure 3. (A) Summary of DEGs identified for the L505 line compared with the L500 control line. Red and green represent up- and down-regulated genes, respectively (padj<0.05, log2FoldChange>1).(B) Summary of KEGG pathway enrichment analysis results for the L505 line

RT-qPCR showed up-regulation of the candidate gene in petioles and male flowers and down-regulation in roots, leaves and shoots (Fig 4A). Amino acid sequence analysis and its orthologs revealed similarities of cucumber protein to DWF4, which is involved in the early steps of brassinosteroids biosynthesis pathway (Fig 4B).

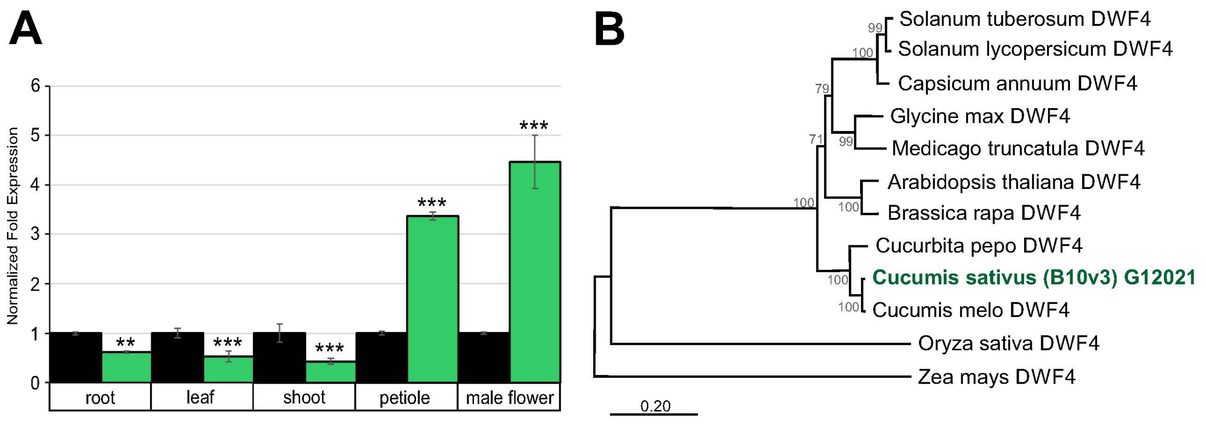

Figure 4. (A) Expression analysis of candidate gene reveled by RT-qPCR. For data normalization, three references *CACS, UBIep*, and *TIP41* were used Significance levels were calculated with Student's t-test p<0.001 (***) (B) Phylogenetic analysis of DWF4 proteins with P450 cytochrome domain

Conclusions

- Fine-mapping and subsequent bioinformatic analyses allowed for identification of a candidate gene for *scp* which encodes CsDWF4 protein with cytochrome P450 domain
- RNA-seq revealed that some up- and down-regulated genes are related to brassinosteroids biosynthesis as well phenylopropanoid biosynthesis and phenyloalanine metabolism
- The expression of the candidate gene in the L505 line showed up-regulation in petioles and male flowers, and down-regulation in roots, leaves and shoots compared to the control L500 line
- These findings provide new insights into the molecular mechanisms underlying the super compact growth of cucumber and offer potential targets for the genetic improvement of plant architecture