Genetic mapping reveals candidate genes controlling plant architecture in cucumber

R. Słomnicka, K. Kaźmińska, M. Cieplak, D. Stokowiec, A. Korzeniewska, G. Bartoszewski

Warsaw University of Life Sciences – WULS, Nowoursynowska 159, 02-787 Warsaw, Poland

Introduction

Plant architecture is one of the most important determinants of nutrient distribution and photosynthesis and has a major impact on crop management and yield efficiency. Identification of key genes and molecular mechanisms that control plant architecture, including plant height and branching is critical for modern breeding programs. In cucumber, only several genes determining altered growth habit at the molecular level have been identified, and there are attempts to develop novel compact cultivars (Liu et al. 2021).
Here, we report research progress on three cucumber lines that exhibit altered growth habit controlled by single recessive genes. The genomes of these lines were previously re-sequenced using the Illumina NovaSeq6000 platform (Słomnicka et al. 2022).

Materials & Methods

Three segregating F2 populations were developed from the crosses between line L500 with normal growth and lines characterized by altered growth habit L504, L505 and L511 due to *cp-2*, *scp* and *sp-2* genes (Fig. 1). For each population, plant phenotyping and DArTSeq genotyping were performed, and next, identified SNPs were used for genetic mapping. Comprehensive analysis of genotyping data allowed to determine genomic regions associated with plant growth habit in all three populations. Then, for the polymorphisms identified within the candidate genes, PCR-based markers were designed and tested in the segregating populations to identify candidate genes controlling plant growth. In addition, for each line transcriptomic analysis was performed.

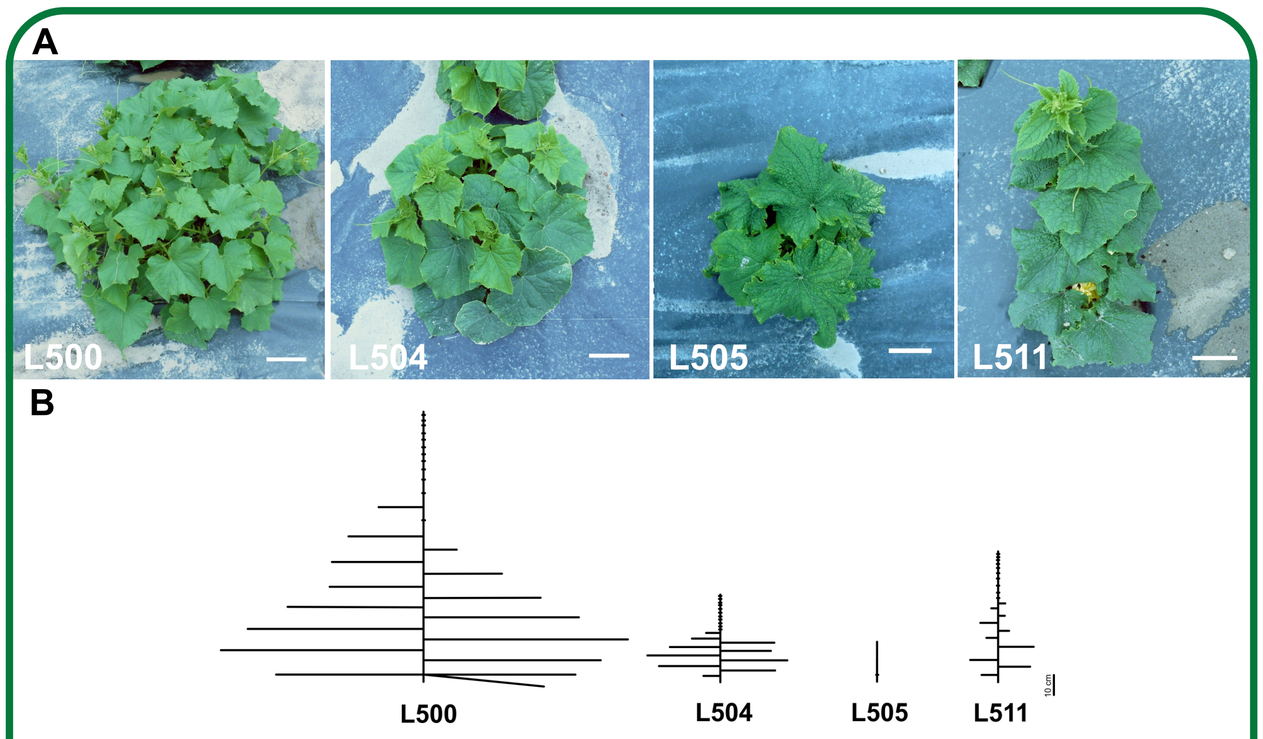


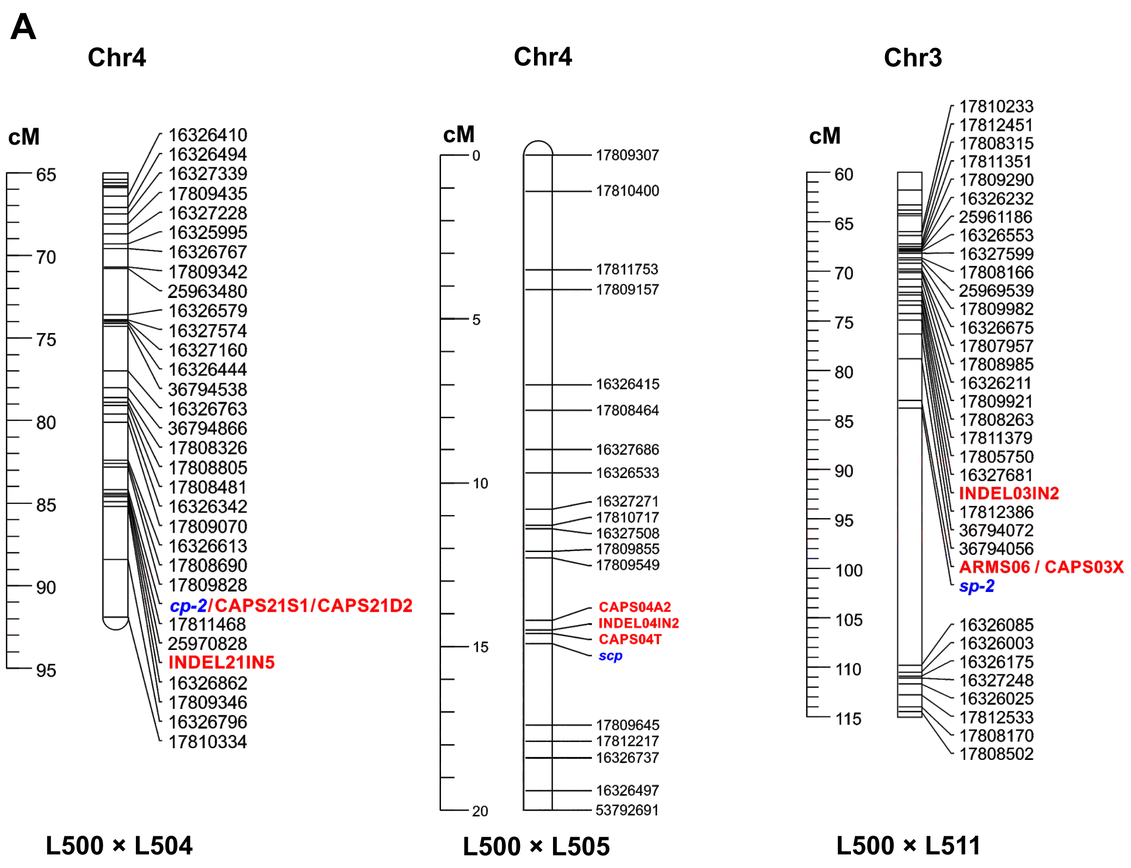
Figure 1. Control inbred line L500 with normal growth habit and inbred lines characterized by altered growth habit grown under field conditions (A). A scheme of the plant architecture representing each line (B). Scale bars, 10 cm.

Results

Phenotyping of F2 individuals and genetic analysis confirmed that the altered growth habit in each cucumber line is determined by single recessive genes (Tab. 1). A total 2,663 SNPs were identified based on DArTSeq genotyping and 1,418 SNPs were used to construct genetic maps for all F2 populations (Fig. 2). Based on the annotation available in the Cucurbit Genomics v2 database (Yu et al. 2022), the genomic regions were mapped and candidate genes related to altered growth habit were identified (Fig. 2 and 3).

Table 1. Segregation and Chi-square analysis of F2 individuals derived from crosses between L500×L504, L500×L505 and L500×L511.

Population	Number of plants		Total	Segregation ratio	χ^2
	normal growth	altered growth			
L500 × L504	220	74	294	3:1	0.06
L500 × L505	263	101	364	3:1	1.47
L500 × L511	246	74	320	3:1	0.60



B

Population	Marker interval (cM)	Flanking markers	Genomic region	No of genes	Protein encoded by candidate gene
L500×L504	87.8-89.4	17809828-17811468	253 kb	17	Kinase domain-containing protein
L500×L505	14.6-17.4	CAPS04T-17809645	717 kb	38	Cytochrome P450
L500×L511	78.0-104.8	ARMS06/CAPS03X-16326085	6.6 Mb	962	Steroid 5-alpha-reductase

Figure 2. Genetic maps of chr 3 and 4 with identified genes related to altered growth habit in three populations L500×L504, L500×L505 and L500×L511 (JoinMap 4.0) (A). Genes associated with altered growth habit are marked in blue. PCR-based markers correlated to each gene are shown in red. Characteristics of genomic regions related to altered growth habit identified for L500×L504, L500×L505 and L500×L511 populations (B).

Results

Genes encoding kinase domain-containing protein, cytochrome P450 and steroid 5-alpha-reductase were selected as candidate genes related to altered growth habit for the L504, L505 and L511 lines, respectively. Single non-synonymous SNPs in the coding sequences of these genes were found (Fig.3A). PCR-based markers were developed for each F2 population to verify polymorphism within or in close proximity to the candidate gene. These markers showed ~99% consistency between phenotypic and genotypic data (Fig. 3B).

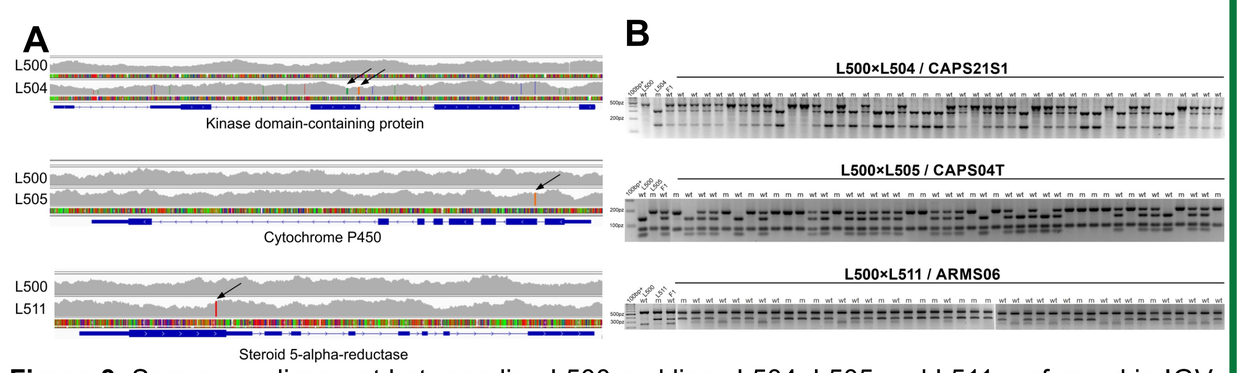


Figure 3. Sequence alignment between line L500 and lines L504, L505 and L511 performed in IGV program for three candidate genes (A). Fragments of amplification profiles obtained by genotyping of CAPS21S1, CAPS04T and ARMS06 markers (B). wt - indicates plant characterized by a normal growth habit and m - indicates plant with an altered growth habit.

Candidate genes identified for L505 and L511 lines play an important role in the brassinosteroid biosynthesis pathway. RNASeq analysis showed some changes in the expression profiles of genes related to brassinosteroid biosynthesis in these lines (Tab. 2).

Table 2. Expression heatmap of crucial genes involved in brassinosteroid biosythesis pathway identified in L505 and L511 lines. The data represents the -log2 FoldChange of raw FPKM values.

Gene abbrev.	Gene ID 9930 V3	-log2FoldChange ≥1.0	
		L505	L511
CYP90B1	CsaV3_5G026940		
CYP724B1	CsaV3_4G005090		
CYP92A6	CsaV3_3G030690		
CYP734A1	CsaV3_1G029710		
DET2	CsaV3_3G034190		
CYPC1	CsaV3_6G046740		
CYPD1	CsaV3_3G043920		
CYP85A1/2	CsaV3_5G038650		

Conclusions

1. Genetic analysis of three F2 populations confirmed that the altered growth habit is determined by a single recessive gene.
2. DArTSeq genotyping resulted in the mapping of genomic regions and identification of candidate genes responsible for the growth habit of cucumber plants in populations:
   - L500×L504 - candidate gene encoding kinase domain-containing protein located on chr4
   - L500×L505 - candidate gene encoding cytochrome P450 located on chr4
   - L500×L511 - candidate gene encoding steroid 5-alpha-reductase located on chr 3
3. RNASeq revealed changes in the expression of genes related to brassinosteroid biosynthesis in L505 and L511 lines.
4. These studies provide new insights into the molecular mechanisms controlling altered growth habit in cucumber.

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 2021-2027", specifically task 33 "Identification of genes associated with altered growth type in cucumber plants (*Cucumis sativus* L.)".

Literature
1. Liu X, Chen J, Zhang X. Genetic regulation of shoot architecture in cucumber. Hortic. Res. 8:143, 2021.
2. Słomnicka R, Kaźmińska K, Bartoszewski G. Assessment of genetic variation in cucumber lines characterized by dwarf phenotype. 6th Polish Congress of Genetics. Book of Abstracts, p.248, Cracow, Poland, 2022.
3. Yu J, Wu S, Sun H et al. CuGenDBv2: an updated database for cucurbit genomics. Nucleic Acids Res 22:gkac921, 2022.