Assessment of genetic variation in cucumber lines characterized by dwarf phenotype

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Introduction

Dwarf plant architecture is an important trait in cucumber (*Cucumis sativus* L.) breeding. Reduced plant growth cultivars have potential to be used in once-over mechanical harvest. Several genes controlling plant growth in cucumber have been identified (Liu et al. 2021).
The aim of this study was to assess genetic variation of cucumber lines characterized by changed shoot architecture and reduced growth (Fig. 1).

Materials & Methods

Genomes of ten inbred lines were re-sequenced. High-quality DNA was isolated and paired-end libraries with 350 bp insert size were constructed. Sequencing was performed using Illumina NovaSeq 6000 platform. Clean reads were mapped on cucumber reference genome B10 v.3 (Osipowski et al. 2020). The genetic variants including SNPs and INDELs were identified and annotated using bioinformatic approach. Selected SNPs and INDELs were confirmed by PCR and sequencing.

Figure 1. Cucumber inbred lines grown under plastic tunnel conditions.

Results

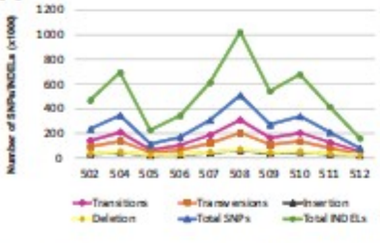
In total from 88 to 145 million of clean reads per line were obtained. The depth of sequencing was from 38x to 61x per line and mapping ratio on average 99.5% (Tab. 1).

Table 1. Summary of the re-sequencing and mapping of the reads for ten cucumber lines characterized by dwarf phenotype.

#	Line	Total reads (Mb)	Mapping reads (Mb)	Mapping ratio (%)	Average depth
1	502	91.28	90.89	99.58	38x
2	504	92.75	92.35	99.58	39x
3	505	91.64	91.33	99.66	40x
4	506	88.71	88.39	99.64	38x
5	507	92.11	91.72	99.57	39x
6	508	92.17	91.74	99.53	40x
7	509	88.36	88.02	99.61	38x
8	510	88.87	88.49	99.57	38x
9	511	90.57	90.22	99.61	39x
10	512	145.28	144.73	99.62	61x

Total number of SNPs varied from 79k to 508k and the number of INDELs from 159k to 1M (Fig. 2A). Calculations based on SNP mutations showed that the observed heterozygosity rate and Ts/Tv ratio were almost equal for all inbred lines (Fig. 2B).

A



B

#	Line	Ts/Tv	Heterozygosity rate
1	502	1.54	0.093
2	504	1.54	0.114
3	505	1.48	0.037
4	506	1.53	0.036
5	507	1.53	0.049
6	508	1.53	0.053
7	509	1.51	0.071
8	510	1.52	0.045
9	511	1.52	0.042
10	512	1.48	0.036

Figure 2. Summary of the SNPs and INDELs for ten cucumber lines characterized by dwarf phenotype.

Acknowledgements
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References
[1] X. Liu, J. Chen, X. Zhang. Genetic regulation of shoot architecture in cucumber. Hortic. Res. 8:143, 2021.
[2] P. Osipowski, M. Pawełkowicz, M. Wojcieszek, A. Skarzyńska, Z. Przybecki, W. Plader. A high-quality cucumber genome assembly enhances computational comparative genomics. Mol. Genet. Genom. 295:177–193, 2020.

Results

The number of exonic SNPs ranged from 3.1k to 22.7k (3.9-4.8% of the total number of identified SNPs) with the highest number of synonymous and non-synonymous SNPs for each line (Fig. 3A). The number of exonic INDELs ranged from 0.9k to 2.2k (0.22-0.59% of total number of identified INDELs). The highest number of frameshift deletions / insertions were indentified (Fig. 3B). Comparative analyzes allowed to identify SNP / INDEL polymorphisms between the studied lines and the reference genome B10 v.3 in some genes related to transcription factors, plant hormone signalling and metabolism of gibberellins or brassinosteroids (Tab. 2).

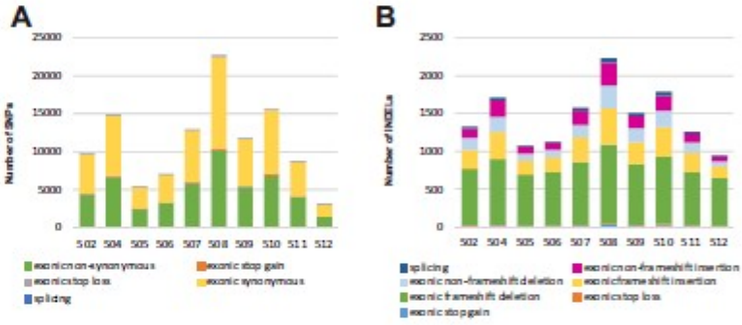

Figure 3. Histograms of the number of exonic SNPs (A) and INDELs (B) obtained by comparison each cucumber line with reference genome B10 v.3.

Table 2. List of selected genes for which SNP / INDEL polymorphisms were verified and confirmed by PCR and sequencing.

Gene	Gene description	Line with polymorphism	Polymorphism type
1	Terminal flower 1-like	506	SNP
2	Cytochrome P450	504	SNP
3	Steroid 5-alpha-reductase	511	SNP
4	Cytochrome P450	505	SNP
5	Cytochrome P450	507	SNP
6	Receptor protein kinase	511	SNP
7	Cullin-1	502, 511	SNP
8	Cytokinin dehydrogenase	508	SNP
9	Ubiquinol oxidase	502, 508, 510	INDEL
10	Terpene cyclase/mutase family member	507	INDEL
11	Non-specific serine/threonine protein kinase	507	INDEL SNP
12	Xyloglucan endotransglucosylase/hydrolase	507, 510	INDEL
13	KH domain-containing protein	504	SNP
14	Xyloglucan endotransglucosylase/hydrolase	502	SNP
15	Protein kinase domain-containing protein	502	SNP

Conclusions

1) Genome for ten cucumber inbred lines characterized by dwarf phenotype were re-sequenced and relatively high genetic variation was detected.

2) SNP/INDEL polymorphisms beetwen the studied lines and the reference genome B10 v.3 in some genes related to transcription factors and plant hormone signalling and metabolism have been identified.

3) This study provides genomic frames for further mapping and identification of novel genes controlling plant growth architecture in cucumber.

Phenotypic evaluation of ten cucumber lines are shown in poster:

PR-4.11 - Kaźmińska et al. - "Phenotypic evaluation of cucumber lines characterized by dwarf plant architecture"