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Polymorphisms in resistance genes within breeding lines of cucumber (*Cucumis sativus* L.)

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INTRODUCTION AND THE AIM OF THE STUDY

Cucumber is an important vegetable crop with high nutritional value and economic importance in many countries. However, its production is largely limited due to various infectious agents, including bacteria, viruses, fungi, and oomycetes. Among these, oomycete, bacterial and fungal infections, causing downy mildew, angular leaf spot disease and powdery mildew pose considerable challenges (Call et al 2012, Słomnicka et al, 2015). Traditionally, chemical treatments have been employed to control diseases in cucumber production. However, this approach has several disadvantages, including potential effects on food safety and environmental pollution. Nowadays, consumers prefer production systems in which chemicals use is limited, like organic production. In organic production, chemical control of diseases is restricted, making disease management particularly challenging. To address this issue breeders aim to develop cucumber cultivars with genetically-determined disease resistances, well-suited for organic production. Resistance breeding in plants relies on introduction of resistance genes into elite breeding material. In the case of cucumber, there is not much data on resistance genes, and only a few disease resistance genes have been identified so far.

The aim of the study was to analyze polymorphisms within major disease-resistance genes in cucumber breeding lines by using genome re-sequencing data. Understanding the genetic diversity of the resistance genes is crucial for successful disease-resistance breeding for organic farming.

MATERIALS AND METHODS

Materials. Genomes of sixteen cucumber lines, used in breeding program of Legutko seed company, aimed to develop cucumber variery for organic production in Polish climatic conditions, were resequenced. Seven genes confering resistance against downy mildew, angular leaf spot disease and powdery mildew, described in the literaturę, were subject for the detailed analysis (Table 1).

Method. Bioinformatic analyses were carried-out based on the data obtained from the resequencing of cucumber lines. The reads were mapped to two reference genomes, Gy14v2.1 and B10v3. Single Nucleotide Polymorphisms (SNPs) were analysed using SAMtools and visulalized using the IGV 2.17.4 software.

Table 1 Cucumber resistance genes analysed in this study.
Diseases: DM-downy mildew, PM-powdery mildew, ALS-angular leaf spot disease

Disease	B10v3 gene ID	Gy14v2.1 Gene ID	Genomic location	Annotation
ALS, DM, PM	Cucsat.G21381	CsGy5G003280	Chr5 : 2147948-2149557	STAY-GREEN, chloroplastic-like
DM	Cucsat.G7534	CsGy1G026860	Chr1 : 25344075-25353861	phosphoinositide 3-kinase regulatory subunit 4
DM	Cucsat.G11688	CsGy4G007450	Chr4 : 5488094-5493007	Caffeoyl-CoA O-methyltransferase
DM	Cucsat.G10958	CsGy7G000130	Chr7: 192521-195951	isoflavone reductase-like
PM	Cucsat.G14408	CsGy1G012830	Chr1 : 8234733 -8240963	MLO-like
PM	Cucsat.G5501	CsGy6G015870	Chr6: 14900023 -14905512	MLO-like
PM	Cucsat.G14914	CsGy5G027670	Chr1: 8286302-8295871	MLO-like

RESULTS

SNPs analyses were carried out on 16 cucumber lines, whose resistance to the disease is currently tested in details. The genomes of the Gy14 line, which is recognized as resistant, and susceptible line B10 were used as the references. Analyses of seven resistance genes revealed heterozygocity in some of them in three lines 375, 386 and 445 suggesting that further inbreding should be considered (Table 2).

Within the genes of resistance to powdery and downy mildew, we observed greater variability. For the genes that determine downy mildew resistance, from 11 to 32 SNPs as reffered to Gy14v2.1 and 1 to 17 as reffered the B10v3 genome were noted. For the major MLO-like genes confering resistance to powdery mildew from 19 to 60 as reffered to Gy14 and from 4 to 24 as reffered to B10 were identified. The analysis allowed us to identify single SNP in the STAY-GREEN gene, which determines resistance to angular leaf spot, downy mildew, and anthracnose. The presence of the susceptibility variant in lines 386 and 431 was noted, suggesting that these lines can be susceptible (Table 2).

CONCLUSIONS

1. The results indicate limited variation in the STAY-GREEN gene conferring multiple-disease resistance within breeding lines with presence of susceptibility alllele in two lines
2. For analyzed DM and PM resistance genes multilbe SNPs depending on the gene were deteced.
3. Some of the lines are not fully homozygous within PM/DM/ALS resistance loci
4. This study contributes to the broader goal of sustainable cucumber production and breeding for organic farming

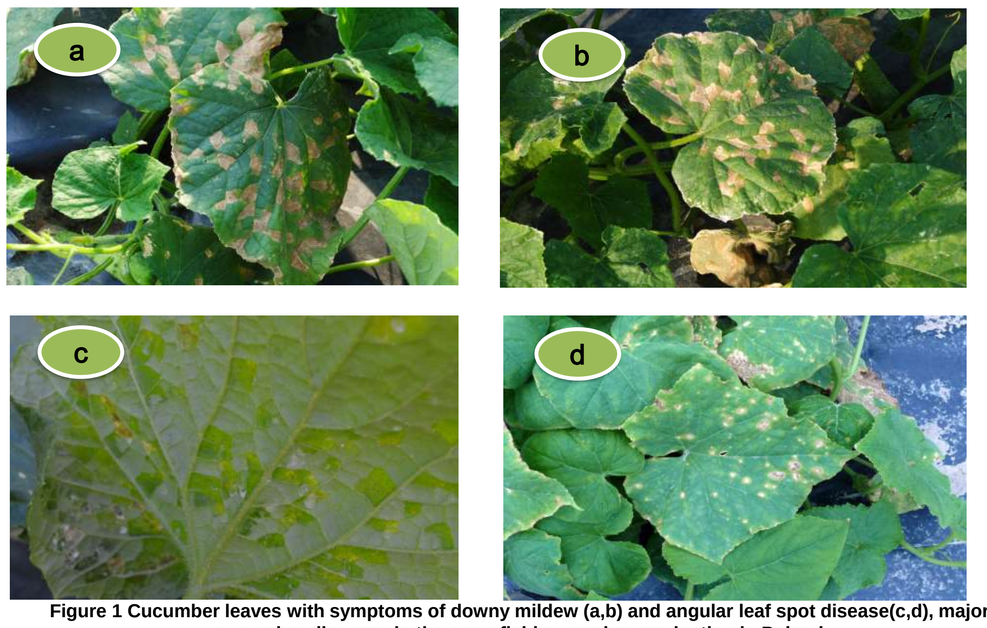

Figure 1 Cucumber leaves with symptoms of downy mildew (a,b) and angular leaf spot disease(c,d), major cucumber diseases in the open-field cucumber production in Poland

Table 2. SNPs within resistance genes in cucumber lines in comparison with two reference genomes Gy14v2.1 and B10 v3. Colored square indicate the occurrence of SNP, a square with stripes indicate heterozygosity

Gene ID	Position	Breeding lines: 367	371	372	375	377	379	383	386	431	439	440	441	442	443	445	447
Cucsat.G7534	ctg1528:4 195 043			■					■								
	ctg1528:4 195 062			■					■								
	ctg1528:4 195 085			■					■								
	ctg1528:4 195 215			■					■								
	ctg1528:4 195 247			■					■								
Cucsat.G11688	ctg1820:3 182 077				■		■	■					■	■			■
	ctg1820:3 184 675				■		■	■					■	■			■
	ctg1820:3 184 687				■		■	■					■	■			■
	ctg1820:3 184 710				■		■	■					■	■			■
	ctg1820:3 185 592				■		■	■					■	■			■
Cucsat.G10958	ctg1681:2 730 374	■	■			■	■	■	■				■	■	■	▤	■
	ctg1681:2 731 002	■	■			■			▤								
	ctg1681:2 732 236	■	■			■			▤								
	ctg1681:2 732 709	■	■			■			▤								■
	ctg1681:2 732 791	■	■			■			■								
Cucsat.G21381	ctg910:2 154 483								■	■						▤	
Cucsat.G14408	ctg1869:7 398 645		■		▤		■						■	■		■	
	ctg1869:7 398 739		■		▤		■						■	■		■	
	ctg1869:7 398 801		■		▤		■						■	■		■	
	ctg1869:7 398 892		■		▤		■						■	■		■	
	ctg1869:7 398 944		■		▤		■						■	■		■	
Cucsat.G14914	ctg1869:7 447 268				▤												
	ctg1869:7 447 296				▤					■						■	
	ctg1869:7 448 383															■	
	ctg1869:7 448 671	■	■		■	■					■	■		■	■		■
Cucsat.G5501	ctg1269:429 897	■	■		■	■					■	■			■		■
	ctg1269:429 907	■	■		■	■					■	■		■	■		■
	ctg1269:429 934	■	■		■	■					■	■		■	■		■
	ctg1269:430 418	■	■		■	■					■	■		■	■		■
	ctg1269:430 516	■	■		■	■					■	■		■	■		■

Gene ID	Position	Breeding lines: 367	371	372	375	377	379	383	386	431	439	440	441	442	443	445	447
CsGy7G000130	Gy14Chr7:193 459	■	■			■			■								■
	Gy14Chr7:193 541	■	■			■			■								■
	Gy14Chr7:194 181	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■
	Gy14Chr7:194 287	■	■			■											
	Gy14Chr7:195 731	■	■	■	■	■			■	■					■	■	■
CsGy4G007450	Gy14Chr4:5 488 243		■	■					▤	■	■				■		
	Gy14Chr4:5 488 364		■	■					■	■	■				■	■	
	Gy14Chr4:5 488 423	■				■											
	Gy14Chr4:5 488 497		■	■					■	■					■	▤	
	Gy14Chr4:5 488 546	■	■	■		■			■	■				■	■	▤	■
CsGy1G026860	Gy14Chr1:25 345 966			■					▤								
	Gy14Chr1:25 346 027			■					▤								
	Gy14Chr1:25 346 175			■					▤								
	Gy14Chr1:25 346 210			■					▤								
	Gy14Chr1:25 346 349			■					▤								
CsGy5G003280	Chr5 : 2147948-2149557								■	■							
CsGy1G012830	Gy14Chr1:8 236 850		■				■										
	Gy14Chr1:8 237 236		■														
	Gy14Chr1:8 237 675		■		■								■				
	Gy14Chr1:8 237 686		■		■		■										
	Gy14Chr1:8 237 720		■		■								■				
CsGy5G027670	Gy14Chr5:31 502 031								■								■
	Gy14Chr5:31 502 123								■								■
	Gy14Chr5:31 502 236								■								■
	Gy14Chr5:31 504 129								■								■
	Gy14Chr5:31 504 145								■								■
CsGy6G015870	Gy14Chr6:14 901 142	■	■			■						■	■				■
	Gy14Chr6:14 901 339	■	■			■					■	■	■				■
	Gy14Chr6:14 901 339	■	■		■	■					■	■	■		■		■
	Gy14Chr6:14 902 259				▤	■					■	■	■				■
	Gy14Chr6:14 902 276	■	■		■	■					■	■	■		■		■

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