



Development of DArTseq markers for genetic mapping of angular leaf spot resistance in cucumber (*Cucumis sativus* L.)



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Introduction

Angular leaf spot (ALS) of cucumber is disease that can result in severe yield losses in cucumber open-field production. It is generally considered that this disease is caused by *Pseudomonas syringae* pv. *lachrymans*. Disease symptoms may vary depending on the bacteria virulence, the host, and environmental conditions (Olczak-Woltman et al. 2007, Ślomsnicka et al. 2015a).

The cucumber RILs mapping population Gy14 × B10 was tested twice under growth chamber conditions in relation to ALS response (Ślomsnicka et al. 2015b, Ślomsnicka et al. 2016). The phenotypic evaluation showed two types of ALS response among RILs: Gy14-type and B10-type. The Gy14-type lines indicated tolerance to ALS with small necrosis and limited, bright chlorosis similar to hypersensitivity response (HR). B10-type lines were susceptible with widespread, water-soaked, angular chlorosis that later became necrotic like paternal line B10 (Figure 1).

Preliminary SSR based genetic mapping suggested that ALS resistance/tolerance gene is located on chromosome 5 (Ślomsnicka et al. 2015b). Interestingly, in cucumber a cluster of genes encoding NBS-LRR proteins were identified on chromosome 5 (Huang et al. 2009, Wang et al. 2013, Yang et al. 2013).

The aim of this work was to genotype 92 RILs of mapping population Gy14 × B10 using DArTseq technology to develop high-density genetic map that will be valuable tool to map ALS resistance gene(s) and also other resistance genes.

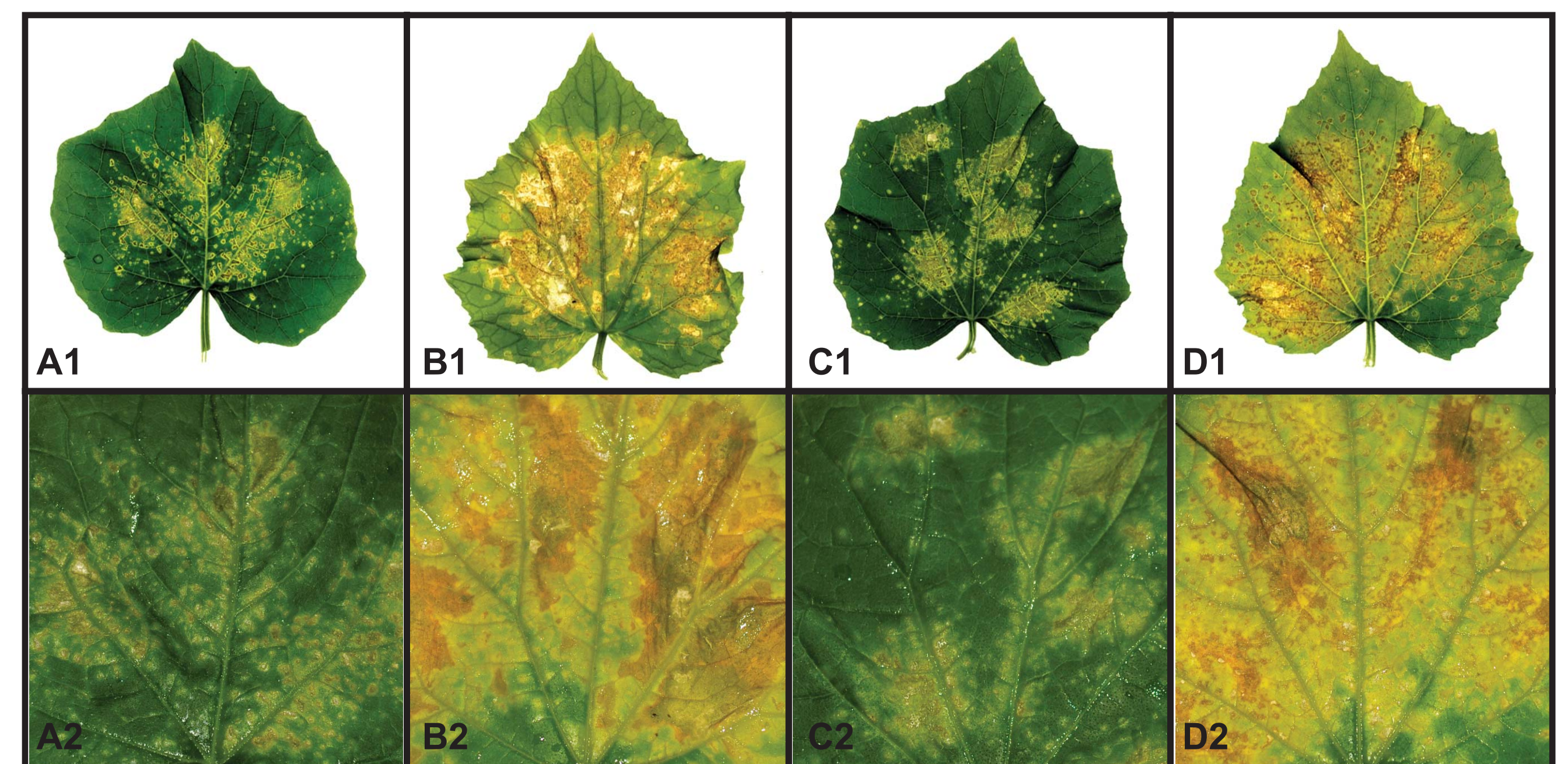


Figure 1. Angular leaf spot symptoms on cucumber leaves seven days after *Pseudomonas syringae* pv. *lachrymans* inoculation with highly virulent strain 814/98.

A - tolerant, maternal line Gy14, B - susceptible, parental line B10, C - tolerant RIL 6 representing Gy14-type, D - susceptible RIL 55 representing B10-type of disease symptoms.

Materials & methods

Gy14 × B10 RILs mapping population was developed by crossing two inbred lines: Gy14 that is showing tolerance to ALS and B10 that is susceptible to this disease. Total genomic DNA was extracted using GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO, USA). Based on RILs phenotyping and SSR markers analysis, 92 RILs generation F₅ and parental lines were chosen for DArTseq genotyping (Canberra, Australia).

DArTseq markers classified as SNPs were aligned using BLAST algorithm and CLC Genomics Workbench v. 9.0 (Qiagen, Germany) against B10 genome contigs corresponding to chromosome 5 (NCBI GeneBank Acc. No. LKU001000000) (Wóycicki et al. 2011). DArTseq markers located on cucumber chromosome 5 were further analyzed by nucleotide BLAST (blastn) search at NCBI.

Results - DArTseq genotyping

A set DArTseq markers, that included *In silico* DArTs and SNPs, was developed for mapping population Gy14 × B10. In total 2161 DArTseq markers distinguishing parental lines were found. Most of the markers 1906 (88,2%) segregated 1:1 as expected for this RILs population and will be used to construct genetic map (Table 1). The polymorphism level revealed by high-throughput DArTseq genotyping was limited. It is probably related to similar genetic background of population parental lines. A set of SNPs was further characterized.

Table 1. The statistics of DArTseq markers developed for mapping population Gy14 × B10

DArTseq markers:	Type of DArTseq markers		
	<i>In silico</i> DArTs	SNPs	Total
- developed during DArTseq analysis	1708	1319	3027
- polymorphic between parental lines	1099	1062	2161
- segregated 1:1 in RILs mapping population	942	964	1906

Literature

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Results - SNPs analysis

Within 964 SNPs all types of SNPs were found. Transitions occurred more frequently than transversions and represented 61,2% of all SNPs. The most frequently occurred SNPs were G>A and C>T (21,3% and 18,4% of all SNPs respectively). T>G and A>C SNPs showed the lowest frequency of occurrence (3,3% and 3,9% respectively). (Table 2).

Over 200 SNPs were mapped on B10 line chromosome 5. Blastn analysis showed that about 15 markers are located within genomic regions encoding proteins that are related to plant response to biotic and abiotic stress (Table 3).

Moreover, a lot of blastn hits indicated similarity to chromosomes 9 and 10 of melon. It confirms syntenic relationships between cucumber chromosome 5 and melon chromosomes 9 and 10. It was postulated that during the cucumber evolution the fusion of chromosomes corresponding to melon chromosomes 9 and 10 took place (Li et al. 2011).

Table 2. The statistics of SNP types

Type of SNP	Number	%
<i>Transitions</i>		
A>G	108	11,2
C>T	177	18,4
G>A	205	21,3
T>C	100	10,4
	590	61,2
<i>Transversions</i>		
A>C	38	3,9
A>T	61	6,3
C>A	58	6,0
C>G	42	4,4
G>C	44	4,6
G>T	46	4,8
T>A	53	5,5
T>G	32	3,3
	374	38,8
Total	964	100,0

Table 3. The summary of blastn search for DArTseq/SNPs markers within genes that are encoding proteins related to plant stress response on chromosome 5

No	Stress-related protein name and description
1	LRR receptor-like protein kinase
2	LRR receptor-like serine/threonine-protein kinase
3	E3 ubiquitin-protein ligase
4	MLP-like protein 43
5	BAG family molecular chaperone regulator 2-like
6	Exocyst complex component SEC6
7	G-type lectin S-receptor-like serine/threonine-protein kinase
8	Glycine-rich RNA-binding protein RZ1C-like
9	Membrane-bound transcription factor site-2 protease homolog

Summary

- Similar genetic background of mapping population parental lines (pickling-types) resulted in limited number of detected SNPs.
- About 15 SNPs located within genes encoding proteins related to plant stress response and located on chromosome 5 were identified.
- DArTseq markers that segregate 1:1 as expected for Gy14 × B10 RILs population will be used to construct genetic map and mapping cucumber angular leaf spot resistance genes.

Acknowledgements

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