Evaluation of a Cucumber RILs Population for Resistance to Angular Leaf Spot

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Abstract. One of the important diseases of cucumber, *Cucumis sativus*, is angular leaf spot (ALS) caused by *Pseudomonas syringae* pv. *lachrymans* (*Psl*). Increased occurrence of this disease in cucumber field production in Poland has caused significant losses over the last few years. The objective of this study was to estimate the ALS resistance level in a RILs population under growth chamber conditions. The tested RILs population was developed by crossing two inbred lines: Gy14 which shows tolerance to ALS and B10 which is susceptible to ALS. Plants were inoculated by spraying with the highly virulent *Psl* strain 814/98, scored (1 to 9 scale), and disease severity index (DSI) was calculated. Two types of reaction to *Psl* were observed, chlorosis on the leaves indicating susceptibility and necrosis with a chlorotic halo indicating tolerance. The results showed that the RILs segregate for ALS resistance/tolerance and thus the Gy14 × B10 RILs population is useful for further inheritance studies and mapping of ALS resistance/tolerance genes.

Keywords: Angular leaf spot disease, *Cucumis sativus*, *Pseudomonas syringae* pv. *lachrymans*, resistance, RILs

Introduction

Angular leaf spot (ALS) caused by the bacterium *Pseudomonas syringae* pv. *lachrymans* (*Psl*) is one of the most important diseases limiting production of field cucumber, *Cucumis sativus* L. Typical ALS symptoms include vein-limited, water-soaked lesions (with or without chlorotic halos) on leaves, which later become necrotic. Water-soaked lesions occur also on fruits, causing the fruits to become misshapen thus resulting in yield reduction (Bradbury 1986, Sherf and Macnab 1986). The first reports describing ALS resistance in cucumber were published by Chand and Walker (1964) and by Dessert et al. (1982). Disease severity, observed in accordance with the number and size of the lesions, differs among cucumber genotypes and has been reported to be a polygenically inherited trait (Chand and Walker 1964, Dessert et al. 1982). By self-pollination and selection of the most resistant plants, Chand and Walker (1964) accumulated genes conferring reduced severity, thereby increasing the level of tolerance to *Psl*. Dessert et al. (1982) distinguished two types of reactions by cucumber plants to ALS based on the symptoms: (1) the occurrence of chlorotic halos around necrotic lesions typical of susceptibility and (2) the lack of chlorotic halos typical of resistance or tolerance. The absence of chlorotic halos was conferred by a single recessive gene *psl* (*pl*). Attempts to find molecular markers associated with ALS resistance gene(s) were ineffective. The RAPD marker, OP-AO07, linked to the locus responsible for the presence/absence of chlorotic halos at a distance of 13 cM, was identified by Olczak-Woltman et al. (2009). The knowledge about inheritance of tolerance/resistance to *Psl* and molecular markers linked to resistance gene(s) are still limited. The objective of this study was to estimate the *Psl* resistance level in a RILs population. The results of the phenotypic evaluation in relation to ALS symptom type will be useful for mapping cucumber gene(s) associated with resistance or tolerance to this disease.

Materials & Methods

The bacterial strain and inoculum preparation

Previously, both virulence and genetic diversity of *Pseudomonas* sp. strains, collected at the Department of Plant Genetics Breeding, and Biotechnology (Warsaw University of Life Sciences, Warsaw, Poland), were studied (Olczak-Woltman et al. 2007, Słomnicka et al. 2015a,b). Based on these studies, the highly virulent *Psl* strain 814/98 was selected and used in this study. *Psl* 814/98 was obtained from the Institute of Plant Protection in Poznań (Poland). To prepare the inoculum, bacteria were grown on King B agar plates at 28 °C for 24 hours. Bacterial colonies were suspended in sterile distilled water and adjusted to OD_{600}= 0.05 to be equal to a concentration of 10^7 CFU·ml^{-1}.

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Plant materials and plant inoculation

Recombinant inbred lines (RILs) of the F_6 generation were tested for *Psl* resistance. The RILs population was developed by crossing two inbred lines: Gy14 that exhibits tolerance to *Psl* and B10 that is susceptible to *Psl*. The F_2 seeds were kindly provided by Prof. M.J. Havey (Univ. of Wisconsin, Madison, WI, U.S.A.) and used further to develop F_6 generation progeny by self-pollination of individual plants at the Wolica experimental field (Department of Plant Genetics, Breeding, and Biotechnology, Warsaw University of Life Sciences, Warsaw, Poland). Cucumber plants were grown in a growth chamber at 25 °C during the day and 22 °C at night, with a 16 h photoperiod. For *Psl*-resistance testing, plants at the 2nd to 3rd leaf stage were inoculated by spraying the abaxial side of each leaf with inoculum, according to Klement et al. (1990). As a control, sterile water was used. Inoculated plants were kept in darkness for 24 hours at 22 °C and relative humidity of 100%, and then for 6 days under typical growth conditions. After 7 days, inoculated leaves were visually scored using a nine-degree rating scale (Jenkins and Wehner 1983) and disease severity index (DSI) was calculated. Each tested line was represented by 16 plants (four replications of four plants in each replication).

Results & Discussion

Parental lines and 110 RILs of the Gy14 × B10 population were characterized for *Psl* resistance. Two types of reaction were observed. B10 was characterized by widespread, water-soaked, angular chlorosis that later became necrosis on leaves. Bacterial exudates on petioles and leaves were also observed. The symptoms covered 50 to 75% and very often even 87% of the leaf surface. The mean DSI of B10 was 4.0. On the leaves of Gy14, small necrotic spots and very limited bright chlorosis were observed. The symptoms appeared on 8 to 25% of the leaf surface and mean DSI was 6.4 (Table 1, Figure 1).

The ALS symptoms observed on Gy14 plants were similar to a hypersensitivity response (HR). Generally, HR is initiated in plants as necrotic lesions in the locally infected tissue and is accompanied by the accumulation of salicylic and jasmonic acids (Yang et al. 2013). The obtained results corresponded well with a previous study aimed at identifying susceptible and tolerant standard lines for *Psl* testing, the DSI for B10 and Gy14 lines being 3.5 to 3.8, and 6.2 to 6.3, respectively (Olczak-Woltman et al. 2008). Among the RILs, two groups were distinguished: tolerant RILs resembling Gy14 ("Gy14 type") and susceptible

Table 1. Disease severity index (DSI) in the Gy14 × B10 F_6 RILs cucumber population and its parents infected with *Pseudomonas syringae* pv. *lachrymans*.

ALS resistance type	Number of lines	DSI range of mean		DSI mean	Standard deviation
		minimum	maximum		
Gy14	Maternal line	6.0	6.8	6.4	0.49
B10	Paternal line	3.3	4.5	4.0	0.61
Gy14 type	58	5.5	6.8	6.1	0.29
B10 type	52	3.0	5.0	4.3	0.45

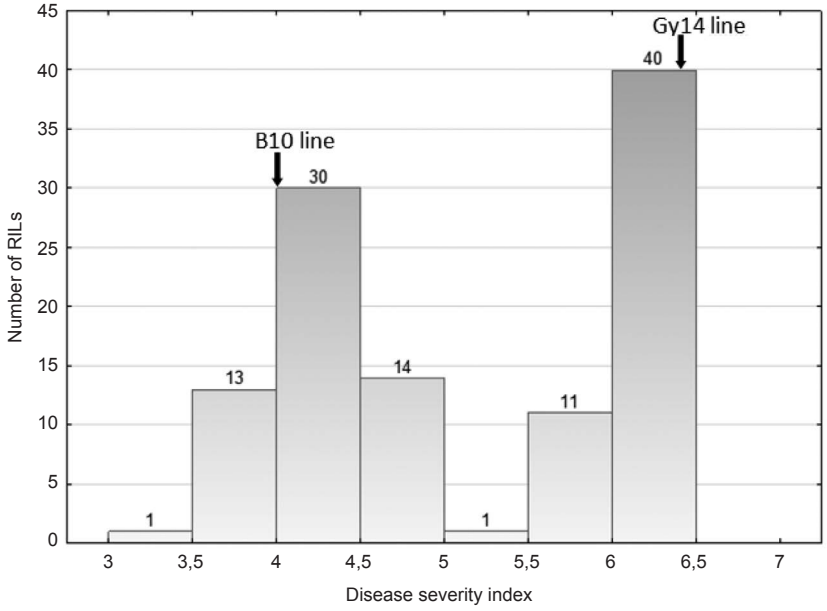

Figure 1. Distribution of disease severity index (DSI) in the Gy14 × B10 F_6 RILs population infected with *Pseudomonas syringae* pv. *lachrymans*.

RILs resembling B10 ("B10 type"). The DSI of lines characterized as "Gy14 type" and "B10 type" ranged from 5.5 to 6.8 and from 3.0 to 5.0, respectively (Table 1, Figure 1). A similar distribution of DSIs in this population was estimated previously at the F_5 generation level (Słomnicka et al. 2015a,b), and it indicates that the RILs of the Gy14 × B10 population are stable and possess a high degree of homozygosity.

The second important indication of cucumber resistance to *Psl* is presence or absence of a chlorotic halo. The chlorotic halo in B10 and "B10 type" RILs was always present. In Gy14 and "Gy14 type" RILs, limited chlorosis and lack of halos were observed. Among 110 RILs, 51 did not possess chlorotic halos, like Gy14, while 58 lines classified as "B10 type" were susceptible, with intense chlorotic halos. One RIL possessed intermediate symptoms. Earlier, Dessert et al. (1982), based on genetic analysis (F_1, F_2, BC, and F_3), indicated that the lack of halos in resistant plants is conferred by a single recessive gene, *psl* (*pl)*, but the number and size of lesions may be controlled by two or more genes (Dessert et al. 1982). Olczak-Woltman et al. (2009), from the cross of B10 × H603 (F_2, BC_1 and BC_2), confirmed this type of inheritance. A recessive mode of inheritance of disease resistance in cucumber was also reported for downy mildew (*dm* gene) and powdery mildew (*pm* gene) (Fujieda and Akiya 1962, van Vliet and Meysing 1977). However, according to recent studies, resistance to downy mildew and powdery mildew is determined by multiple recessive genes (He et al. 2013, Zhang et al. 2013). The phenotypic characterization of the mapping population Gy14 × B10 showed that RILs segregate for ALS resistance type and thus this population can be useful for further inheritance studies and mapping of *Psl* resistance/tolerance genes.

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Literature Cited

Bradbury JF (1986) Guide to plant pathogenic bacteria. London: CAB International Mycological Institute, pp 154 –177.

Chand JN, Walker JC (1964) Inheritance of resistance to angular leaf spot of cucumber. Phytopathology 54: 51–53.

Dessert JM, Baker LR, Fobes JF (1982) Inheritance of reaction to *Pseudomonas lachrymans* in pickling cucumber. Euphytica 31: 847–855.

Fujieda K, Akiya R (1962) Genetic study of powdery mildew resistance and spine color on fruit in cucumber. J Jpn Soc Hort Sci 31: 30–32.

He X, Li Y, Pandey S, Yandell BS, Pathak M, Weng Y (2013) QTL mapping of powdery mildew resistance in WI 2757 cucumber (*Cucumis sativus* L.). Theor Appl Genet 126: 2149–2161.

Jenkins SF, Wehner TC (1983) A system for the measurement of foliar diseases of cucumber. Cucurbit Genet Coop Rep 6: 10–12.

Klement Z, Mavridis A, Rudolph K, Vidaver A (1990) Inoculation of plant tissues. In: Klement Z, Rudolph K, Sands DS (eds) Methods in phytobacteriology. Budapest: Akadémiai Kiadó, pp 94–124.

Olczak-Woltman H, Masny A, Bartoszewski G, Płucienniczak A, Niemirowicz-Szczytt K (2007) Genetic diversity of *Pseudomonas syringae* pv. *lachrymans* strains isolated from cucumber leaves collected in Poland. Plant Pathol 56: 373–382.

Olczak-Woltman H, Schollenberger M, Mądry W, Niemirowicz-Szczytt K (2008) Evaluation of cucumber (*Cucumis sativus*) cultivars grown in Eastern Europe and progress in breeding for resistance to angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*). Eur J Plant Path 122: 385–393.

Olczak-Woltman H, Bartoszewski G, Mądry W, Niemirowicz-Szczytt K (2009) Inheritance of resistance to angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*) in cucumber and identification of molecular markers linked to resistance. Plant Pathol 58: 145–151.

Sherf AF, Macnab AA (1986) Vegetable diseases and their control. New York: Wiley-Interscience.

Słomnicka R, Olczak-Woltman H, Bartoszewski G, Niemirowicz-Szczytt K (2015a) Genetic and pathogenic diversity of *Pseudomonas syringae* strains isolated from cucurbits. Eur J Plant Pathol 141: 1–14.

Słomnicka R, Pietluch A, Łęczycka J, et al. (2015b) Identyfikacja markerów SSR przydatnych do konstrukcji mapy genetycznej i mapowania genów odporności na kanciastą plamistość liści ogórka (*Cucumis sativus* L.) (in Polish). Biuletyn IHAR 276: 93–103.

van Vliet GJA, Meysing WD (1977) Relation in the inheritance of resistance to *Pseudoperonospora cubensis* Rost. and *Sphaerotheca fuliginea* Poll. in cucumber (*Cucumis sativus* L.). Euphytica 26: 793–796.

Yang L, Wang L, Guohua C, Jiang S, Sun L, Li D (2013) Response of tobacco to the *Pseudomonas syringae* pv. *tomato* DC3000 is mainly dependent on salicylic acid signaling pathway. FEMS Microbiol Lett 344: 77–85.

Zhang SP, Liu M, Miao H, et al. (2013) Chromosomal mapping and QTL analysis of resistance to downy mildew in *Cucumis sativus*. Plant Dis 97: 245–251.