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Evaluation of cucumber (*Cucumis sativus*) cultivars grown in Eastern Europe and progress in breeding for resistance to angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*)

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Abstract Increased occurrence of cucumber angular leaf spot, *Pseudomonas syringae* pv. *lachrymans*, has caused significant losses in cucumber, *Cucumis sativus*, yield in Poland in recent years. These losses necessitated evaluation of the level of resistance in cucumber cultivars of mainly Polish breeding, cultivated in Eastern Europe, and initiation of a breeding programme for resistance to this disease. Screening for resistance was performed on 84 cucumber accessions under growth chamber conditions using a highly aggressive strain of *P. syringae* pv. *lachrymans*. Most of the screened accessions were either susceptible or displayed intermediate resistance. The screening resulted in the identification of five F_1 hybrid cultivars moderately resistant to angular leaf spot. The identified F_1 hybrids were self-pollinated up to the F_4 generation. Individuals resistant to angular leaf spot were identified. These individuals can be used as a source of resistance to angular leaf spot in future breeding efforts.

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Abbreviations

CFU	colony-forming unit
DH	double haploid
DSI	disease severity index
RH	relative humidity

Introduction

Cucumber (*Cucumis sativus*) is an important vegetable crop world-wide. One of the most important diseases of cucumber is angular leaf spot caused by the bacterium *Pseudomonas syringae* pv. *lachrymans*. This pathogen is one of the 50 pathovars belonging to the heterogeneous species of *P. syringae* (Young et al. 1996). Angular leaf spot is a very common cucumber disease causing serious fruit yield losses. The symptoms include vein-limited, water-soaked lesions on the cucumber leaves (with or without a chlorotic halo), which later become necrotic, and water-soaked lesions on fruit often causing the fruit to become misshapen (Chand et al. 1963; Bradbury 1986; Sherf and Macnab 1986). Pohronezny et al. (1977) and Khlaif (1995) showed that up to 30–60% of yield reduction can be caused by angular leaf spot (both in fruit weight and fruit number), the degree of severity depending on the cultivar's susceptibility. Only partial resistance to this pathogen has been identified in wild species and varieties in the genus *Cucumis* (Kudela and Lebeda 1997). Besides copper pesticides, chemical treatment is often ineffective. However, copper treatment decreases the fruit quality for preserve produce.

Recently, severe bacterial infections were observed in the open field in Poland, often just before harvest. This necessitated evaluation of the level of resistance to angular leaf spot in a number of Polish cucumber cultivars grown in Eastern Europe. Moreover, an effort was made to obtain cucumber lines that were resistant to angular leaf spot.

Materials and methods

Experimental design

The screened cucumber germplasm consisted of 84 accessions including 11 inbred lines, 14 DH lines, three non-hybrid cultivars, five F_1 cross progenies (cross-breed), and 51 F_1 hybrid cultivars. The screen for resistance to angular leaf spot was conducted under growth chamber conditions during the years 2003–2004. Each entry was tested twice, in two separate experiments, first in the year 2003 and then in 2004. Each experiment was conducted in a completely randomized block design with four experimental boxes serving as replicates. Each replicate consisted of six plants. In total, 48 plants of each accession were screened.

Plant materials

Seeds were sown individually in separate plastic pots filled with peat moss. Plants were maintained under standard growth chamber conditions at 25°C day, 22°C night, with illumination for 16 h from sodium lamps providing 50 W m^{-2}. Names and source or breeder of the tested accessions are given in Table 1. The controls consisted of a susceptible inbred line B, (selected from variety Borszczagowski; B. Kubicki, unpublished), and a resistant inbred line GY14.

Pathogen

A highly aggressive strain 814/98 of *P. syringae* pv. *lachrymans* was used. This strain was chosen out of the collection of strains of field and culture origin. The collection is described in our previous paper (Olczak-Woltman et al. 2007). It included three *P. syringae* pv. *lachrymans* reference strains from plant pathogen culture collections and 25 strains isolated from cucumber leaves showing angular leaf spot symptoms, collected in central and southern Poland in 2001 and 2002. After performing determinative tests, including LOPAT and a pathogenicity test on cucumber, six strains of the 25 collected were classified as *P. syringae* pv. *lachrymans*. However, except 814/98, all the collected strains exhibited very weak aggressiveness. The 814/98 strain is of Dutch origin and was obtained from the Pathogen Bank of the Institute of Plant Protection in Poznań, Poland. This strain caused typical angular leaf spot symptoms on the leaves of the susceptible line B in every repeated test. The symptoms consisted initially of water-soaked and then necrotic lesions, each with a large chlorotic halo, and bacterial ooze on the abaxial side of the inoculated leaf (Olczak-Woltman et al. 2007). Inoculum was prepared by growing the bacterium on King's medium B (King et al. 1954) at 28°C for 24 h. The resulting colonies were suspended in sterile distilled water and their concentration was adjusted to 1×10^7 CFU ml^{-1} using optical density measurement at 600 nm (OD_{600}). OD_{600} of 0.050 was determined to correspond to 1×10^7 CFU using the plate-count technique (Klement et al. 1990). To ensure an even spread of the inoculum on the leaf a single drop of Tween 20 was added to each litre of inoculum.

Plant inoculation and evaluation of disease severity

Cucumber plants at the 2nd to 3rd leaf stage were inoculated by spraying the abaxial side of the fully expanded leaves (Klement et al. 1990). To ensure high RH of 95–100% required during the screen, the plants were placed under polythene covers immediately after inoculation. Plants were kept in the dark at 100% RH (measured with thermohygrograph TZ-18td) for 24 h, followed by 6 days of incubation under light at 50 W m^{-2} and 90% RH. Seven days after inoculation, the inoculated leaves of each plant

Table 1 Grouping of the 84 tested cucumber accessions, with marked source or breeder, into clusters (marked by letters) with similar levels of resistance or susceptibility by the Student–Neuman–Keuls test at α=0.01

		Experiment I			Experiment II		
Tested accession	Source or breeder	Score range[a]	Mean	Homog. group[b]	Score range	Mean	Homog. group
Inbred line B-susceptible cont.	cv. Borszczagowski	3–5	3.8	a	3–5	3.5	a–c
Inbred line H-25	Hocus line	2–5	3.6	a	2–5	4.0	a–f
Sander F_1	Polan-Krakow[c]	1–5	3.7	a	2–6	4.6	f–m
Inbred line Straight 8	USA	2–5	3.9	a–b	3–5	3.8	a–e
Fason F_1	Spojnia-Nochowo	3–5	4.0	a–c	2–4	3.3	a
Polonez F_1	Spojnia-Nochowo	2–6	4.0	a–c	2–6	4.6	e–ł
Wojan F_1	Plantico-Zielonki	3–5	4.1	a–c	3–5	4.2	a–g
Inbred line Trocki	DPGBB[d]	3–5	4.3	a–d	3–5	4.3	b–h
Inbred line 2gg	Mutant, DPGBB	3–5	4.3	a–d	2–5	3.5	a–b
Gracjus F_1	Plantico-Zielonki	3–6	4.4	a–d	2–6	4.3	c–h
Barbakan F_1	Polan-Krakow	3–6	4.4	a–d	3–5	4.6	e–l_1
Malta F_1	Spojnia-Nochowo	3–6	4.5	a–d	3–5	4.6	e–l
Kmicic F_1	Polan-Krakow	3–6	4.6	b–e	4–6	5.5	k–t
Rufus F_1	Polan-Krakow	3–6	4.8	b–f	4–6	4.6	e–l_1
Monika	PNOS Ozarow Maz.	4–6	4.8	b–f	2–5	3.8	a–e
Wawel F_1	Polan-Krakow	2–6	4.8	c–g	3–6	4.6	e–l_1
Moro F_1	Spojnia-Nochowo	2–6	4.8	c–g	2–7	4.9	f–p
Wisconsin 18 SMR	USA	3–6	4.8	c–g	1–6	3.6	a–d
Fortuna F_1	HR Snowidza	4–6	4.9	c–h	4–6	5.4	j–s
Anulka F_1	Polan-Krakow	3–7	4.9	c–h	4–6	4.8	f–m
Dar	DPGBB	3–7	4.9	c–i	4–7	5.5	k–u
Polan F_1	Polan-Krakow	4–6	5.0	c–j	4–6	4.6	e–l_1
Inbred line Monastyrski	DPGBB	3–6	5.0	c–j	3–6	5.0	f–q
Slawko F_1	PlantiCo-Golebiew	4–6	5.0	d–k	3–6	5.0	g–q
Soplica F_1	Plantico-Zielonki	3–7	5.0	d–k	4–6	4.8	f–n
Sremski F_1	Spojnia-Nochowo	4–6	5.1	d–k	3–6	4.6	e–l
Racibor F_1	Polan-Krakow	4–6	5.2	d-l	4–6	4.8	f-n
Bolko F_1	Polan-Krakow	5–7	5.5	e–l_1	4–5	4.4	d–j
Lider F_1	Spojnia-Nochowo	5–7	5.5	e–l_1	5–7	5.4	i–s
Wladko F_1	Polan-Krakow	4–7	5.5	e–m	3–6	4.9	f–p
Calypso F_1	New England Seed	4–7	5.5	e–m	5–8	5.7	m–w
Polkrak F_1	Polan-Krakow	3–7	5.5	e–m	5–7	5.5	k–r
Metro F_1	Polan-Krakow	5–7	5.5	e–m	4–6	5.0	f–q
Tytus F_1	Polan-Krakow	4–7	5.6	f–n	4–6	5.0	g–q
Aladyn F_1	ZO Przyborow	4–8	5.7	f–o	4–8	5.9	p–y
Royal F_1	Clause Semences	4–8	5.7	g–o	4–7	5.5	k–t
DH F 311	DPGBB	5–7	5.7	g–o	4–7	5.6	l–v
Opty F_1	Polan-Krakow	4–7	5.8	g–o	3–6	4.8	f–n
Gomez F_1	PlantiCo-Golebiew	5–6	5.8	g–o	4–6	4.9	f–o
Borus F_1	Polan-Krakow	4–7	5.8	g–p	5–7	5.6	l_1–w
Cezar F_1	PlantiCo-Golebiew	5–6	5.8	g–p	5–7	5.8	n–y
Major F_1	Spojnia-Nochowo	3–7	5.8	g–p	5–7	5.9	o–y
DH I 150	DPGBB	5–7	5.8	g–p	4–7	5.7	m–w
Forum F_1	Polan-Krakow	5–7	5.8	h–q	3–6	4.9	f–o
DH I 503	DPGBB	5–8	5.9	i–q	4–7	5.1	g–q
Izyd F_1	PNOS Ozarow Maz.	5–8	5.9	i–q	5–8	6.2	r–z_1
Natalie F_1	B. Holman	5–7	5.9	j–q	6–7	6.6	w–z_3
Frykas F_1	PlantiCo-Golebiew	5–7	5.9	j–q	4–7	5.7	m–w
Andrus F_1	Polan-Krakow	5–7	5.9	j–q	3–6	4.5	e–k
Julian F_1	Polan-Krakow	5–7	5.9	j–q	4–6	5.2	h–q

Table 1 (continued)

Tested accession	Source or breeder	Experiment I			Experiment II		
		Score range[a]	Mean	Homog. group[b]	Score range	Mean	Homog. group
Chrobry F_1	Polan-Krakow	5–7	6.0	k–q	4–7	5.5	k–u
Hubal F_1	Polan-Krakow	5–7	6.0	k–q	3–6	4.4	d–i
Hermes F_1	PNOS Ozarow Maz.	5–8	6.0	k–q	6–8	6.5	w–z_3
Cross-breed No. 84	USA	5–7	6.0	k–q	3–6	5.3	h–r
Krak F_1	Polan-Krakow	5–7	6.0	k–q	3–6	4.9	f–p
Sremianin F_1	Spojnia-Nochowo	5–7	6.0	k–q	5–7	5.5	k–r
DH I 290	DPGBB	6–7	6.1	l–r	6–8	6.7	x–z_3
Cross-breed No. 90	USA	5–8	6.1	l–r	4–6	4.9	f–o
Cross-breed No. 85	USA	5–7	6.2	l–r	5–6	5.5	k–r
Cross-breed No. 87	USA	5–7	6.2	l–r	5–7	5.9	p–y
DH I 89/37	DPGBB	5–7	6.2	l–r	5–8	6.5	u–z_3
Bazyl F_1	ZO Przyborow	5–8	6.2	l–r	5–8	6.5	u–z_4
DH I 18/1	DPGBB	5–7	6.2	l_1–r	5–9	7.3	z_3–z_4
Aljat F_1	Moravo Seed	5–8	6.2	l_1–r	5–8	6.1	q–z
Cyryl F_1	ZO Przyborow	5–8	6.3	l_1–r	5–8	6.8	y–z_3
Cross-breed No. 82	USA	5–7	6.3	l_1–r	5–7	5.5	k–v
Inbred line GY14—resist. cont.	USA	5–8	6.3	l_1–r	6–7	6.2	r–z_1
Regal F_1	Clause Semences	6–8	6.3	l_1–r	4–7	5.8	n–y
Prymus F_1	PlantiCo-Golebiew	5–8	6.4	l_1–r	4–6	5.4	i–s
DH I 114	DPGBB	5–8	6.4	l_1–r	6–9	6.7	x–z_3
Parys F_1	ZO Przyborow	5–8	6.4	l_1–r	5–7	5.9	o–y
DH I 321	DPGBB	6–7	6.5	m–r	6–9	7.0	z_1–z_3
Inbred line TMG 1	USA	6–8	6.5	m–r	5–8	6.4	t–z_2
DH F 100	DPGBB	6–7	6.6	n–r	5–8	6.3	s–z_1
DH I 506/2	DPGBB	6–8	6.6	n–r	7–9	7.8	z_4
DH I 502/1	DPGBB	6–8	6.6	n–r	7–9	7.9	z_4
Atlas F_1	ZO Przyborow	6–8	6.6	n–r	4–7	5.8	m–x
Inbred line Gy3	DPGBB	6–8	6.6	n–r	6–8	6.5	v–z_3
DH F 310/2	DPGBB	6–8	6.7	o–r	5–8	6.9	z_1–z_3
DH I 504/1/8	DPGBB	6–8	6.7	o–r	6–8	7.2	z_2–z_4
DH I 280	DPGBB	6–8	6.8	p–r	6–8	6.8	z–z_3
Basza F_1	Polan-Krakow	6–8	6.8	q–r	5–8	6.0	q–z
Inbred line H 603	*C. sativus* var. *hardwickii*	6–9	7.0	r	6–8	7.1	z_1–z_3
Inbred line 859/1	Israel	7–9	7.7	s	7–9	7.8	z_4
Means of all accessions			5.7			5.5	

[a] The score range was determined for 24 plants of each accession tested in each experiment

[b] Homogenous groups according to Student–Newman–Keuls test (the means marked by the same letter do not differ significantly)

[c] Polan-Krakow, Spojnia-Nochowo, PNOS Ozarow Maz., Z.O. Przyborow, HR Snowidza, PlantiCo-Golebiew, and Plantico-Zielonki are Polish Seeds and Breeding companies. B.Holman and Moravo Seed are Czech companies

[d] DPGBB—Department of Plant Genetics, Breeding and Biotechnology of Warsaw University of Life Sciences

were scored for disease severity using the following nine-point rating scale: 9 = a few pin-point lesions; 8 = a few lesions on 3–8% of the leaf area, no chlorosis; 7 = necrotic lesions on 8–15% of the leaf area, some lesions on stems, no chlorosis; 6 = large necrotic lesions on 15–25% of the leaf area and on stems, light chlorotic halo; 5 = necrotic or water-soaked lesions on 25–50% of the leaf area and on stems, chlorotic halo; 4 = angular, water-soaked lesions on 50–75% of leaf area, visible bacterial exudates and yellow borders; 3 = water-soaked lesions on 75–87% of the leaf area, with bacterial exudates and extensive chlorosis; 2 = damage to 87–95% of the leaf area, bacterial exudates, extensive chlorosis and necrosis; 1 = damage up to 100% of plant leaves. Plants rated 9 were considered to be highly resistant, 8—resistant, 7—moderately resistant, 6—intermediate, 5—moderately susceptible, 4 and 3 susceptible, 2 and 1 highly susceptible.

Breeding for resistance

In order to obtain lines with increased levels of resistance, five least susceptible to angular leaf spot F_1 hybrid cultivars were identified and one plant from each cultivar was self-pollinated. Fifty-two plants from each of the five F_2 populations obtained through the self-pollination were screened for resistance to angular leaf spot. Six least susceptible plants were selected from among each of the progenies and again self-pollinated. Thirty plants per each F_3 segregating progeny were planted, and evaluated for angular leaf spot resistance. Four least susceptible plants per each F_3 progeny were selected and self-pollinated thus giving rise to F_4 progenies. For each progeny DSI was measured using the following equation:

$$\mathrm{DSI} = (n_1 \times p_1 + n_2 \times p_2 + n_3 \times p_3 + n_4 \times p_4 + n_5 \times p_5 + n_6 \times p_6 + n_7 \times p_7 + n_8 \times p_8 + n_9 \times p_9)/k,$$

where:

$n_{1–9}$ number of plants in each rating category
$p_{1–9}$ disease symptom rating
k number of plants in population (progeny).

Eighteen plants per each F_3 segregating progeny were planted also in open field conditions. Seeds were sown straight into the soil on 1st June 2005. During field growth plants were not inoculated, and no chemical protection was used. Observations were performed twice, on 26th July and 4th August, in comparison to controls B, GY14 and H 603 lines, and to maternal F_1 cultivars of the F_3 progenies. All accessions were sown randomly in rows.

Statistical analysis

The DSI data gathered in the experiments were analysed using two-factor analysis of variance (ANOVA) with blocks regarded as the first factor, accessions as the second factor and single plants as observation units. The tested hypothesis was that accession effects were not statistically significant. This hypothesis was tested using an *F-test* for the ratio of accession mean square and experimental error mean square as the *F* statistic (Table 2). The block effect was tested in a similar manner (Table 2). Groups of accessions with statistically non-significant mean DSI were identified using the multiple mean comparison procedure of Student–Neuman–Keuls at the significance level of α=0.01. The statistical analyses were performed with the aid of the Statgraphics Plus 4.1 for Windows (STSC Inc. 1999) software.

Results

Initial experiments showed that the clearest and strongest symptoms were obtained when the plants were inoculated at the two-fully-expanded leaf stage. The optimum inoculum concentration for the strain 814/98 of *P. syringae* pv. *lachrymans* was 1×10^7 CFU ml^{-1}. Water-soaked, angular, later necrotic spots with bacterial exudates were observed consistently on the susceptible control line B (Fig. 1a) when using this protocol. In both years the lesions on the susceptible control were rated 3.5–3.8 using the nine-point scale. However, the resistant control line GY14 was only moderately resistant with scores of 6.2–6.3 (Fig. 1b). Among the screened accessions, 6% were resistant to angular leaf spot with a DSI>7.0 and without any chlorosis (Fig. 1c), 25% exhibited moderate resistance with DSI between 6.0 and 7.0 and necrotic lesions on 8–25% of the leaf tissue, 43% showed an intermediate level of resistance, with DSI between 5.0 and 6.0 (Fig. 1d), and 26% were susceptible with DSI<5.0, and large areas of chlorosis, necrosis and bacterial exudates.

The ANOVA results for the DSI data are shown in Table 2. The accession effect was found to be significant (P<0.01), indicating the existence of genetic diversity for resistance to angular leaf spot

Table 2 ANOVA results for DSI of cucumber angular leaf spot from two independent experiments carried out for 84 cucumber accessions

Source	*df*		Mean square		*F* ratio		*P*-value	
	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II
Blocks	3	3	2.68	16.22	1.25	6.64*	0.29	0.00
Accessions	83	83	18.04	25.35	8.38*	10.38*	0.00	0.00
Experimental error	249	249	2.15	2.44				
Plants within boxes (sample error)	1680	1680	0.82	0.83				

* $P<0.01$; significance at the level of $\alpha=0.01$

in this germplasm collection and a potential for genetic gain in a breeding programme. The score range and DSI means of the screened accessions are presented in Table 1.

The application of the Student–Neuman–Keuls test at $\alpha=0.01$ allowed for grouping of the tested accessions into clusters with similar levels of resistance/susceptibility. The most susceptible group consisted of the susceptible control B line, H-25, 2gg and Straight 8 lines, as well as cvs Fason F_1 and Wojan F_1. The composition of this group was consistent in both years. Lines 859/1 of *C. sativus* var. *sativus* and H 603 of *C. sativus* var. *hardwickii* were consistently in the most resistant group in both years. Among the resistant and moderately resistant accessions were DH lines obtained from cv. Izyd F_1: DH I 280, DH I 502/1, DH I 504/1/8, DH I 506/2, DH F 310/2, as well as lines GY3 and TMG 1 and cvs Bazyl F_1, Atlas F_1 and Cyryl F_1 (Table 1).

Five F_1 hybrid cvs Atlas F_1, Basza F_1, Bazyl F_1, Cyryl F_1 and Hermes F_1 characterized by lesser susceptibility to angular leaf spot were selected for further work. These cultivars were self-pollinated and the F_2 progenies were evaluated for resistance to angular leaf spot. These F_2 progenies segregated for resistance. On average, 44% in each of these progenies showed

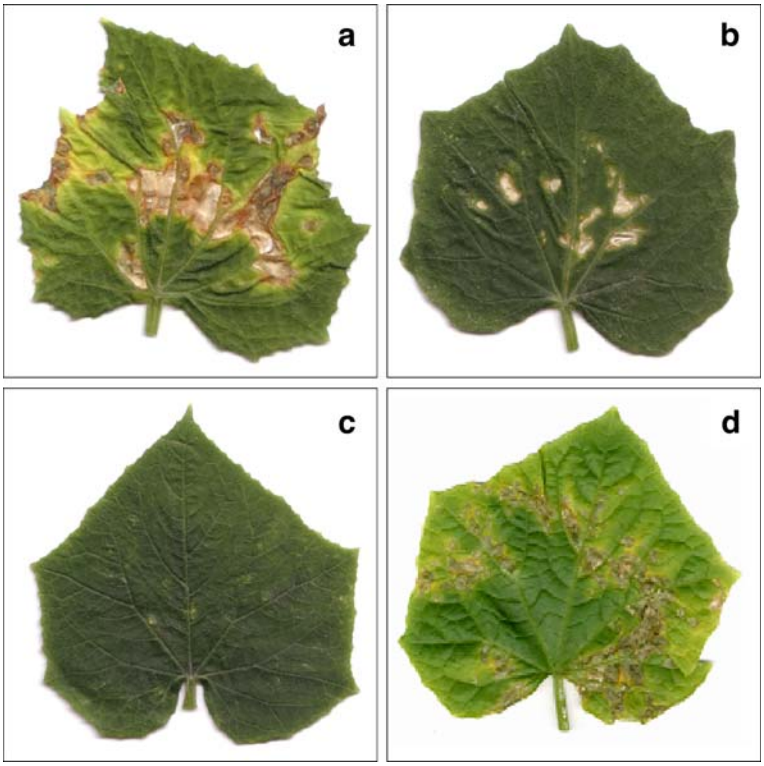

Fig. 1 Angular leaf spot symptoms on four cucumber accessions. **a**, B line; **b**, GY14 line; **c**, 859/1 line **d**, cv. Royal F_1

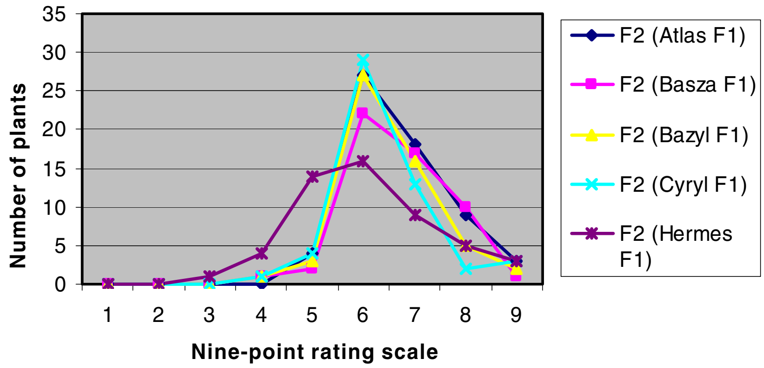

Fig. 2 The resistance of F_2 progenies screened for cucumber angular leaf spot. Number of plants in particular classes of nine-point scale

intermediate levels of resistance, with a DSI of 6.0. The highly resistant and highly susceptible plants were in the least number in each progeny (Fig. 2). The segregation in F_2 generation allowed a selection of several of the most resistant individuals in order to generate F_3 progenies.

In general, the F_3 progenies exhibited higher resistance to angular leaf spot than the F_2 and F_1 generations. The highest increase in resistance was observed among progenies obtained from Hermes F_1, Cyryl F_1 and Bazyl F_1. These progenies had no chlorotic halo, and exhibited only necrotic lesions (Table 3). The field resistance evaluation confirmed that most of the F_3 progenies exhibit higher resistance to angular leaf spot than controls, B and GY14 lines, and F_1 cultivars from which the F_3 progenies were derived (data not shown). The highest level of resistance in field growth conditions was shown by two F_3 progenies obtained from cvs Bazyl F_1 and Hermes F_1, which, similarly to the H 603 line, remained almost without any disease symptoms, and had dark green leaves until September (Fig. 3). The field observations confirmed that the resistance against 814/98 strain obtained in growth chamber conditions was effective also against field *P. syringae* pv. *lachrymans* isolates.

Table 3 Breeding progress for F_2 and F_3 progenies in resistance to angular leaf spot

Progenies	Mean F_1^a	Mean F_2	Mean F_3
Atlas F_1	6.20	6.67	7.07
Basza F_1	6.40	6.68	7.03
Bazyl F_1	6.50	6.50	6.98
Cyryl F_1	6.31	6.38	7.06
Hermes F_1	6.25	6.05	6.85

[a] Mean F_1, F_2, F_3—the average DSI for all progenies in particular generations

Discussion

Only 6% of the 84 tested accessions were resistant to the highly aggressive strain 814/98 of *P. syringae* pv. *lachrymans*. These accessions were scored >7 on a nine-point-scale and had neither chlorotic lesions nor chlorotic halos, exhibiting only small necrotic spots (of up to 8%) on the leaf surface. This resistant group consists of lines H 603 and 859/1, cvs Hermes F_1, Cyryl F_1 and Bazyl F_1 and DH 502/1, DH 504/1/8 and DH506/2 obtained from cv. Izyd F_1. The DH lines provide particularly valuable breeding material, as they are 100% homozygous (Sztangret-Wisniewska et al. 2006). The above listed F_1 hybrid cultivars are also considered to be less susceptible to downy mildew (*Pseudoperonospora cubensis*) of cucumbers (Bartoszak 2004). The association of resistance to angular leaf spot and to downy mildew was observed also for lines H 603, 859/1, DH 502/1, DH 504/1/8 and DH 506/2. These accessions were previously tested for resistance to these two diseases. Similarly, F_3 progenies evaluated in field conditions exhibited not only higher levels of resistance to angular leaf spot, but also to downy mildew when compared to controls. Moreover, accessions susceptible to downy mildew (Shetty et al. 2002): cvs Mieszko F_1, Sremski F_1 and Straight 8 line, were susceptible to angular leaf spot in our screening tests. This suggests that breeding for resistance to angular leaf spot may also help to obtain resistance to downy mildew. Additionally, we noted that the early-harvest cvs Gracjus F_1, Fason F_1, and line B were susceptible to angular leaf

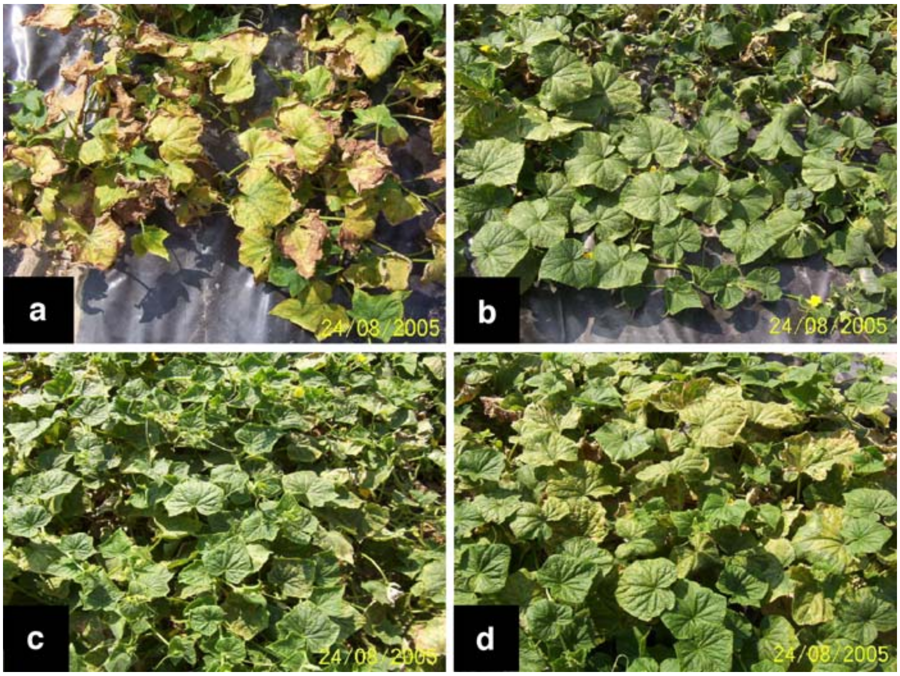

Fig. 3 The field resistance of evaluated cucumber F_3 progenies, derived from F_1 cultivars, together with controls. **a**, B line; **b**, H 603 line; **c**, F_3 generation derived from Bazyl F_1; **d**, F_3 generation derived from Hermes F_1

spot and downy mildew. The late-harvest cvs Bazyl F_1, Cyryl F_1 and line H 603 showed higher resistance to both angular leaf spot and downy mildew. It was also noted that plants with dark leaves were more resistant to both angular leaf spot and downy mildew than those with light-green leaves. It has been previously reported that older leaves are less susceptible to both diseases than young leaves due to the accumulation of salicylic acid in older leaves (Kus et al. 2002). The association of leaf age and resistance makes it imperative for the plant screening to be performed on young plants and leaves, i.e. in the 2nd–3rd-leaf stage.

Five F_1 hybrid cultivars scored as moderately resistant (7 on a nine-point-scale) were self-pollinated in order to obtain breeding lines resistant to angular leaf spot. The self-pollination of commercial cultivars ensured high levels of horticulturally desirable traits in the segregating material. Through two cycles of selection it was possible to identify F_3 progenies that exhibited levels of resistance higher than the resistant control line GY14. The best new progenies were derived from cvs Bazyl F_1, Cyryl F_1 and Hermes F_1. The increase in resistance between the F_2 and F_3 progenies indicated that the accumulation of resistance alleles resulted in less visible disease severity. Most of the tested F_2 genotypes displayed intermediate resistance and only one third were scored >7. As a result of selecting and advancing only the most resistant plants, with a DSI of 8–9, two thirds of plants in the F_3 generations were scored as resistant, with a DSI of 7–9. As only the most resistant plants with a DSI of 9 were selected from among the F_3 generation to obtain the F_4 generation, it is expected that the average level of resistance will be even higher in the F_4 progeny. This confirms the results of Chand and Walker (1964) who also observed the highest increase of resistance between generations F_3 and F_4. The nature of resistance discussed here appears to be determined by multiple loci, the accumulation of which resulted in less disease severity, i.e. number of water-soaked lesions, necrotic and chlorotic spots and bacterial exudates. It is important that the increase in resistance obtained after cucumber inoculations with very aggressive strain 814/98, segregation and selection, was confirmed under field conditions, where the F_3 progenies showed resistance to the field isolates of *P. syringae* pv. *lachrymans*.

Overall, although the majority of the screened cucumber accessions proved to be intermediate in resistance to the 814/98 strain of *P. syringae* pv. *lachrymans*, the existence of genetic diversity in resistance was apparent because the screening was performed on plant material of different origin. The method described here of breeding for resistance to angular leaf spot using developed cultivars as a starting point is valuable, as it resulted in highly resistant plants with good horticultural characteristics in a relatively short time. This is the first comprehensive analysis of Polish cucumber cultivars resistance to angular leaf spot.

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