Utilization of *afila* types of pea (*Pisum sativum* L.) resistant to powdery mildew (*Erysiphe pisi* DC.) in the breeding programs

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ABSTRACT

The yield potential, quality and level of resistance to powdery mildew (*Erysiphe pisi* DC.) of *afila* smooth seeded pea (*Pisum sativum* L.) were tested in the field trials. The cultivars and breeding lines Mozart, Consort-R, AGT-01, Cebeco 1171 and AGT-GH surpassed the control cv. Gotik in the yields of dry seed, in contrast the dry seed yields of Highlight, AGT-KR, Melfort and LU 390-R2 were about 12–27% lower than that of the control. The low seed yield was caused by virus infections (PEMV, BYMV), root diseases (*Pythium*, *Fusarium*), and a low level of thousand seeds weight (TSW). Material crossing with donors possessing high yield potential, a higher TSW, and a higher tolerance to root diseases had a positive effect on the dry seed yield. The main objective of the resistant pea breeding programme is *afila* smooth seeded pea resistant to powdery mildew, with a high tolerance to viruses, root diseases, and lodging, with the stem length of 60 to 75 cm, and with high yield potential.

Keywords: pea; *Pisum sativum*; *afila*; resistance; *Erysiphe pisi*; breeding; genetic resources

Powdery mildew (*Erysiphe pisi* DC., *Ascomycetes*) is a specialised form of *E. pisi* f.sp. *pisi* (Hammarl) Boerema adapted to pathogenesis on pea (*Pisum sativum* L.) only. Seed yield losses of 10–65% caused by powdery mildew under warmer and drier conditions were reported in peas (Heringa et al. 1969, Tiwari et al. 1997b, Nagaraju and Pal 1990).

Pea genetic resources with resistance to powdery mildew were detected in Peruvian peas populations (Harland 1948) and also in the commercial cultivar of wrinkle seeded pea Stratagem derived from Mexican population of Mexique 4 (Pierce 1948, Cousin 1965). The resistance to powdery mildew is controlled by recessive genes *er-1* and *er-2* (Tiwari et al. 1997b, Heringa et al. 1969). Gene *er-1* can bring about full resistance while gene *er-2* provides only leaf resistance. Both genes are inherited independently from each other. The crosses of susceptible and resistant plants result in F_1 generation all susceptible, and segregation of F_2 into susceptible and resistant plants gives a good fit with 3:1 ratio. The disadvantages of the sources of resistance cited were low yield potential, low TSW values and high sensitivity to all other pea diseases. The incidence of different pathogen races was not found (Heringa et al. 1969, Tiwari et al. 1997a, 1998, Vaid and Tyagi 1997).

An extensive breeding program of wrinkle seeded peas resistant to powdery mildew (utilizing sources of Stratagem and Mexique 4) was realized in USA, Canada, and India, and it induced a number of resistant commercial cultivars (Almoto, Aspen, Concord, Dacota, Gypsy, Knight, Melton, Midget, Parlay, Regal, Sentry, Tempter, etc.). Projects related to genes pyramiding of powdery mildew, root rot and virus infections were started in USA and Canada in the nineties. In the hybridization process of new resistant cultivars, the wrinkle seeded lines were used with multiple resistance selected from the cross B880-221 × OSU1026 (Weeden and Providenti 1999), lines 96-2052, 96-2058, 96-2068, 96-2198, 96-2222, 97-261, 97-2154, 97-363, 97-2170, 97-2162 unique in combining genes for resistance (Kraft and Coffman 2000) and cvs. Franklin, Joel, Fallon and Lifter (Germaplasm Resources Information Network 2002).

Canadian and American research teams (Cereal Research Centre, Winnipeg, Morden Research Centre, Manitoba and Washington State University) determined markers of the pea resistance to powdery mildew for both *er-1* and *er-2* genes (Tiwari et al. 1997a, 1998).

The first three commercial cvs. of smooth seeded pea resistant to powdery mildew were registered in Canada (Vaid and Tyagi 1997, Tiwari et al. 1997b, 1998, Warkentin et al. 2000): leaf type cvs. Tara and Tamor and afila type cv. Highlight. Subsequently, new resistant cvs. of smooth seeded peas with improved resistance and yield potential were bred in Canada during the second part of nineties: Melfort, Eclipse, Mozart, Acer, Handel, Minuet, Admiral, Stalwarth, Advantage, Montero and Dominator. Under the European conditions, no afila type of smooth seeded pea cv. resistant to powdery mildew was registered.

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MATERIAL AND METHODS

Comparative trials of the resistance sources. The following powdery mildew resistant lines and cvs. Highlight, Melfort, Mozart, LU 390-R2, Cebeco 1171, Consort-R (resistant plants selected from cv. Consort), AGT-01 (/Gotik × Highlight/ × Consort-R), AGT-GH (Gotik × Highlight) and AGT-KR (Komet × LU 390-R2) were included into the comparative field trials. The susceptible cv. Gotik was used as a control. The negative characteristics of the resistant cvs. Highlight and LU 390-R2 were marked out (low TSW, low dry seed yield, and high susceptibility to the root diseases). Normal and *afila* types of pea were used. *Afila* pea type (*af*) (pea with reduced leaf area) was detected after Goldenberg (1965). The experiment was arranged as a randomised complete block design with four replicates under the conditions of infection field.

Breeding strategy. The possibility of improving the negative characteristics cited was verified by cross with susceptible cvs. Gotik and Komet (high TSW, high dry seed yield). Gotik and Komet were crossed with the resistance sources Highlight and LU 390-R2 in April 1998 (glasshouse). The F_1 generation was multiplied under the field conditions (July 1998) and F_2 generation was sown in the glasshouse (August 1998) and inoculated with powdery mildew (population mixture of *Erysiphe pisi* DC. 1998, location Rapotín).

Plant inoculation method. Powdery-mildew-infested plants were collected before harvest (July/August 1998) from pea field trials. The plant inoculation was done by dusting the susceptible cv. Komet with the collected plants. A number of mycelium colonies appeared. The complete leaves and pods mycelium cover culminated during September. The inoculation of the plants tested was done with conidia dusting in the glasshouse at the end of September. For the successful plant inoculation with powdery mildew, it is necessary to have a fresh and vital inoculum, and the plants tested must have 4–5 internodes. The optimal term of inoculation was at the start of flowering.

Selection process. The selected resistant plants of the *afila* type F_2 generation were harvested in December. The resistant F_3 generation was multiplied in the glasshouse in spring 1999 and F_4 generation under the conditions of infection field (July 1999). The selection was guaranteed of specimens match in the traits required (*afila* type, smooth seed shape, yellow seed colour, plant height 60–75 cm, medium vegetation period, TSW more than 160 g and more than 4 pods per plant). The selected plants of F_5 possessing the characteristics declared were sown in the glasshouse (autumn, 1999) and inoculated for the elimination of prospectively susceptible specimens (population mixture of *Erysiphe pisi* DC. 1999, location Temenice). F_6 generation was multiplied under the field conditions (during 2000) so as to obtain enough seeds for the comparative trials which were established under the field and glasshouse conditions (F_7 generation, 2001).

Yield evaluations. A field test was carried out for the evaluation of the yield potential of the resistance sources and the hybrid combinations obtained (F_8 generation) marked AGT-GH and AGT-KR. 60 seeds were sown on the trials plots of 1.5 m length and 1 m width in 4 replications. Plant infestation was scored on 0–5 scale (0 = without symptoms, 1 = infestation or occurrence 1–5%, 2 = infestation or occurrence 6–20%, 3 = infestation or occurrence 21–30%, 4 = infestation or occurrence 31–75%, 5 = infestation or occurrence 76–100%). The same trial was carried out in a glasshouse. Seeds were sown in to a soil bed, 15 seeds per 1 m of row length in 8 replications. The number of harvested plants, pod number per plant, seed number per pod, seed weight, and TSW were recorded during the harvest. Plots yields were evaluated by the multiple comparison method, 95 and 99% Tukey-*HSD* interval.

RESULTS AND DISCUSSION

The results of the yield trials with resistance sources and hybrid combinations under the field and glasshouse conditions are presented in Table 1. The results proved low

Table 1. Evaluation of seed yield potential of resistance sources and hybrid combinations under glasshouse and field conditions (Temenice 2001)

Genotype	Glasshouse			Field		
	dry seed yield (g/plant)	% of control cv. (%)	TSW (g)	dry seed yield (g/plant)	% of control cv. (%)	TSW (g)
Gotik (S)	2.44	100	207	2.26	100	165
Highlight (R)	1.93	79.1	177	1.12	49.5	118
AGT-GH (R)	3.37	138.1	176	2.26	100	142
Komet (S)	3.33	100	198	4.54	100	183
LU 390-R2 (R)	1.13	33.9	171	0.7	15.4	109
AGT-KR (R)	1.63	48.9	180	1.75	38.5	128

R = resistance source, S = susceptible cultivar

Table 2. Frequency of incidence of pathogens in roots of plants under glasshouse and field conditions

Genotype	Glasshouse			Field		
	Rhizoctonia	*Pythium*	*Fusarium*	*Rhizoctonia*	*Pythium*	*Fusarium*
Gotik (S)	3.5	2.0	1.5	3.0	2.2	1.5
Highlight (R)	2.0	3.5	2.0	2.2	4.5	1.5
AGT-GH (R)	2.2	2.4	1.6	3.0	2.5	1.5
Komet (S)	1.5	2.0	2.5	1.2	1.6	2.5
LU 390-R2 (R)	3.3	3.6	3.0	3.5	4.6	3.0
AGT-KR (R)	2.8	3.0	3.0	3.6	3.5	3.2

yield potentials of the parental resistance sources Highlight and LU 390-R2. The hybrid combination AGT-GH surpassed in the glasshouse both those sources. Under the field conditions, this combination achieved the same yield as cv. Gotik in spite of a high disease pressure and a low TSW value. The second hybrid combination AGT-KR did not achieve the appointed results. The yield of dry seed did not reach as much as 50% of the susceptible cv. Komet under both the field and the glasshouse conditions. The main reason for the low yield potential of the source LU 390-R2 and the hybrid combination AGT-KR was high susceptibility to the root diseases (Table 2). In comparison with the susceptible cvs. Gotik and Komet, both these resistance sources were analysed by microscopic observation of necrotic roots. Consecutively, by the isolation of fungi onto agar medium, an increased frequency of pathogenic fungi *Pythium ultimum*, *Pythium aphanidermatum* and *Aphanomyces euteiches* was found. Moreover, high sensitivity to pathogenic fungi *Rhizoctonia solani*, *Fusarium solani*, *equiseti* and *oxysporum* was found with line LU 390-R2.

Years 2001 and 2002 vary in the presence of diseases under the field conditions. Root diseases complex (*Pythium*, *Aphanomyces*, *Rhizoctonia*, *Fusarium*) prevailed in 2001, however, virus diseases (PEMV, BYMV), rust (*Uromyces pisi*), anthracnoses (*Phoma* and *Mycosphaerella*), and powdery mildew (*Erysiphe pisi*) harmed in 2002 and the incidence of root diseases was low.

The yield trials results confirm the literature references on low harvest potential and higher susceptibility to almost all the pea diseases (Cousin 1965, Heringa et al. 1969, Vaid and Tyagi 1997, Warkentin et al. 2000). Qualitatively different resistance sources, mainly those with higher yield potential, TSW value, tolerance to the complex of root pathogens and to all other diseases (viruses included) are necessary for successful powdery mildew breeding realisation. This issue is recently the object of intensive activities of breeders' teams. The first pea lines of *afila* type close to those characteristics are being acquired.

Field trials for the verification of information about pea resistance of *afila* types of pea resistance sources and their yield potential were based at Šumperk locality in 2002. The results are presented in Table 3. Cvs. Mozart, Consort-R, AGT-01, Cebeco 1171 and hybrid combination AGT-GH surpassed the control cv. Gotik in dry seed

Table 3. Evaluation of the yield potential of the powdery mildew resistant sources; field trials (Temenice 2002)

Genotype	Seed yield per plant				Seed yield per pod			
	g/plant	% of control cv.	95%	99%	g/pod	% of control cv.	95%	99%
Mozart	5.1	134.2	A	A	242.4	130.1	A	A
Consort-R	4.1	107.8	B	AB	203.5	109.2	AB	AB
Cebeco 1171	4.1	107.8	B	AB	197.4	105.9	ABCD	AB
AGT-01	4.0	105.2	B	BC	199.8	107.2	ABCD	AB
AGT-GH	4.0	105.2	B	BC	194.5	104.4	BCD	AB
Gotik (S)	3.8	100	BC	BC	186.3	100.0	BCD	ABC
Highlight	3.4	89.4	BCD	BCD	164.3	88.2	BCDE	BC
AGT-KR	3.1	81.5	CD	BCD	156.4	83.9	CDE	BC
Melfort	3.0	78.9	CD	CD	153.0	82.1	DE	BC
LU 390-R2	2.6	68.4	D	D	136.0	73.0	E	C

Statistic evaluation of yield (ANOVA); multiple comparisons (g/plant and g/plot), classified by genotype; method 95% and 99% Tukey-*HSD* interval

Table 4. Evaluation of the yield potential and quality of *afila* pea resources resistant to powdery mildew, field trials (Temenice 2002)

Genotype	Number pods/plant	Number seeds/pod	TSW	Seed colour	Earliness	Stem length	Diseases root diseases	Diseases viruses PEMV	Diseases powdery mildew
	mean values	mean values	(g)			(cm)		(0–5)	
Mozart	6.7	4.2	177	Y	M	59	2.0	1.6	0
Consort-R	4.1	4.8	206	Y	VE	52	2.7	2.7	0
Cebeco 1171	4.0	5.4	189	G	M	61	3.0	4.0	0
AGT-01	5.3	4.2	191	Y	M	76	2.6	2.5	0
AGT-GH	5.8	4.6	151	Y	M	75	2.9	2.5	0
Gotik (S)	4.5	4.6	183	Y	M	78	2.6	2.7	5
Highlight	5.9	4.1	139	Y	E	62	2.5	2.0	0
AGT-KR	4.3	4.9	148	Y	M	80	3.7	3.8	0
Melfort	4.5	4.4	150	Y	M	51	3.6	3.8	0
LU 390-R2	4.1	4.6	137	Y	M	50	3.5	3.2	0

Earliness: M = medium, E = early, VE = very early; seeds colour: Y = yellow, G = green

yield. The other resistance sources cvs. Highlight, Melfort, LU 390-R2, and the hybrid combination AGT-KR reached lower dry seed yields in comparison with cv. Gotik. The best resistance source to powdery mildew was from the yield potential point of view cv. Mozart. The main reason for its higher seed yield was a high pod number per plant and improved tolerance to viruses and root diseases (Table 4). The disadvantages of cv. Mozart were higher susceptibility to rust (*Uromyces pisi*) and anthracnoses (*Phoma* and *Mycosphaerella*). Good results were achieved with cv. Consort-R (high TSW value and earliness), cv. Cebeco 1171 (lodging resistance, high TSW value, high seed number per pod), and the hybrid combination AGT-01 (high TSW, improved tolerance, height of plants on the level of cv. Gotik).

Only cv. Highlight with defined high pod number per plant, earliness, and higher tolerance to viruses could be utilised in the resistant breeding programmes concerning the sources of resistance with lower yield potential (in comparison with Gotik). The remaining sources of resistance, i.e. cvs. Melfort, LU 390-R2, and the hybrid combination AGT-KR cannot be recommended for breeding utilisation due to their low yield potential, high sensitivity to root diseases, and low TSW value.

The results of the yield tests with the resistance sources from Šumperk locality (2002) can be compared with the results of the yield tests carried out in Canada (Klassen 2002) in 2001 and 2002. 13 resistant *afila* smooth seeded peas were evaluated in 2001. Control cv. Carneval was surpassed in dry seed yield just by three resistance sources (Mozart +6%, Melfort +3% and Cebeco 1171 +2%). The same yield level as that of the control cultivar was found in these resistant sources: Stalwarth, Cebeco 1489, Minuet and Eclipse. Lower yields in the range 5–40% was reached by cvs. Admiral, Montero, Highlight, Handel, Advantage, and Dominator. In the yield tests of 2002, the following cvs. were included: Acer, Mozart, Eclipse, SGL-45, SGL-1977 and SGL-2024. Tests were carried out at two locations – Arborg and Morden. Control cv. Carneval was surpassed at the Arborg locality just by cv. Mozart (8%), cv. Eclipse was on the same level and SGL-1977 had about 4% lower seed yield (Acer – 5%, SGL-2024 – 12% and SGL-45 – 20%). Cultivars Eclipse and SGL-1977 surpassed the control cv. Carneval at the Morden locality by about 7–12%, on the same yield level were cvs. Mozart and Acer, and about 15–16% lower production was found with cvs. SGL-2024 and SGL-45.

It is possible to anticipate an increase in *afila* smooth seeded peas resistant breeding with multiple resistance in the near future. In those new programmes, *afila* pea types resistant to powdery mildew with high TSW and the highest yield potential will be utilised as well as new sources of leaf type pea with multiple resistance to powdery mildew, root rot and virus infections (Fallon, Lifter, Joel, Franklin, etc.).

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REFERENCES

Cousin R. (1965): Resistance to powdery mildew in pea. Ann. Amélior. Plantes, *15*: 93–97.

Germaplasm Resources Information Network. (2002): http://www.ars-grin.gov/cgi-bin/npgs/html/acchtml.pl?1614803.

Goldenberg J.B. (1965): "Afila", a new mutation in pea (*Pisum sativum* L.). Bol. Genet., *1*: 27–31.

Harland S.C. (1948): Inheritance of immunity to mildew in Peruvian forms of *Pisum sativum*. Heredity, *2*: 263–269.

Heringa R.J., Vannorel A., Tazelaar M.F. (1969): Resistance to powdery mildew in pea. Euphytica, *18*: 163–196.

Klassen E. (2002): Internal annual report. S.S. Johnson Seeds Ltd. Winnipeg, Canada.

Kraft J.M., Coffman M.Z. (2000): Registration of pea germaplasm. Crop Sci., *40*: 301–303.

Nagaraju V., Pal A.B. (1990): Character analysis in garden pea lines with variable resistance to powdery mildew and rust diseases. Mysore J. Agr. Sci., *24*: 68–71.

Pierce W.H. (1948): Resistance to powdery mildew in pea. Phytopathology, *37*: 21.

Tiwari K.R., Panner G.A., Warkentin T.D. (1997a): Inheritance of powdery mildew resistance in pea. Can. J. Plant Sci., *77*: 307–310.

Tiwari K.R., Panner G.A., Warkentin T.D., Rashid K.Y. (1997b): Pathogenic variation in *Erysiphe pisi* the causal organism of powdery mildew in pea. Can. J. Plant Pathol., *19*: 267–271.

Tiwari K.R., Panner G.A., Warkentin T.D., Rashid K.Y., Menzies J.C. (1998): Powdery mildew of pea. Genetics of host pathogen interaction and identification of molecular markers for resistance. 3rd Eur. Conf. Grain Leg., Valladolid. Part I, Ses. VIII: 120–121.

Vaid A., Tyagi P.D. (1997): Genetics of powdery mildew resistance in pea. Euphytica, *96*: 203–206.

Warkentin T., Xue A., Sloan A., Rashid K., Ali-Khan S.T., Vera C., Orr D., Turkington K., Clayton G., Loeppky G. (2000): AC Melfort field pea. Can. J. Plant Sci., *80*: 117–119.

Weeden N.F., Providenti R. (1999): Availability of seed from breeding lines containing genes for resistance to powdery mildew, Fusarium wilt races 1 and 2 and least nine virus disease. Pisum Genet., *30*: 33.

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ABSTRAKT

Využití zdrojů rezistence *afila* typů hrachu (*Pisum sativum* L.) proti padlí (*Erysiphe pisi* DC.) ve šlechtitelských programech

V polních pokusech byl srovnáván výnosový potenciál, vlastnosti a zdravotní stav devíti zdrojů rezistence *afila* typů polního hrachu (*Pisum sativum* L.) proti padlí (*Erysiphe pisi* DC.). Ve výnosu semen překonalo kontrolní genotyp Gotik pět zdrojů (Mozart, Consort-R, AGT-01, Cebeco 1171 a AGT-GH). Zbývající zdroje poskytly výnos nižší o 12–27 % (Highlight, AGT-KR, Melfort a LU 390-R2). Příčinou nízkého výnosu byla zvýšená citlivost na virózy (PEMV, BYMV), na kořenové choroby (*Pythium*, *Fusarium*) a nízká hodnota hmotnosti tisíce semen (HTS). Kombinační křížení s donory vyššího výnosového potenciálu, s vyšší HTS a vyšší odolností proti kořenovým chorobám mělo pozitivní vliv na výnos semen. Cílem programu rezistentního šlechtění hrachu v ČR je získání genotypů *afila* typů polního hrachu, rezistentních proti padlí, s vysokou odolností proti virózám (PEMV), proti kořenovým chorobám, se střední raností, se zvýšenou odolností proti poléhání, s délkou stonků 60–75 cm, s vysokým výnosovým potenciálem a s HTS nad 160 g.

Klíčová slova: hrách; *Pisum sativum*; *afila*; rezistence; *Erysiphe pisi*; šlechtění; genetické zdroje

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