Inheritance and linkage relationships of resistance to powdery mildew of peas

Sakr B[1] and Muehlbauer J.F[2]

[1] Améliorateur au laboratoire des légumineuses alimentaires, INRA CRRA Settat, Morocco
[2] Research Geneticist, USDA-ARS, Washington State University, Pullman, WA-99164 USA.

Abstract

Powdery mildew (Erysiphe polygoni DC.; syn. E. pisi DC.) of pea (Pisum sativum L.) has a worldwide distribution and causes economic losses when climatic conditions are conducive to the development of the disease. Resistance is reportedly controlled by a single recessive gene designated as er. However, attempts to locate the gene in the genome have been unsuccessful. In this study we investigated the chromosomal location of the gene for resistance by analyzing joint segregation with 12 morphological markers and eight isozymic markers in six crosses involving five resistant and five susceptible pea lines. Resistance in each of the six crosses was conferred by a single recessive gene. Allelism tests among the five resistant parents indicated they all carried the same gene for resistance. Linkage analysis suggested that er is located on chromosome 7 about 20 map units from Skdh and Oh genes.

Key words : *Pisum, Erysiphe polygoni,* resistance, Genetic Marker, linkage analysis.

Résumé : L'hérédité et les relations de linkage de la résistance du pois à l'oïdium

L'oïdium (Erysiphe polygoni DC.; syn. E. pisi DC.) du pois (Pisum sativum) présente une distribution mondiale et cause des pertes économiquement importantes lorsque les conditions climatiques favorisent le développement de la maladie. La résistance génétique est reportée par un seul gène récessif désigné er. Cependant, la localisation chromosomique de er n'a pas encore eu lieu avec précision malgré les tentatives multiples. Dans la présente étude nous avons investi la localisation chromosomique du gène de résistance à l'oïdium par l'analyse de la ségrégation conjointe de 20 marqueurs génétiques dont 12 morphologiques et 8 isozymiques.

Six croisements entre dix lignées de pois dont cinq sont résistantes et cinq sensibles à l'oïdium ont été utilisés.

Il est constaté que la résistance dans chacun des six croisements est contrôlée par un seul gène récessif. Le test d'allélisme a confirmé que le même gène de résistance se trouve dans les cinq lignées résistantes. L'analyse des linkages suggère que er est localisé sur le chromosome 7 à une distance de 20 unités environ des marqueurs Skdh et Oh.

Mots clés : *Pisum*, *Erysiphe polygoni*, résistance, marqueur génétique, analyse de linkage

ملخّص : توريث مقاومة البازلاء للبياض الدّقيقي و علاقاته الرّابطة بالعلامات الوراثية

صقر ب1.، و موهلباور ج.ف2.

1 : المركز الجهوي للبحث الزّراعي، سطّات، المغرب

2 : جامعة ولاية واشنطن، بلمان، و.م.الأمريكيّة

البياض الدّقيقي عند البازلاء (*Pisum sativum*) يسبّبه *Ersiphe polygoni* D.C.; Syn. E. *pisi* D.C و يتواجد في الكثير من أنحاء العالم. يمكن للمرض أن يتسبّب في إتلاف كبير لمحصول البازلاء و ذلك في حالة توفّر الظّروف الملائمة لتطوّر المرض.

لقد ورد سابقا أن المقاومة الوراثيّة لمرض البياض الدّقيقي توجد تحت تأثير مورث واحد سمي *(er)* موجود على صفة متنحية. لكنّ مكان هذا المورث *(er)* على الكروموزوم لم يتحدّد بعد بالرّغم من المحاولات المتعدّدة. فيما يخصّ الدّراسة الحالية، عملنا على تحديد موضع *(er)* مستعملين طريقة تحليل الانعزالات المشتركة. لقد درسنا العلاقات الرّابطة بين *(er)* و 20 علامة وراثية منها 12 علامة شكلية "morphologique" و 8 علامات أيزوزيمية (isozymiques) مستعملين 6 تهجينات بين 10 سلالات من نبات البازلاء، خمسة منها مقاومة و الخمس الأخرى حساسة لمرض البياض الدقيقي.

يتّضح من خلال هذه الدّراسة أنّ مقاومة البازلاء لمرض البياض الدّقيقي توجد تحت تأثير مورث واحد متنحّي كما ورد في دراسات أخرى. كما أن المورث نفسه يوجد في كل السّلالات الخمسة المقاومة التي استعملت في الدّراسة الحالية. كما أنه اتضح من خلال تحليل الروابط بين *(er)* و العلامات الوراثية أن *(er)* يوجد في الكروموزوم رقم 7 و على بعد 20 وحدة خرائطية تقريبا من العلامتين *Skdh* و *Oh*.

كلمات مفتاحيّة : *Erysiphe polygoni, Pisum*، مقاومة، علامة وراثية، تحليل الانعزالات، تحليل الروابط.

Introduction

Powdery mildew, a fungal disease caused by Erysiphe polygoni DC. *(syn. E. pisi DC.)*, is a major constraint to pea *(Pisum sativum L.)* production throughout the world. The disease adversely affects yield (Gritton and Ebert 1975) and seed quality (Rant and Wangikar 1979).

In Morocco, the disease usually appears late in the growing season and can be particularly severe in late planted fields (El Guilli 1987). Most dry pea cultivars in use in Morocco are susceptible to the disease, but, usually escape economic injury because of early maturity. Early maturity as an escape mechanism is not sufficient in some years when cool, moist conditions prevail and delay crop maturity. The use of fungicides to control the disease is almost nonexistent. Therefore, the development of resistant cultivars would provide farmers with a means of improving pea yields and remove a major production constraint.

The inheritance of resistance to powdery mildew in pea has been widely investigated. However, the number of genes involved in resistance and their chromosomal locations is not clear. Hammarlund (1925) reported that resistance was controlled by four genes acting additively. Later, Harland (1948) reported that the resistance in Peruvian material was conferred by a single recessive gene that he designated as *er*.

Using different cultivars and lines, Pierce (1948), Cousin (1965), Hari *et al.* (1981) and Mishra and Shukla (1983) reported similar results to those found by Harland in 1948. However, under greenhouse conditions, Heringa *et al.* (1969) detected the presence of a second recessive gene for resistance.

The first attempt to locate the gene for resistance was made by Harland (1948) using 'Huancabamba'. He presented evidence suggesting that the gene was located on chromosome 1 and 35 map units from *A*, the major gene for anthocyanin pigmentation of stem axes, flowers, and testa. However, Marx (1971) was unable to show linkage of *er* with *A* or any of six other morphological genetic markers located on chromosome 1. Marx (1971) reported that *er* was 10 map units from *Gty*, the gene for « gritty » testa, 30 map units from *B*, the gene for begonia flower color, and 32 map units from *Och*, the gene for ochraceous colored seed coat. He, therefore, concluded that these genes were all located on chromosome 3. However, the location of *Gty* on chromosome 3 has not been confirmed by linkage tests with other markers on that chromosome. In further studies, Marx (1974 and 1986) confirmed the 10 % *er-Gty* linkage and that *er* and *A* were independent, but he could not confirm the linkage of *er* with *B* or the linkage of er with *Och*. Also, Marx (1986) found independence between *er* and four other genes *(tac, apu, st and bulf)* located on chromosome 3. No linkage of *er* to any biochemical marker has been reported. The recently identified isozymic loci (Weeden and Marx 1984) represent additional markers that could be useful in locating *er* and possibly other genes which confer resistance to powdery mildew.

With the available morphological markers and the recently identified isozyme loci, our objectives were to confirm the inheritance of resistance to powdery mildew in pea and to establish linkage relationships. The identification of tightly linked markers would facilitate the introgression of powdery mildew resistance gene(s) into susceptible pea cultivars.

Materials and methods

Six crosses were made in 1986 between five powdery mildew resistant pea lines and five susceptible lines (Table 1). F1 plants were grown in the greenhouse during the fall and winter of 1986-87. Parental lines and 300 F2 plants were grown in the fall of 1987 in the greenhouse and a powdery mildew epiphytotic was created by shaking spore-bearing parts of infected plants over the plants at the early flowering stage. Parental material and F2 plants were scored for disease reaction two weeks after inoculation. The same material was scored for twelve morphological traits and seven isozymic markers (Table 2). In order to study most of the pea genome, two to five genetic markers were chosen for each chromosome except that easily identifiable genetic markers were not available for chromosome 6. The Pisum gene map as presented by Weeden and Wolko (1990) was used as the reference gene map.

Isozymes were resolved using starch gel electrophoresis. Small samples (50-100 mg) of young leaves taken from each F2 plant were analyzed. The assays were performed according to Weeden and Marx (1984). Aspartate aminotransferase (AAT), leucine aminopeptidase (LAP) and shikimate dehydrogenase (SKDH) were resolved using the tris citrate/lithium borate buffer system. Isocitrate dehydrogenase (IDH) and methyl-umbelliferyl-esterase (EST) were resolved using the citrate/N-(3 aminopropyl)-morpholine buffer system, and 6-phosphogluconate dehydrogenase (6PGD) was resolved using the histidine buffer system.

Seeds harvested from each F2 plant were sown in separate rows in field plots at Spillman Farm, Pullman, WA in the summer of 1987. The soil was a Palouse silt loam (fine, mixed, mesic, pachic Ultic Haploxerol). Natural inoculation of powdery mildew occurred and the material was evaluated for disease reaction. The F3 progeny rows, used to verify previous scoring of F2 plants in the greenhouse, were classified as either susceptible, segregating or resistant. The linkage-1 program of Suiter *and al.* (1983), which uses the maximum likelihood method, was used to determine goodness of fit to expected ratios and to determine joint segregation of resistance with the previously scored genetic markers.

Allelism tests were performed by crossing the five resistant parental lines to each other in all combinations without reciprocals. F1 plants were grown in the greenhouse and inoculated as described above. Heavily infected plants of 'Alaska 81' were collected from the field and used to inoculate the parents and F1 hybrids of the allelism test.

Table 1. Pea lines and genetic markers involved in the inheritance and mapping studies of the gene controlling powdery mildew resistance in peas

Cross	Disease reaction	Parent	Genotype
1	Resistant	PL2318	*a, f/fs, Aat-p*(S), *6pgd-p*(F)
	Susceptible	A480-195-1	*A, F/Fs, Aat-p*(F), *6pgd-p*(S)
2	Resistant	A83-22(4)e	*A, was, gp, F/Fs, Oh, Est-2*(S), *Skdh*(S), *Idh*(S)
	Susceptible	Sentry	*a, Was, Gp, f/fs, oh, Est-2*(F), *Skdh*(F), *Idh*(F)

Table 1. Continued

3	Resistant	H898-8-5	*a, f/fs, St, Tl, 6pgd-c*(S)
	Susceptible	A83-22(11)6	*A, F/Fs, st, tl, 6pgd-c*(F)
4	Resistant	PL2318	*f/fs, Tl, Aat-p*(S), *Aat-m*(F), *Est-2*(F), *6pgd-c*(S), *Lap1*(S)
	Susceptible	A83-22(11)6	*F/Fs, tl, Aat-p*(F), *Aat-m*(S), *Est-2*(S), *6pgd-c*(F), *lap-1*(F)
5	Resistant	Kodiak	*le, N, V, i, R, 6pdg-c*(S), *Skdh*(F), *Lap-1*(S)
	Susceptible	C77-323-2a	*Le, n, v, I, r, 6pgd-c*(F), *Skdh*(S), *Lap-1*(F)
6	Resistant	RP95126	*i, R, Aat-p*(S), *Lap-1*(S), *Skdh*(S), *Est-2*(F)
	Susceptible	C77-323-2b	*I, r, Aat-p*(F), *Lap-1*(F), *Skdh*(F), *Est-2*(S)

Table 2. Description of the morphological* and isozymic** genes used to study possible linkage with *er*

Genes	Chr	Description
A	1	General plant anthocyanin pigmentation
F/Fs	5	More or less sharp and evenly distributed, small violet spots on the seed testa.
Gp	5	Recessive *Gp* produces yellow pods; dominant *Gp* produces green pods.
i	1	Green cotyledons when recessive, dominant *I* givesyellow cotyledons.
le	4	Short internodes, *Le* gives long internodes
n	4	Recessive *N* conditions thick and fleshy pod walls; whereas, dominant *N* conditions thin pod walls.
Oh	2	Dominant *Oh* produces reddish-brown testa; whereas, recessive *Oh* produces green testa.
r	7	Recessive *r* produces wrinkled seeds. Dominant *R* produces round seeds.
st	3	Recessive *st* produces stipules that are lanceolate, slightly bent and greatly in size. Dominant *St* has normal sized stipules.
tl	7	Recessive *tl* converts tendrils to leaflets (acacia) the heterozygote, *Tl/tl* causes flattened tendrils and homozygous dominant *Tl* produces normal tendrils.
	4	Recessive *v* removes most of the sclerenchymatous membrane from the inner pod wall. Dominant *V* has fibrous pod walls.
was	4	Recessive *was* has no wax on both sides of stipules, on the underside of the leaflets or on the pods.
Aat-p	1	Aspartate aminotransferase-plastidic
Aat-m	7	Aspartate aminotransferase-mitochondrial
Lap-	3	Leucine aminopeptidase-1
Skdh	7	Shikimate dehydrogenase
Est-2	7	Methyl-umbelliferyl esterase-2
Idh	1	Isocitrate dehydrogenase
6pgd-c	5	6-Phosphogluconate dehydrogenase-cystolic
6pgd-p	7	6-Phosphogluconate dehydrogenase-plastidic

* Source : Blixt *and al.* 1978
** Source : Weeden and Marx 1987

Results and discussion

Disease development in F1, F2, and F3 progenies

The F1 plants from the six crosses were grown in the absence of powdery mildew in order to produce F2 populations for the genetic study. Symptoms on susceptible greenhouse-grown F2 plants appeared four days after inoculation as small whitish spots on the upper surfaces of the lowest leaves. The disease soon spread to other leaves and stems causing a light to heavy infection. Some of the plants appeared to be resistant with no lesions or only a few small lesions which later disappeared. Susceptible parental plants were fully covered with powdery mildew. Field grown F3 progenies were infected by powdery mildew that occurred naturally and caused severe disease on the susceptible progenies and susceptible parental plants. All the F1 plants used for the allelism test did not develop lesions or developed only a few small lesions indicating that all the resistant parents used in this study carried the same resistance gene.

Inheritance of resistance

Segregation of F2 plants within all six families fit the expected 3 susceptible : 1 resistant ratio (Table 3). The heterogeneity X^2 was small indicating no significant difference among the segregation patterns of the six families. Almost all resistant F2 plants produced F3 progeny rows that were uniformly resistant, while susceptible F2 plants produced F3 progeny rows that were either uniformly susceptible or segregating. The small size of some F3 families could have created some misclassification of these families resulting in some observed differences between F2 and F3 results. Tests for goodness of fit to the expected 1 uniformly resistant F3 family : 2 segregating F3 families : 1 uniformly susceptible F3 family revealed nonsignificant X^2s for all six crosses (Table 4), confirming the hypothesis that a single recessive gene conferred resistance to the disease.

Pierce (1984) and Cousin (1965) previously had reported that resistance to powdery mildew in certain French pea cultivars was conferred by a single recessive gene. Mishra and Shukla (1983) showed that the same is true for several pea germplasm lines from India. Results from the present study on the five resistant lines from the United states are similar and indicate that a single recessive gene was responsible for resistance. None of the lines were immune to the disease as reported by Harland (1948) on Peruvian lines. Resistant plants showed a few small lesions on the leaves as well as on the stems and, therefore, the separate resistance in leaves or stems as reported by Heringa *et al.* (1969) on Peruvian lines was not observed in the material used in this study.

Table 3. Goodness of fit to the expected 3 susceptible : 1 resistant segregation ratio for six F2 families segregating for resistance to powdery mildew

Cross	Number of plants		x^2	Probability
No	Susceptible	Resistant		
1	30	11	0.073	78
2	36	10	0.260	58
3	20	7	0.012	91
4	22	5	0.604	43
5	48	17	0.046	82
6	68	25	0.175	67
Total			1.1700	90
Pooled	224	75	0003	99
Heterogeneity			1.1697	99

Table 4. Goodness of fit to an expected 1 resistant : 2 segregating: 1 susceptible ratio for F3 families from six crosses segregating for resistance to powdery mildew

Cross	Number of F3 Families			x^2	Probability
No	Uniformly Resistant	Segregating	Uniformly Susceptible		
1	10	18	12	0.600	0.75
2	9	26	8	1.930	0.50
3	6	15	6	0.333	0.90
4	6	10	11	3.660	0.25
5	17	29	19	0.876	0.75
6	25	46	20	0.560	0.90
Total	73	144	76	7.959	0.20
Pooled				0.147	0.95
Heterogeneity				7.812	0.75

Chromosomal location of the resistance gene *(er)*

Results of joint segregation analysis of data from individual crosses are presented in table 5. These results indicated linkage of *er* with *Skdh* and *Oh*, both reportedly (Weeden and Wolko 1990) located in the same vicinity on chromosome 7, which indicates that *er* may also be located in that linkage group. Joint segregation of *Skdh* with resistance in crosses 5 and 6 indicated that the two genes were 20 and 25 map units apart in the respective crosses. *Skdh* and *er* were in repulsion and coupling phase in crosses 5 and 6, respectively. Oh, a gene closely linked to *Skdh* (Weeden and Marx 1984) also showed 25 % recombination with resistance in cross 2. However, data on *skdh/er* from cross 2 are not conclusive. While the recombination frequency (RF) was as high as 50 % indicating random assortment, the X^2 value was as high as 12.2 indicating some linkage. The gene *Est-2* previously reported to be located 13 map units from *Skdh* (Weeden and Marx 1987) showed 17 % recombination with Skdh in the present study, but, did not show linkage with *er* in three crosses (2, 4 and 6) suggesting that *er* is dis-

tal to *Est-2*. Also, *Aat-m*, a gene located 17 map units from *Est-2* (Weeden and Marx, 1987) did not show linkage with *er* in cross 4. In the present study, *Aat-m* showed 14 % recombination with *Est-2*.

There was independence between *er* and all the markers tested from chromosomes 1, 3, 4, and 5. None of the four marker genes *(A, i, Aat-p and Idh)* known to be located on chromosome 1 showed linkage with powdery mildew resistance. Also no linkage of resistance was obvious with two markers *(st,* and *Lap-1)* located on chromosome 3, four markers *(le, n, v,* and *was)* located on chromosome 4, five markers *(r, tl F/Fs, Gp and 6pdg-c)* located on chromosome 5 or three markers *(Est-2, Ast-m, and 6pgd-p)* located on chromosome 7, presumably because these latter markers are located quite distant from *er* (Table 5).

Results of the present study differ from those reported by Harland (1948) on the possible association of *er* with *A* on chromosome 1. The loose linkage of 35 map units between *A* and *er* reported by Harland (1948) using Peruvian lines, could not be confirmed by Marx (1971) and also could not be confirmed in this study. This suggested the possibility that the lines used in this study which all carry the same gene for resistance, as confirmed by the allelism test, may have a different gene for resistance than the one reported in the Peruvian material studied by Harland (1948). Another possibility might be a translocation from linkage group 1 to linkage group 7.

Results of the present study also differ from those reported by Marx (1971) who indicated that *er* was tentatively located on chromosome 3. Further attempts to locate *er* on chromosome 3 were unsuccessful (Marx 1974 and 1986).

Resistance to powdery mildew in each of the crosses used in this study was controlled by a single recessive gene. The linkages that were found between resistance and *Skdh*, and *Oh* are not sufficiently close to enable their use as « tags » for transferring resistance in a breeding program. Additional molecular markers on chromosome 7 are needed to confirm the location of *er* in that linkage group. Crosses between the resistant lines used in the present study and those used by Harland (1948) are needed to help define the gene(s) conferring resistance to powdery mildew.

Table 5. Joint segregation of er with genetic markers in F2 progenies from six crosses.

		No. of F2 Progenies with designated Phenotype*								
		Er/-			*er er*					
Loci	Cross	F/D	H	S/R	F/D	H	S/R	x^2	RF**	SE
Chromosome 1										
A	1	19		10	6		5	0.40		
A	2	27		8	8		2	0.03		
A	3	14		5	4		3	0.65		
i	5	4	35	7	3	12	2	1.04		
i	6	19	36	11	4	12	8	3.39		
Aat-p	1	8	14	8	6	3	2	2.79		
Aat-p	4	5	4	7	2	1	1	0.66		
Aat-p	6	11	34	23	7	14	4	3.44		
Idh	2	9	18	8	4	4	4	0.78		

Table 5. Continued

Chromosome 3										
St	3	12		8	6		1	0.54		
Lap-1	4	2	12	8	1	2	2	0.61		
Lap-1	5	4	12	5	4	5	2	1.00		
Lap-1	6	15	37	16	4	12	6	1.52		
Chromosome 4										
le	5	42		5	16		1	0.33		
n	5	32		16	9		8	0.01		
v	5	30		15	14		3	0.47		
Was	2	26		10	7		3	0.02		
le	5	42		5	16		1	0.33		
n	5	32		16	9		8	0.01		
v	5	30		15	14		3	0.47		
Was	2	26		10	7		3	0.02		
Chromosome 5										
F/FS	1	15		14	8		3	1.43		
F/Fs	2	16		20	5		5	0.09		
F/Fs	3	9		11	3		4	0.00		
Gp	2	26		10	8		2	0.24		
6pgd-c	3	5	9	5	2	4	1	0.43		
6pgd-c	4	3	13	6	1	1	3	2.62		
r	5	4	35	7	3	12	2	1.04		
r	6	37	18	5	10	9	3	1.78		
tl	4	9	12	1	3	1	1	2.65		
Chromosome 7										
6pgd-p	1	6	17	7	2	3	5	2.84		
Oh	2	33		3	6		4	6.08	25	0.13
Skdh	2	10	22	4	6	0	4	12.20		
Skdh	5	3	15	5	8	3	1	10.52	20	0.07
Skdh	6	11	24	5	2	4	8	11.31	25	0.06
Aat-m	4	4	8	4	1	2	1	0.00		
Est-2	2	11	19	6	4	3	3	1.77		
Est-2	4	4	13	5	0	4	1	1.20		
Est-2	6	14	38	15	4	15	6	0.27		

*F/D = Homozygous for fast isozyme allele or dominant morphological allele

H = Heterozygous

S/R = Slow isozyme allele or recessive morphological allele

** RF = Recombination frequencies were calculated only when x^2 probability

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