Two powdery mildew resistance mutations induced by ENU in *Pisum sativum* L. affect the locus *er*1

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Abstract The alkylating compounds and strong mutagens methylnitrosourea (MNU) and ethylnitrosourea (ENU) were used to widen the genetic variability of pea (*Pisum sativum* L.) via experimental mutagenesis. Amongst multiple mutations of agronomical interest, for the first time two mutations conferring broad-range resistance to powdery mildew (*Erysiphe pisi* Syd.) were induced in pea. Mutagenic treatments were carried out on seedlings using a technique that ensures very high mutagenic efficiency. Two-hour exposure of cv. Solara seedlings to chemical mutagens resulted in almost non noticeable lethality and sterility in the M1 generation and very high mutation rates: ~39% families with visible mutations in the M2 generation. The influence of the cell cycle phase on the process of mutagenesis was studied in cv. Frilene using a previously developed technique for synchronization of shoot apical meristem cells. The cell cycle phase at which cells were treated apparently did not influence the lethality and sterility in the M1 generation, while the visible mutation rate, assessed in the M2 generation, showed a clear cell cycle dependency. Seedlings treated at the G2 and M phases gave rise to progenies exhibiting the highest mutation rate, over 50% of M2 families with visible mutations. The powdery mildew resistant (PMR) mutant S(*er1mut1*) was induced by treatment of cv. Solara seedlings for 2 h with ENU, while the PMR mutant F(*er1mut2*) was induced by treatment of cv. Frilene seedlings with the same chemical mutagen for 1 h during the G2 phase of the cell cycle. The genetic analysis of the novel PMR mutant lines showed that both resistances are inherited as monogenic recessive traits. The performed genetic complementation analyses revealed that both mutations affect the same locus—*er*1, which determines most of the natural sources of PMR in pea. A project aiming at the isolation of the powdery mildew resistance mutated gene via map based cloning is currently under way.

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Introduction

Experimental mutagenesis has a prominent role in plant breeding. During the past 70 years over 2,250 varieties have been released, derived directly from

new mutants or from breeding programmes involving induced mutants (Ahloowalia et al. 2004; Maluszynski et al. 2000). With 265 mutant varieties released in 32 countries the grain legumes are a representative group among the crops genetically improved by experimental mutagenesis (Bhatia et al. 2001). Thirty-two pea (*Pisum sativum* L.) mutant varieties are presently listed in the FAO/IAEA database (www.mvd.iaea.org/MVD/default.htm) and a large number of induced mutants can be found at the John Innes Pisum Collection (www.jic.ac.uk/germplas/pisum/index.htm).

Among the panoply of mutations those that confer disease resistance are of particular interest. So far, the most classical and prominent example is the recessive mutation *mlo* conferring resistance against all pathotypes of powdery mildew in barley (*Hordeum vulgare* L.), which nowadays is present in most of the commercial varieties of this crop (for review, see van Harten 1998).

Powdery mildew, caused by the biotrophic ascomycete fungus *Erysiphe pisi* Syd, is the most important disease affecting pea production in Portugal (Sousa 1999) and in several other countries, all over the world (Warkentin et al. 1996).

Natural sources of resistance to powdery mildew in pea have been identified in germplasm from all continents, and regardless of their origins all sources of resistance to this disease have shown monogenic recessive inheritance (Harland 1948; Liu et al. 2003; Sharma 2003; Fondevilla et al. 2006). Moreover, one single locus—referred to as *er*1 by Heringa et al. (1969)—seems to be responsible for most of the powdery mildew resistances (PMR) identified so far (Sharma 2003).

In some cases, PMR was proposed to be conferred by the complementary action of two recessive loci (Heringa et al. 1969; Kumar and Singh 1981). Nevertheless, these claims based on the resistance expressed on the lines Mexique 4 and S-143, were not corroborated by the later studies of Tiwari et al. (1997), Vaid and Tyagi (1997), and Sharma (2003), who reported a monogenic recessive inheritance for the resistance present in these two genotypes.

A second recessive powdery mildew resistance locus, *er*2, was identified in lines SVP 951 and SVP 952 (Heringa et al. 1969) and JI 2480 (Ali et al. 1994; Tiwari et al. 1997). This monogenic recessive resistance was shown to be partially or totally broken depending on biotic and abiotic stress factors such as

(leaf) age, field versus glasshouse/growth cabinet growing conditions (Heringa et al. 1969; Tiwari et al. 1997; Fondevilla et al. 2006) and temperature (Heringa et al. 1969; Fondevilla et al. 2006).

Due to the conditional expression of *er*2 and the difficulties found in genetic mapping of this locus, Sharma (2003) assumed the existence of one single powdery mildew resistance locus, *er*, a designation used by Harland (1948) and also adopted by other authors, e.g. Janila and Sharma (2004) and Ek et al. (2005). However, bearing in mind the genotypes used for reference, e.g. Mexique 4, it becomes evident that in these cases *er* is synonymous to *er*1.

Recently an exogenous source of powdery mildew resistance was identified in *Pisum fulvum* by Sharma and Yadav (2003) which these authors assumed to be recessive by analogy with the resistances previously identified in *P. sativum*. More recently Fondevilla et al. (2007, 2008) identified a resistance locus (*Er*3) in *P. fulvum* which they determined to be monogenic and dominantly inherited using interspecific crosses with *P. sativum*.

Herein we describe, in detail, the studies that resulted in the first experimentally induced powdery mildew resistant mutants in *Pisum sativum* L. (Leitão et al. 1998; Pereira et al. 2001) and the genetic characterization of these two novel mutants induced by ENU in the commercial varieties Solara and Frilene.

In one series of experiments four different periods of exposure to the alkylating agents methylnitrosourea (MNU) and ethylnitrosourea (ENU) were assayed in seedlings of cv. Solara. In the second series of experiments the role of the cell cycle in the mutagenic efficiency of ENU was assessed on cv. Frilene.

Materials and methods

Plant material

Two commercial varieties, Frilene and Solara, characterised, respectively, by wild-type leaves and yellow seeds and by semi-afila leaves and green seeds, were used as starting plant material in the experimental chemical mutagenesis program. Genetic complementation crosses were performed between both powdery mildew resistant mutant lines, S(*er1mut1*) and F(*er1mut2*), and between these lines and line E835 homozygous for the powdery mildew

resistance allele *er*1 inherited from line Mexique 4, kindly provided by Dr. Alain Burghoffer, INRA, Versailles, France.

Before treatment with chemical mutagens, seeds were washed once in tap water and detergent and disinfected by immersion for 5 min in 10% (v/v) commercial bleach and 0.5% (w/v) SDS solution. After several washes with distilled water seeds were immersed in autoclaved tap water for 3 h and transferred to Petri dishes containing moistened filter paper for germination in the dark, at 24°C, for 72 h.

Cell synchronisation

The shoot apical meristem cells of cv. Frilene seedlings were synchronised with hydroxyurea as described by Leitão et al. (1986) and illustrated in Fig. 1. Briefly, 72 h old seedlings were placed with cotyledons on the top of perforated plexiglass racks and roots completely immersed in 0.03% (w/v) hydroxyurea solution. The shoot apexes were compactly covered with cotton subsequently moistened to saturation with a 0.015% (w/v) hydroxyurea solution. After 24 h at 24°C in the dark the moistened cotton covering the plumula was removed and seedlings washed for 30 min by immersion in three changes of pre-warmed tap water (24°C). Seedlings were maintained at 24°C in the dark for further treatments.

Mutagenic treatments

Mutagenic solutions were prepared just before use. Methylnitrosourea (MNU) (Sigma Chemical, Co.) solubilised in absolute ethanol and maintained at −20°C until use was diluted to 1 mM in distilled water. Ethylnitrosourea (ENU) (Sigma Chemical, Co.) was weighted, immediately dissolved in 1–2 ml dimethylsulphoxide (DMSO) and diluted to 5 mM with distilled water.

Treatments were carried out on seedlings, a procedure previously found to result in high mutagenic efficiency (Leitão et al. 1987). Seedlings were placed upside-down in glass beakers and the mutagenic solutions added to the level of cotyledons so that shoot apexes but not the roots were covered. Beakers were sealed with parafilm to prevent evaporation and root desiccation. After treatment, the mutagenic solutions were decanted. Seedlings were briefly washed with tap water followed by 5 min in 10% (w/v) sodium thiosulphate and three successive 10-min washes in tap water. The mutagenic solutions and washing waters were inactivated with sodium hydroxide pellets.

Seedlings subjected to similar procedures, but using tap water instead of mutagenic chemicals were used as experimental controls. Three days old Solara seedlings were treated, respectively, for 1, 2, 3 and 4 h with the chemical mutagens. Frilene seedlings were

Fig. 1 Mutagenic treatments of *Pisum sativum* L. seedlings with synchronised shoot apical meristems
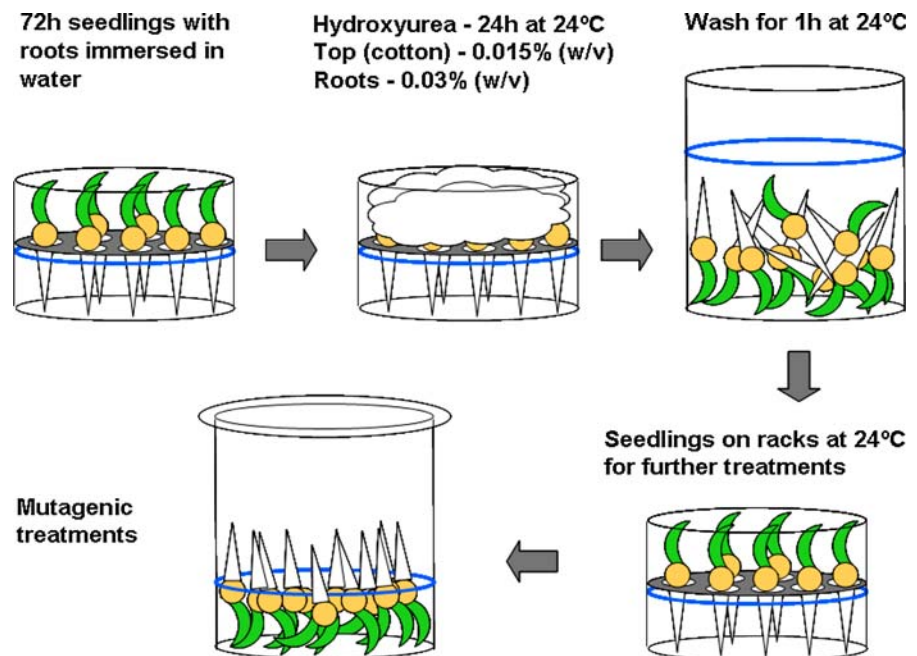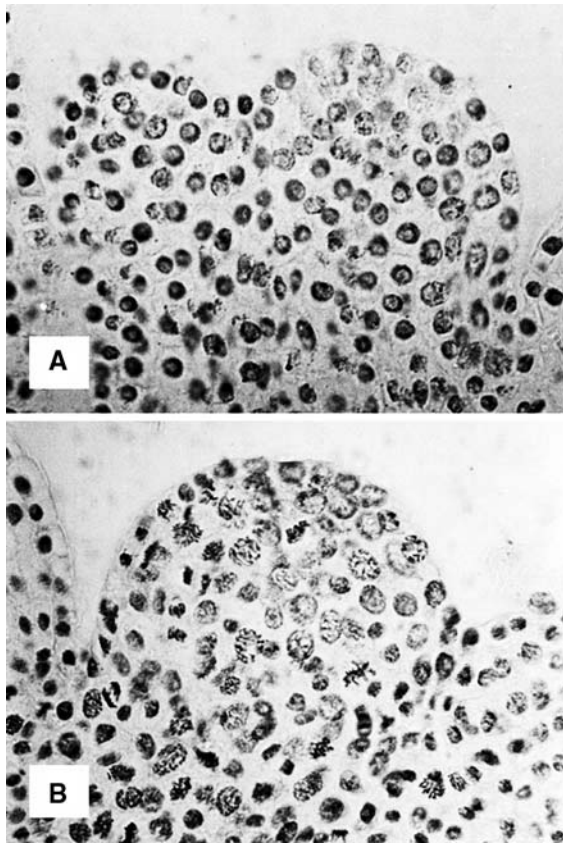
Fig. 2 Synchronised shoot apical meristem of *Pisum sativum* L. **a** 2 h after hydroxyurea release (S phase). Notice the absence of mitotic figures. **b** 8.30 h after hydroxyurea release. Most of the cells are in Mitosis

treated with ENU for 1 h in each different phase of the cell cycle: S, G2, M and G1—corresponding to 2, 7, 8.30 and 13 h after the release of the DNA synthesis inhibitor hydroxyurea, respectively (Figs. 1, 2).

In each treatment 30–40 seedlings were used per tested exposure time or per cell cycle phase. All treatments were repeated three times except for those using MNU, which was performed only twice, as this mutagen was discarded from our pea breeding program.

M1 and M2 analyses

Treated (and control) seedlings were transferred to the field where the M1 generation was analysed for several parameters. Results regarding three main parameters are presented in this article: (i) survival rate—percentage of plants attaining the adult phase normalised by the control value; (ii) sterility—percentage of adult plants with no viable seeds; and (iii) average number of seeds per plant.

Seeds of each M1 plant were collected separately and whenever possible up to 20 plants were sown per M2 family. Only M2 families constituted by 16 or more emerged plants were used for calculation of the visible mutation rate. M2 plants showing chlorophyll and/or morphological mutations were recorded and labelled.

Powdery mildew resistance evaluation

Crosses involving cv. Solara, cv. Frilene, line E835 (*er*1) and the two induced powdery mildew resistant mutant lines, S(*er1mut1*) and F(*er1mut2*), were performed in greenhouses and in the field. The assessment of powdery mildew resistance was performed under field conditions, at the University of Algarve. In order to ensure severe powdery mildew infections the seeds were sown at the beginning of March. In some years, to increase the level of powdery mildew infection, the plants were repeatedly pulverised with a suspension of naturally emerging mycelia and spores of the pathogen. Segregation results were pooled and the concordance of the experimental results with the expected Mendelian segregation ratios was assessed by the χ^2 test.

Results

Duration of exposure to chemical mutagens

The main results of this series of experiments are condensed in Table 1. All MNU and ENU treatments of cv. Solara seedlings resulted in very low M1 lethality as the survival rate was in most cases close to 100%. The lowest survival rate (89%) was attained only after 4 h exposure to 1 mM MNU. The toxic effect of mutagenic treatments was much more marked for plant sterility which clearly increased with the time of exposure to mutagens. Nevertheless, MNU had a more deleterious effect on seed setting than ENU, as 4 h exposure to 1 mM of MNU resulted in ~31% completely sterile plants and an average of 11 seeds per plant, compared to 5% sterile plants and 26 seeds per plant when a five times more concentrated ENU solution (5 mM) was used (Table 1).

Table 1 MNU and ENU mutagenesis of cv. Solara seedlings

	M1				M2		
	Exposure (h)	Survival (%)	Sterility (%)	Seeds per plant	Families (N)	Families with visible mutations (%)	Families with multiple visible mutations (%)
Control 1	1	100	0.0	80	60	0.0	0.0
Control 2	4	99	0.0	76	60	0.0	0.0
ENU 5 mM	1	100	0.0	58	79	20.3	2.5
	2	99	0.0	58	76	39.5	13.2
	3	99	2.1	44	60	20.0	1.7
	4	100	5.0	26	60	23.3	0.0
MNU 1 mM	1	100	0.0	30	36	16.7	0.0
	2	100	2.9	21	32	37.5	6.3
	3	92	20.8	16	34	14.7	2.9
	4	89	30.8	11	34	11.8	0.0

Overall, the procedure used for chemical mutagenesis was very efficient as the high plant survival (over 90%) in the M1 generation was accompanied by high visible mutation rates in the M2 generation—11.6 to 39% families with mutations. For both mutagens the highest mutation rate was observed after 2-h treatments—37.4% (MNU) and 39.3% (ENU). Increasing the time of exposure beyond this threshold resulted in lower mutation rates. A similar tendency was observed for the parameter M2 families with multiple visible mutations, for which the highest frequency was registered when plants were exposed for 2 h to ENU or MNU.

Two-hour exposure also resulted in the widest mutation spectra induced by both chemical mutagens. Despite the apparently wider mutation spectrum induced by ENU in cv. Solara, the comparison of the efficiency of ENU (10 mutation types) versus MNU (4 mutation types) concerning this parameter should bear in mind the lower number of M2 families analysed for the last mutagen (Table 2).

Influence of the cell cycle phase in chemical mutagenesis efficiency

Taking into account the results of the first series of experiments and in order to ensure acute mutagenic treatments within the limits of a cell cycle phase, particularly during the shorter Mitosis (M) phase, all the mutagenic treatments were carried out for 1 h with the less deleterious alkylating agent ethylnitrosourea (ENU). For easy analysis most of the results of these experiments are compiled in Table 3. Mutagenic treatments were performed at the time when most of the shoot apical meristem cells were in S, G2, M or G1 cell cycle phases (Figs. 1, 2; Leitão et al. 1986).

Despite previous treatment with hydroxyurea for 24 h, the survival rate of the M1 generation was very high, about 100%, independently of the cell cycle phase of the cells at the moment of treatment. Full sterile plants were almost no noticeable—only 2.9% for seedlings treated at the G2 phase.

The high efficiency of using seedlings instead of seeds in chemical mutagenic treatments, confirmed in the above described first series of experiments, was reconfirmed in this second series of assays. The very low lethality and low sterility in the M1 generation was followed by a very high visible mutation rate, which depending on the phase of the cell cycle varied from 27 to 58% mutated M2 families.

In spite of the preliminary character of these experiments the results point out a role of the cell cycle phase on the ultimate mutational effect, not only because of the differences in mutation rate in different cell cycle phases, but also because the shape of the curve this rate describes along the cell cycle. Beginning at a lower level (40%) 2 h after the release of the DNA synthesis inhibitor (S phase), the mutation rate attains a peak (58–53%) at the G2 and M phases, decaying to approximately one half of this value some hours later when treatments were carried out at the G1 phase. A different shape of this curve, namely a constant increase or decrease of the mutation rate would lead us to assume the continuously increasing

Table 2 Induced mutation spectra

Mutation type	ENU cv. Solara				MNU cv. Solara				Cell cycle phases (ENU) cv. Frilene				
	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h	H_{yd}	S	G2	M	G1
Albina	✔	✔	✔	✔		✔				✔	✔		✔
Costata				✔									
Chlorina		✔	✔	✔	✔	✔				✔	✔	✔	✔
Chlorotica	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Xantha	✔	✔								✔		✔	✔
Incerata	✔	✔	✔	✔			✔	✔		✔	✔	✔	
Long internodes		✔	✔								✔	✔	✔
Short internodes	✔	✔			✔					✔		✔	✔
Acacia leaves													✔
Dwarf plant		✔		✔									
Fasciata						✔							
Increased number of pods		✔					✔						
Increased number of reproductive nodes											✔		
Abnormal flowers											✔		
PM Resistance		✔									✔		

Table 3 ENU treatments of cv. Frilene seedlings with synchronised shoot apical meristem cells

Treatment	M1		M2		
	Survival (%)	Sterility (%)	Families (N)	Families with mutations (%)	Families with multiple mutations (%)
Control (H$_2$O)	100	0.0	70	0.0	0.0
Hydroxyurea	100	0.0	70	5.7	0.0
S phase	100	0.0	75	40.0	6.7
G2 Phase	100	2.9	79	57.0	15.2
M Phase	94	0.0	80	53.8	11.3
G1 Phase	100	0.0	85	27.1	1.2

or decreasing effect of other factor(s), e.g. the preceding hydroxyurea treatment.

Despite the higher number of different mutations induced when treatments were performed at the G2 phase, the wideness of the mutation spectrum did not show a drastic variation among the cell cycle phases (Table 2).

Powdery mildew resistant (PMR) mutations

Several mutants exhibiting new traits of agronomical interest, e.g. higher number of productive nodes, erected plants with higher number of pods, short-internodes, *fasciata*, etc., were confirmed in later

generations and included in the pea breeding program of the INRB, Elvas.

The use of the mutagenic agent ethylnitrosourea (ENU) was particularly successful in the induction of mutants of agronomical interest. Two hours treatment of cv. Solara and 1 h treatment of cv. Frilene (at the cell cycle phase G2) with ENU resulted, for the first time in *P. sativum* L., in the induction of powdery mildew resistant (PMR) mutants, one mutant in each one of these two commercial varieties.

While the PMR mutant of the cv Frilene is phenotypically very similar to the original cultivar, the PMR mutant induced in cv. Solara, differentiates clearly from the original variety exhibiting a *ramosus*

habit (3–4 basal branches vs. 1–2 branches in the original variety), longer period to flowering, less seeds per pod and smaller seeds than the original cultivar.

Being these the first powdery mildew resistant mutants induced via experimental mutagenesis in *P. sativum* L., several questions raised up that needed to be answered: (i) was the inheritance of these mutations recessive, as suggested by their emergence as single plants among the M2 progenies?; (ii) were the resistances monogenic?; (iii) were the resistant mutations located in the same locus or in different loci?; (iv) were these loci linked or did they segregate independently?; (v) what was the relationship between these mutated genes and the natural sources of resistance known so far?

The crosses displayed in Tables 4 and 5 were performed to answer to these questions.

The cross of the PMR mutant lines with the respective original susceptible cultivar or with the original susceptible cultivar of the other mutant resistant line resulted in totally susceptible F1 populations confirming the recessiveness of both mutations.

The F2 progenies of the crosses between the PMR Frilene-mutant and the original cv. Frilene or cv. Solara segregated according to a very clear 3:1 (susceptible:resistant) mendelian ratio, while the F2 of both crosses involving the Solara-mutant line showed some segregation distortion, however not enough to discard the 3:1 segregation ratio hypothesis confirming that both induced powdery mildew resistances exhibit monogenic recessive inheritance (Tables 4, 5).

The full resistance observed in the F1 and F2 progenies of the genetic complementation (resistant × resistant) crosses performed between both resistant mutant lines and between them and the resistant E835 line carrying the PMR gene *er*1 (Table 4) clearly indicates that: (i) the two PMR mutations have affected one and same locus; and (ii) the mutated locus is *er1* (from Mexique 4) which confers PMR to line E835.

Taking into consideration the above results and the order of their induction the PMR mutant alleles were named *er1mut1* and *er1mut2*, and the respective mutant lines carrying these alleles S(*er1mut*) and F(*er1mut2*), respectively.

Due to its best agronomical performance line F(*er1mut2*) was selected for further research work. This mutant line was crossed again to cv. Solara and large mapping populations useful for the map based cloning of the mutated resistant gene were generated (Table 5).

Table 4 Genetic analysis of induced powdery mildew resistant mutations

Type of cross	Parental lines	Host–pathogen interaction	
		F$_1$	F$_2$
Susceptible × resistant	Frilene × F(*er1mut2*)	Susceptible	Susceptible:Resistant (3:1)
	Solara × F(*er1mut2*)		
	Solara × S(*er1mut1*)		
	S(*er1mut1*) × Frilene		
Resistant × resistant	S(*er1mut1*) × F(*er1mut2*)	Resistant	Resistant
	S(*er1mut1*) × E835 (*er1er1*)		
	F(*er1mut2*) × E835 (*er1er1*)		

Table 5 F2 segregation of induced powdery mildew resistant mutations

Cross	Susceptible plants	Resistant plants	Observed ratio	Expected ratio	χ^2	*P*
Solara × S(*er1mut1*)	55	11	5:1	3:1	2.00	0.10–0.25
S(*er1mut1*) × Frilene	67	13	5:1	3:1	3.52	0.05–0.10
Frilene × F(*er1mut2*)	187	59	3:1	3:1	0.09	0.90–0.95
Solara × F(*er1mut2*)	438	131	3:1	3:1	1.08	0.25–0.50

Discussion

The technique we have implemented, treating seedlings for short periods (1–2 h) instead of soaking dry seeds for long periods of 6 h and over, into chemical mutagens solutions, resulted in very low lethality and sterility among the M1 generation and very high mutation rates in the M2 generation, confirming a previous assay of this technique (Leitão et al. 1987). This procedure that clearly increases the efficiency of the chemical mutagenesis can be used in most plant species, particularly in leguminous plants, as confirmed by some preliminary assays we have performed in *Lathyrus* sp. (results not shown).

Beyond the interest that the synchronization of shoot apical meristem cells (Leitão et al. 1986) could represent for fundamental cell cycle studies, this technique can also be applied to increase mutation rates and mutagenic efficiency as indicated by the results of our assays.

Although the first powdery mildew resistant mutant was induced in cv. Solara in 1997 one year before the induction of the second mutant in cv. Frilene, our further research efforts have been focused on the second mutant which does not differentiate morphologically from the original variety, except for the powdery mildew resistance.

The recessive broad-range PMR conferred by the mutant allele *er1mut2* was reconfirmed during the last years in crosses with the cv. Solara (results pooled in Table 5), which progenies are being used in a program aimed at the map based cloning of the mutated gene (Pereira et al. 2009).

The observation of the host–pathogen interaction phenotype of powdery mildews susceptible and resistant (carrying the allele *er1mut2*) plants, led us to come to conclusions very similar to those presented by Sharma (2003) concerning other powdery mildew resistance sources. Under the field conditions at the University of Algarve, the susceptible plants became totally covered by the whitish-grey mycelium and spores while the resistant plants remain almost immune to the fungus (Fig. 3).

In some years, usually at the end of the vegetation period, there are some resistant plants that show some patches of powdery mildew on the lower foliage, which can suggest some kind of quantitative expression of the resistance. However, this quantitative

Fig. 3 **a** The PMR line F(*er1mut2*) at the top is growing side by side with cv. Solara (*at the bottom of the picture*). **b** Absolutely healthy pods of a powdery mildew resistant plant F(*er1mut2*) and pods of a plant of the original cv. Frilene

expression is illusory and a simple, but attentive, analysis of each plant will allow its qualitative classification in one of two clearly distinguishable classes: resistant versus susceptible.

A much more obvious qualitative expression of the resistance can be observed at the very end of the vegetation period. At that time, disease infected tissues turn black and susceptible plants get a burnt aspect exhibiting strongly contaminated pods and seeds, often not viable, while resistant plants show no marks or scars of the infection and exhibit absolutely healthy pods and seeds (Fig. 3). On other words, powdery mildew resistance evaluations performed during vegetation on the basis of leaf contamination can be undoubtfully confirmed at harvest, on the basis of overall plant appearance and pods and seeds infection. In this regard it is worthy of mention that based on multiple experiments with PMR lines Sharma (2003) arrived to the conclusion that resistant and susceptible plants can be qualitatively distinguished on the basis of infection of the stem, peduncles and pods, ignoring the fungal growth, whatever its intensity, on the foliage.

The induction of two mutations conferring powdery mildew resistance constitutes a new development in the study of resistance to this disease in *P. sativum* L. Nevertheless, some facts deserve reflection and further research.

The first fact is that, the number of analysed M2 families was relatively low. This lead to questioning why no other powdery mildew resistance mutants have been reported so far, since multiple programs on experimental mutagenesis involving large numbers of M2 families, often bulked, have been carried out on *P. sativum* L.

A second fact is that the mutants are two, obtained in two independent experiments in two different cultivars, a double event with a very low probability of occurrence.

The third fact is that both mutations have affected the locus *er1* that determines most of the natural occurring resistances against powdery mildew. Although mutations are expected to occur more often in the locus *er1* than in locus *er2* (natural mutations in locus *er2* seem to be much less frequent), it remains hard to explain the preferential access of ENU to the *er1* locus.

We can speculate that the technique we used for the chemical treatments could facilitate the accessibility of the mutagens to the shoot apical meristem cells

(and to their genomes), and that specific genomic regions could became more or less efficiently targeted due to different chromatin conformations related to specific gene expression in specific developmental moments. In this regard it is interesting to point out that the highest mutation rates were observed when cell were at G2 and M cell cycle phases when chromatin is more compact and DNA is supposed to be less accessible to any chemical compound, suggesting that other important factors like DNA repair could play a major role in the ultimate mutational event. Nevertheless, these and other issues need further research and clarification.

It should be also noted that the induction of two independent mutations in the locus *er1* suggests that the endogenous chances for powdery mildew resistance in *P. sativum* are very slim, apparently restricted to naturally occurring or induced mutations in one single locus (*er*1) or two loci (*er*1 and *er*2), a constraint that concerns pea powdery mildew researchers (Liu et al. 2003).

Aiming at achieving a better understanding of the molecular mechanisms of the recessive powdery mildew resistance in pea, we have been performing molecular markers analyses and the assessment of plant–pathogen interaction phenotypes among large F2 (and F2:3) progenies of the crosses F(*er1mut2*) × Solara and more recently F(*er1mut2*) × line Gp3257 (*P. sativum* var *arvense* L.) aiming at the isolation of the *er1mut2* mutated gene via map based cloning (out of the scope of this article). Molecular markers identified in close vicinity to the resistant locus will be also useful for marker assisted selection in plant breeding programs using the *er1mut2* (mutated) gene to obtain new powdery mildew resistant cultivars.

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