RESEARCH ARTICLE

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Sensitivity of two garden pea genotypes to physical and chemical mutagens

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ABSTRACT

A study on the sensitivity of two breeding lines of garden pea to mutagenic agents was carried out in the Maritsa Vegetable Crops Research Institute, Plovdiv. The purpose was to evaluate the sensitivity of the *Pisum sativum* L. genotypes to physical and chemical mutagens. In the experiment, the pea seeds were irradiated single or combined with ^{60}Co gamma rays (40, 80, 100, 200 and 400 Gy) and Ethyl methanesulfonate (EMS) at concentrations of 0.1 and 0.2 %. Visible morphological changes of the stems and leaves were observed in plant of M_1 generation, such as shorter stems, double petiole, clover-shaped leaves and a couple of tendrils. Single treatments with 100 Gy ^{60}Co induced the highest mutation frequency M.F. = 7.69 for line 88-7, and M.F. = 2.11 for line 97-3, reported in M_2 generation. Higher doses or combined gamma rays and EMS treatments induced more efficiently mutations. Line 88-7 was selected for further mutagenic treatment due to the higher sensitivity assessed.

Key words: gamma rays, EMS, induced mutagenesis, *Pisum sativum* L.

Introduction

Breeding programmes established based on the variability induced by mutagenic treatment are commonly used for improvement of the most productive and well-adapted varieties. The practical use of these programmes is closely associated to the genetic structure and reproductive systems of different plant species (Oldach, 2011; Wani et al., 2014).

Pea is a self-pollinated diploid (2n = 14) annual crop in which the initial lines for mutagenesis are mostly homogenous. The recessive induced mutations can be detected in the segregating M_2 and/or M_3 generations, and it is easy to maintain their homozygous state (Oldach, 2011). An additional advantage of the species for mutagenesis is that it possesses a small number of chromosomes (x=7). Polyploids are absent in the genus *Pisum*. Therefore, pea is a widely used object for genetic and breeding research and studies on experimental mutagenesis (Sidorova, 1981).

The major pea mutant collections include John Innes Collection, Norwich, UK (575 accessions); Plovdiv, Bulgaria (122 accessions); a targeted-induced local lesions in genomes (TILLING) at INRA, Dijon, France (population of 4817 lines) (Smykal, 2014).

The database established by the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) in Vienna, Austria (IAEA, 2011) contains information about 446 mutant varieties from 21 food legume species, which have been officially released between 1950 and 2010 (at least 33 mutant varieties have been registered in pea) (Materne et al., 2011; Oldach, 2011).

In Bulgaria, a great variability of new germplasm in field pea and garden pea was developed through induced mutagenesis and sexual hybridization (Mehandjiev et al., 2002; Mehandjiev et al., 2003; Tomlekova, 2010). Several mutants with improved characters including increased yield, lodging resistance, larger seeds, increased protein concentration, modified maturity and tolerance to economically important diseases were released (Noneva & Mehandjiev, 2005; Kosev, 2012). The newest garden pea variety "Sredets" was developed by combined treatment with gamma rays and EMS (Mehandjiev et al., 2002).

The aim of this study was to evaluate the sensitivity of two homogenous lines of garden pea to mutant agents ^{60}Co and EMS. The generated mutant populations will further contribute to create variability for breeding by induced mutagenesis and to enrich the gene pool of *Pisum sativum* L.

Materials and Methods

The study was conducted in the experimental fields of the Maritza Vegetable Crops Research Institute – Plovdiv, Bulgaria during the period 2013-2015.

Plant material

The mutagenic effect was investigated in two homogeneous lines: 88-7 – with early maturity and line 97-3 – late maturity. From each variant of mutagen treatment 100 dry seeds per genotype were used.

Methods

Two mutagens were used in the experiments: a chemical mutagen - Ethyl methanesulphonate (EMS) (Sigma-Aldrich, Cat. M0880), and a physical mutagen - gamma rays 60Co. The treatment variants applied for the comparison were:

Control – 10 % of the total number of seeds in the experiment served as control. The seeds used for control were soaked for 20 hours in dH2O simultaneously with the EMS-treated seeds.

The lines 88-7 and 97-3 were treated separately with EMS (after being metabolized for 16 h 30 min., seeds were treated with EMS for 3 h 30 min.) or ^{60}Co, and combined - with EMS and gamma rays at the following concentrations/doses variants:

EMS	Gy^{60}Co	EMS + Gy^{60}Co
0.1%EMS	40 Gy^{60}Co	0.1%EMS+40 Gy^{60}Co
0.2%EMS	80 Gy^{60}Co	0.1%EMS+80 Gy^{60}Co
	100 Gy^{60}Co	0.2%EMS+40 Gy^{60}Co
	200 Gy^{60}Co	0.2%EMS+80 Gy^{60}Co
	400 Gy^{60}Co	

Sowing was immediately after mutagenic treatment in a cultivated plot under field conditions together with the control. Seeds were sown in a high four-bed scheme 80 + 20 + 40 + 20 cm / 4-5 cm.

Morphological alterations were observed in M_1 and M_2 mutant generations. All plants with deviations from the characteristic phenotype were marked and the inheritance of the occurred changes was followed in M_3.

Analyzed characters / parameters

Field germination, (%) - 20 days after sowing of all variants, relative to the control;

$$Survival\ (S)\ in\ M1, (\%) = \frac{A}{C} \times 100 \quad (1)$$

where "A" = the number of harvested plants, "C" = the number of seedlings;

$$Mutation\ Frequency\ (M.F.), (\%) = \frac{M}{N} \times 100 \quad (2)$$

where "M" = the number of mutants, "N" = total number of M_2 plants (% of mutations in M_2) (Khursheed and Khan, 2016).

$$Efficiency\ factor\ (C.F.), (\%) = \frac{S \times M.F.}{100} \quad (3)$$

of the mutagenic doses used (Walther, 1969).

- Coefficient of interaction

$$(\boldsymbol{k}) = \frac{(\boldsymbol{a}+\boldsymbol{b})}{(\boldsymbol{a})+(\boldsymbol{b})} \quad (4)$$

in which k = Hypothetical interaction coefficient; (a+b) - the mutation frequency induced by the two mutagens in combined treatments; (a) + (b) - the mutation frequencies induced by the two mutagens when applied alone. The value of k should be one if the interaction is additive. Any deviation from this value would show synergistic or less than additive effects (Khursheed and Khan, 2016).

- Morphological changes.

Results

Visible morphological changes of the stems and leaves were observed in plants of M_1 generation shorter stems, double petiole, clover shape leaves, and a couple of tendrils (Figure 1). The listed M_1 mutations were not confirmed in next mutant generations due to the fact that the plants were sterile and seeds were not collected.

Figure 1. *Mutant plant with a couple of tendrils induced from from the parental line 97-3.*

Induced morphological and physiological mutations were determined in M2 and their inheritance was confirmed in M3. The treatments with 0.1 % EMS single or combined with 40 Gy gamma rays in both lines induced to increasing of the plant high and growth period (Table 1). Possibly the chemical mutagen (in dose 0.1 %) exerted stimulating effect on the phenotype expressions of mentioned parameters. The height of plants, period of growing and weight of 1000 seed were significantly lower compared to the control after treatment with 0.2 % EMS and 40, 100 and 200 Gy 60Co. Mehandjiev et al. (2001) and Khursheed & Khan (2016) described many more mutations induced by the combined treatment compared to the single treatment. The results of the current experiment are shown in Table 2. Line 97-3 with late maturity showed higher field germination - 5.32 % in the treatment with 100

Table 1. *Spectrum of induced mutations after single and combined application of gamma rays and EMS in pea for concrete doses and combinations.*

Treatment	Height of plant, cm / %	Weight of 1000 seed, g / %	Growth period, days / %
LINE 97-3			
Control	66 / 100.00	224 / 100.00	63 / 100.00
0.1 % EMS	-	-	66 / 104.76
40 Gy	61 / 92.42	198 / 88.39	-
100 Gy	-	-	58 / 92.06
LINE 88-7			
Control	67 / 100.00	173 /100.00	61 / 100.00
0.1 % EMS	72 / 107.46	-	-
0.2 % EMS	61 / 91.04	-	-
40 Gy + 0.1 %EMS	68 / 101.49	-	63 / 103.28
100 Gy	-	-	56 / 91.80
200 Gy	-	-	58 / 95.08

Table 2. *Frequency of induced mutations after treatment of seeds of garden pea lines with gamma rays and EMS.*

Treatment	Number of sown seeds	Field germination in M_1, %	Survival of M_1 plants per number of seedlings, %	Mutation Frequency (M.F.) in M_2, %
LINE 97-3				
Control	100	94.00	97.87	
0.1 % EMS	100	42.55	*92.50	0.39
0.2 % EMS	100	46.81	88.64	
40 Gy +0.1 % EMS	100	42.55	90.00	
40 Gy +0.2 % EMS	100	56.38	94.34	
80 Gy +0.1 % EMS	100	43.62	87.81	
80 Gy +0.2 % EMS	100	34.04	93.75	
40 Gy	100	10.64	80.00	1.84
80 Gy	100	11.70	72.73	
100 Gy	100	5.32	100.00	2.11
200 Gy	100	0	0	
400 Gy	100	0	0	
LINE 88-7				
Control	100	83.00	93.98	
0.1 % EMS	100	15.66	92.31	0.62
0.2 % EMS	100	14.46	100.00	0.69
40 Gy +0.1 % EMS	100	12.05	90.00	0.95
40 Gy +0.2 % EMS	100	28.92	83.33	
80 Gy +0.1 % EMS	100	13.25	72.73	
80 Gy +0.2 % EMS	100	18.07	93.33	
40 Gy	100	4.88	75.00	
80 Gy	100	1.20	100.00	
100 Gy	100	2.41	50.00	7.69
200 Gy	100	1.20	100.00	2.94
400 Gy	100	1.20	100.00	

Gy to 56.38% after the combined treatment with 40 Gy + 0.2 % EMS compared to the control.

Germination of the seeds irradiated with 200 and 400 Gy was not observed. The line was characterized by a high percentage of survived plants from 72.73 % after treatment with 80 Gy to 100 % with 100 Gy gamma rays. Mutation frequency per doses and combinations used was the highest (2.11 % and 1.84 %) after irradiation treatment with 100 Gy and 40 Gy 60Co, respectively, and the lowest after 0.1 % EMS treatment. Mutations were not reported after the rest of the doses and combinations, therefore mutation frequency was not calculated.

In the early line 88-7 the field germination was low, and ranged from 1.20 % using 80, 200 and 400 Gy to 28.92 % in combined treatment with 40 Gy + 0.2 % EMS, but germinated plants were characterized by a high percent of survival (Table 2). The observed mutations in M2 were obtained after individual treatment with EMS at concentrations 0.1 % and 0.2 %, after gamma-rays at 100 and 200 Gy doses of treatments, and after combined treatment 40 Gy 60Co + 0.1 % EMS.

The highest percentage of field germination in both lines was established after combined treatment with gamma rays 40 Gy and 0.2 % EMS. Mutations in both lines were found in the variants of treatments with 0.1 % EMS and 100 Gy 60Co.

Data on the effectiveness of the single and combined mutagens calculated in M2 showed the highest efficiency factor (C.F.) 3.85 % for line 88-7 achieved by 100 Gy gamma-rays and 2.94 % by 200 Gy (Figure 2). C.F. of line 97-3 was 2.11 % estimated for 100 Gy and 1.47 % by 40 Gy gamma rays irradiation. The combined mutagen effect was detected only in the variant of treatment with 40 Gy 60Co and 0.1 % EMS in which 0.95 % mutation frequency and efficiency factor of 0.86 % was registered, and the hypothetical interaction coefficient was k = 1.50 %.

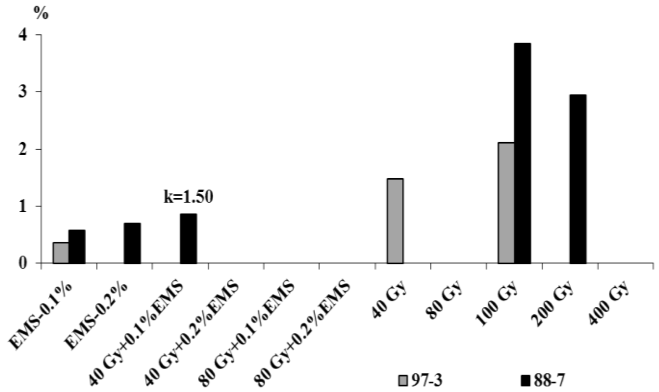

Figure 2. *Efficiency factor (C.F.) of the used doses and concentrations of mutagen agents of lines 97-3 and 88-7, (%); k = hypothetical interaction coefficient.*

Discussion

Based on the reported field germination no significant dependence was found between concentrations used for chemical mutagenesis. The two applied concentrations were low for the selected two genotypes.

A lower germination of line 88-7 was reported in the combined treatment than the germination of line 93-7. The field germination decreased with the increase of the dose of the gamma-rays treatment. Dhulgande et al. (2011) noted that the genotypes respond differently to the applied mutagens and doses.

The number of germinated seeds after chemical and combined treatment in the two different genotypes was the same. For combined treatment, low doses of physical mutagen were used.

Strong dependence between mutagens, doses/ concentrations was not detected for the parameter survived plants for the studied genotypes. Plant survival of 100 % was reported only when the number of seedling was low. For most food legumes, radiation dose varies between 100 Gy and 250 Gy for gamma rays and 0.08 %, 0.16 % and 0.32 % for EMS (Oldach, 2011).

The early maturing line 88-7 was characterized by higher sensitivity (at a lower field germination and higher mutation frequency) compared with the late maturing line 97-3.

In the present investigation mutations were detected and mutation frequency calculated in EMS used alone as a mutagen, in the experiment of the combined mutagens treatment, and in some of the doses of the physical mutagen. Kosev (2012) reported similar mutation frequency (0.31 % – 1.42 %) in four field pea varieties treated with different doses of physical mutagen – 40, 80, 120 Gy gamma rays. The same author reported similar efficiency coefficients to the obtained in this study on pea plants treated with different doses of physical mutagen – gamma-rays.

The combined treatment efficiency established in the present study was lower than the efficiency coefficient reported by Mehandjiev et al. (2001) after the same mutagens and treatments of pea seeds.

The coefficient of interaction was calculated only for the variant of treatment 40 Gy + 0.1 % EMS in which mutations were assessed. The hypothetical interaction coefficient (k) showed that the combined mutagen effect may have important advantage together with single treatment due to possible hyper-additive mutation effects may occur (Mehandjiev et al., 2001; Khursheed and Khan, 2016). When the value of k is 1, therefore the observed effect of the coefficient of interaction is additive. The established deviation of this value in our investigation showed a synergistic effect as it was also stated by Khursheed and Khan in 2016.

Conclusions

Sensitivity of garden pea lines to single or combined treatment with physical (gamma rays) and chemical (EMS) mutagens was established. Line 88-7 was selected for further mutagenic treatment due to the higher sensitivity assessed.

Single treatments with gamma rays induced higher mutation frequency of both investigated lines followed by individual treatments with Ethyl methanesulfonate.

Combined treatments of gamma rays and Ethyl methanesulfonate can lead to a synergistic effect of occurrence of mutations.

The study is a prerequisite for increasing the genetic variability in garden pea. This is of substantial importance in mutation genetics and breeding and will further contribute in the initiated breeding programme.

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