ORIGINAL PAPER

INDUCTION OF CHLOROPHYLL MUTANTS IN COMMON BEAN UNDER THE ACTION OF CHEMICAL MUTAGENS ENU AND EMS

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ABSTRACT

Effect of treatment with different concentrations of N-nitroso-N-ethyl urea (ENU) and etylmethan sulfonate (EMS) on seeds of Bulgarian common bean Dobroudjanski 7, Dobroudjanski 2, Plovdiv 10, Plovdiv 11M and snap bean Tcher Starozagorski varieties, for induction of chlorophyll mutants, was studied. It was established that investigated varieties manifested specific reactions to the treatment with ENU and EMS. Different mutation frequencies and width of mutation spectra were induced under the action of different concentrations of the two applied mutagens. ENU induced chlorophyll mutants with higher frequency in all studied varieties, in comparison to the action of EMS. Sixteen types of chlorophyll mutants were found, for all studied varieties, and mutagenic treatments. Mutant types chlorina (19,8%), xantha (19,3%), viridissima (15,4%) and chimerical leaves (9,1%) were with the highest frequency, comparing to the total number of observed mutants. Results were statistically elaborated by the Fisher's method "φ".

Key words: Mutagens, ENU, EMS, chlorophyll mutants, Phaseolus vulgaris L.

DETAILED ABSTRACT

Common bean is a traditional legume crop in Bulgaria utilized for dry seeds and green pods. The breeding process of common bean in Bulgaria started at the beginning of 20th century.

Breeders use experimental mutagenesis for creation of new varieties of agricultural crops and for obtaining of bigger genetic diversity. Chlorophyll mutants can be used as tests for evaluation of genetic action of mutagenic factors and they are the most frequently observed.

Effect of treatment with different concentrations of N-nitroso-N-ethyl urea (ENU) and etylmethan sulfonate (EMS) on seeds of Bulgarian common bean Dobroudjanski 7, Dobroudjanski 2, Plovdiv 10, Plovdiv 11M and snap bean Tcher Starozagorski varieties, for induction of chlorophyll mutants, was studied and presented in our publication. Mutagens were applied in concentrations as followed: ENU ⇒ $6{,}2 \cdot 10^{-3}$; $3{,}1 \cdot 10^{-3}$; $1{,}55 \cdot 10^{-3}$ M; EMS ⇒ $2{,}5 \cdot 10^{-2}$; $1{,}25 \cdot 10^{-2}$; $6{,}2 \cdot 10^{-3}$ M. Chlorophyll mutants were determined and grouped by their type in M_2-generation using classifications of Lamprecht (2) and Priilin et al. (4). Gaul's method (1) – determination of mutant's number on the basis of 100 M_2 plants was used for determination of mutation frequency. Results were statistically elaborated by the Fisher's method "φ".

It was established that investigated varieties manifested specific reactions to the treatment with ENU and EMS. Different mutation frequencies and width of mutation spectra were induced under the action of different concentrations of the two applied mutagens. ENU induced chlorophyll mutants with higher frequency in all studied varieties, in comparison to the action of EMS, when both mutagens were applied in concentrations induced the same level of plant lethality. Sixteen types of chlorophyll mutants were found, for all studied varieties, and mutagenic treatments. Mutant types chlorina (19,8%), xantha (19,3%), viridissima (15,4%) and chimerical leaves (9,1%) were with the highest frequency, comparing to the total number of observed mutants.

INTRODUCTION

Chlorophyll mutants are used as markers in genetics, physiological and biochemical investigations. Chlorophyll formation in plants is the last result from a long chain of biochemical processes where are involved a lot of loci.

According to Von Wettstein et al. (7) nuclear genes control the biogenesis of plastids. Those authors found that the chlorophyll synthesis in higher plants is realized under the control of nuclear genes by the products of regulatory genes.

Other scientists (6) assert that the chlorophyll synthesis is under the control of nuclear and out-nuclear (cytoplasmic) genes. A lot of chlorophyll mutants who are nuclear controlled showed recessive monogenic inherit.

Mutations in nuclear genes can change genetic resistance of out-nuclear DNA, localized in chloroplasts or in mitochondria.

Chlorophyll mutants are used as tests for evaluation of genetic action of mutagenic factors (1, 5). They are the most frequently observed and can be easily identified factorial mutations in M_2-generation yet.

The aim of the work was to study the effect of N-nitroso-N-ethyl urea (ENU) and etylmethan sulfonate (EMS) on induction of different types chlorophyll mutants and to determine their frequency in five Bulgarian common bean varieties.

MATERIAL AND METHODS

Five-years investigations were carry out with Bulgarian common bean – Dobroudjanski 7, Dobroudjanski 2, Plovdiv 10, Plovdiv 11M and snap bean Tcher Starozagorski varieties. Mutagens were dissolved in buffer solutions with pH 6,0 - for ENU and pH 7,0 - for EMS. They were applied in the next concentrations: ENU ⇒ $6{,}2 \cdot 10^{-3}$; $3{,}1 \cdot 10^{-3}$; $1{,}55 \cdot 10^{-3}$ M; EMS ⇒ $2{,}5 \cdot 10^{-2}$; $1{,}25 \cdot 10^{-2}$; $6{,}2 \cdot 10^{-3}$ M. Control treatments were with buffers pH 6,0 and pH 7,0. Small cuts, with diameter 1 mm, were made on the seed coats and seeds were socked in buffers and mutagenic solutions for 8 hours. Then they were washed well under the tip-water and sowed in the field on the next day.

Chlorophyll mutants were determined and grouped by their type in M_2-generation using classifications of Lamprecht (2) and Priilin et al. (4). Gaul's method (1) – determination of mutant's number on the basis of 100 M_2 plants was used for determination of mutation frequency. Obtained results were statistically elaborated by the method "φ" of Fisher (3).

Inherit of changed traits which appeared in M_2 was verify in M_3-generation and plants with modifications were not used in the next experiments.

RESULTS AND DISCUSSION

Varieties included in our investigations were differed by frequency and spectra of induced chlorophyll mutations (Table 1). Varieties Plovdiv 11 M and Dobroudjanski 2 were with the highest frequency and the most width spectra of induced mutants. The lowest frequency and the narrow spectra of chlorophyll mutants were induced on the plants of the variety Tcher Starozagorski.

Table 1: Frequency and spectrum of induced chlorophyll mutants, confirmed in M_3 generation (average of five years investigations on the same generation in every year).

Treatments	Total number of plants in M_2	Number of mutant types	Total number of mutants	Mutation frequency, in % to the total number of plants in M_2 generation
Variety Dobroudjanski 7				
Control pH 6,0	1026	-	-	-
$6,2 \cdot 10^{-3}$ M ENU	1050	11	50	4,76 ± 0,66 This is the mean errore of data
$3,1 \cdot 10^{-3}$ M ENU	911	10	43	4,72 ± 0,70
$1,55 \cdot 10^{-3}$ M ENU	1258	8	25	1,99 ± 0,39
Control pH 7,0	2250	-	-	-
$2,5 \cdot 10^{-2}$ M EMS	1298	11	46	3,54 ± 0,51
$1,25 \cdot 10^{-2}$ M EMS	1194	9	38	3,18 ± 0,51
$6,2 \cdot 10^{-3}$ M EMS	2231	8	34	1,52 ± 0,26
Variety Dobroudjanski 2				
Control pH 6,0	1044	1	2	0,19 ± 0,13
$6,2 \cdot 10^{-3}$ M ENU	1132	14	66	5,83 ± 0,70
$3,1 \cdot 10^{-3}$ M ENU	1268	12	63	4,97 ± 0,61
$1,55 \cdot 10^{-3}$ M ENU	1200	10	28	2,33 ± 0,43
Control pH 7,0	1295	1	3	0,23 ± 0,13
$2,5 \cdot 10^{-2}$ M EMS	1136	12	56	4,93 ± 0,64
$1,25 \cdot 10^{-2}$ M EMS	1277	11	49	3,84 ± 0,54
$6,2 \cdot 10^{-3}$ M EMS	1224	9	25	2,04 ± 0,40
Variety Plovdiv 11 M				
Control pH 6,0	1243	1	3	0,24 ± 0,14
$6,2 \cdot 10^{-3}$ M ENU	1226	16	91	7,42 ± 0,75
$3,1 \cdot 10^{-3}$ M ENU	1210	13	77	6,36 ± 0,70
$1,55 \cdot 10^{-3}$ M ENU	1265	12	54	4,27 ± 0,57
Control pH 7,0	1112	1	3	0,27 ± 0,15
$2,5 \cdot 10^{-2}$ M EMS	1207	14	74	6,13 ± 0,69
$1,25 \cdot 10^{-2}$ M EMS	1212	13	60	4,95 ± 0,62
$6,2 \cdot 10^{-3}$ M EMS	1224	11	38	3,10 ± 0,49
Variety Plovdiv 10				
Control pH 6,0	1134	1	1	0,09 ± 0,09
$6,2 \cdot 10^{-3}$ M ENU	1084	7	43	3,97 ± 0,59
$3,1 \cdot 10^{-3}$ M ENU	1165	9	48	4,12 ± 0,58
$1,55 \cdot 10^{-3}$ M ENU	1234	8	31	2,51 ± 0,44
Control pH 7,0	1275	-	-	-
$2,5 \cdot 10^{-2}$ M EMS	1228	8	36	2,93 ± 0,48
$1,25 \cdot 10^{-2}$ M EMS	1152	8	29	2,52 ± 0,46
$6,2 \cdot 10^{-3}$ M EMS	1018	4	14	1,37 ± 0,36
Variety Tcher Starozagorski				
Control pH 6,0	1214	-	-	-
$6,2 \cdot 10^{-3}$ M ENU	1032	6	38	3,68 ± 0,59
$3,1 \cdot 10^{-3}$ M ENU	1300	8	51	3,92 ± 0,54
$1,55 \cdot 10^{-3}$ M ENU	1226	6	16	1,30 ± 0,32
Control pH 7,0	1249	-	-	-
$2,5 \cdot 10^{-2}$ M EMS	1001	5	17	1,70 ± 0,41
$1,25 \cdot 10^{-2}$ M EMS	1124	6	24	2,13 ± 0,43
$6,2 \cdot 10^{-3}$ M EMS	1209	6	15	1,24 ± 0,32

Effect of applied mutagenic's concentrations on the frequency and spectra of induced mutants were found for the varieties Dobroudjanski 7, Dobroudjanski 2 and Plovdiv 11 M. For those varieties, the frequency of chlorophyll mutants increased and spectra extended with increasing of applied mutagenic concentrations. Such tendency was not found for varieties Plovdiv 10 and Tcher Starozagorski.

In all studied varieties, ENU induced chlorophyll mutants with higher frequency and more width spectra, only for varieties Dobroudjanski 2 and Plovdiv 11M, in comparison to the action of EMS.

Mutant's types chlorina and xanthoviridoterminalis are presented in figure 1.

Mutants from the types albina (varieties Dobroudjanski 2 and Plovdiv 11M), chlorina (var. Dobroudjanski 2), viridocostata (var. Plovdiv 11M) and xanthomarginata (var. Plovdiv 10) were found in control treatments of some of the studied varieties presented in parenthesis.

The more width spectra of chlorophyll mutants were obtained applying treatment with $3{,}1 \cdot 10^{-3}$ M ENU on varieties Plovdiv 10 and Tcher Starozagorski. Spectra of induced mutants in the same two varieties differed when EMS and concentrations $6{,}2 \cdot 10^{-3}$ and $1{,}55 \cdot 10^{-3}$M of ENU were applied.

Data for induced chlorophyll mutants in all varieties and mutagenic treatments were statistically elaborated by Fisher's "φ" criterion at the highest level of significance ($P > 0{,}01\%$), (Table 2).

It was found, from conducted investigations, that totally sixteen types of chlorophyll mutants were induced for all studied varieties and mutagenic treatments. With the highest frequency to the total number of chlorophyll mutants were the types chlorina (19,8%), xantha (19,3%), viridissima (15,4%) and chimeric leaves (9,1%).

Chlorophyll mutant type's frequencies differed in all studied varieties and mutagenic treatments. Treatments with the lowest concentrations of the two applied mutagens (EMS and ENU) induced the smallest number of mutants from different types in all varieties. It was found differences in total number and spectra of induced chlorophyll mutant types for different studied varieties.

In all years of investigations, were not found mutants from the type claroviridis – in variety Tcher Starozagorski; aurea – in Plovdiv 10 and Tcher Starozagorski; lutea – in Dobroudjanski 7, Plovdiv 10 and Tcher Starozagorski; xanthomarginata – in Tcher Starozagorski; xanthoviridoterminalis – in Dobroudjanski 2, Plovdiv 10 and Tcher Starozagorski; viridomarginata – in Dobroudjanski 7, Plovdiv 10 and Tcher Starozagorski; viridocostata – in Plovdiv 10 and Tcher Starozagorski, and viridoterminalis – in Dobroudjanski 7 and Tcher Starozagorski.

Mutants from the type anthocyana purpurea were found only in variety Plovdiv 11 M.

The highest diversity of chlorophyll mutants was found in varieties Plovdiv 11M (16 types) and Dobroudjanski 2 (14 types).

CONCLUSIONS

On the base of conducted investigations can be concluded that:

1. Specific reactions of Bulgarian common and snap bean varieties were discriminated to treatments with N-nitroso-N-ethyl urea (ENU) and etylmethan sulfonate (EMS).
2. Different mutation frequencies and spectra were induced from N-nitroso-N-ethyl urea (ENU) and etylmethan sulfonate (EMS) in all studied varieties.
3. The highest mutation frequency and spectrum of chlorophyll mutants were found in varieties Plovdiv 11M and Dobroudjanski 2.
4. Sixteen different types of chlorophyll mutant types were discriminated in all studied Bulgarian common and snap bean varieties.

Figure 1: Chlorina (A) and xanthoviridoterminalis (B) mutant's types

Table 2: Significance of results

Treat-ments	Control pH 6,0	$6{,}2 \cdot 10^{-3}$ M ENU	$3{,}1 \cdot 10^{-3}$M ENU	$1{,}55 \cdot 10^{-3}$M ENU	Control pH 7,0	$2{,}5 \cdot 10^{-2}$ M EMS	$1{,}25 \cdot 10^{-2}$M EMS	$6{,}2 \cdot 10^{-3}$ M EMS
Variety Dobroudjanski 7								
F_f		101,19	92,16	45,21		116,88	100,63	67,21
P	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%
Variety Dobroudjanski 2								
F_f		84,78	75,58	26,24		74,43	56,58	22,02
P	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%
Variety Plovdiv 11 M								
F_f		126,53	104,85	63,95		90,29	67,85	36,12
P	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%
Variety Plovdiv 10								
F_f		65,40	69,53	39,59		73,19	61,12	31,70
P	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%
Variety Tcher Starozagorski								
F_f		129,41	99,19	31,72		37,78	50,29	29,49
P	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%	P > 0,01%

F_{st} (3,8 – 6,6 – 10,8) = P (0,5 – 0,1 – 0,01%)

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