INDUCED MUTAGENESIS IN WHEAT AT VARIOUS PLOIDY LEVELS

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Different wheat species with 2x = 14, 28 and 42 were treated with ethylene imine (EI) and gamma-rays, and the M_1 damage and M_2 mutation frequency recorded. The resistance towards mutagenic treatment, in general, increased with ploidy level, but in each ploidy group the cultivated varieties were tolerant than the wild and primitive forms of the same species or ploidy level. It was also observed that the manifestation of mutagenic damage is expressed differently for different parameters taken for recording the extent of injury. There was no direct correlation between the sensitivity expressed through M_1 injury and the mutation frequency recorded in M_2, although in hexploid bread wheat a more sensitive variety (Bezostaya-1) was also more mutable than the other variety of the same species (Beltskaya-32).

INTRODUCTION

One of the important directions in mutational selection is the study of the role of genetic features of an organism and, in particular, the role of the level of its ploidy in resistance to the action of mutagen factors and in mutational process of plants.

Till recently the comparative reaction of ploidy series in wheats to the action of radiation and chemical mutagens has been poorly studied.

In this respect it was decided to study, first, the sensitivity to physical and chemical mutagens of a number of species and varieties of wheats belonging to various ploidy groups ($2n=14$, $4n=28$, $6n=42$); secondly, to study the mutability level and the nature of mutation variability of analysed wheats, and also to induce mutations with economically valuable traits and properties for utilization in selection.

MATERIALS AND METHODS

Seeds of species with different chromosome numbers were taken as the initial material for investigations: representatives of 42-chromosome common wheat: *Tr. aestivum* (variety Bezostaya-1 = B-1 and Beltskaya-32 = *B*-32), *Tr. macha*; 28-chromosome durum wheats; *Tr. araraticum, Tr. timopheevi,* and *Tr. durum* (Variety Novo-Michurinskaya); 14-chromosome wheats: *Tr. boeticum* and *Tr. monococcum.*

Ethyleneimine at concentrations 0.005; 0.01 and 0.02 per cent were used as chemical mutagen, and the irradiation was conducted with gamma-rays Co^{60} in doses 10 and 15 kR.

RESULTS AND DISCUSSION

Germination analysis of the treatments of the experiment showed that the investigated species and varieties have a clear-cut specific reaction to the action of

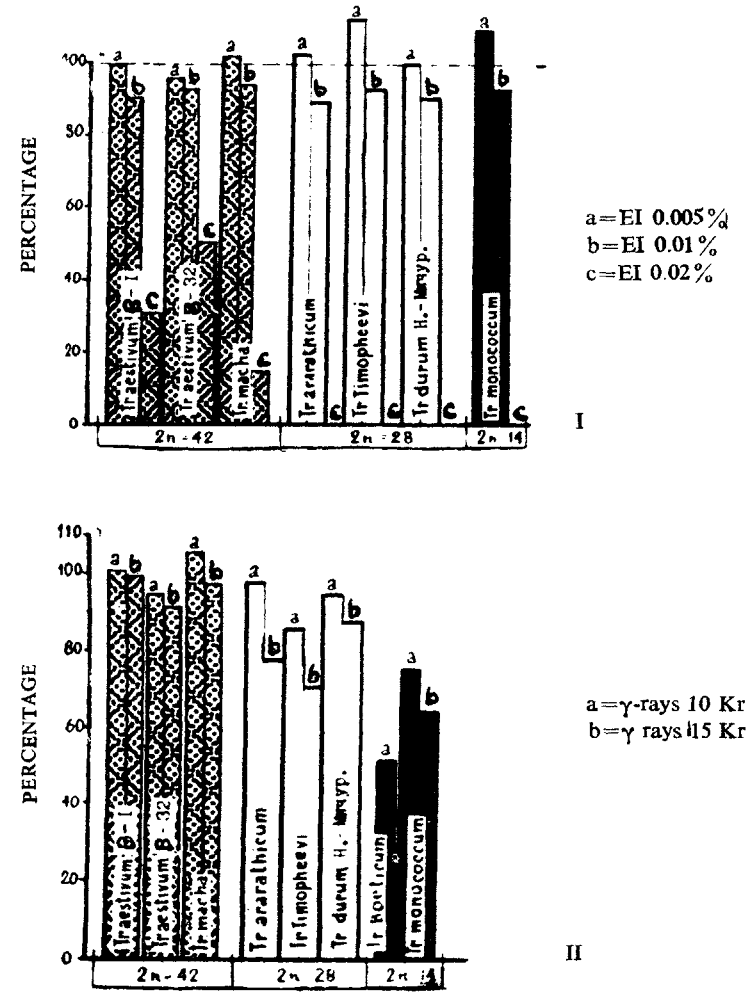


FIG. 1. Dependence of field germinating ability of species and varieties of wheats on the mutagen action (in % to control) (I) Under the influence of ethyleneimine, and (II) Under the influence of gamma-irradiations.

various mutagens (Fig. 1). As it may be seen from the Fig. 1, EI in a low concentration (0.005 per cent) leads to some stimulation of seed germination in wild and primitive species. But EI in a concentration 0.02 per cent in all species $2n$ and $4n$ groups results in complete lethality of seeds, while in hexaploid wheats 13.3–52.5 per cent of seeds remain alive.

For plant height of two-week's seedlings treated with EI there are no differences in the dependence upon ploid plants (Fig. 2). Upon gamma-irradiations, a significant growth inhibition is observed in some tetraploid and diploid species as compared with hexaploids. However, the height indices in *Tr. monococcum* appeared to be at the same level with the indices of hexaploid wheats. This suggests that our

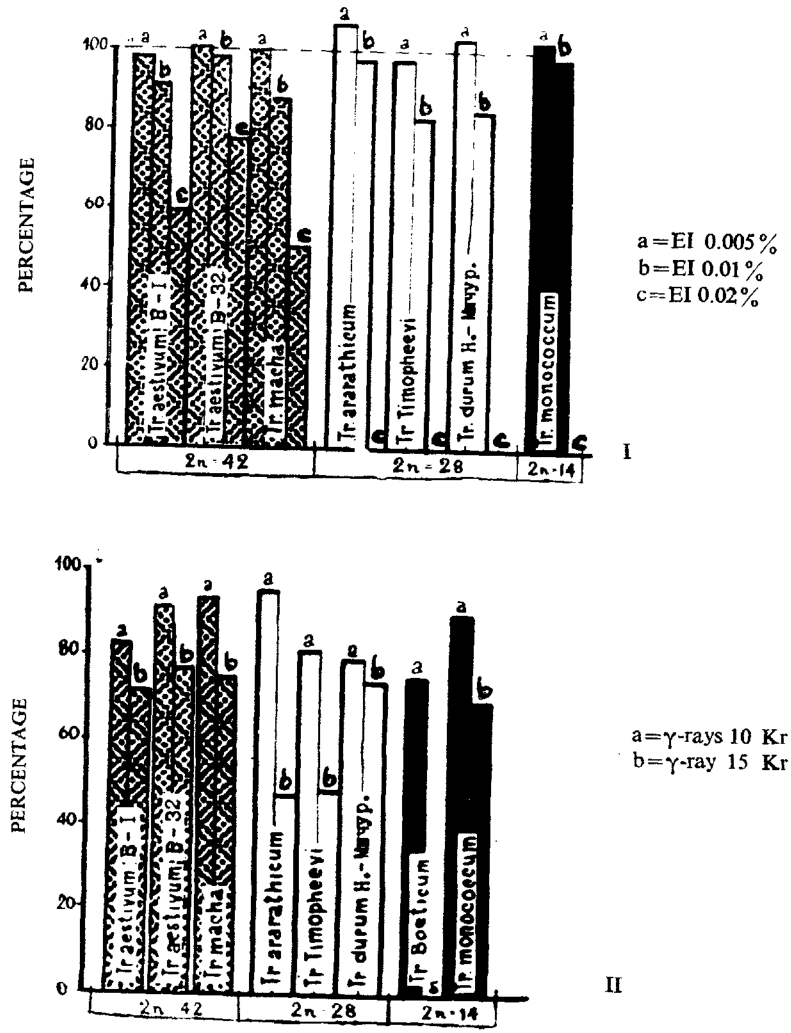
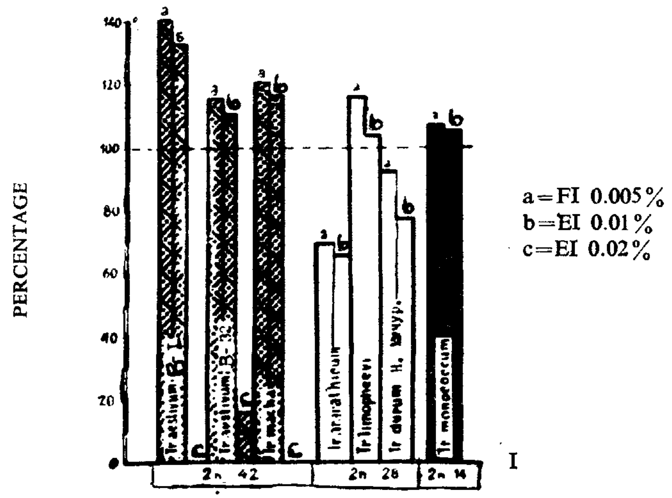


FIG. 2. Dependence of height of seedlings of various species and varieties of wheats on the action of (I) ethyleneimine, and (II) gamma-irradiations (in % to control).

ideas on the influence of radiation upon various-ploidy plants, if judged by a single criterion, should mainly depend on those representatives which are taken for the experiment.

Various species and varieties differed by their ability to restore normal growth and development. The restoration to the normal height of plants was observed before and at the stooling phase in *Tr. aestivum* and at later developmental phases in *Tr. macha*. In di- and tetraploid wheats in some treatments of the experiments (EI 0.01 and gamma-rays 15 kR), when the damage was very strong, differences with control were observed till the end of vegetative period.



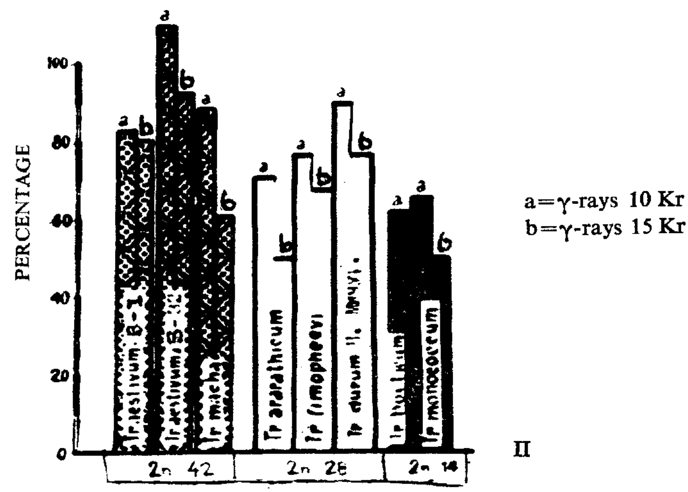


FIG. 3. Dependence of plant survival of various species and varieties of wheats on the action of (I) ethyleneimine, and (II) gamma-irradiations (in % to control).

The analysis of the data of conducted experiments, where plant survival served as a criterion, shows that representatives of hexaploid wheats appeared to be the most resistant to EI (Fig. 3). Diploids were on the second place, and tetraploid wheats were least resistant. In the treatments with gamma-irradiation on the whole for this criterion, a decrease in the resistance from hexaploid to diploid wheats is observed.

Primitive and wild forms of all chromosome groups were less resistant for all criteria as compared with the cultivated forms.

The account of sterility degree showed that cultivated diploids in all studied treatments had a higher sterility level as compared with tetraploid and hexaploid wheats.

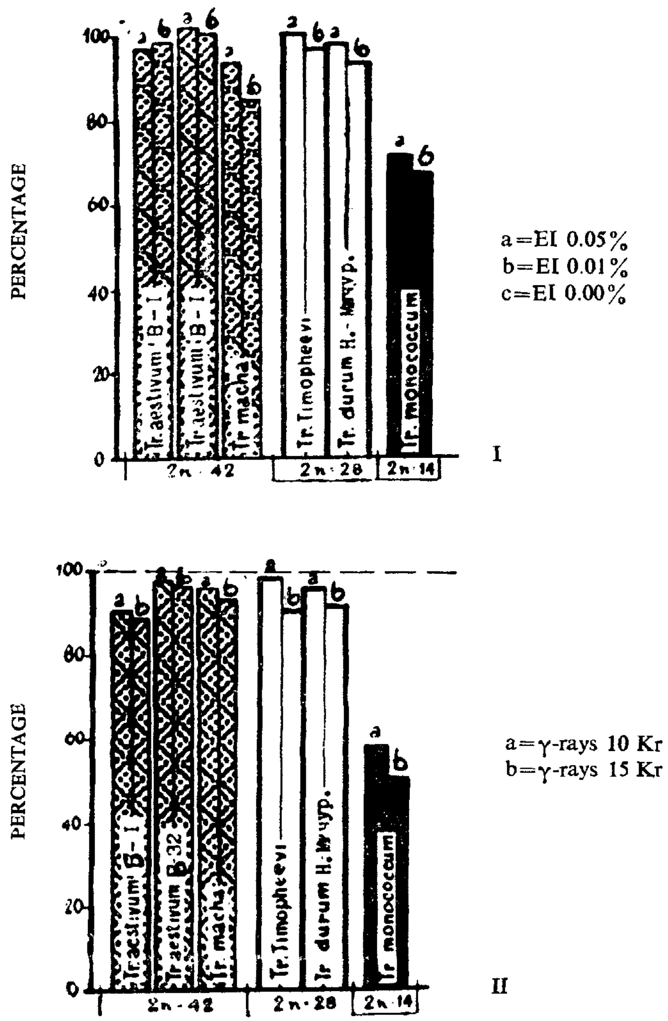


FIG. 4. Dependence of flower fertility of wheats of various ploidy levels on the action of (I) ethyleneimine and (II) gamma-irradiations (in % to control).

(Fig. 4). This phenomenon was observed in treatments of both EI and gamma-irradiation.

Fertility level of flowers in hexa- and tetraploid wheats in all treatments was almost at the same level. Gamma-irradiation caused a greater reduction in fertility as compared with EI treatment.

It may be concluded from the data obtained that the mutagen response determined by various criteria, changes not in the same way during vegetation in various wheat species and varieties.

The lack (absence) of complete correlation between various criteria, determining the response by the different representatives of ploidy groups to mutagens was also observed.

Consequently, it may be concluded that one criterion is hardly sufficient when plant sensitivity to mutagens is determined. Only the combination of a number of indices and its analysis may give a correct characteristics to sensitivity.

Among the plants raised in M_2 families during vegetative period, changed forms of plants were isolated. The frequency after examination of their heredity in M_3 is given in Table I.

TABLE I

The frequency of mutations confirmed after third generation (% of mutant families)

Mutagen	2n = 14		2n = 28		2n = 42		
	Tr. boeoticum	*Tr. monococcum*	*Tr. ararathicum*	*Tr. durum* (Novo-Mitchurinskaya)	*Tr. macha*	*Tr. aestivum*	
						(Bezostaya-1)	(Beltskaya-32)
Control	0	0	0	2.0 ± 1.4	—	5.0 ± 2.2	0
EI 0.005	—	0	0	2.0 ± 1.0	0	8.5 ± 2.0	3.5 ± 1.7
EI 0.01	—	0	0	1.3 ± 0.7	0	13.3 ± 2.0	4.7 ± 1.2
γ-rays 10	0	0.5 ± 0.5	0	0	—	14.7 ± 2.2	7.3 ± 1.5
γ-rays 15	—	0	0	1.0 ± 0.7	—	11.5 ± 2.3	11.0 ± 2.2

Analysis of the data on the second and third generations shows, that among wild and primitive species of all ploidy wheats (*Tr. boeoticum, Tr. ararathicum, Tr. macha*) no mutant forms appeared, except chlorophyll mutations. Among cultivated 14- and 28-chromosome species single visible mutations were found in *Tr. monococcum:* (0.5 per cent), and in the variety Novo-Mitchurinskaya the frequency of mutant families for experimental treatments was 1-2 per cent.

The highest number of hereditary changed forms was noted in *Tr. aestivum.* In varieties Bezostaya-1 and Beltskaya-32 the mutation frequency induced by EI-treatment in concentrations 0.005 and 0.10 per cent was respectively 8.5–13.3 and 3.5–4.7 per cent ; and it was 14.7–11.5 and 7.3–11 per cent after seed irradiation in doses 10 and 15 kR.

Thus, the variety Bezostaya-1 due to its biological peculiarities appeared to be more mutable, than the variety Beltskaya-32. It is of interest that this variety was more sensitive to the mutagenic treatment of seeds and that the frequency of heredity changes decreased with increasing radiation dose from 10 to 15 kR, while the maximum rate of mutations in the variety Beltskaya-32 was obtained at 15 kR. The lack of complete dependence of the frequency of visible mutations on radiation dose and EI concentrations may be evidently explained by the elimination of cells with mutations due to large damages in the chromosome apparatus in cells of a more radiosensitive variety; this agrees well with the data of other investigators (Shkvarnikov and Cherny 1961; Mozhaeva 1961; Zoz and Makarova 1964 ; Zoz *et al.* 1965).

A tendency to a higher mutability is also observed in common wheats in the treatments with gamma-rays; but there are cases when EI in the concentration 0.01 per cent induced, in fact, the same number of mutations in the variety Bezostaya-1 as in the case of gamma-irradiation (13.3 and 14.7 per cent).

The data obtained testify that mutational variability of plants significantly depends not only on specificity of the treatment factor and ploidy level, but also on the genotype of a species, form, living conditions and culture degree.

Results of our experiments are in good agreement with the data of other investigations (Natarajan, Sikka and Swaminathan 1958; Mak-Key 1958, 1967; Volodin 1965; Kolajskina and Tarankina 1969), who observed a higher mutability of common wheats as compared with diploid and tetraploid species, under the influence of various treatments.

Various mutability of species in a ploidy series of wheats, obtained in our experiments, may be evidently explained by phylogenetic differences between the representatives of the genus *Triticum.* It is known, for example, that species *Tr. aestivum* in natural conditions is characterised by higher mutability, than di- and tetraploid forms. Proceeding from the literature data and the results of our own experiments it may be concluded that various forms and varieties even of the same species, which differ for general frequency of spontaneous mutations, also differ for induced mutability rate. So, the variety of common wheat Bezostaya-1 in control had a rather high mutation frequency (5.0 per cent), while no spontaneous mutations were observed in the variety Beltskaya-32. The same regularity in mutability remained in the experimental variants, where the variety Bezostaya-1 in all EI concentrations and irradiation doses differed by a higher mutating frequency as compared with Beltskaya-32. Different mutability of varieties of the same species is evidently due to their genotypical peculiarities.

The absence of variability in wild and primitive wheats in our experiments is evidently a result of a limited hereditary basis, caused by the limited areas of their distribution.

An important moment in the elucidation the specificity of mutational variability of wheats with different chromosome sets is the question concerning the survival of mutant cells and the further phenotypical mutation expression. It is not possible to judge from the beginning of mutational process about frequency and spectrum of observed mutations, as the most part of the mutant cells is eliminated, and the level of elimination depends on the ploidy.

A more expressed difference in the elimination level of chromosome mutations in relations to the ploidy of wheats is discovered in our experiments during the scoring of an indirect index, i.e. flower sterility of ear. In all the experimental treatments a higher flower sterility of the first order is marked in a diploid species (Fig. 4). Thus, the high survival of mutations in hexaploid species in the course of natural cell selection probably provides their better phenotypical preservation and expression.

Our experiments failed to reveal direct dependence between the sensitivity of M_1 plants and the frequency of mutations in M_2. The analysis of the data shows although common wheats (6*n*) on the whole are more resistant to direct mutagen action, at the same time they differ significantly from individuals of other ploidy groups by high mutability. Tetraploid wheats are less mutable than common wheats despite they are characterised by a higher sensitivity to mutagens in M_1.

In the varieties *Tr. aestivum* (Bezostaya-1 and Beltskaya-32) as much as 7-10 various mutant types more were recorded as compared with wheat species 4*n*(*Tr. durum*) and 2*n* (*Tr. monococcum*). Also, a difference in the induction of separate mutation types was observed in the varieties of common wheats. In the variety Bezostaya-1 the frequency of such mutations as squareheadness, speltoidy, lack of a wax layer on ears appeared to be comparatively high, but these were very rarely observed in the variety Beltskaya-32. Some mutant types, i.e. those, which are resistant to mildew and rust, were rarer in the first variety and more frequent in the variety Beltskaya-32.

Analyses showed that ethyleneimine and gamma-irradiation more often induce not one mutation per plant, but change at once to two and more traits, i.e. the expression of multiple mutations is observed.

In diploid and tetraploid wheats in the treatments with gamma-irradiation (10 and 15 kR), all changed families are characterized by multiple mutations. The highest per cent of the families with multiple mutations among soft wheats was marked in the treatments with gamma-irradiation and EI (0.01 per cent) in the less mutable variety Beltskaya-32. It varied from 81, 82 and 100 per cent from the total number of mutations. In the variety Bezostaya-1 (as a whole, more mutable) in the same treatments of the experiment the percentage of families with multiple mutant traits varied from 72.50 to 78.26 per cent.

Hence, the frequency of multiple mutations to a greater extent depends on the variety than the mutagen; and there is no direct correlation between the frequency of multiple mutations and mutability of a variety. Multiple mutations were characterised by simultaneous changes in such traits as ear density, height of stem, rust resistance, early ripening, changes in the protein content, lodging resistance. There were cases when certain mutations were accompanied by others, but always the same mutations. Thus, the mutation falcate, drooping ear in the variety Bezostaya-1 was always accompanied by lack of wax layer on leaves and a decreased protein content in grain. In the variety Beltskaya-32 the mutation "early ripening" is very often accompanied by lodging resistance, that may be caused by either simultaneous mutation of several genes in the same cell, or pleiotropic action of the mutating gene.

The data obtained in our experiments on two varieties of soft wheats, show that the mutation frequency of useful traits from the total number of mutations is higher in the variety Beltskaya-32 in all treatments of the experiment with El and γ-irradiation (51–65 per cent). In the variety Bezostaya-1 in the same treatments of the experiments, the mutation frequency was 36–44 per cent. After gamma-irradiation, the frequency of useful changes in all the experimental treatments is practically higher than in EI treatments.

The description of the best mutants characterised by separate economically valuable traits; is given in Table II. For the trait of stem height, both high and low

TABLE II

Characteristics of the productivity elements in mutant lines

No of mutants & original variety	Type of changes	Height of plants (cm)	Productive tillering	Number of grains in main ear	Weight of grains (g) of main ear	Weight of grains (g) of one plant	Weight of 1000 grains
Bezostaya-1 control	—	90.0	6.9	37	1.7	7.2	43.8
43	late, large ear	94.0	6.9	45	2.1	8.9	41.2
53	late	95.0	7.2	45	2.1	8.0	44.8
239	large ear	80.0	6.2	41	2.2	8.4	46.8
368	,, ,,	100.3	9.2	39	2.1	9.7	42.0
444	dense ear	90.2	7.2	50	2.5	8.6	41.8
455	larger ear	92.7	7.8	44	2.0	8.8	45.4
474	dense ear	91.3	6.8	45	2.2	8.9	45.7
Beltskaya-32, control	—	129.0	7.1	40	1.6	7.1	37.9
605	resistant to rust, lodging, early	110.7	9.1	37	1.7	7.9	44.4
835	lodging resistant, large, dense ear	122.0	9.0	54	2.6	10.8	44.2
858	lodging resistant, early	126.3	8.0	40	1.8	9.1	42.7
924	large ear	128.7	9.6	49	2.4	9.5	38.4
944	resistant to rust and mildew	118.0	10.0	43	1.6	8.4	34.8
947	,,	122.0	9.9	39	1.8	8.6	38.9
983	lodging resistant, large ear	133.3	6.9	44	2.2	9.2	44.0

productive mutants were obtained. Except such morphologically changed forms, the forms with a changed protein content in a grain were also obtained.

The protein content in most of productive mutants, the variety Bezostaya-I changed within the limits from —1.27 per cent to +1.53 per cent as compared with the control, the maximum protein content in some mutants was (No. 239, 358) 17.10 per cent.

At present the primary task of breeding for grain quality is the breeding of wheat varieties not only with high protein content, but also with its balance for amino acid content. In this respect, the amino acid content of some productive forms, with high protein content in flour, was studied.

In Table III the data on the content of essential amino acids, which are of great interest are listed.

These data show that the total number of essential amino acids and the number of some amino acids in certain cases, vary significantly. For the total quantity of

TABLE III

Content of essential amino acids in flour of mutant lines

No. of mutant and original variety	Amino acids — Per cent content in 100 g of absolutely dry matter								
	Lysine	Histidine	Arginine	Threonine	Valine	Methionine	Isoleucine	Leucine	Phenyl alanine
Bezostaya-1 *control*	0.30	0.31	0.52	0.37	0.39	0.13	0.50	0.95	0.64
No. 53	0.28	0.41	0.56	0.36	0.77	0.19	0.65	1.17	0.68
No. 215	0.27	0.33	0.57	0.41	0.68	0.13	0.56	1.07	0.62
No. 239	0.30	0.33	0.51	0.47	0.64	0.13	0.53	1.02	0.67
No. 359	0.26	0.33	0.58	0.39	0.74	0.15	0.58	1.06	0.70
No. 455	0.25	0.25	0.52	0.42	0.65	0.11	0.54	0.99	0.65
Beltskaya-32, *control*	0.30	0.47	0.60	0.47	0.67	0.16	0.65	1.15	0.74
No. 599	0.24	0.44	0.59	0.51	0.84	0.18	0.68	1.24	0.91
No. 605	0.24	0.34	0.55	0.40	0.90	0.22	0.90	1.33	0.76
No. 947	0.24	0.43	0.58	0.37	0.87	0.20	0.71	1.26	0.74
No. 983	0.33	0.31	0.67	0.93	0.75	0.20	0.59	1.12	0.69

essential amino acids, all mutant lines exceeded the original variety Bezostaya-1 by 0.27–0.96 per cent. The sum of essential amino acids in the lines No. 53, 215, 239 359, 455 varied within 4.38 and 5.07 per cent. While the original variety had 4.11 per cent.

Mutant lines of the variety Beltskaya-32 differ by a less number of essential amino acids. For some mutant lines (No. 599, 605, 947, 983) this index exceeds the control by 0.38–0.62 per cent.

The analysis of the data on the qualitative structure of protein shows that an increase in the quantity of essential amino acids of mutant forms, selected from the variety Bezostaya-1 and Beltskaya-32, occurs mainly due to valine and leucine, and in other forms—due to isoleucine, threonine and phenylalanine.

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