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Induction of Winter Wheat Plant Structure Mutations by Chemomutagenesis

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Abstract. The objectives of our investigations are to describe the variation by muta-tions of stem architecture of the modern Ukrainian winter wheat varieties accord-ing to their interactions with mutagen nature, concentration and genotype-mutagen interaction specific. 7 modern Ukrainian winter wheat varieties and one line were treated by 1.4-bisdiazoatsetilbutan water solution at 0.1 and 0.2% concentrations. Types of visible mutations like as high and short stem, dwarfs and semi-dwarfs, changes in a waxy bloom, stem thickness have been investigated. New genetic- and breeding-value mutant lines have been obtained in terms of research program. Main components for mutation induction successful were geno-type-mutagen interaction and peculiarities of genotype as a subject of mutagen action (due to discriminant and factor analyses). By mutation occurs (in sense of mutation rate and spectra) genotypes can be subdivided on several groups. Effect of recurrent mutagenesis was identified as decrease of mutation rate under mutagen action by variety Kalinova, which obtained with same mutagen. Other genotypes reaction on mutagen action depends on genotype specific only, not kind of treatment. Bisdiazoatsetilbutan as a mutagens for creation new material by plant height and stem structure has been shown as less successful than gamma-rays and nitrosoalkylureas, but this mutagen may be used for special investigation by some types of mutation induction. But it was more specific in a mutagen-genotype interaction and more clearly demonstrate some effects of recurrent mutagenesis regarding nature of chemical agents. As for example action on variety Sonechko with great number of mutations by waxy bloom. These peculiarities may be caused by previous chemical mutagens damages of DNA in complex with recurrent action of bisdiazoatsetilbutan. Genotype-mutagen interaction and classification of mutant material are possible by rate of high and short stem, with and without waxy bloom forms. Concentration affected on rate of high stem forms. Mutagen cannot induce dwarf and semi-dwarf forms in significant amount and completely unpromising in this regard. Our research will be further focused on transformation of scientific and technological achievements and mutagenic mech-anism of bisdiazoatsetilbutan on plant at the molecular level in the recent future.

Keywords: winter wheat; mutagen; 1.4-bisdiazoatsetilbutan.

Introduction

Experimental mutagenesis has been used successful in main crops improvement for obtaining new agronomical value traits. Induced mutations in winter wheat have been obtained for morphological and quantitative characters by treatment with different types of mutagens (Nazarenko, Lykholat, Grigoryuk, & Khromykhl, 2018). The main purpose of using mutagens has been to induce genetic variation of agronomic important traits. Grain yield and quality, as complex polygenic traits, is highly affected regarding the complex of difference traits of plants architecture (height, thickness, waxy bloom) (Nazarenko, Beiko & Bondarenko, 2019). More than 3500 varieties of plants obtained either as direct mutants or derived from their crosses and 2700 mutant varieties of different plants including cereal crops have been released throughout the world through direct or indirect use of mutation breeding (IAEA, 2018). Mutation breeding has been successfully utilized for the improvement of crops as well as to supplement the efforts made using traditional methods of plant breeding. Induced mutation is the ultimate source to alter the genetics of crop plants that may be difficult to bring through cross breeding and other breeding procedures (Kolakar, Nadukeri, Jakkeral, Hanumanthappa, & Gangaprasad, 2018).

Bread wheat (Triticum aestivum L.) with the annual production of about 752 million tons (in 2018), is one of the world's most important cereal crops. Common wheat (Triticum aestivum L.) is a major staple food crop that feeds about 40% of the world's population. Wheat production and utilization accounts for ~28% of the global cereal crops. Consequently, wheat supplies approximately one-fifth of human calories in a variety of forms. Wheat will remain a crucial component of human nutrition, and increasing its production is therefore an important requirement for food security. Wheat consumption has been steadily increasing due to population expansion and urbanization (Hongjie, Timothy, Mc Intoshc, & Yang, 2019; Li, Timothy, Mc Intoshc, & Zhou, 2019; Shan, Adnan, & Basir, 2018).

Winter wheat is an important crop, suited to the typical weather conditions in the current climate. In a changing climate the increased frequency and severity of adverse weather events, which are often localized, are considered a major threat to wheat production (Harkness, Semenov & Areal, 2020).

The improvement of grain productivity and its components of winter wheat through exploiting of mutagens lead to creation of new varieties with improved traits. The use of induced mutations has become an important technique to optimize plant structure for bioproductivity (Naveed, Nazir, Abdu, Raza, & Muhammad, 2015).

The present studies were therefore undertaken to investigate the effects of chemical supermutagen (1.4-bisdiazoatsetilbutan) on so yield and quality associated trait as plant height and structure of stem.

Plant height is one of the main agronomic traits related to plant architecture and grain yield in cereals. Tiller number and plant height are pointed out as two major agronomic traits in cereal crops affecting plant architecture and grain yield (Ellis et al., 2004). In researches of chines scientists NAUH167, a new mutant of common wheat landrace induced by ethylmethyl sulfide treatment, exhibits higher tiller number and reduced plant height was attributed to the decrease in the number of cells and their length. Genetic analysis showed that the high-tillering number and dwarf phenotype were related and controlled by a partial recessive gene (Xu et al., 2017).

Dwarfing and semi-dwarfing mutations have mutual effects. As for example, dwarfing gene Rht5 was associated with a plant height reduction, delaying heading date by 1 day, increasing the number of fertile tillers plant-1, while reducing the number of spikelets spike-1 and number of grains spike-1. The results of this study could be useful for proper use of Rht5 dwarfing gene in breeding programs to improve lodging tolerance, yield potential in wheat and increase efficiency of marker assisted selection for agronomic traits (Daoura, Chen, Du, & Hu, 2014).

One strategy to meet this challenge is to raise grain productivity by optimizing plant structure. As a sample of this investigation, the reduced height 8 (Rht8) semi-dwarfing gene is one of the few, together with the Green Revolution genes, to reduce stature of wheat (Triticum aestivum L.), and improve lodging resistance, without compromising grain yield. Rht8 is widely used in dry environments where it increases plant adaptability. Morphological analyses show that the semi-dwarf phenotype of Rht8 lines is due to shorter internodal segments along the wheat culm, achieved through reduced cell elongation (Gasperini et al., 2012).

The development of winter wheat mutants not only provided new genetic resources for wheat improvement, but also facilitated our understanding of the regulation of these traits at the molecular level. Identification of a dwarf mutant with a compact spike, NAUH164, produced from ethyl methyl sulfonate treatment of wheat variety Sumai 3, has reduced plant height and shortened spike length. Dwarfness and compact spike were controlled by a single dominant gene that was designated Rht23 (Chen et al., 2015).

Regarding 47 wheat varieties carrying different Rht alleles screening for their ability to emerge from deep sowing, and for detailed physiological characterization in the field the modern wheat lines have been shown differences in early developmental stages were associated with grain yield, as indicated by a reduction of 37.3% in the modern cultivars (Amram et al., 2015). But reducing by grain productivity at modern investigations not always characteristic for dwarf winter wheat varieties with typical gibberellinresponsive (GAR) dwarfing genes, such as Rht12. In investigations of chines researches (Chen, Hao, Condon, & Hu, 2014) plant height of the tall lines was not affected significantly by GA3 treatment. Plant biomass and seeds shape of the GA3-treated dwarf lines was significantly increased compared with untreated dwarf plants while there was no such difference in the tall lines. This effect has addictive value effect Rht12 dwarf plants developed faster than control plants and reached double ridge stage 57 days, 11 days and 50 days earlier and finally flowered earlier by almost 7 days while the tall lines. Both possibilities are confirmed by several investigations (Fellahi, Hannachi, Oulmi, & Bouzerzour, 2018; Hans, Anthony, & Matthew, 2019; Lingling et al., 2019).

The objectives of our investigations are to describe the genotypic variation of new mutant winter wheat lines by plant height and structure, investigation of role genotype-mutagen interactions at formation of new trait. The most target objects are developing relations between genotype and nature of chemical mutagen, mutagen concentration. Second our purpose to estimate recurrent mutagen effect and its suitability for future plant improvement process.

Material and methods

Winter wheat seeds (approx. 14% moisture content, in brackets method of obtaining varieties or used mutagens) of Favoritka, Lasunya, Hurtovina (mutation and mutation-recombination varieties regarding IAEA classification, radiomutans), line 418, Kolos Mironovschini (hybrid varieties), Sonechko and Kalinova (mutation varieties, chemomutant), 'Voloshkova' (mutation variety, termomutagenesis – low plus temperature at plant development stage of vernalizaion) of winter wheat (Triticum aestivum L.) were soaked with solutions of chemical mutagen 1.4-bisdiazoatsetilbutan (DAB) 0.1 and 0.2%. Each treatment was comprised of 1,000 wheat seeds. Exposition of chemicals mutagens was 18 hours. These concentrations and exposure are trivial for the breeding process that has been repeatedly established earlier (Nazarenko, 2015; Spencer-Lopes, Forster, & Jankuloski, 2018). Non-treated initial varieties and national standard by grain yield Podolyanka were used as a control for mutation identified purpose by all traits changes.

Treated seeds were grown in rows with inter and intrarow spacing of 50 and 30 cm, respectively, to raise the M1 population. The untreated seeds of mother varieties and standard (parental line/variety) were also planted after every ten rows as control for comparison with the M1 and next generations populations. Mutant populations and families rows were grown in three replications with checkrows of untreated varieties in every tenrow interval (Shu, Forster, & Nakagava, 2013).

In M2–M3 generations mutation families have been selected via visual estimation. The sowing was done by hand, at the end of September, at a depth of 4–5 cm and with a rate of 100 viable seeds to a row (length 1.5 m), interrow was 15 cm, between samples 30 cm, 1–2 rows for sample with controlrows of untreated varieties and standard in every twenty-sample interval.

Estimation of total characteristics and heritability of changed traits was conducted from 2014 to 2018 years (M4–M8 generations). The controls were national standard by productivity Podolyanka and initial variety. The working methods in the breeding trials are satisfied to state variety exam requests. The trial was set up as a randomized block design method with three replications and with a plot size of from 5 to 10 m^2 in 2–3 replications (Shu et al., 2013).

Experiments were conducted on the experiment field of Dnipro State Agrarian-Economic University (village Oleksandrovka, Dnepropetrovsk district, Dnipro region, Ukraine). Normal cultural practices including fertilization were done whenever it is necessary. Weeds were manually removed where necessary, and fungicides and insecticides were applied to prevent diseases and insect damage. Evolution was conducted during 2011–2018 years.

Mathematical processing of the results was performed by the method of analysis of variance, the variability of the mean difference was evaluated by Student's t-test, the grouping mutants cases was performed by cluster and discriminant analysis, factor analyses was conducted by module ANOVA. In all cases standard tools of the program Statistic 8.0 were used.

Results

Total size of population 12000 families at second–third generation (include controls) and represented by variants of mutagen treatment at table 1. Investigations are conducted with trivial mutagen concentrations for breeding purposes.

From M2–M3 generations (from all experiments, included all variants with other mutagens) 1.482 potential productivity winter wheat mutation lines and 5.862 lines with mutation changes were determined overall. In all variants 500 families have been investigated, all concentrations are optimal for plant surviving. General rate of mutations was up to 14.0% under DAB 0.2% action (Sonechko) and to 9.8% under DAB 0.1% action (Sonechko too) (table 1), but for the most part of genotypes was on the level 7–9% for DAB 0.2% and 6–8% for DAB 0.1%. Regarding this date difference between concentrations of DAB not so strong as between doses of gamma-rays at previous investigations (Nazarenko et al, 2019).

The lowest general mutation rate was 2.8% (DAB 0.1%) and 5.8% (DAB 0.2%) (at both cases for chemomutant Kalinova, which

Table 1. Rates of mutations (general and by plant structure) at second–third generations

Trial	Kolos Mironovschini	Kalinova	Voloshkova	Sonechko	Favoritka	Hurtovina	Lasunya	Line 418
General rate of mutations								
Control	0.4	1.2	1.8	0.8	0.6	0.8	1.4	0,8
DAB, 0.01%	5.6*	2.8*	7.8*	9.8*	6.2*	5.8*	5.8*	3.8*
DAB, 0.025%	8.8*	5.8*	8.6*	14.0*	7.8*	6.8	9.6*	7.6*
Rate of mutations by plant structure								
Control	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
DAB, 0.01%	1.4*	0.4	2.4*	3.0*	1.2*	2.0*	1.2*	0.8
DAB, 0.025%	2.4*	1.6*	2.6	4.8*	1.4	2.2	1.0	3.0*

obtained with DAB action). DAB is no so active as mutagen by general rate of mutations (in spite of other chemical and physical mutagens), but from the dates its depends on genotype-mutagen interaction (especially for chemomutants Kalinova and Sonechko, both varieties are underlined by their mutations activity) and can be changed according to nature of initial variety.

Regarding rate of plant structure mutations action of mutagen was equal at low-average and average level of mutagen concentrations (low for Kalinova, average for Sonechko) and depended more on initial genotype, than concentration (due to factor analyses). Group of plant structure mutations includes next types plant height mutations (at our case high stem, short stem, semi-dwarf), waxy bloom intensity (intensive, weak, without waxy bloom, types of mutations are depended on morphological traits of initial variety), thick and thin stem. Rate of this group of mutations was varied from 0.4% (Kalinova, DAB 0.1%) to 4.8% (Sonechko, DAB 0.2%). We can see similar situation by genotype reaction on mutagen as for a general rate of mutations. Generally, the rate of mutations by this group was on the level 1–3%, difference between concentrations statistical reliable for varieties Kolos Mironovschini, Kalinova, Sonechko, line 418, between control and DAB 0.1% for Kolos Mironovschini, Voloshkova, Sonechko, Favoritka, Hurtovina, Lasunya.

From these investigations fact of decreasing general mutation rates and number of mutations by plant structure (at second case only partly) for variety Kalinova, which obtained after DAB action has been developed. But other chemomutant, which obtained by nitrosoureas, Sonechko showed highest general rate of mutations with statistically significance and great number of mutations by plant structure.

We can subdivided initial material by the method of breeding as radiomutans (Favoritka, Hurtovina, Lasunya), chemomutants (Kalinova and Sonechko), thermomutants (low plus temperature at plant development stage of vernalizaion has been used as mutagen factor) (Voloshkova) and forms, obtained after hybridization (Kolos Mironovschini, line 418) (table 2–4).

For first group (table 2) similar number and types of mutations was characterized to all concentrations, but reaction of genotypes was differing among the genotypes. For Favoritka as variety short-stem mutations are less characterized, than for other. Highstem mutants were more possible and prevalent under other types. Rates of mutations are not high, variety Favoritka characterized by more types of mutations, dwarfs mutations were absence at all genotypes and only for one genotypes under both concentrations of DAB mutations by waxy bloom intensity were presence. Higher mutability

Table 2. Spectrum of mutations under DAB action. Radiomutants

№	Trait	Control		DAB, 0.1%		DAB, 0.2%	
		lines	%	lines	%	lines	%
Variety Favoritka							
1	high stem	1	0.2	5	1.0	4	0.8
2	short stem	1	0.2	1	0.2	2	0.4
3	semi-dwarf	0	0	0	0	1	0.2
4	total	2	0.4	6	1.2	7	1.4
Variety Hurtovina							
1	high stem	1	0.2	4	0.8	4	0.8
2	short stem	1	0.2	3	0.6	3	0.6
3	semi-dwarf	0	0	1	0.2	0	0
4	intensive waxy bloom	0	0	1	0.2	2	0.4
5	weak waxy bloom	0	0	1	0.2	2	0.4
6	total	2	0.4	10	2.0	11	2.2
Variety Lasunya							
1	high stem	1	0.2	2	0.4	3	0.6
2	short stem	1	0.2	3	0.6	2	0.4
3	semi-dwarf	0	0	1	0.2	0	0
4	total	2	0.4	6	1.2	5	1.0

Table 3. Spectrum of mutations under DAB action. Chemomutants

№	Trait	Control		DAB, 0.1%		DAB, 0.2%	
		lines	%	lines	%	lines	%
				Variety Kalinova			
1	high stem	1	0.2	2	0.4	5	1.0
2	short stem	1	0.2	0	0	1	0.2
3	weak waxy bloom	0	0	0	0	1	0.2
4	without waxy bloom	0	0	0	0	1	0.2
5	total	2	0.4	2	0.4	8	1.6
				Variety Sonechko			
1	high stem	1	0,2	5	1.0	9	1.8
2	short stem	1	0,2	2	0.4	2	0.4
3	weak waxy bloom	0	0	4	0.8	7	1.4
4	without waxy bloom	0	0	4	0.8	6	1.2
		2	0.4	11	3.0	24	4.8

Table 4. Spectrum of mutations under DAB action. Recombinant genotypes

№	Trait	Control		DAB 0.1 %		DAB 0.2 %	
		lines	%	lines	%	lines	%
				Variety Kolos Mironovschini			
1	high stem	1	0.2	7	1.4	7	1.4
2	short stem	1	0.2	0	0	1	0.2
3	thick stem	0	0	0	0	1	0.2
4	thin stem	0	0	0	0	1	0.2
5	weak waxy bloom	0	0	0	0	1	0.2
6	without waxy bloom	0	0	0	0	1	0.2
7	total	2	0.4	7	1.4	12	2.4
				Variety Voloshkova			
1	high stem	1	0.2	2	0.4	2	0.4
2	short stem	1	0.2	5	1.0	5	1.0
3	weak waxy bloom	0	0	3	0.6	3	0.6
4	without waxy bloom	0	0	2	0.4	3	0.6
5	total	2	0.4	12	2.4	13	2.6
				Line 418			
1	high stem	1	0.2	2	0.4	5	1.0
2	short stem	1	0.2	1	0.2	5	1.0
3	semi-dwarf	0	0	0	0	1	0.2
4	weak waxy bloom	0	0	1	0.2	2	0.4
5	without waxy bloom	0	0	0	0	2	0.4
6	total	2	0.4	4	0.8	15	3.0

was inherited for variety Hurtovina, but not so discrepancy as at the cases of other mutagens. Seldom mutations of stem thickness cannot be observed at all cases. Semi-dwarf mutations were seldom and under both concentrations of mutagen.

For DAB as for mutagen high stem mutations are more typical than other types and in general only this type of mutations was on the same level as proper changes for previous investigated mutagens.

For second group (table 3) lower rate of mutations was developed for variety Kalinova at all concentration. In spite of nitrosoalkylureas action at previous investigation variety Sonechko shows as highest genetic activity under DAB action. It witnessed that DAB action as chemical substance is more specify on DNA than nitrosoalkylureas. We observed all type of mutations without only rare semi-dwarf mutations. Mutations by waxy bloom were typical for Sonechko and this type of mutations, seldom for other genotypes, took first place for this variety by rate of mutations. High stem mutations are often too as at previous cases.

Regarding table 4 the same situation was observed as for mutants from tables 2 and 3. We have only one new low-active genotype line 418 under 0.1% DAB action, but this variety demonstrated activity on the same level as other two varieties under 0.2%

DAB action. At all cases for all genotypes concentration DAB 0.2% was more suitable for mutation induction by plant structure, but for variety Voloshkova difference was not statistical significance. High stem mutations typical for variety Kolos Mironovschini, for other varieties high and short stem mutations, mutants by waxy bloom was typical for variety Voloshkova, but less than for Sonechko. Mutations by stem thickness can be observed only for Kolos Mironivschina genotype.

Regarding dates of table 2–4 there is no any statistically reliable difference between rates in these groups more than inside each group. Rate of this type of mutations varied from 0.4 (Kalinova) to 3.0% (Sonechko) for DAB 0.1% and from 1.0% (Lasunya) to 4.8% (Sonechko) for DAB 0.2%. As we can see from the tables, higher rates and more types of this group mutations were characterized chemomutant Sonechko, varieties like Kalinova, Favoritka, Lasunya were less sensitive to this type of mutagen action (second and third more at 0.2% concentration).

General mutation rate to all types' mutation has been increased concentration growth. High level of changeability was correspon-0ded to higher concentrations of DAB. But tendency was not so clear as for previous mutagens for investigated type of mutations and for the most part of genotypes was no reliable statistic difference between 0.1 and 0.2% concentrations.

DAB action are more useful for high-stem type of mutations then gamma-rays and nitrosoureas, but not effective to the other types mutation induction. DAB as mutagen was more specify in its action are more depends on mutagen-genotype interaction than mutagens at previous investigations (gamma-ray, nitrosoalkylureas)

Regarding cluster analysis genotypes by the plant structure mutations rate can be subdivided on one main group and three genotypes with specify individual response. Main group consists of five genotypes (Favoritka, Lasunya, Hurtovina, Voloshkova, line 418). These varieties were obtained primary by action of physical mutagens. Only one, line 418, was obtained by crosses with wild wheat relative form. Two chemomutants (Sonechko and Kalinova) show individual reaction on DAB action according to genotype-mutagen interaction.

One other genotype, Kolos Mironovschini, developed by their reaction due to high variability by high rate of high stem mutations without any variability by other types of plant structure mutations.

Cluster analyses (Fig. 1) confirmed complicated and complex character of mutagen-genotype interaction. But we can classify with high precision genotypes by their response on mutagen action.

Individual responsibility on DAB action for two chemomutants gives us an opportunity to identify genotypes by their reactions not only on mutagens by nature, but on difference types of chemical mutagens too. At our previous investigations this varieties were correspondenced to the same group, just as by DAB action to the difference group.

According to dates of table 5 (results of discriminant analysis) two parameters can be used for identification of mutagen DAB action high-stem mutations and short-stem mutations. Regarding other types it's not possible. Especially interested in semi-dwarf mutations which have a key value for identification for gamma-rays (Nazarenko et al, 2019). Value of partial Lamba is enough only for using two first traits as features for precision analysis.

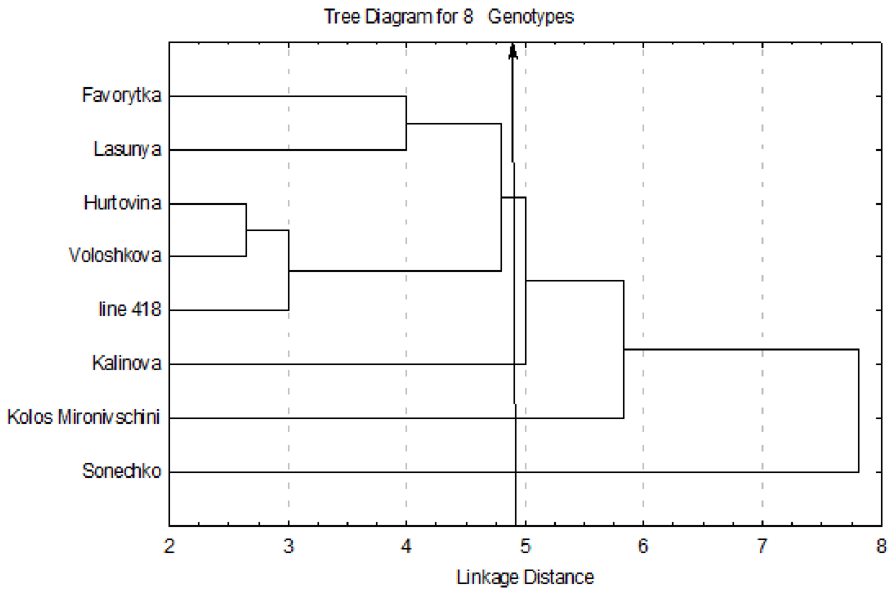

Fig. 1. Results of cluster analysis for winter wheat varieties

Table 5. Discriminant function analysis summary

Parameters	Wilks' – Lambda	Partial – Lambda	F-remove – (7.10)	p-level
High stem	0.092278	0.090568	2.526855	0.096209
Short steam	0.091727	0.100378	2.426415	0.094534
Semi-Dwarf	0.039969	0.772769	0.420068	0.868923
Intensive waxy bloom	0.053178	0.580817	1.031020	0.466448
Weak waxy bloom	0.035680	0.865661	0.221696	0.971146
Without waxy bloom	0.039146	0.789026	0.381980	0.892819

Table 6. Factor structure matrix correlations variables – canonical roots

	Root 1	Root 2	Root 3	Root 4	Root 5	Root 6
High stem	–0.161224	0.355788	0.084194	0.255750	–0.646154	0.204953
Short steam	0.470332	–0.369566	0.079807	0.107926	–0.472296	0.575308
Semi-Dwarf	–0.128165	–0.172724	0.022804	–0.560197	–0.581431	0.532118
Intensive waxy bloom	–0.169117	–0.379708	0.835858	0.274080	–0.134613	–0.010796
Weak waxy bloom	0.496220	0.370347	0.296030	0.279244	–0.584397	0.106829
Without waxy bloom	0.522491	0.506766	0.123625	0.228237	–0.465909	0.313148
Thick stem	–0.258848	0.108530	–0.184271	0.709113	–0.341246	0.237917

As for canonical roots analysis we can see just the same situation for all main canonical roots (table 6) and only fig. 1 for canonical root 1 and 2 interaction was enough for full image of situation.

Due to this date only second trait (short-stem mutations) can be used for all genotypes classification, first one (high-stem mutations) may be used only in complex with second one. Mutant material can be classified in a complex analysis with next success (table 7) full (100%) for variety Kalinova, enough for varieties Hurtovina, Lasunya, Sonechko, Voloshkova, Kolos Mironovschini. Total percent of identification under root 1 and root 2 interaction was 62.5%, which gives as the opportunity to identify enough mutants' cases for statistical reliable analysis.

Otherwise, our experimental dates by high-stem and short-stem mutations give us possibility to classify mutant material by genotype-mutagen interaction and identify effect of recurrent mutagenesis for variety Kalinova as evident.

From the table 8 (correlation matrix of mutant traits) great relate between mutations by waxy bloom has been developed. This relationship can be explained by the high rate of this type of mutations only for the variety Sonechko, not for other. As for our opinion, genotypes specify in occurrence of high-stem mutations was explained significance correlation between this trait and waxy bloom mutations.

Only one more case has been developed strong enough relation between high-stem and short-stem mutation. This case can be predicted by facts of relatively equal probabilities at the occurrence of both mutations types for certain genotypes (such as line 418, Hurtovina, Lasunya). The relationships between other types of mutations is insignificant and is of no interest either from the point of view of identifying the fact of recurrent mutagenesis, or to developed genotype-mutagenic specificity.

Factor analysis (untransforming, table 9, significance factors in bold) has been shown two factors (genotype and concentration), which affected on mutations rates by several types with statistical significance. First factor genotype of mutagen action subject influenced on rates of high and short stem, weak and without waxy bloom mutations. Second one concentration of DAB was weaker by influence and effective changed rates of short and thickness stem types of mutations (but second one was very rare and didn't have any importance for our investigations).

After factor matrix rotation (table 10) also two factors was verified, but for this case factor concentration of mutagen influenced on rate of high stem mutation, not on short stem changes. This kind of

Table 7. Classification matrics – canonical roots

Genotype	Percent of classification
Favoritka	33.3
Hurtovina	66.7
Lasunya	66.7
Kalinova	100.0
Sonechko	66.7
Line 418	33.3
Voloshkova	66.7
Kolos Mironovschini	66.7
Total	62.5

Table 8. Correlations

Parameter	High stem	Short steam	Semi-Dwarf	Intensive waxy bloom	Weak waxy bloom	Without waxy bloom
High stem	1.00	0.06	0.12	0.10	0.56	0.55
Short steam	0.06	1.00	0.34	0.23	0.48	0.42
Semi-Dwarf	0.12	0.34	1.00	0.07	–0.11	–0.11
Intensive waxy bloom	0.10	0.23	0.07	1.00	0.10	–0.15
Weak waxy bloom	0.56	0.48	–0.11	0.10	1.00	0.95
Without waxy bloom	0.55	0.42	–0.11	–0.15	0.95	1.00

Table 9. Factor loadings (unrotated)

Parameter	Genotype	Concentration
High stem	**–0.765249**	0.204622
Short steam	**–0.475373**	**–0.499688**
Semi-Dwarf	–0.001049	–0.216411
Intensive waxy bloom	–0.067556	–0.181307
Weak waxy bloom	**–0.906909**	–0.321197
Without waxy bloom	**–0.895056**	–0.257958
Thick stem	–0.340702	**0.903443**
Thin stem	–0.340702	**0.903443**
Expl.Var	2.671916	2.173392
Prp.Totl	0.333990	0.271674

Table 10. Factor loadings (varimax raw)

Parameter	Genotype	Concentration
High stem	**0.655243**	**–0.445122**
Short steam	**0.613366**	0.315356
Semi-Dwarf	0.072229	0.204005
Intensive waxy bloom	0.123473	0.148965
Weak waxy bloom	**0.962096**	0.004760
Without waxy bloom	**0.930087**	–0.051052
Thick stem	0.024319	**–0.965244**
Thin stem	0.024319	**–0.965244**
Expl.Var	2.617897	2.227411
Prp.Totl	0.327237	0.278426

analyze more proper for experiment results regarding less relation to genotype specify but more closely related to DAB action and tendency of rate increasing.

Thus, in the group of plan structure mutations, DAB is characterized by the following rates of changes of some traits – only one case of a thick stem mutant was noted (Kolos Mironovschini, DAB 0.2%); thin stem – also only one case in the same variant, i.e. mutations in stem thickness occur for only one variety; high-stem mutants induce by DAB in a relatively large number and at all variants, rate from 0.4 to 1.8%, on average according to variants 0.9%, more or less uniformly for all varieties, but with a significant advantage for the varieties Kolos Mironovschini and Sonechko; short stems – high probability of occurrence, but not for all variants, at average 0.5%, rate for some variants up to 1.0%, which is significantly lower than in the case of gamma-rays; semi-dwarf mutation is rare, not more than 0.2% for the Favoritka, Hurtovina, Lasunya, line 418; dwarfs were not appear at all.

Intensive wax bloom only two cases in the Hurtovina variety, the probability of occurrence is minimal; weak waxy bloom more highly probable, but absent in the Favoritka variety, up to 1.4%, on average 0.3%, i.e. less frequency than for all other mutagen agents; absence of wax bloom was average probability up to 1.2%, i.e. more frequency in some variants, but depends on the genotype of the subject of mutagenic action and occurs mainly in the varieties of Sonechko and partially Voloshkova, completely absent in varieties Favorite, Hurtovina, at average 0.2%.

Discussion

In terms of "green revolution" first problem which had to be solved was the question of plant architecture changing for new semi-dwarf forms (Pingali, 2019) with modified ration between grains (in form of spike size and length) and other parts of plant in favor of a spike (Hongjie et al, 2019). This process is providing up to now and remains of the key question in area of plant productivity, nutrient elements utilization, grain quality (regarding higher grain protein quality and quantity in modern short stem and semi-dwarf forms) (Le Gouis, Oury, & Charmet, 2020). This problem concerns not only winter wheat but other cereals too (Essam, Badrya, & Aya, 2019).

Complicated genetic control and several only main metabolic pathways for regulation of plant height makes this problem very difficult and variable (Würschum, Langer, & Longin, 2015). Experimental mutagenesis is well-known as the most prompt and successful method, which can be used in this area for rapid and reliable solution. Only on this way new forms with gens of dwarfism have been obtained (Xicun, Xia, & Wenjian, 2016; Çelik et al., 2018).

In spite of the previous investigation in our experiment it has been shown new possibilities in induction of mutant with changes in plant structure. Short height forms are important for grain productivity and efficient nutrient sources utilization (Shan et al, 2018), changes in waxy bloom need for drought tolerance and plant energetic balance improvement for future climates changes challenges (Saifu-Malook et al, 2015; Harkness et al, 2020), thickness of stem can be used for lodging resistance (Pavlista, Hergert, Baltensperger, & Knox, 2010). Plant height is an important trait that influences the yield and sustainability of wheat productions. It is also an important objective for agronomic breeding and a critical indicator to represent the status of plant growth and nitrogen absorption in the vegetative stage (Tengcong, Jian, Yujing, & He, 2020).

Our investigations was focused on genotype-mutagen interaction in terms of induction these types of mutations and possibilities of recurrent mutagenesis. First way counted on in previous investigation partly and not for modern forms (Spencer-Lopes et al, 2018), second in our time preferably exploit for ornamental (Hiroyasu, 2018), medicinal and aromatic crops (Kolakar et al, 2018).

Investigations showed some genotypes can be provided great number in one of types of mutations with variability by other types on the level of other genotypes. Chemical changes after mutagen action on DNA level are deeper than researches think early and can be shown in their consequences as changes of mutation rates under second action many generations later. It's one of the new effects of genotype-mutagen interaction.

Increase of mutagen concentration of DAB didn't provide leap in mutation rate and this value more depended on genotype. Rate grows only for some genotypes, but for other remained at the same level.

Most of the lines released so far have been developed from a mutation in combination with the direct selection (Hallajian, 2016). In the present era chemical mutagenesis with screening on phenotype and genotype level have resulted in the creation of new and wide paradigm in the utilization of mutations for crop improvement (Spencer-Lopes et al, 2018).

Effects of DAB which has been shown in this investigations can be used in complex of molecular methods for screening genetic structures (Ermias & Dembele, 2016; Juhi, Alisha, Vacha, Sonali, & Rupesh, 2019) which responsible for regulation of mutation activity in case of chemical mutagenesis.

Conclusion

Due to results of our investigations DAB as a mutagens for creation new variation material on plant height and stem structure has been shown as less successful than gamma-rays and nitrosoalkylureas, but this mutagen may be used for special investigation by some types of mutation induction. In complex with proper genotype (Sonechko) it possible to increase rate of mutations by waxy bloom. DAB as a mutagen more specific in a mutagen-genotype interaction and more clearly demonstrate some effects of recurrent mutagenesis regarding nature of chemical agents. It can be associated with special interactions between DNA after chemical mutagen action and DAB action.

Some mutant lines with short stem and changes in waxy bloom has been obtained both as for perspective new varieties and the sources for winter genetic-value collection for possible future changing plant architecture. Three traits appeared significant influence of genotype as a key component for mutation breeding success, all times genotype-mutagen interaction regarding results of factor analyze was significance in its influence on mutation rates. Genotype-mutagen interaction and classification of mutant material are possible by rate of high and short stem, with and without waxy bloom forms. Concentration affected on rate of high stem forms.

DAB as a mutagen were less effective to mutants, which obtained with same action (Kalinova), but effective for other chemomutants (Sonechko). DAB as mutagen can produce high rate of high-stem mutations. There are no concentrations of DAB useful of dwarfs' mutations.

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