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MUTAGEN DEPRESSION AFTER RECURRENT CHEMICAL MUTAGEN ACTION AT FIRST WINTER WHEAT GENERATION

SUMMARY

The strategy of investigation combined the effects of mutation depression evident at first generation on cell and plant level and peculiarities of recurrent mutagen action. The main purposes of investigations in this area were determination genotype-mutagen interaction for modern Ukrainian winter wheat varieties, identify less sensitive for DAB (1,4-bisdiazoatsetilbutan) genotypes and evolution recurrent mutagenesis as a method in difference alteration on first step of mutation breeding program, which limited next stages by quantity of material for selection. Here we report cytogenetic, plant growth and development characteristics of mutation induction variability of the new wheat varieties and some relationships between means of plants grows and developments, morphometrical parameters, cytogenetic characteristics and different concentrations and types of mutagens at first generation after DAB treatment.

Keywords: chemical mutagenesis, winter wheat, chromosomal aberrations, 1,4-bisdiazoatsetilbutan,

INTRODUCTION

DAB (1,4- bisdiazoatsetilbutan) as a mutagen is not so widely used for breeding practice as previous investigated in our experiment mutagens (gamma-rays and nitrosoalkylureas), but it more suitable for reverse genetic approach in modern functional genomics and for programs for genetic improving indigenous cultivars in South-East Asia (FAO-IAEA programs for coordinating investigations in chemical mutagens) (Shu *et al*, 2011). DAB as a mutagen factor is traditionally related to radio-mimetic chemical mutagens substances due to similarity of mutagen effects appearance amid this mutagen and physical mutagens like as gamma- and x-rays.

Mutagen effects on cell and whole plant level are the key factors which limited either winter wheat productivity for agricultural purpose or number of

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families for breeding program in obtaining next generation material for identification and selection of mutants. Consequences of mutagen action on cell level (chromosomal aberrations) are closely connected with future mutation rate. Influence of mutagen factors action is depended on next parameter: physiological parameter of mutagen action object, genotype of object, type of mutagen action (acute, chronicle), nature of mutagen, doses or concentration of mutagen, fractional of dose or concentration, time of exposure, concentration or appearance of free active oxygen, temperature and other environmental conditions (Zhang et al, 2015; Nazarenko, Kharytonov, 2016).

This article is a part of our investigation of recurrent mutagen treatment of winter wheat varieties. In previous parts we developed effects of genotype-mutagen interaction after gamma-ray irradiation and nitrosomethilurea (NMU) nitrosoethilurea (NEU) (Nazarenko, 2017a; Nazarenko, Izhboldin, 2017)

Recurrent mutagenesis includes the exposure to mutagen action of progeny of plants that had been treated in previous generation. The strategy of treating the progeny of previously treated plants is well-known as recurrent action. Investigators studied a wide range of mutagens including different types of physical mutagens (different types of radiation) and the chemical mutagen (EMS); the alternation of EMS with irradiation was also studied. The results of these experiments did not bear out the expected results and were at best mixed. In most cases, radiosensitivity, mutation rate and spectra remained unaffected with repeated irradiation of subsequent generations. In our investigations we used other types of chemical mutation factors (nitrosoalkylureas, DAB) and alteration these mutagens with gamma-rays. We obtained new results according to reduce radiosensetivity, mutagen depression after recurrent mutagenesis and determined some new laws for recurrent mutagen action. In case of mutagen alteration we ran on with trivial, normal reaction on mutagen action (Chaima *et al*, 2012, Nazaenko, 2017b).

DAB are related to special group of mutagens “supermutagens” (as classified by Rapoport). Special ability of this group is induction mutations on level of comparable mutagen without high damages, which influence on survival ability of plant material (Jovtcheva et al, 2002; Özel et al, 2015). Supermutagens induct 50-60 times more mutations than relevant by their consequences for surviving and plant development doses of gamma rays or fast neutrons (Albokari, 2014). But DAB in spite of previous chemical mutagens (nitrosoalkylureas), by its action more similar to physical mutagens (like as gamma-rays) than for other chemical mutagens and don’t so site-specific.

Other feature (general for all chemical mutagens) is induction of gen mutations on peculiar DNA-sequence rather than structural changes. It is depends on chemical nature of specific mutagen. That’s why chemical mutagenesis is one of the important methods for modern genetics investigations (as for example for reverse genetics, for different types of tilling’s methods). We can predict (in certain limits) more probably types of future mutations with higher rates (according to preferable DNA sequences for mutagen action) (Juchimiuk-

Kwasniewska, 2002; Natarajan, 2005).

Mutagenic effects of chemicals have been assessed by both analysis of chromosomal aberrations (Rakhmatullina and Sanamyan, 2007) and investigation plant development and grows at first generation under field conditions.

Parameters traditionally used to estimate the degree of plant injury in the M_1 generation are: 1. Seedling height, determined at a particularly stage soon after germination. 2. Root length, determined soon after germination in controlled environment conditions. 3. Emergence under field conditions or germination. 4. Survival under field or controlled environment conditions. 5. Number of florets, flowers or inflorescences per plant. 6. Number of florets or flower parts per inflorescence. 7. Number of seed set. 8. Number of seeds per plant (Khaled *et al*, 2016).

Mutated plants typically show reduced fertility, mainly caused by chromosomal changes during meiosis. Plant surviving, pollen fertility and yield structure were studied for identification of mutagen depression evident at first generation (Karthika and Subba, 2006; Nazarenko, 2017b).

Most of the observed effects in the M_1 generation are physiological. Plant injuries in the M_1 generation are indicative of the degree of the effects of mutagens on plants and can be determined quantitatively in various ways. Physical injury is commonly measured using such parameters as reductions in germinability of seeds, growth rates of seedlings, vigor, sterility and even lethality of plants.

Chromosomal abnormalities in irradiated mitotic cells range from breaks, through exchanges, laggards and anaphase bridges, dicentric and centric ring formations, terminal fragments with telomeric signal at only one end and interstitial fragments that appear as double minutes without any telomeric signals (Rakhmatullina and Sanamyan, 2007). For crops like wheat, individual tillers (side branches) originate from different cells of the embryo of the treated seeds. If an aberration occurs in one of these cells, it will be carried in the tiller developed from that cell (Bolzarn and Bianchi, 2006; Huaili *et al*, 2005; Shu *et al*, 2011).

The main purposes of investigations in this area were determination genotype-mutagen interaction for modern ukrainian winter wheat varieties, identify less sensitive for DAB genotypes and evolution recurrent mutagenesis as a method in difference alteration on first step of mutation breeding program, which limited next stages by quantity of material for selection.

MATERIALS AND METHODS

Seeds of (in brackets method of obtaining varieties or used mutagens) Favoritka, Lasunya, Hurtovina (irradiation of initial material by gamma rays), line 418, Kolos Mironovschiny (field hybridization), Sonechko (chemical mutagenesis, nitrosodimethilurea (NDMU) 0.005%) and Kalinova (chemical mutagenesis, DAB 0.1%), Voloshkova (termomutagenesis – low plus temperature at plant development stage of vernalizaion has been used as mutagen

factor) of winter wheat (*Triticum aestivum* L.) were subjected to chemical mutagen 1,4- bisdiazoatsetilbutan (DAB) – 0,1 and 0,2 % presoaked. Each treatment was comprised of 1000 wheat seeds. Exposition of chemicals mutagens was 18 hours. These concentrations and exposure are optimal for the breeding process that has been repeatedly established earlier. (Ahloowalia *et al*, 2004; Nazarenko, 2016). Non-treated varieties were used as a check for each variety.

Treated seeds were sown in rows with inter and intra-row spacing of 50 and 15 cm, respectively, to raise the M_1 population. M_1 plant rows were grown in three replications with check-rows of untreated varieties in every ten-row interval. Data on seed germination and surviving plants were recorded considering whole plots of M_1 population. Data on yield structure components (plant height, general number of culms, number of productive culms, spike length, spikelets per spike, number of grain per spike, grain weight per spike and plant, 1000 grains weight) were taken from 50 randomly selected plants of each treatment representing more or less all types of morphological plants (Sanamyan *et al*, 2010).

The seeds used in this study were of the M_0 generation. After mutagen treatment dry seeds were germinated in Petri dishes under 24 – 72 hours (depends on presoaking and mutagen action), temperature $+25^0$C. After wards central primary roots were cut and fixed in solution of alcohol and acetic acid (in proportion 3:1) for 24 hours. Fixation material was stored in 70% alcohol solution under temperature 2 ^{0}C (20 – 25 roots per variant). Cytological analysis was carried out by the standard method at temporary press-time preparations of root tips (1 – 1.5 mm) stained with acetocarmine (has been prepared by Remsderh). Tissue maceration (if it needs for analysis) was carried out at 45% solution of acetic acid (during 5 minutes on bane-marie under 60^0C). Anaphase of cell division was observed by light microscope JNAVAL. No less than 900 cells in proper phases of mitosis were observed in each variant, number of samples were about 20 – 25 per variant (Lifang *et al*, 2001; Rank *et al*, 2002; Natarajan, 2005; Nikolova *et al*, 2015).

Mathematical processing of the results was performed by the method of analysis of variance, the variability of the mean difference was evaluated by ANOVA. Used the standard tools of the program Statistica 8.0 for factor analysis (ANOVA module).

RESULTS AND DISCUSSION

Analysis of grows and development of plants

In M_1 population, observations were recorded seed germination and plant surviving, pollen fertility, plant height, spikes/plant, spike length, kernels/spike, 1000-grain weight, yield/plant (table 1 – 2). Standard error (±SE) values of the treated populations are at tables too.

The results on germination of seeds, survival rate of plants derived from treated and untreated seeds are tabulated (Table 1). Germination and survival

abilities of seeds reduce compared to untreated seeds of the initial variety in all cases.

Germination and survival abilities of seeds reduce compared to untreated seeds of the initial variety in all cases except one (Hurtovina, DAB, 0.1 %). Plant survival ability ranges from 85 (Favoritka) to 80% (Kolos Mironivschini, Hurtovina) at 0.2% DAB, while it ranged from 98 to 92% under untreated control. Some varieties have been shown this parameter without significant difference from difference concentration (between 0.1 and 0.2 – Kalinova, Sonechko, Favoritka, Lasunya).

In general, the correlation between the concetration value and survival abilities of plants is on least level from other mutagens (-0.5).

Table 1. Main parameters of grown of winter wheat plants at M_1 generation

Trial	Germination, %	Survival after winter, %	Germination, %	Survival after winter, %
Variety	Kolos Mironivschini		Kalinova	
Check	98±0.57	91±0.93	94±0.94	88±0.98
DAB, 0.1%	85±1.03*	84±0.96*	84±0.51*	83±0.68*
DAB, 0.2%	82±0.63*	80±1.01*	81±0.79	81±0.78
Variety	Voloshkova		Sonechko	
Check	92±0.57	87±0.93	94±0.94	89±0.98
DAB, 0.1%	84±0.96*	84±1.10*	84±1.02*	84±1.10*
DAB, 0.2%	80±0.74*	79±0.90*	84±1.06	83±1.10
Variety	Favoritka		Hurtovina	
Check	98±0.57	91±0.93	92±0.94	84±0.98
DAB, 0.1%	84±0.92*	84±0.98*	85±0.71*	84±1.10
DAB, 0.2%	86±0.98	85±1.11	81±0.86*	80±1.10*
Variety	Lasunya		Line 418	
Check	98±0.57	94±0.93	93±0.94	92±0.98
DAB, 0.1%	86±1.11*	86±0.90*	89±1.14*	88±1.10*
DAB, 0.2%	84±1.70	83±1.16	85±0.61*	84±1.09*

* - difference is statistically significance from check at $P_{0.05}$

Correlation between the concentration of mutagens and pollen fertility was -0.68 (table 2). It was significantly lower in comparison to gamma-ray and nitrosoalkylureas. For some varieties such as Favoritka, Hurtovina, Lasunya there were not any statistically difference between DAB 0.01 and untreated check. As we can see from these tables parameters of surviving and pollen fertility are not so responsible on mutagen action (in spite of previous mutagens) and, as a consecutive, only partly suitable for evolution mutagen depression in case DAB.

Table 2. Pollen fertility after mutagen action, %

Trial	Kolos Mironivschini	Kalinova	Voloshkova	Sonechko	Favoritka	Hurtovina	Lasunya	Line 418
Check	95.0	93.1	89.7	96.7	95.7	98.6	96.8	93.0
DAB, 0.1%	95.4	90.0*	73.4*	90.5*	93.1	95.9	94,2	89.7*
DAB, 0.2%	88.3*	88.7*	70.0*	88.8*	89.4*	90.8*	91.5*	88.1*

* - difference is statistically significance from check at $P_{0.05}$

All parameters of the crop yield structure have been studied. Components such as plant height, 1000 grain weight, grain weight per plant, number of grains per spike, grain weight per spike, general number of culms, number of productive culms, spike lengths have been developed. Only three (plant height, grain weight per spike, and 1000 grain weight) showed statistically difference level of mutagen depression under any concentration action. But for this type of mutagen sometimes we observed luck of mutagen depression under DAB 0,1 and (for one variety) DAB 0.2, in spite of other mutagens (Nazarenko and Kharytonov, 2016; Nazarenko, 2017b).

Regarding the plant height, correlation between the concentration and the indicator constituted -0.68, (high invert correlation). This parameter decreases if the concentration increases. Gradual decrease in height is a tendency, but we ran on with substantial differences between the varieties. Variety Kalinova has not been shown depression effect under any concentration of DAB.

The indicator of grain weight per spike was not informative in case of DAB, 0.1 %. The correlation coefficient was -0.52. It was reliable only under DAB 0.02 % concentration.

The thousand grain weight is the most reliable for mutagen depression evalution (similar as other mutagens). We observed depression in all variants except Kalinova, DAB 0.1 The correlation coefficient was -0.71.

Variety Kalinova are less sensitive to DAB as mutagen compared with other genotypes for all variants.

Table 3. Correlation between DAB concentration and some components of yield structure of M_1 varieties.

Parameter	Plant height	Number of culms	Spike lengths	Number of spikelets	Number of grains per spike	Grain weight per spike	Grain weight per plant	1000 grain weight
Concentr.	-0.68	-0.20	0.06	-0.08	-0.42	-0.52	-0.41	-0.71

Chromosomal aberrations analysis

At table 4 we represent dates of the results of next parameters analyzed: general number of observing mitosis in primary roots tips, number of cells in appreciate phase with visible chromosomal aberrations rearrangements, total rate of chromosomal aberrations. As we can see from table 1 frequencies of aberrations were changed from 3,3 % (Kalinova, DAB 0,1 %) to 14,2 % (Voloshkova, DAB 0,2 %) percent from total number of cells in division in experimental microscope samples. All the variants are statistically substantially dissimilar from each other and from the check.

Lower frequency of aberrations in any cases peculiar to varieties obtained by chemical mutation breeding (Sonechko, Kalinova).The higher frequency of aberrations has been obtained by used DAB 0,2 as usual.

Rates of chromosomal aberrations was statistically lower when we used DAB for varieties obtained with chemical mutagenesis. The same situation we observed in case of varieties Kalinova and Sonechko, when NMU and NEU have been used in our previous investigations. Therefore, we can affirm DAB as mutagen by the nature not so depends on object genotypes as nitrosoalkylureas and less damaging mutagens with lower rates. Varieties Sonechko and Kalinova are less sensitive to DAB action. DAB as a mutagen initiated least rates of chromosome damages than nitrosoalkylureas or gamma-rays (Nazarenko, 2016). We can range mutagens in next sequence by its genetic activity (from least to pick) DAB → NEU → NMU → gamma-rays.

From the Table 1 we can see that the higher rates of chromosomal changes in any cases characteristic for variety obtained by mutation breeding with using of thermomutagenesis (Volochkova). All other varieties (field hybridization and after irradiated) were similar by its reaction on DAB – rate of chromosomes rebuildings on 9 – 10 percent. According to this fact recurrent mutagenesis is acceptable method for exploit DAB as a mutagen if we exploit physical mutagens or classical breeding methods for obtaining objects of action (for example DAB after gamma-rays in our pattern or vice versa).

We developed next types of aberrations of chromosomes after investigation of spectra in our samples: chromosomal bridges and double-bridges, fragments of chromosomes and double-fragments, micronucleus, lagging chromosomes. Cases with complicated aberrations (two or more kinds of changes in one mitosis) and ratio fragments till bridges were counted up singly (Table 5). Number of any type of chromosomal changes was leaped with dose ascended (correlation coefficients is on 0,6 – significantly lower than for other mutagens). Previously we observed this evident in our investigations, when more bridges than fragments have been induced with gamma-rays (fragments-bridges ratio lower than 1) too (Nazarenko, 2017a). In this case, like as nitrosoalkylureas, more fragments and double-fragments were caused by DAB (fragments-bridges ratio more than 1) (Nazarenko and Izhboldin, 2017)). We will be able to use this parameter for identify difference between gamma-rays action and chemical mutagenesis in case of unknown mutagen factor.

Table 4. Frequency of chromosomal aberrations in M_1 generation of winter wheat varieties

Variable	Mitosis, number	Chromosomal aberrations		Mitosis, number	Chromosomal aberrations	
		n.	%		n.	%
	Favoritka			Line 418		
Check	984	19	1.93±0.31	962	11	1.14±0.11
DAB, 0.1%	1048	139	5.92±0.69*	906	106	4.01±0.64*
DAB, 0.2%	934	179	10.13±1.03*	983	188	8.99±0.88*
	Lasunya			Hurtovina		
Check	1056	15	1.42±0.19	1034	12	1.16±0.11
DAB, 0.1%	1019	121	5.52±0.69*	1005	143	6.00±0.74*
DAB, 0.2%	844	161	10.19±1.06*	1022	223	11.17±1.05*
	Sonechko			Voloshkova		
Check	1026	8	0.78±0.04	1003	31	3.09±0.34
DAB, 0.1%	1027	56	5.78±0.33*	1002	142	7.99±0.80*
DAB, 0.2%	981	108	8.64±0.51*	912	207	14.20±1.11*
	Kalinova			Kolos Mironivschini		
Check	1047	9	0.86±0.11	909	10	1.10±0.13
DAB, 0.1%	1009	106	3.30±0.14*	1016	129	5.78±0.73*
DAB, 0.2%	851	133	7.60±0.43*	917	190	10.26±1.02*

* – difference statistically significant on $P_{0,01}$

Number of complicated (or combined) aberrations was significantly lower as well as micronucleus and lagging chromosomes then for previous mutagens. Generally, when concentration of DAB was increased the rate of fragments and bridges also has increased. But other situation was for other types. We couldn't observe these types for variety Kalinova at all, only fragments and bridges. For line 418 complicated aberrations are also absent, but present other types. For varieties Favoritka, Lasunya, Hurtovina (all varieties are radiomutants) in some variants under DAB action complicated aberrations are absent too. On the other hand micronucleus, lagging chromosomes are absent for varieties Sonechko and Hurtovina.

The results of three-factor analysis ("genotype", "dose (concentration)" and "mutagen"; in general scheme of analyze we include our date from previous investigation of chemical mutagens and gamma-rays action (Nazarenko and Kharytonov, 2016) shown us that, prevalently, on the rate of chromosome aberrations factor "dose" influenced, then "genotype", then the "mutagen". The part of second and third factors are increased as compared with previous investigations. Thus, we developed that repeated exposure to the similar mutagen

(for example, DAB on the variety obtained by the action of this mutagen) leads to a substantially lower rate of chromosomal aberrations, absence some types of rarely aberrations in spectra.

CONCLUSIONS

The most informative parameters to determine the degree of mutagenic depression in the first generation for plant growth and development were germination and survival rates, pollen sterility, 1000 grain weight. But these parameters are partially suitable for mutagen depression evolution in case of DAB. The least level of mutagen depression by morphometrical indicators we observed in case of Kalinova. Therefore, chemical mutation varieties are less sensible for to same chemical mutagens both either for DAB or for nitrothoalkylureas

DAB as a mutagen substantially lower in chromosomal aberration induction in comparison with previous mutagens (gamma-rays and nitrosoalkylureas). We ranged mutagens in next sequence (from least to pick) DAB → NEU → NMU → gamma-rays. We can predict less quantity of mutations if we use DAB for mutation breeding purpose.

Chemical mutations varieties Kalinova and Sonechko are less sensitive to recurrent mutagenesis with this mutagen. In spite of other chemical mutagens DAB was less peculiar in genotype-mutagen interaction by the level of chromosomal rebuildings, but more peculiar in spectra of aberrations. Complicated (or combined) aberrations haven't been observed for Kalinova and we have not developed micronucleus, lagging chromosomes after DAB action for Kalinova and Sonechko.

Comparing between bridges and fragments after DAB action confirmed reliability of fragments-bridges ratio (prevalence of fragments under bridges for chemical mutagens and opposite situation for gamma-rays) for mutagen nature identification. In general, the rate of any kind of chromosomal aberrations is linearly increased with increase dose of the mutagen.

Recurrent mutagenesis is acceptable method for exploit DAB as a mutagen if we exploit physical mutagens or classical breeding methods for obtaining objects of action.

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