

Rates and spectra of chromosome aberrations in winter wheat cells after dimethylsulfate action

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In the article we report about our investigation of cytogenetic parameters in chromosomal complex of the new Ukrainian winter wheat varieties and some relations amid value of cytological indexes and different concentrations of DMS (dimethylsulfate). We used the anaphases method for the investigation of chromosomal rearrangements and determination the mutagen action on plants and identification the nature of mutagen. We studied combined the sensitivity of genotype by the cytological analysis of mutagen treated population of wheat and revealed the reaction of different varieties depending upon the way of its origin. This was done for determining its connections and differences on cytogenetics level together with specificity of mutagen action on cell level. Dry seeds of seven varieties and one line of winter wheat were subjected to DMS in 0.0125, 0.025, and 0.05 % concentrations, which are the most useful for winter wheat in terms of genetic investigation programs. We investigated the rates and spectra of chromosomal aberrations in winter wheat (primary roots of tips cells) during the mitosis. We identified the following types of chromosomal rearrangements: chromatid and chromosome bridges, single and double fragments, micronuclei, and lagging chromosomes. Investigation of DMS action confirmed reliability of fragments-bridges ratio (prevalence of fragments under the bridges for chemical mutagens and vice versa for physical mutagens) for the mutagen nature identification.

Key words: chromosomal aberrations; recurrent mutagenesis; dimethylsulfate; wheat; genotype

Introduction

DMS (dimethylsulfate) as a mutagen is widely used for special investigations in plant genetics structure, it the one of the most often used for reverse genetic approach mutagens in modern functional genomics (other exploited substance is related to DMS (in the same group of chemical mutagens) ethilmethansulphat, EMS (Shu et al., 2011). DMS as a mutagen factor is traditionally related to the same family of mutagens (supremutagens), which used in our previous investigations (Nazarenko, 2016; Nazarenko, Izhboldin, 2017; Nazarenko, 2017) due to mechanism of action on DNA-level, but with a little distinguishes (Eiges, 2013).

Mutagen effects on cell and whole plant level (named as mutagen depression) are the key factors which limited either winter wheat productivity for agricultural purpose at first generation (due to some modern investigations at next generations too (Mangi et al., 2016) or quantity of materials obtained for next stapes of mutation breeding programs after mutagen action for identification and selection of mutants. Consequences of mutagen action on cell level (chromosomal aberrations) are closely connected with future mutations 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, 2016).

This article is a forth 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 nitrosomethylurea (NMU), nitrosoethylurea (NEU), 1,4- bisdiazotsetilbutan (DAB) (Nazarenko, 2016; Nazarenko, Izhboldin, 2017; Nazarenko, 2017). In spite of previous mutagens DMS didn't use in breeding of varieties, which we used for investigation.

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. 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 (Ali Sakin et al., 2005; Waugha, 2006). In our investigations we used other types of chemical mutation factors and alteration these mutagens with gamma-rays. And we obtained other valuable results according to reduce radiosensitivity, mutagen depression but also decrease in mutation rate in case of the same mutagen. In case of mutagen alteration we collide with trivial, normal reaction on mutagen action (Nazarenko, 2017).

In modern science practice three main methods exploited for investigation mutagens action on chromosomal level. First, the oldest one, is used as observance different types of chromosomal aberration appearance in mitotic cells during cell division. Second, FISH (fluorescence in situ hybridization), is based on involved hybridization of chromosome specific DNA probes to metaphase preparations followed by detection with immuno-staining procedures. This technique allows detection of translocations involving different painted chromosomes (Jovtcheva et al., 2002). Third one is the micronucleus (MN) test in detecting small micronuclei compared with untreated and treated objects (Juchimiuk-Kwasniewska et al., 2011; Jankowicz-Cieslak et al., 2017).

In our investigations we used first method due to preferences of this trivial approach which are simplicity, objectivity of the results, the reliability and the ability to assessment the impulse of integrated wide variety of mutagens by nature (Karthika, Subba, 2006; Albokari, 2014).

Analysis of variability of chromosomal aberrations after mutagen action of any type of mutagens by anaphases method is one of the well-known and the most precision methods which we can exploited for determine fact of mutagen action, identify nature of mutagen (Lifang et al., 2001; Adlera et al., 2004; Natarajan, 2005; Ukai, 2006). For example, this method is widely used as for determine of radionuclide's pollution of environment, its level, danger of this pollution as for identification optimal doses of radiation and concentrations of chemical mutagens in breeding practice (Ahloowalia et al., 2004; Nazarenko, 2017). A relation amid clastogenic adaptation shown in descending of chromatid type of changes, micronuclei and changes in number of chromosomes in cells, and the clastogenic effect has been appeared (Grant, Owens, 2001; Bignold, 2005).

Influence of different types of chemical mutagens or any type ionizing radiation according to this method can be analyzed by summarized number and kinds of chromosomal damages (Rakhmatullina, 2007). Both structural and numerical aberrations occur spontaneously due to intraneous and extraneous factors and appear in terms of last mitotic cell division phases (Nikolova et al., 2015).

Changes in chromosome number and structure in mitotic cells is initiated after mutagen action. Chromosomal changes rank 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 changes in irradiated mitotic cells (Bolzar'n, Bianchi, 2006; Rakhmatullina, 2007).

Joining with gene mutations, chromosomal aberrations in the form of different structure rebuilding (deletions, inversions, translocations, etc) were generates (Shu et al, 2011). Translocations are the most important for mutation breeding kind of changes on chromosome level, which are possibly result from the rejoining of broken chromosomes districts. Cytological aberrations observed in cells mitosis include the production of micronuclei and other types of chromosomal changes (Ukai, 2006; Shu et al., 2011).

All the effects, which are summarized above have led to many practical protocols. In vitro "chromosome aberration test" in cultured mammalian cells and in vivo "micronucleus assay" in bone marrow cells have been incorporated in the guide lines of mutagenic testing by national and international regulatory bodies. Several international inter-laboratory studies were carried out in 1970s and 1980s to standardize the test protocols and these exercises are still going on (Natarajan, 2002).

The main purposes of investigations are determining mutagen factor damage ability at cell level and capability for producing difference types of chromosomal aberrations and, in perspective, mutations in next generations, definition of cytogenetic variability of the modern Ukrainian winter wheat varieties and relations between chromosomal rearrangements and different concentration of DMS.

Material and methods

Winter wheat seeds (*Triticum aestivum* L.) (approx. 14% moisture content) of (in brackets method of obtaining varieties or used mutagens) Favoritka, Lasunya, Hurtovina (irradiation by gamma rays), line 418, Kolos Mironovschiny (field hybridization), Sonechko and Kalinova (chemical mutagenesis), Voloshkova (termomutagenesis – low plus temperature under vernalization has been used as mutagen factor) were subjected to dimethylsulfate (DMS) – 0.0125, 0.025 and 0.05 % 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.

The seeds used in this study were of the M_0 generation. After mutagen treatment dry seeds were germinated in Petri dishes under 24–48 hours (depends on presoaking and mutagen action), temperature +25 °C. Afterwards central primary roots were cut off (when these length up to 10 – 15 mm.) 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 °C (30–35 roots per variant). We conducted cytological analysis in trivial way at temporary press-time preparations of primary roots tips (1–1.5 mm length) stained with acetocarmine (has been prepared by Remsderh). Tissue maceration was conducted at 45% solution of acetic acid (during 5 minutes under 60°C). 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).

Statistical analyze obtaining dates was conducted by the method of multi-factor and discriminant analysis, the variability of the parameter difference was evaluated by ANOVA, used the standard tools of the program Statistica 8.0. Data in tables presented like mean \pm standard error.

Results and discussion

In Table 1 we represent data of next parameters analyzed: general number of observing mitosis in primary roots tips, number of cells in appreciate phase with visible chromosomal aberrations rearrangements, and total rate of chromosomal aberrations. As we can see the frequency of aberrations were changed from 9.96 % (Lasunya, DMS 0.0125 %) to 30.0 % (line 418, DMS 0.05 %) from total number of cells in division in experimental microscope samples. All the variants are statistically different from each other and from the control.

Table 1. Rate of chromosomal aberrations in winter wheat division cells

Variant	Mitosis, number	Chromosomal aberrations		Mitosis, number	Chromosomal aberrations	
		number	percent		number	percent
		Favoritka			418	
Control	984	19	1.9 \pm 0.3	962	11	1.1 \pm 0.1
DMS 0.0125%	1001	127	12.7 \pm 1.1*	850	85	10.0 \pm 1.0*
DMS 0.025%	911	174	19.1 \pm 1.3*	939	178	19.0 \pm 1.4*
DMS 0.05 %	564	147	26.1 \pm 1.6*	1009	302	30.0 \pm 1.9*
Variety		Lasunya			Hurtovina	
Control	1056	15	1.4 \pm 0.2	1034	12	1.2 \pm 0.1
DMS 0.0125%	1004	100	9.96 \pm 0.9*	1010	110	10.89 \pm 1.0*
DMS 0.025%	1017	163	16.02 \pm 1.3*	895	161	17.99 \pm 1.4*
DMS 0.05%	717	166	23.14 \pm 1.5*	581	142	24.44 \pm 1.6*
Variety		Sonechko			Voloshkova	
Control	1026	8	0.8 \pm 0.1	1003	31	3.1 \pm 0.3
DMS 0.0125%	1014	101	10.0 \pm 1.0*	1016	104	10.2 \pm 1.0*
DMS 0.025%	985	145	14.7 \pm 1.1*	892	153	17.2 \pm 1.3*
DMS 0.05%	509	99	19.5 \pm 1.3*	511	129	25.3 \pm 1.5*
Variety		Kalinova			Kolos Mironivschini	
Control	1047	9	0.9 \pm 0.1	909	10	1.1 \pm 0.1
DMS 0.0125%	1010	101	10.0 \pm 1.0*	1040	124	11.9 \pm 1.1*
DMS 0.025%	917	157	17.1 \pm 1.2*	892	173	19.40 \pm 1.5*
DMS 0.05%	649	137	21.1 \pm 1.4*	639	177	27.70 \pm 1.7*

* – difference statistically significant on P_{0,05}

In spite of previous investigation the level of rates of aberrations in any cases peculiar to varieties obtained by chemical mutation breeding (Sonechko, Kalinova) didn't differ from other varieties and we can predict that rates of mutations at next generations wouldn't change. The higher frequency of aberrations has been obtained by used DMS 0.005 % (as usual for any mutagen factor in less than critical concentration).

Rates of chromosomal aberrations in all cases were on comparable level. No one variety didn't stand out against other. Influence of DMS is compared with gamma-rays (in spite of other chemical mutagens) and, not only by aberration rates, but by parameters of spectra of chromosomal aberrations is very similar to gamma-irradiated. We can range mutagens in next sequence by its genetic activity (from lowest to peak) DAB (Nazarenko, 2017) \rightarrow NEU \rightarrow NMU (Nazarenko, 2016) \rightarrow gamma-rays (Nazarenko, Izhboldin, 2017) \rightarrow DMS.

From Table 1 we cannot identify in any way varieties or group of varieties more or less sensitive to DMS action. Changeability on cell level was on comparable mean for all genotypes. Distinct from previous investigation not recurrent, only repeat mutagen action doesn't depend on object genotype.

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 2).

Number of any type of chromosomal changes was leaped with concentration ascended (correlation coefficients 0.87 was significantly higher than for other chemical mutagens, comparable with gamma-rays). In this case, like as for nitrosoalkylureas (Nazarenko, 2016) and DAB (Nazarenko, 2017), more fragments and double-fragments were caused by DMS action (fragments-bridges ratio more than 1), except some variants with difference concentration of DMS, which depends on genotype (variety) (sometimes equally or slightly less than 1, as for example at any concentrations for variety Voloshkova)) (Nazarenko, 2016; Nazarenko, Izhboldin, 2017; Nazarenko, 2017).

But in the most cases 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 2. Types of chromosomal rebuildings at winter wheat first generation

Variant	Fragments		Bridges		Fragments / bridges	Micronucleus, lagging		Complicated aberrations	
	(single and double)		(chromosome and chromatide)			number	percent	number	percent
	number	percent	number	percent					
Favoritka, control	6	54.6	5	45.5	1.2	0	0.0	2	18.2
DMS 0.0125%	65	51.2	60	47.2	1.1	2	1.6	8	6.3
DMS 0.025%	74	42.5	81	46.6	0.9	19	11.0	19	10.9
DMS 0.05 %	72	49.0	63	42.9	1.1	12	8.2	41	27.9
Lasunya, control	4	26.7	11	73.3	0.4	0	0.0	0	0.0
DMS 0.0125%	47	47.0	51	51.0	0.9	2	2.0	8	8.0
DMS 0.025%	84	51.5	73	44.8	1,2	6	3.7	17	10.4
DMS 0.05 %	88	53.0	76	45.8	1.2	2	1.2	21	12.7
Hurtovina, control	7	58.3	5	41.7	1.4	0	0.0	0	0.0
DMS 0.0125%	53	48.2	55	50.00	0.9	2	1.8	9	8.2
DMS 0.025%	78	48.5	74	46.0	1.1	9	5.6	27	16.8
DMS 0.05 %	71	50.0	60	42.3	1.2	11	7.8	38	26.8
Line 418, control	6	54.6	5	45.5	1.2	0	0.0	2	18.2
DMS 0.0125%	49	57.7	30	35.3	1.6	6	7.1	8	9.4
DMS 0.025%	89	50.0	76	42.7	1.2	13	7.3	21	11.8
DMS 0.05 %	137	45.4	141	46.7	0.9	24	7.9	43	14.2
Kolos Mironovschiny, control	5	50.0	5	50.0	1.0	0	0.0	0	0.0
DMS 0.0125%	61	49.2	59	47.6	1.0	4	3.2	17	13.7
DMS 0.025%	80	46.2	85	49.1	0.9	8	4.6	22	12.7
DMS 0.05 %	87	49.2	83	46.9	1.1	7	4.0	19	10.7
Sonechko, control	6	75.0	2	25.0	3.0	0	0.0	0	0.0
DMS 0.0125%	56	55.5	43	42.6	1.3	2	2.0	12	11.9
DMS 0.025%	60	41.4	66	45.5	0.9	19	13.1	34	23.5
DMS 0.05 %	41	41.4	35	35.4	1.2	23	23.2	54	54.6
Kalinova, control	2	22.2	7	77.8	0.3	0	0.0	0	0.0
DMS 0.0125%	59	58.4	40	39.6	1.5	2	2.0	12	11.9
DMS 0.025%	88	56.8	64	41.3	1.4	3	1.9	26	16.8
DMS 0,05 %	80	58.4	46	33.6	1.7	11	8.0	38	27.7
Voloshkova, control	16	51.6	13	41.9	1.2	2	6.5	5	16.1
DMS 0.0125%	50	48.1	48	46.2	1.0	6	5.8	14	13.5
DMS 0.025%	65	42.5	64	41.8	1.0	24	15.7	41	26.8
DMS 0.05 %	59	45.7	60	46.5	1.0	10	7.8	40	31.0

Number of complicated (or combined) aberrations was significantly higher as well as micronucleus and lagging chromosomes then for previous mutagens. Moreover, in some cases number of complicated aberrations was more than for gamma-rays. But for some varieties we cannot observe any difference with this parameter between DMS 0.025 % and 0.05 %. Generally, when concentration of DMS was increased the rate of fragments and bridges also has increased. Complicated aberrations for this type of action is a value parameter for mutagen influence evaluation. Significance of parameters was satisfied by discriminant analyze (Table 3).

Table 3. Results of discriminant analysis parameters of rate and spectra of chromosomes rearrangements

Parameter in model	λ	F-remove (4,51)	p-level
Rate of aberrations	0.61	9.01	0.00
Fragments (single and double)	0.44	7.12	0.01
Bridges (chromosome and chromatide)	0.31	6.03	0.02
Micronucleus, lagging chromosomes	0.07	0.89	0.35
Complicated aberrations	0.22	5.83	0.04

The results of two-factor analysis ("genotype" and "concentration" shown us that, prevalently, on the rate of chromosome aberrations factor "concentration" influenced, the "genotype". Genotype strong influenced on parameter fragments/bridges ratio ($F= 18.92$; $F_{cr}= 4.92$; $p\text{-level } 0.01$; $F= 12.64$; $F_{cr}= 4.92$; $p\text{-level } 0.01$).

Thus, we developed that repeated exposure to the other mutagen (DMS on the variety obtained by the action of other mutagen) doesn't lead to important difference between genotypes.

Thereby, investigation of DMS action confirmed reliability of fragments-bridges ratio (prevalence of fragments under bridges for chemical mutagens and vice versa for gamma-rays) for mutagen nature identification. Complicated (or combined) aberrations is a valuable parameter for dose evaluation under DMS action. Genotype-mutagen interaction has been shown in bridge – fragments ratio. Previous rules for other mutagens was confirmed for DMS (with some peculiarities).

Conclusions

To sum it up, DMS as a mutagen substantially stronger in chromosomal aberrations induction in comparison with previous chemical mutagens (DAB and nitrosoalkylureas) and compare with gamma-rays by its action on cells. We ranged mutagens in next sequence (from least to pick) DAB → NEU → NMU → gamma-rays, DMS. We can predict high level of gen changes if we use DMS for mutation breeding purpose.

Repeated action of chemical mutagen doesn't lead to the same consequences for mutation varieties as recurrent action. There isn't some significant difference between genotypes regarding the method of breeding.

Compared the bridges and fragments after DMS action we confirmed the reliability of fragments-bridges ratio (prevalence of fragments under the bridges for chemical mutagens and opposite situation for gamma-rays) for mutagen nature identification; but for this mutagen the other situation could be observed in case of some genotypes (0.9 – 1.0). In spite of other mutagens, for DMS the genotype-mutagen interaction has been revealed in such a way. In general, the rate of chromosomal aberrations is linearly increased with concentration of the mutagen.

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