

Ecological Consequences of Increasing Crop Productivity

Plant Breeding and Biotic Diversity

Editors

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CHAPTER 23

CHEMICAL MUTAGENESIS AS A METHOD OF BASELINE GENETIC DIVERSITY ENLARGEMENT IN PLANT BREEDING

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23.1 INTRODUCTION

Biological potential of a field depends totally on the variety of genetic system, which is manifested by the degree of effective use of ecological potential, which is created by humans with technical equipments; hence, rentable technical and soil-reclamation operations depend on it.

Vavilov [1] defined breeding as an evolution directed by human power. Breeding is based on the selection of plants with the changes caused by changes in their genotype. Understanding of the variability is of fundamental importance in studying the baseline, which is the most important part of breeding.

Wide use of modern methods of studying genetic effect enables us to penetrate into the most important processes, which make the base of heredity, to discover its regularity, as well as to solve one of the main tasks of breeding—creating the genetic diversity of baseline [2].

Gene and chromosome mutations are the main source of all heredity variabilities and the materials for evolution and breeding [3]. According to the law of homologous series proposed by M.I. Vavilov, the world collection of breeders can be reproduced by means of mutation [4].

Mutations are heredity change, making genetic base of variability and the raw material for selection. As Vavilov affirms [5], mutations are significant in winter wheat forms created by natural and artificial hybridization.

Mutation variability underlies any baseline for breeding, as the initial primary changeability arises only as a result of mutation. Breeding and artificial selection are based on the processes of spontaneous changeability of genes and chromosomes. Spontaneous mutational changeability is important in evolution of an organic world, although it arises with low frequency [6–8].

Primitive breeding, based on the selection of spontaneous mutations and hybrids for thousands of years, have created valuable forms of crops hard to be improved, even with the use of modern breeding methods.

Mutations are displayed in changes of plant organs (root, stem, leaf, inflorescence, flower, fruit, and seeds) and their study gives rich material for taxonomy. Experimental mutagenesis use enables to disclose all the possibilities of species in forms on the basis of received polymorphism and to create rich collections of genetically diversity of some plants species on the basis of the received mutations.

Mutation process is a “supplier” of new elementary material; it supports genetic heterogeneity of natural populations. However, plant breeding could not be based on using only spontaneous mutations as their frequency is quite low. The induced mutagenesis is a powerful method of solving multiple theoretical and practical tasks of genetics and breeding.

Methods of induced mutagenesis complement all the other parts of initial material studied, as they enable to involve the variegated material in the form of gene and chromosome mutations into the breeding, although the mutations are just a baseline as they can give rise to new varieties only after they have undergone strict selection, in some cases even with hybridization [9].

Experiments obtaining mutations and their applications in plants breeding have attracted attention only after discovering high mutagen activity of ionizing radiation in mushrooms [9, 10] and higher plants [11].

Rapoport [12–15] and Auerbah [16] discovered a lot of new highly active mutagens. In his further scientific works, starting from 1957 at the Institute of Chemical Physics of the USSR Academy of Sciences, I.A. Papoport discovered another large amount of highly active substances—supermutagens, which cause hundreds and thousand times more mutations compared with nonorganic chemical compounds. Williams [17] accentuates the importance of mutations for breeding, stating that plant breeding is based on the definition and use of mutations that strengthen their characteristics in the desired way.

Nowadays, hundreds of chemical compounds with mutagen properties are known. Soyfer [18], while studying chemical mutagens action mechanism, discovered alkylate compound reaction with both nitrogen base and DNA phosphate groups. These reactions can finally result in destroying purine base, which may cause spot mutation and DNA sugarphosphatic skeleton breakage, which causes chromosome aberration.

Chemical mutagens are strong factors inducing changes in genetic material.

Primary damages of DNA molecules with chemical mutagens include disturbance in molecule structure (acridine inclusion and phosphorus group alkylation), transformation of DNA normal base into atypical one (either because of their chemical modification or because of their change in the process of replication on the structural analogue), and destruction of bases. These damages can be repaired, saved, or transformed into new changes of molecules and exhibited through change of their characteristics. Specificity of these changes can exhibit in the initial physiological and genetic effects: early or distant death of plants; growth and development slowdown or stimulation; and frequency of chromosome reformation.

Induced mutations make a wide spectrum of forms by changing any agricultural and farm-valuable features of crops. That is why quite an easy way to work out the weak points in plant breeding of any crop seemed possible. That is, a perspective of improvement of single characteristic with simultaneous saving, the complex of other useful practical characteristics in breeding varieties was considered. Yet, it turned out that working out the weak points, that is to improve one or a few characteristics possible is not always possible. Mutation changes through mutation effect and other interaction of genes often envelop in the entire organism. As a result, undesirable agricultural or breeding features may arise along with the newly developed ones.

Research aims. The research aims at detecting cytogenetic activity of chemical mutagens depending on the genotype variety and proving the possibility of selection and identification of winter wheat lines of mutagen origin, selected for morphological characteristics in generations of genetically unstable mutants.

23.2 MATERIALS AND METHODOLOGY

Investigated cytogenetic and mutagen action of eight alkylate DNA compounds: *N*-nitrozo-*N*-methyl urea (NMU), *N*-nitrozo-*N*-ethyl urea (NEU), *N*-nitrozodimethyl urea (NDMU), dimethyl sulfate (DMS), diethyl sulfate (DES), nitrozomethylbiuret (NMB), 1,4-bisdiazoacetylbutane (DAB), and ethylene imine (EI). The mutagens

synthesized at the Department of Chemical Mutagenesis of the Institute of Chemical Physics (Moscow) were kindly granted for our research. Mutagen influence of these compounds was investigated in Ukrainian and foreign varieties and in the variety mutants M_3 – M_7 induced by us. During the research period (1981–2012), we studied 50 variety samples that underwent the influence of mutagens.

Dry seeds of the variety samples were soaked in room temperature for 4–18 h in water solutions with mutagen concentration values of 0.05, 0.025, and 0.0125 percent. After soaking, the seeds were washed with water for an hour, air-dried, and 500 seeds of each variant were sowed in the field and 100 seeds—in Petri dish for cytological analysis. The soaked seeds were the control.

Cytogenetic activity of mutagens was studied by frequency of cytogenetic aberrations in meristem cells of initial roots of seeds and soaked in mutagen solutions. Then they were fixed in solution of Karnua changed fixer on temporary acetocarmine substances in late anaphase and early telophase. Minimum 600 cells were examined for each variant.

M_1 plants were observed from their germination to maturity. Changed forms account in M_1 plants were accounted on their height and the amount of changed families was counted in M_2 .

We chose plants with morphological changes, made individual biometrical analysis, and the seeds were sowed for the heredity checking in M_2 and further generations. The rest of M_1 plants were gathered, individual analysis was made, and spike sown (main spike) that guaranteed avoiding mechanical infestation with other forms.

Seeds from individually chosen plants in M_1 – M_3 plants with morphological changes were sowed in baseline nursery for their further examination compared with initial varieties.

In our research study with constant mutagens, we used individual selection for breeding and agricultural valuable characteristics; in nonstable, the continuous selection by pedigree method in combination with mass selection of plus-variants in 39 generations after selection of mutant plant was used.

Identification of genotypes by electrophoresis spectrum of gliadins was conducted by the results of the analysis of 100 kernels of each line. Electrophoresis of gliadins in polyacrilamide gel (PAAG) was performed in “Molecular markers” laboratory of Bila Tserkva National Agrarian University.

Biometrical analysis was made by well-known methods on an average sample of 20–30 plants. Statistic analysis was performed by methods of descriptive statistics, correlation, and variance analysis with “Statistic” v.6.0 program.

23.3 RESULTS AND DISCUSSION

Strong chemical mutagens are characterized with their different influence on plants cells. Many of them have cytotoxic impact on all levels of cell organization. Mechanism of cytotoxic effect shows a wide range of interaction of chemical agents that can enter into reaction with both gene material and cytoplasmic enzymes. So, toxic influence of mutagens can be a reason for structural damage of chromosomes or for inactivation of essential enzymes.

Molecules of mutagen while penetrating through cell membrane enter the metabolic balance of organism and condition different disorders in the in-cell balance. As chemical mutagens influence on viability is revealed more intensive on the first stage of growth and development of M_1 plants, we also studied intensity of roots growth initially in the period of seed germination in laboratory conditions besides well-known criteria of plant sensitivity to mutagens (field germination of seeds, survival, and sterility of plants).

By the treatment by mutagen, water penetrates the seed tissue first, with mutagen molecules to follow. Individual field of mutagen molecules consists of main physical and chemical constants and expects not only specific interaction with gene material but also with structural elements of nucleo- and cytoplasm for each mutagen as well [19].

Mutations arise as a result of mutagen interaction with cell genetic material. On the way to this contact, mutagens interfere the metabolism balance and can cause different abnormalities of in-cell balance and injury of genome part. That is why the first processes that take place directly after seeds soaking in mutagen solution cause injury on the cell level and violation in chromosome structure.

Chromosome aberrations are important in genetic variation, monitoring, and evolution and are one of the main characteristics of mutagen genetic activity. We studied the influence of six mutagens on five varieties by frequency of chromosome aberrations. Mutagens break a chromosome apparatus and normal behavior of mitosis in initial roots cells. The violation frequency depends on the concentration of mutagen. Direct relation was detected in all varieties and mutagens: chromosome aberration frequency increases with the increase in mutagen concentration. For example, NEU 0.0125, 0.025, or 0.5 percent concentration conditioned from 3.7 percent of chromosome aberrations in Illichivka variety to 6.4 percent—in Krasnodarska 46 one, from 8.2 percent in Illichivka variety to 10.9 percent in Polisska 70 one and from 14.1 percent in Myronivska 25 variety to 18.7 percent in Illichivka, respectively. Ethylene imine in these varieties conditioned more violations in chromosome structure compared with other varieties (Table 23.1).

Mutagens also differ in their genetic activity. Thus, 0.05 percent NMB conditioned from 18.2 ± 1.5 to 31.8 ± 1.8 percent in five varieties, while DES of the same concentration—only from 12.3 ± 1.3 to 16.7 ± 1.5 percent of chromosome aberrations. NMB and EI revealed the highest activity by their influence on mitotic cell apparatus of all the studied mutagens.

Mutagen activity is revealed uneven in different genotypes (Table 23.1). In Polisska 70, Krasnodarska 46, and Illichivka varieties, the highest percentage of chromosome aberrations was conditioned with NMB 0.05 percent concentration while in the seed of these varieties treated with NMB and EI 0.0125 percent EI concentration solution, it conditioned violation of chromosome structure with higher frequency than with NMS.

Our data match with the those of the other scientists in some way. By comparing the aberration activity of *N*-nitrozo-*N*-alkilurea (NMU, NDMU, NEU, NDEU, and NMB) on spring wheat varieties (Leningradka, Diamant), they had higher activity of NMU in all the concentrations. The rest mutagens, especially in NMB, had aberration frequency increase reliably only in tough doses.

Analysis of correlation between chromosome aberration frequency and field germination in our experiments revealed the inverse relationship. Correlation coefficients in different varieties ranging from $r = -0.473$ in Poliska 70 variety to $r = -0.704$ in Mironivska 25 prove the correlation dependence of field germination and chromosome aberration frequency in initial root meristem of mutagen-treated seeds.

Use of chemical compounds that cause mutations gave the breeders a powerful method of growth of diversity increase and enabled them to create valuable forms of crops. Collections of valuable mutants of barley [20, 21], buckwheat [22, 23], maize [24], wheat [25–27], and other valuable crops were created in a rather short time. Creating and saving a rich genetic fund of mutants is an urgent task as it is the material base for theoretical research development and practical breeding.

Identification of varieties, hybrids, and genotypes of cultural plants is one of the most important problems of agriculture. Varieties were initially differentiated only by most visible morphological characteristics. As scientific breeding methods arose, methods of genotypes, fast identification and variety purity identification of sowing materials have become significant in the process of new breeding material and varieties creation.

Thorough genetic studying of induced mutations is a guarantee of their successful use as induced mutants are new forms with changed genetically systems which have disturbed linkage between an organism and the environment and correlation between some features, created in the initial varieties by natural and artificial selection in breeding.

Selection of mutants in mutational breeding is based on morphological characteristics solely. However, the leveling of mutagen origin lines by the phenotype does not reflect its internal genetic structure. Search for molecular markers for identification of mutants is an urgent issue. Nowadays, use of molecular genetic markers for studying the tendencies of forming adopted gene complex in the process of selection, detecting the relationship between allele variant cluster of genes of reserve protein with loci, controlling the level of expression of quantitative characteristics, [28] for identification of genotypes and estimating the varietal purity [29] are introduced. Yet, these research studies were made on breeding varieties, intervarietal and interspecific hybrids, and lines of hybrid origin. The available literature does not submit a lot of publications on the usage of molecular markers for identification of breeding material of mutagen origin.

Among the biochemical gene markers, the ones controlling the reserve proteins have been studied better than the others. Reserve proteins of winter wheat—gliadins and high-molecular glutenins—have played a significant role in genetically breeding research studies of this culture lately. They have the only of the most informative genetic markers among the well-known ones [30]. Proteins are primary products of genetic systems, and protein electrophoretic activity is finally determined by the se-

quence of gene nucleotide structure. That is why proteins are markers of both single structural genes and their associations. Allele variants of proteins are usually inherited codominantly; their content is not predetermined with growing conditions and is detected comparatively easily by electrophoresis method [31].

We started the research studies on identification by electrophoretic spectrum of gliadins in 2001 and they have been carried over until now. Collection of mutants used as a baseline was set up in the previous years at the Department of Genetics, Breeding and Seed production of Bila Tserkva National Agrarian University. In 1998, S. Vasilkivskiy by the method of chemical mutagenesis bred from Czech Republic Roxana variety diazoacetylbutane 0.025 percent concentration induced and selected 432/5-dwarf mutant, which was not stable. The lines, which gave rise to new ones, were bred in its generations.

The 432/5 mutant belongs to real dwarf species by its morphological characteristics. The result of electrophoresis (Figure 23.1) results in conclusion about heterogeneity by number, intensity, and electrophoretic activity of compounds.

The M 432/5 contains no new components, which are missing in Roxana variety.

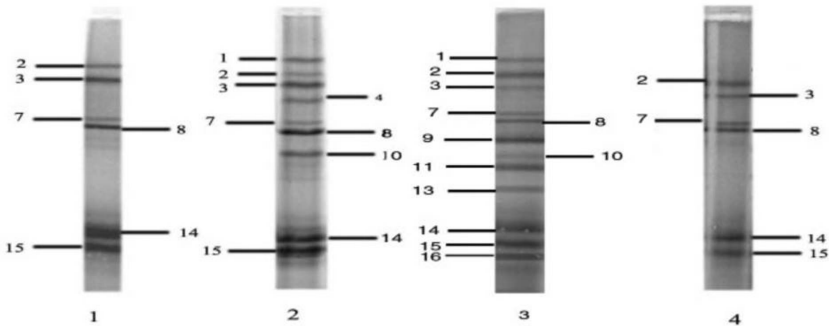


FIGURE 23.1 Electrophoretic spectra of winter wheat gliadins for 432/5 mutant.

In their practical work with mutants, research studies reject the forms, which in their fourth–fifth generations give splitting with deviations in their quantity correlations from the theoretically expected. We managed to work out a way of using new source of genetic changeability and to offer the method of working with nonstable mutants attributed to years of research at the Department of Genetics, Breeding and Seed Production of Bila Tserkva National Agrarian University [32, 33, 34].

In 1999–2005 in posterity of M 432/5 dwarf erythrospERMum form, plants were individually chosen, different in their anatomy morphological and agricultural features, one of them gave rise to a new 710 erythrospERMum line, another—for 712 erythrospERMum line.

Presence of the same components in these lines points on their affinity, affinity with 432/5 mutant and with Roxana initial variety. Absence of some components proves their difference in both genetic and morphological levels.

Cytogenetic activity of mutagens in M_0 by their chromosome aberration frequency in winter wheat correlates with their concentration and exposition of seed soaking. The highest activity on the influence on cells mitotic apparatus reveal NMU and EI, and the least number of chromosome structure violation was caused by DEU and NMB. The method of gliadine electrophoresis in polyacrylamide gel can be used in genetic identification of mutant origin winter wheat lines.

KEYWORDS

- **Chemical mutagenesis**
- **Chromosome aberrations**
- **Electrophoresis**
- **Gliadins**
- **Molecular markers**
- **Mutations**
- **Polyacrylamide gel**

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