Creation of mutant populations of barley (*Hordeum vulgare* L.) and flax (*Linum ussitatissimum* L.) induced by the chemical mutagen of the phosphemide

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Abstract: The effectiveness of barley and flax seed treatment with the chemical mutagen Phosphemidum has been studied for sensitivity in the first generation (M_1) and for the mutability in the second (M_2) and third generations (M_3). Optimal concentrations of the mutagen for treatment of barley (0.002, 0.01 %) and flax (0.005, 0.01 %) seeds for obtaining mutant populations were determined. The change in chlorophyll content in leaves measured by a SPAD 502 optical meter (Minolta Camera Co., Ltd., Tokyo, Japan) during different phenological phases was the informative criterion for evaluating the response of the species studied to the effect of the mutagenic factor. It has been shown that chlorophyll accumulation in the leaves of plants in the control and experimental variants is similar and continues until the stage of milky ripeness in barley and flowering phase in flax. Chlorophyll degradation is accelerated by Phosphemidum.

Key words: barley; flax; mutant population; mutagen concentration; sensitivity; mutability.

1. Introduction

Genetic erosion of plants leads to a reduction in the number of cultivars and, therefore, to a decrease in the productivity of agrocenoses in changing environmental conditions (Aleksanyan, 2007; Mark van de Wouw, 2009). In this regard, the key role is given to the genetic diversity of plants, for widening of which creation of induced mutants are promising in breeding programs. Cultivars increase biodiversity, provide the source material for selection, which contributes to the conservation and an efficient use of plant genetic resources. The use of induced mutants in selection programs led to the creation of 3222 cultivars of 170 plant species in more than 60 countries of the world (FAO, 2015). High results were achieved using well-known chemical mutagens (DMS, HMM, HEM, EI, etc.). The significance of research in this area considerably increases with the induction of mutations by means of a new or an insufficiently studied mutagenic factor. This is especially important for the northern regions characterized by harsh (often extreme) soil and climatic conditions and limited resources of cultivated plant species.

The aim of our research is to develop the scientific basis for the use of the Phosphemidum chemical mutagen in the treatment of barley (*Hordeum vulgare* L.) and flax (*Linum usitatissimum* L.) seeds.

2. Materials and methods

The objects of the study are three barley samples: Zernogradsky 813 (k-30453, Russia, var. *erectum*), Dz02-129 (k-22934, Ethiopia, *var. nigripallidum*), C.I. 10995 (k-30630, Peru, var. *sinicum*), and three varieties of flax: Yarok (k-8282, Belarus), Ottava 770 B See (k-4035, Canada), Velizhzhsky Kriazh (k-5398, Russia), differing in origin, morphological characters and biological properties. Dry seeds were treated with an aqueous solution of Phosphemidum (diethylenimide 2-amidopyrimidyl phosphoric acid) at the following concentrations: barley, 0.002 % ($2 \cdot 10^{-3}$ *M*) and 0.01 % ($1 \cdot 10^{-2}$ *M*); flax, 0.005 % ($5 \cdot 10^{-3}$ *M*), 0.01 % ($1 \cdot 10^{-2}$ *M*) and 0.1 % ($1 \cdot 10^{-1}$ *M*), with exposure for 3 hours. Then the seeds were washed in running tap water for 40 minutes (Weisfeld, 2010, 2016). 300 seeds of each barley sample and 200 seeds of each flax sample were treated by the mutagen. Seeds kept in distilled water were used as the control. Sowing in the field to obtain the first generation (M_1) was carried out in blocks for each variant of seeds in four repeats. M_1 plants were harvested individually. Sowing of the second generation (M_2) was carried out by families (the offspring of inflorescences from M_1 plants). The modified forms according to morphological characters and biological properties were selected from the control and mutagen-treated populations by means of visual observations and descriptions of M_2 plants during the growing season. In the third generation (M_3), seeds collected from plants with phenotypic deviations from the initial sample in M_2 were sown by families, and the percentage of families, in which the altered character was inherited, was calculated.

Statistical processing of experimental data was performed using STATGRAPHICS, STATISTICA 7 (StatSoft) software. The standard error of means (S_x) and the significance of differences according to Student's criterion were calculated. The "box-whiskers" diagrams were constructed according to the method of John Tukey (Field et al., 2012).

3. Results and discussion

The effectiveness of the mutagenic factor was determined according to the sensitivity of barley and flax to the effects of Phosphemidum in seed treatment in the first generation (M_1), the mutation frequency and spectrum in the second (M_2) and third (M_3) mutant generations in controlled laboratory conditions (thermostat, climate chamber, vegetation shelves) and in the field experiment. Although new molecular methods for detecting mutant forms have been developed (e. g., the TILL-

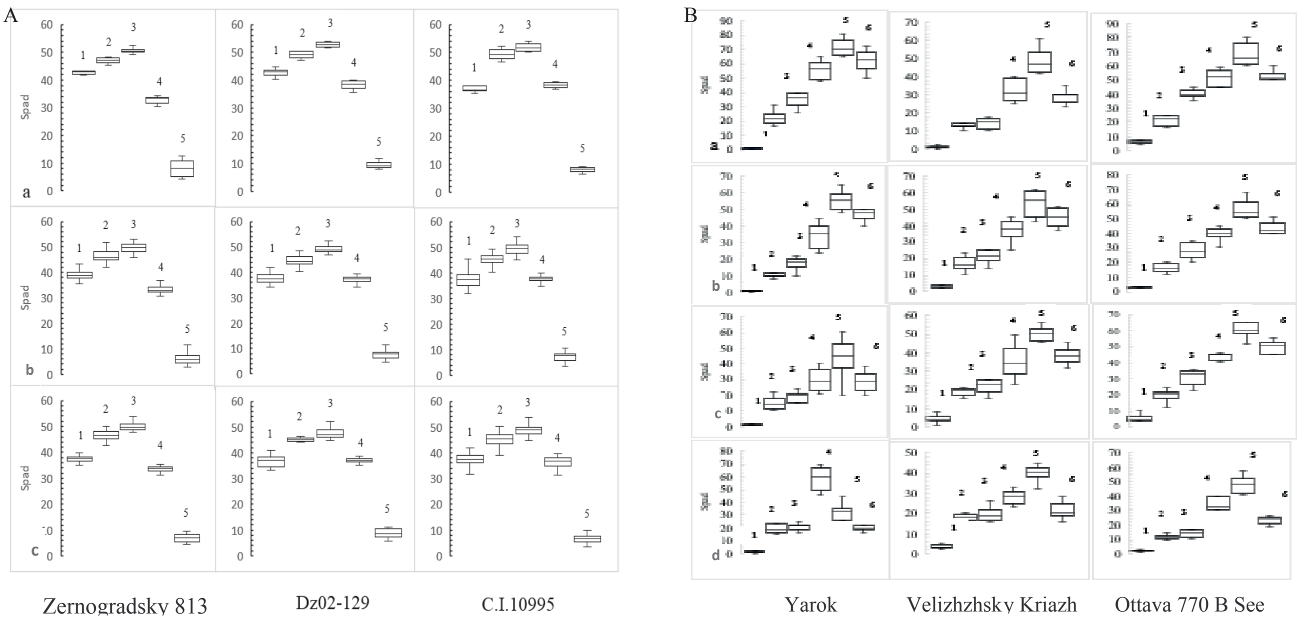

Figure1. Accumulation and degradation of chlorophyll in barley (A) and flax (B) leaves in control and mutant populations.
Stages of barley plant development: 1, tillering; 2, stem elongation; 3, milky ripeness; 4, wax ripeness; 5, full ripeness.
a, control; *b*, 0.002 %; *c*, 0.01 %.
Stages of flax plant development: 1, germs; 2, start of leaf spiral; 3, quick growth; 4, budding; 5, flowering; 6, green ripening.
a, control; *b*, 0.005 %; *c*, 0.01%; *d*, 0.1%.
Notation: "—", arithmetic mean; "□" is the standard error, ±; "˩" is the minimum value of the attribute (min); "˥" is the maximum value of the attribute (max).

ING method) (Till et al., 2003, 2006), a prerequisite for their use is the presence of a mutant population, the size of which depends on the type of mutagen, its concentration, time and conditions of explant treatment.

The analysis of field germination of seeds and plant survival of barley and flax of the M_1 generation allowed us to determine the concentrations of Phosphemidum optimal for the growth and development of barley (C.I. 10995), 0.002 and 0.01 %, and of flax (Yarok, Velizhzhsky Kriazh), 0.005 and 0.01 %. Semi-lethal concentrations include: 0.01 % for barley samples (Dz02-129, Zernogradsky 813) and 0.1 % for flax varieties (Ottava 770 B See), since seed germination after treatment with Phosphemidum was below 50 %.

The physiological status of seeds and the variability of plant morphometric parameters in ontogenesis are found to be the informative criteria. The express diagnostics of chlorophyll content in the leaves of plants at different stages of ontogenesis was tested. Based on the readings of the SPAD 502 chlorophyll optical counter (Minolta Camera Co., Ltd., Tokyo, Japan), significant differences were found in the accumulation and degradation of chlorophyll in the control and variants with Phosphemidum (Figure 1).

Positive correlations of chlorophyll content in leaves with other selection-valuable characters (plant height, leaf area, yield, biological properties of seeds) provide more complete information about the interaction of the factors 'genotype' and 'environment' and their contribution to the overall phenotypic variability. The genetic activity of a mutagen is determined by the frequency and spectrum of mutations. In our study, mutational changes that occur under the effect of Phosphemidum were diverse in their display and affected the stem, leaves, ear, physiological parameters of plant growth and development; system mutations were encountered as well. In the studied species, induction of plants with early ripening, large inflorescence, and an increase in the length of the stem were most frequently observed. The specificity of mutational changes was noticed. For example, only in barley sample C.I. 10995 the mutagen treatment led to the emergence of a new variety. In variants with a mutagen concentration of 0.01 %, stunted forms of plants with high resistance to lodging were obtained (9 points). In flax cultivars in variants with the mutagen, plants with a stem mass substantially exceeding the control were found. Using Phosphemidum allowed us to obtain plant forms with an increased number of seeds in a capsule. We consider mutational changes in the color of flowers (from white to shades of pink and blue), the seed coat (from yellow with different shades to dark brown), the shape and degree of opening of the box as a promising starting material for flax selection.

4. Conclusion

Reducing plant genetic diversity decreases the possibilities of the population supply with a variety of high-quality food products (McCouch et al., 2013). Therefore, the identification of new resources to increase the genetic diversity of cultivated plants and reduce their vulnerability to changing environmental conditions is extremely important. It is evident that the further development of mutation selection is largely determined by the search for new mutagens and the development of the technology for their application in plants. The task of selecting the optimal concentrations of the mutagen solution, providing the maximum possible yield of mutant forms with selection-valuable characters remains difficult to solve. The damaging action of the mutagen, leading to loss of seed viability, death of seedlings or mature plants, is often the obstacle to solve this task. This may be due to differences in the chromosome set (*Hordeum vulgare* L., 2n = 14; *Linum usitatissimum* L., 2n = 30), which should be taken into account in experimental studies.

Phosphemidum contains two Ethyleneimine groups, and is a derivative of Ethyleneimine, a known and widely used mutagen. Our results suggest that Phosphemidum is an effective chemical mutagen for producing plants with new or improved characters (Bome et al., 2017; Korolyov, Bome, 2018; Korolyov et al., 2019). The created mutant forms are a valuable source material for selection and genetic programs and for enriching the genetic diversity of agricultural plants. Identification of barley sample C.I. 10995 plants with a complex of new morphological characters that determine their belonging to another variety, the breadth of variation in flower color and the structure of the capsule in flax confirm the hypothesis by I.A. Rapoport (1993) on a high efficiency of chemical mutagens, especially alkylation compounds (Ethyleneimine and its derivatives), in the creation of rare mutations.

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Conflict of interest. The authors declare no conflict of interest.