**Conclusions**. Herbicide Pulsar 40 is the most suitable for soybean. Post-emergence application of Pulsar 40 in pea and chickpea crops without inoculation significantly reduced the 1000-seed weight. The greatest gain in the yield from seed inoculation with Rizobophyte was achieved in chickpea. There was no significant effect of the factors under investigation on the protein and fat contents in seeds.

*Key words*: pea, chickpea, soybean, inoculation, weeds, herbicide, yield, 1000-seed weight, protein, fat

UDK631.9:633.15:541.142

DOI:10.30835/2413-7510.2018.134376

### MORPHOLOGICAL PARAMETERS OF MAIZE ENDOSPERMAL MUTANTS SEEDLINGS AND EFFECT OF SEED STORAGE REGIMES ON THEM

Shyianova T.P., Tymchuk S.M., Boguslavskyi RL. Plant Production Institute nd. a V.Ya. Yuriev of NAAS, Ukraine

According to the reaction of morphological parameters of seedlings from the grains of maize endospermal mutants to the influence of the regimes that simulate a longterm storage – accelerated aging, freezing and combined regimes, the highest longevity is distinguished by *ssp. indentata* seeds, as well as carriers of the genes *ae* and *su*<sub>1</sub>, low durability – carriers of the genes *wx* and *se*, and the lowest – carriers of the gene *sh*<sub>2</sub>.

*Key words*: maize, seeds, seedlings, morphology, endospermal mutants, longevity, accelerated aging, freezing.

**Introduction.** The progress in the breeding of maize which is one of the leading grain crops in the world, is associated with genetic regulation of the endosperm composition and structure. This enables fuller utilizing the wide potential of this crop as a source of raw materials for different industries and consumption areas, especially taking into account that endosperm structure mutations are natural and their use is not connected with overcoming legislative restrictions and human prejudices [1, 2, 3]. The *wx* endosperm mutation carriers starch of which consists almost entirely of amylopectin, *ae* mutants which are characterized by significantly increased proportion of amylose in starch, *su*<sub>1</sub>mutants with increased content of water-soluble polysaccharides in the endosperm, *se* mutants which are characterized with very high content of sucrose in grain, are the most actively used [4, 5]. However, in Ukraine, the effects of endosperm mutations are not properly used in crop and breeding practices. Insufficient longevity of stored seeds of endosperm mutants compared to traditional dent and flint forms is among the reasons for this. Therefore, not only the creation of such maize lines, but also their storage in collections of breeding institutions and genetic banks providing preservation of high sowing qualities of stored seeds is gaining importance.

**Literary review, problem formulation.** It is known from literature that seeds of endosperm mutants are often less long-living upon storage give weaker sprouts compared to seeds of traditional dent and flint forms [6]. This is particularly clearly evident in the  $sh_2$  endosperm mutation carriers [7, 8] germinating under stress conditions [9]. It was established that reduced germination intensity of forms carrying this mutation or other mutations can be increased by selection [10, 11]. This is in good agreement with some authors' conclusions about hereditary control of seed longevity and germination intensity [12].

<sup>©</sup> T.P. Shyianova, S.M. Tymchuk, RL. Boguslavskyi. 2018.

ISSN 1026-9959. Селекція і насінництво. 2018. Випуск 113.

It is believed that accelerated aging is a convenient and informative method that allows evaluating seed longevity upon natural aging and predicting it upon storage. This method consists in short-term incubation of seeds under high humidity and air temperature [13, 14, 15].

The international standards recommended the temperature  $-18\pm2^{\circ}$ C for long-term storage of seeds, which ensures the seed longevity [16]. This regime is also used in the National Repository of Plant Gene Pool Accessions of Ukraine which functions at the Plant Production Institute named after V.Ya. Yuriev of NAAS. In addition, previous studies [17] demonstrated a possibility of increasing the germinability of seeds which decreased it after accelerated aging, by incubation under - 18±2 °C and thus reducing negative effects of aging. However, responses of seeds to these factors vary depending on the genotype [18]. Effects of accelerated aging and freezing of seeds of different maize endosperm mutations carriers have not been elucidated so far. This limits prospects for developing the most efficient storage technologies for this category of starting material for breeding.

In the previous article [19], we described the response of seeds of different corn endosperm mutants to two storage regimes in terms of the germination energy and germinability.

Our purpose was to determine effects of different regimes simulating long-term storage on morphological parameters of seedlings from the seeds of maize endosperm mutants.

**Material and methods.** Seeds of six simple F<sub>1</sub> hybrids and 12 parent lines belonging to different carriers of endosperm mutations regulating contents and ratio of major carbohydrate fractions in grain: *wx, ae, su<sub>1</sub>, se, sh<sub>2</sub>* and dent type (ssp. *indentata*) were taken as the test material: mid-early hybrid Vympel and its parents PHK26M,  $rac{O}Kh523ZM$ , FAO 270 were used as references for comparison with the others. Carriers of the *ae* endosperm mutation were the hybrid AE392/AE800 and its paternts PAE392,  $rac{O}AE800$  (mid-ripening, FAO 350); carriers of the waxy endosperm mutation – hybrid Binom and its parents PVK69,  $rac{O}VK64$  (mid-ripening, FAO 320). Sugar corn was represented by carriers of the *su*<sub>1</sub> mutation – hybrid Dmytryk and its parents PMS401,  $rac{O}MS266$  (midearly, FAO 260); the *se* mutation (recessive modifier the *su*<sub>1</sub> gene) – hybrid Snihova Koroleva and its parent lines PSE854,  $rac{O}SE843$  (mid-early, FAO 290) and the *sh*<sub>2</sub>mutation – hybrid SS386 / SS389 and its patents PSS386,  $rac{O}SS389$  (mid-early, FAO 250) [20].

All endosperm mutants were characterized by decreased content of starch compared to wild type corn, but among them, the wx mutation carriers had the highest average starch content and the  $sh_2$  mutation carriers – the lowest one. The maize endosperm mutants were clearly divided into two groups by the average content of amylose in starch. Group I comprised the wx mutation carriers (almost amylose-free starch unlike wild type maize). Group II included the carriers of other mutations (*ae*,  $su_1$ , se, and  $sh_2$ ); they had higher amylose content in starch, and this effect was particularly pronounced in the *ae* mutation carriers compared to wild type dent maize.

The experiments were carried out with the seeds harvested in 2012–2014. 2012 and 2013 were characterized by increased average daily temperature with sufficient or excessive (at the end of vegetation) rainfall. Such conditions were favorable for grain filling, however, they delayed water evaporation from grain and full ripeness and favored formation of partly empty ears. 2013 was favorable for the spread of major diseases (common smut, stem rots, kernel rot) and corn pests (European corn borer and corn earworm). 2014 was dry, which contributed to fast ripening and low consumption of water by grain. Since the ranking of indices coincided in the three years, the article presents averaged data over the three years.

The experiments were conducted in three replicas, with 100 seeds for each.

The longevity of seeds of maize lines and hybrids with a moisture content of 7–10% was studied in three models. Model 1 –«accelerated aging»: seeds of each form were acceleratedly aged by B.S. Likhachiov's method [15], which simulates natural aging during long-term storage. The seeds were placed in hermetically sealed foil bags and kept under 37°C for 30 days. Model 2 - «freezing»: exposure under -18  $\pm$  2 °C for 1 month. This temperature is used in the National Repository of Ukraine for long-term storage of the plant gene pool accessions seeds. Model 3 – «combined mode»: after «accelerated aging», the seeds in the same sealed foil bag were exposed under room temperature for 4 hours and then placed in a freezer under -18  $\pm$  2 °C for 1-month storage.

Before the onset of each experiment and at each of its stages, the laboratory germination energy and germinability were evaluated in compliance with the State Standard 4138-2002 [21]. The seeds were sprouted in distilled water under  $25 \pm 1^{\circ}$ C. Concurrently with evaluation of the geminability, morphological parameters were measured: sprout length, number and total length of primary roots [22]. The seeds of the same lines and hybrids (with the same moisture content) stored in paper bags under room temperature were taken as the control in the laboratory tests. The significance of differences between the means was estimated by t-test at probability level P = 95% [23].

The effects of accelerated aging, freezing and combined mode on the morphological parameters of seedlings were estimated by the *I* index [24]:

$$I = \frac{\left(\overline{X}_1 - \overline{X}_2\right)}{\left(\overline{X}_2\right)} \times 100 \%$$

where:  $\overline{X}_1$  - experimental average indicator,  $\overline{}_2$  - control average indicator. A positive *I* means an increase in the parameter, and a negative *I* – a decrease.

**Results and discussion.** There were differences in the responses of dent and mutant maize seeds to accelerated aging, freezing and combined regimes manifested in the morphological parameters of seedlings (Figs. 1–6).

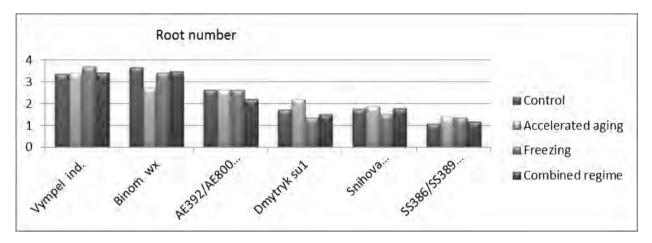


Figure 1. Root number in seedlings from seeds of F<sub>1</sub> corn hybrids – endosperm mutation carriers after different storage regimes, 2012–2014

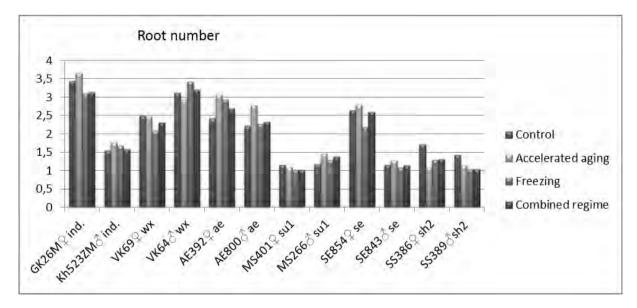


Figure 2. Root number in seedlings from seeds of parent lines of F<sub>1</sub> corn hybrids – endosperm mutations carriers after different storage regimes, 2012–2014

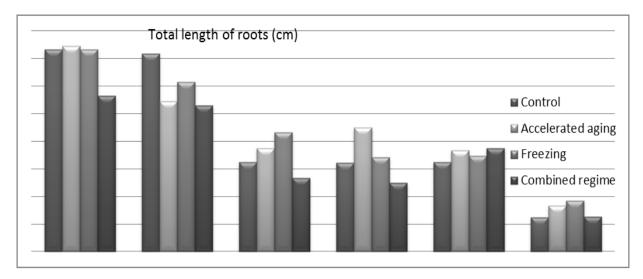


Figure 3. Total length of roots in seedlings from seeds of F<sub>1</sub> corn hybrids – endosperm mutation carriers after different storage regimes, 2012–2014

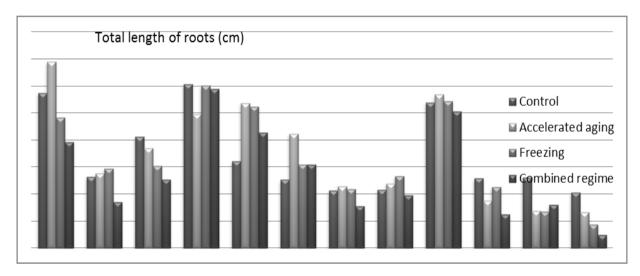


Figure 4. Total length of roots in seedlings from seeds of parent lines of F<sub>1</sub> corn hybrids – endosperm mutation carriers after different storage regimes, 2012–2014

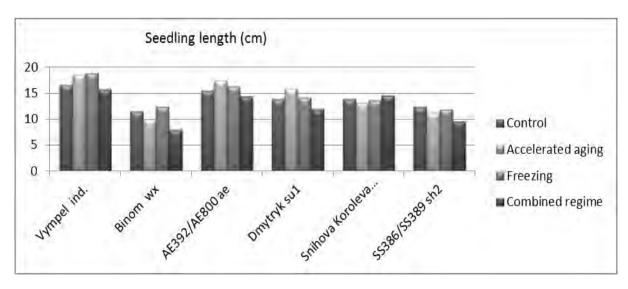


Figure 5. Length of seedlings from seeds of F<sub>1</sub>corn hybrids–endosperm mutation carriers after different storage regimes,2012–2014

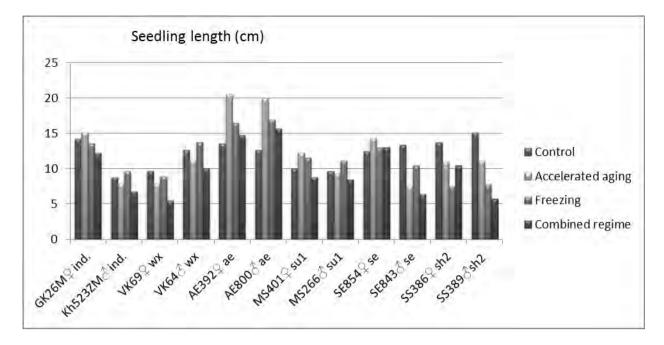


Figure 6. Length of seed lings from seeds of parent lines of F<sub>1</sub> corn hybrids – endosperm mutation carriers after different storage regimes,2012–2014

*Control regime*. The tested accessions significantly differed in the morphological parameters of seedlings.

*Primary root number.* Among the hybrids, the *wx* mutation carrier Binom and the representative of dent subspecies (ssp. *indentata*) Vympel had the greatest root number  $(3.7 \pm 0.26 \text{ and} 3.4 \pm 0.30, \text{ respectively})$ , and the *sh2* mutation carrier SS386/SS389 – the smallest  $(1.1 \pm 0.21)$ . Other hybrids had the root number ranging within  $1.7 \pm 0.55 - 2.6 \pm 0.10$  (Dmytryk, AE392/AE800 and Snihova Koroleva). Of the parent lines, the lines  $3^{\circ}$ VK64 (*wx*) and  $2^{\circ}$ HK26M (ssp. *indentata*) had the greatest number of roots - from  $3.1 \pm 0.25$  to  $3.4 \pm 0.25$ ; the group with medium root numbers (from  $2.2 \pm 0.08$  to  $2.6 \pm 0.32$ ) comprised lines  $2^{\circ}$ SE854 (*se*),  $2^{\circ}$ VK69 (*wx*),  $2^{\circ}$ AE392 and AE800 $3^{\circ}$  (*ae*); the smallest root numbers (from  $1.2 \pm 0.82$  to  $1.7 \pm 0.08$ ) were observed in the lines  $2^{\circ}$ MS401,  $3^{\circ}$ MS266 (*su*<sub>1</sub>),  $3^{\circ}$ SE843 (*se*),  $2^{\circ}$ SS386,  $3^{\circ}$ SS389 (*sh*<sub>2</sub>) and  $3^{\circ}$ Kh523ZM (ssp. *indentata*).

Comparing the hybrids with their parent lines in the control, we noticed that no hybrid exceeded its parents by the root number. Two groups of accessions can be distinguished. In the group I the both parent lines had the roots numbers equal to their hybrid or one of the parent lines inferior to the hybrid. So, the hybrid Vympel (ssp. *indentata*) and its female line  $\bigcirc$ HK26M had the identical root numbers of  $3.4 \pm 0.25$  but its male line  $\bigcirc$ Kh523ZM had a significantly (t> 3, P=95 %) smaller root number ( $1.6 \pm 0.15$ ). In the accessions with the *wx* endosperm mutation, the line  $\bigcirc$ VK64 was almost at hybrid Binom's level ( $3.1 \pm 0.25$  and  $3.7 \pm 0.26$ , respectively), and the line  $\bigcirc$ VK69 was inferior to them ( $2.5 \pm 0.47$ ). In accessions with the *ae* and *su*<sub>1</sub>endosperm mutations, hybrids also had almost the same roots number as a parent lines: *ae* - hybrid AE800/AE392 and lines  $\bigcirc$ AE392,  $\bigcirc$ AE800 ( $2.6 \pm 0.10$ ,  $2.4 \pm 0.06$ ,  $2.2 \pm 0.08$ , respectively); *Su1*- hybrid Dmytryk and lines  $\bigcirc$ MS401,  $\bigcirc$ MS266 ( $1.7 \pm 0.55$ ,  $1.2 \pm 0.82$ ,  $1.2 \pm 0.19$ , respectively).

In the group II, hybrids were inferior either to both parents or to one of them. Of the *se* endosperm mutation carriers, hybrid Snihova Koroleva and line  $\Im$ SE843 had the smallest root numbers (1.8 ± 0.21 and 1.2 ± 0.25, respectively) compared to the line  $\Im$ SE854 (2.6 ± 0.32), with significant difference (t> 3). Of the *sh*<sub>2</sub> endosperm mutation carriers, hybrid SS386/SS389 had the smallest number of roots (1.1 ± 0.21), and both parent lines  $\Im$ SS386,  $\Im$ SS389 had insignificantly exceeded it by the root number (1.7 ± 0.08 and 1.4 ± 0.30, respectively).

*Total length of roots (cm).* In the control, among maize endosperm mutant hybrids, the longest roots were recorded in Vympel (dent type,  $36.7 \pm 6.03$ ) and Binom (*wx*,  $35.9 \pm 8.16$ ); the shortest – in hybrid SS386/SS389 (*sh2*,  $6.2 \pm 0.41$ ); the difference was significant (t> 3, P=95%).

Other hybrids had similar total length of roots ranging within  $16.1 \pm 3.07 - 16.3 \pm 3.65$ . Parent lines were divided into two groups. Group I had the greatest total length of roots:  $\bigcirc$ HK26M (ssp. *indentata*),  $\bigcirc$ VK64,  $\bigcirc$ VK69 (*wx*) and  $\bigcirc$ SE854 (*se*) (28.7 \pm 9.44, 30.3 \pm 5.10, 20.6 \pm 7.46, 26.9 \pm 5.81, respectively). Group II included the remaining lines with lower values ranging within 10.3  $\pm 1.13 - 16.1 \pm 1.7$ , the difference between the groups was significant (t = 2, P=95%).

When we compared hybrids with their parent lines in the control, the accessions were categorized into the following groups. In the group I, hybrids significantly (t> 3, P=95%) exceeded both parent lines by the total length of roots. This group included dent corn (lines  $\Diamond$  Kh523ZM – 13.2 ± 3.55,  $\heartsuit$  HK26M – 28.7 ± 9.44, hybrid Vympel – 36.7 ± 6.03); *wx* mutants (lines  $\heartsuit$  VK69 – 20.6 ± 7.46,  $\Diamond$  VK64 – 30.3 ± 5.1, hybrid Binom – 35.9 ± 8.16) and *su*<sub>1</sub> mutants (lines  $\heartsuit$  MS401 – 10.6 ± 2.0,  $\Diamond$  MS266 – 10.8 ± 1.58, hybrid Dmytryk – 16.1 ± 3.07). In group II which included accessions with *ae* mutation, hybrid AE800/AE392 was comparable with one of its parent lines  $\heartsuit$  AE392 (16.2 ± 0.41 and 16.1 ± 1.7, respectively), and the other parent line  $\Diamond$  AE800 was significantly (t> 2) inferior to them (12.6 ± 1.13). In group III, hybrids were significantly (t> 2, P=95%) inferior to one or both parent lines: these are the *se* mutation carriers (hybrid Snihova Koroleva 16.3 ± 3.65 and lines  $\heartsuit$  SE854 – 26.9 ± 5.81,  $\Diamond$  SE843 – 12.8 ± 2.78) and the *sh*<sub>2</sub>mutation carriers: hybrid SS386/SS389 (6.2 ± 0.41) was significantly inferior to its parent lines –  $\heartsuit$ SS386 (13.0 ± 2.48),  $\Diamond$ SS389 (10.3 ± 1.13).

Seedling length (cm). In the control, of the hybrids, Vympel (ssp. indentata) and AE800/AE392 (ae) had the longest seedlings:  $15.5 \pm 0.42$  and  $16.6 \pm 1.48$ , respectively. Hybrid Binom (wx) had the shortest seedlings:  $11.4 \pm 4.52$ . The carriers of other mutations SS389/SS386 (sh<sub>2</sub>), Dmytryk (su<sub>1</sub>), Snihova Koroleva (se) had intermediate seedlings ( $12.3 \pm 2.04$ ,  $13.8 \pm 6.2$  and  $13.9 \pm 2.2$ , respectively). Of the parent lines,  $\Im$ SS389 had the longest seedlings ( $15.1 \pm 1.5$ ) and the su<sub>1</sub>mutation carriers  $\Im$ MS401 ( $10.1 \pm 3.25$ ) and  $\Im$ MS266 ( $9.7 \pm 2.68$ ),  $\Im$  VK69 (wx,  $9.7 \pm 4.3$ );  $\Im$ Kh523ZM (dent type,  $8.7 \pm 2.2$ ) – the shortest seedlings. The following mutants had intermediate seedlings:  $sh2 - \Im$ SS386 ( $13.8 \pm 2.5$ );  $se - \Im$  CE854 ( $12.5 \pm 1.9$ ),  $\Im$  SE843 ( $13.3 \pm 2.05$ );  $ae - \Im$ AE392 ( $13.6 \pm 1.55$ ),  $\Im$ AE800 ( $12.7 \pm 2.0$ ). The seed ling length also differed between male and female lines: in the wx mutation carriers  $-\Im$ VK64 ( $12.6 \pm 2.3$ ) and  $\Im$ VK69 ( $9.7 \pm 4.3$ ) and in dent maize -  $\Im$ HK26M ( $14.2 \pm 4.5$ ) and  $\Im$ Kh523ZM ( $8.7 \pm 2.2$ ).

When we compared the seedling length in hybrids with their parent lines, two groups of accessions can be distinguished. In group I, the seedling length in hybrids was significantly higher (t> 2, P=95%) than that in both parent lines. These are dent hybrid Vympel (16.6 ± 1.48) and its parent lines  $\bigcirc$  HK26M (14.2 ± 4.5),  $\bigcirc$  Kh523 ZM (8.7 ± 2.2); the *su*<sub>1</sub> mutation carriers, hybrid Dmytryk (13.8 ± 6.2) and lines  $\bigcirc$  MS266 (9.7 ± 2.7) and  $\bigcirc$  MS401 (10.1 ± 3.3); and the *ae* mutants, hybrid AE800/AE392 (15.5 ± 0.4) and lines  $\bigcirc$  AE392 (13.6 ± 1.6) and  $\bigcirc$  AE800 (12.7 ± 2.0). In group II, seedlings of hybrids were comparable with those of its parent lines, or one of the lines had longer seedlings. These are the following mutants: *wx* - hybrid Binom (11.4 ± 4.5) and its parent lines  $\bigcirc$  VK69 (9.7 ± 4.3) and  $\bigcirc$  VK64 (12.6 ± 2.3); *se* - hybrid Snihova Koroleva (13.9 ± 2.2) and its parents  $\bigcirc$  SE854 (12.5 ± 1.9) and  $\bigcirc$  SE843 (13.3 ± 2.1); *sh2* - hybrid SS389/SS386 (12.3 ± 2.04) and its parent lines  $\bigcirc$  SS386 (13.8 ± 2.5) and  $\bigcirc$  SS389 (15.1 ± 1.5).

In general, the corn accessions with different endosperm structure under investigation were ranked in the control according to the three morphological parameters of seedlings as follows: high values of morphological parameters (the root number, their total length and seedling length) were intrinsic to hybrid Vympel and line  $\mathcal{P}HK26M$  (dent type). High or intermediate number and total length of roots with intermediate or short seedlings were observed in hybrid Binom and its parent  $\partial VK64$  (*wx*). Intermediate number and total length of roots with long seedlings were seen in the *ae* mutation carriers, hybrid AE800/AE392 and its parent lines  $\mathcal{P}AE392$ and  $\partial AE800$ . Low number of roots and short seedlings with intermediate total length of roots were intrinsic to lines  $\mathcal{P}$  MS401 and  $\partial MS266$  (*su*<sub>1</sub>). Long seedlings with various number and total length of roots is a common feature for parental lines – the *se* mutants  $\mathcal{P}$  SE854 and  $\partial SE843$  and also *sh*<sub>2</sub> mutants  $\mathcal{P}SS386$  and  $\partial SS389$ . Hybrids with the *su*<sub>1</sub> and *sh*<sub>2</sub> genes were characterized by intermediate values of all the three morphological parameters. There was a certain pattern between hybrids and parent lines. Hybrids AE800/AE392 (*ae*) and Dmytryk (*su*<sub>1</sub>) significantly (t> 2) exceeded their parent lines by all the three morphological parameters; Vympel (dent type) and Binom (*wx*) significantly (t> 2, P=95%) exceeded their parent lines only by two parameters – the number of roots and their total length. Hybrid Snihova Koroleva (*se*) was inferior to its female line in terms of two morphological parameters (root number and length), and hybrid SS389/SS386 (*sh2*) – in terms of the three morphological features (seedling length, root number and length).

*Accelerated aging*. The effect of accelerated aging on seeds depended on the hybrid or line genotype. In general, stimulating effects were observed in 29 of 54 cases, i.e. in the majority; in 5 cases there was no response; depressing effects were recorded in 19 cases (Figs. 1–6).

Significantly (t> 2, P=95%) depressing effects for the three morphological parameters of seedlings were observed in the *wx* mutation carriers F<sub>1</sub> hybrid Binom (I = -12.9% – -25.5%) and line  $\partial VK$  64 (-5.8% – -18.3%); sh2 lines  $\partial SS389$  (-19.7% to -35.5%) and  $\Im SS386$  (-21.0% to -47.5%). Reduction in two indices – the total length of roots and the seedling length – was noticed in female line  $\Im VK69$  (*wx*, I = -10.2% and -19.1%, respectively), male line  $\partial SE843$  (*se*, I = -32.2% and -43.6%, respectively). Accelerated aging did not affect the root number in the former, and in the latter this parameter increased by 10.3%.

Accelerated ageing had significant (t> 2, P=95%) stimulating effects on two or three parameters in the *ae* mutation carriers: hybrid AE800/AE392 (I for the root number was 0%, for the other two parameters – 15.5% and 12.8%) and lines  $\bigcirc$  AE392 (I = 26.3% – 66.9%) and  $\bigcirc$  AE800 (I = 25.2% – 67.5%). Similar tendency was seen in hybrid Dmytryk (*su*<sub>1</sub>) (I = 15.1% – 39.7%), parent lines  $\bigcirc$  HK 26 M (dent type, I = 6.4% – 19.9%),  $\bigcirc$  SE854 (*se*, I = 5.4% – 14.8%). In hybrid SS386/SS389 (*sh*<sub>2</sub>), stimulatory effects were observed for two morphological traits – the root number and their total length: I = 27.8% and 33.9%, respectively. The remaining hybrids and lines showed ambiguous responses to accelerated aging: some parameters were stimulated, others - inhibited or did not respond.

The advantage of hybrids over parent lines after accelerated aging by all the three morphological features (t> 3, P=95%) was recorded in the  $sh_2$  mutation carriers (SS389/SS386); by two features (the root number and their total length) - in the  $su_1$  mutation carriers (Dmytryk). The hybrid was inferior to its parent lines by all the three indices in the *ae* mutation carriers (AE800/AE392). Thus, this hybrid was more homeostatic than its parent lines. Other hybrids were comparable with their parental lines or occupied an intermediate position between them by one, two or three indices.

**Freezing.** Significant (t> 2, p=95%) stimulating effects on the total length of roots and the seedling length were observed in the *ae* mutation carriers: hybrid AE800/AE392 (I = 33.3% and 5.4%, respectively) and its parent lines  $\bigcirc$ AE392 (I = 63.0 % and 21.0%, respectively) and  $\bigcirc$ AE800 (I = 22.7% and 33.6%, respectively). The effect in line  $\bigcirc$ AE392 was also stimulating for the root number (I = 20.6%). In addition, a clear, though weaker, stimulating effect of freezing was seen in lines  $\bigcirc$ Kh523ZM (dent type, I = 7.7 – 11.2%, and  $\bigcirc$ MS266 (*su*<sub>1</sub>, I = 10.3 – 23.6%).

Significant reduction in the three indices under investigation was observed in lines  $\Im$ SS386 and  $\Im$ SS389 (*sh*<sub>2</sub>): I = -24.6 - -58.3%; weaker - in  $\Im$ SE843 (*se*): I = -6.9 - -21.5%.

The remaining hybrids and lines showed ambiguous responses to freezing: some indices were stimulated, others – inhibited or did not respond. The total number of indices in this category of accessions was 21, and stimulating effects were observed in 11 cases (most), no response – in 3 cases; and depressing effect – for 7 indices.

No hybrid showed exceeded its parent lines by all the three parameters after freezing. Hybrid Vympel (dent type) again exceeded both lines of its group by the total length of roots and the seedling length, hybrids Dmytryk ( $su_1$ ) and SS389/SS386 ( $sh_2$ ) – by the total length of roots (t> 3, p=95%). The remaining hybrids were comparable with both lines or occupied an intermediate position in terms of one, two or three morphological traits.

**Combined regime.** Significantly depressing (t> 2, p=95%) effects on the three traits (the seedling length, number and total length of roots) were registered in the *wx* mutations carriers–

hybrid Binom (I = -5.2 - -30.2%) and line  $\heartsuit VK69$  (I = -7.2 - -43.4%);  $su_1$  mutants – hybrid Dmytryk (I = -12.1 - -22.7%) and line  $\heartsuit MC401$  (I = -12.2 - -26.8%);  $sh_2$  mutants – lines  $\heartsuit SS386$  (I = -23.4 - -39.0%) and  $\circlearrowright SS389$  (I = -26.8 - -77.0%); dent line  $\heartsuit HK26M$  (I = -9,0 - -31,7%). Some parameters were depressed in the following accessions: the total length of roots and length of seedlings– in lines  $\circlearrowright X523ZM$  (dent corn, I = -35.5 and -23.0%, respectively),  $\circlearrowright SE843$  (*se*, I> 50% for the both indices); the length of seedlings – in line  $\circlearrowright VK64$  (*wx*, I = -20.8%).

Stimulating effects (t> 2, p=95%) in the three indices were observed in the *ae* carriers: parent lines  $\bigcirc$  AE392 (I = 8.3 - 32.5%) and  $\bigcirc$  AE800 (I = 4.5 - 23.2%), while their hybrid responded to the combined regime by lowering all the three morphological parameters. The total length of roots was also increased in hybrid Snihova Koroleva (*se*, I = 15,2%).

In other accessions, the responses to the combined regime were various: of the total of 18 cases, stimulating effects were noticed in 6 cases; reduction - in 8 cases, no effect - in 3 cases.

Of the hybrids under investigation, Vympel (dent) exceeded its parents after the combined regime by all the three indices. The remaining hybrids were equal to the both parent, inferior to them or ranked intermediate by one – three morphological traits.

It was expected that hybrids would be characterized by smaller absolute values of the factor effect index compared to their parents, hence, be more homeostatic [25]. Concerning the response to accelerated aging, this occured in the *ae* mutation carriers – AE800 / AE392; after freezing, by the number and total length of roots – in AE800/AE392 (*ae*), Snihova Koroleva (*se*) and SS389/SS386 (*sh*<sub>2</sub>) with seedlings lengths equal to those in the lines; by seed ling length – in Dmytryk (*su*<sub>1</sub>); after the combined regime – in Vympel (dent corn), SS389/SS386 (*sh*<sub>2</sub>) (in the latter, the seedling length was only equal to that in \$S386); to a certain extent – in AE800/AE392 (*ae*), in which the total length of roots and seedling length were lower than those in parents or at least not exceeded them.

Summarizing the study results on the responses of the seedling morphological parameters to the factors that simulate the processes of storage of corn seeds with different endosperm structures, we established the following: All the morphological parameters were reduced under the influence of the three factors in the parent lines – the *sh*<sub>2</sub> mutation carriers,  $\Im$ SS386 and  $\Im$ SS389. Hybrid SS386/SS89, on the contrary, had either increased or unchanged indices. Reduction in the majority of indices under accelerated aging and combined regime was shown for all the *wx* mutation carriers: hybrid Binom and its parents  $\Im$  VK69 and  $\Im$ VK64. Positive effects of all the three factors on almost all morphological parameters were noticed in the *ae* mutation carriers: parent lines  $\Im$ AE392 and  $\Im$ AE800. Positive effects of accelerated aging and freezing were observed in these lines and their hybrid AE800/AE392.

Hybrids exceeded parent lines by the majority of morphological parameters in dent accessions (Vympel); by the total length of roots – in the  $su_1$  mutation (Dmytryk) and  $sh_2$  mutation (SS389/SS386) carriers.

In general, hybrids AE800/AE392 (*ae*) and SS389/SS386 ( $sh_2$ ) were more homeostatic than their parent lines.

The correlation analysis showed that the number of roots, their total length and length of seedlings positively correlated with the germinability in the accelerated aging, freezing and combined regime experiments: r = 0.47-0.64. Relatively high correlations were found between the germinability and the effects on the seedling length after accelerated aging and freezing: r = 0.72 and 0.75, respectively; the coefficient was somewhat lower after the combined regime: r = 0.64. The correlation coefficients from sub-moderate to moderate, also positive, were noticed between the germinability and the number of roots and their total length in all the three experimental variants: r = 0.27 - 0.63. Consequently, the stress factors under investigation had similar effects on the seedling length. At the same time, the correlations between the initial (control) germinability and morphological parameters of seedlings after influence of the three studied factors were weak: r did not exceed 0.35. Thus, the initial germinability cannot predict the development of seedlings after influence of these stress factors.

**Conclusions.** Summarizing the results of studying the response of the seedling morphological characteristics to the factors that simulate the processes of storage of corn seeds with different endosperm structures, we established the following:

The three factors suppressed all the morphological characteristics in the parent lines  $-sh_2$  mutation:  $\Im$ SS386 and  $\Im$ SS389. At the same time, hybrids, on the contrary, more often had increased indices, and only in one case a hybrid reduced them (the seedling length under the combined regime). Decrease in the majority of morphological characteristics under accelerated aging occurred in the *wx* mutation carriers: hybrid Binom and its parental lines  $\Im$ VK69 and  $\Im$ VK64. Positive effects of two stress factors (accelerated aging and freezing) were noted for all the morphological characteristics in the *ae* mutation carriers: hybrid AE800/AE392 and parent lines  $\Im$ AE392 and  $\Im$ AE800. The remaining hybrids and lines showed ambiguous responses to the three stress factors: some indices were stimulated, others – inhibited or did not respond.

After accelerated aging and freezing, hybrids to various degrees exceeded their parent lines by two morphological characteristics (seedling length and root number) in dent accessions (Vympel); by number and total length of roots – in the  $sh_2$  mutation carriers (SS389/SS386); and by the total length of roots and seedling length – in the  $su_1$  carriers (Dmytryk).

In general, hybrids AE800/AE392 (*ae*) and SS389/SS386 ( $sh_2$ ) were more homeostatic as compared to their parent lines.

The highest seed longevity upon storage (at seed moisture of 7%-10%) was observed in dent corn and the *ae* and *su*<sub>1</sub> mutations carriers; low longevity – in the *wx* and *se* mutations carriers, and the lowest longevity – in the mutation *sh*<sub>2</sub> carriers.

### Список використаних джерел

- 1. Smith A.M., Martin C. Starch biosynthesis and the potential for its manipulation. Biosynthesis and manipulation of plant products. Plant biotechnology. In: D. Grierson, editor. Glasgow: Blackie and Son Publ., 1993. Vol. 3. P. 1–54.
- 2. Hannah L.C., Giroux M., Boyer C.D. Biotechnological modification for sweet corn and maize improvement. Sci. Hortic. 1993. Vol. 55. P. 177–197.
- Ferguson V.L. High amylose and waxy corns. Specialty corns. In: A.R. Hallauer, editor. Boca Raton, Fl.: CRC Press, 1994. P. 55–779.
- Nelson O.E., Pan D. Starch synthesis in maize endosperm. Ann. Rev. Plant Physiol. Plant Mol. Biol. 1995. V. 46. P. 475–496.
- 5. Tracy W.F. History, breeding, and genetics of super-sweet corn. Plant Breeding Review. 1997. V. 14. P. 189–236.
- 6. Лінник Ю.О., Тимчук С.М., Чупіков М.М., Кузьмишина Н.В. Витривалість насіння різних підвидів кукурудзи до старіння. Таврійський наук. вісник.2010. Вип. 69. С. 67–74.
- 7. Wilson Jr.D.O., Mohan S.K., Ratcliff S.L., Knapp A.D. Effect of harvest and conditioning on vigor of shrunken 2 sweet corn seed. J. Prod. Agric.1994. V. 7.P. 335–339.
- Parera C.A., Cantliffe D.J., McCarty D.R., Hannah L.C. Improving vigor in shrunken-2 corn seedlings. J. Amer. Soc. Hort. Sci. 1996. V. 121. P. 1069–1075.
- 9. Douglass S.C., Juvik J.A. Cold soil emergence and variation in sweet corn kernel carbohydrate reserves. Hort. Sci. 1987. V. 22. P. 1097–1102.
- 10. Rowe D.E., Garwood D.L. Effects of four maize endosperm mutants on kernel vigor. Crop Science. 1978. V.18. P. 709–712.
- 11. Yousef G.G., Juvik J.A. Enhancement of seedling emergence in sweet corn by markerassisted backcrossing of beneficial QTL. Crop Sci. 2002. V. 42.P. 96–104.
- 12. Revilla P., Velasco P., Malvar R.A. et al. Variability among maize inbred lines for seed longevity. Genet. Res. And Crop Evol. 2006. V. 53. P. 771–777.
- Woltz J., Tekrony D. Accelerated aging test for corn seed. Seed Technol. 2001. V. 23. P. 21– 34.
- Miloševic M., Vujakovic M., Karagic D. Vigour tests as indicators of seed viability. Genetika. 2010. Vol. 42. No. 1. P. 103–118.

- 15. Лихачев Б.С. Некоторые методические вопросы изучения биологии старения семян. Сельскохозяйственная биология. 1980. Том XV. №6. С. 842–844.
- 16. Стандарты банков генов генетических ресурсов растений для производства продовольствия и ведения сельского хозяйства. ФАО, 2015, 180 с.
- 17. Лінник Ю.О. Вплив від`ємної температури на показники життєздатності насіння. Вісник Полтавської державної аграрної академії. 2010. № 3(58). С. 175–179.
- 18. Douglass S.C., Juvik J.A. Cold soil emergence and variation in sweet corn kernel carbohydrate reserves. Hort. Sci. 1987. V. 22. P. 1097–1102.
- 19. Шиянова Т.П., Тимчук С.М., Богуславський Р.Л. Довговічність насіння форм кукурудзи з різною структурою ендосперму. Генетичні ресурси рослин. 2015. № 17. С. 87–98.
- 20. Евростандарт. Семена кукурузы. Классификация гибридов кукурузы по ФАО. URL: http://eurostd.com/?p=97.
- 21. Насіння сільськогосподарських культур. Методи визначення якості. ДСТУ 4138-2002 [Чинний від 2004-01-01]. К.: Держспоживстандарт України, 2003.173 с.
- 22. Лихачев Б.С. Морфологическая оценка проростков и силы роста семя. Селекция и семеноводство. 1977. № 3. С. 67–68.
- 23. Лакин Г.Ф. Биометрия. М.: Высшая школа, 1990. 350 с.
- 24. Диагностика устойчивости растений к стрессовым воздействиям (методическое руководство). Л., ВИР, 1988. 228 с.
- 25. Gustafson A. The effect of heterozygosity on variability and vigour. Hereditas. 1946. V. 32. P. 263–284.

### References

- 1. Smith AM, Martin C. Starch biosynthesis and the potential for its manipulation. Biosynthesis and manipulation of plant products. Plant biotechnology. In: D Grierson, editor. Glasgow: Blackie and Son Publ., 1993. Vol. 3. P. 1–54.
- 2. Hannah LC, Giroux M, Boyer CD. Biotechnological modification for sweet corn and maize improvement. Sci. Hortic. 1993; 55: 177–197.
- 3. Ferguson VL. High amylose and waxy corns. Specialty corns. In: AR Hallauer, editor. Boca Raton, Fl.: CRC Press, 1994. P. 55–779.
- 4. Nelson OE, Pan D. Starch synthesis in maize endosperm. Ann. Rev. Plant Physiol. Plant Mol. Biol. 1995; 46: 475–496.
- 5. Tracy WF. History, breeding, and genetics of super-sweet corn. Plant Breeding Review. 1997; 14: 189–236.
- 6. Linnyk YuO, Tymchuk SM, Chupikov MM, Kuzmishina NV. Tolerance of seeds of different corn subspecies to aging. Tavriyskyi naukovyi visnyk. 2010; 69: 67–74.
- 7. Wilson JrDO, Mohan SK, Ratcliff SL, Knapp AD. Effect of harvest and condition in gon vigor of shrunken 2 sweet corn seed. J. Prod. Agric.1994; 7: 335–339.
- 8. Parera CA, Cantliffe DJ, McCarty DR, Hannah LC. Improving vigor in shrunken-2 corn seed-lings. J. Amer. Soc. Hort. Sci. 1996; 121: 1069–1075.
- 9. Douglass SC, Juvik JA. Cold soil emergence and variation in sweet corn kernel carbohydrate reserves. Hort. Sci. 1987; 22: 1097–1102.
- 10. Rowe DE, Garwood DL. Effects of four maize endosperm mutants on kernel vigor. Crop Science. 1978; 18: 709–712.
- 11. Yousef GG, Juvik JA. Enhancement of seedling emergence in sweet corn by marker–assisted backcrossing of beneficial QTL. Crop Sci. 2002; 42: 96–104.
- 12. Revilla P, Velasco P, Malvar RA et al. Variability among maize inbredlinesforseed longevity. Genet. Res. And Crop Evol. 2006; 53: 771–777.
- 13. Woltz J, Tekrony D. Accelerated aging test for corn seed. Seed Technol. 2001; 23:21–34.
- 14. Miloševic M, Vujakovic M, Karagic D. Vigour tests as indicators of seed viability. Genetika. 2010; 42(1): 103–118.
- 15. Likhachev BS. Some methodological issues of research into seed aging biology. Selskokhoziaystvennaia biologiia. 1980; XV(6): 842–844.

- 16. Standards of gene banks of plant genetic resources for food production and agriculture. FAO, 2015, 180 p.
- 17. Linnyk YuO. Influence of negative temperature on viability indices of seeds. Visnyk Poltavskoyi derzhavnoyi agrarnoyi akademiyi. 2010; 3(58): 175–179.
- 18. Douglass SC, Juvik JA. Cold soil emergence and variation in sweet corn kernel carbohydratereserves. Hort. Sci. 1987; 22: 1097–1102.
- 19. Shyianova TP, Tymchuk SM, Boguslavskyi RL. Longevity of corn seeds with different structure of endosperm. Genetychni resursy Roslyn. 2015; 17: 87–98.
- 20. Euro-standard. Corn seeds. FAO Classification of corn hybrids.URL: http://eurostd.com/?p=97.
- 21. Seeds of agricultural crops. Methods for determining quality. DSTU 4138-2002. 2004-01-01. Kyiv: Derzhspozhyvstandart Ukrayiny, 2003. 173 p.
- 22. Likhachev BS. Morphological evaluation of seedlings and germination energy of seeds. Selektsiia I semenovodstvo. 1977; 3: 67–68.
- 23. Kakin GF. Biometrics. Moscow: Vysshaia shkola, 1990. 350 p.
- 24. Diagnosis of plant resistance to stress factors. Leningrad: VIR, 1988. 228 p.
- 25. Gustafson A. The effect of heterozygosity on variability and vigour. Hereditas. 1946; 32: 263–284.

# МОРФОЛОГІЧНІ ПОКАЗНИКИ ПРОРОСТКІВ ЕНДОСПЕРМОВИХ МУТАНТІВ КУКУРУДЗИ ТА ВПЛИВ НА НИХ РЕЖИМІВ ЗБЕРІГАННЯ НАСІННЯ

Шиянова Т.П., Тимчук С.М., Богуславський Р.Л. Інститут рослинництва ім. В.Я. Юр'єва НААН, Україна

- Ендоспермові мутанти кукурудзи є джерелом цінної сировини для споживання і різних галузей промисловості. Тому проблема довговічності їх насіння є актуальною для зберігання в генбанках, селекційних і насінницьких установах.
- **Метою дослідження** є визначення впливу різних режимів, моделюючих довгострокове зберігання, на морфологічні показники проростків з насіння ендоспермових мутантів кукурудзи (довжина ростка, кількість корінців та їх сумарна довжина).
- **Матеріали і методи**. Матеріалом було насіння шести простих гібридів F<sub>1</sub> та їх 12 батьківських ліній носіїв ендоспермових мутацій: *wx, ae, su<sub>1</sub>, se, sh<sub>2</sub>*; контроль зубоподібний тип (ssp. *indentata*). На насіння діяли прискореним старінням за методом Б.С. Лихачева (1978), проморожуванням (30 діб) та комбінованим режимом прискорене старіння + проморожування.
- **Обговорення результатів.** Зниження показників усіх морфологічних ознак під дією трьох чинників показали носії мутації  $sh_2 \Im SS386$  і  $\Im SS389$ . Їх гібрид F<sub>1</sub>, як правило, перевищував показники, у нього знизилася лише довжина ростка при комбінованому режимі. Зниження більшості показників морфологічних ознак під дією прискореного старіння спостерігалося у носіїв *wx*: Бином,  $\Im BK69$  та  $\Im BK64$ . Позитивний вплив прискореного старіння та проморожування відмічено на показники всіх трьох морфологічних ознак у носіїв *ae*: AE800/AE392,  $\Im AE800$ . Перевищення за довжиною ростка та кількістю корінців гібрида над батьківськими лініями після прискореного старіння та проморожування проявилося у гібрида зубоподібного типу Вимпел, а за кількістю та сумарною довжиною корінців – у гібрида носія  $sh_2$  SS389/SS386; за сумарною довжиною корінців та довжиною ростка – у носія  $su_1$  Дмитрик. У цілому у порівнянні з батьківськими лініями гібриди AE800/AE392 (*ae*) та SS389/SS386 (*sh*<sub>2</sub>) є більш гомеостатичними.
- **Висновки.** Найвищою довговічність була у насіння кукурудзи ssp. *indentata* та носіїв генів *ae* і *su*<sub>1</sub>, низькою у носіїв генів *wx* і *se*, а найнижчою у носіїв генів *sh*<sub>2</sub>.

Ключові слова: кукурудза, насіння, проросток, морфологія, ендоспермовий мутант, довговічність, моделювання старіння, проморожування

# МОРФОЛОГИЧЕСКИЕ ПОКАЗАТЕЛИ ПРОРОСТКОВ ЭНДОСПЕРМАЛЬНЫХ МУТАНТОВ КУКУРУЗЫ И ВЛИЯНИЕ НА НИХ РЕЖИМОВ ХРАНЕНИЯ СЕМЯН

Шиянова Т.П., Тимчук С.М., Богуславский Р.Л. Институт растениеводства им. В.Я. Юрьева НААН, Украина

- Эндоспермальные мутанты кукурузы являются источником ценного сырья для направлений потребления и разных отраслей промышленности. Поэтому проблема долговечности их семян является актуальной для хранения в генбанках, селекционных и семеноводческих учреждениях.
- **Цель исследований:** определить влияние разных режимов, моделирующих длительное хранение, на морфологические показатели проростков из семян эндоспермальных мутантов кукурузы (длина ростка, количество корней и их суммарная длина).
- **Материалы и методы**. Материалом были семена шести простых гибридов F<sub>1</sub> и 12 их родительских линий – носителей эндоспермальных мутаций: *wx, ae, su<sub>1</sub>, se,sh<sub>2</sub>*; контроль – зубовидный тип (ssp. *indentata*). На семена воздействовали ускоренным старением по методу Б.С. Лихачева (1978), промораживанием (30 суток) и комбинированным режимом – ускоренное старение + промораживание.
- **Обсуждение результатов.** Снижение показателей всех морфологических признаков под действием трех факторов отмечено у носителей мутации  $sh_2$  SS386 и SS389. Их гибрид F<sub>1</sub>, как правило, повышал показатели, уменьшилась только длина ростка при комбинированном режиме. Снижение большинства показателей морфологических признаков под действием ускоренного старения наблюдали у носителей *wx*: Бином, QBK69 и BK64. Положительное влияние ускоренного старения и промораживания отмечено на показатели всех трех морфологических признаков у носителей *ae*: AE800/AE392, QAE392, AE800. Превышение по длине ростка и количеству корешков гибрида над родительскими линиями после ускоренного старения и промораживания проявилось у гибрида зубовидного типа Вымпел, а по количеству и суммарной длине корешков у гибрида носителя  $sh_2$  SS389/SS386; по суммарной длине корешков и длине ростка у носителя  $su_1$  Дмитрик. В целом по сравнению с родительскими линиями гибриды AE800/AE392 (*ae*) и SS389/SS386 ( $sh_2$ ) были более гомеостатичными.
- **Выводы.** Наиболее высокую долговечность наблюдали у семян кукурузы ssp. *indentata* и носителей генов *ae* и *su*<sub>1</sub>, низкую у носителей генов *wx* и *se*, а наиболее низкую у носителей генов *sh*<sub>2</sub>.

**Ключевые слова**: кукуруза, семена, проросток, морфология, эндоспермальный мутант, долговечность, моделирование старения, промораживание

## MORPHOLOGICAL PARAMETERS OF MAIZE ENDOSPERMAL MUTANTS SEEDLINGS AND EFFECT OF SEED STORAGE REGIMES ON THEM

Shyianova T.P., Tymchuk S.M., Boguslavskyi RL. Plant Production Institute nd. a V.Ya. Yuriev of NAAS, Ukraine

- Endospermal mutants of maize are a source of valuable raw materials for various industries and directions of consumption. Therefore, problem of their seeds longevity is relevant for storage in genebanks, breeding and seed-growing establishments.
- The aim and tasks of the study was to determine effect of different storage regimes on morphometric characteristics of seedlings from seeds of maize endosperm mutants.
- **Material and methods.** Seeds of six simple hybrids F1 and 12 their parental lines carriers of endospermal mutations: wx, ae,  $su_1$ , se,  $sh_2$ , and dentate type are used as the material. The seeds were affected by accelerated aging using the method of B.S. Likhachev (1978), freezing during a month and combined regime accelerated aging + freezing.

- **Results and discussion.** The decrease in the indices of all morphometric traits under influence of three factors was shown by carriers of the mutation  $sh_2 \ SS386$  and  $\ SS389$ . Their F1 hybrid, as a rule, increased the indices and reduced only length of the seedling under the combined regime. Reduction of most indicators of morphometric traits under accelerated aging was observed in carriers *wx*: Binom,  $\ VK69$  and  $\ VK64$ . Positive effect of accelerated aging and freezing was observed on the indices of all three morphological characters in *ae* carriers: AE800/AE392,  $\ AE392$ ,  $\ AE800$ . The excess of the hybrid over the parent lines in length of seedling and number of roots after accelerated aging and freezing manifested in the dentate hybrid Vympel, in the number and total length of roots in carriers of *sh*<sub>2</sub> SS389 / SS386; in total roots length and seedling length from the carrier *su*<sub>1</sub> Dmytryk. In general, the hybrids AE800/AE392 (*ae*) and SS389/SS386 (*sh*<sub>2</sub>) are more homeostatic than the parent lines.
- **Conclusions.** The highest longevity is characterized for the seeds of dentate maize and carriers of *ae* and  $su_1$ , comparatively low longevity–carriers of *wx* and *se*, and the lowest carriers of  $sh_2$ .

*Key words:* maize, seed, seedling, morphology, endospermal mutants, longevity, modeling of aging, freezing