

THE DECLINE IN VIABILITY OF CORN SEEDS (*INBRED LINES*) UNDER INFLUENCE OF THE STORAGE DURATION IN “OPEN WAREHOUSE”

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Abstract. The objective of this research was to determine the changes in quality of maize (*Zea mays* L.) seed in three years storage and in four genetically different *Inbred Lines*, during storage, under open storage conditions. The complexity of producing inbred lines to produce hybrids with a number of attributes such as adaptability, disease resistance and drop, high production potential has in practice led to them being produced in quantities to meet the needs over many years, therefore subject to longer shelf life. Four experimental Inbred lines, each represented by four seed lots produced by the Agricultural Research and Development Station Turda, /Romania, were evaluated. Seeds (untreated) were stored for 36 months, under open storage conditions and for the seed performance was evaluated the time of “initial-phase” and every 12 months by seed viability (germination). The experimental results show a large variation of the recorded germination, both in the line with the duration of the storage, but also between them, in each analyzed stage. After a 36-month retention period, they recorded very low germination values, for example, the LC363 line had the final germination of $G_{36} = 33\%$, fact that highlights the different conservation capacity of each seed's initial traits and that this is a genetic feature, influenced differently by the duration and conditions of retention.

Keywords: maize, seed viability, seed vigour, storage conditions.

INTRODUCTION

Since the beginning of agriculture, man has been preoccupied with keeping agricultural products in the form of beans for food and for propagation. The preservation of agricultural products, especially seeds, requires increased attention to maintain essential features and reduce loss. According to the information in the field, over 25% is lost each year (Iyoti and Malik, 2013) of the amount of grain harvested worldwide, largely due to inadequate storage. The quality of agricultural crop production reflects biological features, environment, quality of applied cultivation technology, harvesting, processing and, last but not least, conservation management. The following parameters are of fundamental importance for keeping the seed quality during storage: the moisture content of the seed to be kept, the temperature and relative humidity in the storage medium, the seed quality (initial quality) at the time of storage and the destructive attack of microorganisms and insects. In order to limit the action of these factors on the normal seed aging process, with consequences on seed quality, it is necessary to know, control and regulate this process in order to prolong the useful life of seed lots. The most commonly used and most applicable method of storage for all seeds used as biological material in the world and in the country is dry storage in open warehouses.

Open area is considered the place of storage where the seed water content fluctuates depending on relative humidity and storage temperature. These storage systems do not

require cooling installations, which are expensive, but suitable concrete constructions, such as silos or wall sheds, best insulated to outside temperature variations, provided with aeration and control systems in different points of the seed mass parameters. In order to maintain the viability of the seeds, it is necessary to maintain the proper condition of phytosanitary hygiene (free of pathogens, nematodes, mites, insects and rodents in these deposits).

Production in maize increased with the introduction and use of seeds for the hybrid culture, first in the US in 1933, and in our country they expanded into culture after 1954, bringing production increases of more than 50% to the varieties of corn. Inbred lines are characterized by their combined ability to cross-hybrid hybrids with high production capacity, which requires it to be highly hereditary, uniformity and viability. In maize, a high level of stability and uniformity is achieved after several generations of repeated self-pollination when most places become homozygous. The homozygous state influences the phenotypic appearance of the plants, reflecting their great uniformity. At the same time, the reaction to the storage conditions increases (Olaru and Marghitu, 1987). The moment of harvest, fixed by physiological maturity, influences seed quality, germination and vigor, as well as good preservation. Inbred lines are more susceptible to drying temperatures, in the form of cobs, compared to commercial hybrids, requiring moderate drying regimes (Moldovan et al., 2015).

Non-observance of the drying dynamics, in addition to the deterioration of the aleuronic layer (the living layer of the endosperm), the cracking of the embryonic spindle, two detrimental reactions may be triggered, the Maillard reaction and free radical oxidations (Murariu et al. 1988), both with effects on proteins and effects on reaching the vitreous stage of the cytoplasm in the seed. The complexity of producing inbred lines with good combinatorial capacity and valuable inbred characters has determined that in practice they are produced in quantities that meet the needs for many years. The ultimate goal is to achieve safe and quality productions, for which the entire genetic system competes through various mechanisms, acting on physiological, biochemical processes, structural components, all in interaction with the environment, permanently changing, throughout life, from seed – to plant and again to seed.

MATERIAL AND METHODS

The conditions in which the experiments took place. The research presented in this paper began in 2013, at SCDA Turda, Romania, and ITCSMS Cluj, Romania, during 2013-2016. Storage (open space), thermally insulated, providing small variations of environmental parameters in space, at the major changes of the outside, with a temperature variation during the year, 8-28°C and relative variation, 25-75% humidity with the possibility of applying natural aeration.

Factors and experimental design. Taking into account the importance of inbred lines in the practice of improvement, four inbred lines (parental forms) of simple commercial hybrids or plowing forms of trilinear hybrids were taken for analysis.

- Inbred lines (A):

A₁- Line LC 223 NrfT

A₂- Line LC 223 NrfC

A₃- Line LC 363

A₄- Line LC 763

-storage duration (B) with graduations: “before sowing-2013” is the initial moment of research; after 12 months; after 24 months; after 36 months.

- The experimental design: Completely Randomized Design (RCD) of tipe: 4x4 in four rehearsals

RESULTS AND DISCUSSION

Study of the viability of seed lots

In the present study, the four inbred lines under study were from the 2013 harvest, stored dry and untreated, in the "open" warehouse. It was considered as the initial moment of research, the stage "before sowing 2013". Combination factor and mean germination of the four repetitions, for each variant, are given in Table 1.

Table 1

Combination of studied factors in the experiment

| Inbred lines | Duration of storage | | | |
|--------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| | <i>before sowing</i> | <i>after "12 months"</i> | <i>after "24 months"</i> | <i>after "36 months"</i> |
| LC 223 Nrf.T | a ₁ b ₁ = 93,25 | a ₁ b ₂ = 94,00 | a ₁ b ₃ = 88,00 | a ₁ b ₄ = 87,25 |
| LC 223 Nrf.C | a ₂ b ₁ = 91,50 | a ₂ b ₂ = 86,00 | a ₂ b ₃ = 80,75 | a ₂ b ₄ = 63,00 |
| LC 363 | a ₃ b ₁ = 94,00 | a ₃ b ₂ = 92,50 | a ₃ b ₃ = 64,50 | a ₃ b ₄ = 33,00 |
| LC 763 | a ₄ b ₁ = 92,50 | a ₄ b ₂ = 85,00 | a ₄ b ₃ = 78,50 | a ₄ b ₄ = 80,00 |

We can see a decrease in average germination for all lines, with the aging of seeds, but more evident in the stage, "after 24 months".

In order to study the influence of factors, inbred lines and duration of preservation on viability, another research scheme has been approached, which is based on the calculation of the so-called "deviations" of individual values and not on the average square deviations.

Following the procedure, the results are given in Table 2, in which it can be seen from the application of the criterion "F" the very significant influence on the viability of the seed line - (represented by the standard germination) of the main factors but also of their interaction.

The experimental error was based on the determinations of the limit differences by known methodologies. We also present the technique of making simple or cumulative comparisons between factor levels.

error variance:

$$S_E^2 = \frac{N \cdot \delta_E}{df_E} = \frac{64 \cdot 2,176}{45} = 3,095; \quad (1)$$

δ_i -deviația valorilor individuale

- * comparisons between the simple or cumulative levels of the factors use the relationship (2):

$$F_c = \frac{n_1 \cdot n_2}{n_1 + n_2} * \frac{(\bar{m}_1 + \bar{m}_2)^2}{s_E^2} > F_t(df_1=1; df_2=df_E) \quad (2)$$

- * we compare Level 2 with Level 4 of the Inbred Lines Factor; Following the application of the relationship (2) results:

$$F_c = \frac{16 \cdot 16}{16 + 16} * \frac{(80,313 - 84)^2}{3,095} = 35,1 > F_{tabel} = 4,05 ;$$

which means that there are significant differences between the action level 2 and level 4 of the "inbred lines".

The experimental results show a large variation of recorded germination, both in the case of a line along with the duration of the storage, but also between them, at each analyzed

stage, which shows their increased sensitivity to the storage duration and last but not least of the storage conditions (Table 3)

Table 2

Highlithing of factors influence by other statistic methods

| Cause of variability | Deviation of partial averages [δ_i] | Degree of freedom [df] | Average deviation [δ_i] | Test "F" |
|--|--|------------------------|----------------------------------|-----------------------|
| Repetition (δ_R) | $\delta_R = (\sum \bar{x}_{Ri}^2) / r - \bar{\bar{x}}^2 = 0,219$ | 3 | | |
| Inbred lines (δ_L) | $\delta_L = (\sum \bar{x}_{Li}^2) / e - \bar{\bar{x}}^2 = 50,355$ | 3 | 16,785 | 346,79 ^{xxx} |
| Duration of storage (δ_Δ) | $\delta_\Delta = (\sum \bar{x}_{\Delta i}^2) / d - \bar{\bar{x}}^2 = 112,255$ | 3 | 37,418 | 773,09 ^{xxx} |
| Factorial variability ($\delta_{L\Delta}$) | $\delta_{L\Delta} = (\sum \bar{x}_{(L\Delta)i}^2) / l * d - \bar{\bar{x}}^2 = 243,541$ | 15 | - | - |
| Interaction: L x Δ ($\delta_{L\Delta}$) | $\delta_{L\Delta} = \delta_{L\Delta} - \delta_L - \delta_\Delta = 80,931$ | 9 | 8,992 | 185,78 ^{xxx} |
| Error (δ_E) | $\delta_E = \delta - \delta_R - \delta_{L\Delta} = 2,176$ | 45 | 0,0484 | - |
| Total (δ) | $\delta = (\sum x_i^2) / N - \bar{\bar{x}}^2 = 245,936$ | 63 | | |

Table 3 shows the separate influence of inbred lines and retention time on germination. Within the "storage duration" factor, the "before sowing" control was taken and line 363 was taken, followed by the calculation of the differences and the appreciation of the meanings.

Table 3

The separate influence of inbred lines and storage duration on germination

| Inbred lines | Duration of storage | | | | Average germination (%) | Relative germination n (%) | Difference | Significance |
|--------------------------|---------------------|------------|------------|------------|--|----------------------------|------------|--------------|
| | „initially | „12 months | „24 months | „36 months | | | | |
| LC 223 NrfT | 93,25 | 94,00 | 88,00 | 87,25 | 90,625 | 127,64 | 20,62 | ** |
| LC 223 NrfC | 91,50 | 86,00 | 80,75 | 63,00 | 80,31 | 113,11 | 9,31 | *** |
| LC 363 | 94,00 | 92,50 | 64,50 | 33,00 | 71,00 | 100 | - | Mt |
| LC 763 | 92,50 | 85,0 | 78,50 | 80,00 | 84,00 | 118,30 | 13,00 | * |
| Average germination (%) | 92,81 | 89,37 | 77,93 | 65,81 | LSD _{5%} = 1,31; LSD _{1%} = 1,75; LSD _{0,1%} = 2,28 | | | |
| Relative germination (%) | 100 | 96,29 | 83,97 | 70,90 | | | | |
| Difference | - | 3,43 | 14,87 | - 27 | | | | |
| Significance | Mt. | ooo | ooo | ooo | | | | |

LSD_{5%} = 1,26; LSD_{1%} = 1,69; LSD_{0,1%} = 2,20

Limit differences for the combined effect: LSD_{5%} = 2,50; LSD_{1%} = 3,35; LSD_{0,1%} = 4,38

We note the good behavior with the LC 223Nrf.T line storage length, over the entire storage period followed by the LC 763 line but the poor storage resistance of the LC 223 Nrf C and LC 363 lines after the "36 months" storage period.

Quantification and nature of factors effects on germination using polynomial orthogonal coefficients. It was highlighted the very significant action of the studied factors

on germination, which was previously demonstrated by the method of deviations. There is a question of the nature of the effects that exist between the "standard germination" of the inbred lines and the "shelf-life" factor. To divide a square of deviations of a factor having (P-1) degrees of freedom, in sums related to the linear, namely quadratic and residual component, the polynomial orthogonal coefficients. After calculating these components, it is found that both the storage duration and the interaction duration factor are the predominantly linear part (Table 4).

Table 4

The nature of effects of „storage duration” factor on inbred lines germination capacity

| Cause of variability | Degree of freedom [df] | Sum of squares [SP] | Square of averages [s_i^2] | Test 'F' |
|----------------------------------|------------------------|---------------------|--|------------|
| Repetition (σ_R) | 3 | 14,01 | $s_R^2 = \delta_R * N / df_R = 4,67$ | |
| Inbred lines (L) | 3 | 3222,72 | $s_L^2 = \delta_L * N / df_L = 1074,24$ | 347,08*** |
| Duration of storage (Δ) | 3 | 7184,31 | $s_\Delta^2 = \delta_\Delta * N / df_\Delta = 2394,77$ | - |
| linear (Δ_L) | (1) | 6835,75 | 6835,75 | 2208,64*** |
| quadratic (Δ_Q) | (1) | 301,89 | 301,89 | 97,54** |
| residual (Δ_{rez}) | (1) | 46,67 | - | - |
| Interaction: L X Δ | 9 | 5179,59 | $s_{L*\Delta}^2 = \delta_{L*\Delta} * N / df_{L\Delta} = 575,51$ | - |
| L X Δ_L - linear | (1) | 4217,96 | 4217,96 | 1362,83*** |
| L X Δ_Q - quadratic | (1) | 831,422 | 831,422 | 268,63*** |
| L X Δ_{rez} - residual | 7 | 130,208 | - | - |
| Error | 45 | 139,275 | $s_E^2 = \delta_E * N / df_E = 3,095$ | |

After applying the meanings, it is noted that both linear and square components, in the case of the storage duration and the interaction factor, are significant, this shows that both components are important in describing line behavior during storage.

At the same time, the meanings of both components show that the response of the lines relative to the factor, the retention time, can't be quantified strictly after a linear dependence.

There is a possibility to further study to determine which of the inbred lines in combination with the storage duration factor give a predominant response after a non-linear dependence, in which case the response can't be estimated, and the line reactions are unpredictable, otherwise, unpredictable components are of greater importance than those predictable.

Regarding the resistance and the preservation of the initial features in the inbred lines stored in dry state in open warehouses, we find a lower preservation of the original qualities than in the case of the hybrids (Barbos et al, .2016)

CONCLUSIONS

- the high variability (almost 35%) of the germination value of the "orthodox" seed of the studied lines during preservation, shows the different conservation capacity of the initial seminar characteristics of each and that it is a genetic characteristic influenced by the different from the duration and storage conditions;

- the intensity of seed decline may be reduced by controlling and directing the fundamental parameters that characterize the storage conditions;

- of the recorded results show a slight conservation of their initial features, so after a 36-month retention period, they recorded very low germination values, for example the **LC 363** line had the final germination of $G_{36} = 33\%$;

- the increased sensitivity of the inbred lines to the storage conditions requires a much closer observation and management of the parameters of the storage medium to ensure their good conservation;

- using the *orthogonal polynomial coefficients*, their behavior was estimated during storage;

- the linear component of the factor, *the storage duration* of 6835.75 is approximately 22 times the non-linear part, while in the interaction the duration of the x-line storage is reduced to approximately **5 times (4217,96 / 831,422)**, but both components for the duration of the storage and interaction factors are significant;

- the result of significance shows that the estimation of their behavior during the preservation cannot be estimated, in other words, the reactions of the inbred lines under the given experimental conditions are unpredictable, which means that the unpredictable components are of greater importance than those predictable within the effects of the factors.

REFERENCES

1. Bărbos A., Gr. Moldovan, Otilia Micu, 2016. The influence of genotype, treatment and storage period on ageing process of hybrid corn seeds, Agriculture-Science and Practice Journal 97-98, 70-75.
 2. Iatoi, S.A, Malik, C.P, 2013. Seed deterioration: A review Vol. 2 No.3; Internațional Journal of life Sciences Biotechnology.
 3. Morad Shaban, 2013. Study on some aspects of seed viability and vigor. International journal of Advanced Biological and Biomedical Research. Volume I, issue 12, 1692-1697.
 4. Murariu Daniela, și colab., 1998. Dereglări la nivelul unor componente celulare provocate prin îmbătrânirea forțată a semințelor de porumb. Vol.V. Cercetări de genetică
 5. Olaru, C., Valeria Marghitu, 1987. Bazele tehnice ale producerii, condiționării și păstrării semințelor agricole. Ed. Scrisul Românesc, Craiova.
 6. Maniu , Al., Voda, Gh, V., 2006. Proiectarea statistică a experiențelor. Ed. Economică Bucuresti.
 7. Moldovan, Gr., Bria, N., Barbos, A., 2015. The influence of the conditioning system on seed quality. Volumul: "International SYMPOSIUM- ISB-INMA the Aricultural and Mechanical Engineering". October-2015. University Politehnica of Bucharest.
- *** ISTA Seedling Evaluation, 3 rd Edition, 2003.
*** SR 1634/1999 Seminte pentru însamantare. Determinarea germinației.
*** AOSA, 1983. Seed vigor testing handbook an seed test