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EFFECT OF NEGATIVE TEMPERATURE SHOCK ON THE PRIMARY REZISTANCE OF MAIZE HYBRIDS

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ABSTRACT

The paper presents the results of assessing the resistance of 14 maize hybrids to a negative temperature shock (NTS) of -4°C for 16 hours. The germination and growth seedlings parameters of maize hybrids were studied. Germination of maize seeds at optimal temperature of 26°C for 5 days was used as a control, and pretreatment of maize seeds with NTS followed by germination and subsequent growth at 26°C for 5 days was considered experimental. Pretreatment of maize seeds with NTS made it possible to identify and evaluate the primary resistance of each hybrid to stress, and according to the degree of constitutive frost resistance of each hybrid, the distribution and classification of the studied hybrids into 3 zones was carried out, including hybrids with high, medium and low frost resistance. Also, the effect of NTS on the morpho-physiological traits of the maize seedlings are shown.

INTRODUCTION

Maize represents one of the main agricultural crops in most countries of the world. Maize (*Zea mays* L.) originating from areas with tropical and subtropical temperatures, in the Republic of Moldova regions is often exposed to the action of a various environmental factors, including extreme temperatures. Due to the phenomena of global climate change, the area under maize cultivation continues to expand in the northern regions and high altitudes (Borozan et al. 2019). Growing maize, a thermophilic crop, under conditions of prolonged periods of low temperature, as well as short cooling events that often occur in spring, greatly affects the initial phases of germination, subsequent growth and productivity (Waqas et al. 2021). Numerous literature data have demonstrated that low temperatures, non-freezing, temperatures induce different types of morphological, physiological and molecular damages in maize throughout the developmental phases of ontogeny (Caus et al. 2022, Waqas et al. 2021, Zhou et al. 2022). As for the influence of freezing temperatures, maize is sensitive to these conditions at all phases of the growing cycle, with the exception of dry seeds (Miedema 1982). In most cases, maize like other crops exposed to extreme temperatures, develops resistance to them as a result of adaptation to the created conditions. The ability of each hybrid to adapt to adverse temperature conditions will be determined by the degree of its capacity to modify metabolism in accordance with the accompanying stresses (Waqas et al. 2021, Zhou et al. 2022), so it is important to take into account the specifics of the interaction of the genotype with the environment (Borozan et al. 2021, Muntean et al. 2022). Maize breeding also pays attention to the risk of damage caused by adverse frost conditions that may occur in some regions.

The purpose of this work was to study the effect of pretreatment of seeds of various maize hybrids with a negative temperature shock on the germination and growth of seedlings at an early stage of ontogenesis.

MATERIAL AND METHODS

In the experiments, 14 maize hybrids from the selection of the Institute of Crop Science "Porumbeni", Republic of Moldova were used. The following hybrids were used: Porumbeni (P.) 427, P. 310, P. 374, P. 180, P. 176 MRf, P. 220, P. 221, P. 230, P. 243, P. 305, Bemo (B.) 203, B. 235, Alimentar (Alim.) and Farmec. Before being placed for germination, the seeds of controls and experimental variants were selected for uniformity and immersed in water at a temperature of 4°C for 36 hours. Previously, it was shown that after this operation, maize seeds are uniformly and well prepared for germination. Further, the seeds of experimental variants freed of water were exposed to negative temperature shock (NTS) by incubation for 16 hours in a climatic chamber RUMED-3401 (Germany) at temperature of –4°C. While the control seeds, also freed of water, were kept in a refrigerator at a temperature of +4°C at this time. Thereafter control and experimental seeds were simultaneously sown in containers on wet cotton discs and set for germination and growth in the thermostat, in the dark, at optimal temperature of 26°C and the relative air humidity of 70-85%. Incubation lasted 120 hours. Every 24 hours, during 5 days, the evolution of seed germination was monitored. The seed was considered germinated when the length of the radicle reached 2-3 mm. At the end of the incubation period, for each variant, the final percentage of germinated seeds was determined as a percentage (%) of the total number of tested seeds, the length of radicles and the height of epicotyls were measured. The experiments were performed in three repetitions. In each repetition, 50 seeds were used. Each experiment was repeated at least three times. The data were statistically processed by determining the mean value and the standard deviation of the mean was calculated using the program "Statistics 7".

RESULTS AND DISCUSSIONS

The response of plants exposed at the initial stages of ontogenesis to the action of the unfavorable factor is defined as primary (specific) resistance or constitutive (Dascaliuc 2021, Ştefîrţă et al. 2021). In order to determine the primary resistance of various maize hybrids to the action of a negative temperature shock (NTS), preliminary experiments were carried out to determine the value and duration of the NTS effect on the seeds, so that after the negative temperature shock, the seed germination percentage was 50%. For this, seeds of P. 427 were subjected to the action of NTS of -6°C during 16 hours. The resulting percentage of seed germination was 16 % with NTS against 98% in the control. Thus, in order to evaluate the constitutive resistance of maize hybrids to the action of negative temperature in the following experiments, a dose of hypothermic shock of -4°C with a duration of 16 hours was used. The germination percentages of maize seeds of investigated hybrids exposed to NTS at the freezing temperature of -4°C for 16 hours are shown in figure 1. The obtained results demonstrate significant differences in the germination percentage of the seeds of the maize hybrids that were pretreated with NTS. Application of NTS seeds before germination causes subsequent inhibition of the germination process at optimal temperature (figure 1). The results showed that the level of inhibition of seed germination by NTS in the analyzed hybrids is different, and the decrease in germination of maize seeds is the more significant, the lower is the resistance in the juvenile phase of the genotype to the action of NTS. Depending on the degree of percentage inhibition of seed germination under the influence of NTS, the hybrids can be divided into 3 zones according to their primary resistance

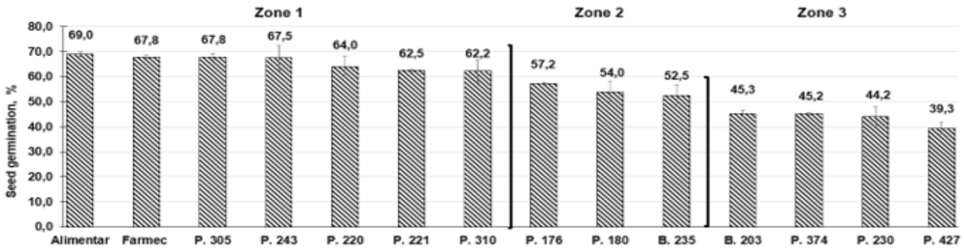

Figure 1. Effect of a negative temperature shock of -4°C (NTS) applied to seeds of different maize hybrids before germination for 16 h on the final percentage of subsequent seed germination under normal conditions of 26°C.
Seedling age - 5 days.

to negative temperature conditions. The percentage of seed germination after NTS of hybrids in zone 1 is 69.0- 62.2%, in zone 2 there are hybrids with a germination rate of 57.2- 52.5%, and in zone 3 there are hybrids with the lowest degree of germination – 45.3- 39.3%. So, after applying a hypothermic shock of -4ºC for 16 hours, hybrids Alimentar, Farmec, P. 305, P. 243, P. 220, P. 221 and P. 310 can be included in the group of highly resistant genotypes of zone 1. Hybrids P. 176, P. 180

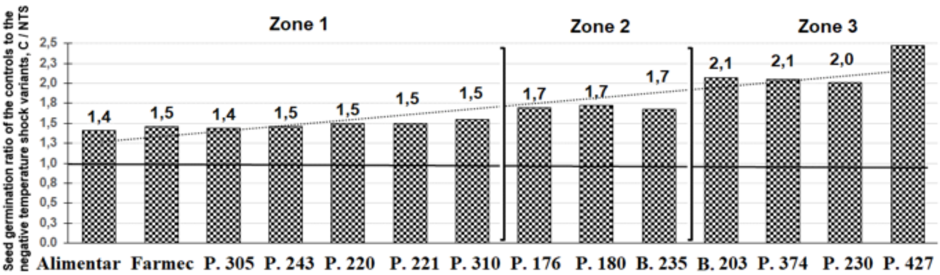

Figure 2. The ratio of the germination percentage of the control seeds (C, %) to the germination percentage of the experimental variants (NTS, %), C / NTS.

and B. 235 from zone 2 showed medium resistance, and seeds of hybrids P. 203, P. 230 and P. 427 from zone 3 showed the least thermotolerance to NTS application.

Also, the ratio between the germination percentage of the control seeds to the germination percentage of the experimental variants with NTS was determined (figure 2). According to the obtained values of this ratio, the studied maize hybrids can also be divided into 3 zones, but the distribution of the values of this ratio was inverse compared to the values in figure 1 with the germination percentage of experimental seeds with NTS. Hybrids from zone 1 with high germination resistance show the lowest ratio – 1.4- 1.5, the index value of 1.7 was for hybrids from zone 2 with medium germination resistance, and hybrids with low resistance from zone 3 are characterized by the highest index value of 2.0- 2.1.

Figure 3. Appearance at the first day of germination of different maize germs, control (C) and experimental (NTS), the seeds of which were pretreated with NTS at – 4°C for 16 hours.

The pretreatment of maize seeds with NTS, which affects the final percentage of germination, also influences the growth of the sprouts. Observations have shown (figure 3) that already on the first day of germination with the appearance of the sprouts, differences between the control and the experimental can be distinguished, in variants with NTS the length of the sprouts is visually smaller than in the control of the analyzed hybrids. This growth inhibition was observed up to day 5 when the seedlings were harvested.

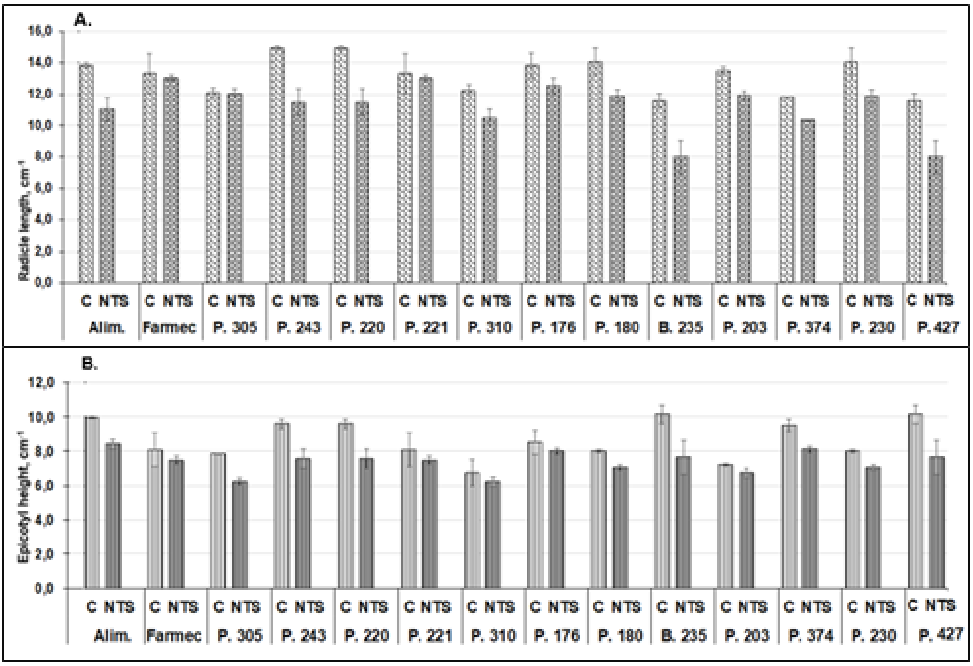

Figure 4. Effect of negative temperature shock (NTS) on radicle length (A) and epicotyl height (B) of 5-day-old seedlings grown from seeds pretreated with NTS of -4°C for 16 h, followed by germination and growth at normal temperature 26 °C.

From the results on the seedling growth parameters presented in figure 4A and 4B can be observed that NTS affects the growth of both radicles length and epicotyls height in all investigated hybrids. Under optimal conditions (control), the length of the seedling radicles varied within 12.6 cm (B.235), 14.9 cm (P. 220), while with NTS - within 8.0 cm (B. 235, P. 427) ...13.0 cm (Farmec, P. 221). And the radicle length values of all other hybrids are distributed within indicated parameters (figure 4A). An inhibitory effect of NTS on the height of epicotyls was also recorded. The values of height epicotyls in experimental variants with NTS were within 6.2 cm (P. 310, P. 305) … 8.0 and 8.4cm (P. 176 and Alim.). While in controls the epicotyls height varied within 6.8 cm (P. 310), 7.2 cm (P. 203) … 10 cm (P. 427, P. 235, Alim.). All other hybrids by epicotyls height are distributed within the indicated parameters (figure 4B).

Table 1

The ratio between the values of the radicle length and epicotyl height of the controls to those of the experimental variants with NTS.

Maize hybrids	C/NTS Radicles, cm^{-1}	C/NTS Epicotyls, cm^{-1}
Alimentar	**1.25**	**1.19**
Farmec	1.02	1.08
P. 305	1.00	1.26
P. 243	**1.30**	**1.27**
P. 220	**1.30**	**1.27**
P. 221	1.02	1.08
P. 310	1.17	1.09
P. 176	1.10	1.06
P. 180	1.18	1.13
B. 235	**1.45**	**1.33**
P. 203	1.13	1.07
P. 374	1.14	1.18
P. 230	1.18	1.13
P. 427	**1.45**	**1.33**

The ratio between the values of the radicle length and epicotyl height of the controls to those of the experimental variants (table 1) shows that this ratio is greater than 1.

If, in the case of the influence of NTS on seed germination, it was possible to distribute the hybrids in a clear order corresponding to the degree of inhibition of germination, whereas according to the rate of subsequent growth of radicles and epicotyls of seedlings grown from seeds pretreated with NTS, the hybrids studied cannot be arranged in a distinct order. But nevertheless, as indicated above, the hybrids P. 427 and B. 235, classified in zones 3 and 2 (figures 1.2) with the lowest germination thermotolerance level, showed the highest ratio of the growth rate of radicles and epicotyls of the control variants to the experimental ones (C/NTS), which amounted to 1.45 and 1.33, respectively (table 1). Also, the percentage of inhibition of radicle and epicotyl growth in seedlings of hybrids (P. 427, B. 235) of the NTS variants was the highest and amounted to 30 and 25%, respectively. The percent inhibition of radicle and epicotyl growth by NTS was about 23 and 21% (P. 220 and P. 243) and 20 and 17% (Alimentar), respectively. Thus, NTS applied to seeds before germination then retained seedling growth at the optimal temperature in some hybrids, although the latter demonstrated a relatively high percentage of germination after NTS. The obtained different results on the effect of seed pretreatment with NTS on subsequent germination rate at the optimum temperature, on the one hand, and on the growth of seedlings under optimal conditions, on the other hand, can probably be explained by the properties of parental forms used for selection (Borozan et al. 2021), or these differences may be related to the role of endogenous phytohormones (Kong et al. 2020), involved in respective processes.

CONCLUSIONS

Specific approach of using pretreatment of seeds of 14 maize hybrids with a negative temperature shock (NTS) of -4°C during of 16 hours made it possible to highlight and evaluate the primary resistance of each hybrid to the frost stress conditions.

Depending on the degree of percentage inhibition of seed germination under the influence of NTS, the hybrids studied can be divided into 3 zones according to their primary resistance to negative temperature conditions. The percentage of seed germination after NTS in hybrids of the 1st zone was 69.0-62.2 %, in hybrids of the 2nd zone – 57.2-52.5 %, and the hybrids of the 3rd zone had the lowest degree of germination – 45.3-39.3 %.

The obtained different results on the effect of seed pretreatment with NTS on subsequent germination at the optimum temperature, on the one hand, and seedling growth, on the other hand, can, probably, be explained by the properties of the parental forms used to obtain the corresponding hybrids.

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