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EFFECTS OF HYPOTHERMIC STRESS APPLIED TO SEEDS BEFORE GERMINATION ON THE PARAMETERS OF THE PHOTOSYNTHETIC APPARATUS OF MAIZE PLANTS.

Cauș Maria[1*], Platovschii Nicolai[1]
[1]State University of Moldova, Institute of Genetics, Physiology & Plant Protection
**Corresponding author: e-mail*: mcaus2021@yahoo.com

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ABSTRACT

In maize (*Zea mays* L.) plants grown from seeds pre-treated with negative temperature stress (NTS) of -4°C for 16 hours before germination, followed by growth in dark and light conditions, the content of chlorophyll pigments (Chl) in leaves, the chlorophyll content index (CCI) and some gas exchange parameters were assessed. Combined application of NTS and illumination conditions to seedling growth showed that 6-day-old NTS maize seedlings grown in the dark were etiolated, contained carotenoids and traces of Chl *a* and Chl *b*. Whereas in green seedlings grown in the light, NTS decreased Chl *a*, Chl *b* and carotenoids. NTS also reduced the CCI in1st, 2nd and 3ed leaves of plants grown in the light for 17 days. While in the 1st, 2nd and 3ed leaves of control plants that grew in the light, the CCI changed dynamically, showing a maximum on the 9th day of growth and then decreased. The influence of NTS also affected the parameters of gas exchange in leaves, causing a decrease in CO2 exchange, real CO2 absorption and total respiration.

INTRODUCTION

Humanity is currently facing serious climate changes, which are a problem for agriculturists. Plants are subject to sudden changes in temperature, especially in spring, when under natural conditions soil temperatures can be low, even below zero degrees. For temperate heat-loving plants, including maize, such conditions represent a difficult challenge. Low soil temperatures during early maize planting can negatively impact seed germination, delaying the process, with further reduction in seedling vigor and subsequent growth due to the extreme conditions they were exposed to. (Zhou et al. 2022). A lot of scientific works have been carried out comparing the effect of low positive temperatures on the germination of various maize cultivars (Mira et al. 2021, Cauș et al. 2022). Studies on cold-tolerant maize genotypes have been demonstrated maize to be highly sensitive to cold stress and chilling during seed germination and the seedling phase, which can lead to reductions in plant vigor and grain production (Riva-Roveda et al. 2016, Mira et al. 2021, Zhou et al. 2022, Cauș et al. 2023). As climate change intensifies, the negative effects of extreme temperature fluctuations are becoming more frequent, making the study of maize's tolerance to low temperatures and frosts increasingly important. Various studies have investigated the effects of temperature stresses on plant productivity traits, including photosynthetic capacity, chlorophyll content and their significant role in maize yield (Savitch et al. 2011, Liu C, 2019).

The aim of the work was to determine the effect of negative temperature stress applied to seeds before germination on the performance of the photosynthetic apparatus of the resulting maize plants.

MATERIAL AND METHODS

In this study, we used Bemo 203 hybrid maize seeds offered by the Public Institution "National Center for Research and Production of Seeds", RM. The experiments were conducted under controlled laboratory conditions. Before germination, maize seeds were placed for imbibition in distillate water at 5°C for 36 hours. Next, a part of the seeds (experimental), freed from water, were subjected to negative temperature stress (NTS) of -4°C for 16 hours, and the other part of the seeds (control), also freed from water, were placed in a refrigerator at a temperature of 5°C. Subsequently, control and experimental seeds were sown simultaneously in containers on wet cotton discs and set for germination in a thermostat, in the dark, at an optimal temperature of 26°C and relative air humidity of 70-85%. After 48 hours of incubation control and experimental germinated seeds were planted into pots with soil. A part of control and experimental pots with seedling were left for subsequent growth in the dark at 26°C and relative air humidity of 70-85%, while the other part of pots with seedlings (control and experimental) were placed for growth in a chamber with controlled conditions of lighting, aeration, humidity and temperature of 26°C. Then, after six days of growth, samples were taken from seedlings grown in the dark and in the light to determine chlorophyll pigments. And the seedlings that grew in the light were left for further growth up to 17 days. During growth, the chlorophyll content index, the content of chlorophyll pigments, and CO2 exchange and respiration were also measured in the leaves of 17-day-old plants.

Determination of the concentration of photosynthetic pigments**.** The concentrations of chlorophylls (Chl) *a,* Chl *b)* and carotenoids in leaves were analyzed using 100% acetone, and the absorbance of extracted solution was recorded at 662 nm, 644 nm and 440.5 nm using a UV-Vis spectrophotometer Agilent 8453. The chlorophyll contents were calculated according to the formulas of (Holm 1954, Wettstein 1957). *The chlorophyll content index* (CCI) was determined using a chlorophyll counter CCM-200Plus***.***

Respiration and CO2 exchange were determined with PTM-48A apparatus. 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

Studies of the influence of environmental factors on the content and composition of pigments in plant leaves have shown that light is one of the most important factors in the plant's life, determining the rate of their growth and development, participating in the formation of plastids, including chloroplasts, as well as in the biosynthesis of chlorophylls (Armarego-Marriott et al. 2020, Zhan et al. 2024).

The effect of combined application of NTS to seeds before germination and subsequent plantlets growth under dark or light conditions demonstrated significant differences in the external appearance of maize seedlings (figure 1). Both control (C) and experimental (NTS) 6-day-old maize seedlings grown in the dark are morphologically etiolated and appear taller than the light-grown seedlings, which are more vigorous and greener (figure 1.1 and 1.2). Experimental seedlings (NTS) were smaller than the control ones in both growth conditions - in the dark or under light (figure 1.1 and 1.2).

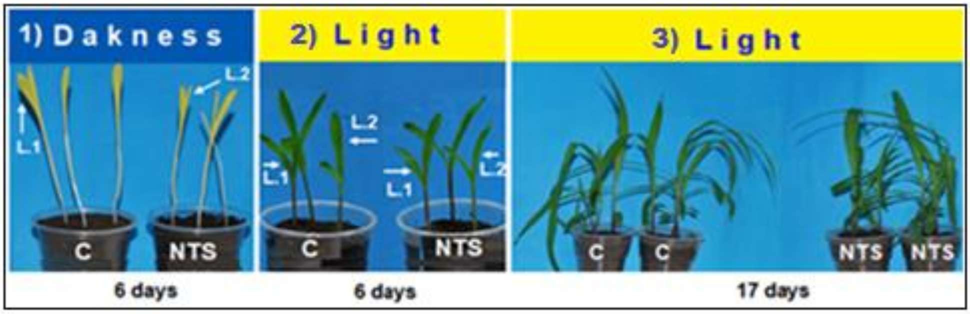

Figure 1. Maize seedlings, control (C) and experimental (NTS), the seeds of which were pre-treated with negative temperature stress (NTS) at -4°C for 16 hours and grown in the dark (1) or in the light (2) for 6 days, as well as plants (C, NTS) which continued to grow in the light during of 17 days.

The results showed that both control and experimental seedlings grown in the dark and that were etiolated contained traces of Chl *a* and *b*, but the carotenoid content was higher in both treatments (figure 2). However, both control and experimental 6-day-old seedlings grown in the light are more vigorous, with dark green colored leaves, compared to the plant of the same age grown in darkness. Upon illumination, de-etiolation occurs, characterization by the transition from etioplast (non-green plastids) to chloroplast, and, at the seedling level, a switch to photomorphogenic growth (Armarego-Marriott et al. 2020). Studies have shown that in maize seedlings during de-etiolated, light controls circadian rhythm, chloroplast development and autotrophic establishment (Zhan et al. 2024).

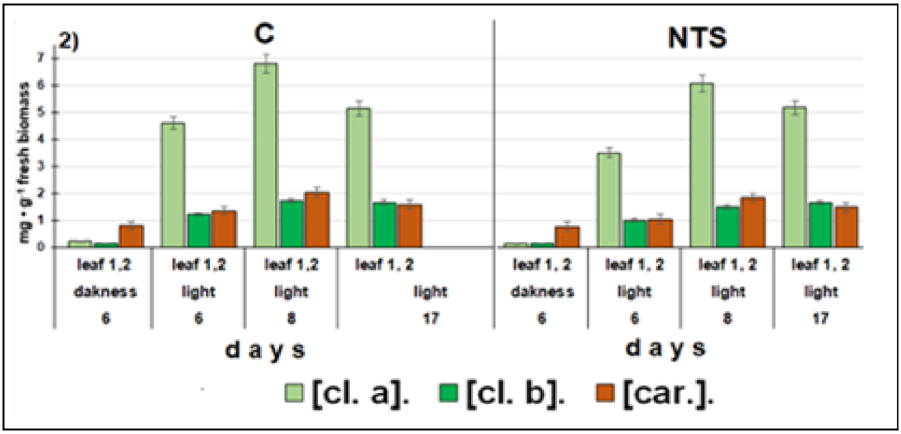

Figure 2. Effect of negative temperature stress (NTS) of -4°C for 16 hours applied to the maize seeds before germination and the subsequent cultivation of plants from respective seeds in soil under controlled laboratory conditions on the chlorophyll *a* and *b*, as well as carotenoids in leaves during the growth.

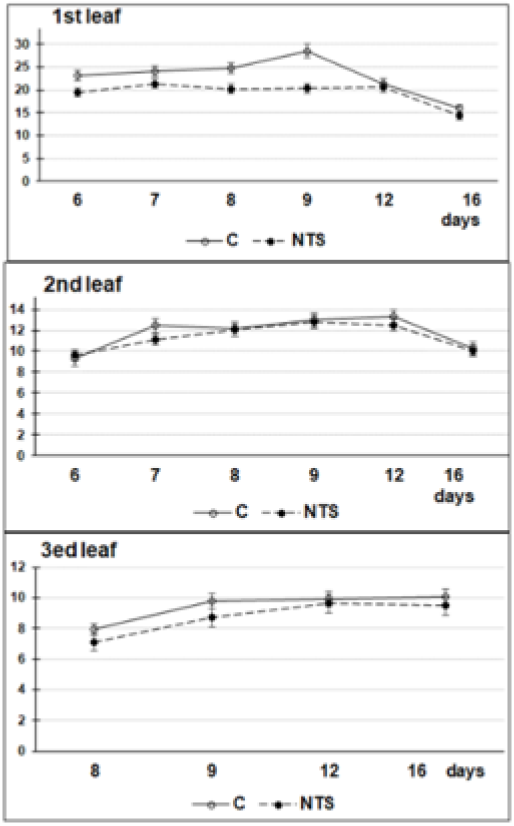

Figure 3. The dynamics of changes in the chlorophyll content index (CCI) in leaves the1^{st}, 2^{nd} and 3^{ed} of Bemo 203 hybrid maize seedlings, grown from seeds pretreated with NTS before germination and subsequent growth in soil under laboratory conditions at 26°C.

A comparative analysis of changes in pigment content in the leaves of control and experimental seedlings grown in the light shows (figure 2) that their content changes dynamically, reaching a maximum level on the 9th day, after which the pigment content decreases. It is also noteworthy that NTS had a significant negative effect on the Chl *a* content in maize seedlings, while the content of Chl *b* and carotenoids was affected to a lesser extent (figure 2). Thus, the NTS applied to seeds before germination affects subsequently growth of resulting maize seedlings, causing disturbances in the formation of chlorophyll pigments and carotenoids in leaves.

An important indicator for evaluating plant growth is the chlorophyll content index (CCI), which makes it possible to indirectly evaluate the activity of the photosynthetic apparatus and plant productivity (Liu et al. 2019). CCI can be measured using the fast non-destructive optical method, which provides valid estimates of the content relative of leaf chlorophyll compared to traditional and chemical methods (Ciganda et al. 2009).

Our determinations of the chlorophyll content index in the leaves of maize plants are shown in figure 3. The chlorophyll content index values in the 1^{st} leaf, both in the control and STN, are higher compared to those in the 2^{nd} and 3^{ed} leaves. The maximum value of the chlorophyll index in the 1^{st} leaf, the control variant, was recorded on the 9^{th} day, after which it starts to decrease, reaching a minimum on the 17^{th} day of plant growth. These results are in agreement with the changes in the content of chlorophyll pigments shown in figure 2. The high values of the chlorophyll index in the 1^{st} leaf, which are in the range of 20-27cci (figure 3) and those of the chlorophyll *a* and *b* content (figure 2) indirectly prove that the 1^{st} leaf produces biochemical compounds, which are translocated for the formation of the 2^{nd} leaf. At the same time, the chlorophyll index values in the 2^{nd} and 3^{ed} leaves are at a lower level, compared to the 1^{st} leaf, in the 2^{nd} leaf, the chlorophyll index is 10-13cci, and in the 3^{ed} leaf, the values of this index are in the range of 7-10cci. These data demonstrate that the

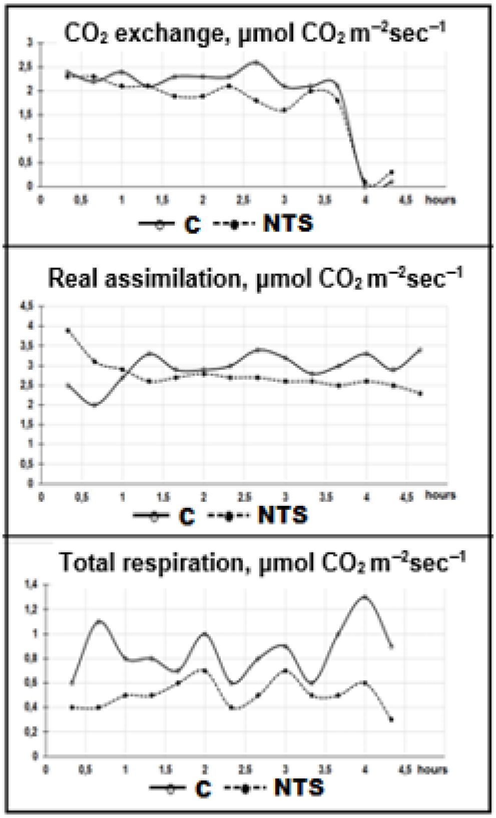

Figure 4. Gas exchange in the 3ed leaves of intact maize, grown from seeds pre-treated with NTS of -4°C for 16 hours. The measurements were performed on leaf 3 of 17-day-old maize plants.

organic compounds formed in the 1st leaf are allocated for the formation and growth of the 2nd leaf, and the biochemical compounds from the latter are directed towards the formation of 3ed leaf. Also, it can be seen that if in 1st leaf the chlorophyll indexes in the control and experimental variant with NTS are significant differences, in the 2nd and 3ed leaves these differences are not significant. Since chlorophyll content is considered an important physiological indicator that reflects the growth status of crops (Li et al. 2024), our data show how the dynamics of changes in the level of chlorophyll index occur as maize leaves form, grow and develop.

The growth and development of plants depend on the net CO2 assimilation, the contents of chlorophyll and carbohydrates that would ensure the energy balance in the development of these processes (Khangura et al. 2020). Figure 4 presents the results regarding gas exchange parameters such as CO2 exchange, real CO2 assimilation and total respiration in maize plants, grown from NTS - pretreated seeds. The obtained data indicate that the dynamics of changes in the studied parameters in plants grown from seeds that were pre-treated with NTS at a temperature of -4°C for 16 hours before germination occurred at a lower level compared to the controls (figure 4). Leaves of unstressed maize have, in our experiments, a rate of CO2 exchange evolution which is smaller (about 2-2,5 μmol CO_2 $m^{-2}sec^{-1}$) in comparison to the real assimilation (2,6-3,6 μmol CO_2 $m^{-2}sec^{-1}$). These results are consistent with the findings of (Fryer et al.1998), who showed that the capacity of field-grown maize leaves to assimilate CO_2 was decreased in May, when cooling periods were experienced, and increased significantly as what temperature increased by June.

CONCLUSIONS

It has been shown that maize plants grown in the dark contain carotenoids in both control and experimental variants, against the background of the absence of chlorophyll (Chl) *a* and *b*.

In the seedlings grown from seeds pretreated with NTS and the subsequent growth in the light, it was established that NTS reduced the content of Chl *a*, Chl *b* and carotenoids, as well as the chlorophyll content indices in leaves 1, 2 and 3.

NTS also affected the parameters of gas exchange in leaves, causing a decrease in CO2 exchange, real CO2 absorption and total respiration.

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