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Study on the cold tolerance of maize (*Zea mays* L.) inbred lines in Phytotron

Eszter Csepregi-Heilmann[1] – Ágnes Áldott-Sipos[1] – Anita Mészáros[1] – Anett Klaudia Kovács[2] – Tamás Spitkó[1,*] – Csaba Szőke[1] – János Pintér[1] – Tamás Berzy[1] – Adrienn Széles[3] – Csaba L. Marton[1]

[1]Maize Breeding Department of the Agricultural Institute of Centre for Agricultural Research, Martonvásár

[2]Hungarian University of Agriculture and Life Sciences

[3]Institute of Land Use, Engineering and Precision Farming Technology, University of Debrecen, Hungary

[*]Correspondence: spitko.tamas@atk.hu

SUMMARY

Maize has come a long way from the tropics to the temperate zone. In the beginning, the spreading of maize was prevented by its sensitivity to cold. Improved cold tolerance at germination is one of the most important conditions for early sowing. The advantage of cold tolerant hybrids is that they can be sown earlier, allowing longer growing seasons and higher yields, due to the fact that the most sensitive period in terms of water requirements, flowering, takes place earlier, i.e. before the onset of summer drought and heat.

In Martonvásár, continuous research is carried out to improve the cold tolerance of maize. In the present experiment, the cold tolerance of 30 genetically different maize inbred lines was investigated in a Phytotron climate chamber (PGV-36). The aim of our research is to identify cold tolerant lines that can be used as parental components to produce proper cold tolerant hybrids and/or as sources of starting materials for new cold tolerant inbred lines. After observing and evaluating changes in phenological traits under cold-test, the results of the cold-tolerance traits of interest have been used to highlight several inbred lines that could be good starting materials for further research on genetic selection for cold tolerance.

Keywords: *maize; cold-test; Phytotron; cold tolerance*

INTRODUCTION

Today, maize is one of the most widely cultivated crops in the world, thanks to its excellent adaptability and high yield potential (Nagy, 2021). With an area of 206 million hectares, it was the second largest cereal crop after wheat in 2021 (Faostat, 2023). The climatic conditions of Hungary and the seed export destination countries make it necessary to study the cold tolerance of maize and continuously improve it (Marton et al., 2013).

For a long time, maize's sensitivity to cold prevented the plant from spreading to the cooler regions of the north. This plant of tropical origin, with high temperature and water requirements, but with a wide genetic variance that allows the production of early cold-tolerant hybrids in temperate climates (Berzsenyi, 2012). In northern growing regions, tolerance to lower temperatures is still a major problem due to low temperatures and water scarcity (Zydelis et al., 2018). The appearance of short growing season varieties seems to solve the problem of growing under low temperature conditions (Marton and Tóthné, 2017; Gombos, 2021). Soane et al. (2012) and Spiertz (2014) also confirm that climate change, new varieties, and a short growing season have made it possible, while growing demand has encouraged the spread of maize cultivation outside the traditional growing zones, to northern regions with a cooler climate. In the last 50 years, the northward expansion of maize in Europe has increased more than in the previous 500 years combined. In 1960 the northern boundary was the imaginary Paris-Prague-Kiev line (Marton and Tóthné, 2017).

Since the optimum temperature for maize is around 30 °C for the whole growing season (Lehenbauer, 1914), the term "low temperature" is used to mean a wide range of temperatures when interpreting cold tolerance. The literature generally distinguishes between three ranges: (1) freezing: below 0 °C, (2) low, non-freezing: 0–6 °C, (3) sub-optimal: 6–20 °C (Marton and Tóthné, 2017). Maize is sensitive to frost at all stages of development except at the dry kernel stage (Marton and Tóthné, 2017). There is no genetic variation in the freezing temperature range. As a result of -1; -2 °C frost, the above-ground parts of the plant are damaged, its leaves turn yellow, but it does not freeze and regenerates quickly (Marton, & Tóthné, 2017; Gombos, 2021; Nagy, 2021), if the apical meristem in the soil of the stem remains viable (Marton and Tóthné, 2017). In the low-temperature range, cell membranes are disrupted, with the severity of damage depending on temperature, duration of cold exposure, developmental stage and, in part, genotype (Marton and Tóthné, 2017; Nagy, 2021). In most research, the minimum temperature required for development is defined as 8–10 °C (Nagy, 2021; Gombos, 2021) but there are other opinions as well. According to Sánchez et al. (2014), the minimum average temperature required for the growth and development of maize is 6.2 °C, while other authors say this value is 8 °C (Fischer et al., 2014). Adaptation to low temperatures means that maize is resistant to cold throughout its growing season, resistant to soil fungi and capable of vigorous growth and development. The suboptimal temperature zone results in wide genetic variability, which favors selection for cold tolerance and a short growing season. One of the most important condition for early sowing is the improvement of cold tolerance

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during germination (Szundy et al. 2005, Marton and Tóthné, 2017).

MATERIALS AND METHODS

At the Agricultural Institute of Centre for Agricultural Research, 30 genetically diverse inbred lines (marking: T1–T30) were tested for cold tolerance in two replicates in a PGV-36 chamber in Phytotron under controlled conditions. A long daytime setting was used from day ten. 16 hours light and 8 hours dark period. Humidity was not under control. A cold-test method was used to observe adaptation to low temperatures: seeds were incubated at 8 °C for 10 days, and then at 13.5 °C for 30 days after regeneration were observed the initial development of the plants to 3–4 leaf stage. In sterilized soil, twenty genotypes were placed in M10 boxes with ten to ten seeds per box. As a standard, two well-known W401 cold tolerant and W64A cold sensitive lines were used for comparison. The soil was irrigated to 70% water capacity after sowing. Water loss was continuously replenished. Tapwater was used for irrigation. During the duration of the experiment, we studied the percentage of plants at emergence, the number of days from sowing to emergence, and then at 3–4 leaf stage, we measured the fresh and dry weight. We have calculated the cold-test index for the lines of cold experiment. The formula used for the calculation:

$$\text{Cold Test Index (CT index)} = \left(\frac{percentage\ of emergence}{days\ from\ sowing\ to\ emergence}\right)$$

The control study was performed in the Phytotron, PGV-36 chamber at a constant temperature of 23 °C. The lightning and the humidity setting was the same in both chamber.

Agrobase RCBD (Randomized Complete Block Design) ANOVA Analysis was used for statistical evaluation.

The aim of our research was to identify cold tolerant lines that could be used as parental components to produce good cold tolerant hybrids and/or as sources of starting materials for new cold tolerant inbred lines.

RESULTS AND DISCUSSION

Among the tested traits, the results of the cold tolerance experiment showed a significant difference (LSD=4.25***) in the development of the percentage of emergence compared to the control conditions. The low temperature caused only 62.8% of the plants to emergence (*Figure 1*), which is a loss of 30.2% compared to the number of plants that germinated in the control study. There were significant differences in emergence rates between lines (*Figure 2*).

Figure 1. **Evolution of the emergence percentages in relation to the two treatments**

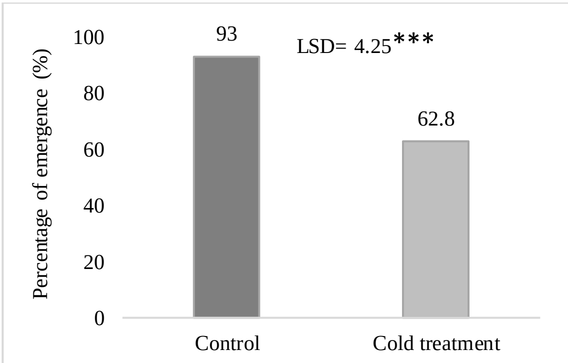
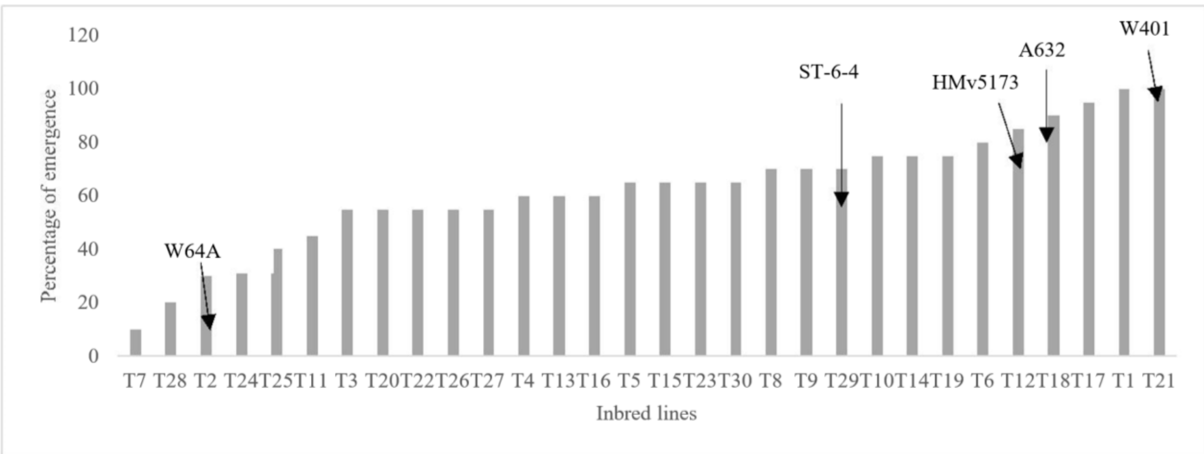


Figure 2. **Evolution of the emergence percentages of the tested lines in the cold tolerance experiment**



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The number of days from sowing to emergence was significantly higher (LSD=0.61***) in the low-temperature test compared to the control (*Figure 3*). On average, plants emergence at a constant temperature of 23 °C were in 7.6 days (range 5 to 8 days). In contrast, the plants in the cold tolerance treatment were an average of 25.6 days (range 22 to 29 days) (*Figure 3 and 4*).

There is a remarkable difference between inbred lines in the number of days from sowing to emergence. The cold tolerant line W401 emerged in the first one-third, in 24 days, while the cold sensitive line W64A had a delayed emergence, lasting nearly 27 days (*Figure 4*).

Figure 3. **Number of days from sowing to emergence by lines**

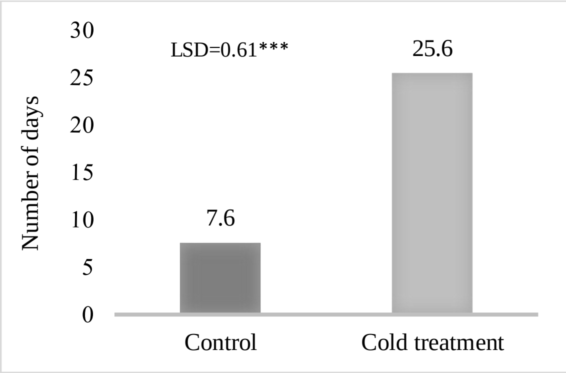
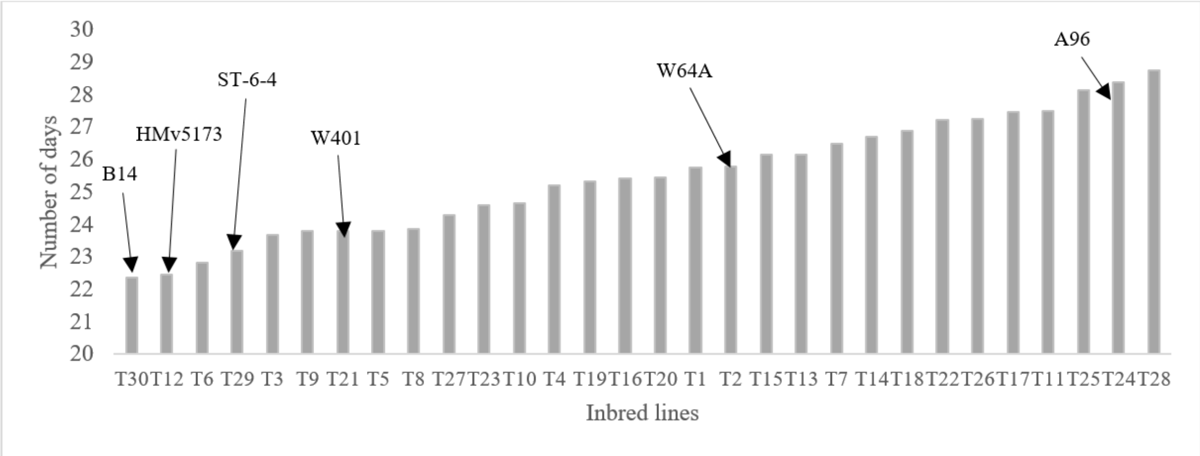


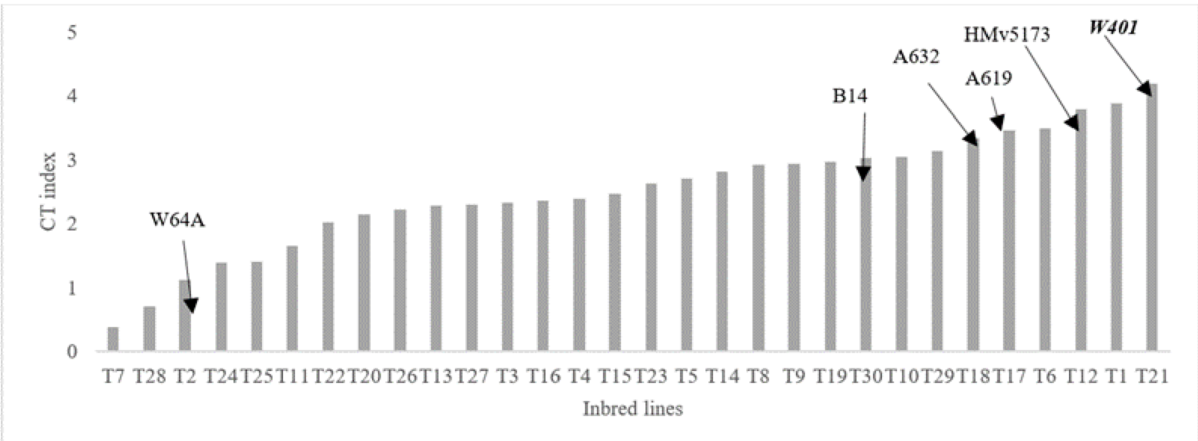
Figure 4. **Number of days from sowing to emergence in the cold tolerance experiment by lines**



The most important indicator for cold tolerance is the so-called cold-test index. It gives decisive information about the plant's response to cold. The cold-test value is the ratio of the percentage of emergence to the number of days from sowing.

The higher the numerator – and the lower the denominator – the higher the CT value, i.e. the better the cold tolerance of the line. In *Figure 5*, the position of the lines used as standards (W401 and W64A) confirms the characteristic described in the literature, their adaptation to low temperatures that W401 is a cold tolerant line and W64A is a cold sensitive line.

Figure 5. **The Cold Test value of cold tolerance test lines**



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The effect of cold stress on fresh and dry mass was also investigated. At low temperatures, the individual fresh weight of plants reached 63% of the individual fresh weight of plants growing under optimal conditions (*Figure 6*). *Figure 7* shows the evolution of individual fresh weight of inbred lines at cold experiment.

The dry matter content of the plant provides information on its health status. Looking at the inbred lines as a function of this (*Figure 8*), there are healthy lines that respond well to cold stress with a dry matter content of less than 10% (W401, ST-6-4, A632 waxy), and there was inbred line which showed a dry matter content 17% (14-10-43-103).

Figure 6. **Evolution of average individual fresh weight in the context of treatments**

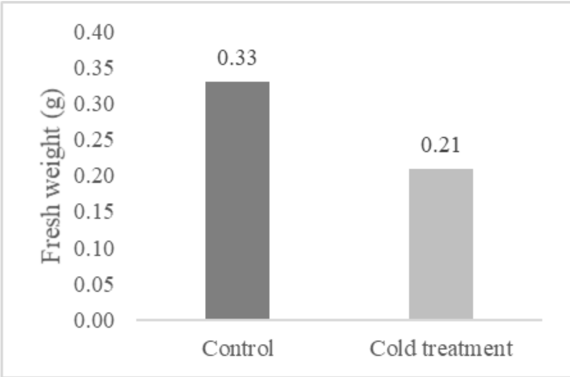


Figure 7. **Evolution of the individual fresh weight of the cut stock of the inbred lines in the cold tolerance experiment at 3–4 leaf stage**

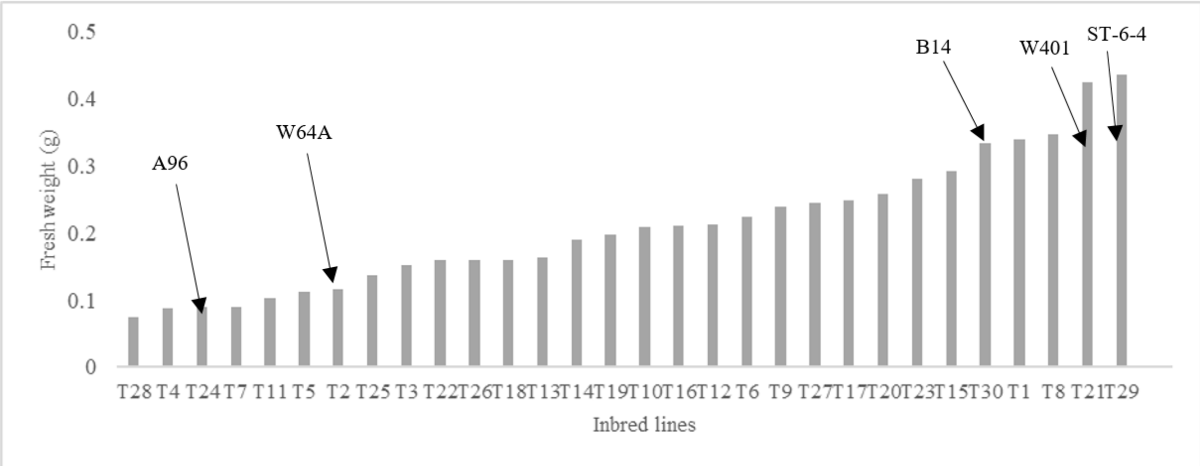
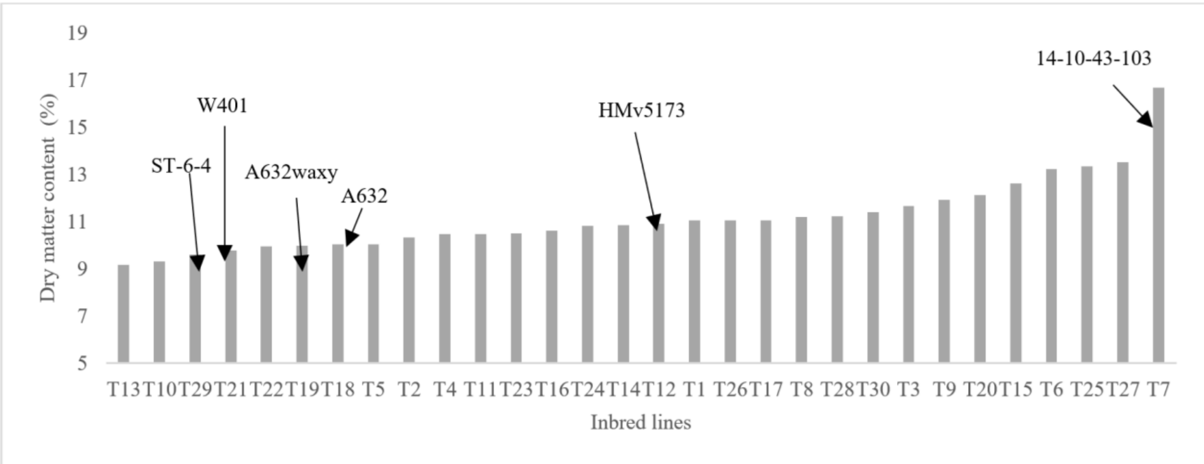


Figure 8. **Distribution of the percentage dry matter content of the inbred lines in the cold tolerance experiment**



CONCLUSIONS

30 inbred lines with different genetic backgrounds were tested for cold tolerance in two replicates in the Phytotron, in PGV-36 chamber, in the Agricultural Institute of the Centre for Agricultural Research in Martonvásár. During the tests, remarkable differences were recorded between the inbred lines in some phenological traits.

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Characteristics determining the cold tolerance: the percentage of emergence, the number of days from sowing to emergence and the CT index. In addition to the standard inbred line *W401*, the outstanding lines in terms of the traits mentioned were the lines *HMv5173, ST-6-4, A632*. These lines, in addition to showing good cold tolerance, combine well. They are the parental components of several commercially available hybrids. The cold tolerance of line A632 is also confirmed by the literature (Eagles, 1982; Dőry et al., 1990; Brandolini, 2000). We will continue to test the cold sensitivity of additional inbred lines preparing the raw materials for genetic selection.

ACKNOWLEDGEMENTS

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