

Investigation of antioxidant activity in maize during low temperature stress

Tibor Janda^{1*}, Eszter Imola Kósa², Gabriella Szalai¹, Emil Páldi¹

¹Agricultural Research Institute, Hungarian Academy of Sciences, Martonvásár, Hungary, ²Department of Botany and Plant Physiology, Georgikon Faculty of Agriculture, University of Veszprém, Keszthely, Hungary

ABSTRACT Young maize inbred lines and their hybrids were tested for chilling tolerance using the chlorophyll fluorescence induction technique. The genotypes were ranked based on the decrease in the F_v/F_m parameter after chilling stress at 5°C. The activities of certain antioxidant enzymes were determined in control and cold-treated plants. The results suggest that although there are differences between the genotypes in the activities of almost all the antioxidant enzymes, these differences do not reflect the differences in chilling tolerance.

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KEY WORDS

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The low temperature susceptibility of young maize plants is one of the most important limiting factors for expanding the maize production area. For maize plants even temperatures below 12-15°C may induce chilling stress (Pál and Nagy 2002; Holá et al. 2003). If growth and development take place at below-optimum temperature, several important life functions will suffer inhibition, including the photosynthetic apparatus, which is particularly susceptible to the negative effects of low temperature. Low temperature-induced photo-inhibition also has an important role in the chilling damage to young maize plants (Janda et al. 1994).

Growth at low temperature may increase the concentration of reactive oxygen species (ROS), which may cause damage to membrane lipids, proteins and nucleic acids, leading to the death of the cells (Apel and Hirt 2004). The ability to adjust their antioxidant systems to changing ROS concentrations may be vital to all species under stress conditions (Kocsy et al. 1997, 2001; Foyer et al. 2002).

The aim of the present work was to investigate whether the activity of certain antioxidant enzymes was indicative of the chilling tolerance of young maize plants.

Materials and Methods

Maize hybrids (H1-H6) and their parental inbred lines (L1-L9) [(H1: L5xL7; H2: L3xL4; H3: L5xL2; H4: L1xL2; H5: (L8xL9)xL4 and H6: L5xL6)] were used in the experiments at the 4-leaf stage. Plants were grown for 3 weeks at 22/20°C and 75% relative humidity with a 16/8-hour light-dark periodicity. The cold treatment was carried out in the same growth chamber. The chlorophyll fluorescence induction parameters of the youngest fully expanded leaves were determined at room temperature (22°C) in complete darkness using a pulse amplitude modulated fluorometer (PAM-2000; Walz, Effeltrich, Germany). The activities of the antioxidant enzymes

were measured photometrically as described earlier (Janda et al. 2003). The t-test method was used to compare enzyme activities in control and cold-treated plants.

Results and Discussion

In the first series of experiments 9 young inbred maize lines (L1-L9) and their hybrids (H1-H6) were cold treated at a constant 5°C for 1 week, after which the plants were returned to normal growth temperature. During this period the F_v/F_m chlorophyll fluorescence induction parameter was measured after 1, 2 and 7 days of cold treatment, and 1 day after the recovery phase. This parameter is widely used as a rapid indicator of chilling-induced photoinhibition in cold-sensitive plants (Janda 1998; Lootens et al. 2004). To determine differences in chilling tolerance between the genotypes the plants were ranked after each measurement, and the final order was created based on the average ranks. Based on statistical probes chilling tolerance were relatively poor for lines L6, L7 and L8, medium for lines L1, L2, L3 and L4 and relatively good for L5 and L9. In contrast to the inbred lines, the chilling tolerance of the hybrids did not differ from each other significantly, except for L8xL9, which had significantly less chilling tolerance than the others. Furthermore, as also reported by other authors (Körnerová and Holá 1999; Holá et al. 2003, 2004), hybrids usually showed better cold tolerance than their parental lines.

Exposure to low temperature may increase the amount of ROS both in cold-sensitive and cold-tolerant plants. The activities of antioxidant enzymes were measured in the leaves of 3-week-old inbred maize lines and their hybrids before and after 1 day of cold treatment carried out at 5°C. There was no significant change in the catalase activity in the inbred lines after 1-day chilling stress. Comparing the genotypes, catalase activity was relatively high in lines L2, L4 and L5, and was very low in L3. Among the hybrids the

*Corresponding author. E-mail: jandat@mail.mgki.hu

highest activity could be detected in H3. A change in the catalase activity after 1-day chilling stress at 5°C occurred only in hybrid H2. With the exception of L9 the glutathione reductase activity increased in all the inbred lines tested. High activity could be measured in L2, L4, L7 and L8. Similarly to catalase, L3 exhibited very low glutathione reductase activity. In the hybrids there was a significant increase in H5, H1 and H4. However, the highest activity both before and after the chilling stress could be detected in the L8xL9 genotype. The highest ascorbate peroxidase activity was detected in lines L1 and L4. After the cold treatment there was a slight increase in the activity of this enzyme in lines L1, L4, L6 and also in L7, in spite of the fact that the activity was very low in this line. There was also an increase in the activity of this enzyme in hybrids H3, H5, H1, H4 and H2. The latter, H2, showed substantially higher activity compared to the other hybrids. Guaiacol peroxidase activity increased only in line L4. In the other lines the changes were not statistically significant. Among the lines, L1 showed the highest activity. The other lines did not differ significantly from each other. The hybrids H1 and L8xL9 also exhibited a slight increase in the activity of guaiacol peroxidase. Although there was no significant change in the glutathione-S-transferase activity in any of the lines tested, its activity was relatively high in lines L1 and L5, and especially so in line L4. There was a slight increase in the glutathione-S-transferase activity in the hybrids L8xL9, H5, H1, H2 and H4.

In the second set of experiments two selected inbred lines (L3 and L4), where the differences in the antioxidant activities were the most pronounced, and their hybrid (H2) were selected for more detailed investigations. The genotypes were cold treated at 15, 10 and 5°C for 1, 2 and 7 days. Mild stress (15°C) caused an increase in the glutathione reductase activity even after 1 day and in the ascorbate peroxidase activity after 1 week. Severe stress may cause a decrease in the enzyme activities, suggesting the occurrence of degradation processes.

These results show that although there are differences between the genotypes in the activities of almost all the enzymes, these differences do not correlate with differences in chilling tolerance.

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