Proceedings of the 7th Hungarian Congress on Plant Physiology, 2002
S2-P11

Volume 46(3-4):99-100, 2002
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Role of some N-containing compounds in chilling tolerance of maize

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ABSTRACT The paper reports on the effect of low temperature on the synthesis of a number of N-containing compounds in five inbred maize lines with different degrees of chilling tolerance. The compounds analysed are used as stress markers on the basis of their response to abiotic stress factors. The aim of the experiments was to obtain a better understanding of the role of these compounds in maize chilling tolerance. The results indicate that changes of various magnitudes occur in the quantities of putrescine, agmatine, glycine-betain and proline as the result of chilling treatment. The quantitative changes are correlated with the chilling tolerance of the given inbred maize lines. The alternative pathway of polyamine synthesis plays a substantial role in the development of the defence mechanism against low temperature.
Acta Biol Szeged 46(3-4):99-100 (2002)

Maize, which is of subtropical origin, is one of the most important crops in Hungary. However, various aspects of the climate of the temperate zone may have an unfavourable influence on maize plant development and frequently reduce yield. If growth and development take place at suboptimum temperatures various types of physiological damage may occur (Miedema 1982). Some of these effects only become visible during the warmer period after chilling (Szalai et al. 1996). Previous results indicate that the greatest damage is caused by low temperature during the early stages of development. Like other abiotic stress effects, low tempera-ture induces changes in many of the processes involved in the plant metabolism (Alscher and Cumming 1990; Katterman 1990). As the result of low temperature various genes are expressed, leading to the synthesis of the functional and regulatory proteins required for the biosynthesis of certain osmolytes (sugars, polyamines, amino acids, glycine-betain etc.), for the protection of membranes or macromolecules, or to stimulate the activity of antioxidant enzymes (Shinozaki and Yamaguchi-Shinozaki 1997). Some of the changes induced by chilling exhibit a positive correlation with the low temperature-induced synthesis of certain organic compounds (Lásztity et al. 1994; Rácz et al. 1996 Janda et al. 1996; Szalai et al. 1997), so these compounds are regarded as reliable stress markers. In the present investigations the synthesis of four N-containing compounds (putrescine, agmatine, proline and glycine-betain) was traced following low temperature treatment in five inbred maize lines with various degrees of chilling tolerance.

Materials and Methods

In the course of the experiments five inbred maize lines were used (Co 158, F 564, F7 cmsC, Mo 17 and W 152 R). Preliminary studies carried out by breeders indicated that F7 cmsC and F 564 had chilling tolerance both at emergence and in the young plant stage, while W 153 R was chilling-sensitive at germination, and Mo 17 and Co 158 in both development stages. The disinfected seeds were sown three to a pot in a 2:2:1 mixture of meadow soil, compost and sand, sterilised prior to sowing. The plants were raised for six weeks with 16 h illumination, day/night temperatures of 20/15°C and 70% relative humidity. The light intensity was 420 µmol/m^2. The six-week-old plants were exposed to various lengths of chilling treatment (1, 3, 5 days at 8ºC). For the chemical analysis 1 g samples were taken from the middle of the third leaf, from both sides of the main vein.

Amino acids were determined by the HPLC method (Bartók et al. 1994). Polyamines were analysed by a modified HPLC method (Bencsik et al. 1998). The activity of arginine decarboxylase, the key enzyme in the alternative biosynthesis pathway, was determined according to Kaur-Sawhney et al. (1982). Glycine-betain was analysed by pyrolysis gas chromatography as described by Hitz and Hanson (1980).

Results and Discussion

Changes in the quantities of a number of N-containing compounds (putrescine, agmatine, proline, and glycine-betain) were analysed in five inbred maize lines after various periods (1, 3, 5 days) of chilling treatment (8ºC). Among the polyamines, only the values of putrescine and agmatine are reported, since these had the most important role in chilling tolerance. A comparison of the results indicates that chilling treatment led to a substantial rise in the total quantity of the two polyamines in three inbred lines (F7 cmsC, W 153 R and F 564). This increase was almost 200% in F7 cmsC and approx. 170% in W 153 R. In the other two lines (Co 158, and Mo 17), however, no polyamine accumulation could be observed; in fact, a reduction was recorded after three days of chilling treatment. A similar tendency was observed in the case of the amino acid proline, except that in line F 564 there was a reduction after five days of chilling treatment, at which time no proline at all could be detected in line Mo 17.

An analysis of the results obtained for glycine-betain indicated that an accumulation of this N-containing compound could only be observed in lines F7 cmsC and W 153R. By the end of the chilling treatment this increase was as high

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as 400% in F7 cmsC and slightly more than 200% in W 153 R. In line F 564 there was a slight increase on the third day, but this declined again by the fifth day. A considerable reduction was detected for the other two lines (Co 158 and Mo 17), so that no glycine-betaine could be observed after five days for the former, and after only three days for the latter.

A comparison of the data for the control plants shows that the joint quantity of the two polyamines was practically the same in all five lines. The situation was less clear-cut for proline, since less free amino acid was detected in the control plants of Mo 17 than in the other lines over the average of five biological replications. With respect to glycine-betaine, the control plants of two of the lines (Co 158 and Mo 17) had approx. 100% lower glycine-betaine contents than those of the other three lines.

The results published here are the first part of a long series of experiments designed to determine whether the changes occurring in these N-containing compounds could be used to characterise the chilling tolerance of the inbred maize lines which are so important in developing new hybrids. In other words, to discover the extent to which these compounds are involved in the development of protection mechanisms against low temperature in the C_4 plant, maize. The N-containing compounds examined in the present work are generally found to accumulate and behave as osmolytes in plant cells as the result of various types of abiotic stress, and are thus used in many cases as stress markers. The chilling-induced accumulation of polyamines is well known. That of proline occurs as the result of many types of abiotic stress, so it is not stress-specific. Previous reports on stress physiology suggest that glycine-betain plays an important role chiefly in defence against drought and salt stress, while no results have yet demonstrated a correlation between low temperature and glycine-betain content in cereals.

The experimental results indicate that the inbred lines tested gave differing responses to low temperature treatment. In lines with good or moderate chilling tolerance there was a well-defined increase in polyamines and proline, which was not observed in chilling-sensitive lines. In the case of glycine-betain it was found unexpectedly that even before chilling treatment there was considerably more glycine-betain in chilling-tolerant genotypes than in sensitive ones. The results obtained for glycine-betain must be treated with certain reservations, though in many cases the reduction in a compound as the result of stress may be just as good a marker as an increase. In experiments carried out in recent years the chilling tolerance of these same lines was characterised with the aid of chlorophyll fluorescence induction parameters (Janda et al. 1994) and the results were in good agreement with those reported here. In order to obtain a better understanding of the interactions between the low temperature-induced synthesis of certain compounds and the chilling tolerance of maize, experiments will be required using lines for which data are available not only from the traditional cold test but also for other agronomic traits.

In conclusion it can be said that polyamines may play a decisive role in chilling tolerance, while further studies will be required to determine the extent to which certain polyamine biosynthesis pathways (Tiburcio et al. 1997) contribute to the development of defence responses to low temperature. Nothing is yet known about the role of glycine-betain in chilling tolerance and it would be advisable to use the up-to-date, but more expensive Fourier transformation NMR spectroscopic methods to clarify this.

Acknowledgments

The authors are gratefully indebted to Zsuzsa Kóti and Edit Kövesdi for their technical assistance. This work was supported by grants from the Hungarian National Scientific Research Foundation (OTKA T32653, T37195, T37987).

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