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ABSCISIC ACID ACCUMULATION AND EXTENT OF INJURY IN TWENTY MAIZE INBRED LINES UNDER CHILLING CONDITIONS

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Introduction

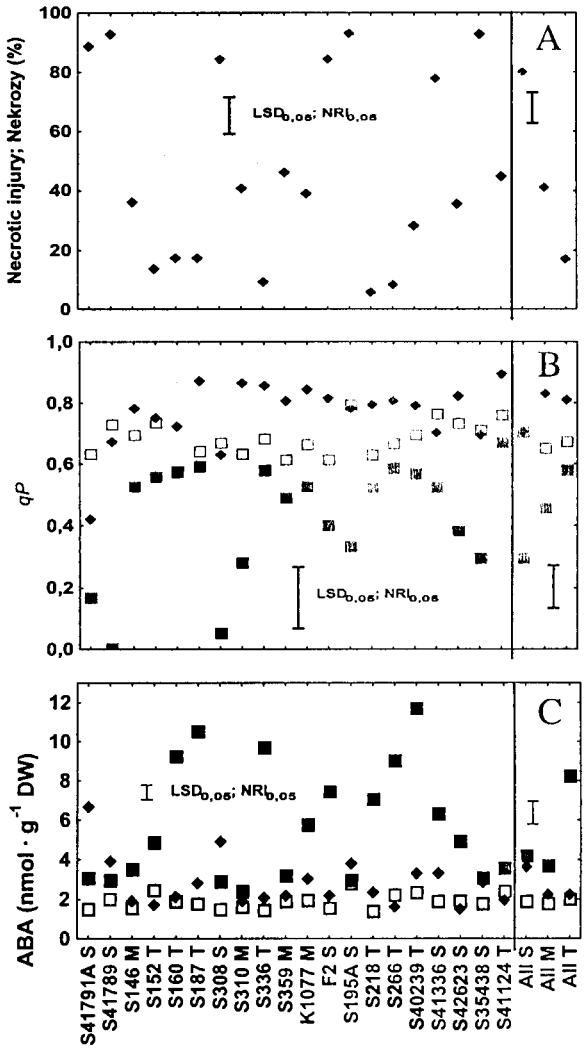
Under chilling conditions (0–10°C) abscisic acid (ABA) accumulates in maize seedlings. This accumulation was found to be faster and greater in chilling tolerant genotypes than in sensitive ones [CAPELL, DÖRFFLING 1993; JANOWIAK, DÖRFFLING 1996]. On the basis of these results the hypothesis was developed that CT in maize is related to the ability for fast and pronounced formation of ABA as a protective agent against chilling injury. This hypothesis has been discussed controversially in the recent literature [RISTIC et al. 1998]. The aim of the present study was therefore, to reinvestigate the role of ABA in CT of maize, using twenty new maize inbred lines, currently used in breeding programs with defined degree of chilling tolerance.

Material and methods

Plant material and growth conditions

Experiments were carried out on twenty maize inbred lines with well-defined degree of chilling tolerance [JANOWIAK, ADAMCZYK 1999]: 8 chilling tolerant lines (T), 8 sensitive (S) and 4 medium tolerant ones (M) (Fig. 1). These maize lines are currently used in breeding programs in Plant Breeding Station in Smolice, Poland.

Seeds, obtained from Dr J. Adamczyk, were sown in the third decade of April, 2000, into pots (5 L volume) filled with soil substrate (peat, sand and clay in a ratio of 2 to 1 to 1, v/v/v). For each line, three pots with nine plants in each were established. The plants were grown in the vegetation hall near Kraków, under natural climatic conditions, which were typical in early spring with temperature drops to nearly zero during the nights, and big night-day amplitudes. Seedlings at the third leaf stage were transferred for 2 days to a growth room with 4°C and 16 h photoperiod, light intensity 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (high pressure sodium lamps, Philips SON-T AGRO, 400 W), humidity 40–50%. After chilling treatment plants were recovered at 20°C for the next 2 days.



□ Control; Kontrola ■ Chilling 2d at 4°C; Chłodzenie 2d w 4°C

◄► Recovery 2d at 20°C after chilling; Regeneracja 2d w 20°C po chłodzie

Fig. 1. Necrotic injury (A), chlorophyll fluorescence photochemical quenching (*qP*; B) and ABA content (C) in 20 maize inbred lines which were chilled for two days at 4°C and afterwards recovered at 20°C for two days. Necrotic injury was estimated after two days of recovery, *qP* and ABA content were measured before the chilling treatment (control), immediately after the chilling treatment and after 2 days of recovery

Rys. 1. Uszkodzenia nekrotyczne (A), fotochemiczne wygaszanie fluorescencji chlorofilu (*qP*; B) i poziom ABA (C) u 20 linii wsobnych kukurydzy, których siewki chłodzono 4 dni w 4°C a następnie poddano regeneracji przez 2 dni w 20°C. uszkodzenia nekrotyczne szacowano po 2 dniach regeneracji, *qP* i poziom ABA mierzono bezpośrednio przez (kontrola), po zakończeniu chłodzenia oraz po 2 dniach regeneracji

Measurements of ABA level, chlorophyll fluorescence and necrotic injury

On the day before transferring the plants to 4°C in a growth room, on the day after chilling and after recovery measurements of chlorophyll fluorescence (effective quantum yield of PSII (Ψ_{PSII}), photochemical quenching *qP*), and determinations of the ABA content by ELISA according to the protocol of WALKER-SIMMONS [1987] in the third leaves were performed. Necrotic injury (NI) of seedlings was estimated visually 2 d after chilling treatment.

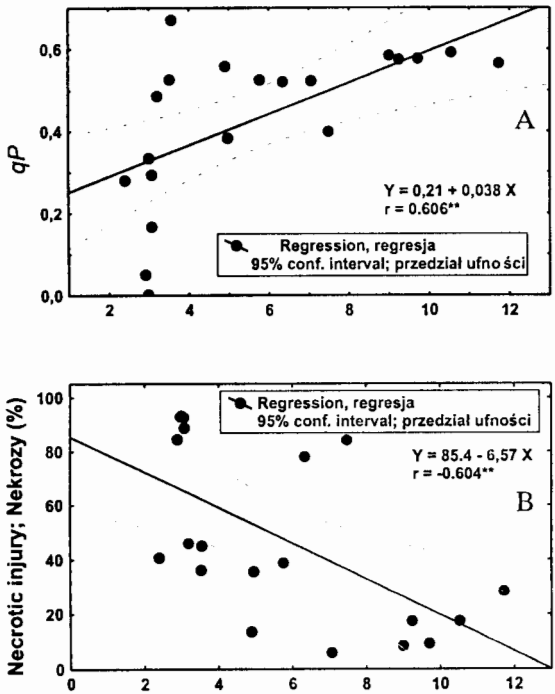


Fig. 2. Correlation between ABA content in the 20 maize inbred lines after two days of chilling at 4°C, chlorophyll fluorescence photochemical quenching (*qP*) after two days of chilling (A), and necrotic injury after two days of recovery (B). Each point is the mean of at least nine ABA determinations from three independent samples, nine qP measurements and ten necrotic injury estimations

Rys. 2. Korelacja między poziomem ABA po 2 dniach chłodzenia w 4°C a fotochemicznym wygaszaniem fluorescencji chlorofilu (*qP*) po 2 dniach chłodzenia (A) i uszkodzeniami nekrotycznymi po 2 dniach regeneracji (B) u 20 linii wsobnych kukurydzy. Każdy punkt jest średnią co najmniej dziewięciu pomiarów ABA w trzech niezależnych próbach, dziewięciu pomiarów qP i dziesięciu oznaczeń uszkodzeń nekrotycznych

Statistical evaluation

A fully randomised experimental design was used. The data are mean values of at least nine replicates. Least statistical differences (LSD) were calcu-

lated at the level of error probability 5% (p 0.05) and are presented in the figures. Correlation coefficients presented in the figures are statistically significant at p 0.05 (**).

Results and conclusions

Chilling of the seedlings for two days at 4°C resulted in significant differences between the genotypes and genotype groups in the manifestations of chilling injury measured as reduction of *qP* immediately after the stress treatment and as development of necrotic leaf areas two days later (Fig. 1). ABA levels in the non-chilled seedlings differed not significantly among the 20 genotypes investigated. But, accumulation of ABA induced by low temperature was significantly higher in the chilling tolerant genotypes than in the chilling sensitive ones (Fig. 1, lower part). The increase in ABA content in the eight tolerant genotypes (T) was statistically higher than that in the sensitive ones (S). A significant negative correlation between ABA content and percent necrotic injury ($r = -0.604^{**}$) existed. Similarly, *qP* was significantly and positively correlated with ABA level during chilling (0.606**, Fig. 2).

Thus, the study clearly indicates a relationship between leaf ABA level and extent of injury in maize, according to the hypothesis that CT in maize is related to the ability for the accumulation of ABA under chilling stress conditions. Data of Ψ_{PSII} for the 20 genotypes and further discussion on the relationship between chilling tolerance and the capacity to synthesise ABA in maize seedling under chilling conditions are presented in the paper JANOWIAK et al. [2002].

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Key words: abscisic acid, chilling tolerance, chlorophyll fluorescence, maize

Abbreviations: ABA = abscisic acid; CT = chilling tolerance; ELISA = enzyme linked immunosorbent assay; LSD = least significant difference; *qP* = photochemical fluorescence quenching; $\Psi_{PS\ II}$ = effective quantum yield of PS II.

Summary

The role of ABA in chilling tolerance (CT) of maize seedling has been reinvestigated using a set of new maize inbred lines with defined differences in CT. Under chilling conditions (4°C) the ABA level increased in all lines investigated. This increase was significantly higher in the chilling tolerant lines than in sensitive ones. Between ABA level and extent of chilling injury a significant relationship was observed. ABA level correlated negatively with percent necrotic injury (–0.604**) and positively with *qP* (0.606**). Thus, the study clearly indicates a relationship between the ABA accumulation under chilling stress and extent of injury in maize according to the hypothesis that CT in maize is related to the ability for the accumulation of ABA under chilling stress conditions.

AKUMULACJA KWASU ABSCYSYNOWEGO A POZIOM USZKODZEŃ W WARUNKACH CHŁODU U 20 LINII WSOBNYCH KUKURYDZY

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Słowa kluczowe: kwas abscysynowy, odporność na chłód, fluorescencja chlorofilu, kukurydza

Streszczenie

Badano rolę ABA w odporności na chłód siewek kukurydzy na nowym zestawie linii wsobnych kukurydzy o ustalonych różnicach w odporności na chłód. W warunkach chłodu (4°C) poziom ABA wzrastał u wszystkich badanych genotypów. Wzrost ten był istotnie wyższy u linii odpornych na chłód w porównaniu z wrażliwymi. Między poziomem ABA a stopniem uszkodzeń chłodowych obserwowano istotną korelację. Poziom ABA korelował ujemnie z procentem uszkodzeń nekrotycznych (–0,604**) a pozytywnie z *qP* (0,606**). Prezentowane badania

wskazują więc na zależność między akumulacją ABA w warunkach stresu chłodowego a rozległością uszkodzenia u kukurydzy zgodnie z hipotezą, że tolerancja na chłód u roślin kukurydzy związana jest ze zdolnością do akumulacji ABA w warunkach stresu chłodowego.

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