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Stomatal reactions of two different maize lines to osmotically induced drought stress

R. STIKIĆ* and W.J. DAVIES**

*Department of Agrochemistry and Plant Physiology, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Yugoslavia**
*Department of Biological Sciences, Institute of Environmental and Natural Sciences, Lancaster University, Bailrigg, Lancaster, LA1 4YQ, UK***

Abstract

Two maize lines differing in drought resistance were grown at different drought stress induced by polyethylene glycol (PEG 10 000) solutions with osmotic potentials of -0.20, -0.40 and -0.80 MPa in the semipermeable membrane system. During the five days soil water content decreased (from 0.43 to 0.29, 0.25 and 0.23 g cm^{-3} for three PEG solutions, respectively) as well as leaf water potentials (ψ_w; from - 0.54 to -0.76, -1.06 and -1.46 MPa). These values were not significantly different between the investigated lines, indicating that a controlled and consistent soil moisture stress was achieved. Soil drying induced an increase in the ABA content of leaves and xylem of both lines and the effects on stomatal conductance were greater in drought susceptible line (B-432) compared to drought resistant line (ZPBL-1304). To test possible difference in stomatal sensitivity to xylem ABA between lines and to assess any ABA *vs.* ψ_w interaction, roots were fed with 10, 50 and 100 mmol m^{-3} ABA solutions in another set of experiments. These results showed that manipulation of xylem ABA affected stomata of both lines similarly. Comparison of stomatal sensitivity to drought-induced and applied ABA demonstrated that drought treatment affected stomata of investigated lines by differentially increasing their sensitivity to xylem ABA, thus confirming an interaction between chemical signalling and hydraulic signalling.

Additional key words: abscisic acid, genotypic differences, PEG-10 000, stomatal sensitivity, *Zea mays* L.

Introduction

It is well known that shoot dehydration influences stomatal behaviour and shoot growth. In recent years great emphasis has been placed on the possibility that stomatal aperture and shoot growth of droughted plants may be also controlled by chemical messages, some of which originate in dehydrated roots. Although some controversy about the identity of such chemical messages still persists (Munns and King 1988, Munns 1990), substantial evidence suggests a key role for abscisic acid (Dodd *et al.* 1996).

Drought experiments conducted in the field (Wartinger *et al.* 1990, Tardieu *et al.* 1991, Tardieu and Davies 1992) and in controlled environments (Zhang *et al.* 1987, Zhang and Davies 1989a, 1990a,b) provided evidence that increased xylem ABA concentration can be largely root-sourced and may be quantitatively sufficient to account for observed reductions in stomatal conductance and growth. Further investigations suggest, however, that drought-induced changes in sensitivity of stomatal responses to ABA may be as important as changes in concentration alone in controlling the stomatal response to soil drying.

Tardieu and Davies (1992) suggested that in the field, plant water status might have a considerable influence on the way stomata behave. They showed that low water potential in maize leaves sensitised stomata to ABA signals such that a given dose of xylem ABA was more effective in the restriction of stomatal conductance

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Abbreviations: ABA - abscisic acid; PEG - polyethylene glycol; ψ_w - leaf water potential; ψ_0 - osmotic potential of PEG solution.
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Fax: (+381) 11 193 659, e-mail: rstikic@eunet.yu

at low than in high water potentials. The same tendency was evident with epidermal tissue of *Commelina communis* incubated in different ABA and PEG solutions. These results suggested that hydraulically modified sensitivity to an ABA signal is an important component of the signal transduction mechanism. Such a mechanism may be of crucial importance for the regulation of gas-exchange in plants exposed to drought in the field. Further evidences for the chemical-hydraulic interaction came from Trejo and Davies (1994).

Nevertheless, the importance of an ABA *vs.* ψ_w interaction has been challenged by results of Correia *et al.* (1995). These authors were not able to use this hypothesis to account for afternoon closure in grape stomata. Similarly, Tardieu *et al.* (1996) could not demonstrate an interaction between ABA and ψ_w when these variables were manipulated in attached and detached leaves of sunflower. Tardieu and Simmoneau (1998) concluded that stomatal control of sunflower leaves only depended on the concentration of ABA in the xylem sap and that leaf water status behaved as a consequence of water flux through the plant, controlled by stomatal conductance. They have described this type of stomatal behaviour as "anisohydric", the contrary of "isohydric" behaviour described for maize plants. The differences in stomatal "strategy" between these groups of plants they attributed to the degree of influence of leaf water status on stomatal control, for a given level of the chemical signal.

Comparisons between soil drying experiments are difficult to make, especially in the field. Different environmental factors (temperature, soil water content, soil nutrients, soil compaction) may influence stomatal reaction. During a soil drying, water availability can change dramatically over a short period of time. Thus, for further validation of any ABA and water potential interaction it would be desirable to perform the assay in a system in which the reactions of intact plants are investigated, but without the influence of other varying environmental factors and where the water status of the soil is held at a constant and controlled value.

The semipermeable membrane system seems to be an appropriate method for this purpose. Although this method was proposed by Zur (1966) 30 years ago, it has apparently not been used frequently, as there are few literature reports of its use (Tingey and Stockwell 1977, Sikurajapathy *et al.* 1983, Kidder and Behrens 1991).

From an agronomic standpoint it is difficult to predict the importance of stomatal sensitivity to drought. Ludlow *et al.* (1990) suggested that the effect of drought on yield depends on the rainfall pattern during the season. Under unirrigated conditions, a sensitive genotype may be able to conserve water as a result of partial stomatal closure and redistribution of canopy expansion. However, Blum *et al.* (1991) showed that well-developed chemical signalling retarded growth, increased tiller mortality and reduced biomass in wheat. Despite the intensive selection work on producing maize lines and wheat cultivars which differ in drought-induced bulk leaf ABA accumulation (Quarrie 1991, Pekić *et al.* 1995), differences in stomatal sensitivity to drought of these genotypes have not been investigated.

The aim of this study was to investigate stomatal response of different maize lines to drought and possible chemical signals. Plants were exposed to drought by using the semipermeable membrane system as a convenient assay for screening for genotypic differences in drought reactions. To compare stomatal sensitivity to endogenously-generated ABA with an effect of applied ABA, we repeated Zhang and Davies' (1990a) experiment but in this case our control of plant water status allowed an assessment of any ABA *vs.* ψ_w interaction.

Materials and methods

Plants: Genotypes used for the assays were two inbred maize (*Zea mays* L.) lines provided by the Maize Research Institute (Belgrade, Yugoslavia). These lines have previously been classified as drought resistant (ZPBL 1304) and drought susceptible (B-432) according to germination tests and visual estimation under field conditions (Pekić and Quarrie 1988).

Seeds were geminated in *John Innes No. 2* compost in a greenhouse, with temperature varying between 22 °C and 28 °C (day) and 12 °C and 15 °C (night). The photosynthetic photon flux density (PPFD) during the 14-h photoperiod was 100 - 200 μmol m^{-2} s^{-1} (fluorescent lamps). Humidity was not controlled. At the one leaf-stage plants were selected for uniformity and transferred to the growth cabinet (day/night temperature 25/18 °C, PPFD of 250 μmol m^{-2} s^{-1} provided by Hg vapour lamps (*LB 250 H*, *Philips*, Lawbay, UK) and 14-h photoperiod. After about three weeks, when the plants were at the 3-leaf stage, they were transferred into specially prepared dialysis membrane cylinders for the root drying treatment.

Soil drying experiment: Dialysis membrane cylinders were built from *Spectra/Por 1 Molecular Porous Dialysis Membrane* (*Spectrum*, Lubbock, USA) cut into 20 cm lengths and clamped to flat perspex discs. The cylinders were filled with a sterilised soil (*John Innes No. 2* compost) and in each cylinder one plant was placed. The plant-membrane system was placed in a large plastic tank and immersed up to the soil level in distilled water for equilibration to the soil water field capacity. After this period for the half of the tubes, distilled water was replaced by PEG solution (water stress treatment). The fungicide *Benlate* (*ICI Garden Product*, Surrey, UK) was added to the solutions (0.15 g dm^{-3}) to prevent microbial degradation of the membrane.

Three level of soil water deficit (Kidder and Behrens 1991) were generated by transferring the membrane cylinders in polyethylene glycol 10 000 (PEG 10 000, *Sigma Chemical Co.*, Poole, UK) with osmotic solutions of -0.20, -0.40 and -0.80 MPa. Osmotic potential (ψ_o) of these solutions was measured with a *Wescor 5100* vapour pressure osmometer (*Wescor*, Logan, USA). The plants were established for 5 d under control or treatment conditions.

Soil water content was expressed on a volume basis by multiplying the gravimetric soil water content by the mean of bulk soil density (Zhang and Davies 1989a). Mean soil bulk density in soil column was 0.952 g cm^{-3}.

Measurement of physiological variables: The abaxial stomatal conductance of fully expanded leaves was measured with a porometer (*AP-4*, *Delta T Devices Ltd.*, Cambridge, UK) at 10.30 (4 h after beginning of the photoperiod). After measurements of stomatal conductance, the leaf water potential measurements were taken using a pressure chamber (*Soil Moisture Equipment*, Santa Barbara, California, USA). When the leaf water potential values were noted, the lamina of the 3rd leaf was freeze-dried, ground to a fine powder and incubated overnight at 5 °C with distilled, deionized water for ABA analysis.

Xylem sap was extracted with a pressure chamber using the procedure described by Zhang and Davies (1989b). Plants were detopped (first at a higher position to relieve xylem tension) fitted in the pressure chamber, pressurised to 0.5 - 1.0 MPa and approximately 0.5 cm^3 of sap was collected. Xylem sap was frozen and stored until analysis. ABA concentration in xylem and leaves samples was determined using RIA with the *AFRC MAC 252* antibody according to a protocol of Quarrie *et al.* (1988).

ABA-feeding to part of the maize root system: Plants at the three-leaf stage were transferred (with attached soil) in 80 mm diameter pots from which the bottoms were removed and replaced with a piece of plastic mesh (5 mm diameter holes). Pots were inserted into 400 cm^3 plastic beakers containing 100 cm^3 Hoagland's nutrient solution. The mesh at the bottom of the pots was positioned about 10 mm above the surface of the solution. Plants remained in this condition for approximately 10 d until a substantial amount of root (about 20 % of the total mass) was established outside of the pot and dangling in the nutrient solution. The nutrient solution was replenished every day. The whole procedure was performed according to the procedure described by Zhang and Davies (1990a).

ABA concentration of 10, 50 and 100 mmol m^{-3} of (±)-synthetic ABA (*Lancaster Synthesis*, Morecambe, UK) was fed to the plants via the nutrient solution. Starting two days after ABA-feeding, measurements of stomatal conductance were made as described above. After this, xylem and leaf samples were analysed for ABA using the procedure described by Quarrie *et al.* (1988).

Statistical analysis: Means of variables measured in both experiments were discriminated using unpaired *t*-tests in *Sigma Plot for Windows Version 1.0* (*Jandel Scientific*, Erkhart, Germany). For comparison of genotype *vs.* treatment interactions two way *Analysis of Variance ANOVA* was performed.

Results

As a result of the PEG treatments, volumetric soil water contents decreased from 0.43 g cm^{-3} (control) to 0.29 g cm^{-3} (ψ_o = - 0.20 MPa), 0.25 g cm^{-3} (ψ_o = -0.40 MPa) and 0.23 g cm^{-3} (ψ_o = - 0.80 MPa). The water potential of plants of both lines decreased from around -0.54 MPa (control) to around -0.76, -1.06 and -1.46 MPa, respectively.

As a result of the drought treatments stomatal conductance of both lines decreased significantly ($P < 0.01$) and the depression was more expressed in line B-432 than in line ZPBL-1304 (Fig. 1*A*). The drought treatments significantly increased ($P < 0.001$) xylem ABA concentration (Fig. 1*B*). Compared to the xylem ABA content increase, leaves of both lines were less sensitive to soil drying treatments, since their bulk content in the highest stress condition (ψ_o = -0.80 MPa) increased by only *ca.* 3 fold (Fig. 1*C*). *ANOVA* did not show any significant genotypic differences between these traits.

Xylem ABA concentration in ABA-feeding experiment was calculated as (+)-ABA, presuming that maize roots did not discriminate between (+)-ABA and (-)-ABA during uptake from synthetic (±)-ABA solution (Zhang and Davies 1989b, 1990a). ABA-feeding induced a reduction in stomatal conductance and an increase in xylem ABA concentration and bulk leaf ABA content (Fig. 2). *ANOVA* analysis did not reveal any significant genotypic differences between investigated lines.

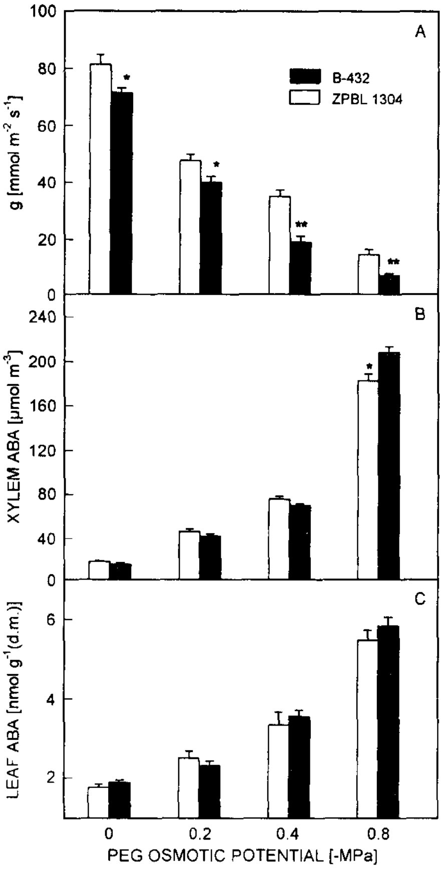

Fig. 1. Stomatal conductance (*A*), xylem ABA concentration (*B*) and bulk leaf ABA (*C*) content of maize lines ZPBL-1304 and B-432 exposed to PEG- induced drought stresses. Means ± S.E, $n = 10$, * and ** indicated differences between investigated lines which are significant at $P < 0.05$ and $P < 0.01$, respectively.

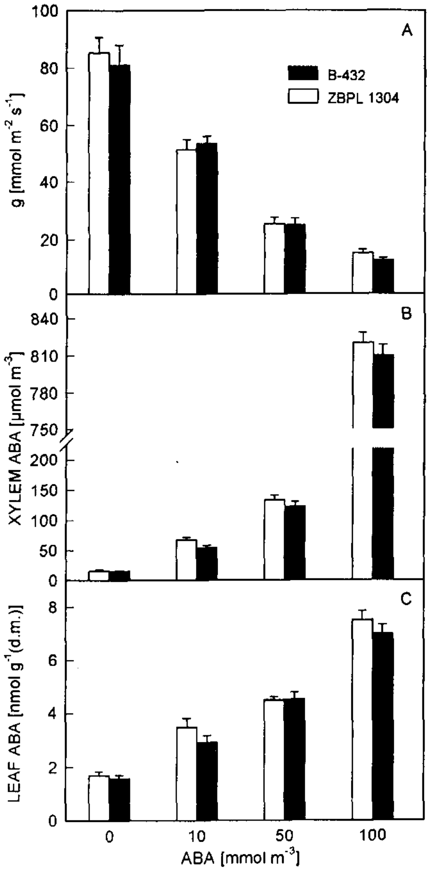

Fig. 2. Stomatal conductance (*A*), xylem ABA concentration (*B*) and bulk leaf ABA content (*C*) of maize lines ZPBL-1304 and B-432 fed with different concentrations of synthetic (±) ABA solutions. Means ± SE, $n = 10$.

In order to test the influence of reduced water potential on stomatal sensitivity to xylem ABA the mean stomatal conductance values for each treatment were plotted against corresponding mean xylem ABA concentration (Fig. 3*A*,*B*) and the plots from this graph were then been fitted by using the second order of regression (*SigmaPlot for Windows*) with logarithmic transformation. These results showed a highly significant correlation of stomatal conductance with xylem sap ABA. The slopes of these regressions and correlation coefficients were then used to compare the sensitivity of stomata to xylem ABA in drought and ABA-feeding experiments. Obtained equations for mean values obtained in PEG experiments were: $y = 215.26 - 129.99 \log x + 18.28 (\log x)^2$, $r^2 = 0.93$ (line ZPBL-1304) and $y = 195.95 - 128.95 \log x + 20.90 (\log x)^2$, $r^2 = 0.93$ (line B-432). The equations obtained for ABA-feeding experiments were: $y = 228.91 - 145.91 \log x + 24.83 (\log x)^2$, $r^2 = 0.93$ (line ZPBL-1304) and $y = 213.91 - 135.57 \log x + 22.73 (\log x)^2$, $r^2 = 0.95$ (line B-432). These results indicated that the stomata of both lines was more sensitive to drought than to ABA-feeding. These equations also reveal genotypic differences in stomatal sensitivity to xylem ABA, but only for drought treatments. Alternatively, stomatal sensitivity to xylem ABA was analysed by expressing stomatal conductance data in relative units (as mean % of control values) and plotting them against mean xylem ABA concentration. Plots were then fitted by the second order of regression (Fig. 3*C*,*D*). From this graphs it may be seen that similar stomatal reduction obtained from the medium stressed and 10 mmol m^{-3} ABA-feeded plants (*ca.* 60 % of control) were induced by significantly ($P < 0.001$) lower xylem ABA concentration in droughted (*ca.* 45 µmol m^{-3}) than in ABA-feeded plants (*ca.* 60 µmol m^{-3}). Obtained data also showed that the stomata of line B-432 had more sensitive response to drought-induced xylem ABA than line ZPBL-1304. These differences were more expressed in medium-high and high stress conditions. In medium-high stress conditions the lower stomatal conductance in line B-432 (32 % of control) then in line ZPBL-1304 (43 % of control) was induced by similar xylem ABA concentration (75.8 and 70.2 µmol m^{-3} for ZPBL-1304 and B-432, respectively). In high stress conditions lower stomatal conduction of line B-432 (10 % of control) than in line ZPBL-1304 (18 % of control) was induced by higher xylem ABA content (209 µmol m^{-3}) then in line B-432 (183 µmol m^{-3}).

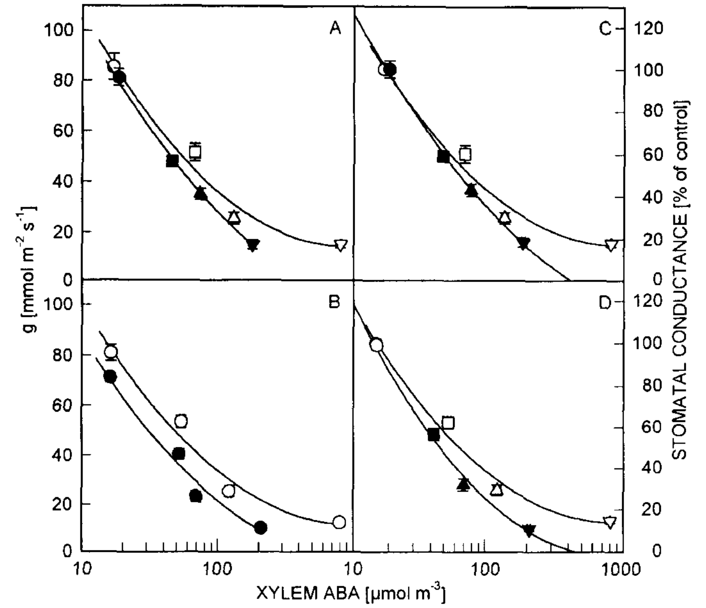

Fig. 3. Relationships between mean stomatal conductance and mean xylem ABA concentration in lines ZPBL-1304 (*A*) and B-432 (*B*) and between relative leaf conductance (mean values in % of control) and mean xylem ABA in lines ZPBL-1304 (*C*) and B-432 (*D*). Plants were exposed to PEG-induced drought (*close circles* - control, *close squares* - medium stress, *close triangles* - medium-high, and *reverse close triangles* - high stress) or fed with synthetic (±)-ABA solutions (*open circles* - control, *open squares* - 10 mmol m^{-3}, *open triangles* - 50 mmol m^{-3} and *reverse open triangles* - 100 mmol m^{-3}). The *solid lines* show the second order regression fitted in *SigmaPlot for Windows 1.0.*

However, comparison of results obtained from ABA-feeding plants did not reveal such a significant genotypic difference. Therefore, it could be concluded that decreased water potential during the drought treatment affected stomata of investigated lines by differentially increasing their sensitivity to xylem ABA.

Discussion

PEG solutions, with osmotic potentials varying between -0.20 and -0.80 MPa, lowered the volumetric water content of soil on the other side of a dialysis membrane from 0.43 to 0.23 g cm^{-3}. The magnitude of this changes in soil water content and leaf water potentials are similar to those obtained by growing *Setaria viridis* and *Panicum milliaceum* plants in similar semipermeable membrane conditions (Kidder and Behrens 1991). Soil water content values are also similar to those obtained with maize plants expossed to 6, 9 and 12 d of drought in large soil columns and when decreased soil water content substantially increased the root ABA concentration, enhanced xylem ABA content and induced partial stomatal closure (Zhang and Davies 1989a).

In a large soil column as well as in the field, soil dries slowly and progressively and soil water content is often very low in shallow soil horizons. In the semipermeable membrane cylinders, soil water content is uniform down through the soil column. It might, therefore, be argued that soil water conditions in the semipermeable membrane system are not comparable with a realistic field situation. Nevertheless, as Zur (1966, 1967) noted, two important experimental requirements are achieved, namely: *a*) control of water supply is sustained at the required level and *b*) rapid replenishment of the soil water content that keeps pace with the plant root extraction rate. This means that during the whole experimental period, soil water content values in the semipermeable membrane as well as leaf water potentials, were not significantly different between investigated lines.

Comparison of the results obtained in the soil drying study with those of the ABA-feeding experiment (Fig. 3), showed that in both investigated lines a reduced leaf water potential increased stomatal sensitivity to xylem ABA. The degree of stomatal closure detected in the leaves of both lines exposed to medium stress conditions (mean decrease to 56 % of control) did not differ from the plants treated with 10 mmol m^{-3} ABA (mean decrease to 62 % of control), but it was promoted by a significantly lower xylem ABA concentration. Data obtained from the plants fed with higher ABA concentration (50 and 100 mmol m^{-3}) showed that with a greater increase in xylem ABA content (mean 7.6 and 49 fold) stomata remained more open than those of severely stressed plants. These results, similarly to the results of Correia and Pereira (1995), showed that the decrease in stomatal conductance of droughted plants was not mimicked by feeding-ABA plants even at the ABA concentrations which were two or four fold higher then those in the xylem sap of stressed plants.

Results support Tardieu and Davies' (1992, 1993) hypothesis that the sensitivity of the stomatal response to xylem ABA is enhanced by a reduction in leaf water status. Wilkinson and Davies (1997) have provided an explanation of this interaction. Increases in the pH of xylem sap as the soil dries will be expected to increase sensitivity of stomata to xylem ABA, since a greater proportion of the ABA arriving in the leaf will be expected to reach sites of action on the guard cells.

We have also confirmed differences in stomatal sensitivity between lines but only under more severe drought conditions. When mean stomatal conductances were calculated in relative units, stomata of line B-432 were shown to be about 10 % more responsive to medium-high and high stress than those of line ZPBL-1304. Differences in leaf water potential were not detected between lines. These results indicate that the semipermeable membrane system is suitable for screening for genotypic differences in stomatal responses to an ABA signal or indeed any other chemical signal.

We have shown an increase in the bulk leaf ABA content of the investigated lines but there was no significant genotypic difference in drought-induced ABA accumulation, as has previously been shown for these lines (Pekić and Quarrie, 1987; 1988). However, these reported and more recent results of same authors (Pekić *et al.* 1995) indicated that the capacity for drought-induced ABA accumulation in the maize leaves depends on type and duration of stress.

It is important to note that in well-watered conditions, both investigated lines, similarly to *Phaseolus vulgaris* plants (Trejo and Davies, 1991), had a significantly higher ABA content in the leaves than hybrid used for Zhang and Davies' (1989b, 1990a) experiments. Since similar high ABA values were detected in other inbred maize lines in well-watered control conditions and in the irrigated field (Pekić and Quarrie 1987, Capel and Dörffling 1993), some important questions arise concerning physiological relevance of such high amounts of leaf ABA. It may be that these high amounts of the hormone are not relevant for stomatal regulation and are related to the characters that allow the plant to survive under severe stress. Wilkinson *et al.* (1998) suggested that ABA might also have a role to play in modifying the water relations of the well watered plants. Davies and Gowing (1999) reported that a variety of changes in the edaphic environment could lead to an alkalinisation of xylem sap. Without the presence of ABA in the xylem sap this change will open stomata to a value that is greater than that set by the prevailing climatic conditions.

In conclusion, our results confirm a hydraulic influence on stomatal sensitivity to xylem ABA and significant genotypic differences between investigated lines. Further investigation of the relevance of these data for adaptation and resistance of maize to drought would be very worthwhile.

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