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Xylem embolism and drought-induced stomatal closure in maize

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Abstract Water relations during drought and xylem vulnerability to embolism were studied on four maize (*Zea mays* L.) genotypes having contrasting grain yields under drought conditions. Drought provoked a drop in xylem pressure, leaf water potential and whole-plant transpiration. Transpiration was reduced to a minimum value when xylem pressures reached ca. -1.6 MPa. This value corresponded to the threshold xylem pressure below which xylem embolism developed to a substantial degree in leaf midribs. Therefore, xylem embolism always remained low in leaf veins, even when plants exhibited clear water-stress symptoms. This suggests that stomatal closure during drought contains xylem embolism to a minimum value. Cavitation resistance was not related to grain yield under drought conditions for the four genotypes evaluated. However, it can be speculated that an increase in cavitation resistance by cultural practices or genetic selection may increase drought survival in maize.

Keywords Cavitation · Hydraulic conductance · Stomata · Water potential · Water stress · *Zea* (water relations)

Abbreviations *E*: whole-plant water loss · P_{xyl} : negative xylem pressure · P_{cav} : threshold xylem pressure for cavitation · PLC: percent loss of hydraulic conductance · RSWC: relative soil water content · Ψ_{leaf} : leaf water potential · Ψ_{soil} : soil water potential

Introduction

Sap is transported under negative pressure (tension) in plant xylem conduits. This state being physically unstable, cavitation events are likely to occur if air enters into vessels (Pickard 1981). Cavitation is a serious threat for plants because it lowers the conductive capacity of the xylem, and can thus impair water supply to transpiring leaves.

The analysis of cavitation events in woody plants has received considerable attention during the past two decades. Two major findings have emerged from these studies. First, xylem pressure (P_{xyl}) is the triggering parameter for water-stress-induced cavitation. Cavitation occurs only when the xylem pressure drops below a specific threshold value (P_{cav}) determined by the porosity of pit membranes. The second important finding was that for most woody species, P_{cav} values are only slightly more negative than the P_{xyl} values experienced by each species when leaf transpiration is high (Tyree and Sperry 1988). As a consequence, when soil water content is depleted, plant transpiration has to be reduced in order to avoid excessive cavitation events (Jones and Sutherland 1991; Saliendra et al. 1995). These data on woody plants have thrown new light on the understanding of stomatal function in trees and their resistance to drought.

So far, the analysis of cavitation events in crop plants has received very little attention (Tyree et al. 1986; Neufeld et al. 1992; Buchard et al. 1999). The evidence for stomatal control of cavitation in crops awaits experimental confirmation. Similarly, the significance of xylem cavitation in drought resistance remains largely unexplored for such species (Neufeld et al. 1992). The objectives of this study were to analyze the relations between xylem cavitation, water-loss regulation and drought resistance in four different maize (*Zea mays*) genotypes. Maize was chosen because previous studies had suggested that cavitation events are of common occurrence, even for moderately water-stressed plants (Tyree et al. 1986; McCully et al. 1998). Maize was thus

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a species likely to exhibit undocumented relationships between hydraulic and stomatal traits.

Materials and methods

Plant material and growth conditions

Experiments were conducted on four non-genetically modified maize (*Zea mays* L.) genotypes. Two genotypes ('Aprilia' and 'Belonia') were obtained courtesy of Pioneer Hi-Bred International (Aussonne, France). Two more genotypes ('Furio', Novartis; LG 24-47, Limagrain) were provided by the Laboratory of Genetics (INRA, Clermont-Ferrand, France). Aprilia and Furio genotypes are considered more 'drought resistant' than, respectively, Belonia and LG 24-47. Plants were planted on 12 March 2001 in 5-l pots containing a mixture of Limagne soil, peat and scoria. The pots were placed in a greenhouse where air temperature was always maintained above 15 °C. Two seeds were planted in each pot, but after 1 month, the less-developed plant was removed (leaving a total of 10 plants per genotype). On 6 June, eight plants (two replicates per genotype) were transferred to a growth cabinet for the first experiment. At this stage, plants were flowering and all leaves were fully expanded. Air temperature (25 ± 1 °C), air relative humidity ($65 \pm 3\%$) and light irradiance ($300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) were automatically controlled in the cabinet. The experiment was repeated on 11 and 25 June giving a total of six replicates for each genotype. The four remaining plants were kept in the greenhouse for occasional sampling (vessel length determination, vulnerability curves).

Relative soil water content (RSWC)

Pots were watered to field capacity and their initial weight (w_i) was determined at the beginning of each experiment (0.1 g resolution). The plants were then allowed to dehydrate and the pots were weighed periodically (w). At the end of the experiment, the pots were oven-dried at 80 °C (w_f). RSWC was then computed as: $\text{RSWC} = (w_i - w) / (w_i - w_f)$.

Plant water relations

Whole-plant water loss (E , $\text{mmol s}^{-1} \text{plant}^{-1}$) was estimated by weighing pots periodically (usually early in the morning, midday and late afternoon). To account for the differences in leaf area between plants, E was normalized by the maximum E value recorded for each plant. Because air temperature, air humidity and light intensity were constant in our study, E was a proxy of the leaf-to-air stomatal conductance to vapor water.

Leaf water potential (Ψ , MPa) was measured with a pressure chamber on terminal leaf parts (about 10 cm long). For the first two experiments, xylem pressures (P_{xyl}) were estimated by measuring Ψ on terminal leaf parts enclosed for at least 6 h in aluminum bags (Turner 1981; Wei et al. 1999). This assumes (i) that the Ψ of the terminal leaf part was in equilibrium with the Ψ in the xylem at the entrance of the bag and, (ii) that the sap osmotic potential was negligible. For the third experiment, Ψ was on measured unbagged leaves when the lights were on (Ψ_{leaf}) or 4 h after the lights were turned off. In the latter situation, Ψ_{leaf} was supposed to be representative of the soil water potential (Ψ_{soil}) in the root zone (Turner 1981; Améglio et al. 1999).

Xylem embolism

When xylem conduits cavitate, the initial vacuum becomes rapidly filled with gas and an embolism forms. The percent loss of hydraulic conductance (PLC) due to air blockage is an indirect estimate of the percentage of cavitated vessels (Sperry et al. 1988;

Cochard et al. 2000). Xylem embolism was measured on leaf midrib segments. Segments were about 2 cm long and 0.5 cm wide and contained 15–20 large metaxylem vessels. Since midribs have a U-shaped profile, leaks were prevented by filling the cavity with mastic and wrapping the segment with Teflon tape. The segment was then gently inserted into exacanal tubing (Merk Eurolab, Nogent-sur-Marne, France) and the PLC value was measured with a prototype of the new Xyl'em apparatus (INRA license, <http://www.instrutec.fr>) following Sperry et al. (1988). In short, PLC was computed as: $100 \times (1 - Lp_x^i / Lp_x^s)$ where Lp_x^i is the initial xylem conductance and Lp_x^s is the saturated conductance. Xylem saturation was achieved by a series of short perfusions at 0.15 MPa.

The degree of xylem embolism was determined at the end of each experiment on leaves located near the ears. The leaves were excised from the plants under water. Embolism levels were also measured on entire excised leaves (including leaf sheath) allowed to dehydrate on a laboratory bench. This was necessary to obtain higher degrees of leaf dehydration and induce sufficient embolism. The terminal part of each leaf was enclosed in an aluminum bag and P_{xyl} was determined just before sampling for embolism estimates. Xylem vulnerability curves were obtained by plotting PLC against P_{xyl} values.

Our previous experience with embolism detection in monocot leaves (*Festuca arundinacea* Schreb.; Martre et al. 2001) was unsuccessful because this species has large air spaces in its mesophyll that conduct water after a perfusion at 0.1 MPa. To test the relevance of the PLC determination in maize, the following experiment was done. Leaf blades were cut at their base from plants grown in the greenhouse. Leaves were excised during the day and then allowed to dehydrate on a laboratory bench for about 5 min. This caused air to enter the full length of the vessels cut open at the base of the leaf. PLC values were then determined, as above for midrib segments excised under water, at different locations along the blade. In theory, the PLC value should be close to 100% at the base of the blade and progressively decrease to the native-state level depending on vessel length (Cochard et al. 1994). Maximum vessel length in the leaf blade was determined following the air-injection method of Ewers and Fisher (1989).

Results

Water relations during drought

When potted plants were allowed to dehydrate in a growth chamber, three distinct successive phases were observed. For soil RSWC values above 0.7, E (Fig. 1), Ψ_{soil} (Fig. 2a), P_{xyl} (Fig. 2b) and Ψ_{leaf} (Fig. 2c) were close to their maximum value before treatment. When RSWC was further decreased to 0.5, the values of the above parameters decreased linearly. At this stage, leaves were starting to exhibit distinct leaf curling, characteristic of water stress in maize. For RSWC values below 0.5, all parameters leveled down. E was reduced to ca. 10% of its initial value, which corresponded to the value measured in full darkness (cuticular transpiration). Water potentials within the plants tended to equilibrate at a value close to -1.6 MPa. At this level of dehydration, leaves at the base of the plants were yellowing. The four different genotypes responded similarly.

Xylem embolism

The results of the experiment designed to test the relevance of the hydraulic technique to measure xylem

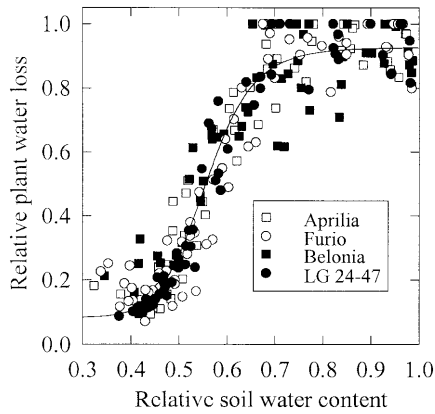


Fig. 1 Relative plant water loss versus relative soil water content in maize (*Zea mays*). Different symbols correspond to four different genotypes. Open symbols correspond to more “drought-resistant” genotypes

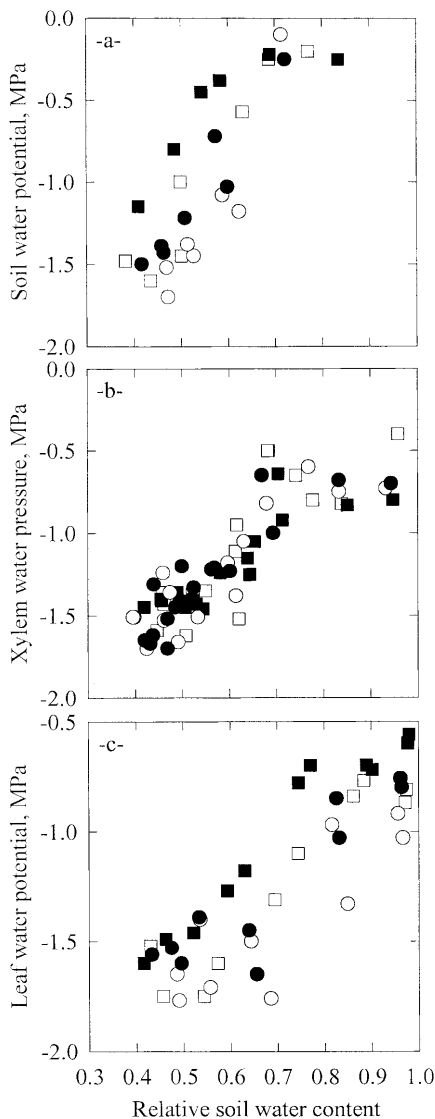


Fig. 2 Soil water potential (a), xylem pressure (b), and leaf water potential (c) versus relative soil water content in four genotypes of maize. Symbols as in Fig. 1

embolism in maize midribs are shown in Fig. 3. PLC values were close to 100 at the air-exposed base of the blade and decreased progressively in the more distal segments to 0 PLC at a distance consistent with the maximum vessel length as determined by the air-injection technique (Fig. 3, insert). These results conformed to our predictions, and therefore validated the hydraulic technique for maize leaf veins. Substantial differences were found between genotypes, Aprilia tending to have the longest vessels (about 20 cm) and Furio the shortest ones (about 10 cm).

The amount of xylem embolism measured in the leaf veins of potted plants always remained low (below 10 PLC). Substantial losses of conductance were only detected in veins of yellowing leaves. Excised leaves exposed to very high water deficits exhibited up to 60 PLC. The dependence of PLC on xylem pressure is shown in Fig. 4. Embolism was less than 5 PLC for P_{xy1} in the range 0 to -1.0 MPa and developed appreciably only for P_{xy1} values below ca. -1.5 MPa. The differences between genotypes were not noteworthy.

Discussion

The objective of the study was to analyze the water relations of maize during water stress with respect to its hydraulic characteristics. Maize water relations during water stress have been extensively documented (e.g. Bennett et al. 1986, 1987; Tyree et al. 1986; Tardieu et al. 1992). The relationships between relative soil water content (RSWC), leaf water potential (Ψ_{leaf}) and leaf water loss (E) reported in this study are consistent with the data in the literature. Complete stomatal closure at a Ψ_{leaf} value between -1.5 and -2 MPa seems to be a general pattern for maize plants.

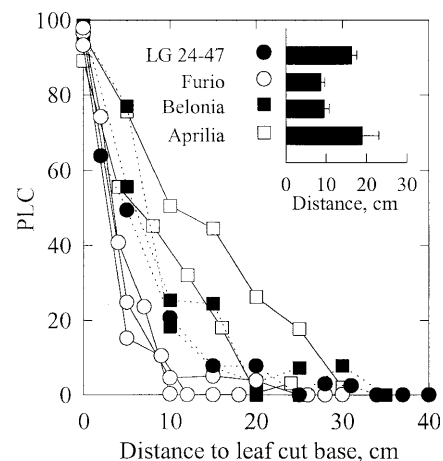


Fig. 3 Change in percent loss of conductance (PLC, y-axis) in the xylem of maize leaves exposed to air at their cut base. Symbols as in Fig. 1. The different lines represent eight different leaves. The mean (\pm SE) maximum vessel length as determined by the air-injection technique for each genotype is shown in the insert

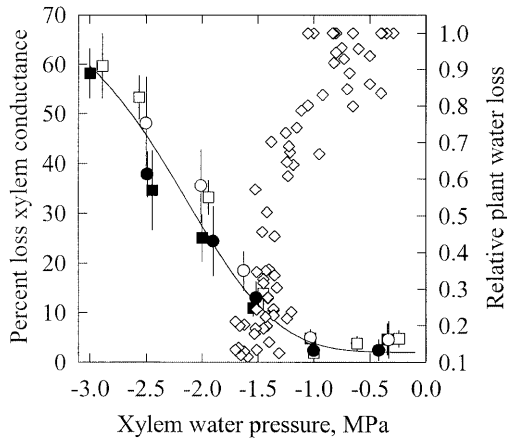


Fig. 4 Percent loss of xylem conductance and relative plant water loss (transpiration) in maize leaf midribs versus xylem pressure. For xylem conductance, the symbols are as in Fig. 1. The error bars represent \pm SE. For relative plant water loss (*open diamonds*), the data are combined from Figs. 1 and 2b, all genotypes being pooled

Much attention has been paid in the recent literature to the identification of parameters involved in stomatal control. Although this issue is not entirely solved, it seems that both leaf water status and abscisic acid (ABA) concentration play a role in this control (Tardieu and Davis 1993). However, much less attention has been paid to the physiological parameters controlled by the stomatal closure during water stress. For woody plants, maintenance of the conductive capacity of xylem has been recognized as a major trait that could drive stomatal closure during water stress (Sperry 2000; Cochard et al. 2002). Our objective was to evaluate such a behavior in maize.

In Fig. 4, the dependence of E on P_{xyl} is superimposed on the vulnerability curve for maize midribs. E was a proxy for stomatal conductance in our study because light irradiance, air temperature, and humidity were maintained constant in the chamber. Although the cyclic variations in air humidity caused by the air conditioner were substantial ($\pm 3\%$), they were too rapid (period 10 min) to have a measurable effect on maize water relations. It is clear from this graph that stomata were completely closed (and transpiration reduced to cuticular values) before appreciable levels of embolism had formed in the xylem. This was consistent with the fact that the degree of xylem embolism always remained below 10% in situ. It can be predicted that, for drought-exposed plants, any increase in transpiration would have decreased P_{xyl} and caused substantial xylem embolism. Therefore, our results on maize plants in this study demonstrate that the avoidance of xylem cavitation might also drive stomatal closure in a herbaceous plant. The mechanisms behind the correlation shown in Fig. 4 are not yet unraveled. Some authors (Salleo et al. 2000) have suggested that hydraulic signals originating from early cavitation events in the xylem conduits might trigger the stomatal closure. A causal relationship between xylem cavitation and stomatal regulation might

exist. However, it is worth noting that other physiological parameters are altered simultaneously with the stomatal closure. In walnut, for instance, stomatal closure was correlated with bulk leaf turgor loss (Cochard et al. 2002). Variable hydraulic conductances in the roots (Henzler et al. 1999) or in the xylem itself (Zwieniecki and Holbrook 1998) may also play an important role in such a mechanism. This point clearly deserves further study.

Our measurements of xylem embolism in maize are inconsistent with previous reports on this species. Tyree et al. (1986) used ultrasonic acoustic sensors to detect cavitation events in internodes. They found that in drought-subjected plants as many as 50% of vessels had embolisms. McCully et al. (1998) used a cryo-scanning electron microscope (cryo-SEM) to visualize embolisms in roots. They found embolisms in up to 70% of late-metaxylem vessels of well-watered plants by midday. In contrast, in our study the amount of xylem embolism, determined hydraulically, remained below 10%, even for highly stressed plants. Recent information from the acoustic and cryo-SEM methods may suggest that the results obtained by these procedures may be vitiated by technical problems. Indeed, Ritman and Milburn (1988) and Cochard and Tyree (1990) have reported that many ultrasonic acoustic emissions are produced by non-vessel xylem conduits (fibers or tracheids) in the early stages of dehydration. Furthermore, Cochard et al. (2000, 2001a, 2001b) have reported that the large numbers of air-filled vessels seen with the cryo-SEM technique are artifacts of the freezing procedure.

A second objective of our study was to evaluate the implication of hydraulic traits in the drought resistance of different maize genotypes. Our results show virtually no genotypic variability in terms of water-loss regulation or xylem vulnerability to embolism. The two pairs of genotypes we evaluated were provided by maize breeders on the basis of their grain yield under drought conditions. Schmidhalter et al. (1998) have shown that, in maize, growth is reduced before any appreciable change in water relations has occurred. Therefore, there is probably little reason for growth performance under drought conditions to be correlated with hydraulic traits, such as cavitation avoidance, that manifest at much higher water stress levels. However, we believe that cavitation avoidance is a trait that may pertain to the ability of maize to survive severe droughts. Drought survival is a feature that may receive more attention in the future if water available for irrigation is limited or if maize is planted in more arid regions. From the knowledge that has been acquired on woody plants, we can predict that plants that are more cavitation resistant may survive longer during a severe drought. In this eventuality, we can think of two different approaches to confer higher cavitation resistance in maize. We know from experiments on woody plants that growth conditions such as nutrient status (Ewers et al. 2000), water availability (Alder et al. 1996), soil porosity (Hacke et al. 2000) and light availability (Cochard et al. 1999) may

substantially modulate xylem vulnerability. Some cultural practices may therefore confer higher cavitation resistance to maize plants. The second approach is on a genetic level. Cavitation resistance is a trait closely associated with wall anatomy (Sperry and Tyree 1988) and many genes coding for wall structure have already been identified. Some of these genes probably code for the primary wall structure of the pit membrane and may thus be strongly implicated in vessel vulnerability to cavitation. *Sorghum* is a species closely related to maize but much more drought resistant. It may be of interest to compare these two species from both a hydraulic and a genetical point of view in order to identify genes involved in plant drought resistance.

In conclusion, the analysis of maize water relations and hydraulic traits during drought suggests the implication of stomata in the control of xylem embolism. Stomatal closure prevented the development of xylem tensions below the point of incipient cavitation. In this respect, maize behaved like many woody plants studied so far. Although cavitation resistance was not related to growth performance under drought conditions in the four genotypes evaluated, we believe that an increase in cavitation resistance may help maize to survive prolonged periods of drought. We hope that these results will convince crop physiologists of the great importance of hydraulic traits in the understanding of crops water relations during drought.

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