Comparison of *Zea diploperennis* and *Zea mays* under water stress conditions

Alberto-Daniel GOLBERG, Jean-François LEDENT & Charles RENARD

Université Catholique de Louvain, ECOP (Grandes Cultures), 2, place Croix-du-Sud, B 1348 Louvain-la-Neuve, Belgique

SUMMARY

Effects of cessation of watering on leaf water potential (ψ_1), leaf diffuse conductance (G) and dry matter were observed in *Zea diploperennis* (Z.D.) and maize, under controlled conditions. A more rapid decrease of soil water content was observed in containers where Z.D. was grown, indicating a higher transpiration in Z.D. than in maize. Despite that difference, Z.D. was found to maintain constant G during 2 weeks after ψ_1 started to decline, whereas in maize G values were not maintained. Moreover recovery to control G values occurred within 2 days in Z.D., but not in maize. Dry matter and leaf area were less reduced by drought in Z.D. than in maize. At equivalent leaf potential value, CO_2 exchange rate (CER) and G were less reduced by water stress in Z.D. than in maize.

Additional key words : *Water potential, leaf conductance, photosynthesis.*

RÉSUMÉ

Relations hydriques de Zea diploperennis et Zea mays, soumis à la sécheresse.

Les effets d'un arrêt de l'arrosage furent étudiés chez *Zea diploperennis* (Z.D.) et chez le maïs en conditions contrôlées. Le contenu en eau du sol des conteneurs où croissait Z.D. baissa plus rapidement, indiquant une transpiration plus élevée chez Z.D. (par rapport au maïs). Malgré cela, la conductance foliaire (G) de Z.D. fut maintenue à sa valeur initiale pendant les deux premières semaines qui suivirent, alors que chez le maïs une chute de G fut observée rapidement. De plus, lors de la reprise des arrosages, les valeurs de G du maïs ne se rétablirent pas rapidement au niveau des témoins comme ce fut le cas chez Z.D. Finalement, la masse sèche et la surface foliaire du maïs furent plus affectées par la sécheresse. A potentiel hydrique foliaire équivalent, la photosynthèse et G de Z.D. furent moins réduites par le stress hydrique que chez le maïs.

Mots clés additionnels : *Potentiel hydrique, conductance foliaire, photosynthèse.*

I. INTRODUCTION

Zea diploperennis Iltis, Doebley, Guzman is a primitive relative of *Zea mays* L. discovered recently (ILTIS *et al.*, 1979). The species crosses easily with maize and might therefore be used to bring new germplasm into a maize breeding programme (GALINAT, 1980 ; GAY, 1984). For this reason, an investigation was started on physiological characters of *Zea diploperennis* (which will be referred to as Z.D.) in comparison with maize.

Since we intend to study in future the behaviour of interspecific hybrids between maize inbred lines and Z.D. we chose as a first step to compare Z.D. with one maize inbred line. In this paper we examine, under controlled conditions, the effect of water stress on photosynthetic capacity (CO_2 exchange rate, CER), stomatal opening (leaf conductance, G), and dry matter. Photosynthetic rates per unit leaf area may differ among genotypes, and this character might have some value in

maize breeding (CROSBIE *et al.*, 1981 ; MOROT-GAUDRY *et al.*, 1981).

On the other hand, water can be a very important factor limiting productivity (MORIZET *et al.*, 1984 ; ALGANS & DESVIGNES, 1984) and CER of maize can be drastically affected by water stress (MCPHERSON & BOYER, 1977).

II. MATERIALS AND METHODS

A. Plant material, conditions of growth

Seeds of Z.D. (*Zea diploperennis*) were harvested in 1983 on plants grown in the botanical garden of INIREB (Xalapa, Mexico). Maize seeds were commercial seeds on inbred line F_1 from I.N.R.A., France.

TABLE 1

Water stress treatments and physiological measurements.

Traitements (contrainte hydrique) et mesures de paramètres physiologiques.

	Beginning of cessation of watering (days after sowing)	Number of expanded leaves at onset of treatment	Duration of treatment (days)	Harvest (days after sowing)	Measurements [1]	Leaves measured (position on the shoot 1 = uppermost expanded leaf)	Number of leaves measured for each date and treatments
	Début de l'arrêt de l'arrosage	Nombre de feuilles déployées au début du traitement	Durée du traitement (jours)	Récolte finale (jours après le semis)	Mesures	Feuilles mesurées (1 = dernière feuille déployée)	Nombre de feuilles mesurées
Containers	25	6	13	40	θ_w, G^1, ψ_l, ψ_s	1 or 2	5
Pots	54	8-9	8	Plant discarded after measurement	G, CER, ψ_l	2 or 3	6

[1] = θ_w : soil water content (percentage on dry weight basis) ; Teneur en eau du sol (sur la base du poids sec).
G : leaf diffusive conductance ; Conductance stomatique.
ψ_l : leaf water potential ; Potentiel hydrique foliaire.
ψ_s : leaf osmotic potential ; Potentiel osmotique foliaire.
CER : CO_2 exchange rate ; Taux d'échange du CO_2 (photosynthèse).

After germination in Petri dishes at 24 °C, seeds were transplanted in containers. An additional series of seeds was transplanted to pots. Pots and containers were placed in growth chambers (PGV 36, from Controlled Environment).

Containers were 80 × 50 cm by 64 cm depth. There were four containers (two for each species). Each contained a bottom layer of gravel (10 cm thick) and a top layer (40 cm) of a fifty-fifty sand/loam mixture. There was no opening in the containers for drainage. Water was supplied through perforated pipes buried 35 cm below the soil surface. The quantities provided were not recorded. In each container plants were placed in 2 rows 34 cm apart.

In the growth chamber plants were kept under a 10 h photoperiod and 21° (day), 15° (night) temperatures. Air relative humidity was 60 % (day) and 80 % (night). Photosynthetic photon flux density on plant tops was 250 µmol m^{-2} s^{-1}.

The pots used for the additional series of plants (16 pots per species) had a 24 cm diameter and contained a mixture of 25 % sand, 25 % peat, 50 % loam. In each pot the number of shoots was limited to three : three plants in the case of maize, or one plant trimmed to its main shoot and two tillers in the case of Z.D. Other tillers were removed as soon as they became visible.

B. Water stress treatments

Watering was withheld on half the containers and pots as indicated in table 1. The other half was used as control. We tried to maintain soil water content of controls at 80-90 % field capacity (f.c.). Field capacity was considered to correspond to pF = 2.5, and permanent wilting point to pF = 4.2. From curves of pF as a function of θ_w, field capacity in containers appeared to correspond to θ_w = 8.7 % and permanent wilting point to θ_w = 5.7 %.

C. Environmental and physiological measurements

The water content of the soil in the containers was determined gravimetrically on 3 samples. No measurements of water content were made on the soil in pots. Leaf diffusive conductance (G) was measured using a diffusive porometer (Delta T, Cambridge). Total conductance was obtained summing G on adaxial and abaxial surface. In the case of Z.D. measurements were soon limited to the abaxial surface since G on adaxial surface was found to be negligible, as in a previous study (GOLBERG & RENARD, unpublished data). Leaf water potential (ψ_l) was determined on leaf parts 5 cm long using a membrane press (J-14 Press, Crump, Raleigh). With a hydraulic pump, a pressure is applied on a flexible membrane, pushing the leaf material against the translucid wall of a chamber. The pressure applied when sap flows out of the leaf and change in the leaf coloration occurs is recorded. A close relationship exists between the results obtained with this method and the measurements made with a pressure bomb (RENARD & NDAYISHIMIE, 1982).

CO_2 exchange rates (CER) were measured using a slightly modified version of a rapid CO_2 pulse method described by SHIMSHI (1969).

Light was provided by 4 mercury vapour lamps of 400 W each. A transparent tank through which water was flowing was placed as a screen between lights and plants. Air containing 385 µl l^{-1} CO_2 + $^{14}CO_2$ with a specific activity of 61 406 dpm/ml was passed through a miniature assimilation chamber (8 mm diameter). Clamping the chamber on the leaf for 20 s released an air flow (speed 3.9 cm s^{-1}) on both sides of the enclosed leaf part. Air temperature was 20 °C, and RH 50 %.

Immediately afterwards the treated leaf part was punched out and subjected to chemical digestion with Soluene before being processed for counting in a Packard Tricarb liquid scintillation counter. We are aware of conflicting results in the literature concerning comparison of CER measured by this method and with the infra red gas analyzer (IRGA) : see for instance VAN DIJCK & LEDENT, 1984 ; KEMP & BLACKLOW, 1984. The

method is simple and convenient (portable equipment, rapidity) and it seems sufficient when no absolute values of CER are needed.

With a few exceptions, physiological measurements were taken at least daily after cessation of watering. Measurements of the different parameters (table 1) were made successively on the same leaves. G were measured at different hours per day but since the same trend through days was observed only daily averages will be discussed. For measurements of photosynthesis plants in pots were removed from the growth chamber at 9 a.m. and brought to the laboratory. They were placed 30 minutes under a PPFD (photosynthetic photon flux density) of 500 μmol m^{-2} s^{-1} then PPFD was increased to 1 200 μmol m^{-2} s^{-1} and measurements started 30 minutes later. After measurement of photosynthesis, plants in pots were discarded.

Leaf area of all leaf laminae was measured at the end of the experiment with an electronic leaf area meter (AAM-7, from Hayashi Denko). Dry weights of different plant parts (laminae, stems,...) were determined after drying at 80 °C for 48 h.

III. RESULTS

A. Soil water content

Soil water content, θ_w (percentage on dry weight basis) of controls in containers showed erratic variations between 75 % and 120 % f.c. When water was witheld θ_w decreased very regularly in a linear fashion, to reach 50-60 % of f.c. just before watering was resumed. The decrease was faster in the case of Z.D. (fig. 1). A θ_w value of 5.6 % was reached 8 DCW (Days after Cessation of Watering) in the case of Z.D. whereas in maize it took 12-13 DCW to reach that value. Thus transpiration by Z.D. was higher than for maize. This result may be explained by the higher leaf area of Z.D.
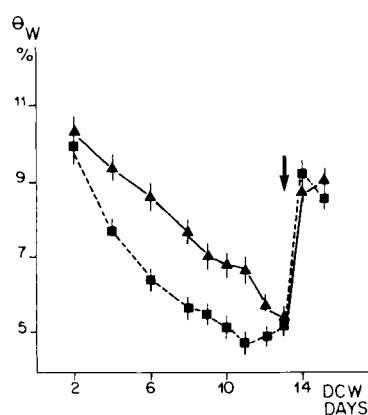

Figure 1

Soil water content, θ_w (% on a dry weight basis) in containers with Zea diploperennis (Z.D.) ■, or maize ▲, with time (in days after cessation of watering DW).
Vertical lines correspond to $s_{\bar{x}}$ of each mean presented. The arrow indicate resumption of watering.

Teneur en eau du sol, θ_w (p. 100 sur la base du poids sec) dans les récipients contenant Zea diploperennis (Z.D.) ■, ou le maïs ▲, en fonction du temps (en jours, après l'arrêt des arrosages, DCW).
Les lignes verticales correspondent à $s_{\bar{x}}$ (écart-type de la moyenne). La flèche indique la reprise des arrosages.

(see below) and/or a higher transpiration rate per unit leaf area. No measurements of soil water content were made on plants growing in pots.

B. Leaf water potential

In containers ψ_l of controls remained around – 0.2 MPa at all times. Cessation of watering had no effect on ψ_l, ψ_s or ψ_p (pressure potential) (data not presented) during an initial period (IP) of 6 DCW. The following days a drop of ψ_l was observed and values of 1.2 and – 1.4 MPa (Z.D. and maize respectively) were finally reached. Plots of ψ_l versus θ_w are presented in figure 2. The association of ψ_l with θ_w was looser in the case of Z.D. (r = 0.89** for maize and r = 0.65 N.S., p $\leq$ 0.05 for Z.D. ; corresponding slopes were 0.15 N.S. and 0.25**). Fitting the equations $\ln(-\psi_l) = a + b\theta_w$ gave values of b = – 0.54 and – 0.26, a = 3.30 and 0.93, r = – 0.95** and 0.61 N.S., for maize and Z.D. respectively. In plants grown in pots stressed maize showed significantly lower ψ_l than Z.D. starting 3 days after cessation of watering.
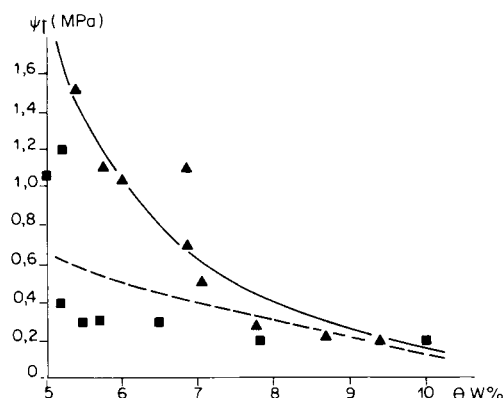

Figure 2

Leaf water potential ψ_l (MPa) in relation to soil water content θ_w (%) ; experiment in containers. Values of ψ_l calculated from the model $\ln (-\psi_l) = a + b\theta_w$ are also presented.

Potentiel hydrique foliaire ψ_l (Mega Pascals, MPa) en fonction du contenu en eau du sol θ_w (p. 100) ; expérience en conteneurs. Les valeurs de ψ_l prédites à partir de ln ($-\psi_l$) = a + bθ_w sont également indiquées.
■ : Zea diploperennis - - - - - ▲ : Zea mays ——————

C. Leaf diffusive conductance

In containers, leaf diffusive conductance (G, fig. 3) of plants deprived of water remained relatively constant during the initial period (IP) of 6 DCW for both species. Differences between maize and Z.D. appeared afterwards : after a small decrease at day 6 (DCW) G of Z.D. remained remarkably constant (around 0.12 cm s^{-1}) whereas in maize a continuous decrease to 0.05 cm s^{-1} was recorded. Only on day 13, a decrease of G (to values of 0.05 cm s^{-1}) was observed for Z.D., i.e. one week later than for maize. Thus, despite lower soil water contents, Z.D. maintained higher G than maize.

Only a sample of control values for G are presented in figure 3 to improve readability. G values started from similar values and showed the same trend with time in

both species. An increase of G with time, associated with the reproductive stage, has been reported in other species such as millet (HANSON *et al.*, 1983). In maize plants growing in pots complete stomatal closure (G ≃ 0) was observed at the 4th DCW. In Z.D. on the contrary G remained little affected until the 6th day, when a drop was recorded, but even at that stage stomata were not completely closed (G ≠ 0). Plots of G versus ψ_l for the period starting after cessation of watering are presented in figure 4 (plants in containers). Linear regression fitted to the data in figure 4 gave a higher slope in the case of maize   (b = 0.17** cm s⁻¹ MPa   for   maize   and b = 0.09** for Z.D.). Moreover the relationship was looser in the case of Z.D. : corresponding coefficients of correlation were indeed r = 0.89** and 0.59** (for maize and Z.D. respectively). Comparable results were obtained with plants growing in pots : b = 0.18** and 0.12 N.S. ; r = 0.90** and 0.57 N.S. (maize and Z.D. respectively). Thus a decrease in ψ_l had less effect on G in Z.D. than in maize.

D. Recovery

When watering was resumed, recovery was very fast for ψ_l and its components : for both species return to control values took less than 2 days. Recovery was also fast for G values in Z.D. but not in maize (fig. 3).

E. CO₂ exchange rate

CER of maize decreased rapidly after cessation of watering (plants in pots). It reached nil values the 4th day after the onset of treatment. In Z.D. the decrease was more gradual and the last day of measurement CER still reached 50 % of the rate observed in the controls.

In maize where stomatal conductance (G) was drastically affected by cessation of watering a relatively close association existed between CER and G (r = 0.81**, d.f. 13).
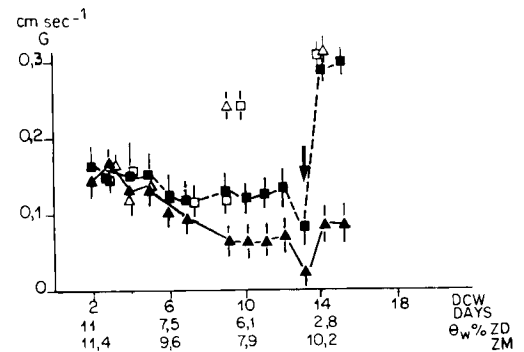

Figure 3

Leaf diffusive conductance G (cm s⁻¹) with time (DCW) ; experiment in containers. Corresponding values of θ_w for Z.D. (Zea diploperennis) and Z.M. (Zea mays) are also given in abscissa.

Conductance stomatique G (cm s⁻¹) en fonction du temps (DCW) : expérience en containers. Les valeurs correspondantes de la teneur en eau du sol θ_w sont également indiquées en abscisse.

Z.D. (Zea diploperennis)		Maize, maïs (Z.M.)	
Control	Stressed	Control	Stressed
Témoin	Stressé	Témoin	Stressé
▫	■	▲	△

Vertical lines correspond to $s_\bar{x}$.
Les lignes verticales correspondent à $s_\bar{x}$.

In Z.D. where stomatal opening was less affected the corresponding correlation was not significant (r = 0.26 N.S. ; d.f. 28). Correlations between CER and ψ_l gave similar results (r = 0.82**, and 0.26 N.S. for maize and Z.D. respectively).

Plots of CER as a function of G or ψ_l showed a great dispersion of CER values at some G or ψ_l values. Heterogeneity within the leaf (small assimilation chamber : 8 mm diameter) and sampling of leaves differing in positions and therefore age and microclimate might explain partly this result. To homogenize the results CER values obtained for second uppermost leaves were discarded if they differed more than 30 % from the value obtained for uppermost leaves, moreover we
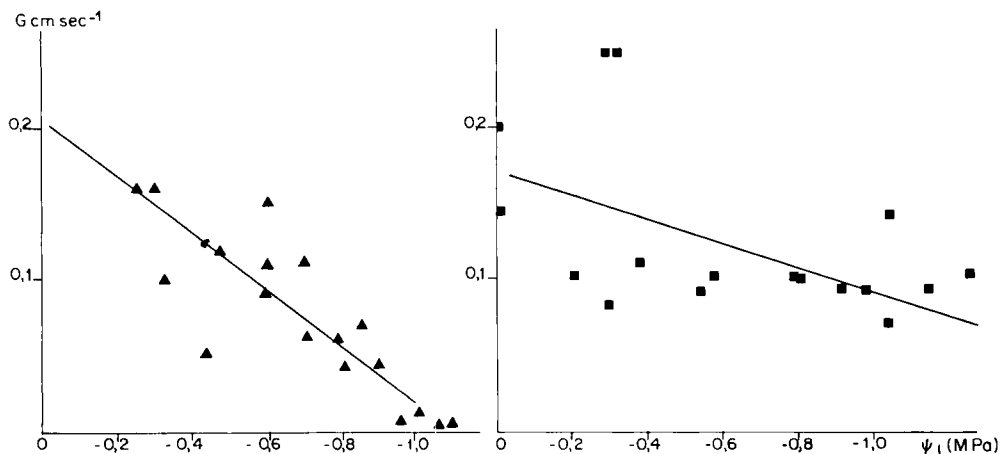

Figure 4

Leaf diffusive conductance G (cm s⁻¹) in relation to leaf water potential (ψ_l), plants in containers. Regression equations are G = 0.17 ψ_l + 0.20 for Zea diploperennis and G = 0.9 ψ_l + 0.17 for maize.

Conductance stomatique G (cm s⁻¹) en fonction du potentiel hydrique foliaire (ψ_l), plantes en conteneurs. Les équations de régression sont G = 0,17 ψ_l + 0,20 pour Zea diploperennis et G = 0,09 ψ_l + 0,17 pour le maïs.
■ : Zea diploperennis - - - - -   ▲ : Zea mays ———

discarded data from pots where a difference of more than 30 % was observed between the shoots (tillers or plants within the same pot). The screened data are presented in figure 5 (relationship of CER vs ψ_l) and figure 6 (relationship of CER with G). Correlations calculated on screened data were 0.93** (maize) or 0.31 N.S. (Z.D.) for the relation CER, G, and 0.78** (maize) or 0.49* (Z.D.) for the relation CER, ψ_l. They lead to the same conclusions as those reached from the correlations on rough data.
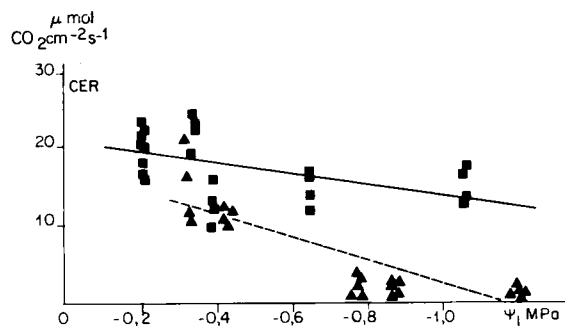

Figure 5

Relationship between CER and ψ_l (screened data). CER was measured at 20 °C, under a 1 200 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux density.

Relation entre CER et ψ_l (données criblées). CER a été mesuré à 20 °C sous une densité de flux de photons de 1 200 $\mu moles\ m^{-2}\ s^{-1}$.
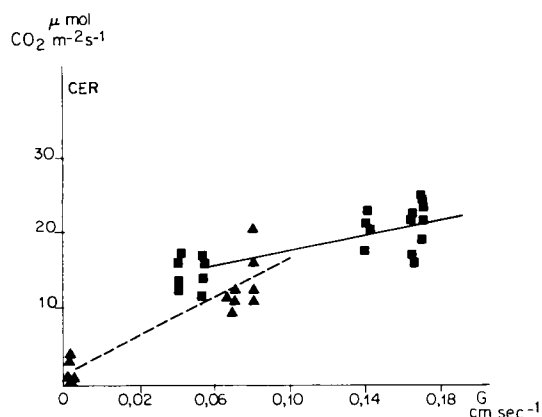
■ : Zea diploperennis   ▲ : Zea mays

Figure 6

Relationship between CER and G (screened data). See explanation and symbols in figure 5.

Relation entre CER et G (données criblées). Voir les explications et les symboles à la figure 5.

Figure 5 indicates that CER of maize was completely inhibited when ψ_l was about – 1 MPa (– 10 bar).

Similar (BEADLE et al., 1973) but also lower values (– 2 MPa, MCPHERSON & BOYER, 1977) are found in the literature hence these numbers should not be taken as applying generally.

CER of Z.D. was less affected since at ψ_l values of – 1 MPa it still reached about 50 % of the control value. The same result is obtained with G.

F. Leaf area and dry matter

Drought reduced total shoot dry weight by 42 % in maize and by 22 % in Z.D. (plants growing in contai-

ner, table 2). The interaction treatment × species was highly significant. Therefore dry matter was more reduced by drought in maize than in Z.D., a result found also for stomatal conductance. Leaf area was also more reduced in maize (29 % in maize, versus 23 % in Z.D.). The higher leaf area values for Z.D. were associated with its tillering capacity (3-6 tillers per plant in containers and pots respectively) and occurred despite a smaller leaf area per shoot (a mean surface area per leaf lamina of 2.1 dm² (maize) and 0.6 dm² (Z.D.) was observed on plants in pots. Number of tillers in Z.D. was reduced 14-20 % by drought.

TABLE 2

Effect of water stress on leaf area and total dry weight (experiment in containers).

Effet d'une contrainte hydrique sur la surface foliaire et le poids sec total (expérience en conteneur).

| | Zea mays | | Zea diploperennis | | LSD (ppds) |
	Control	Stressed	Control	Stressed	p ≤ 0.05
Leaf area/plant (dm²) Surface foliaire/plante (dm²)	17.1	12.2	22.5	17.3	6.87
Total shoot dry weight (g) Poids sec des parties aériennes (g)	21.5	12.5	20.8	16.6	2.10
Nb tillers/plant Nombre de talles par plante	0	0	3.6	3.1	0.42

Dead plant material was carefully harvested separately. The very low amounts obtained indicate that the drought treatments had little effect on tissue death in these experiments.

IV. CONCLUSIONS

Higher leaf diffusive conductances (G) were maintained in Z.D. than in maize, when watering was withheld. This result was observed although, during the drought period, soil in containers with Z.D. reached lower soil water content (θ_w) than in containers with maize. In fact the lower soil water content obtained with Z.D. appears to be the result of a higher transpiration of Z.D., in relation with higher G and larger leaf area. Higher G in Z.D., despite higher loss of water, indicates a better maintenance of turgor in this species. Whether this may be attributed to differences in elasticity of cell walls or to other mechanisms such as osmotic adjustments etc... remains an open question.

The maintenance of a higher transpiration rate when water becomes limited is not, per se, an advantage.

Maintenance of photosynthetic rates is. Higher CER were observed in Z.D. during the stress period.

The final dry weights obtained were consistent with the lesser reduction of CO_2 exchange rate (CER) in Z.D. plants subjected to water shortage. Maintenance of higher G was favourable to CER and dry matter production.

The disadvantage of maize, as far as CER is concerned, seems to be associated with greater effect of water shortage on G, affecting growth through a close association of CER with G.

Such a close association is not observed in Z.D., but this is probably because of its higher G values, corresponding to the part of the response curve of CER to G which tends to plateau.

Reduction of photosynthesis at low water potentials is known to be due to stomatal closure, but also to other factors related to chloroplast activity and structure (VIEIRA DA SILVA, 1976 ; FARQUHAR & SHARKEY, 1982 ; KRIEG & HUTMACHER, 1986).

Measurements of fluorescence induction seemed to indicate, however, that the primary photochemistry of photosystem II was not affected and was not a cause for the reduction of CER in these experiments (GOLBERG et al., 1986).

Under our conditions and with the material we used Z.D. (Zea diploperennis) appeared to be more drought-tolerant than maize : leaf diffusive conductance was better maintained and less reduction in dry matter occurred. Field-grown plants of different age might however differ markedly from plants grown indoors in pots or containers because of root systems, stomatal behaviour, etc... Our results should therefore not be extrapolated. It can be concluded, however, that the species appears to present some interest and it seems worthwhile to continue the investigations with interspecific crosses and field trials.

REFERENCES

Algans J. L., Desvignes P., 1984. Action d'un déficit thermique et d'un déficit hydrique simultanés sur le rendement du maïs et ses composantes, 477-482. In Gallais A. : « Physiologie du maïs ». Communication au Colloque Physiologie du maïs. Royan 15-17 mars 1983. I.N.R.A., Paris.

Beadle C. L., Stevenson K. R., Neumann H. H., Thurtell G. W., King K. M., 1973. Diffusive resistance, transpiration and photosynthesis in single leaves of corn and sorghum in relation to leaf water potential. Can. J. Plant Sci., 53, 537-544.

Crosbie T. M., Pearce R. B., Mock J. J., 1981. Recurrent phenotypic selection for high and low photosynthesis in two maize populations. Crop Sci., 21, 736-740.

Farquhar G. D., Sharkey T. D., 1982. Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol., 33, 317-345.

Galinat W. C., 1980 ; Diploperennis, a premaize teosinte of value in corn breeding. Maize Genet. Coop. Newsl., 54, 120.

Gay J. P., 1984. Fabuleux maïs, histoire et avenir d'une plante. Ed. « Association générale des producteurs de maïs ». France. 295 p.

Golberg A. D., Ledent J. F., Lannoye R., 1986. Fluorescence induction and water stress in maize and Zea diploperennis. Arch. int. Physiol. Biochim., 94, pp. 48-00.

Hanson I. E., Mahlakshmi V., Alagarswamy G., Bidinger F. R., 1983. An association between flowering and reduced stomatal sensitivity to water stress in pearl millet (Pennisetum americanum (L.) Leeke). Ann. Bot., 52, 641-648.

Iltis H. H., Doebley J. F., Guzman M. R., Pazy B., 1979. Zea diploperennis (Gramineae) : a new teosinte from Mexico. Science, 203, 186-188.

Kemp D. R., Blacklow W. M., 1984. Estimates of leaf photosynthesis with gas flow and times of feeding and sampling when measured by $^{14}CO_2$ in small unventilated cuvettes. Photosynthetica, 18, 344-349.

Krieg D. R., Hutmacher R. B., 1986. Photosynthetic rate control in sorghum (Sorghum bicolor) : stomatal and nonstomatal factors. Crop Sci., 26, 112-117.

Morizet J., Pollacsek M., Togola D., 1984. Tolérance à la sécheresse de 4 variétés de maïs. Essai de mise en évidence des mécanismes impliqués, 493-501. In Gallais A. : « Physiologie du maïs ». Communication au Colloque Physiologie du maïs. Royan 15-17 mars 1983. I.N.R.A., Paris.

McPherson H. G., Boyer J. S., 1977. Regulation of grain yield by photosynthesis in maize subjected to a water deficiency. Agron. J., 69, 714-718.

Morot-Gaudry J. F., Farineau J., Rocher J. P., Jolivet E., 1981. Modifications dans les premières étapes de l'assimilation photosynthétique du carbone chez le maïs sous l'effet du génotype. Agronomie, 1, 739-744.

Renard C., Ndayishimie V., 1982. Etude des relations hydriques chez Coffea arabica L. : comparaison de la presse à membrane et de la chambre à pression pour la mesure du potentiel hydrique foliaire (ψ). Café Cacao Thé, XXVI, 27-30.

Shimshi D., 1969. A rapid field method for measuring photosynthesis with labelled carbon dioxide. J. Exp. Bot., 20, 381-401.

Van Dijck E., Ledent J. F., 1984. Comparison of photosynthetic rates measured with IRGA and a rapid $^{14}CO_2$ pulse method. Arch. int. Physiol. Biochim., 92 (5), PF 32.

Vieira da Silva J., 1976. Water ultrastructure and enzymatic activity, 207-224. In Lange O. L., Kappen L., Schulze E. D. : Ecological studies analysis and synthesis. Vol. 19 : Water and Plant Life. Springer-Verlag, Berlin, Heidelberg.