Short communication

Leaf gas exchange and fluorescence of two teosinte species: *Zea mays* ssp. *parviglumis* and *Z. diploperennis*

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Abstract

This study compared the leaf blade characteristics, pigment composition, photosystem II (PSII) photochemistry, and photosynthetic rates (P) of a multi-stemmed annual (*Zea mays* ssp. *parviglumis* Iltis & Doebley) and a perennial (*Z. diploperennis* Iltis, Doebley & Guzman) teosinte species growing in a glasshouse under analogous nutritional and environmental conditions. Similar chlorophyll contents and ratios implied comparable efficiencies of photon energy capture and electron flow through PSII in both species. Maximum photochemical efficiency (*Fv/Fm*) was about 0.75 at predawn and declined linearly to a minimum at midday of about 0.65, implying down-regulation of photosynthesis. By contrast, maximum *P* (*Pmax*) was 11.5% higher in the annual compared to the perennial teosinte, signifying that wild maize germplasm could potentially be used for improving the photosynthetic efficiency of cultivated maize. Future studies, however, should assess a larger number of teosinte species for their differences in leaf area per plant, growth habit, grain yield, and leaf photosynthesis.

Keywords: Chlorophylls, Down-regulation, Photochemical efficiency, Photosynthesis, Wild maize.

The genus *Zea* includes a group of annual and perennial wild relatives of corn (known as teosinte, *Zea* ssp) besides the domesticated corn (*Zea mays* ssp *mays* L.), native to Mexico and Central America (Buckler and Holtsford, 1966; Doebley, 2004). There are five *Zea* species and four subspecies of *Z. mays*. Contrasting the plant architecture of cultivated corn with that of its closest wild relatives (teosinte), reveals the phenotypic evolution of the domesticated corn with branch internode contraction and highly modified inflorescence (Iltis, 1983). This selection process seems enormously complex and involves a multiplicity of genes that control tillering, kernel size, composition, palatability, and leaf size (Doebley, 2004). Teosinte and cultivated maize hybridize in nature as well as artificially, a fact that facilitates genetic improvement of this crop (Ellstrand et al., 2007). Preservation and assessment of genetic material of wild relatives is, therefore, important for genetic improvement of this crop (Evans, 1977). Changing land use, however, has reduced and fragmented the teosinte habitats, in turn reducing the genetic diversity of the wild relatives of corn (Wilkes, 2007). We selected two teosintes with contrasting growth habits: the multi-stemmed annual (*Z. mays* ssp. *parviglumis* Iltis & Doebley), the closest relative of cultivated corn and a perennial teosinte (*Z. diploperennis* Iltis, Doebley & Guzman), which is less related to corn (Buckler and Holtsford 1996). We hypothesized that given their contrasting growth habits, plant carbon gain and photosynthetic efficiency may be different. Our objective was to compare the leaf blade characteristics, PS II photochemistry, and photosynthesis in both teosintes growing under similar nutritional and environmental conditions. This information would provide insights for cultivated corn improvement, and would also encourage research on teosinte genotypes.

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Zea mays ssp. *parviglumis* was collected from El Salado (Mazatlan, Sinaloa, 1,300 m elevation, 17º25'N, 99º30'W), and *Z. diploperennis* from the flat valley bottoms at Las Joyas (El Chante, Jalisco, 1,900 m elevation, 19º35'N, 104º17 W). Both the annual and the perennial species were grown in a glasshouse (5 L pots filled with a well fertilized potting mix: 50% organic soil and 25% each of sand and clay) for 50 days (June-July 2008) at the Simon Bolivar University (Caracas, Venezuela). The glasshouse had natural sunlight and photoperiod (12 h) during the experimental period. There were a total of 24 pots with one plant per pot (i.e., 12 plants per species). The plants remained vegetative during the experiment, and measurements were made under natural irradiance conditions. Leaf gas exchange and fluorescence measurements were made on the two most recent and fully expanded leaves of each plant using a portable open gas analyzer system (Model LCA-2, Analytical Development Co., Hoddesdon, Herts, UK) inside the glasshouse (photosynthetic photon flux density, PPFD: 100 to 2000 μmol m^{-2} s^{-1} and ambient CO_2 concentration (*Ca*): 360 μmol mol^{-1}). Chlorophyll *a* fluorescence (initial, *Fo*; maximum, *Fm*; and variable, *Fv*) was measured simultaneously in dark adapted leaves for 30 min using a chlorophyll fluorometer (Model OS-30p, Opti-Sciences, Hudson, USA), provided with white leaf clips to avoid overheating of the leaf tissue. The gas exchange and fluorescence measurements were carried out on consecutive days until the full range of PPFD measurements was completed. Afterwards, the sample leaves used for measurements from each plant were harvested separately and subdivided into two sub-samples per plant, after excluding the mid-vein. One of the subsamples (0.01 m^2) was used to measure leaf area and dry mass after being oven-dried at 60ºC until constant weights, from which the leaf dry mass to area ratio (*Sw*) was computed. Three pooled samples (species-wise) were ground for nitrogen (N) analysis by Kjeldahl method. The second subsample of about 0.003 m^2 was frozen to determine chlorophylls *a* and *b* (*Chl a*, *Chl b*) and its ratio (*Chl a/b*) following the procedures of Lichtenthaler and Wellburn (1983). The data were analyzed for normal distribution and equality of variance, to enable species comparison. When the data were normal, a *t*-test comparison was made,

whereas a Mann-Whitney Rank Sum Test was used when the normality test failed ($p \leq 0.05$). All the analyses were performed using Sigma Stats 3.1 and SPSS 10.0 software for Windows.

Leaf blades of annual and perennial teosinte showed comparable values of *Sw*, *N*, and *Chl a*, *Chl b*, *Chl a+b*, and *Chl a/b* (Table 1). Similar *Chl a/b* in both species suggests comparable efficiencies in photon capture and electron flow through PS II. *Fm* and *Fv* declined significantly ($p \leq 0.01$) from predawn to midday in both species; however, *Fo* remained unchanged (Table 1). As irradiance increased, the progression of *Fv/Fm* tended to decline linearly in both annual and the perennial teosinte (Fig. 1). Efficiency and stability of PS II can be ascertained by comparing the diurnal changes in *Fv/Fm* ratios; typical values for mature healthy tissue range from 0.74 to 0.85 (Roháèek 2002). Midday depression in *Fv/Fm* is the result of reversible or irreversible photo-inhibition (Demming-Adams and Adams, 1992). Reversible photoinhibition represents an acclimatory down-regulation without PS II damage. Midday reduction of *Fv/Fm* as a consequence of changes in *Fm* and *Fv* without significant changes in *Fo* indicates reduction of photochemical efficiency due to photo-protective mechanisms (Kitajima and Butler, 1975). The xanthophyll cycle-dependent non-radiative energy dissipation may play a central role in leaf photo-protection as a high energy exchange in non-photochemical quenching (NPQ; Demming-Adams and Adams, 1992). In both teosinte species, the *Fv/Fm* recovered overnight, which further suggested the reversibility of photo-inhibition (data not shown). The photosynthetic para-meters in corn races are sensitive to temperature and irradiance (Duncan and Hesketh, 1968). In this study, plants were grown in a tropical environment experienced high temperatures and irradiances. Thus, air and leaf temperature raised progressively from 29.5 ± 0.3 and 27.0 ± 0.2ºC at low PPFD up to 32.0 ± 0.7 and 31.7± 0.6ºC at midday, respectively. These conditions may favor the interception of more photons than the leaf can use in carbon assimilation, consequently triggering the photo-protection mechanism.
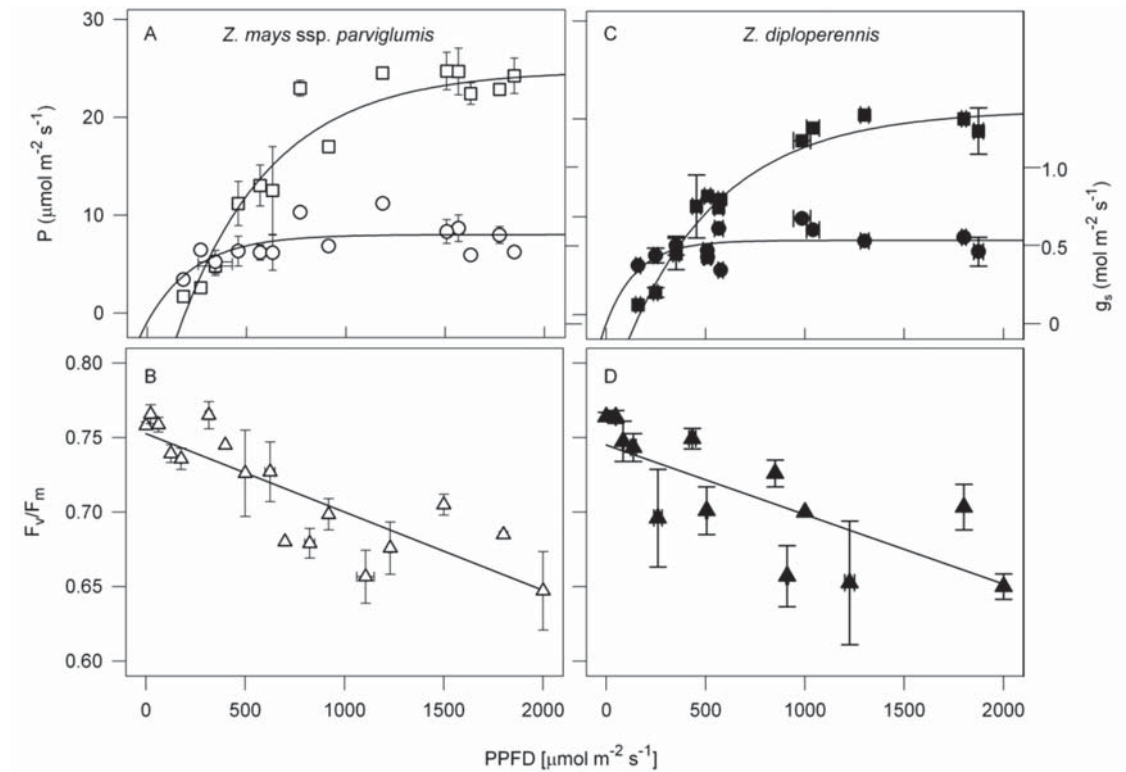
Net photosynthesis (*P*) increased with PPFD in both

Figure 1. A,C: Net photosynthesis (*P*; squares) and stomatal conductance (g_s; circles); B,D: ratios of variable to maximum fluorescence (F_v/F_m) as a function of photosynthetic photon flux density (PPFD) in annual (*Z. mays* ssp. *parviglumis*: A,B; open symbols) and perennial teosinte (*Z. diploperennis*: C,D; closed symbols). Values are mean ± standard error of measurements taken in 12 plants per species. Linear regressions for F_v/F_m as a function of irradiance were statistically significant at $p \leq 0.01$ in both species.

Table 1. Leaf characteristics measured in annual (*Zea mays* ssp. *parviglumis*) and perennial teosinte (*Z. diploperennis*).

Parameter	*Z. mays* ssp. *parviglumis*	*Z. diploperennis*
S_w (g m^{-2})	57.8 (1.5)	55.8 (2.9)
N (g kg^{-1})	38 (2)	36 (2)
Chl_a (mg m^{-2})	353 (14)	332 (17)
Chl_b (mg m^{-2})	84 (6)	79 (4)
Chl_{a+b} (mg m^{-2})	437 (19)	411 (21)
$Chl_{a/b}$	4.3 (0.2)	4.2 (0.1)
P_{max} (mol m^{-2} s^{-1})	22.9 (0.9)*	20.4 (1.3)
Φ (mol CO$_2$ mol^{-1} quanta)	0.012 (0.001)	0.014 (0.001)
PD F_o	64 (2)	62 (3)
PD F_m	266 (10)	265 (12)
PD F_v	202 (7)	203 (10)
MD F_o	56 (6)	58 (8)
MD F_m	170 (13)	174 (11)
MD F_v	114 (12)	116 (9)

Leaf mass to leaf area ratio (S_w), nitrogen (*N*), Chlorophylls (*Chl*) *a*, *b* and their ratio, light saturated photosynthesis (P_{max}), photosynthetic quantum yield (Φ) and predawn (*PD*) and midday (*MD*) fluorescence parameters (initial, F_o; maximum, F_m; and variable, F_v). Values are mean (*SE*) of measurements taken in twelve plants per species. Significant differences at $p \leq 0.05$ are indicated by asterisk (*).

species but the annual species (*Z. mays* ssp. *parviglumis*) had higher values than *Z. diploperennis* (Table 1; Fig. 1). Saturation PPFD for *P* in both teosinte species was about 1,500 μmol m^{-2} s^{-1}. Differences in *P* of about 11% could not be attributed to contrasting stomatal conductance (g_s) and/or apparent quantum yields, which were similar for both species (Table 1; Fig. 1). Nonetheless, the possibility of differential carboxilation capacity or ribulose-1,5-biphosphate regeneration in the chloroplast of the bundle sheath cells could not be precluded. *Pmax* in both species were also below those in cultivated maize under temperate and tropical conditions (Sobrado, 1990). Although differences in *Pmax* between the two multi-stemmed teosintes were modest, it may impact the whole plant carbon gain and grain yield. These results suggest that wild maize can provide a germplasm base for improving the photosynthetic efficiency of cultivated maize. Future studies should, therefore, focus on a larger number of the reported teosinte races (Buckler and Holtsford, 1966; Doebley, 2004), and its differential leaf area, growth duration and habit, grain yield, and photosynthentic potential.

Acknowledgements

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