Effects of drought on photosynthetic performance and water relations of four *Vigna* genotypes

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Abstract

The effect of drought on plant water relations and photosynthesis of *Vigna glabrescens* (Vg) and *Vigna unguiculata* (cvs. 1183, EPACE-1 and Lagoa), which differ in their drought resistance, was compared. With the increase of drought severity, Vg showed a more gradual stomatal closure and maintained significantly higher levels of stomatal conductance (g_s) and photosynthetic activity (P_N) than the other genotypes even when minimum relative water content (RWC) values were observed. Furthermore, Vg was the only genotype able to accumulate significant amounts of proline already under moderate water deficit, what could explain the lower osmotic potential (ψ_s) values observed in these plants. The three *V. unguiculata* cultivars presented a similar stomatal control under increasing water deficit. A mesophyllic impairment of photosynthetic capacity (P_{max}) was detected for cv. 1183 from the beginning of drought onset (85-75 % RWC) while in the Vg plants the values remained unaffected along the whole drought period, indicating that P_N decrease observed in this genotype is mainly a consequence of stomatal closure. Such P_{max} maintenance suggests the existence of a high mesophyllic ability to cope with increasing tissue dehydration in Vg.

Additional key words: drought; gas exchanges; water relations.

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Abbreviations: g_s - stomatal conductance to water vapour; P_{max} - photosynthetic capacity; P_N - net photosynthetic rate; PPFD - photosynthetic photon flux density; RWC - relative water content; ψ_p - pressure potential; ψ_s - osmotic potential; ψ_w - water potential.
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Introduction

Drought frequently causes rapid stomata closure, with the reduction of water loss through transpiration, the decrease of internal CO_2 concentration, and the decline in leaf photosynthetic rate. Concomitantly, inhibition or damages in the primary photochemical and biochemical processes may occur (Björkman and Powles 1984, Kaiser 1987, Lawlor 1995). Since P_{max} reflects the result of those mesophyllic impairments, its determination allows to evaluate the non-stomatal limitations of photosynthesis and hence the degree of drought tolerance of the photosynthetic machinery.

Osmotic adjustment is one of the most important adaptive mechanisms to dehydration in many crops. Osmotic adjustment could be advantageous at transient shortage of water due to highly variable rainfall, whereas a sensitive stomatal response could be more successful in regions where crop growth depends entirely on a small amount of stored water (Wright *et al.* 1983).

The aim of this work was to analyse the effects of drought on photosynthesis and water relations in *V. glabrescens* and three cultivars of *V. unguiculata* (1183, EPACE-1, and Lagoa), which were previously selected in the field for their different degrees of drought resistance. The search for specific characters conferring drought tolerance is important in breeding programs of protein-rich crops, such as *Vigna*.

Materials and methods

Plants: *Vigna glabrescens* (Vg, drought tolerant) and *V. unguiculata* (cv. 1183, drought sensitive; cv. EPACE-1, drought tolerant; cv. Lagoa, not tested) were used. Vg is a wild species originally from the Philippines. Cv. 1183 is cultivated in China, cv. EPACE-1 was obtained in Brazil, and cv. Lagoa is a highly productive cultivar originally from Portugal. After germination, plants were grown in pots, in a mixture of vermiculite:*Triohum-Tray* substrat (4:5) and were irrigated with modified (two-fold micronutrients) Hoagland and Snyder (1933) solution, twice a week. For each experiment 15 pots of each cultivar were placed in a semi-controlled greenhouse, under natural irradiance (PPFD up to 800-900 μmol m^{-2} s^{-1}), daily temperatures 25-35 °C, and relative humidity between 70 (morning) and 40 % (late afternoon). Dehydration was progressively induced in six weeks-old plants by withholding irrigation for 10 to 12 d. The measurements described below were made on mature leaves.

Water relations: RWC was calculated according to Čatský (1960) in samples of 10 foliar discs of 0.5 cm^2 each, as RWC = [(FM-DM)/(TM-DM)]×100, where FM is the fresh mass of the discs, TM is the mass after overnight rehydration of the discs in a humid chamber at room temperature, and DM is the mass after drying at 80 °C for 24 h. Leaf water potential (Ψ_w) was determined on the petiole of the central leaflet immediately after excision from the plant, using a pressure chamber (Scholander *et al.* 1965). To obtain cell sap, three leaf discs of 0.5 cm^2 per sample were homogenized in Eppendorf tubes. The samples were allowed to equilibrate for 20 min, and the osmotic potential of the cell sap (Ψ_s) was measured using a dew point hygrometer (*HR-33 T*, *Wescor*, USA) equipped with *C-52* leaf chambers. Ψ_w and Ψ_s were assumed to be a measure of bulk leaf water potential and bulk leaf osmotic potential, respectively, and the difference between Ψ_w and Ψ_s was calculated as an estimate of pressure potential (Ψ_p), according to Schackel and Hall (1983). The fitted lines for Ψ_w/RWC were obtained for RWC >70 % and < 80 %. Points between 70 and 80 % RWC were considered to represent an intermediate transition level and were included in both fittings.

Net photosynthetic rate and stomatal conductance: P_N and g_s were measured at a PPFD of 1300-1600 μmol m^{-2} s^{-1} in three attached leaves from different plants, using a portable photosynthetic system *LI-6200* (*LI-COR*, Nebraska, USA). These measurements were done between 10:00 and 11:00 h. P_N and g_s were calculated according to the equations of Caemmerer and Farquhar (1981).

Photosynthetic capacity measurements: For P_{max} measurements, leaf discs were submitted to saturating CO_2 (6–8 %) and irradiance (1700 μmol m^{-2} s^{-1}), using an oxygen electrode *LD2/2* (*Hansatech*, Kings Lynn, U.K.), which enabled to study the non-stomatal limitation of photosynthesis under water stress (Chaves 1991).

Proline and soluble sugars′ contents: Proline content was measured according to Bates *et al.* (1973), using 8 foliar discs of 0.5 cm^2 extracted in 2.5 cm^3 methanol. Soluble sugars were quantified according to Ashwell (1957).

Statistical analysis was made using a two-way ANOVA, applied to the various measured and calculated parameters, followed by a Tukey test for mean comparison between genotypes or degrees of dehydration (for a 95 % confidence level).

Results

Plant water status and water relations: The four genotypes followed a similar general pattern of dehydration with two well-defined phases (Fig. 1). However, in the first phase, corresponding to Ψ_w between -0.3 and -1.5 MPa, the Ψ_w decline in Vg was accompanied by smaller RWC decreases than in the *V. unguiculata* cultivars. In the second phase (Ψ_w between -1.5 and -2.4 MPa), Vg reached lower Ψ_w than the remaining genotypes (Fig. 1).

Under increasing water stress, a significant decline in Ψ_s values was observed in all the genotypes already for S1 conditions (RWC 75-65 %); these values remained stable under severe water deficit, S2 (RWC <60 %) (Table 1). Vg showed larger decreases (*ca.* 63 %) of Ψ_s than the three *V. unguiculata* cultivars.

Cv. Lagoa plants under S1 showed higher Ψ_p values than the remaining genotypes, and were the only ones that presented non-significant decreases in relation to control (Table 1). Under S2, Lagoa and Vg still showed positive Ψ_p values, contrary to cvs. EPACE-1 and 1183 (Table 1).

Net photosynthesis and stomatal conductance measurements: A good linear correlation was found in the four genotypes between g_s and RWC (Fig. 2). Vg presented a lower slope and reached its minimal g_s [*ca.* 100 mmol(H_2O) m^{-2} s^{-1}] at lower RWC (74 %) than was observed in *V. unguiculata* cultivars. Among the latter, g_s in cv. 1183 was 90-80 mmol(H_2O) m^{-2} s^{-1} at RWC 82 %, while g_s in cv. Lagoa was 50 mmol(H_2O) m^{-2} s^{-1} at RWC 77 %.

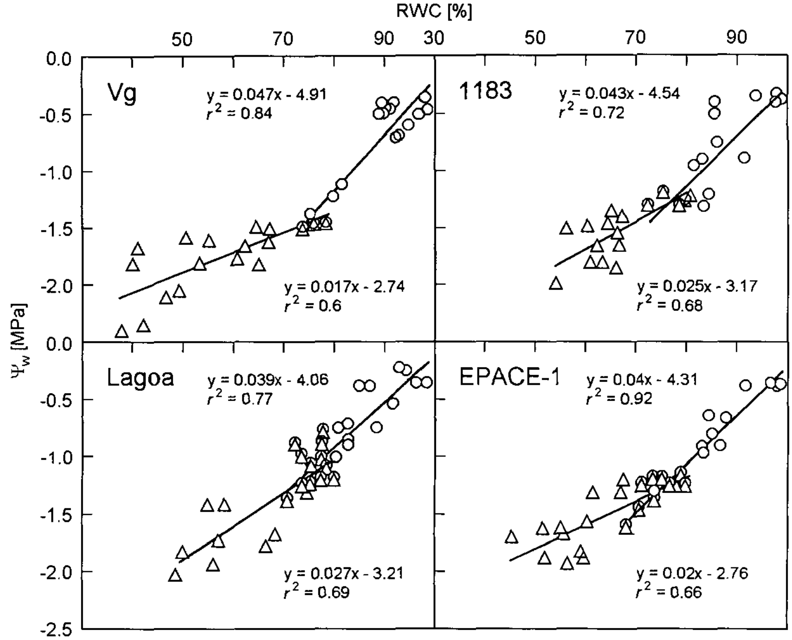

Fig. 1. Relation between leaf water potential (Ψ_w) and relative water content (RWC) in *Vigna glabrescens* (Vg) and in *Vigna unguiculata* cultivars (Lagoa, 1183, and EPACE-1). Each point represents an individual measurement.

The stomatal closure was reflected in P_N. Minimum P_N and g_s were found below RWC of 75 % for Vg, while stomatal closure and photosynthetic activity drastically declined at RWC around 77, 80, and 82 % for cvs. Lagoa, EPACE-1, and 1183, respectively (Figs. 2 and 3). As regards the relation between P_N and g_s, cv. Lagoa showed the highest maintenance in P_N values as g_s decreased, with a significant P_N reduction only for g_s values below 150 mmol(H_2O) m^{-2} s^{-1} (Fig. 3). Cv. EPACE-1 showed a similar pattern, although changes in P_N occurred for g_s values below 250 mmol(H_2O) m^{-2} s^{-1}. Vg and 1183 presented a gradual decline in g_s and P_N values, suggesting a higher control of g_s over P_N along a larger RWC range.

Photosynthetic capacity: In well-watered plants (RWC > 85 %), P_{max} was similar for Vg and the three *V. unguiculata* cultivars (Fig. 4). The imposed water stress did not significantly affect P_{max} in Vg plants during the whole stress period. On the contrary, cv. 1183 showed a strong decline to 27 % at the early stages of dehydration (RWC 85–75 %), while EPACE-1 and Lagoa presented significant decreases (to 25 % and 10 %, respectively) only under intermediate drought (RWC 75-65 %) (Fig. 4).

Table 1. Water (Ψ_w), osmotic (Ψ_s), and pressure (Ψ_p) potentials [MPa] in *Vigna glabrescens* (Vg) and in *Vigna unguiculata* cultivars (Lagoa, 1183, and EPACE-1) submitted to three drought conditions: C (RWC > 85 %), S1 (RWC 75-65 %), and S2 (RWC < 60 %). Means ± SE (n=3). Different letters express significantly different results between dehydration levels in the same genotype ($^{a, b, c}$) or between genotypes with the same dehydration level ($^{r, s, t}$).

Genotype	Treatment	Ψ_w	Ψ_s	Ψ_p
Vg	C	$-0.43 \pm 0.04^{a/r}$	$-1.05 \pm 0.10^{a/r}$	$0.62 \pm 0.07^{a/r}$
	S1	$-1.55 \pm 0.04^{b/r}$	$-1.71 \pm 0.02^{b/r}$	$0.16 \pm 0.06^{b/r}$
	S2	$-1.41 \pm 0.13^{b/rs}$	$-1.65 \pm 0.19^{b/r}$	$0.24 \pm 0.19^{b/r}$
Lagoa	C	$-0.34 \pm 0.03^{a/r}$	$-1.09 \pm 0.04^{a/r}$	$0.74 \pm 0.02^{a/r}$
	S1	$-1.08 \pm 0.04^{b/r}$	$-1.51 \pm 0.06^{b/r}$	$0.44 \pm 0.03^{a/r}$
	S2	$-1.39 \pm 0.01^{c/r}$	$-1.53 \pm 0.09^{b/r}$	$0.14 \pm 0.01^{b/r}$
1183	C	$-0.37 \pm 0.02^{a/r}$	$-1.03 \pm 0.06^{a/r}$	$0.66 \pm 0.05^{a/r}$
	S1	$-1.27 \pm 0.07^{b/rs}$	$-1.47 \pm 0.05^{b/r}$	$0.20 \pm 0.05^{b/r}$
	S2	$-1.48 \pm 0.01^{b/rs}$	$-1.46 \pm 0.05^{b/r}$	$-0.02 \pm 0.10^{c/r}$
EPACE-1	C	$-0.38 \pm 0.01^{a/r}$	$-1.09 \pm 0.14^{a/r}$	$0.71 \pm 0.15^{a/r}$
	S1	$-1.39 \pm 0.12^{b/rs}$	$-1.53 \pm 0.07^{b/r}$	$0.14 \pm 0.12^{b/r}$
	S2	$-1.64 \pm 0.02^{c/s}$	$-1.42 \pm 0.13^{ab/r}$	$-0.22 \pm 0.13^{c/r}$

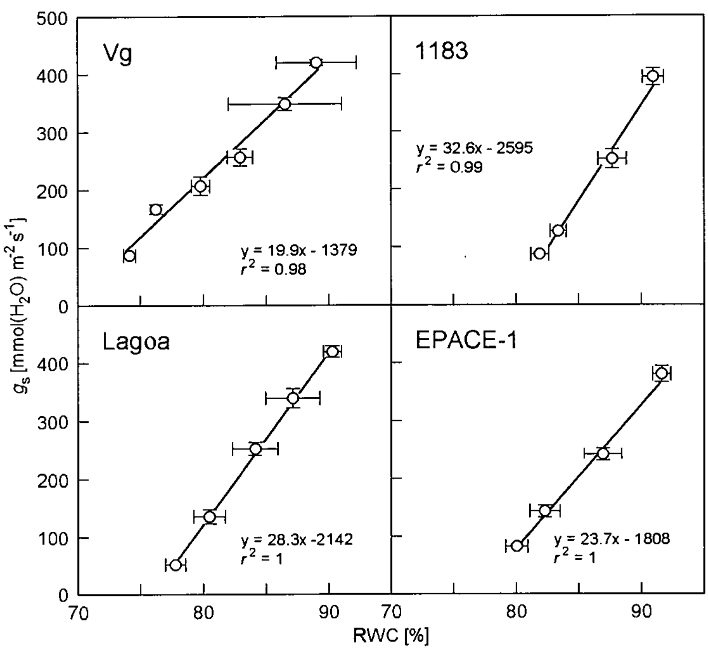

Fig. 2. Changes in stomatal conductance (g_s) of *V. glabrescens* (Vg) and *V. unguiculata* (cultivars Lagoa, 1183, and EPACE-1) in response to decreasing relative water content (RWC). Means ± SE (n=3 to 8). Linear regression fits the range of values shown in the figure.

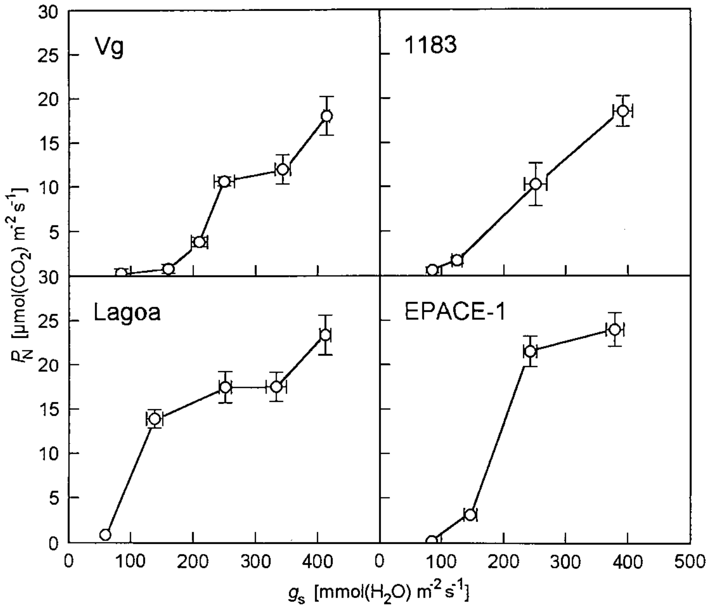

Fig. 3. Relation between stomatal conductance (g_s) and net photosynthetic rate (P_N) in *Vigna glabrescens* (Vg) and in *Vigna unguiculata* cultivars (Lagoa, 1183, and EPACE-1). Means ± SE (n= 3 to 8).

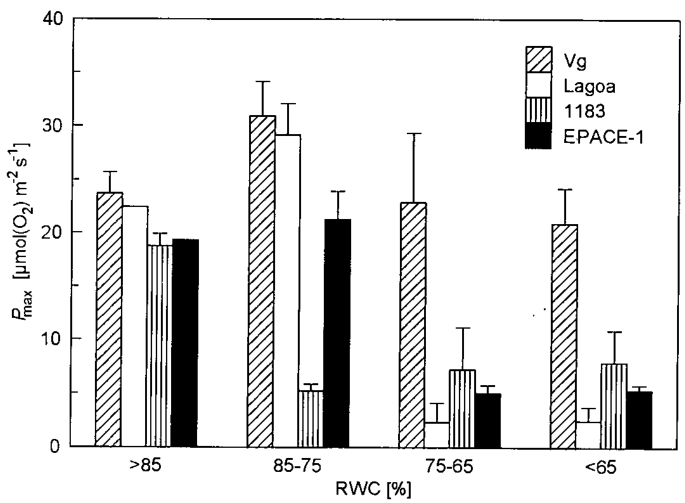

Fig. 4. Changes in photosynthetic capacity (P_{max}) of four *Vigna* genotypes submitted to different levels of dehydration. Means ± SE (n=3).

Proline and soluble sugar contents: Vg showed a significant increase in proline content already under intermediate drought (S1), while the remaining genotypes only presented significant increases under S2 (Table 2).

Table 2. Proline [mmol kg^{-1}(DM)] and soluble sugars' [kg kg^{-1}(DM)] contents in *Vigna glabrescens* (Vg) and in *Vigna unguiculata* cultivars (Lagoa, 1183, and EPACE-1) submitted to three drought conditions: C (RWC > 85 %), S1 (RWC 75-65 %), and S2 (RWC < 60 %). Means ± SE (*n*=3). For meaning of letters see Table 1.

Genotype	Treatment	Proline	Soluble sugars
Vg	C	185.6±25.6$^{b/r}$	129.2±24.4$^{a/r}$
	S1	341.2±48.6$^{a/r}$	83.8±3.7$^{a/r}$
	S2	312.0±71.7$^{a/r}$	89.9±23.1$^{a/rs}$
Lagoa	C	163.8±32.6$^{ab/r}$	100.5±12.7$^{a/r}$
	S1	136.8±8.4$^{b/s}$	103.6±24.1$^{a/r}$
	S2	276.4±24.7$^{a/r}$	71.6±2.5$^{a/rs}$
1183	C	118.1±41.4$^{b/r}$	64.2±9.1$^{b/r}$
	S1	118.6±24.7$^{b/s}$	197.4±10.8$^{a/r}$
	S2	286.5±46.2$^{a/r}$	209.6±29.7$^{a/r}$
EPACE-1	C	152.3±16.9$^{a/r}$	109.8±23.0$^{a/r}$
	S1	151.6±32.1$^{a/s}$	85.6±19.8$^{a/r}$
	S2	228.9±9.5$^{a/r}$	62.9±13.7$^{a/r}$

Leaf soluble sugars slightly decreased already under S1 for Vg and EPACE-1, but only under S2 conditions for cv. Lagoa. As regards this parameter, the cv. 1183 showed an opposite tendency since a threefold increase was observed both under S1 and S2 (Table 2).

Discussion

As regards Ψ_w/RWC along the drought period, the *V. unguiculata* cultivars followed a similar dehydration pattern. *V. glabrescens* presented a slower dehydration rate than the remaining genotypes at the initial stages of drought, which may favour the maintenance of transpiration for a longer period. The decreases of g_s and RWC were highly correlated in the four tested genotypes. However, in *V. unguiculata* cultivars stomata were probably more sensitive to water deficit, since stomatal closure occurred at higher RWC than in Vg. An extreme drought avoidance has already been reported for *V. unguiculata* under field conditions (Türk and Hall 1980), stomatal closure and paraheliotropism being important components of this drought response (Lopez *et al.* 1987). In Vg the control of stomatal opening probably assumes an important role in the prevention of tissue water loss only under more severe dehydration. Vg, and to a certain degree also cv. Lagoa, maintain significant photosynthetic activity and stomatal opening at RWC values already limiting for cvs. 1183 and EPACE-1. This suggests the presence of dehydration tolerance in the former genotypes.

The decrease in Ψ_S values observed in the four tested genotypes is within the range of that observed for other legumes, namely *Vigna radiata* (Zhao *et al.* 1985), *Phaseolus* (Markhart 1985), and *Lupinus* (Turner *et al.* 1987). Small decreases of Ψ_S

have been previously described for *V. unguiculata* (Türk and Hall 1980, Schackel and Hall 1983). In the present study it is unclear if the decreases of Ψ_s observed in the four genotypes are true osmotic adjustment or if they result from a concentration of the cell sap due to tissue dehydration, as previously suggested for *Macroptilium atropurpureum* (Wilson *et al.* 1980), *Phaseolus vulgaris*, and *Phaseolus acutifolius* (Markhart 1985). However, since under S1 Vg presented Ψ_s values lower than the *V. unguiculata* cultivars, the lowest dehydration rate in the initial phase of drought onset, and higher amounts of proline under S1, we assume that some osmotic adjustment has occurred. This might have contributed to the lower stomatal closure observed in this genotype under increasing drought severity, since plants that adjust osmotically can maintain higher stomatal conductance at low leaf water potentials than plants that do not adjust (Turner and Jones 1980). We have recently found that endogenous abscisic acid accumulation induced by drought in Vg does not occur in early dehydration stages but rather under moderate stress conditions, contrarily to what happens in cvs. EPACE-1 and 1183 (Campos *et al.*, unpublished).

The magnitude of P_N decreases accompanying g_s reductions varied in the four genotypes. P_{max} values in Vg plants were not affected along the whole drought period, indicating that the P_N decrease observed in this genotype is probably a consequence of stomatal closure. Such P_{max} maintenance suggests the existence of a high mesophyllic ability to cope with increasing tissue dehydration in this genotype.

A significant increase in content of soluble sugars was found only in droughted plants of cv. 1183. There have been contradictory reports on the effect of water stress on photosynthate partitioning. A general depletion of leaf sugar and starch would be expected, but alternatively soluble sugars may accumulate in water stressed leaves while starch is depleted (Zrenner and Stitt 1991). Accumulation of soluble sugars in wheat is an important adaptive response to water deficit (Munns and Weir 1981), and the total concentration of sugars approximately doubles with the development of water deficits in sorghum (Jones *et al.* 1980). In the cv. 1183 accumulation of soluble sugars could have played an osmoregulatory role. However, a lower osmotic potential was not found when compared to EPACE-1 and Lagoa under the same dehydration conditions. Thus in cv. 1183 some metabolic impairment may be present, affecting sugar consumption in the leaf or its translocation. Under a low consumption of assimilates, accumulation of saccharides in source tissue may inhibit photosynthesis by feedback processes (Evans 1993). Such mechanism might contribute to the strong P_{max} decline observed in this genotype at early dehydration stages.

In summary, the mechanisms underlying drought responses in the studied genotypes reflect distinct strategies among species. Stomatal closure seems to assume a more important role in cvs. EPACE-1, 1183, and Lagoa than in Vg. Indeed, under similar dehydration conditions the latter presents higher g_s values, what might be related with its better ability to perform osmotic adjustment, namely through proline accumulation, reflected in the lower Ψ_s at intermediate drought. Furthermore, the absence of significant decreases in P_{max} also suggests that Vg has better drought adaptation characteristics, while the cv. 1183 is most susceptible to dehydration. Cvs. Lagoa and EPACE-1 showed intermediate behaviours.

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