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Growth response of major USA cowpea cultivars II. Effect of salinity on leaf gas exchange

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Abstract

In an effort to elucidate the physiological processes involved in cowpea differential growth response of four major USA cowpea cultivars (CB5, CB27, 8517 and 7964) to increasing salinity, we investigated the effect of salinity on leaf gas exchange of net photosynthetic rate per unit leaf mass (Pnm) and per unit leaf area (Pna), and stomatal conductance (gs) of the four cowpea cultivars. The experiment was set up as a standard split-plot design in which cowpea plants were grown in greenhouse sand tanks irrigated with nutrient solutions. Seven salinities ranging from 2.6 to 20.5 dS m^{-1} were constructed based on Colorado River water salt composition with NaCl, $CaCl_2$ and $MgSO_4$ as the salinization salts. Light-saturated Pnm, Pna and gs of fully expanded trifoliage were examined at the vegetative growth and flowering stages, and the data were analyzed using a split-plot analysis of variance (ANOVA) model. We found a highly significant ($P \leq 0.0001$) reduction of Pnm, Pna and gs due to salinity. The responses of Pnm, Pna and gs to salinity could be further described by a general model of $\log(y) = a_1 + a_2x + a_3x^2$, where y represents either Pnm, Pna, or gs; a_1, a_2 and a_3, empirical constants; x, salinity. We found that Pnm was more sensitive to salinity than Pna. Additionally, we found that increasing stomatal closure with increasing salinity might limit Pnm or Pna. While we did not find any significant difference ($P > 0.05$) of Pnm and Pna among the four cultivars, we did find a significant difference ($P \leq 0.05$) in gs. No significant salt × cultivar interaction effect ($P > 0.05$) was found with Pnm, Pna and gs indicating that the four cowpea cultivars have the same response pattern of their leaf gas exchange to salinity.

Keywords: Cowpea; Photosynthesis; Salinity; Salt tolerance; Stomatal conductance; *Vigna unguiculata*

1. Introduction

Salinity is a major factor reducing total crop yields. It is believed that salinity reduces yield in about 50% of cultivated land [1–3]. Recently, to expand worldwide crop production interest in desert agriculture has increased [4,5]. In Southern California, the desert valleys have become major producers of vegetables [6]. Covers crops are important in maintaining soil productivity and environmental quality [7,8] and may be useful in desert agriculture [5]. Cowpea has become a cover crop of some interest as it may be suitable for growth during summer months in desert valleys when vegetables are not usually grown because it is adapted to high temperatures and drought [9,10].

Unfortunately, in desert regions salinity is a widespread and prevalent problem. Soil salinity issues are of increasing concern in the Coachella Valley of California where the Colorado River has been a major source of irrigation water for many years [11]. An earlier study [12] indicates that salt stress produces a strong, non-linear reduction effect on cowpea biomass accumulation, and that various cowpea cultivars may be differentially affected by increasing salinity.

However, little information is available on the basic controlling physiological processes involved in the differential response of cowpea cultivars to salinity. In our earlier work [12], we concluded that salt tolerance is mainly concerned with leaf area and dry weight such that the leaves might be likely targets for investigation. In the present study, we examined the photosynthetic response of four cowpea cultivars of differing salt tolerances to increasing levels of salinity to (1) determine if there might be a differential response of photosynthesis to salinity among cowpea cultivars, and (2) to determine if this could explain the differences in salt tolerance.

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2. Materials and methods

2.1. Experiment design

The cowpea experiment was set up as a standard split-plot design, with salt as the main plot variable, and cowpea cultivar as the subplot variable. This experiment was a part of a larger cowpea salt tolerance investigation which consists of 12 cultivars of cowpea subjected to seven different salinity levels of simulated Colorado River drainage water (Table 1). These solutions reflect the predicted composition of Colorado River drainage waters of various salinities after long-term irrigation (steady state). Short-term transient compositions would result in greater Ca/Mg ratios. The entire experimental design of this study used four California cowpea (*Vigna unguiculata* L. Walp) cultivars: CB5 (California Blackeye 5), CB27 (California Blackeye 27, New variety), 8517 (DLS), 7964 (DLS) and was replicated across four plots (1 plot = 1 sand tank with one salinity, 28 sand tanks in total). In all, there were 112 (4 (cultivars)*7 (salt levels)*4 (replicates)) observations for each leaf gas exchange variable. CB27 is a new cultivar which has great heat tolerance and broad-based resistance to *Fusarium* wilt and root-knot nematodes. CB5 is a commercially important cultivar. DLS-labeled cultivars exhibit delayed leaf senescence. The leaf biomass accumulation responded to salinity differentially among the four cultivars [12].

2.2. Planting

Four cowpea cultivars were planted in greenhouses in each sand tank based on a random cultivar map generated by SAS PLAN procedure, and were mixed randomly along with other eight cowpea cultivars used in another salt tolerance study [12]. The greenhouses were located at Riverside, California (lat. 33°58′24″N, long. 117°19′12″W). The tanks measured 1.2 m × 0.6 m × 0.5 m deep and contained washed sand having an average bulk density of 1.2 Mg m^{-3}. At saturation, the sand has an average volumetric water content of 0.34 m^3 m^{-3}. The planting spaces were arranged on two rows (20 cm apart) in one tank. Two seeds were sown half-inch deep and 17 cm apart on each row. After germination, the plants were thinned to one at each space.

2.3. Growth condition

During the course of this study, the air temperature ranged from 32 to 35 °C throughout day and from 15 to 18 °C at night. Relative humidity ranged from 43 to 52%. The temperature, radiation, and humidity were automatically recorded hourly at a point slightly above the plant canopy. Plants were irrigated three times daily with a base nutrient solution (BNS) made up with City of Riverside municipal water. The BNS composition was a modified (~80% strength) Hoagland solution consisting of (in mol m^{-3}): 2.5 $Ca(NO_3)_2$, 4.0 KNO_3, 2.0 KCl, 3.0 NH_4NO_3, 0.36 KH_2PO_4, 1.5 $MgSO_4$, 0.10 Fe as sodium ferric diethylenetriamine pentaacetate, 0.023 H_3BO_3, 0.015 $MnSO_4$, 0.0012 $ZnSO_4$, 0.0003 $CuSO_4$, 0.0001 H_3MoO_4 [13]. For this experiment, the BNS served as the control treatment. Each irrigation was of 15 min duration. From prior experience, we found this allowed the sand to become completely saturated, after which the solution drained into 765 L reservoirs for reuse in the next irrigation cycle. Water lost by evapotranspiration was replenished automatically each day with deionized water to maintain constant electrical conductivities in the solutions. The pH was adjusted weekly using concentrated H_2SO_4 and maintained between 6.5 and 7.5.

2.4. Salt treatment

Salt treatment consisted of irrigation waters prepared to simulate the increasingly saline waters derived from Colorado River water using NaCl, $CaCl_2$ and $MgSO_4$ as the salinization salts. Salinization commenced 7 days after planting and continued for up to six consecutive days until the highest salt level was achieved. Equivalent amounts of salts were added to BNS incrementally each day to avoid osmotic shock to the seedlings. Final ion compositions are shown in Table 1. The final electrical conductivities of the irrigation waters (EC_i) were: 2.6 (control), 3.7, 5.4, 8.3, 12.1, 17.3, and 20.1 dS m^{-1}. Irrigation solutions was stored in reservoirs (volume = 765 L each) in a basement underneath the sand tanks and were recycled through the growth period.

2.5. Measurement of leaf gas exchange

Net photosynthetic rate per unit area (Pna), and stomatal conductance (gs) of the most recent fully expanded trifoliage

Table 1
Salt levels and composition for irrigation solution constructed to simulate increasing Colorado River water salt compositions

Salinity level (dS m^{-1})	Osmotic potential (MPa)	Ca	Mg	Na (meq L^{-1})	K	SO_4	Cl
2.55	−0.08	5.0	3.0	0.0	6.0	3.0	2.0
0.74	−0.15	5.0	6.0	10.5	6.0	6.0	12.5
5.36	−0.19	7.8	12.1	20.9	6.0	12.1	25.7
8.31	−0.31	15.1	25.3	43.6	6.0	25.3	53.7
12.14	−0.47	22.7	38.8	66.9	6.0	38.8	86.6
17.28	−0.69	35.2	62.3	107.0	6.0	62.3	139.2
20.45	−0.82	40.7	77.8	134.0	6.0	77.8	171.9

meq L^{-1}: milliequivalent per liter. Note: Solutions were prepared using Riverside tap water having about (meq L^{-1}): 3.3 Ca, 1.6 Na, 0.1 K, 0.83 Cl, 1.36 NO_3 and 1.44 SO_4, which were not included in the above table.

were measured between 9:00 and 16:00 h using a Li-Cor 6400 Photosynthesis System. Leaf gas exchange measurements were taken during two growth stages: (1) vegetative growth stage, 31–32 days after planting; (2) flowering stage, 45–46 days after planting. The following conditions for leaf gas exchange measurements were used: photosynthetic photon flux density, 1200 μmol photon $m^{-2}\,s^{-1}$; chamber CO_2 concentration, 380 μmol CO_2 mol^{-1}; chamber temperature, 28–29 °C; chamber vapor concentration, 20 mmol H_2O mol^{-1}. Each leaf used for Pna and gs measurement was sampled and its area was measured immediately using Li-Cor 3100 Leaf Area Meter (LiCor, Lincoln, NE). The leaf sample was oven-dried at 45 °C for 1 week, weighed using a Mettler AC 100 balance. Specific leaf weight (SLW) was determined by dividing leaf dry weight by leaf area. Net photosynthetic rate per unit mass (Pnm) was calculated by dividing Pna by SLW. Pnm and SLW were not measured at the vegetative growth stage because the leaves of the plants grown at the high salinity treatment (20.5 dS m^{-1}) were too small to sample.

2.6. Data analysis

The initial analysis of both the gas exchange and leaf weight data was performed using a standard split-plot analysis of variance model. The main factor salt effect was then partitioned into linear, quadratic, and higher order terms to further quantify the (continuous) main effect of salinity and to equate the split-plot model to a transformed version of the van Genuchten and Hoffman [14] nonlinear salt tolerance equation.

In each analysis, the split-plot model was fit to log transformed response data. The specific model was defined as:

$$\log(y_{ijk}) = \mu + \mathrm{B}_i + \mathrm{S}_j + \mathrm{SB}_{ij} + \mathrm{C}_k + \mathrm{SC}_{jk} + \xi_{ijk} \quad (1)$$

where y represents the appropriate response (Pnm, Pna, gs, or SLW) data, B, S, and V represent the blocking ($i = 1, \ldots, 4$), salinity level ($j = 1, \ldots, 6$), and cultivar ($m = 1, \ldots, 4$), respectively, and ξ_{ijk} represents the residual mean square error (RMSE). The analysis was performed on the log transformed data in order to stabilize the variance and facilitate the use of the transformed van Genuchten and Hoffman [14] salt tolerance equation [15]. In Eq. (1), the main plot experimental error was estimated by the salinity by block (SB) interaction component and used to test the overall salinity (S) effect, as well as the partitioned (linear, quadratic, and higher-order) salinity terms. Likewise, the RMSE was used to test for significant sub-plot cultivar (C) and salinity by cultivar (SC) interaction effects [16].

The nonlinear salt tolerance equation described by van Genuchten and Hoffman [14] is defined as

$$y = \delta \exp(\alpha x - \beta x^2) \quad (2)$$

where y represents the particular response data under study, x the salinity, and δ, α and β are empirical parameters which must be estimated from the salt tolerance data [14]. Under a log transformation, Eq. (2) becomes a quadratic regression equation:

$$\log(y) = \log(\delta) + \alpha x - \beta x^2 + \varepsilon = \beta_0 + \beta_1 x + \beta_2 x^2 + \varepsilon \quad (3)$$

where ε represents the model error, which is assumed to be additive under the transformation. When response data is collected across multiple cultivars and subject to random block effects imposed by a split-plot design, Eq. (3) can be respecified into the following mixed-linear model

$$\log(y_{ijk}) = \beta_0 + \mathrm{C}_k + \beta_1 x_j + \beta_2 x_j^2 + (\eta_i + \theta_{ij} + \xi_{ijk}) \quad (4)$$

where η, θ, and ξ represent the random block, block by salinity, and residual variance components [17]. Note that Eq. (4) assumes that there are no significant salinity by cultivar interaction effects. Additionally, if there is no significant cultivar effect (4) can be reduced to (3) where $\varepsilon = \eta_i + \theta_{ij} + \xi_{ijk}$. In either situation, both (3) and (4) are special cases of Eq. (1) where $\beta_0 = \mu$, $\eta_i = \mathrm{B}_i$, $\theta_{ij} = \mathrm{SB}_{ij}$, and the salinity effect (S_j) is re-expressed as a continuous quadratic equation with the higher-order terms adsorbed into the appropriate variance components.

Based on the split-plot test results obtained from Eq. (1), either Eq. (3) or (4) were fit to the various leaf gas exchange measurements for each cultivar. The resulting (back-transformed) salt tolerance equations were then used to estimate the salinity levels associated with the maximum leaf gas exchange values (Y_{max}), the 50% reduction point in Y_{max} (C_{50}), and to display graphically the final estimated salt tolerance curves against the actual data points. SAS version 8 software was used to perform all of these statistical analyses [18].

3. Results

We found a highly significant ($P < 0.0001$) salt effect on Pna, Pnm, gs and SLW for all four cowpea cultivars examined (Table 2). The ANOVA analyses showed that the partitioned (linear + quadratic) salinity effect was highly significant ($P < 0.0001$), but higher order salinity effects were not significant ($P > 0.05$) for all the leaf gas exchange variables of the four cultivars at either the vegetative growth stage or during flowering period (Table 2). There were also no significant salinity × cultivar interaction effects detected in any of the analyses. Hence, a log transformed quadratic equation of the non-linear salt tolerance model (Eq. (3) or (4)) can be used to adequately describe the response of cowpea Pna, gs, Pnm, and SLW to salinity stress.

At the vegetative growth stage, we found no significant cultivar effect associated with the Pna ($P > 0.05$) (Table 2). Therefore, we utilized Eq. (3) to generate a single Pna–salt response curve combining all four cultivars (Fig. 1). We should note, however, that we did find a significant cultivar effect for gs ($P = 0.02$). Thus, we employed Eq. (4), having unique intercepts (maximum gs estimate: 1.27, 1.24, 1.05 and 1.03 mol H_2O $m^{-2}\,s^{-1}$ for CB5, 7964, CB27 and 8517, respectively) but giving the same general response pattern, to generate the predicted curves for the four cultivars for gs

Table 2
Summary of standard split-plot ANOVA model *F*-test effect results for leaf net photosynthetic rate (Pnm, per unit mass; or Pna, per unit area), stomatal conductance (gs) and specific leaf weight (SLW) of four cowpea cultivars, 7964, 8517, CB5 and CB27

	(log Pnm)		(log Pna)		(log gs)		(log SLW)	
	F-test	$P > F$	*F*-test	$P > F$	*F*-test	$P > F$	*F*-test	$P > F$
Vegetative growth stage								
Salt	–	–	52.87	<0.0001	76.62	<0.0001	–	–
Cultivar	–	–	0.60	0.6205	3.53	0.0198	–	–
Salt × cultivar	–	–	0.90	0.5835	1.39	0.1705	–	–
Partitioned main plot salinity effects								
Salt (linear + quadratic)	–	–	154.00	<0.0001	228.29	<0.0001	–	–
Salt (higher order)	–	–	2.28	0.1008	0.79	0.5452	–	–
Flowering stage								
Total salt	40.19	<0.0001	23.72	<0.0001	81.87	<0.0001	39.00	<0.0001
Cultivar	0.67	0.5765	1.78	0.1604	2.27	0.0892	0.43	0.4307
Salt × cultivar	1.53	0.1174	0.90	0.5858	0.91	0.5681	1.24	0.2632
Partitioned main plot salinity effects								
Salt (linear + quadratic)	115.98	<0.0001	69.40	<0.0001	243.60	<0.0001	108.40	<0.0001
Salt (higher order)	0.17	0.9534	0.89	0.4907	1.00	0.4339	1.17	0.3574

(Fig. 2). The common maximum Pna estimate and individual (cultivar-specific) gs estimates derived from these fitted salt tolerance equations are given in Table 3.

At flowering stage, we did not find any significant cultivar effects for Pnm, Pna, gs and SLW ($P > 0.05$) (Table 2). Therefore, we again utilized Eq. (3) to generate a single salt–Pnm, Pna, gs or SLW response curve combining all four cultivars (Figs. 3–6). Note that since SLW increased with the increasing salinity levels (Fig. 6), its quadratic term has a positive instead of negative parameter.

It appears that increasing biomass accumulation on a per unit area basis is a physiological characteristic of cowpea foliage in response to salt treatment. Thus, Pna might not be the most insightful measure of photosynthesis. Therefore, we decided to measure photosynthesis on a mass basis, Pnm (Fig. 5). As in the case with Pna, we found no significant cultivar effect.

Since it appeared that cowpea leaf gas exchange-salt response pattern appears unchanged by salt stress duration (24–25 days after salinization versus 38–39 days after salinization) or the growth stage (vegetative versus flowering) (Figs. 1–4), we decided to compare the photosynthetic data between the vegetative growth stage and the flowering stage derived from the salt tolerance model. The model predicted similar maximum Pna or gs values for both growth stages (Table 3). The model predicted maximal Pna (Y_{max}) for the vegetative stage of 24.1 μmol CO_2 m^{-2} s^{-1} versus 24.0 μmol CO_2 m^{-2} s^{-1} for the flowering stage. In addition, the model predicted similar C_{max} (salinity level resulting in a maximum leaf gas exchange) of 6.1 dS m^{-1} compared to 6.0 dS m^{-1} for the vegetative stage and the flowering stage, respectively, and C_{50} values (salinity level resulting in a 50% reduction of the maximum leaf exchange), 17.5 dS m^{-1} versus to 17.8 dS m^{-1} for the vegetative stage and the flowering stage, respectively. Our calculations for gs obtained for the two growth stages followed a similar pattern (Table 3).

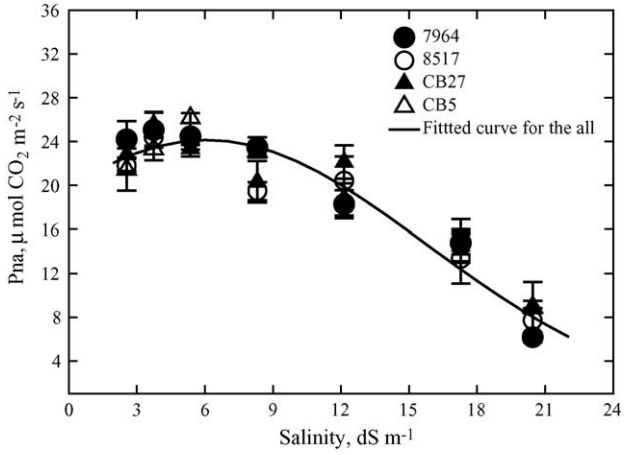

Fig. 1. Leaf net photosynthetic rate on area (Pna) of four cowpea cultivars at vegetative growth stage in response to salinity levels of irrigation water. Bars represent ±1 standard error ($n = 4$ leaves, one leaf per plant per sand tank).

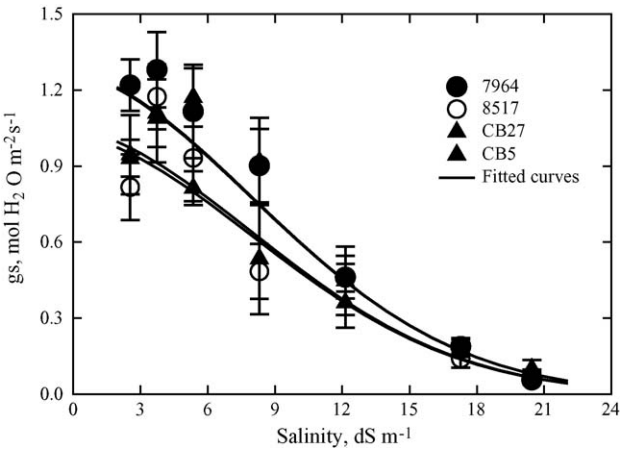

Fig. 2. Leaf stomatal conductance (gs) of four cowpea cultivars at vegetative growth stage in response to salinity levels of irrigation water. Bars represent ±1 standard error ($n = 4$ leaves, one leaf per plant per sand tank).

Table 3
Summary of model-predicted maximum values (Y_{max}) of cowpea leaf net photosynthetic rate (Pnm, per unit mass; or Pna, per unit area), stomatal conductance (gs) and the salinity level that could result in a Y_{max} (C_{max}) or a 50% reduction of the Y_{max} (C_{50}) for four cowpea cultivars of 7964, 8517, CB5 and CB27

	Pnm			Pna			gs		
	Y_{max} (μmol CO_2 g^{-1} s^{-1})	C_{max} (dS m^{-1})	C_{50} (dS m^{-1})	Y_{max} (μmol CO_2 m^{-2} s^{-1})	C_{max} (dS m^{-1})	C_{50} (dS m^{-1})	Y_{max} (mol H_2O m^{-2} s^{-1})	C_{max} (dS m^{-1})	C_{50} (dS m^{-1})
Vegetative growth stage	–	–	–	24.1	6.1	17.5	1.03–1.28	0	9.6
Flowering stage	0.73	2.3	12.6	24.0	6.0	17.8	1.20	0	10.0

4. Discussion

An earlier study reported the effect of photosynthesis in cowpea and concluded that there were different responses of net assimilation and stomatal conductance at different salinity levels within the same species and cultivar and not merely between species as previously believed [19]. In an effort to elucidate the physiological processes involved in cowpea salt response and its cultivar difference, we investigated the effect of salinity on leaf gas exchange of net photosynthetic rate (CO_2 fixation rate) per unit area, and stomatal conductance per unit area (controlling CO_2 or H_2O to diffuse into or out of leaves) of four major USA cowpea cultivars: CB5, CB27, 8517 and 7964. These cultivars were chosen because in our previous study [12], they displayed a differential response in leaf area to salinity with CB5 being the most tolerant and 7964 the least tolerant; CB27 and 8517 showed an intermediate response. Leaf net photosynthetic rate per unit leaf mass was also examined, because salt stress might affect leaf specific weight (SLW, mass per unit area), and Pna might not reflect the difference of leaf photosynthetic rate caused by different SLW. Leaf mass measures its enzymatic system for CO_2 fixation.

Salinity treatment results in a progressive decline in growth among the four cultivars with CB5 being the most tolerant, 7964 the least tolerant, and CB27 and 8517 of intermediate tolerance as judged by C_{50} values [12]. However, in our studies on net assimilation at the vegetative stage, we found no significant cultivar effect on Pna, nor did we measure any significant salinity × cultivar interaction effects on either Pna or gs ($P > 0.05$) (Table 2) indicating that differences salt tolerance among cowpea cultivars may not be related to differences in photosynthetic activity. This interpretation is supported by the observation that we did not find any significant cultivar or salinity × cultivar interaction effects for Pnm, Pna, gs and SLW ($P > 0.05$) (Table 2) at the flowering stage either.

Consistent with this view is the observation that cowpea growth decreases almost linearly at first with increasing salinity [12]. However, Pna remains largely unaffected by salinity, with perhaps a slight increase, until about 9 dS m^{-1} (Fig. 2). Beyond this point, photosynthesis decreases linearly. This observation is the same regardless of whether photosynthesis was measured at the vegetative stage or the flowering stage (Figs. 2 and 3). The Pnm curve more closely resembles the growth pattern of cowpea under increasing levels of salinity (Fig. 5). However, the point at which photosynthesis decreases by 50%, the C_{50} value, is 12.5 dS m^{-1}. This is much higher than the C_{50} value for growth based on leaf area of our most tolerant cultivar, CB5 (C_{50} = 7.4 dS m^{-1}) [12].

Another interesting finding is the discrepancy among C_{max} values for Pna, Pnm, and gs. The C_{max} for Pna is 6.0–6.1 dS m^{-1}; for Pnm, 2.3 dS m^{-1}; and for gs, 0 dS m^{-1} (Table 3). When gs decreased from the observed highest values to ~0.6 mol H_2O m^{-2} s^{-1} (salinity levels increasing

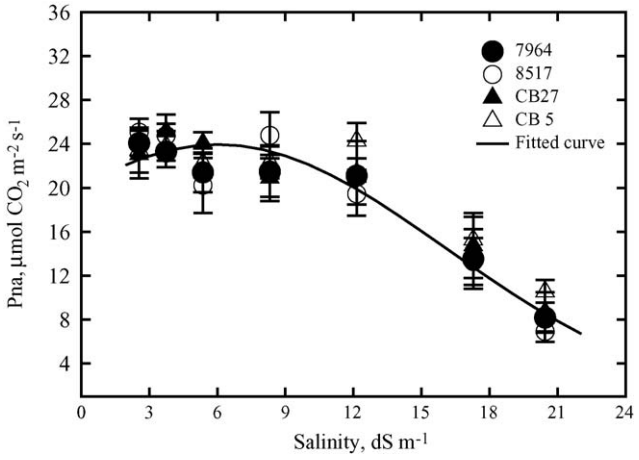

Fig. 3. Leaf net photosynthetic rate on area (Pna) of four cowpea cultivars at flowering stage in response to salinity levels of irrigation water. Bars represent ±1 standard error (n = 4 leaves, one leaf per plant per sand tank).

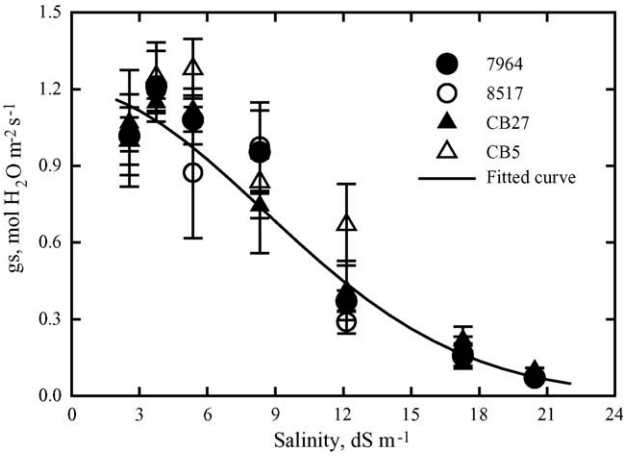

Fig. 4. Leaf stomatal conductance (gs) of four cowpea cultivars at flowering stage in response to salinity levels of irrigation water. Bars represent ±1 standard error (n = 4 leaves, one leaf per plant per sand tank).

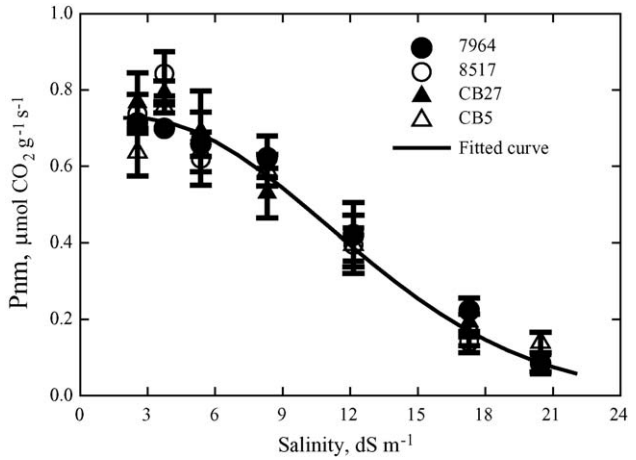

Fig. 5. Leaf net photosynthetic rate on mass (Pnm) of four cowpea cultivars at flowering stage in response to salinity levels of irrigation water. Bars represent ±1 standard error ($n = 4$ leaves, one leaf per plant per sand tank).

from around 3–9 dS m^{-1}), Pna did not vary much (Figs. 1–4) and Ci remained fairly constant (data not shown). It is believed that in cowpea decreases in leaf conductance with drought are not related to changes in bulk leaf water status. Rather, stomatal closure is mediated by changes in the root water status via effects on the flow of information from root to shoot, possibly hormonal [20]. It is possible that salinity elicits the same, or similar, flow of information.

Pnm decreased from its maximum value (Fig. 5) as salinities increased. Apparently, Pnm is more sensitive to salt stress than Pna. From a speculative viewpoint, there may be in cowpea a non-stomatal salt limitation on leaf photosynthesis or perhaps some direct affect of salinity on leaf enzymatic CO_2 fixation reactions. Future research is planned to investigate this possibility. This non-stomatal salt limitation effect may explain the lower C_{50} for Pnm (12.6 dS m^{-1}) than for Pna (17.7–17.8 dS m^{-1}) (Table 3).

In agreement with other reports in the literature [21,22], the decrease in Pna with increasing salinities was clearly associated with the decrease in gs (Figs. 2 and 4). This finding indicates that stomatal closure with increasing salt stress may be a factor in limiting leaf photosynthesis by limiting CO_2 flux into leaves. This view is supported by the observation that Pna declined with decreasing Ci (intercellular CO_2 concentration) as gs decreased from ~0.6 mol H_2O m^{-2} s^{-1} (data not shown).

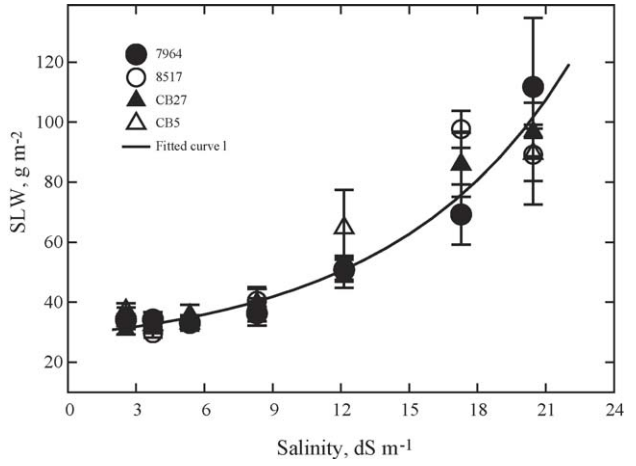

Fig. 6. Specific leaf weight (SLW) of four cowpea cultivars at flowering stage in response to salinity levels of irrigation water. Bars represent ±1 standard error ($n = 4$ leaves, one leaf per plant per sand tank).

It appears that in cowpea, growth is affected by low to moderate levels of salinity. However, Pna in cowpea does not seem to be influenced by low to moderate levels of salinity. While Pnm may be influenced by low to moderate salinity levels, the high C_{50} values indicate that a decrease in Pnm alone does not account for the pattern of growth reductions observed. With this in mind, our results are consistent with the notion that salinity tolerance is a complex phenomenon brought about by a range of physiological processes [23]. In tomato [24] photosynthetic rate also does not seem to be the first factor which limits plant growth under salinity. These researchers suggested that differential distribution and use of photoassimilates may be involved in the response of tomato to increasing salt stress.

In conclusion, we found a highly significant ($P < 0.0001$) reduction of Pnm, Pna and gs due to salinity. The responses of Pnm, Pna and gs to salinity could be quantitatively described by a salt-tolerance model of $\log(y) = a_1 + a_2x + a_3x^2$, where y represents either Pnm, Pna, or gs; a_1, a_2 and a_3 are empirical constants; x represents salinity. Based on a comparison of the salinity responses of Pna and Pnm, we found that Pnm is more sensitive to salinity than Pna. This finding indicates that salinity may directly affect leaf photosynthetic carbon fixation reactions. While we did not find any significant difference ($P > 0.05$) of Pnm and Pna among the four cultivars, we did find a significant difference ($P > 0.05$) in gs. However, no significant salt × cultivar interaction effect ($P > 0.05$) was found on Pnm, Pna and gs indicating that the four cowpea cultivars have the same response pattern of their leaf gas exchange to salinity. Based on our results, we conclude that while salinity may affect photosynthesis and thereby overall yield, differences in photosynthesis do not explain the cultivar difference in cowpea with respect to salt tolerance. Future work will investigate the possible involvement of differential carbon allocation in salt tolerance among cowpea cultivars.

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