Lima Bean Growth, Leaf Stomatal and Nonstomatal Limitations to Photosynthesis, and ^{13}C Discrimination in Response to Saline Irrigation

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ABSTRACT. Soil salinization is a widespread problem severely impacting crop production. Understanding how salt stress affects growth-controlling photosynthetic performance is essential for improving crop salt tolerance and alleviating the salt impact. Lima bean (*Phaseolus lunatus*) is an important crop, but little information is available on its growth and leaf gas exchange in relation to a wide range of salinity. In this study, the responses of leaf gas exchange and whole plant growth of lima bean (cv. Fordhook 242) to six salinities with electrical conductivity (EC) of 2.9 (control), 5.7, 7.8, 10.0, 13.0, and 15.5 $\text{dS}\cdot\text{m}^{-1}$ in irrigation waters were assessed. Significant linear reduction by increasing salinity was observed on plant biomass, bean yield, and leaf net carbon assimilation rate (A). As EC increased from the control to 15.5 $\text{dS}\cdot\text{m}^{-1}$, plant biomass and A decreased by 87% and 69%, respectively, at the vegetative growth stage, and by 96% and 83%, respectively, at the pod growth stage, and bean yield decreased by 98%. Judged by the linear relations, the reduction in A accounted for a large portion of the growth reduction and bean yield loss. Salinity also had a significantly negative and linear effect on leaf stomatal conductance (g_s). Leaf intercellular CO_2 concentration (C_i) and leaf ^{13}C isotope discrimination (Δ^{13}) declined in parallel significantly with increasing salinity. The A - C_i curve analysis revealed that stomatal limitation [L_g (percent)] to A increased significantly and linearly, from 18% to 78% and from 22% to 87% at the vegetative and pod-filling stages, respectively, as EC increased from the control to the highest level. Thus, relatively nonstomatal or biochemical limitation [L_m (percent), $L_m = 100 - L_g$] to A responded negatively to increasing salinity. This result is coincident with the observed Δ^{13} salt-response trend. Furthermore, leaf carboxylation efficiency and CO_2 -saturated photosynthetic capacity [maximum A (A_{max})] were unaffected by increasing salinity. Our results strongly indicate that the reduction in lima bean A by salt stress was mainly due to stomatal limitation and biochemical properties for photosynthesis might not be impaired. Because stomatal limitation reduces A exactly from lowering CO_2 availability to leaves, increasing CO_2 supply with an elevated CO_2 concentration may raise A of the salt-stressed lima bean leaves and alleviate the salt impact. This is supported by our finding that the external CO_2 concentration for 50% of A_{max} increased significantly and linearly with increasing salinity at the both growth stages. Leaf water use efficiency showed an increasing trend and no evident decline in leaf chlorophyll soil plant analysis development (SPAD) readings was observed as salinity increased.

Soil salinization is a widespread problem affecting $\approx 20\%$ of world irrigated land, posing a threat to crop production (Munns et al., 2020). Because most crops are glycophytes and sensitive to salt stress, it is expected that a low level of salinity may cause a significant reduction in crop growth and production (Acosta-Motos et al., 2017; Maas and Hoffman, 1977). Reduction in crop growth is generally a consequence of growth-related physiological responses, such as reduced photosynthetic performance, mineral imbalance and alterations of water relations, and carbon allocation and utilization (Chaves et al., 2009; Grattan and Grieve, 1999; Munns, 1993; Negrão et al., 2017). Leaf photosynthetic

carbon assimilation directly determines plant carbon gain or growth, and thus is a primary focus for understanding physiological mechanisms of, and improving crop salt tolerance (Chaves et al., 2011). Reduced growth of various crop species is generally associated with a reduction in leaf carbon assimilation under salt stress (Bayuelo-Jiménez et al., 2012; Koyro, 2006; Melgar, et al., 2008; Rouphael et al., 2017; Seemann and Critchley, 1985; Stoeva and Kaymakanova, 2008; Wilson et al., 2006). It is compelling that leaf photosynthesis is a central growth-controlling physiological process for plants to respond to salt stress.

Leaf photosynthesis is composed of a physical CO_2 diffusion process for carbon supply and a sequence of physiological and biochemical processes for carbon fixation. Its net rate or net CO_2 assimilation rate (A) can be defined as $A = g_s (C_a - C_i)$, where g_s is leaf stomatal conductance to water measuring stomatal opening, and C_a and C_i are the ambient and sub-stomatal intercellular CO_2 concentrations, respectively (von Caemmerer and Farquhar, 1981). Stress factors can affect A directly or through influencing g_s , and the response of A to drought or salt stress is highly complex (Acosta-Motos et al., 2017; Chaves et al., 2009; Negrão et al., 2017).

Similar to drought stress, under salt stress, a reduction in g_s was generally reported (Chaves et al., 2009; Flexas et al., 2004;

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Pérez-Pérez et al., 2007; Rouphael et al., 2017). This is usually a result from osmotic effect occurring in the first phase of plant salt stress response through time (Munns and Tester, 2008). Once salinity imposes in the soil, it lowers down soil osmotic potential and slows down root water uptake and transport to leaves and thus induces leaf water deficit. Followed is the loss of leaf guard cell turgor pressure and a reduction in g_s . This reduction is likely a feedback signal for plants to reduce water loss through leaf transpiration by stomata closure and thus reduce ion influx. Under this salt osmotic effect in the soil, other plant cells also lose their turgor pressure so that their expansion rate is reduced, which retards shoot and root growth. Parallel to the g_s reduction by salinity, a decline in A was observed on several crop species (Bethke and Drew, 1992; Brugnoli and Lauteri, 1991; Delfine et al., 1999; Escalona et al., 1999; Gibberd et al., 2002; James et al., 2002; Jiang et al., 2006; Moradi and Ismail, 2007; Rouphael et al., 2017; Stoeva and Kaymakanova, 2008; Wilson et al., 2006). Hence, stomatal closure reduces CO_2 supply to leaves and may impose a CO_2 limitation to photosynthesis. As salt stress is prolonged, the second, or salt toxic phase of plant response to salinity starts when salt accumulates to toxic concentrations in the old leaves (Munns and Tester, 2008). High salt level in cells if the salts (in most cases, Na^+ and Cl^-) are not compartmentalized into vacuoles, are very toxic to cytoplasm, and may impair biochemical properties of photosynthesis, such as activities of carboxylation key enzyme of Rubisco and other enzymes for Calvin cycle, of light-harvesting components and electron transfer to cause nonstomatal limitation to A (Chaves et al., 2011; Munns and Tester, 2008). Such a biochemical or nonstomatal limitation to photosynthesis was observed on a number of crop species (Brugnoli and Björkman, 1992; Delfine et al., 1999; Gibberd et al., 2002; James et al., 2002; Killi and Haworth, 2017). Visually, early leaf senescence and leaf chlorosis from chlorophyll degradation can be seen under severe salt toxic effect.

Salt stress can reduce A predominantly through stomatal limitation (Brugnoli and Lauteri, 1991; Downton et al., 1985), or through both stomatal and nonstomatal responses to salinity (Hichem et al., 2009; Seemann and Critchley, 1985) or mainly from nonstomatal limitation (Ball and Farquhar, 1984; Bethke and Drew, 1992; Dunn and Neales, 1993; Gibberd et al., 2002). Under drought stress, the response can be dominated by stomatal limitations accounting for $\approx 50\%$ to 75% of the total limitation depending on species and stress progress (Flexas et al., 2004; Killi and Haworth, 2017; Ni and Pallardy, 1992; Olsovska et al., 2016; Wilson et al., 2000). Within the same species, like sunflower (*Helianthus annuus*), contrasting results were observed that g_s and stomatal limitation might be affected or not by salt stress depending on what mechanisms the cultivars adopted in response to salt stress and cultural conditions (Katerji et al., 1994; Rivelli et al., 2002; Steduto et al., 2000). The relative importance of stomatal or nonstomatal limitations to photosynthesis may depend highly on species and on their capacities to limit salt uptake into the leaves, to exclude or to store the toxic salt ions in safe places of vacuoles; and on salt stress composition and duration as well as cultural conditions (Centritto et al., 2003; Flexas et al., 2004; Munns and Tester, 2008). The analysis of A to C_i response or $A-C_i$ curve provides a nondestructive technique for quantifying how much the reduction in A may come from stomatal limitation due to the reduction of CO_2 availability compared with biochemical or nonstomatal limitation (Farquhar and Sharkey, 1982; Jones, 1985, 1998; Sharkey et al., 2007; Stinziano et al., 2017). The

assessment of the relative contribution of stomatal to nonstomatal control of photosynthesis under salt stress is essential for minimizing the negative stress impact and revealing how much the irreversible or permanent damage may occur from the impairment of photosynthetic metabolic components under salt stress (Chaves et al., 2009, 2011; Flexas et al., 2004). The significance lies in that stomatal opening is reversible on the removal of stress so long as g_s was not severely reduced. A recovery of g_s may reverse the reduction in A , enabling a recovery in growth (Centritto et al., 2003; Chaves et al., 2009; Cornic, 2000; Delfine et al., 1999; Flexas et al., 2004; Loreto et al., 2003; Tezara et al., 1999).

In C3 plants, leaf ^{13}C isotope discrimination (Δ^{13}) is indeed primarily caused by the process of CO_2 diffusion from the atmosphere to the chloroplasts and by carboxylation of the rate-limiting enzyme, Rubisco, during photosynthesis (Farquhar et al., 1982). The cause of variation of Δ^{13} is complex. Rubiscos from different species or cultivars may have different values of Δ^{13} (Guy et al., 1993; McNevin et al., 2007). Leaf Δ^{13} was reported to increase with the increase in C_i/C_a (Farquhar et al., 1989). The decrease in Δ^{13} was observed on a number of C3 plant species as the increase in drought stress (Arndt and Wanek, 2002; Evans et al., 1986; Monti et al., 2006). Soil salinity or salt treatment was observed to cause significant decrease in Δ^{13} of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) (Jiang et al., 2006; Shaheen and Hood-Nowotny, 2005). It is proposed that stomatal limitation on A may exist if Δ^{13} is linearly and positively correlated with g_s (Brugnoli and Lauteri, 1991; Ehleringer, 1990; Massonnet et al., 2007). Integrating examination of Δ^{13} under salt stress with leaf gas exchange study may reveal more information for our understanding of how leaf photosynthetic performance responds to salt stress.

Lima bean (*Phaseolus lunatus*) is an internationally important legume and a major crop in several regions, and its annual acreage is $\approx 16,200$ ha in the United States (Kee et al., 2004). Salt tolerance varies in a quite big range from salt sensitive to moderately salt tolerant in legume, and lima bean was rated as moderately salt tolerant (Maas and Grattan, 1999). Unlike common bean [*Phaseolus vulgaris* (salt sensitive)] and soybean [*Glycine max* (moderately salt tolerant)], lima bean has not received much research (Akande and Balogun, 2007; Santos et al., 2009; Da Silva Souza et al., 2018) and little information is available for our understanding of how abiotic stresses affect its growth and growth-related physiological processes. With regard to salt stress effect on leaf photosynthesis, only one research work was found that reports the response of lima bean leaf gas exchange to a short range of irrigation solution salinity (Pereira Filho et al., 2019). No research was found in interpreting lima bean stomatal and nonstomatal limitations to its carbon assimilation under salt stress. Our hypothesis is that being a glycophytic crop, once subjected to salt stress, lima bean might not be able to maintain its normal photosynthetic performance and its g_s would decrease with increasing salinity, which could reduce A . The reduced A reduces carbon source strength for growth resulting in growth decline and yield loss. The aims of this study were thus to 1) characterize the responses of lima bean biomass accumulation and leaf gas exchange to a series of salinities imposed through irrigation water, 2) measure and analyze leaf $A-C_i$ curves at the vegetative and pod-filling stages to evaluate lima bean stomatal and nonstomatal limitations to A , and 3) to determine whether the limitations change with increasing irrigation water salinity. In addition, Δ^{13} was also

evaluated to provide more information on lima bean leaf gas exchange characteristics under salt stress.

Materials and Methods

PLANT MATERIALS AND GROWTH CONDITIONS. The experiment was conducted outdoors at the U.S. Department of Agriculture-Agricultural Research Service, U.S. Salinity Laboratory, Riverside, CA (lat. 33°58'24"N, long. 117°19'12"W) using a sand tank culture system. Each sand tank was a rectangular cylinder made of concrete wall 6-cm thick having an internal dimension of 2.0 m (length) × 0.81 m (width) × 0.88 m (depth) and filled with loamy sand up to a level 0.1 m below the top of the tank. The sand had an average bulk density of 1400 kg·m⁻³, and an average volumetric water content of 0.34 m·m⁻³ at saturation. Six salinities were randomly assigned to 24 sand tanks in a one-factor design, and one salinity was replicated across four sand tanks. The 24 tanks were situated in 4 × 6-m space arrangement. Lima bean (cv. Fordhook 242) seeds (Johnny's Selected Seeds, Fairfield, ME) were directly planted into the sand medium (≈4 cm deep) on 7 July 2010 in two rows (11 cm apart) along the tank depth, 18 seeds in a row. As the plants grew, they were thinned by removing every other plant in each row.

The irrigation system is a type of volumetric lysimeter for each sand tank with irrigation solutions stored in individual reservoirs (1740-L capacity for each). One reservoir served one sand tank. The reservoirs were situated in a basement underneath the sand tanks and the solution was pumped from each reservoir via 5.1-cm polyvinyl chloride (PVC) pipe to the tanks to completely saturate and leach the sand for irrigation. The drained solution flowed back by gravity through a subsurface drainage system to the reservoirs to maintain a uniform and constant profile of salinity in sand and to be reused in the next irrigation cycle. Water lost through evapotranspiration was replenished with municipal tap water of the City of Riverside, CA. Irrigation uniformity was achieved through three 2.54-cm PVC pipes installed across the tank length on the sand surface, each of the three pipes had two straight rows (1 cm apart) of small holes (1.6-mm diameter, 5 cm apart) to deliver the irrigation water. The irrigated water amount in a 6-min duration was 296 ± 5.5 L with a leaching fraction of 0.94 ± 0.004 based on five times of testing with every tank (n = 5 × 24), and thus the irrigation flow rate was ≈50 L·min⁻¹ for one tank. Note: the irrigation and leached water amounts were measured with each tank/lysimeter sensor system (Poss et al., 2004). Salt stress

treatments were designed to simulate saline drain waters often present in the inland valleys of southern California and essentially representing salt concentrations of the Colorado River water (predominately NaCl with significant Ca²⁺, Mg²⁺, and SO₄²⁻) with six targeted salinities with EC values of 2.5 (control), 5, 7.5, 10, 13, and 16 dS·m⁻¹. The control was a modified ≈1/2 strength Hoagland base nutrient solution (BNS) consisting of (added amounts in mM): 1.75 CaCl₂, 4.0 KNO₃, 0.5 KCl, 2.0 NH₄NO₃, 3.0 MgSO₄, 0.5 Na₂SO₄, 5.0 NaCl, 0.36 KH₂PO₄, 0.15 Fe as sodium ferric diethylenetriamine pentaacetate, 0.023 H₃BO₃, 0.03 MnSO₄, 0.0024 ZnSO₄, 0.0006 CuSO₄, and 0.0002 H₃MoO₄, and was made with the tap water. The simulations and predictions of the salt compositions followed Suarez and Šimunek (1997) and the solution salt composition is shown in Table 1. CaCl₂, KCl, MgSO₄, Na₂SO₄, and NaCl were salinizing salts and all the weighed salts were added to the BNS once to achieve all the targeted EC. As a pre-salinization type of experiment, salt treatments with all the six salinities commenced at the same time right after the seeds were sown. The measured EC values of the irrigation waters were the averages from 12 times of measurement roughly once per week throughout the growth season up to near pod maturity stage as: 2.9 (control), 5.7, 7.8, 10.0, 13.0, and 15.5 dS·m⁻¹. Solution pH was adjusted three times for all the 24 reservoirs in the first 2 months of growth period using concentrated H₂SO₄ and was maintained between 6.5 and 6.8 throughout the entire experiment. Plants were irrigated three times daily at 0900, 1300, and 1700 HR with a duration of 3 min each time.

MEASUREMENTS. Two plants from each sand tank were harvested at 22 to 23 d after planting (DAP) at the vegetative growth stage and at 68 to 72 DAP at pod-filling or pod growth stage. The harvested plants were immediately brought to the laboratory and their root zone up to the stem base washed with tap water and then rinsed with deionized water. The surface water was blotted dried using paper wipes (Tech Wipes; Horizon Industries, Tyler, TX). The matured pods from two plants from each tank were harvested at 117 to 122 DAP. All the samples were oven-dried at 70 °C for 7 d, then weighed.

The most recent fully expanded trifoliate leaves (two to four leaves per plant per tank) exposed to sunlight were taken at 21 DAP at the vegetative growth stage and at 67 DAP at the pod growth stage. The sampled leaves were immediately washed with deionized water and blotted dried using paper wipes (Tech Wipes), deep-frozen at -80 °C and then freeze-dried in a freeze dryer (FreeZone6; Labconco, Kansas City, MO) for 72 h. The dried samples were ground in a Wiley mill to pass a 40-mesh (0.635 mm) screen. The ground samples (0.35 mg each) were weighed and analyzed for carbon isotope abundance using an elemental analyzer (Nario Pyro Cube-Isoprime 100; Elementar America, Ronkonkoma, NY). Δ¹³ was calculated using the equation of Δ¹³ = (δ¹³_{Catm} - δ¹³_{Cplant})/(1 + δ¹³_{Catm}) according to Farquhar et al. (1989), where δ¹³_{Catm} and δ¹³_{Cplant} are the mean carbon isotopic abundances of atmospheric air and leaves, respectively, expressed in molar ratio of ¹³C/¹²C in thousandths [relative to or deviated from the international Pee Dee Belemnite standard (Craig, 1957)]. δ¹³_{Catm} has an approximate value of -8‰.

Table 1. Salinizing ion composition in millimoles of charge (mmol_c) per liter of the irrigation solutions as the salt treatments in simulating increasing salinities [measured as electrical conductivity (EC)] typical of those present in saline tailwaters encountered in the Inland Valley of southern California that typically represent concentrations of Colorado River water.^z

Targeted EC (dS·m ⁻¹)	Ca ²⁺	Mg ²⁺	Na ⁺	SO ₄ ²⁻	Cl ⁻	Achieved EC (dS·m ⁻¹)
	(mmol _c ·L ⁻¹)					
2.5	3.5	6.0	6.0	7.0	9.0	2.9
5.0	7.5	14.0	25.0	15.0	32.5	5.7
7.5	12.5	23.0	40.0	24.0	52.5	7.8
10.0	16.5	32.0	55.0	33.0	72.0	10.0
13.0	24.5	44.5	77.0	46.5	101.0	13.0
16.0	32.0	58.5	98.0	60.5	130.0	15.5

^zNot including the salt composition of the municipal tap water of City of Riverside, CA (mmol_c·L⁻¹): 3.2 Ca²⁺, 0.8 Mg²⁺, 1.7 Na⁺, 1.2 SO₄²⁻, and 0.6 Cl⁻.

Fig. 1. Correlations of leaf chlorophyll (a+b) [Chl(a+b)] with leaf SPAD meter readings of lima bean (cv. Fordhook 242), grown in outdoor sand tanks and irrigated with saline waters of the six different salinities.

The responses of A , g_s , leaf transpiration rate (Tr) to salinity, and the response of A to C_a in the chamber were measured between 0900 and 1630 HR on sunny days using a photosynthesis system (LI-6400; LI-COR Biosciences, Lincoln, NE) at the experimental sand tank site outdoors using intact leaves. All the measurements were taken on the most recent fully expanded trifoliate exposed to sunlight, and the sample size was two leaves from two plants from every one of four sand tanks for each treatment. The measurement conditions were as the following: photosynthetically active radiation, $1400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photons, provided by a red light-emitting diode source emitting at 670 nm (LI-COR Biosciences); operational or chamber ambient CO_2 concentration, $370 \mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 (for gas concentration, $\text{mol}^{-1} = \text{mol}^{-1}_{\text{air}}$ other than specifically noted); chamber air temperature, 24.0 to 32.7°C ($28.2 \pm 0.06^\circ\text{C}$), which resulted from a rough temperature control by the chamber cooling fan to blow the chamber to prevent its temperature from going too high; leaf to air vapor pressure deficit, 1.23 to 4.01 kPa ($2.32 \pm 0.02 \text{ kPa}$); sample cell relative humidity, 39% to 69% ($51\% \pm 0.16\%$, 1225 measurements $\pm 1 \text{ SE}$). Instantaneous leaf water use efficiency (WUE) was calculated using $WUE = A/Tr$.

The C_a settings stepwise from the beginning to end were $400, 300, 200, 50, 400, 550, 750, 1000, 1250, 1500$, and $1850 \mu\text{mol}\cdot\text{mol}^{-1}$, which were entered into an automatic program in the photosynthesis system (LI-6400) for A - C_i curve measurement. C_i was obtained through calculation using the measured values of A , Tr , and g_s under a given C_a by the machine according to von Caemmerer and Farquhar (1981). The execution of the automatic program brought C_a to each of the set C_a values via controlling CO_2 supply from an external CO_2 cylinder (12 g capacity). Once leaf gas exchange readings became stable, the program recorded the readings and promptly started the next measurement at the next C_a level. Usually it took 2 min for the gas exchange variables to reach a steady-state after C_a was changed (according to our observation, after lima bean leaf gas exchange reached a steady-state, further time elapse of several minutes made little change on the readings).

Leaf SPAD chlorophyll readings were taken six times across the whole leaf blade vertically and horizontally avoiding the main vein on each of the leaves for the gas exchange measurement using a chlorophyll meter (SPAD-502; Minolta, Osaka,

Japan) and the data were averaged for a leaf as an estimate of its chlorophyll content. Our meter calibration data showed that the SPAD chlorophyll readings were highly ($r^2 = 0.93$) correlated in a linear relation with dimethyl sulfoxide-extracted chlorophyll ($a + b$) concentration (extraction duration: 25 h under dark) for lima bean leaf discs (leaves sampled from the plants that received all the salt treatments) based on a method described in Chappelle et al. (1992) (Fig. 1).

ESTIMATION OF STOMATAL AND NONSTOMATAL LIMITATION. The data of each A - C_a and A - C_i curve were fitted into a non-rectangular hyperbola Eq. [1] (Jones, 1983) by a nonlinear regression fitting using Table Curve 2D (Systat Software, 2002).

$$A = d \left\{ C_{\text{CO}_2} + a - [(C_{\text{CO}_2} + a)^2 - (bC_{\text{CO}_2} - c)]^{0.5} \right\}, \quad [1]$$

where C_{CO_2} is either C_i or C_a ; a , b , c , and d are the parameters defining the response function and were found by the fitting. Rearranging Eq. [1] gives

$$C_{\text{CO}_2} = (2adA - A^2 + d^2c) / (bd^2 - 2dA) \quad [2]$$

when

$$C_{\text{CO}_2} \rightarrow \infty, A = A_{\text{max}} = bd/2 \quad [3]$$

where A_{max} is a maximum A at saturated CO_2 .

When

$$A = 0, C_{\text{CO}_2} = c/d = \Gamma \quad [4]$$

where Γ is CO_2 compensation point.

The derivative of the Eq. [1] for C_i is given as

$$\frac{\partial A}{\partial C_i} = \left\{ 2d[C_i^2 + (2a - b)C_i + a^2 + c]^{0.5} + bd - 2ad - 2dC_i \right\} / \left\{ 2[C_i^2 + (2a - b)C_i + a^2 + c]^{0.5} \right\} \quad [5]$$

using an online derivative calculator (Number Empire, 2010).

The sensitivity of A to C_i variation (g^*) was calculated from Eq. [5] using an operational value of C_i for A that was when C_a was at the ambient external CO_2 concentration ($370 \mu\text{mol}\cdot\text{mol}^{-1} \text{CO}_2$). The relative contributions of stomatal [L_g (percent)] and nonstomatal [L_m (percent)] limitations to A were given (Jones, 1985; Steduto et al., 2000) as

$$L_g = 100g^* / (g_{sc} + g^*) \quad [6]$$

$$L_m = 100 - L_g \quad [7]$$

where g_{sc} is leaf stomatal conductance to CO_2 . In the calculation, g_s for water was converted to g_{sc} using $g_{sc} = 0.625 g_s$.

The leaf carboxylation efficiency (α), was derived from Eq. [5] when $C_i = 0$, or

$$\alpha = 2d[(a^2 + c)^{0.5} + bd - 2ad] / [2(a^2 + c)^{0.5}]. \quad [8]$$

STATISTICAL ANALYSIS. All the variables were first checked for normality distribution using SAS (version 9.2; SAS

Fig. 2. Effect of irrigation water salinity on (shoot+root) dry biomass of lima bean plant (cv. Fordhook 242) at the vegetative growth stage (A), at the pod growth stage (B), and bean (pod removed) yield (C) [days after planting (DAP)]. Values are means ($n=8$ plants), and bars represent ± 1 SE. The smooth curve represents the best-fitted relationship obtained by a regression analysis of the pooled mean values.

Institute, Cary, NC) UNIVARIATE Procedure. Significance of salt effect on growth, leaf SPAD readings, leaf gas exchange variables, relative stomatal and nonstomatal limitations, and all the other related parameters/variables was analyzed taking salinity as the main effect in one-way analysis of variance at $P \leq 0.05$ using SAS GLM procedure with a standard split-plot test format. Significant differences among salinities (for SPAD reading at the pod-filling stage) were analyzed using SAS GLM procedure with Bonferroni multi comparison method at $P \leq 0.05$. The significant difference of all the tested variables between the two growth stages at each salinity were analyzed at $P \leq 0.05$ using SAS TTEST procedure. SAS statistical software package (version 9.2) was used for executing all the preceding SAS procedures. The mean regression of the vari-

ables with salinity or with g_s was performed using SigmaPlot (version 11; Systat Software, San Jose, CA).

Results

GROWTH AND BEAN YIELD. Lima bean growth and matured bean yield were significantly ($P < 0.0001$) affected by salinity (Fig. 2A–C). The total plant dry mass as shoot + root steadily decreased with increasing salinity of EC in irrigation water from 2.9 (control) to 15.5 $\text{dS}\cdot\text{m}^{-1}$. The growth and yield formation response patterns were all significantly and well correlated with EC in a linear relation having r^2 of 0.95 ($P = 0.001$) and 0.99 ($P < 0.0001$) at vegetative growth stage and pod growth stage, respectively, and of 0.99 ($P < 0.0001$) for bean yield. The EC values causing 50% reduction in maximum (EC_{50}) for total plant dry mass at the vegetative growth stage and at the pod growth stage, and for bean yield were 8.4, 7.7, and 8.0 $\text{dS}\cdot\text{m}^{-1}$, respectively. Also, the photographs in Fig. 3 clearly showed the salt response of lima bean plant growth decline at both growth stages. The plant size visually decreased with increasing salinity, and green leaves were seen on all the plants, which well represented the sand tank experimental plant growth status under the salt treatments.

LEAF GAS EXCHANGE AND Δ^{13} . Salinity significantly ($P < 0.0001$) inhibited lima bean g_s and A at the ambient CO_2 condition (Fig. 4A–D). As salinity increased from the control level of 2.9 $\text{dS}\cdot\text{m}^{-1}$ to the next high value of 5.7 $\text{dS}\cdot\text{m}^{-1}$, g_s decreased dramatically. As salinity increased further, g_s continued to decrease steadily. When the EC reached 15.5 $\text{dS}\cdot\text{m}^{-1}$, g_s dropped to the lowest observed value (Fig. 4A and B). Parallel to the g_s response, A decreased steadily from the highest to the lowest values as the salinity increased from 2.9 to 15.5 $\text{dS}\cdot\text{m}^{-1}$ (Fig. 4C and D). The response pattern of g_s and A to salinity were well described by linear relations in both the vegetative growth and pod growth stage with r^2 of 0.87 ($P = 0.006$) and 0.90 ($P = 0.004$), respectively for g_s , and of 0.96 ($P = 0.001$) and 0.98 ($P = 0.0002$), respectively, for A (Fig. 4A–D). When salinity increased to 15.5 $\text{dS}\cdot\text{m}^{-1}$ from the control level, A and g_s were reduced by 69% and 88%, respectively, at the vegetative growth stage, and by 83% and 93%, respectively, at the pod growth stage. At salinity levels of 7.8, 10.0, and 13.0 $\text{dS}\cdot\text{m}^{-1}$, g_s was significantly ($P \leq 0.05$) lower at the pod growth stage than that at the vegetative growth stage (Fig. 4A and B). Generally, no significant ($P > 0.05$) difference of A was found between the two growth stages at each salinity level except at EC 13.0 $\text{dS}\cdot\text{m}^{-1}$ ($P = 0.031$) (Fig. 4C and D). The EC_{50} values of g_s were 5.9 and 4.9 $\text{dS}\cdot\text{m}^{-1}$, and of A , 11.2 and 9.1 $\text{dS}\cdot\text{m}^{-1}$, at the vegetative growth stage and at the pod growth stage, respectively.

C_i was also significantly affected by salinity ($P < 0.0001$) at both growth stages, showing decreasing trends with increasing of salinity, which well and significantly fitted linear relations with r^2 of 0.77 ($P = 0.021$) and 0.90 ($P = 0.004$) at the vegetative growth stage and pod growth stage, respectively (Fig. 4E and F). C_i was significantly lower ($P \leq 0.05$) at the pod growth stage than at the vegetative growth stage under EC 7.8, 10.0, and 13.0 $\text{dS}\cdot\text{m}^{-1}$ (Fig. 4E and F). The decrease in A with increasing salinity did not cause a decline in WUE, instead the increasing salinity either did not significantly affect WUE ($P > 0.05$) at the vegetative growth stage or significantly increased WUE in a linear relation (r^2 of 0.88, $P = 0.006$) at the pod growth stage (Fig. 4G and H). The highest WUE occurred at a

Fig. 3. Visible growth response of lima bean (cv. Fordhook 242) plants to irrigation water salinities in electrical conductivity (EC) at the vegetative and pod growth stages [days after planting (DAP)].

high EC of 13 or 15.5 $\text{dS}\cdot\text{m}^{-1}$, which was consistently found at both growth stages (Fig. 4G and H). At salinity levels of 2.9, 7.8, 10.0, and 13.0 $\text{dS}\cdot\text{m}^{-1}$, WUE was significantly ($P \leq 0.05$) higher at the pod growth stage than that at the vegetative growth stage (Fig. 4G and H). Δ^{13} was affected significantly ($P \leq 0.05$) by salt stress at both growth stages and decreased roughly in a parallel trend with the decrease in C_i but in the opposite direction to WUE change. Δ^{13} had a significant negative linear relation to salinity with r^2 of 0.70 ($P = 0.038$) and 0.85 ($P = 0.009$) at the vegetative growth stage and pod growth stage, respectively. Δ^{13} appeared higher at the pod growth stage than that at the vegetative growth stage, but the significant difference was found only in the control group (Fig. 4I and J). Leaf chlorophyll content, measured as leaf SPAD readings, changed little with increasing salinity from the control to 15.5 $\text{dS}\cdot\text{m}^{-1}$ showing no significant ($P > 0.05$) salt effect at the vegetative growth stage (Fig. 4K). At the pod growth stage, significant ($P > 0.05$) difference of leaf SPAD for salt effect was found only between the control, 2.9 $\text{dS}\cdot\text{m}^{-1}$ and the highest EC, 15.5 $\text{dS}\cdot\text{m}^{-1}$, and leaf SPAD values were significantly ($P \leq 0.05$) higher than that at vegetative growth stage at salinity levels of 2.9, 5.7, and 10.0 $\text{dS}\cdot\text{m}^{-1}$ (Fig. 4K and L).

Apparently, the reduction in A was significantly ($P \leq 0.05$) associated with the reduction in g_s under the salt treatments in a linear relation with r^2 of 0.90 ($P = 0.004$) and 0.91 ($P = 0.003$) at the vegetative growth stage and at the pod growth stage, respectively (Fig. 5A and B). Correspondingly, Fig. 5C and D showed that C_i changed with g_s in a significant ($P \leq 0.05$) linear relation at the both growth stages with r^2 of 0.80 ($P = 0.016$) and r^2 of 0.98 ($P = 0.0001$) for the vegetative growth stage and pod growth stage, respectively. Stomatal closing may have a role in reducing C_i and thus limiting leaf CO_2 assimilation. As stomates closed or g_s decreased, leaf carbon isotope discrimination also decreased significantly, roughly in a linear relation with r^2 of 0.65 ($P = 0.054$) and 0.66 ($P = 0.050$) for the vegetative growth stage and pod growth stage, respectively (Fig. 5E and F).

STOMATAL AND NONSTOMATAL LIMITATIONS TO PHOTOSYNTHESIS. Figure 6A and B show the response of A to

the change of C_i in a wide range from the low values of C_i that brought A down to near zero to the high values of C_i that brought A up to reach a maximum at a plateau at $C_i = \approx 900 \mu\text{mol}\cdot\text{mol}^{-1}$. The data were pooled and included all the measurements on the control and salt-treated plants in the six salinities as indicated. One data set was from one leaf and was for one A - C_i curve, and all the data sets were individually well fitted into a non-rectangular hyperbola Eq. [1] with r^2 values between 0.91 and 0.99 (most r^2 values were greater than 0.98, and only one r^2 value was 0.91). The fitted equations (eight curves per treatment, one curve per leaf) were used for quantitatively estimating L_g , L_m , and the other related photosynthetic parameters.

Salinity significantly ($P < 0.0001$) increased stomatal limitation to A , which was consistently observed at the two growth stages. Figure 7A and B show that L_g (given by Eq. [6]) increased from 18% to 78% at the vegetative growth stage and from 22% to 87% at the pod growth stage as salinity increased from 2.9 to 15.5 $\text{dS}\cdot\text{m}^{-1}$. Reversely, L_m (given by Eq. [7]) decreased from 82% to 22% at the vegetative growth stage and from 78% to 13% at the pod growth stage as salinity increased from 2.9 to 15.5 $\text{dS}\cdot\text{m}^{-1}$ (Fig. 7C and D). The response pattern was well fitted into a linear relation with r^2 of 0.89 ($P = 0.005$) and 0.99 ($P < 0.0001$) at the vegetative growth stage and at the pod growth stage, respectively. At salinity levels of 7.8, 10.0, and 13.0 $\text{dS}\cdot\text{m}^{-1}$, L_g was significantly ($P \leq 0.05$) higher and L_m , significantly ($P \leq 0.05$) lower at the pod growth stage than that at the vegetative growth stage (Fig. 7A–D). No significant ($P > 0.05$) salinity effect on leaf carboxylation efficiency, α (given by Eq. [8]) was found at either growth stage. Also, no significant ($P > 0.05$) difference of α between the two growth stages was found at each salinity level except at the salinity level of 5.7 $\text{dS}\cdot\text{m}^{-1}$ ($P = 0.027$) (Fig. 7E and F).

EXTERNAL CO_2 CONCENTRATION AND PHOTOSYNTHETIC CAPACITY. Overall, Γ (given by Eq. [4]) showed an increasing trend with increasing salinity for the both growth stages and a significant linear relation was found between Γ and salinity with r^2 of 0.85 ($P = 0.009$) at the vegetative growth stage (Fig. 8A and B). Also, no significant ($P > 0.05$) difference of Γ between the two growth stages was found at each salinity level except at the salinity level of 13.0 $\text{dS}\cdot\text{m}^{-1}$ ($P = 0.05$) (Fig. 8A and B). Apparently, lima bean leaves were reaching their maximum photosynthetic capacity for carboxylation as C_a or C_i increased to saturating level. More importantly, salinity did not significantly ($P > 0.05$) affect or reduce A_{max} (given by Eq. [3]), which was observed at both growth stages. A_{max} either had a slightly increasing trend at the vegetative growth stage or showed little variation at the pod growth stage as salinity increased from the control to 15.5 $\text{dS}\cdot\text{m}^{-1}$ (Fig. 8C and D). A_{max} was lower at the pod growth stage than that at the vegetative growth stage at each salinity level but the significance ($P = 0.021$) was found only at the salinity level of 13.0

Fig. 4. Repponses of stomatal conductance [g_s (A and B)], net photosynthetic rate [A (C and D)], intercellular CO_2 concentration [C_i (E and F)], water use efficiency [WUE (G and H)], carbon 13 isotope discrimination [Δ^{13} (I and J)], and SPAD readings (K and L) of lima bean (cv. Fordhook 242) leaves to irrigation water salinity at the vegetative growth and pod growth stages [days after planting (DAP)]. Values are means (n = 8 leaves, 1 leaf per plant; except for Δ^{13} , where n = 4 plants, two to four leaves per plant), and bars represent ± 1 SE. The smooth curve represents the best-fitted relationship obtained by a regression analysis of the pooled mean values. *Indicates a significant difference ($P \leq 0.05$) of the variable between the two growth stages at a salinity level, and without this mark means there was no such a significant difference found.

dS·m⁻¹ (Fig. 8C and D). With increasing salinity from the control to 15.5 dS·m⁻¹, an external CO_2 concentration for leaves to reach 50% of its A_{max} ($C_{a50\%A_{\text{max}}}$) increased significantly ($P \leq 0.05$) and almost by twice, from 682 to 1140 and from 527 to 1074 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 at the vegetative growth stage and at the pod growth stage, respectively (Fig. 8E and F). The response pattern was also well described by a linear equation with r^2 of 0.77 ($P = 0.021$) and 0.88 ($P = 0.006$) for the vegetative growth stage and pod growth stage, respectively (Fig. 8E and F). No significant ($P > 0.05$) difference of $C_{a50\%A_{\text{max}}}$ between the two growth stages was found at each salinity

level except for the control, where $C_{a50\%A_{\text{max}}}$ was significantly ($P = 0.032$) higher at the vegetative growth stage than that at the pod growth stage (Fig. 8E and F).

Discussion

GROWTH RESPONSE AND LIMA BEAN SALT TOLERANCE. Maas and Hoffman (1977) used soil saturation extract salinity electrical conductance (ECe) to classify crop salt tolerance. We used $\text{ECe} = 0.472\text{EC}$ for the same tank culture system (Ors and Suarez, 2016) to convert the EC of irrigation water to ECe. Based on the ECe values from the conversion and the Maas and Hoffman (1977) salt response model, the lima bean growth salt response slopes were 12.5%, 13.3%, and 13.2% decrease at the vegetative growth stage, the pod growth stage, and pod maturity stage, respectively, where the slope corresponds to the yield decrease per 1 unit ECe increase. These values are all in the “moderately sensitive” category based on the chart provided by Maas and Hoffman (1977). Our results also indicated that the calculated threshold ECe at which lima bean yield decreases could be at or below 2.7 dS·m⁻¹. Thus, we define lima bean salt tolerance as moderately sensitive.

According to the determined linear salt response relations, the percent reductions in A and in biomass accumulation per unit EC increment beyond a threshold salinity level ($\text{EC} = 0$) were 4.5% and 5.9%, respectively, at the vegetative growth stage, and 5.5% and 6.5%, respectively, at the pod growth stage. As a result, the reduction in A might account for $\approx 76\%$ and 85% of growth reduction of lima bean at the vegetative stage and at the pod growth stage, respectively, under an assumption that all the leaves on the plants uniformly responded to increasing salinity. Further analysis of biomass accumulation in different parts of plants under salt stress may reveal how carbon partitioning into leaves, stems, pods, and roots might be altered in association with photosynthesis reduction under salt stress. Our hypothesis stands that lima bean growth reduction induced by salt stress was to a great extent attributed to the effect of salinity on the primary physiological performance, photosynthesis, although salt osmotic effect might directly retard cell expansion to directly reduce lima bean growth. As salt stress persisted, it appeared that reductions in growth and A could be getting worse as indicated by the observed lower A and g_s , and higher L_g as well as the apparent lower EC_{50} values of total plant biomass at the pod growth stage compared with those at the vegetative growth stage. The data of ion accumulation in the leaves of plants from the two growth stages may provide more information for our understanding of the effect of salt stress duration on lima bean growth and physiological performance (Bayuelo-Jiménez et al., 2012; James et al., 2002).

LEAF GAS EXCHANGE RESPONSE AND STOMATAL LIMITATION TO PHOTOSYNTHESIS. Our results clearly showed that lima bean A and g_s decreased steadily in parallel with increasing salinity, similar to the reported effects of salinity on photosynthesis for several crop species such as carrot (*Daucus carota*), mandarin lime (*Citrus limonia*), common bean, cowpea (*Vigna unguiculata*), cotton (*Gossypium hirsutum*), olive (*Olea europaea*), rocket (*Eruca sativa*), spinach (*Spinacia oleracea*), and tomato (*Solanum lycopersicum*) (Brugnoli and Lauteri, 1991; Centritto et al., 2003; Delfine et al., 1999; Gibberd et al., 2002; Hnilíčková et al., 2017; Melgar et al., 2008; Seemann and Critchley, 1985; Wilson et al., 2006; Wu et al., 2010). Pereira Filho et al. (2019) also reported the same response trend of lima

Fig. 5. Lima bean (cv. Fordhook 242) leaf net photosynthetic rate [A (A and B)], intercellular CO_2 concentration [C_i (C and D)], and carbon 13 isotope discrimination [Δ^{13} (E and F)] in relation to leaf stomatal conductance (g_s) at the vegetative growth and pod growth stages [days after planting (DAP)]. Values are means ($n = 8$ leaves, 1 leaf per plant), and bars represent ± 1 SE. The smooth curve represents the best-fitted relationship obtained by a regression analysis of the pooled mean values.

bean A and g_s to irrigation water salinity in a range from 1.1 to $5.1 \text{ dS} \cdot \text{m}^{-1}$. The salt treatment in our study covered a wide range of salinity that well induced a characteristic response of lima bean leaf photosynthetic performance from the observed highest at the control to the lowest at the highest treatment salinity of $15.5 \text{ dS} \cdot \text{m}^{-1}$. Under this high salinity, stomata almost completely closed with g_s values mostly $\approx 0.04 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \text{ H}_2\text{O}$. The decreased g_s could effectively reduce lima bean transpiration water loss. As a result, even though A was reduced, the leaves of salt-stressed plants could still keep the same high instantaneous WUE as that of the control plants.

In kidney bean (*P. vulgaris*) and wheat plants, it was observed that an increase in soil salinity might reduce the accumulated biomass WUE (ratio of growth biomass over the amount of water used by crop in a certain period) when salinity was greater than $4 \text{ dS} \cdot \text{m}^{-1}$ (Khataar et al., 2018). However, this might not be necessarily true for the instantaneous WUE because other components like night transpiration and dark respiration might reduce WUE to a great extent (Medrano et al., 2015; Yang et al., 2019). The reduction in transpiration water

loss may not only alleviate plants from water stress resulting from root zone salinity, but also slow down the ion transport from roots to shoots via transpiration flow, thus reducing salt amounts delivered to plants and protecting cells from ion toxicity (Acosta-Motos et al., 2017; Chaves et al., 2009; Greenway and Munns, 1980; Yang et al., 2019). The stomatal closing in reducing water loss while keeping WUE from declining appears a physiological adaptive mechanism for lima bean plants to cope with salt stress. The decrease in C_i and in Δ^{13} with decreasing g_s strongly implies that stomatal closing reduced CO_2 diffusion into lima bean leaves and caused the reduction in A .

The analysis of A - C_i curve further, for the first time (based on the results from our wide searching in the literature), revealed that the reduction in A of lima bean plants under salt stress was primarily caused by the decrease in g_s in response to increasing salinity. This was because L_g increased with increasing salinity that accounted for A reduction. Also because of the increased L_g , relative L_m decreased and became less and less important in controlling leaf photosynthesis with increasing salinity, indicating that metabolic factors might not be impaired by the salt stress. Thus, L_m had little to do with the reduction in A . This explains why carboxylation efficiency was unaffected by the increasing salinity from the control ($2.9 \text{ dS} \cdot \text{m}^{-1}$) to $15.5 \text{ dS} \cdot \text{m}^{-1}$. Stoma-

tal response appeared a key process in controlling lima bean photosynthetic performance, and the reduction in A under the salt stress was mainly due to stomatal limitation, which is well supported by our two times of observation results at the vegetative growth stage and at the pod-filling stage. This is also consistent with the results from observations on barley, common bean, cotton, olive, rice (*Oryza sativa*), and wheat, in which the salt stress affected A mainly via reducing g_s to lower down CO_2 transport from outside to leaf intercellular space and/or via reducing mesophyll conductance (g_m) for CO_2 transport from intercellular space to carboxylation site, but had little effect on their leaf biochemical capacity for A (Brugnoli and Lauteri, 1991; Centritto et al., 2003; James et al., 2002; Pérez-López et al., 2012; Wang et al., 2018). Flexas et al. (2004) pooled the reported data including many different species and origins, and different leaf habits and environmental conditions to compare their leaf capacities/activities of a series of metabolic components (initial Rubisco activity, ATP content, total soluble protein content, nitrate reductase activity) for photosynthesis based on g_s monitored under drought or salinity stress

Fig. 6. Leaf A - C_i response pattern with all the measurement data of lima bean (cv. Fordhook 242) irrigated with saline waters in six salinities at the vegetative growth stage (A) and at the pod growth stage (B) [days after planting (DAP)].

(most data came from drought stress experiments). They found that the metabolic component activities/contents started to have a steep decrease only when g_s dropped down to a certain threshold [generally lower than $0.1 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \text{ H}_2\text{O}$ (Flexas et al., 2004)].

Our results support the conclusion drawn by Flexas et al. (2004) that drought and salt stress, especially under medium-high salinity level, predominantly affect diffusion of CO_2 in leaves through a decrease of g_s and g_m , but not the biochemical capacity to CO_2 assimilation. The significantly lower g_s observed and higher stomatal limitation at the pod growth stage than that at the vegetative growth stage suggests that as salt stress is prolonged, lima bean g_s would be further reduced, imposing more stomatal limitation to A .

Δ^{13} RESPONSE IN RELATION TO STOMATAL LIMITATION. When CO_2 availability to leaves is reduced by an increased stomatal limitation, C_i/C_a may decrease. As a result, in a number of plant species under drought or salt stress, Δ^{13} was often observed to decrease with increase in the stress level (Arndt and Wanek, 2002; Brugnoli and Lauteri, 1991; Celik and Tekeli, 2017; Downton et al., 1985; Ehleringer, 1990; Shaheen and Hood-Nowotny, 2005; Veccelli and de Siqueira, 2017). This is an indication that leaf photosynthesis becomes less discriminating to carbon isotope under stress. In the case of stomatal limitation, Δ^{13} should be linearly and positively correlated with g_s (Brugnoli and Lauteri, 1991; Ehleringer, 1990), explaining our result on the significant correlation between Δ^{13} and g_s , especially when the carboxylation enzymatic system was not impaired. Under this condition, the salt-stressed leaves may demand higher CO_2 supply or higher C_a to perform the same level of photosynthesis that is performed by non-salt-stressed leaves under lower C_a . This is supported by the results that $C_{a50\%A_{\max}}$ and Γ were raised by increasing salinity. So long as there is a high CO_2 supply, the salt-stressed leaves might be capable of performing a maximum CO_2 assimilation rate equal to that a non-salt-stressed leaf commonly can achieve. It has been pointed out by Flexas et al. (2004) that virtually higher C_a might be needed for increasing the CO_2 concentration gradient to compensate for the CO_2 supply reduction owing to salt stress-caused stomata closing and thus for restoring leaf photosynthesis to non or less reduced status.

ELEVATED CO_2 AND ALLEVIATION OF SALT EFFECT. The salt stress of reducing A was overcome in olive trees by exposing

leaves to very high C_a or inducing their stomata to reopen, thus A was restored (Centritto et al., 2003). Flexas et al. (2009) reported that stomatal limitation to hybrid grape (*Vitis berlandieri* $\times$ *Vitis rupestris*) leaf photosynthesis induced by moderate or severe drought stress was eliminated by at least half after re-watering. Although there was no incidence of biochemical limitation on A in this case, the recovery of leaf photosynthesis was slow, taking 7 d. Elevated growth CO_2 was observed to be able to facilitate recovery of rice leaf photosynthetic performance from water deficit effect after reflooding (Widodo et al., 2003), to reduce negative salinity effect on reed (*Phragmites australis*) leaf

photosynthetic performance (Eller et al., 2014) and stomatal limitation on barley leaf photosynthesis (Pérez-López et al., 2012). Therefore, enhancing CO_2 supply to leaves of salt-stressed lime bean plants either by removing CO_2 diffusion resistance through reopening stomata or increasing ambient CO_2 concentration might alleviate the salt stress impact on A thus on growth and production for an improved salt tolerance. On the other hand, elevated CO_2 may reduce g_s . However, this CO_2 effect on g_s is usually relatively small compared with the stress effects on g_s (Xu et al., 2016), and thus effective alleviation of drought or salt stress effect on photosynthesis by elevated CO_2 were observed. Tezara et al. (1999) argued that high CO_2 concentrations might not be able to reverse the stress effect on A , especially when leaf photosynthetic capacity is impaired, as for the case of sunflower though an elevated CO_2 was reported to alleviate the nonstomatal limitation through increasing Rubisco carboxylation activities (Eller et al., 2014; Pérez-López et al., 2012). Elevated CO_2 alleviation effect under salt stress is scarcely reported, and different species may show different results (Xu et al., 2016). It is not easy to elevate crop field (an opening system) CO_2 concentration for a certain long period. Despite this disadvantage, the recovery response of salt stress reduced leaf photosynthesis to elevated CO_2 needs to be further investigated because the atmosphere CO_2 concentration is climbing and salt stress on crop growth is aggravating.

LEAF SPAD READING RESPONSE. No significant salt stress response found in leaf chlorophyll concentration measured as SPAD readings (in most cases) suggested that the salt stress might not cause leaf chlorophyll degradation and leaf photosynthetic processes other than CO_2 diffusion might not be affected by the salt stress (Acosta-Motos et al., 2017). Apparently or significantly higher leaf SPAD readings at the pod growth stage compared with that at the vegetable growth stage might come from the increase in specific leaf weight (SLW) as plants grew into the later stage (SLW was either significantly or appeared higher at the pod growth stage than that at the vegetative growth stage at each salinity; and averaged for all the salinities, SLW was $58.7 \text{ g} \cdot \text{m}^{-2}$ at the pod-filling stage and $51.7 \text{ g} \cdot \text{m}^{-2}$ at the vegetative stage, detailed data not shown). However, the higher SPAD readings did not bring higher A at the pod growth stage. To further explain this, information on leaf ion accumulation and chlorophyll fluorescence is needed.

Fig. 7. Responses of lima bean (cv. Fordhook 242) leaf relative stomatal limitation to photosynthesis [L_g (A and B)], nonstomatal limitation to photosynthesis [L_m (C and D)], and photosynthesis carboxylation efficiency [α (E and F)] to irrigation water salinity at the vegetative growth and pod growth stages [days after planting (DAP)]. Values are means ($n = 8$ leaves, 1 leaf per plant), and bars represent ± 1 SE. The smooth curve represents the best-fitted relationship obtained by a regression analysis of the pooled mean values. *Indicates a significant difference ($P \leq 0.05$) of the variable between the two growth stages at a salinity level, and without this mark means there was no such a significant difference found.

RELIABILITY OF $A-C_i$ CURVE ANALYSIS. $A-C_i$ curve analysis is an *in vivo* approach valuable for studying stomatal and nonstomatal limitations to photosynthesis. Its reliability can be affected mainly by two problems. One is patchy stomatal closing and the other one, the increase of the relative importance of leaf surface cuticular transpiration, both of which might cause an error in C_i calculation (Boyer et al., 1997; Cheesemann, 1991). However, as Flexas et al. (2004) pointed out, the patchiness was not a universal phenomenon based on water stress experiments and the effects of patchy-induced and cuticular-associated errors on C_i calculation were not large or significant until g_s was lower than $0.03 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \text{ H}_2\text{O}$ (Flexas et al., 2002). Our $A-C_i$ data sets were generated under conditions that g_s was greater than or equal to $0.04 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \text{ H}_2\text{O}$, which must minimize the effects from the two problems and thus very high correlation results were obtained from a nonrectangular regression between A and C_i for every $A-C_i$ curve data set. With regard to g_m , it is a component in controlling CO_2 diffusion from the substomatal cavity or intercellular space to chloroplasts. The g_m might be included in the liquid phase and the salt stress might reduce g_m to limit A (Acosta-Motos et al., 2017; Flexas et al., 2004; Loreto et al., 2003; Yang et al., 2019). In this study, the differential method was used to derive L_g and L_m , which minimizes the effect of liquid-phase CO_2 transport on the A/C_i relationship (Jones, 1985). Further research is needed to understand g_m limitation on lima bean photosynthesis and to get a complete picture of diffusive resistance limiting CO_2 uptake in lima bean plants

under salt stress. Moreover, in most cases, ignoring g_m changes might add some uncertainty only when the conclusion is that there might be an increase in nonstomatal limitation caused by stress (Chaves et al., 2009; Flexas et al., 2004), which was not the case in this study.

In conclusion, this study has provided new information on lima bean leaf gas exchange in response to saline irrigation waters. Our results from comprehensive measurements and data analysis at the vegetative and pod growth stages clearly showed that g_s was a key parameter in controlling lima bean leaf photosynthesis in response to salt stress. The reduced A by the stomatal limitation appears an important cause for the observed lima bean growth decline and yield loss under the salt stress, although likely the salt osmotic effect might also reduce lima growth to a great extent (Bayuelo-Jiménez et al., 2012; Munns and Tester, 2008). Even though g_s decreased as salinity increased, which limited photosynthesis dramatically, such response might be considered a benefit for salt-sensitive crops to deal with salt stress, minimizing leaf water use and ion uptake and thus minimizing the impairment of leaf photosynthetic apparatus. Once external CO_2 concentration is elevated or salt stress is removed, the leaves of previously salt-stressed plants may be able to perform photosynthesis at a high level, comparable to that of non-salt-stressed plants. This is a meaningful physiological mechanism providing possibilities for sustainable crop production practices to improve lima bean production and WUE in salt-affected land or in dry or semidry areas.

Fig. 8. Lima bean (cv. Fordhook 242) leaf CO_2 compensation point [Γ (A and B)], maximum leaf net CO_2 assimilation rate [A_{max} (C and D)] and external CO_2 concentration for 50% of A_{max} [$C_{a50\%A_{\text{max}}}$ (E and F)] in response to irrigation water salinity at the vegetative growth and pod growth stages [days after planting, DAP]. Values are means ($n = 8$ leaves, one leaf per plant), and bars represent ± 1 SE. The smooth curve represents the best-fitted relationship obtained by a regression analysis of the pooled mean values. *Indicates a significant difference ($P \leq 0.05$) of the variable between the two growth stages at a salinity level, and without this mark means there was no such a significant difference found.

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