Journal of Agronomy and Crop Science

J. Agronomy & Crop Science (2010) ISSN 0931-2250

SALINITY STRESS

Salt Tolerance is Associated with Differences in Ion Accumulation, Biomass Allocation and Photosynthesis in Cowpea Cultivars

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Keywords
carbon gain; gas exchange; growth and allocation; salt stress; *Vigna unguiculata*

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Accepted October 27, 2009

doi:10.1111/j.1439-037X.2009.00412.x

Abstract

Cowpea is widely cultivated in arid and semi-arid regions of the world where salinity is a major environmental stress that limits crop productivity. The effects of moderate salinity on growth and photosynthesis were examined during the vegetative phase of two cowpea cultivars previously classified as salt-tolerant (Pitiúba) and salt-sensitive (TVu). Two salt treatments (0 and 75 mM NaCl) were applied to 10-day-old plants grown in nutrient solution for 24 days. Salt stress caused decreases (59 % in Pitiúba and 72 % in TVu) in biomass accumulation at the end of the experiment. Photosynthetic rates per unit leaf mass, but not per unit leaf area, were remarkably impaired, particularly in TVu. This response was unlikely to have resulted from stomatal or photochemical constraints. Differences in salt tolerance between cultivars were unrelated to (i) variant patterns of Cl^- and K^+ tissue concentration, (ii) contrasting leaf water relations, or (iii) changes in relative growth rate and net assimilation rate. The relative advantage of Pitiúba over TVu under salt stress was primarily associated with (i) restricted Na^+ accumulation in leaves paralleling an absolute increase in Na^+ concentration in roots at early stages of salt treatment and (ii) improved leaf area (resulting from a larger leaf area ratio coupled with a larger leaf mass fraction and larger specific leaf area) and photosynthetic rates per unit leaf mass. Overall, these responses would allow greater whole-plant carbon gain, thus contributing to a better agronomic performance of salt-tolerant cowpea cultivars in salinity-prone regions.

Introduction

Salinity is a major environmental stress that limits crop productivity, particularly in arid and semi-arid regions where soil salt content is naturally high and rainfall can be insufficient for leaching salt excess. During the onset and development of salt stress within a plant, several major processes such as photosynthesis, protein synthesis and lipid metabolism may be impaired, but the earliest response is usually a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stress intensifies (Heuer 2006). Growth inhibition has been linked to both osmotic effects that provoke water deficit and specific ion effects that may cause toxicity and mineral deficiencies (Neumann 1997). Moreover, photosynthetic rates are usually lower in plants exposed to salinity and especially to NaCl, but it is still unclear whether these low rates are also responsible for the reduced growth observed in salt-treated plants. In any case, the precise mechanisms of photosynthetic limitation under saline conditions remain unresolved (Steduto et al. 2000, Heuer 2006). Overall, photosynthetic rates may decrease as a result of stomatal closure or more directly through the effects of salt on the photosynthetic apparatus (Heuer 2006). Nonetheless, there is no unified concept of the non-stomatal events that constrain photosynthesis. For example, impairments in (i) photosystem II activity (Everard et al. 1994, Neocleous and Vasilakakis 2007), (ii) chlorophyll content (Seemann and Chritchley 1985, Neocleous and Vasilakakis 2007) and (iii) the activities of key enzymes involved in the photosynthetic carbon reduction cycle (Seemann and Chritchley 1985, Reddy et al. 1992) have been reported as a consequence of salt accumulation in chloroplasts.

Cowpea [*Vigna unguiculata* (L.) Walp] is widely cultivated in arid and semi-arid regions of the world, both under irrigation and dry-farming conditions (Ehlers and Hall 1997). When the crop is grown in saline soils or irrigated with saline brackish water, its productivity often decreases (Assis Júnior et al. 2007). Cowpea cultivars showing contrasting responses to excess salts have been identified (Murillo-Amador et al. 2002a, Costa et al. 2003, Murillo-Amador et al. 2006a). Contrasting responses in terms of vegetative growth among salt-tolerant and salt-sensitive cowpea cultivars have been mostly associated with variant patterns of ion accumulation in leaves (Costa et al. 2003). However, despite some efforts in the last 20 years to understand the effects of salt stress on cowpea photosynthesis (Kannan and Ramani 1988, Larcher et al. 1990, Plaut et al. 1990, Murillo-Amador et al. 2006a, Wilson et al. 2006), it is unclear whether or not salt-induced inhibition of photosynthesis is indeed related to salt accumulation in leaves. Other important questions about the physiological and morphological bases of salt tolerance in cowpea remain to be ascertained. For example, is salt tolerance associated with differences in dry matter allocation? How do relative growth rate (RGR) and its components change in response to salt stress? How, and to what extent, do photosynthesis and associated processes contribute to decreased growth rates? Answers to these questions should expand our understanding of how to establish criteria and indices of selection for the development of more salt-tolerant cowpea genotypes. In this study, we combined detailed growth analysis, water relations, photosynthetic evaluations and ion accumulation in both leaf and root tissues using two cowpea cultivars with contrasting tolerance to salt stress.

Materials and Methods

Plant material and growth conditions

Two cowpea [*Vigna unguiculata* (L.) Walp] cultivars displaying variant tolerance to salt stress were used: Pitiúba (salt-tolerant) and TVu 2331 (salt-sensitive), as previously documented (Costa et al. 2003). Seeds were surface-sterilized in 1 % sodium hypochlorite for 5 min, followed by rinsing three times in distilled water, after which they were sown in vermiculite wetted with distilled water. Five days after sowing, uniform seedlings were transplanted into trays containing half-strength Hoagland's nutrient solution and acclimated for 5 days. Subsequently, seedlings of each cultivar were transferred to 3-l pots containing the same nutrient solution and randomly assigned to two salt treatments: 0 (control) and 75 mM NaCl (salt stress). This salt concentration was used in an attempt to create a moderate salt stress as it has been shown to cause severe growth inhibition in cowpea plants, while allowing them to complete their reproductive growth cycle (Dantas et al. 2002, Lacerda et al. 2006). Salt additions (at a rate of 25 mM per day, starting 24 h after transferring the plants to the pots) were split over time in an attempt to avoid osmotic shock. All nutrient solutions were replaced every 6 days and kept aerated. Their pH was checked daily and adjusted to 5.5 with 0.1 N NaOH or 0.1 N HCl when necessary. The amount of transpired water was checked daily by weighing the pots and was replaced with distilled water. The experiment was conducted in Fortaleza (3.74ºS, 38.58W), northeastern Brazil, under greenhouse conditions with mean mid-day photosynthetic photon flux (PPF) of about 1500 μmol m^{-2} s^{-1}, temperature of 31.2 ± 5.8 °C and relative humidity of 72 ± 10 %.

Ion determination

Leaf (the third fully expanded leaf from the apex) and root (one-third of the apical portion) samples were freeze-dried as described in Azevedo Neto et al. (2006). The lyophilized tissues (100 mg) were ground in 10 ml of distilled deionized water at 25 °C for 1 h. The resulting homogenate was centrifuged at 3000 ***g*** for 5 min. Supernatants were then filtered through a qualitative filter paper. An aliquot of this filtrate was used for Na$^+$ and K$^+$ determination by flame photometry (B-462, Micronal S/A, São Paulo, Brazil) according to Malavolta et al. (1989). Chloride (Cl$^-$) was determined as described in Gaines et al. (1984).

Growth measurements

On the day when plants were transferred to individual pots (day 0), as well as on days 3, 10, 17 and 24, five plants per cultivar and treatment were harvested and separated into leaves, roots and stems. Leaf area was measured using an LI-3000 leaf area metre (LI-COR, Inc. Lincoln, NE, USA), and the dry mass (DM) of the plant parts was determined in oven-dried tissues at 70 °C for

Table 1 Three-way analysis of variance (F values) of the effect of cultivar (CV), treatment (TR) and time on ion accumulation (Na^+, Cl^-, and K^+), growth and allocation traits (TB, total biomass; LA, leaf area; RGR, relative growth rate; NAR, net assimilation rate; LAR, leaf area ratio; SLA, specific leaf area; LMF, leaf mass fraction; SMF, stem mass fraction and RMF, root mass fraction), photosynthesis traits (A_{area} = rate of net photosynthesis per unit leaf area, A_{mass} = rate of net photosynthesis per unit leaf mass, g_s = stomatal conductance, C_i/C_a = internal-to-ambient CO_2 concentration ratio (C_i/C_a), and F_v/F_m = variable-to-maximum fluorescence ratio), and water relation traits (RWC, relative water content; and Ψ_s, osmotic potential)

Traits	CV	TR	Time	CV × TR	CV × Time	TR × Time	CV × TR × Time
Na^+_{leaf}	0.0 (ns)	53.0**	8.0**	1.3 (ns)	4.7**	3.1*	0.1 (ns)
Cl^-_{leaf}	0.5 (ns)	359.6**	26.1**	2.0 (ns)	1.9 (ns)	11.4**	1.2 (ns)
K^+_{leaf}	45.4**	1.7 (ns)	58.5**	2.3 (ns)	5.8 (ns)	9.5**	3.5*
Na^+_{root}	28.2**	450.8**	4.0**	6.3**	0.5 (ns)	7.1**	1.1 (ns)
Cl^-_{root}	0.6 (ns)	660.9**	21.8**	0.4 (ns)	5.9**	6.5**	6.3**
K^+_{root}	56.3**	43.2**	21.5**	0.5 (ns)	7.7**	4.1**	1.4 (ns)
TB	6.2*	139.2**	687.3**	0.6 (ns)	2.0 (ns)	26.1**	1.9 (ns)
LA	81.2**	407.7**	603.3**	11.2**	20.1**	51.2**	6.7**
RGR	2.7 (ns)	45.0**	112.9**	0.7 (ns)	1.6 (ns)	8.5**	1.5 (ns)
NAR	0.0 (ns)	8.0**	36.5**	0.0 (ns)	2.5 (ns)	3.4*	2.7*
LAR	135.7**	174.1**	125.2**	21.6**	38.6**	10.1**	6.6**
SLA	108.5**	150.6**	9.3**	9.1**	15.5**	5.5**	0.7 (ns)
LMF	1.0 (ns)	0.1 (ns)	108.6**	7.3**	3.3*	0.7 (ns)	2.5 (ns)
SMF	5.1*	4.7*	152.1**	6.2**	6.0**	3.4*	0.1 (ns)
RMF	0.6 (ns)	35.3**	21.7**	8.8**	22.1**	3.1*	1.2 (ns)
A_{area}	9.2**	11.2**	7.7**	2.2 (ns)	0.4 (ns)	2.0 (ns)	0.4 (ns)
A_{mass}	108.2**	449.2**	5.5**	24.2**	12.8**	8.1**	0.7 (ns)
g_s	0.1 (ns)	100.6**	20.3**	0.4 (ns)	2.3*	8.5**	1.4 (ns)
C_i/C_a	4.6*	12.0**	9.5**	0.6 (ns)	0.5 (ns)	1.6 (ns)	0.9 (ns)
F_v/F_m	3.9*	0.2 (ns)	7.5**	0.2 (ns)	0.6 (ns)	2.3*	1.0 (ns)
RWC	0.0 (ns)	85.7**	30.1**	2.8 (ns)	3.9*	11.2**	10.9**
Ψ_s	2.9 (ns)	112.2**	22.0**	0.9 (ns)	1.5 (ns)	1.7 (ns)	1.8 (ns)

CV, cultivar; TR, treatment; TB, total biomass; LA, leaf area; RGR, relative growth rate; NAR, net assimilation rate; LAR, leaf area ratio; SLA, specific leaf area; LMF, leaf mass fraction; SMF, stem mass fraction; RMF, root mass fraction; RWC, relative water content.

Level of significance: $^*P \leq 0.05$; $^{**}P \leq 0.01$; absence of an asterisk denotes a non-significant (ns) effect.

72 h. Based on these data, the following growth traits were calculated: total biomass (TB), leaf mass fraction (LMF), specific leaf area (SLA), leaf area ratio (LAR), stem mass fraction (SMF), root mass fraction (RMF), RGR and net assimilation rate (NAR, on a leaf area basis), as described previously (Dias et al. 2007).

Water relations

Ten leaf discs (1 cm diameter) per replicate, collected at 8:00 a.m. from the second fully expanded leaves (from the top), were used for quantifying the relative water content [RWC; Čatský (1960)]. For osmotic potential (Ψ_s) estimations, 10 leaf discs per replicate were also collected, flash frozen in liquid nitrogen and kept at −25 °C until use. Cell sap was obtained by hand-pressing leaf tissues (enclosed in a piece of muslin cloth) using a syringe, and then a 10 μl aliquot was brought into a microosmometer (Vapro 5520, Wescor, Logan, USA) for Ψ_s measurements. These parameters were evaluated at 3, 10, 17 and 24 days after treatment imposition.

Photosynthetic variables

The rate of net carbon assimilation per unit leaf area (A_{area}), stomatal conductance to water vapour (g_s) and internal-to-ambient CO_2 concentration ratio (C_i/C_a) were measured at 08:00–09:00 h under artificial, saturating PPF (1200 μmol m^{-2} s^{-1}) with a portable open-system infrared gas analyzer (LCi, ADC, Hoddesdon, UK). Chlorophyll (Chl) *a* fluorescence was measured in dark-adapted (30 min) leaves with a portable fluorometer (Plant Efficiency Analyzer, Hansatech, King's Lynn, Norfolk, UK) at a PPF of 1500 μmol m^{-2} s^{-1} for 10 s with an emission peak of 650 nm. The initial and maximum Chl *a* fluorescence were measured, and from these values the variable-to-maximum fluorescence ratio (F_v/F_m) was calculated. This ratio has been used to express the maximum PSII photochemical efficiency. Gas exchange and Chl *a* measurements were made regularly until 23 day from the start of salt additions (DSA). The net carbon assimilation per leaf dry matter (A_{mass}) was estimated as the product of A_{area} and SLA (in this case,

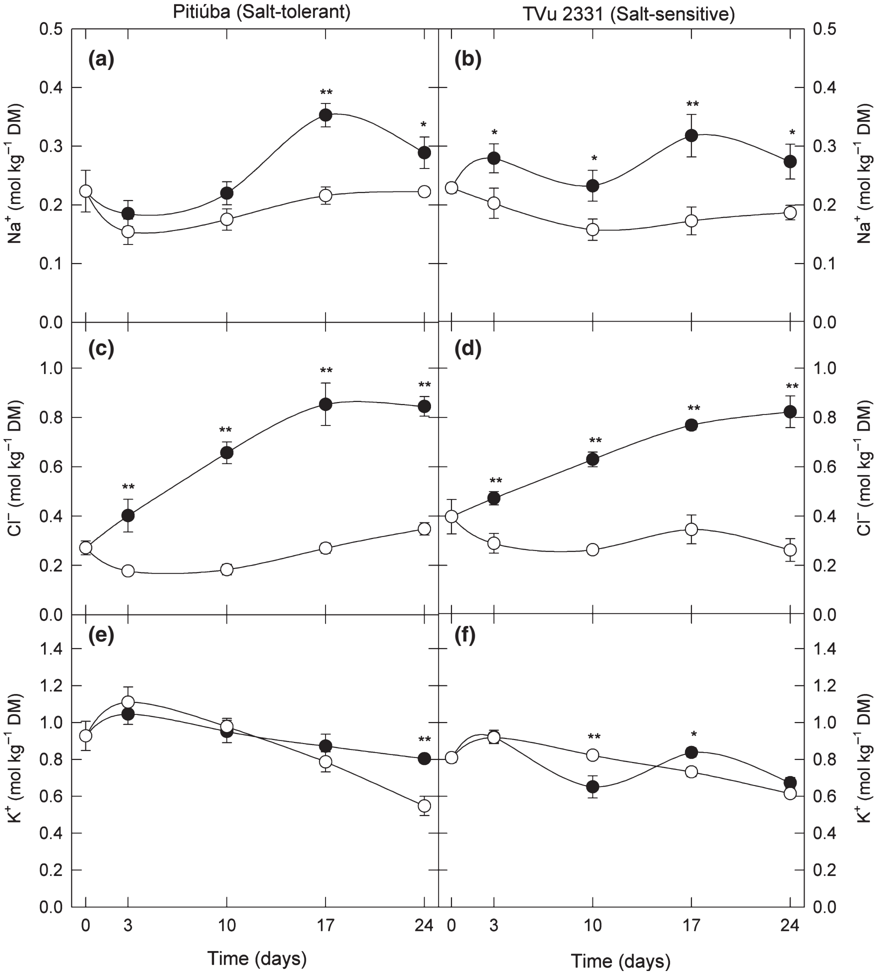

Fig. 1 Leaf concentration of Na^+ (a, b), Cl^- (c, d) and K^+ (e, f), at different times from the start of salt addition, of two cowpea cultivars grown in nutrient solution containing 0 (control, o) or 75 (salt stress, •) mm NaCl. Each point is the mean ± S.E. of five replicates. Statistical differences between treatments are denoted by $*P \le 0.05$ and $**P \le 0.01$ (Student's *t*-test).

SLA was estimated through regression analysis as growth and photosynthetic measurements were performed on different days).

Experimental design and statistical analysis

The plants were distributed over a completely randomized single-plant plot design, with four treatment combinations (two cultivars and two salt treatments) and five replicates. For ion content data, each replicate represented the mean of three determinations. Differences in morphological/physiological traits among cultivars, salt treatments and time were tested by three-way analysis of variance (using ln-transformed dry mass or leaf area data). The Student's *t*-test was used to compare differences in salt treatments at the same DSA, and the Pearson product moment correlation was calculated for some variables. All data were computed using the electronic data sheet Gnumeric 1.8.2 in a Linux platform, according to Sokal and Rohlf (1988).

Results

Ion accumulation

Salt stress affected the concentration of ions in leaves and particularly in roots (Table 1). In leaves, Na^+ concentration (Fig. 1a,b) began to increase earlier (at 3 DSA) in TVu 2331 (hereafter referred to as TVu) than in Pitiúba (17 DSA) (significant cultivar × time and stress × time interactions). In roots (Fig. 2a,b), a rise in Na^+ concentration was evident from 3 DSA onwards in both cultivars under salt stress, with greater absolute concentrations in Pitiúba than in TVu (significant cultivar × stress and stress × time interactions). Salt stress caused an augmentation of Cl^- concentration, which was similar, regardless

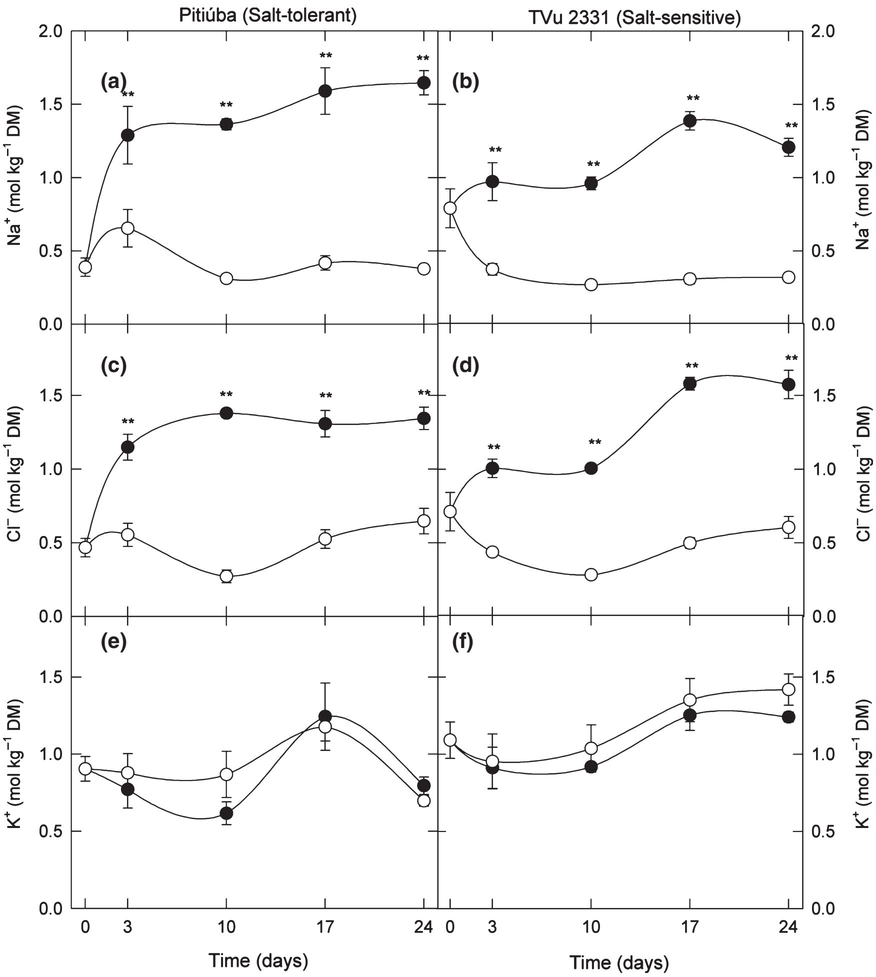

Fig. 2 Root concentration of Na^+ (a, b), Cl^- (c, d) and K^+ (e, f), at different times from the start of salt additions, of two cowpea cultivars grown under control (o) and salt stress conditions (•). See further details in legend to Fig. 1.

of cultivar, in both leaves (Fig. 1c,d) and roots (Fig. 2c,d) from 3 DSA onwards. Salt additions were accompanied by a significant increase in K^+ concentration in Pitiúba leaves at the end of the experiment (thus leading to a decrease in Na^+/K^+ ratio; data not shown). In TVu, no consistent treatment effect on leaf K^+ concentration could be found (Fig. 1e,f). Overall, root K^+ concentration tended to be lower in salt-treated plants than in control plants throughout the experimental period, although it did not differ significantly at the end of the experiment irrespective of cultivar, when the absolute values of root K^+ concentration were larger in TVu than in Pitiúba independent of treatments (Fig. 2e,f).

Plant growth and biomass allocation

Overall, salt stress strongly impaired biomass accumulation, although this effect was similar in either cultivar as indicated by the non-significant cultivar × stress interaction for the ln-transformed data of plant dry mass (Table 1). Significant decreases in biomass accumulation were evident from 17 DSA onwards. At the end of the experiment, biomass had decreased by 59 % in the salt-tolerant Pitiúba and by 72 % in the salt-sensitive TVu, as compared with their respective control plants (Fig. 3a,b). In any case, Pitiúba accumulated more biomass (40 %) than TVu under salt conditions. Salt stress also led to decreases in RGR (Fig. 3e,f), but this effect was independent of cultivar and time, as indicated by the non-significant cultivar × stress and cultivar × time interactions (Table 1). Despite the fact that both the morphological (LMF and SLA, and thus LAR) and physiological (NAR) components of RGR changed with time, only the former varied between cultivars (Table 1, Figs 3 and 4). In fact, regardless of treatments and cultivar, significant relationships could be observed between RGR and NAR, as well

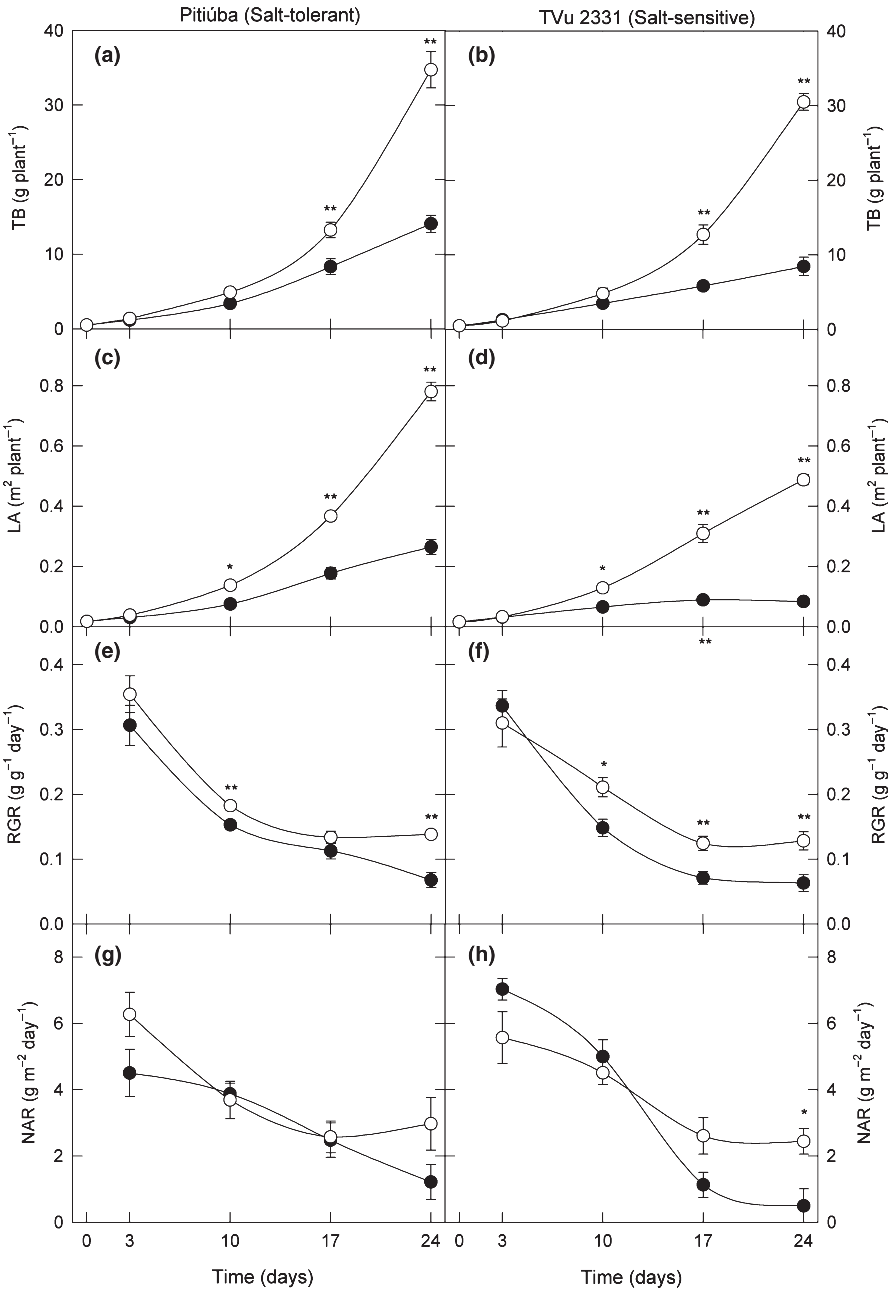

Fig. 3 Growth traits, i.e. total dry biomass (TB; a, b), total leaf area (LA; c, d), relative growth rate (RGR; e, f) and net assimilation rate (NAR; g, h) at different times from the start of salt additions, of two cowpea cultivars grown under control (o) and salt stress conditions (•). See further details in legend to Fig. 1.

as between RGR and LMF (Table 2). The correlation between RGR and LAR was also significant, with the exception of Pitiúba grown under control conditions. In this case, both NAR and LMF had a positive effect on RGR; however, a decrease in LMF was accompanied by an unexpectedly slight increase in SLA (see Fig. 4c,e), resulting in a non-significant correlation of RGR with LAR (Table 2).

Biomass allocation patterns changed remarkably with time and varied between cultivars and treatments. This could be inferred from the significant cultivar × stress, cultivar × time and time × stress interactions for LAR, SLA, LMF, RMF and SMF, although the last interaction was not significant for LMF (Table 1). Changes in biomass allocation started to occur earlier in Pitiúba than in TVu. At 10 and 17 DSA, Pitiúba allocated proportionately

Table 2 Correlation coefficients for relationships between relative growth rate (over time) and net assimilation rate (NAR), leaf area ratio (LAR), LMF (leaf mass fraction) and specific leaf area (SLA) of two cowpea cultivars grown in nutrient solutions containing 0 (control) or 75 (salt stress) mm NaCl. (n = 20)

	Relative growth rate			
	Pitiúba		TVu	
Variables	Control	Stress	Control	Stress
NAR	0.68**	0.70**	0.54 *	0.84**
LAR	0.35 (ns)	0.86**	0.66**	0.83**
LMF	0.69**	0.73**	0.70**	0.69**
SLA	–0.60**	0.16 (ns)	0.40 (ns)	0.77**

TVu, salt-sensitive; NAR, net assimilation rate; LAR, leaf area ratio; LMF, leaf mass fraction; SLA, specific leaf area.
Level of significance: *P ≤ 0.05; **P ≤ 0.01; absence of an asterisk denotes a non-significant (ns) correlation (P > 0.05).

more biomass to roots and less to stems. At the end of the experiment, significant changes were found for SMF and LMF, which were respectively lower and larger in salt-treated than in salt-untreated plants (Fig. 4). In TVu, LMF tended to be lower in stressed plants with an opposite trend for RMF, whereas SMF was unresponsive to the treatments. From 17 to 24 DSA, SMF increased steeply at the expense of reductions in RMF and LMF irrespective of treatment (Fig. 4).

As a common response, salt stress led to decreases in both leaf area (Fig. 3c,d) and SLA (Fig. 4c,d) in both cultivars, which started at 10 DSA. It is noteworthy, however, that whereas the rate of leaf area gain was positive from 10 DSA onwards in Pitiúba, it was negligible in TVu, as can be deduced from Figure 3c,d. This difference was associated with (i) a trend of lower LMF coupled with lower SLA in TVu (as a consequence, LAR decreased progressively in TVu, but remained fairly constant in Pitiúba over time) (Fig. 4) and (ii) an intense leaf senescence in TVu, but not in Pitiúba, as was particularly observed at the end of the experiment (data not shown). At this time, salt-induced decreases in total leaf area were 66 and 83 % in Pitiúba and TVu, respectively, but the absolute values of leaf area in the salt-stressed plants were approximately two times greater in Pitiúba. It should be mentioned that leaf area was 60 % larger in unstressed plants of Pitiúba than in control individuals of TVu (Fig. 3c,d).

Water relations

Salt increments led to similar decreases in RWC and Ψ_s in both cultivars (Table 1, Fig. 5). However, regardless of cultivar, salt stress did not affect RWC at the end of the experiment; in Pitiúba, the effects of lack of salt on RWC were even evident from 17 DSA onwards (Fig. 5).

Gas exchange

The rate of net CO_2 assimilation per leaf area (A_{area}) varied between cultivars, salt treatments and sampling dates, but no significant interaction between these factors could be detected (Table 1). Nonetheless, salt addition impaired A_{area} only marginally, although this effect was apparently more pronounced in TVu than in Pitiúba (Fig. 6a,b). By contrast, salt stress caused remarkable reduction in the rate of net CO_2 assimilation per leaf mass (A_{mass}), which decreased earlier after salt additions and to a greater extent in TVu than in Pitiúba (Fig. 6c,d). The significant interaction effects for A_{mass} largely reflected differences in SLA (Table 1). However, the mean percentage decreases in SLA from 3 DSA onwards (19 % in Pitiúba and 28 % in TVu) were lower than those in A_{mass} (22 % in Pitiúba and 38 % in TVu).

Salt stress strongly reduced the stomatal conductance (g_s) of both cultivars, which started to occur from 5 and 8 DSA onwards in Pitiúba and TVu respectively (Fig. 6g,h). In any case, no significant cultivar effect for g_s could be detected (Table 1). The internal-to-ambient CO_2 concentration ratio (C_i/C_a) was slightly affected (it did not decrease below 0.7) by salt stress in TVu, but that ratio tended to be lower in salt-treated Pitiúba, as found from 17 DSA onwards when the C_i/C_a ratio was significantly lower in these plants as compared with their control counterparts (Fig. 6e,f).

Discussion

In this study, we analysed the effects of moderate (non-lethal) external salinity on growth and photosynthesis during the vegetative stage of two contrasting cowpea cultivars. Our results showed that improved salt tolerance may be primarily associated with restricted Na^+ accumulation in leaves, as had already been reported for cowpea (Murillo-Amador et al. 2002b, Costa et al. 2003), paralleling an absolute larger Na^+ concentration in roots, as shown for Pitiúba. Some mechanisms, such as reduced Na^+ delivery to the shoot, tissue- and organ-specific Na^+ compartmentation and recirculation of Na^+ from the leaves back to the roots (Shabala and Cuin 2008) may be involved in this response. In any case, no evidence that salt tolerance in cowpea is related to differences in Cl^- accumulation was found, contrary to what was previously documented by Murillo-Amador et al. (2002b, 2006a). Furthermore, we found no evidence that differences in bulk tissue K^+ concentration contribute to salt tolerance, as has been often reported (Shabala and Cuin 2008). Moreover, differential physiological responses between cultivars were unlikely to result from differences in leaf water relations, as judged from the similar values of RWC

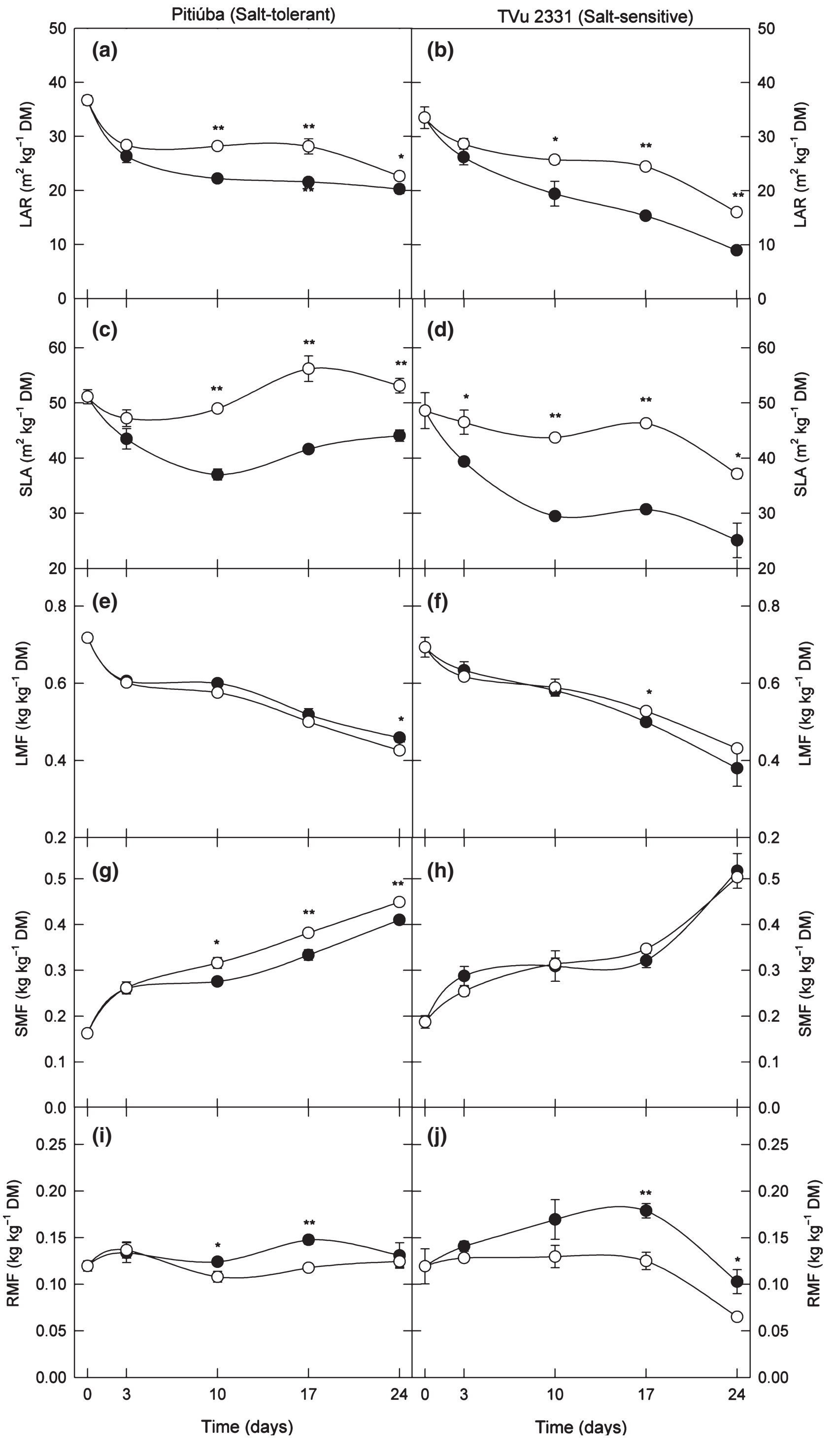

Fig. 4 Allocation traits, i.e. leaf area ratio (LAR; a, b), specific leaf area (SLA; c, d), leaf mass fraction (LMF; e, f), stem mass fraction (SMF; g, h) and root mass fraction (RMF; i, j) at different times from the start of salt additions, of two cowpea cultivars grown under control (o) and salt stress conditions (•). See further details in legend to Fig. 1.

and Ψ_s in salt-treated plants, differently from the related species *Vigna radiata* 24 h after salt stress (Panda and Khan 2009).

Changes in growth rates were mainly attributable to variations in both physiological (NAR) and morphological (LMF, but not necessarily LAR) components of RGR under control conditions, and this response persisted under saline conditions. Compared with TVu, salt-stressed Pitiúba plants showed an improved biomass allocation to leaves probably at the expense of a lower

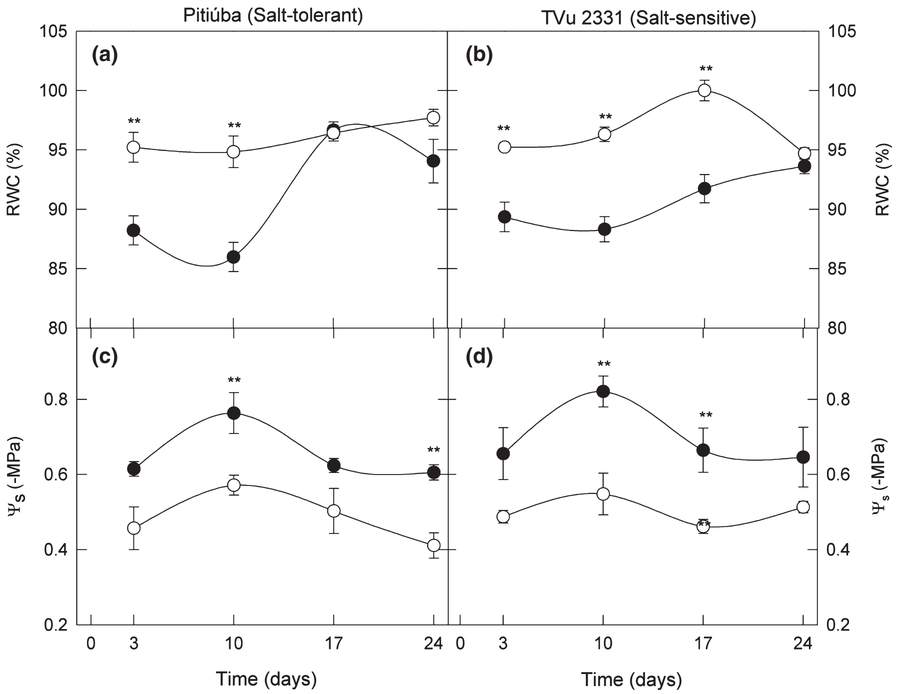

Fig. 5 Leaf relative water content (RWC; a, b) and osmotic potential (Ψ_s; c, d), at different times from the start of salt additions, of two cowpea cultivars grown under control (o) and salt stress conditions (•). See further details in legend to Fig. 1.

allocation to support structures (i.e. lower SMF). In addition, the larger SLA of Pitiúba implies that properties of their leaves were altered by salt stress in such a way that they (i) would allow lower self-shade per leaf area unit and (ii) would create a relatively short path from stomata to chloroplasts over which CO_2 must diffuse (Flexas et al. 2008). Overall, these morphological changes would improve photosynthetic rates on a whole-plant level. Moreover, differences in biomass allocation may play a significant role under field conditions as maintenance of leaf area is crucial for allowing good crop yield in salt-treated plants (Heuer 2006).

The minor effects of salt stress on A_{area} must be thoroughly investigated. As SLA was reduced, a greater amount of photosynthetic machinery per unit leaf area is to be expected, which may potentially mask any decreases in A_{area}. In fact, the decline in A_{mass}, which was only partially explained by decreases in SLA, suggests that the capacity for mesophyll cells to fix CO_2 was impaired by salt stress. This is unlikely to have been associated with photochemical constraints because the F_v/F_m ratio was unresponsive to salt stress (data not shown) or with stomatal limitations to photosynthesis because : (i) regardless of treatment, photosynthetic rates and g_s were significantly unrelated to one another ($P > 0.05$; data not shown), possibly because the lowest value of g_s ($\sim$0.5 mmol m^{-2} s^{-1}) that we measured was relatively elevated and might sustain an adequate CO_2 flux into leaves (see Flexas et al. 2006); (ii) the largest percentage decreases in g_s occurred only in Pitiúba in which photosynthesis was less impaired than in TVu under salt conditions; and (iii) the C_i/C_a ratio was kept at high values, paralleling the relatively high g_s in salt-treated plants. However, these findings are in disagreement with interpretations of several investigators (e.g., Everard et al. 1994, Netondo et al. 2004, Debez et al. 2006, Wilson et al. 2006, Zhao et al. 2007, Youssef and Awad 2008) who reported that stomatal closure is a prime constraint to photosynthesis by limiting CO_2 flux into leaves in salt-stressed plants. Nonetheless, our results are similar to those reported by Murillo-Amador et al. (2006b) who, also studying cowpea plants, did not find significant effects of salt stress on g_s, transpiration rate, net photosynthesis or intercellular CO_2. In this study, we cannot dismiss a possible negative effect of mesophyll conductance (g_m) on CO_2 flux into the carboxylation sites (g_m often decreases in salt-stressed; Flexas et al. 2008). In addition, impairments in photosynthetic capacity should also be related to biochemical limitations to photosynthesis at the chloroplast level. In any case, the significant cultivar effect on A_{mass} and the significant cultivar × salt interaction effect on A_{mass} suggest that differences in salt tolerance among cowpea cultivars might be partially associated with differences in photosynthetic performance, contrary to what was reported earlier for this species (Wilson et al. 2006).

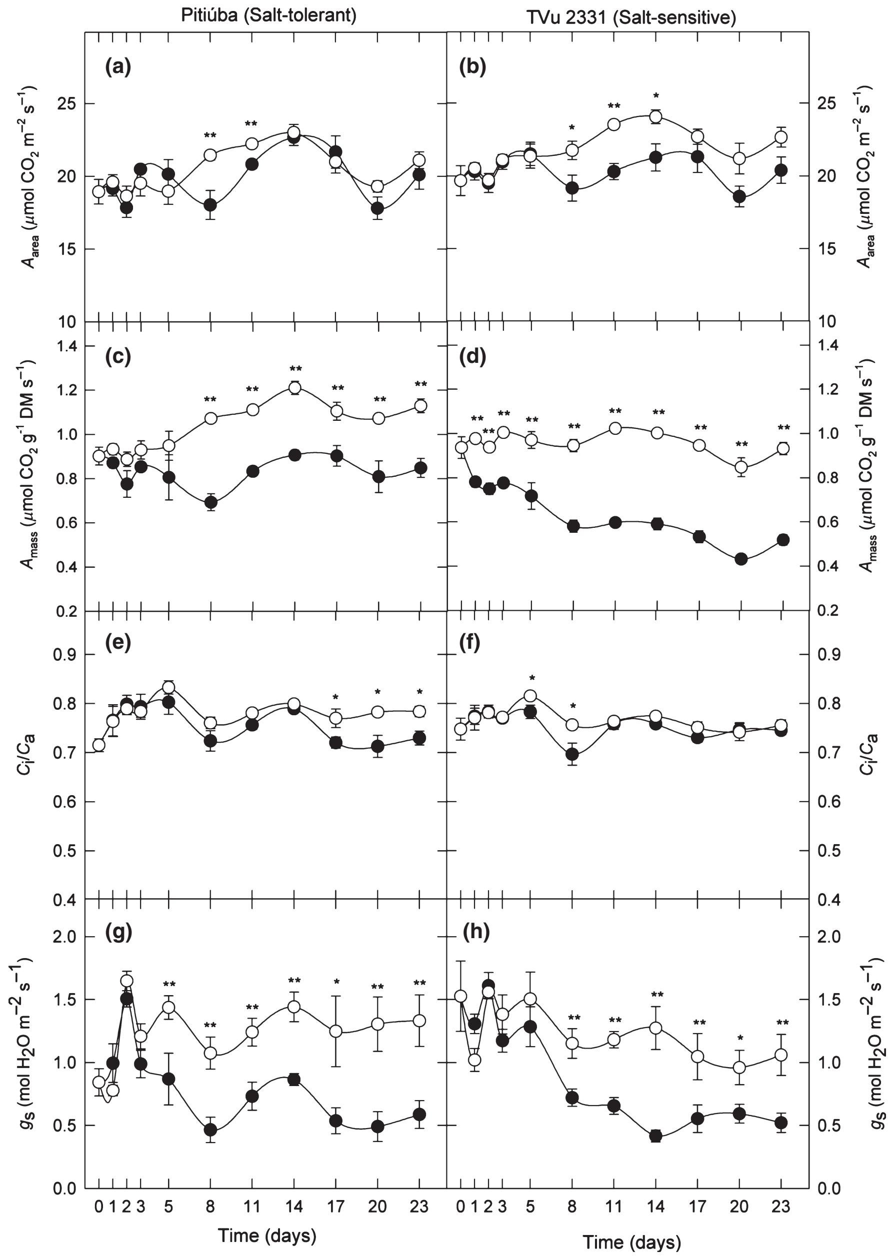

Fig. 6 Net CO$_2$ assimilation rate per leaf area (A_{area}; a, b), net CO$_2$ assimilation rate per leaf dry mass (A_{mass}; c, d), internal-to-ambient CO$_2$ concentration ratio (C_i/C_a; e, f) and stomatal conductance (g_s; g, h), at different times from the start of salt additions, of two cowpea cultivars grown under control (o) and salt-stress conditions (•). See further details in legend to Fig. 1.

In summary, we conclude that differences in salt tolerance between cowpea cultivars under the present experimental conditions were unrelated to changes in RGR and NAR, but could be associated with maintenance, within given limits, of favourable morphological (LAR and its components – LMF and SLA) and physiological (A_{mass}) traits. This is possibly related to lower leaf Na$^+$ accumulation, as shown for Pitiúba, under salt conditions. Overall, these responses, coupled with lower rates of leaf senescence, would allow improved maintenance of leaf area and greater whole-plant carbon gain, thus contributing to a better agronomic performance of salt-tolerant cowpea cultivars under salt-stress conditions (Assis Júnior et al. 2007). In conclusion, although our results may be useful in elucidating physiological mechanisms associated with salt stress, which are relevant in cowpea breeding programmes, field research under representative saline environments must be conducted not only to validate our findings, but also to create some benefit to the cowpea growers in salinity-prone regions.

Acknowledgements

The authors are thankful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Instituto Nacional de Ciência e Tecnologia em Salinidade (INCTSal) for financial support and to the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) for a scholarship to S.C. Praxedes during his PhD studies.

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