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Salinity and Temperature Effects on CO_2 Assimilation in Leaves of Avocados

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Abstract: Young avocado plants were cultured in a glasshouse and subjected to salt stress or were well watered at Maseno University between September 2003 and September 2004. Three-month-old avocado seedlings were grown in 4.5 L pots containing soil and daily irrigated with 300 mL of NaCl concentrations of 0 (control), 15, 30, 45 and 60 mM NaCl. Growth conditions in the greenhouse were: temperature: min/max 20/40°C and relative humidity: min/max 50/95%. The data collected included shoot height, number of leaves per plant, stem diameter growth, root fresh and dry weight, shoot fresh and dry weight, chlorophyll concentration and gas exchange parameters. There were significant differences in growth parameters measured except plant height. A slight increase in shoot height and stem diameter was observable at low salt concentrations (15 and 30 mM NaCl) within the first sixteen days of salt application. Chlorophyll concentration decreased with increasing salt concentration of the growth medium. Photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO_2 concentration decreased significantly with increasing salt concentration. The decrease in photosynthetic rate was only partially to stomatal closure. Salt stress was found to cause irreversible and visible damage, such as leaf tip burn, leaf scorching and death of the plants.

Key words: Chlorophyll, dry biomass, temperature, NaCl

INTRODUCTION

Plants vary widely in their salinity tolerance. Exposure of plants to high salt levels results in oxidative damage and ion toxicity. Avocado (*Persea americana* Mill.) is a salt sensitive tree crop (Hofshi, 1998; Shalhevet, 1999; Micklebart and Arpaia, 2002) and is often grown in areas of relatively low rainfall and saline soils (Micklebart and Arpaia, 2002). It is specifically sensitive to chloride toxicity with some rootstocks, notably Mexican, being more sensitive than others (Guatemalan and West Indian). Avocado culture has markedly benefited by grafting on salt resistant rootstocks and has opened many otherwise unsuited regions for cultivation (Oster and Arpaia, 1992; Mckersie and Leshem, 1994). Salinity is the second greatest limiting factor to avocado growth and yield (Hofshi, 1998; Ashraf *et al*., 2002). Three major hazards are associated with salinity; osmotic stress, ion toxicity and mineral deficiencies (Reinhardt and Rost, 1995; Belkhodja *et al*., 1999; Hasegawa *et al*., 2000; Netondo *et al*., 2004a). There are very few scientific studies on the physiological and growth responses of avocado seedlings to salinity, especially for soil-grown seedlings, yet growth media and environmental factors can influence salt effects on root systems (Reinhardt and Rost, 1995). It has also been established that plant ecological adaptations vary with the physiological age of the plant organs and their developmental history (Fetene *et al*., 1997). The objective of this study was to determine the influence of salinity on growth and gas exchange in leaves of young avocado over a range of temperature and salinity levels.

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MATERIALS AND METHODS

Plant Material and Growth Conditions

The study was conducted in a glasshouse at Maseno University, between September 2003 and September 2004. Day per night temperatures were maintained at 35/25±5°C, min/max relative humidity maintained at 50/95% and no supplemental lighting was provided. The local soils are well drained, acidic with high extractable Ca and K. Soil organic carbon and phosphorus content are 1.8% and 4.5 mg kg^{-1}, respectively. The pH of the soil ranges between 4.6 and 5.4. Three-month-old avocado plants (Persea americana Mill.) were transplanted in 4.5 L plastic pots filled with local soil. Diammonium phosphate (DAP) fertilizer was applied as recommended for three-month-old avocado plants. Plants were subjected to five salinity levels (0 (control), 15, 30, 45 and 60 mM NaCl) and were daily irrigated with 300 mL of NaCl of each concentration administered in a step-wise fashion, adding daily increments of 15 mM until the desired concentration was reached. The abbreviations S0, S1, S2, S3 and S4 were used to denote the salinity levels of 0 (control), 15, 30, 45 and 60 mM NaCl respectively. The pots were arranged in a Completely Randomized Design (CRD) on a bench. The application rate of saline solution was adequate to ensure more than 30% drainage of applied solution through perforations at the bottom of the pots. Weeds and pests were controlled using recommended standard cultural methods. The experiments were repeated twice with four replicates of each treatment.

Growth Parameters

Data for growth and gas exchange parameters were recorded once every week before and after the commencement of the salinity treatments. Shoot height was measured using a meter rule, from the stem base up to the shoot apex. The number of fully expanded mature leaves per plant on the main stem and branches were counted and recorded once every week. The change in growth of the stem diameter was determined in each plant by measuring the diameter at a height of 10 cm from the stem base using a vernier caliper. At the end of the experiment the plants were harvested and their roots and shoot regions separated. Roots were rinsed in tap water after soaking, then were blotted dry on paper towels and fresh weights were determined. The roots and shoots were then oven-dried at 65°C to constant dry weights, for at least 48 h, after which their dry weights were determined.

Chlorophyll Content

The fourth fully expanded leaves were harvested at the end of the experiment. The Chlorophyll concentration was determined in 80% acetone extract on a spectrophotometer (Model Novaspec II). Total chlorophyll, Chl a and Chl b were calculated by the equations of Arnon (1949).

Gas Exchange Measurements

An open portable infrared gas analyzer system (CIRAS-1, PP Systems, Stotfield, Hitchin, Herts, UK) was used to measure gas exchange and leaf temperature. Gas exchange was determined from an area of 2.5 cm^2 of the fully expanded sun-exposed fifth leaf (from the shoot apex) of the plant in each treatment between 0900 and 1230 h. Photosynthetic rates were measured at 26 to 37°C. Air flow rate through the cuvette was 200 mL min^{-1}. Ten consecutive measurements were taken at 3 sec intervals. Measurements were made inside the glasshouse commencing on the seventh day after initiation of salt treatment and were done once per week.

Statistical Analysis

Data were subjected to analysis of variance using the general linear model of statistical analysis systems. Statistical significance, where, indicated is at least at 0.05 or 0.001 level determined by analysis of variance.

RESULTS

Growth and Chlorophyll Content Analysis

Shoot heights growth increased within the first 16 days after initiation of saline water irrigation at 15 mM NaCl concentration level. There were significant ($p \leq 0.05$) differences in shoot heights as a result of the interaction of salinity treatment and duration of salt exposure. Shoot heights did not differ significantly ($p \geq 0.05$) among treatments. Salinity showed a tendency to influence shoot height growth, though shoot height growth decreased at 60 mM NaCl, by about 82% of control plants (Fig. 1). Analysis of variance of data for leaf number per plant showed that NaCl in the rooting medium had adverse effects on leaf growth and initiation of new leaves. Significant ($p \leq 0.05$) differences in leaf number between treatments occurred especially from day 23 to day 40. Decline in this parameter was not consistent with salinity treatments throughout the experimental period. Low salinity levels initially stimulated leaf growth in the first 16 days, followed by a gradual decline (Fig. 2). At 60 mM NaCl concentration, the leaf growth declined much more severely at about 58% of control plants. Leaf tip burn and leaf chlorosis were also visible. Stem diameter increased constantly within the first sixteen days for the 15, 30 and 45 mM NaCl treatments levels after initiation of NaCl irrigation (Fig. 3).

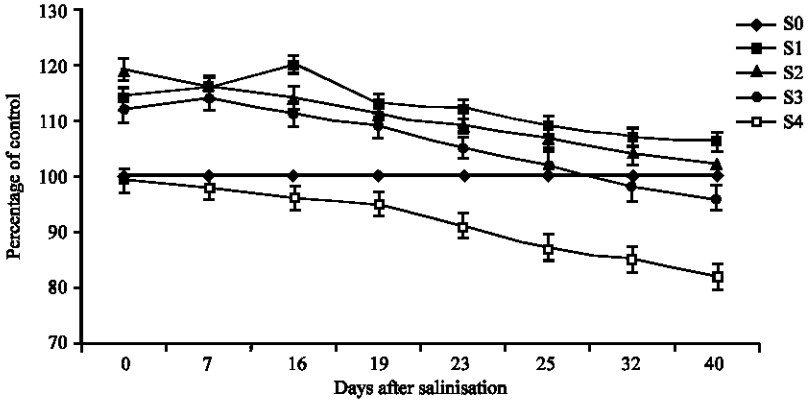

Fig. 1: Shoot height of avocado rootstocks irrigated with saline water for 40 days. Each point represents the mean relative to control of four replications±SE

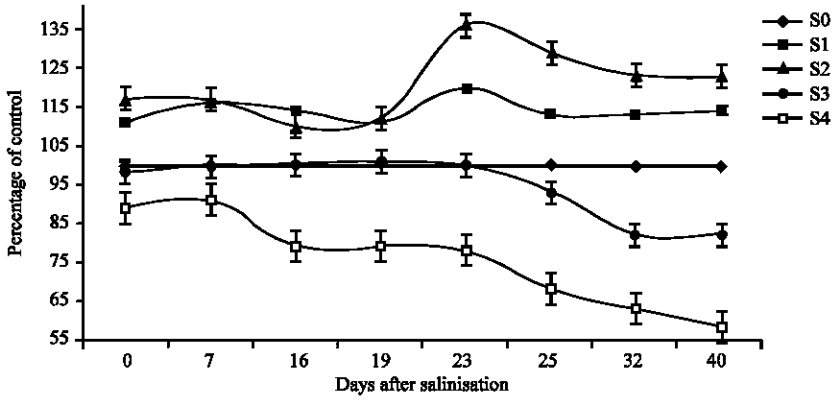

Fig. 2: No. of leaves per plant of avocado stocks irrigated with water for 40 days. Each point represents the mean relative to control four replications±SE

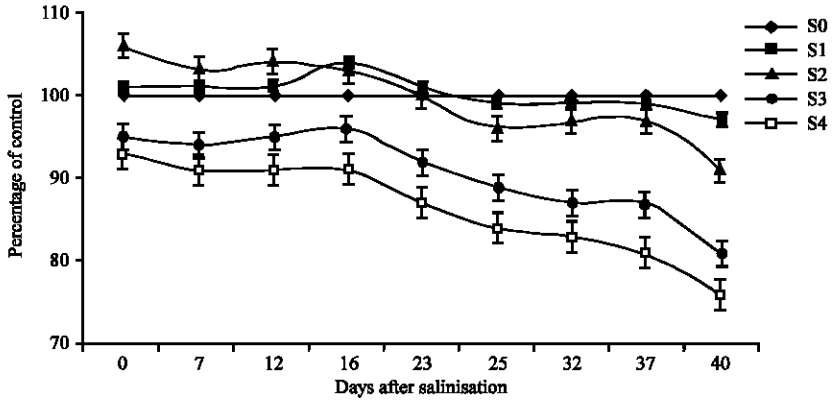

Fig. 3: Stem diameter of avocado rootstocks irrigated with saline water for 40 days. Each point represents the mean relative to control of four replications±SE

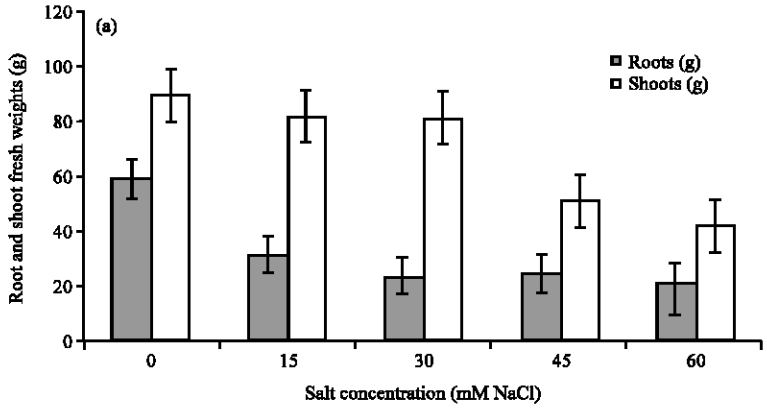

Fig. 4a: Effect of NaCl salinity on fresh weight avocado roots and shoots after 40 days of salinisation. Means of four replicates±SE

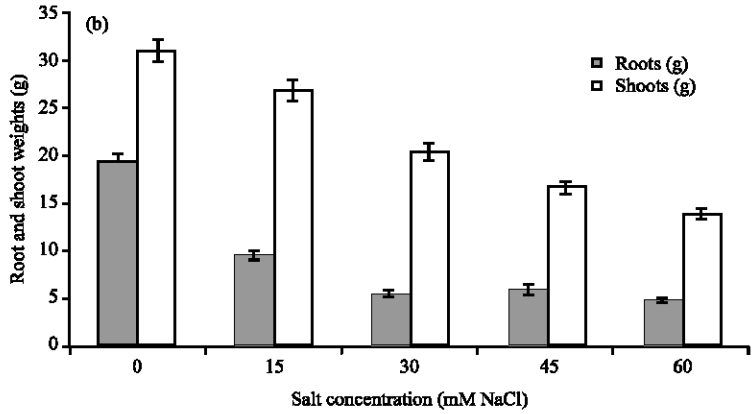

Fig. 4b: Effect of NaCl salinity on dry weight of avocado shoots and roots after 40 days of salinisation. Means of four replicates±SE

Table 1: Effect of saline water irrigation on leaf chlorophyll content 40 days since the initiation of the experiment

Treatments	Chlorophyll a	Chlorophyll b	Total chlorophyll (t Ch)
NaCl (mM)			
0	140.0[a]	32.0[a]	172.0[a]
15	117.0[ab]	26.5[ab]	143.5[ab]
30	93.8[b]	19.1[b]	110.0[b]
45	86.9[c]	18.4[c]	105.2[c]
60	75.8[c]	14.9[c]	90.7[c]

[z]: Letter(s) show significant differences at $p<0.05$. Mean separation within columns by Duncan's multiple range test

Table 2: Analysis of variance for CO_2 assimilation rate and related parameters

Parameters	Source	df	MS	F	Pr>F
CO_2 assimilation rate	Treatment (S)	4	13.424375	21.56	NS
	Error a	15	0.62264583		
	Time (T)	7	13.10199107	30.94	SF
	SXT	28	1.732125	4.09	NS
	Error b	105	0.42340774		
Stomatal conductance	Treatment (S)	4	205.021875	3.12	NS
	Error a	15	65.7354167		
	Time (T)	7	508.134821	7.96	SF
	SXT	28	66.382589	1.04	SF
	Error b	105	63.835417		
Transpiration rate	Treatment (S)	4	0.04239063	2.33	NS
	Error a	15	0.01818917		
	Time (T)	7	0.75737143	106.64	SF
	SXT	28	0.02486027	3.50	SF
	Error b	105	0.00710202		
Intercellular CO_2 concentration	Treatment (S)	4	519910.802	10.56	NS
	Error a	15	49249.497		
	Time (T)	7	414188.909	19.59	SF
	SXT	28	72156.921	3.41	SF
	Error b	105	21143.938		
Leaf temperature	Treatment (S)	4	24.444125	71.51	NS
	Error a	15	0.3418125		
	Time (T)	7	150.27542	247.41	SF
	SXT	28	4.020554	6.62	SF
	Error b	105	0.607384		

SF: Significant, NS: Not Significant at $p\leq0.05$, or $p<0.001$, respectively

However, a significant ($p\leq 0.05$) decline in stem diameter growth was noted after 32 days of salt treatment. Stem diameter growth at 60 mM NaCl concentration reduced over time to about 76% of control plants. The dry weight of roots did not decline as rapidly with salinity stress as fresh weight (25 and 37% of control plants, respectively) at 60 mM NaCl treatment (Fig. 4a, b). Generally, the shoot fresh and dry weights declined significantly ($p\leq 0.05$) and consistently with increasing NaCl level of the growth medium (Fig. 4a, b). Shoot fresh weight was more sensitive to NaCl salinity than dry weight, which was 47 and 45% of control plants at 60 mM NaCl concentration, respectively (Table 1). Chlorophyll a an b contents and total chlorophyll (t Chl,) generally had a decreasing trend with increasing levels of NaCl in the growth medium. Total chlorophyll and Chl a were more in the leaf than Chl b.

Gas Exchange

CO_2 assimilation rate was lower in plants grown under salinity than in controls. Salinity treatments had significant effect on CO_2 assimilation rate over time ($p\leq 0.001$) (Table 2) after 40 days of irrigating with saline water. At 60 mM NaCl, plants were more affected by salinity compared to all other levels. Stomatal conductance of salinised plants decreased significantly ($p\leq 0.001$) over time in contrast to control plants (Table 2). Transpiration rate (E) decreased in response to increasing salt concentration in the irrigation water. Significant differences in transpiration rates were evident in all the days during experimental period ($p\leq 0.05$). This parameter was not consistent with the salinity

Fig. 5: Effects of NaCl Salinity on growth and development of avocado plants after 40 days of saline water irrigation

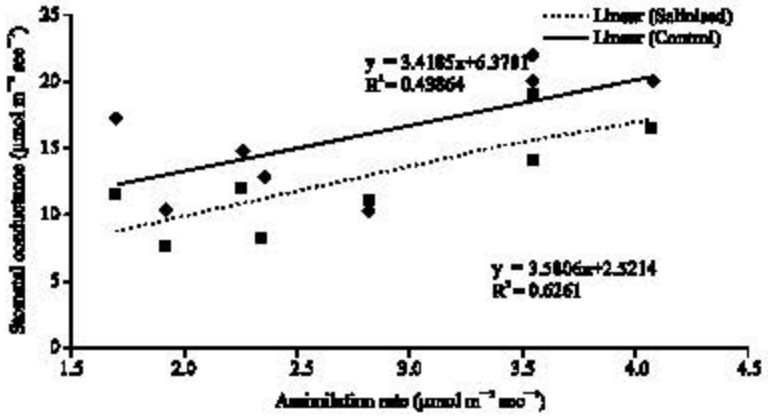

Fig. 6: Relationship between CO_2 assimilation and stomatal conductance in control and salt stressed avocado plants (60 mM NaCl). Data points represent mean of four values measured on the fifth leaf from the shoot apex

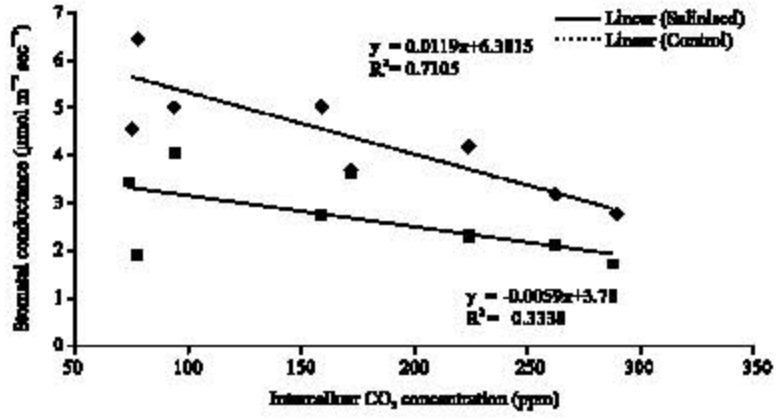

Fig. 7: Relationship between Assimilation rate and Intercellular CO_2 concentration in control and salt stressed avocado plants (60 mM NaCl). Data points represent mean of four values measured on the fifth leaf from the shoot apex

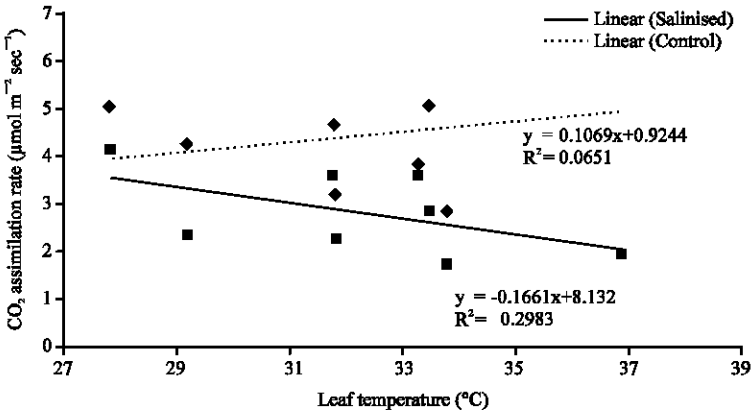

Fig. 8: Influence of leaf temperature on CO_2 assimilation rate in control and salt stressed avocado plants (60 mM NaCl). Data points represent mean of four values measured on the fifth leaf from the shoot apex

treatments throughout the experimental days. A linear relationship between CO_2 assimilation rate and stomatal conductance was found (Fig. 6). However, CO_2 assimilation rate for a similar stomatal conductance value was always higher in control plants than in salinized plants. Assimilation rate continued to decrease as intercellular CO_2 concentration increased (Fig. 7). Figure 8 shows the influence of leaf temperature on CO_2 assimilation rate under salt stress conditions. The results indicate that about 7% of the observed decreases in CO_2 assimilation were due to temperature ($R^2 = 0.0651$, control plants) and about 30% ($R^2 = 0.2983$, salinised plants) of the decreases were due to the interaction between temperature and salt stress.

DISCUSSION

Salt tolerance is the ability of plants to maintain growth in saline environments (Mickelbart and Arpaia, 2002) and is commonly expressed as the yield decrease for a given level of soluble salts in the root zone as compared to the yield of non-saline controls (Alian *et al*., 2000). Growth at high salinity resulted in reductions in dry matter production of both shoot and root tissues (Fig. 4a, b). Both dry and fresh weights of shoot and root tissues decreased with increase in concentration of salt. However, the reduction in the case of shoots was not pronounced. Root growth of avocado was more sensitive to salinity than shoot growth at high salt concentration, in good agreement with the reports by Shalhevet (1999) and Crowley and Smith (1999).

There was a marked change in the relative allocation of dry weight to different plant components (Fig. 4b). The dry matter content of salinised plants was slightly lower than controls. At low salinity root growth may not decrease at all, while shoot growth declines (Greenway and Munns, 1980; Munns and Termaat, 1986) or shoot growth may even increase (Dudeck *et al*., 1993). Growth at the highest salinity level resulted in reduced leaf number per plant by about 58% of control plants. The reduction in shoot dry weight could be associated with lower leaf production and development of smaller leaves. Increased leaf death and leaf fall were also evident during the study. Leaf production and expansion may be the processes particularly sensitive to salinity (Cramer *et al*., 1994; Lutts *et al*., 1996).

Excess sodium showed up as an interveinal leaf burn and necrosis. Typical toxicity symptoms were leaf burn, scorch and dead tissues along the outside edges of leaves in contrast to the symptoms of chloride toxicity, which normally occurred initially at the extreme leaf tip (Fig. 5). The oldest leaves

exhibited the greatest level of leaf burn and necrosis. Excessive accumulation may cause burning of the leaf tips or margins, browning and premature yellowing of leaves (Shalhevet, 1999). The results indicate that, this species does not exclude NaCl, as do some salinity tolerant plants but accumulates these electrolytes, probably to provide osmotic adjustment (Hasegawa *et al*., 2000).

Rapid root growth inhibition by high NaCl levels has been observed for most crops (Reinhardt and Rost, 1995). Salt stress inhibited root growth in Spider plant (*Cleome gynandra*) (Mwai, 2001), although root growth was less pronounced and consistent compared to the shoot responses. Roots might seem the part of the plant most vulnerable as they are directly exposed to salt or to drying soil but their growth rate is not affected as that of shoots (Munns, 2002). Present results agree with the findings of Reinhardt and Rost (1995) who noted the most common salt stress effect to be the general stunting of plant growth. Salt stress is known to alter cellular metabolic activities such as normal protein synthesis (Lutts *et al*., 1996) and could be one of the reasons for the observed decreases in fresh and dry weights at higher salt levels in this study.

The results of chlorophyll concentration (Table 1) indicated that Chl a was higher than Chl b in the salt treated avocado plants. This shows that NaCl salinity induced a marked decrease in Chl b within the salt treatment range. Earlier findings by Lutts *et al*. (1996) have indicated that Chl b is associated with PS II antenna and its reduction may suggest structural damage of the PS II reaction centres. Salinity may inhibit the synthesis of chlorophyll or accelerate its degradation (Netondo *et al*., 2004b). Reduction in chlorophyll concentration due to salinity may be partially responsible for the reduced photosynthetic rates of avocado plants during the experimental period (Table 2) and may explain the reduction in biomass accumulation in this study (Fig. 4b). Reduction in photosynthetic capacity is usually associated with the decline in growth caused by salinity (Munns and Termaat, 1986; Sibole *et al*., 2003). Closure in stomata may account for the decreased stomatal conductance and may have been responsible for the reduction in transpiration rates in salinised plants. Sodium chloride accumulation in leaves has been correlated well with reduced photosynthetic activity and with ultra-structural and metabolic damage (Yeo and Flowers, 1986; Netondo *et al*., 2004a). High intercellular CO_2 concentration in salt stressed avocado plants (Fig. 7) imply reduced activity of the chloroplasts to fix CO_2 and may help to explain the low photosynthetic rate in salinised than control avocado plants. The accumulation of NaCl can reduce photosynthetic efficiency of the leaves before they die (Fig. 5) and this, in association with early senescence, may contribute to the ever-decreasing growth and low photosynthetic rate (Ashraf *et al*., 2002; Munns, 2002; Netondo *et al*., 2004a). The results in Fig. 8, suggest that reduction in photosynthetic rate of avocado plants was influenced by temperature, but also other factors such as chloroplast impairment or because of sensitivity of Rubisco enzyme to chloride ions toxicity (Seemann and Critchley, 1985; Soussi *et al*., 1998) played a role to inhibit photosynthetic rate.

We concluded that, the reduction in photosynthetic rate and growth of avocado seedlings associated with salinity stress is likely to be a consequence of a number of differing effects of salt on plant processes, such as net photosynthesis with combination of other factors such as reduced pigment content and impaired PS II photochemistry. The study indicated that an appreciable proportion of the reduction in growth could be accounted for by the reduction in biomass accumulation, leaf necrosis and reduced number of leaves, through death and fall. The current study confirms previous results, which have demonstrated that avocado plants are sensitive to NaCl salinity (Mickleart and Arpaia, 2002). The results of this study indicated that avocado seedlings are sensitive to high salinity levels (45 and 60 mM NaCl). In particular, avocado plants showed no adverse effects on growth if NaCl concentration was lower than 30 mM NaCl. Salt stress was found to cause irreversible and visible damage, such as leaf tip burn, leaf scorching and death. These findings indicate a need for further studies concerning water relations and chlorophyll fluorescence induction kinetics and various fluorescence parameters to detect salt effects before visible plant damage occurs.

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