Photosynthetically Active Radiation Influence on Gas Exchange Parameters of Avocado Seedlings Growing Under Saline Conditions

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Abstract: Under natural conditions, photosynthesis and stomatal conductance are normally limited by Photosynthetically Active Radiation (PAR), temperature, water stress and salinity. A study was conducted at Maseno University, Kenya in February, 2005 to investigate on growth and physiology of avocado seedlings tolerance to salinity under naturally illuminated glasshouse conditions. Plants were grown in 4.5 L plastic pots containing soil and were subjected to either 0 (control) or 60 mM NaCl salinity treatments. Growth parameters, net photosynthesis, stomatal conductance, transpiration rate, Water Use Efficiency (WUE) and leaf chlorophyll content were all reduced by the salinity of the growth medium. Intercellular CO₂ concentration and leaf chloride content were increased by the salt treatment. The study demonstrated that PAR and salinity play a substantial role in limiting net photosynthesis in this variety of avocado (Puebla). The greater intercellular CO₂ concentration at medium salt levels may indicate impairment of photosynthetic apparatus. Based on observations of water use efficiency it can be concluded that the stressed plants somehow had an elaborate mechanism of water conservation. More research is needed for different avocado rootstocks and soil types over a wide range of environmental conditions.

Key words: Photosynthetically active radiation, saline conditions, stomatal conductance

INTRODUCTION

Salt stress is one of the major environmental stresses that cause decreases in growth and photosynthesis (Munns and Termaat, 1986; Netondo et al., 2004b; Musyimi et al., 2007). Many arid and semi-arid regions in the world contain soils and water resources that are too saline for most important crops. Salinity affects plants through osmotic stress, ion toxicity, oxidative stress and mineral deficiencies (Reinhardt and Rost, 1995; Hasegawa et al., 2000; Netondo et al., 2004a). Accumulation of solutes in roots leads to lowering of the osmotic potential of the root, which maintains the driving force for extracting soil water under water deficit conditions. Salt stress induces cellular accumulation of damaging active oxygen species hence damaging membrane lipids, proteins and nucleic acids. Photosynthesis is a major plant process which contributes to plant growth and productivity and may be reduced by stomatal closure restricting the supply of CO₂ to the photosynthetic apparatus in leaves (Ashraf et al., 2002). Closure will limit photosynthetic activity thereby total plant dry matter accumulation. A decrease in photosynthesis can be an indirect consequence of the impaired physiology growth of the plant growing under saline conditions (Greenway and Munns, 1980; Aro et al., 1993; Munns, 2002).

Changes in photosynthetic parameters and chlorophyll content could potentially be used as tools for screening for salt tolerance in plants. The more tolerant cultivars exhibit fewer disturbances in photosynthetic processes (Munns, 2002). Under natural conditions, photosynthesis and stomatal conductance are normally limited by PAR, temperature, (Tanaka et al., 2000), Varsden Heuvel and Davenport, 2005), water stress and salinity. Reduction in photosynthesis is directly related to stomatal conductance, though non-stomatal factors are also associated with lower photosynthetic capacity in salt treated plants (Tan and Buttery, 1986; Shalhevet, 1999; Ashraf et al., 2002; Netondo et al., 2004b). In a shaded environment, root development and energy budgets are stressed due to low PAR (Steinke and Stier, 2003). Growth and photosynthesis are particularly important under saline condition since resistance to external salinity is much influenced by plant vigour. The more vigorous the plant growth under non-saline conditions, the greater is its resistance to salt. Photosynthetic performance in plants is usually enhanced by additional environmental factors such as high PAR, water availability and soil fertility (Tan and Buttery, 1986; Reinhardt and Rost, 1995; Hofshi, 1998). Determining the dependence of gs and Pn on light irradiance (PAR) are important measurements in understanding the physiology of plant growth and development (Bongi and Loreto, 1989; Whiley and
Avocado (*Persea americana* Mill.) is one of the most important export fruit crops grown in Kenya (HCDA, 1996) and is a salt sensitive tree crop (Hosfai, 1998; Mickelbart and Arpaia, 2002). Growth rates of avocado trees on avocado rootstocks have been shown to reduce during active growth flush under stress (Mickelbart and Arpaia, 2002). Earlier study by Mickelbart and Arpaia (2002) has indicated that sensitivity to salinity among avocado cultivars is reflected in different growth reductions and leaf necrosis. Schaffer and Whiley (2003) have indicated that stomatal conductance is a more reliable early indicator of stress in avocado than measurements of leaf water content, leaf water potential or growth variables. Development of rapid screening techniques to identify salt tolerant individuals has been difficult due to lack of fundamental understanding of the physiology of the growth reduction due to high salinity and because of the complexity of the interactions between salinity and environmental factors. Photosynthetically active radiation and temperature have been shown to be the primary determinants of plant productivity (Kumudini, 2004). There is an interaction of effects of salinity and light upon photosynthesis (Drake and Read, 1981). The current interest in utilization of saline soils and home gardens in Kenya necessitates knowledge of the physiology of salt stressed avocado seedlings and responses to low PAR that could help in making rational crop management decisions, since an environmental or genetic factor that influence growth can do so by way of its effects on physiological plant processes. Documentation is lacking on the potential interactive effects between salinity and low irradiance on gas exchange and quality avocado seedlings from the literature. There is no data at all on intercellular CO₂ concentration that would aid the interpretation of the results of salinity on photosynthesis.

The objective of this study was to determine the interactive effects of salinity and low irradiance on growth and physiology of avocado seedlings. Data obtained may explain the physiological mechanisms by which salt and low irradiance affects avocado growth and development and could help improve the management techniques of avocado seedlings in shaded environments such as home gardens in arid and semi arid areas where irrigation with saline water is possible.

**MATERIALS AND METHODS**

**Plant material and growth conditions:** The experiment was conducted in February, 2005 at Maseno University, Kenya. Three month-old avocado plants (*Persea americana* Mill.) were selected on the basis of uniformity of size and transplanted in 4.5 L plastic pots filled with soil. Diammonium Phosphate (DAP) fertilizer was applied as recommended for three-month-old avocado plants (20 g per pot DAP). The pots were arranged in a complete randomized design on a bench. After two weeks, plants in containers were irrigated daily with either 300 mL saline water or tap water as a control treatment. The saline treatments were administered in step-wise fashion, adding daily increments of 15 mM until the desired concentration of 60 mM was reached to avoid shocking the plants. 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 were controlled manually. Karate was used to control leaf mites. Plants, which averaged 39±2 cm, were maintained inside a greenhouse. The growing conditions during the experimental period were: average temperature: Night/day 20/41°C, relative humidity: min/max 50/95%. Maximum PAR measured on the upper leaf surface was about 200 µmol m⁻² sec⁻¹. The experiment was repeated twice and similar results to those represented here were obtained.

**Growth parameters:** Shoot height was measured using a meter rule from the stem base to shoot apex. Stem diameter was measured using a caliper 10 cm from the stem base and leaf number per plant were established by counting the fully expanded leaves. At the end of the experiment the plants were harvested and root and shoot parts were separated. The roots were washed in tap water before drying. All the plant samples were then oven dried at 60°C to constant dry matter, after which their dry matter were determined using an electronic weighing balance to determine treatment effects on root mass (Denver Instrument, model XL-3100D) and root-shoot ratios calculated.

**Chlorophyll analysis:** Chlorophyll was extracted from fourth leaf from the shoot apex with 80% acetone and absorbance values determined on a spectrophotometer (Model Novaspec II, Pharmacia Biotech, Cambridge, England) at 663 and 645 nm, respectively. Total chlorophyll, Chl a and Chl b were calculated as described by Arnon (1949).

**Leaf chloride content analysis:** 0.1 g of finely ground oven dried tissue was digested over night with 25 mL of 0.1 M HNO₃ at room temperature according to Sibole et al. (2003). Chloride content of the leaves was determined from the aqueous extract by titration with silver nitrate.

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Gas exchange measurements: An open mode portable photosynthesis system with a datalogger (CIRAS-1, PP Systems, Stotfold, Hitchin, Herts, UK) was used to measure gas exchange and environmental parameters (net photosynthesis, transpiration rate, stomatal conductance and substomatal CO₂ concentration, PAR and air temperature). Gas exchange was determined from an area of 2.5 cm² of the fully expanded sun-exposed fifth leaf (from the shoot apex) of the plant in each treatment between 09:00 and 12:30 h. Readings were taken under steady-state conditions (60-90 sec after closing the leaf chamber). The specifications during measurements were, Cuvette air temperature varied from 30 to 36°C, Vapor pressure deficit 1.2-2.7 kPa and the CO₂ concentration in the system ca. 500 ppm. Measurements commenced on the seventh day after stressing the plants and were done once per week.

Water Use Efficiency (WUE): Water use efficiency was calculated according to Ashraf et al. (2002).

Statistical analysis: The data was analyzed using the SAS computer statistical package. All data were subjected to analysis of variance (ANOVA) to test whether there were significant differences between the treatments. Means were compared at (p<0.05). Regression analysis was used to determine the relationship (R² values) between Pₙs, PAR and stomatal conductance.

RESULTS AND DISCUSSION

Growth analysis: During the first three weeks of the study plants appeared healthy. From the fourth week leaf tip burn, leaf margin burn and chlorosis appeared followed by leaf defoliation thereafter. During the first sixteen days after the application of salt, growth was not visibly different to the control plants but after the 19th day, it was obvious that growth was reduced by saline irrigation. At the end of the study salt stress was found to have reduced the plant growth significantly. Shoot heights did not differ significantly (p>0.05) between the control plants and salt treated plants. Salinity reduced shoot height at about 74% of the control plants (Table 1). Stem diameter was also reduced by salinity even though significant differences between the treatments were evident after 32 days of saline irrigation (data not shown). The stem diameter was 63% of the control plants.

There were significant (p<0.05) differences in leaf number per plant between control and salt treated plants that occurred by 23rd day since the initiation of salt treatments. By the end of the experiment the number of leaves per plant for salt treated plants was 70% of the control plants. Salinity was found to have significant influences over the root and shoot growth. At the end of the study, both the root and shoot dry matter were 50 and 23% of control plants, respectively. Because of the greater depression in shoot growth, the root: Shoot ratio was lower for the plants growth under saline conditions. The root:shoot ratio of salt treated plants was 56% of the control plants (Table 1). Effects of salt treatment on the pattern of dry matter accumulation of these plants were generally similar to the fresh weight patterns (data not presented), although fresh weight was more sensitive to NaCl salinity.

Generally salinity reduced plant growth and Pₙ in avocado. Shoot height and stem diameter were affected by salinity leading to stunted growth of the plants. Avocado leaves were more sensitive to stress during flushing when there is high percentage of young leaves in agreement with the results by Schaffer and Whiley (2003). Young plants partition assimilate from lower leaves to roots during early vegetative growth. Reduction in Pₙ may be linked to the high amounts of chloride ions in the avocado leaves leading to increased incidence of leaf burn, necrosis leaf margins and defoliation. This may also account for decreased transpiration rates in the salt treated plants due to reduced number of leaves (Table 1). Reduction in shoot and root dry matter could as well have resulted from defoliation hence reduced allocation of photosynthates to roots during saline water treatments. Root damage and root death due to salt injury may decrease sink activity leading to decreased Pₙ. Increased cell wall thickness and thick cuticular leaf surface due to salinization may also have reduced the rate of transpiration (Hasegawa et al., 2000; Musyimi et al., 2007).

Leaves of salt treated plants had significantly (p<0.05) lower chlorophyll content than control plants.

<table>
<thead>
<tr>
<th>Table 1: Shoot height, stem diameter, shoot dry matter, root dry matter, root:shoot ratio, chlorophyll content</th>
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<tbody>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>Shoot height (cm)</td>
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<tr>
<td>Stem diameter (mm)</td>
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<tr>
<td>Leaf number plant⁻¹</td>
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<tr>
<td>Shoot dry matter (g)</td>
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<tr>
<td>Root dry matter (g)</td>
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<tr>
<td>Root:shoot ratio</td>
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<td>Chl a (mg g⁻¹ fresh weight⁻¹)</td>
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<tr>
<td>Chl b (mg g⁻¹ fresh weight⁻¹)</td>
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<tr>
<td>Chlorophyll a+b</td>
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<tr>
<td>Chloride ions (mg g⁻¹ fresh weight⁻¹)</td>
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</table>

1: chl: Total chlorophyll concentration, Chl a: Chlorophyll a, Chl b: Chlorophyll b, Chl a+b: Chlorophyll a+b ratio (chl), chl a+b ratio and leaf chloride ions (CT) of salt stressed and control (non-stressed) avocado seedlings. All data are means of four replications±SE. Different letter(s) in the same row show significant differences at p<0.05 with t-test.
damage by the low incidental PPF. Injury of the root tips of the avocado plants during the saline irrigation may eliminate a major sink [roots] for photosynthesis reducing the needs for photosynthetic activity; hence the high intercellular CO₂ concentration. In a previous study, Apple plants treated with 250 mg L⁻¹ Apogee had a lower leaf stomatal conductance (gs) 17 days after treatment compared to control plants. The decline in gs, which occurred in the early afternoon hours, was attributed to a possible feedback inhibition of photosynthesis P₅₀ caused by the buildup of starch in the Apogee treated plants (Glenn and Miller, 2005).

Water use efficiency of salt treated plants was lower than that of control plants, about 72% of control plants. The plants must photosynthesize in order to grow but open stomata imply salt uptake as well as water loss, hence the relationship between photosynthesis and transpiration. Water use efficiency was decreased by the salinity treatment, in a similar manner to that reported in some cultivars of wheat (Ashraf et al., 2002). There were significant differences between the control and salt treated plants, which was attributed to low stomatal conductance of the salinised plants, a mechanism for water conservation (Richardson and McCree, 1985). Partial stomatal closure is essential to conserve water by reducing E. When evaporation from the leaf surface is limited by water stress, the absorbed radiation is partitioned more to warming the leaf above air temperature than evaporating water. Water use efficiency falls with advancing leaf senescing as net photosynthesis declines faster than water loss due to poor stomatal control in older leaves. The quantity of water transpired per unit fresh weight, irrespective of the carbon efficiency with which it is transpired, is of major relevance to the response of the plant to the salt. The PAR dependence of P₅₀ measured in this study (Fig. 1) indicate that even very low light levels may reduce (P₅₀) of avocado plants growing under saline conditions more than those growing in non-saline environments. The study show that PAR triggered the inhibition of P₅₀ in avocado seedlings (Fig. 1), which implies that of the observed 38% variations in salinised plants at 60 mM; about 37.9% of the variations were due to salinity, since 0.06% variations were due to PAR. The findings are also supported by the observation that salt treated avocado plants had lower stomatal conductance (Fig. 2) as compared to control plants. Decrease in chlorophyll content during the study may have contributed to decrease in irradiance harvesting and hence reduction in photosynthetic potential of the plants. Chlorophyll a/b ratio was reduced by salinity unlike the reports of Mickelbart and Arpaia (2002), where salinity did not affect Chl a/b ratio. High intercellular CO₂

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**Table 2: Different gas exchange parameters of salt stressed and control avocado seedlings**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Tap water)</th>
<th>Salt stressed (60 mM NaCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₅₀ (μmol m⁻² s⁻¹)</td>
<td>6.370±0.64ᵇ</td>
<td>2.780±0.11ᵇ</td>
</tr>
<tr>
<td>gₛ (mmol m⁻² s⁻¹)</td>
<td>30.00±2.5ᵇ</td>
<td>14.47±0.58ᵇ</td>
</tr>
<tr>
<td>Ci (ppm)</td>
<td>169.0±9.0ᵇ</td>
<td>500.0±5.0ᵇ</td>
</tr>
<tr>
<td>E (mmol m⁻² s⁻¹)</td>
<td>2.840±0.6ᵇ</td>
<td>1.740±1.0ᵇ</td>
</tr>
<tr>
<td>WUE</td>
<td>5.180±0.6ᵇ</td>
<td>3.740±0.02ᵇ</td>
</tr>
</tbody>
</table>

ős: Inter cellular CO₂ concentration, ppm: Parts per million, WUE: Water use efficiency; (P₅₀) Net photosynthetic rate, (gₛ) stomatal conductance, (Ci) inter cellular CO₂ concentration, (E) transpiration rate and water use efficiency of leaves of salt stressed and control (non-stressed) avocado seedlings; the CO₂ concentration in the system ca. 580 ppm. All data are the means of four replications±SE. Different letter(s) in the same row show significant differences at p<0.05.
Fig. 1: Relationship between photosynthetic rate and photosynthetically active radiation

Fig. 2: Relationship between photosynthetic rate and stomatal conductance

concentration of the salt stressed avocado plants than of control plants would explain the hypothesis that reduced $P_s$ values observed in this study were due to losses in leaf chlorophyll content, reduced activity of photosynthetic apparatus or even damage of photosystem II, since the chloroplast demand for CO$_2$ was decreased at low stomatal conductance (Table 2). Differences in $P_s$ between the control treatment and salt treated plants may have also been due to changes in leaf chlorophyll concentration, temperature, humidity and lower PAR.

These results indicate that salt stress can cause a rate limitation in photosynthetic system even at very low PAR. Factors such as root morphology, water and nutrient uptake and source-sink relationships for carbohydrate energy could affect gas exchange and water use efficiency of the avocado seedlings (Musyimi et al., 2007). The low $P_s$ values in avocado are expected based on previous data by Schaffer et al. (1987 and 1991) of orchard trees in Florida (7-10 μmol CO$_2$ m$^{-2}$ s$^{-1}$) at PPF of

400-600 μmol quanta m$^{-2}$ s$^{-1}$. The evolution of avocado as a small gap colonizing, under storey forest species (Whiley and Schaffer, 1994), may have resulted from inconsistently carbon fixation rates or alternative carbon fixing mechanisms (Hofshi, 1998). The results suggest a relation between low PAR values and net photosynthetic rate of avocado plants growing under saline conditions.

CONCLUSIONS

The data obtained guarantee further inquiry into avocado carbon fixing mechanism under field conditions. Farmers who choose to grow this variety of avocado (pueblo) should avoid areas with low irradiance and higher salinity levels of about 60 mM and above.

REFERENCES


