



International Journal of Botany

ISSN: 1811-9700

science
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Responses of Mango (*Mangifera indica* L.) Root Stock Seedlings to Water Stress

Elizabeth Luvaha, G.W. Netondo and G. Ouma

Department of Botany and Horticulture, Maseno University, P.O. Box 333, Maseno, Kenya

Abstract: The present studies were aimed at investigating the effect of different watering regimes on the gas exchange parameters (stomatal conductance, CO₂ assimilation and transpiration rates) and chlorophyll content of young Mango (*Mangifera indica*) rootstock seedlings. The hypothesis was that different levels of water deficit affect the gas exchange parameters and chlorophyll content of the mango rootstock seedlings. Six-month-old Mango (*Mangifera indica* L.) rootstock seedlings were grown in polythene pots in a greenhouse at Maseno University, Kenya from December 2003 to March 2004 and subjects to four different watering regimes (namely daily, twice in a week, once in a week and once in two weeks). These treatments were in a Completely Randomized Design (CRD) with six replications. The parameters determined were stomatal conductance, transpiration rate, CO₂ assimilation rate and intercellular CO₂ concentration. All the gas exchange parameters were determined by infra Red Gas Analyser (PP systems). The results showed that increasing water stress reduced the physiological parameters particularly at the later days of plant growth due to stomatal and non-stomatal factors. Leaf chlorophyll content however was slightly increased since the chlorophyll pigments may have been resistant to dehydration. It is concluded that increase in water stress (increasing level of water deficit) reduces the gas exchange parameters of mango (*Mangifera indica*) rootstock seedlings but slightly increased chlorophyll content due to an adaptive mechanism.

Key words: Assimilation rate, intercellular CO₂ concentration stomatal conductance, transpiration rate

INTRODUCTION

Environmental factors, such as soil moisture content can have a profound effect on plant metabolism and development. When drying soil causes water absorption to lag behind transpiration loss water deficit develops in the plant which, if unrelieved will result in permanent wilting and finally dehydration and death.

This deficit is typically characterized by decreases in water content, turgor and total water potential, partial or complete closure of the stomata and reduction in the rate of cell enlargement and growth (Kramer, 1983). Crop water deficit or stress is the rate of interaction between factors in the rhizosphere, the plant and the atmosphere in relation to the amount of moisture available to plants. Repeated water deficits can reduce the sensitivity of the stomata to low water potentials. The acclimation of photosynthesis in cotton (*Gossypium hirsutum* L.) has been attributed to altered stomatal response to water potential (Mathews and Boyer, 1984). Water deficit in the dryland areas may cause wilting of seedlings immediately after germination. It may give rise to undeveloped seeds for plants at development stages and in fruit trees, to premature fruit drop. A plant will suffer water deficit when its water potential decreases as a result of decreasing

water content (Prioul *et al.*, 1984) water deficit has greater effects on the plant during seedling establishment and floral development. Exposure to moisture deficit at the seedling stage confers some degree of hardening against current and later drought periods (Ashsley, 1993). However, the degree of hardening varies within varieties and species. Water deficit affects the rate of transpiration through its effect on the stomata. Ackerson (1981) observed an inverse relationship between transpiration rates and water deficit. When soil water content decreases, there is a decline in the plant water potential and the plant experiences a water deficit. Therefore the amount of water transpired reflects the amount of water in the plant and the evaporative demands of the atmosphere. Reduced transpiration rate is an important physiological indicator of water deficit (Xu *et al.*, 1995).

Previous studies on effect of water deficit has been mainly on annual crops but very few studies have been on perennial crops particularly at the seedling stage. The nursery industry in Kenya has a problem in regulating water application to ensure its high productivity because there are no documented studies on how water deficit affects the growth of seedlings especially of mango. Mango is a very important fruit crop mainly grown in the arid and semi-arid parts of Kenya.

The present study will apply modern porometry to investigate how water deficit affects gas exchange parameters. It will also apply standard methodologies to determine chlorophyll content as affected by water deficit. The objectives of the present studies are to find the effect of different watering regimes on the gas exchange parameters of young Mango (*M. indica*) rootstock seedlings and to find the effect of water deficit on the chlorophyll content of mango rootstock seedlings. The hypothesis of the study are that different levels of water deficit affect stomatal conductance, transpiration rate and that different levels of water deficit affect the chlorophyll content of Mango rootstock seedlings.

MATERIALS AND METHODS

The study was conducted in the greenhouse at Maseno University (O° 1' N-O° 2' S and 34° 2SE 34° 47' S, absl. 1500 m). It receives an annual mean precipitation of 1750 mm with a bimodal pattern of distribution. The mean temperature is 28.7°C with a relative humidity of 40% (Netondo, 1999). The soils, which are classified, as acrisols are deep, reddish brown, friable clay with the pH ranging from 4.5-5.5, soil organic carbon and phosphorus contents are 1.8% and 4.5 mg kg⁻¹, respectively (Netondo, 1999). The minimum and maximum temperatures inside the greenhouse were 26±6 and 35±6°C and 5%. Overripe mango (Ngowe cultivar) fruits were collected from Luanda market about 5 km from Maseno, Kenya. The seeds were extracted and then planted in a nursery bed. Six months after germination, the seedlings were transplanted into 20 L polythene pots measuring approximately 20 cm in diameter and 30 cm in height. The composition of the soil mixture was prepared as the rooting medium (1 sand; 2 loam: 3 compost manure). The pots were perforated at the bottom to allow for proper drainage of water in order to avoid water logging and were placed on a table in the greenhouse. All the agronomic practices such as weeding, pest control and fertilizer application were observed a part from watering which was controlled. The experimental set up was a Completely Randomized Design (CRD) comprising four treatments with six replications. Treatments commenced four weeks after transferring the seedlings to the greenhouse. The four treatments were: watering daily (W), watering twice in a week (X), watering once a week (Y) and watering once in two weeks (Z). Analysis of data was done using the SAS Statistical package to obtain analysis of variance and separation of means which was done by using the Least Significant Difference (LSD) at p<0.05). Measurement of parameters was taken for a period of 4 months during the duration of the experiment. The parameters determined

were CO₂ concentration. These measurements were taken on the most recently emerged, fully expanded and well exposed leaves under bright light using on infrared Gas Analyzer (CIRAS, 1-PPsystem, Stortfield, Hitchin, Herts, 2001) chlorophyll content was measured using the methods of Arnon (1949) and Coombes *et al.* (1987) as described by Netondo (1999) and Ashraf *et al.* (2002). The fifth fully expanded leaf was sampled from all treatments. In the laboratory, 0.5 g of the fresh leaf tissue was measured and cut into small pieces into a separate bottle. Ten milliliter of 80% acetone was added and then the set up was kept in the dark for 7 days for chlorophyll to be extracted by the acetone. One milliliter of the filtered extract was then diluted with 20 mL of 80% acetone and the absorbance of chlorophyll was read at 645 and 663 nm using a spectrophotometer (Model Novaspec II) to determine chlorophyll a(chla), chlorophyll b (chl b) and total chlorophyll (t chl) of the leaf tissue. The respective chlorophyll content in milligrams (mg) per gram (g) of leaf tissue collected was calculated using the method of Arnon (1949) as described by Netondo (1999).

RESULTS

Transpiration: The rate of transpiration was higher in the well-watered plants compared to the extremely stressed plants on all the days, except on D0 and D14 (Fig. 1). The lowest rates of transpiration in all treatments were observed on D14 when the temperatures in the greenhouse were lowest (24°C) and the humidity was higher (40%) than observed on all other days of data collection. On D0, there was no significant differences between the treatments. However on D14 there was a significant difference (p<0.05) between the treatments. At D56 a decline in the rate of transpiration was observed under extreme water stress (treatment Z) as compared to W, X and Y where an increase in transpiration was observed. Highly significant differences (p<0.001) were observed between the treatments at D28, D42, D56 and D70 (Fig. 1).

Stomatal conductance: The trend in stomatal conductance is almost similar to that of transpiration. The stomatal conductance was highest in the well-watered plants (W) and lowest in the extremely water stressed plants (Z) on all days except on D0 and D28. At D0 there was no significant difference between treatments. However, a highly significant difference (p<0.001) occurred on D14 and D42. The same case was observed between D56 and D70 (Fig. 2).

There was a steady increase in CO₂ assimilation with time except On D42 where a decline occurred. In the highly stressed plants (Z) a decline was observed from

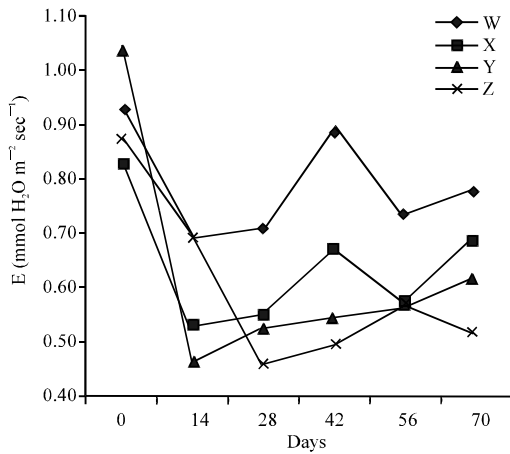


Fig. 1: The effect of water stress on the rate of transpiration (E) in mango rootstock seedlings. Values represent means of six replications

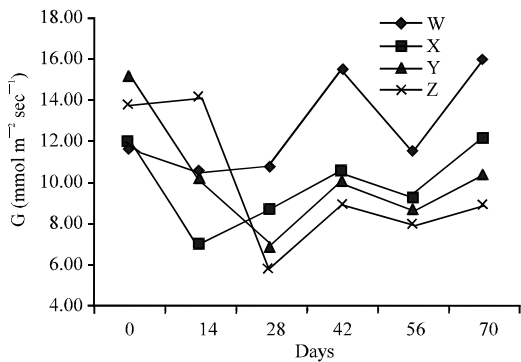


Fig. 2: The effect of water stress on leaf stomatal conductance (g) of mango rootstock seedlings grown at Maseno University, Kenya. Values represent means

D56 to D70. Significant differences ($p \leq 0.05$) occurred between particular treatments on certain days. Plants that were highly stressed (treatment Z) apparently had a higher photosynthetic rate than W and Y, which received more water (Fig. 3).

Intercellular CO_2 Concentration (Ci): The results for the effect of water stress on intercellular CO_2 concentration are shown in Fig. 3. There was an initial increase in Ci among the treatments from D0 to D28 except in treatment 2 when a decline was first observed at D14 then the Ci concentration rose up. A significant difference ($p \leq 0.05$) was observed between the treatments at D28. Generally Ci concentration was higher in the highly stressed plants (Z) as compared to W, X and Y. A highly significant difference ($p \leq 0.001$) was also observed among treatments

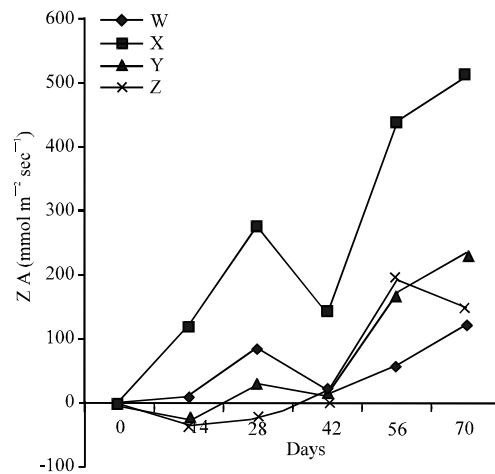


Fig. 3: The effect of water stress on the rate of CO_2 assimilation (A) on the leaves of mango rootstock seedlings. Values represent means of six replications CO_2

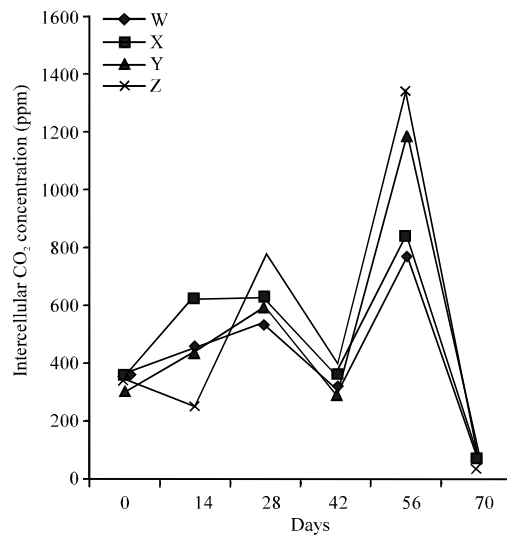


Fig. 4: The effect of water stress on intercellular CO_2 concentration (Ci) on mango rootstock seedlings. Values represent means of six replications

at D56 where the more stressed plants (Y and Z) had a higher Ci than W and X (Fig. 4).

Leaf temperature: The general trend of the graph shows a higher leaf temperature in treatment Y and Z which were the most stressed as compared to W and X. A highly significant difference ($p \leq 0.001$) was observed between treatments on all the days except at D0 (Fig. 5).

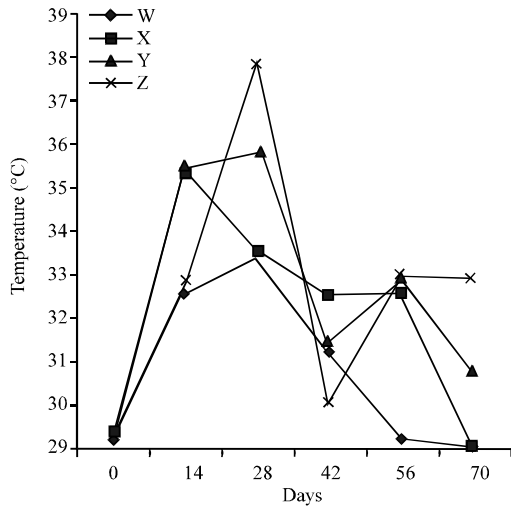


Fig. 5: Effect of water stress on leaf temperature of mango rootstock seedlings grown at Maseno University, Kenya. Values represent means of six replications

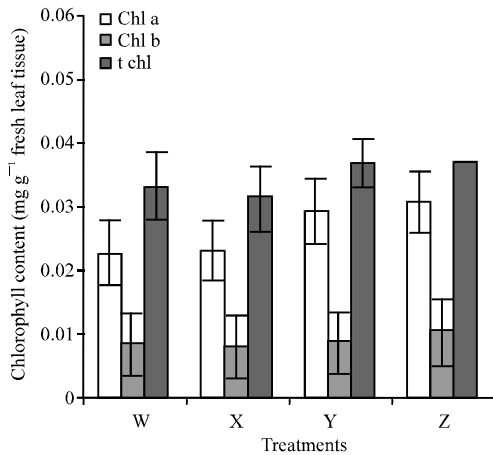


Fig. 6: The effect of water stress on chlorophyll extracted from fresh leaves of mango rootstock seedlings. Values represent means of six replications

Chlorophyll content: There was a steady rise in chlorophyll a and total chlorophyll content with increase in water stress as shown in Fig. 6. Values for chlorophyll b however remained almost constant in all treatments except in Z when there was a slight increase. Total chlorophyll showed a general increase with stress, especially between treatment Y and Z.

DISCUSSION

The reduction in transpiration with increasing water stress was apparently caused by stomatal closure. These

are characteristic responses of the stomata to environmental stress (Sharp and Boyer, 1986). Similar observations have been reported in tomato (Xu *et al.*, 1995) and in wheat (El Hafid *et al.*, 1998) and in plants subjected to water or drought stress (Bradford, 1983) Abscissic acid (ABA) plays a role in stomatal behavior in responses to these stresses and its accumulation during such periods has been previously reported (Bradford, 1983). The opening and closure of stomata controls the rate of transpiration and is regulated by cell turgidity, which is affected by water content in the cells. The rate of transpiration is also affected by environmental factors such as radiation and relative humidity and wind. The fluctuations observed in transpiration rates on specific days such as D42 (Fig. 2) can be attributed to the daily changes in these factors. However we did not determine these parameters in our study but we recommend future studies on water deficit to measure these parameters. Higher air temperatures increase the rate of transpiration and so is wind (Jones, 1992). All these factors promote evaporation of water vapour from the leaves which cause cooling. Leaf temperature however increased as water stress increased and transpiration decreased. This can be explained by the fact that closure of the stomata increases the stomatal resistance which affects the energy balance of the leaf since transpirational cooling is decreased (Netondo, 1991). This causes elevation of leaf temperature. Transpiration cools the plant by loss of latent heat of vaporization. Under extreme stress some seedlings wilted particularly after D46. The plants were unable to absorb capillary water which is scarce and tightly held by the soil particles due to low soil water potential. Other reasons for reduction in transpiration rate under increasing water stress may be morphological (increased in cell wall thickness and cell wall lignification) (Netondo, 1999) reduced leaf expansion and area (Richardson and McCree, 1985). Similarly, CO₂ assimilation rate was also reduced by water stress showing a correlation between it and transpiration which depends on the opening and closing of stomata to allow exit of water vapour and entr of CO₂, respectively. Reduction of CO₂ assimilation rate has been attributed to stomatal closure during exposure to stressful environmental conditions (Hofshi, 1998). Stomatal closure has also been indicated as the limiting factor in photosynthetic activity in fruit trees such as apple and peach seedlings in response to water deficit (Tan and Buttery, 1998). However, non stomatal factors such as inhibition of photochemical activity during period of stress may be the limiting factors of photosynthetic activity (Belkhdja *et al.*, 1999). In several instances decreased mesophyll conductance has also been observed suggesting inhibition of photosynthesis at the

biochemical level (Downtown *et al.*, 1985) CO₂ assimilation rate is affected by both stomatal and non-stomatal factors. In the present study it appears that the photosynthetic apparatus may have been resistant to dehydration since there was no decline in net assimilation rate under extreme stress. The lower rate of increase in CO₂ assimilation under water stress may be attributed to reduced stomatal conductance. However, in order for the stomatal closure to have an effect on transpiration and CO₂ assimilation, CO₂ assimilation must at least be limited by the rate of CO₂ diffusion through the stomata (Boyer, 1976). Another important factor which may have profound effect on the CO₂ assimilation rate in plants is the internal CO₂ concentration (C_i). In this study C_i seemed not to be affected by water stress without a corresponding decline in C_i and this could be due to non-stomatal effects on the photosynthetic process possibly an increase in mesophyll resistance. Similar results have also been reported in wheat (Kecheva *et al.*, 1994). A reduction in C_i can be very detrimental to the photosynthetic process especially in the presence of Rubisco enzyme which has a high affinity for oxygen (O₂) when the intercellular CO₂ concentration is low. Therefore, under low C_i, photosynthesis is limited by the Rubisco enzyme. For many species, C_i tends to remain constant over a range of environmental conditions, including water stress (Wong *et al.*, 1985).

Stomatal conductance was affected by water stress as photosynthetic rate and transpiration. The reduction in the leaf water potential may have led to the development of a water deficit in the leaves causing the guard cells to lose turgor and hence the size of the stomatal pores to reduce.

Further, the increased stomatal resistance may have led to reduced water transport in the leaves further causing a decrease in the stomatal conductance. Reduction in stomatal conductance decreases transpiration and photosynthesis (Tereza *et al.*, 2002), as is also demonstrated in the present study. In certain plants, stomatal conductance declines even before severe water stress sets in thereby avoiding desiccation during drought. This has been observed in *Quercus ilex* (Fortelli *et al.*, 1986).

There was higher Water Use Efficiency (WUE) in the stressed plants as compared to well watered plants. WUE is defined as the ratio of the leaf photosynthesis to transpiration (A/E) measured simultaneously (Smith *et al.*, 1998) or the carbon gained during the photosynthesis in the relation to the water lost during transpiration (Jones, 1992). Similar results were obtained in Papaya (*Carica papaya*) by Clemente and Marler (1996). There is an adaptive behavior in plants

growing in arid areas. However, there is a consequence of maximizing WUE by minimizing water loss. Carbon gain is in fact not maximized. Water conservation at the expense of carbon acquisition is probably an adaptation for mango in water-limited areas.

Chlorophyll content is another factor that affects the photosynthetic process in green plants. In our study, however, chlorophyll a was more resistant to dehydration; it increased slightly with water stress as compared to chlorophyll b, which was constant. The slight increase in total chlorophyll pigments in these leaves were somewhat resistant to dehydration. Another possible explanation may be that chlorophyll synthesis was induced by water stress, especially chlorophyll a.

CONCLUSIONS

Clear results show that the rate of transpiration reduced with increasing water stress. Apart from water stress, transpiration is also controlled by other atmospheric factors such as temperature humidity and air movements (wind). These factors are however controlled in the greenhouse. As transpiration reduced, leaf temperature increased. This confirms that the transpiration process cools the leaf surface by reducing the latent heat of vaporization. Drying and shedding of lower leaves, observed under extreme stress in this study alone is a mechanism for water conservation. Other mechanisms involved in mango seedlings water conservation in drought prone areas need to be established.

The rate of CO₂ assimilation was significantly reduced by water stress. Other factors that also reduced under extreme water stress were the intercellular CO₂ concentration and the chlorophyll content. A reduction in transpiration coupled with an increase in CO₂ assimilation implies that photosynthesis was largely controlled by non-stomatal factors. Continued CO₂ assimilation under water stress is only possible in the drought tolerant plants. This implies that the photosynthetic apparatus is resistant to drought. Increase in chlorophyll a may be due to the continued synthesis of this pigment even under stress conditions, however, further research is required to reach this kind of conclusion.

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